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Insect Meal: A Future Prospect of Source of Protein and Trace Minerals in Practical Feeding of Pigs

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ABSTRACT

Increasing consumption of animal-derived foods is driving higher utilization of protein sources, especially soybean meal and fish meal, thereby straining feed availability. As a renewable resource, insects provide a rich supply of digestible protein, essential amino acids, and important minerals, making them a promising substitute for conventional feeds.Their production can be integrated into circular bio-economy systems through the use of organic waste streams, reducing both feed costs and environmental impact. Among the various insect species assessed, the silkworm pupae (*Bombyx mori*), housefly larvae (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), and black army fly larvae (Hermetiaillucens) have demonstrated the most potential for inclusion in swine diets. Studies indicate that insect meals can support growth performance, nutrient utilization, immune response, and gut health, while also partially replacing conventional protein sources without compromising productivity. However, large-scale adoption is challenged by regulatory gaps, variation in nutrient profiles, consumer perception, and cost of mass production. Addressing these constraints through standardized processing, safety guidelines, and value-added innovations will determine the role of insects as a viable protein resource in practical pig feeding systems.

KEYWORDS: Insect meal, Micronutrients, Protein source, Sustainability, Swine nutrition.

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INTRODUCTION

Rapid demographic and socioeconomic changes are predicted to sharply elevate the consumption of meat and animal protein worldwide in the coming years (FAO, 2013). Pork remains the most consumed meat worldwide and will be a key contributor in addressing this demand. However, the livestock sector faces challenges in securing sustainable feed resources. Rising costs, competition with human diets, and ecological issues are reducing the sustainability of conventional protein feeds such as soybean meal and fishmeal (Henry et al., 2015; Makkar et al., 2014). This situation underlines the demand for alternative sources that balance nutritional adequacy with environmental sustainability.

Owing to their rich nutrient composition—characterized by quality protein, balanced amino acids, functional fats, and substantial concentrations of iron and zinc—edible insects are being explored as sustainable feed alternatives (Finke, 2002; Spranghers et al., 2017; Lu et al., 2022). These characteristics are especially relevant in pig production, where issues like iron-deficiency anaemia in piglets and zinc-dependent growth or health problems continue to be of concern. Furthermore, insect farming can be integrated into circular production systems, as insects can efficiently convert organic by-products and side streams into nutrient-dense biomass (Newton et al., 2005; Oonincx et al., 2015), thereby reducing reliance on conventional crops and lowering waste generation.

Despite their promise, the incorporation of insects into pig diets remains at a developmental stage. Knowledge gaps persist in areas such as nutrient digestibility, bioavailability of minerals, ideal inclusion rates, and long-term impacts on

growth, health, and product quality (Biasato et al., 2019; DiGiacomo & Leury, 2019; Hong & Kim, 2022; Ringseis et al., 2021; Yoo et al., 2019). In addition, factors including consumer acceptance, safety considerations, regulatory constraints, and production costs influence their adoption on a commercial scale (Garofalo et al., 2019; Makkar et al., 2014).

Insects as Feed Resources for Pigs

Common Insect Species for Pig Nutrition

Several insect species have been investigated as feed resources for pigs, and studies reveal marked differences in their nutrient profile, digestibility, and ease of mass production (Biasato et al., 2019; Makkar et al., 2014). Among them, certain species have gained greater attention due to their ease of mass production, high protein content, and established safety profile. Insects considered for animal feeding are generally grouped into several taxonomic orders: Diptera (e.g., black soldier fly, housefly) Coleoptera (e.g., mealworm), Megadrilacea (e.g., earthworms), Lepidoptera (e.g., silkworm), and Orthoptera (e.g., grasshoppers, locusts, crickets) (Oonincx et al., 2015). These species have been identified as practical and resource-efficient replacements for commonly used protein feeds, offering a cost-effective option for animal production (Hong et al., 2022). Beyond their substantial protein contribution, insects also supply essential minerals and bioactive molecules, which add further value to their role in pig nutrition (Spranghers et al., 2017).

1. Black Soldier Fly (*Hermetiaillucens*)

Considering the diversity of insects assessed as potential feed ingredients,feed applications, the black soldier fly (BSF) has received the greatest research attention in animal nutrition.The larvae are capable of transforming food scraps and other organic residues into a nutrient-dense, protein-rich biomass. BSF larvae meal contains 40–45% crude protein and 30–35% fat, with favorable levels of lysine and methionine (Makkar et al., 2014). The fat fraction is particularly rich in lauric acid, which possesses antimicrobial properties (Spranghers et al., 2017). The digestibility of BSF protein in pigs has been reported to be comparable to soybean meal, making it a promising alternative feed ingredient (Cullere et al., 2016). One study reported that iron and zinc content of larvae was 1.4 mg per kg DM and 108 mg/kg DM respectively (Arango Gutiérrez et al., 2004).

2. Housefly (*Musca domestica*)

Their nutrient profile typically includes 40–60% crude protein and 15–20% fat, while the essential amino acid composition closely resembles that of fishmeal (Finke, 2002; Makkar et al., 2014). Their rapid life cycle and ability to grow on diverse substrates enhance feasibility for mass rearing. Incorporation of housefly larvae meal in pig diets, even at levels displacing nearly half of standard protein meals, has not been associated with negative growth responses (DiGiacomo & Leury, 2019; Hong & Kim, 2022).

3. Mealworm larvae (*Tenebrio molitor)*

Mealworms are widely studied within the Coleoptera order. Nutrient analyses show that insect larvae typically provide a high protein content, ranging from about 45–55%, along with lipid levels that may reach nearly 30% heir amino acid composition, particularly in sulfur-containing amino acids, is favorable for monogastric animals (Spranghers et al., 2017; Lu et al., 2022). The lipid profile of mealworms, dominated by unsaturated fatty acids, has the potential to affect fat deposition characteristics in swine. Large-scale rearing is feasible under controlled conditions, although costs remain higher than BSF (Oonincx et al., 2015). While mealworms consume a wide variety of foods in nature, rearing practices commonly employ wheat bran or flour as a base substrate, sometimes enhanced with protein-rich additives like soybean meal, yeast, or skimmed milk powder (Makkar et al., 2014). Their larvae provide a valuable source of trace minerals, with reported concentrations of iron (≈66.9 mg/kg) and zinc (≈104.3 mg/kg) (Spranghers et al., 2017).

4.Earthworm (*Eisenia fetida* and others)

Earthworms, belonging to Megadrilacea, have long been evaluated as unconventional protein sources. The nutrient profile often ranges from 55–65% digestible protein, accompanied by appreciable amounts of calcium, magnesium, and iron. However, large-scale production remains challenging due to slow growth and rearing difficulties (Ijaiya and Eko, 2009). Limited feeding trials in pigs indicate potential as a supplementary protein source (Biasato et al., 2019). Unlike earthworms that ingest soil together with organic matter, epigeic types feed solely on organic substrates. Their meal provides about 63% protein, making it a potential protein supplement for monogastric animal diets. Apart from its higher protein content it is also rich source of iron

(1050–2990 mg/kg) and could be the possible source of iron supplementation in the diet of pigs (Spranghers et al., 2017).

5. Grasshoppers*(Locusta migratoria)*

Natural settings such paddocks, ponds, croplands, and meadows are good places to gather grasshoppers (GH) (Khusro et al., 2012). Utilizing these insects as feed not only provides a sustainable protein source but also helps in reducing dependence on chemical pesticides for their control. In this way, insects that are otherwise considered pests can be converted into a valuable and low-cost protein supplement for poultry diets, particularly in resource-limited regions. Grasshopper meal is a good source of protein (29 to 77.1%) and particularly rich source of Zn (17.34 mg/100 g DM) and Fe (16.19 mg/100 g DM)) possible a better alternative of protein with additional advantage of higher iron and zinc content in the diet of pigs (Makkar et al., 2014, Newton et al., 2005).

6.Locust (*Schistocerca gregaria*)

Locusts, often described as desert locusts, migratory locusts, brown locusts, or red locusts, are utilized in animal nutrition under the term *locust meal*. In pig and poultry feeding, they can be offered either in live form or after processing. Common processing methods include boiling, drying, and subsequently grinding them into meal, which improves handling, storage, and incorporation into diets (Khusro et al., 2012). Locust meal is recognized as a rich source of crude protein (52–76%) and also supplies appreciable amounts of minerals, particularly iron (8.3–13.7 mg/100 g DM) and zinc (14.6–18.6 mg/100 g DM) (Khusro et al., 2012).

7.Crickets (*Gryllustestaceus walker*)

Crickets are commonly found in habitats such as paddy fields and fallow lands. They are highly adaptable and can thrive on diverse organic substrates, including forage-based diets, agricultural by-products, residues from the feed industry (such as spent grains and mung bean sprout waste), as well as various weeds. These low-cost and sustainable feed sources make cricket farming economically viable. Crickets are also relatively easy to rear under farm conditions, which explains their popularity in countries like Thailand, where approximately 20,000 farmers are engaged in cricket production (FAO, 2013). In terms of nutrition, crickets stand out for having a high crude protein content, often between 55% and 73%. Crickets are good source of Zn (515–1032 mg/kg) and Fe (31–100 mg/kg) which makes it's as a good alternative for protein and minerals in the diet of pigs (Makkar et al., 2014; Lu et al., 2022).

8. Silkworm

Different silkworm species reported in literature include ***Bombyx mori Linnaeus, Antheraea assamensis, Antheraea mylitta, Antheraea paphia*** and ***Samia cynthiaricini*** (Makkar et al., 2014). Silkworm pupae are obtained in bulk as waste material after silk is extracted from the cocoons by spinning or reeling processes (Khatun et al., 2005). The larval stage of the moth ***Bombyx mori*** is commonly known as the silkworm and is the primary source of global silk production, contributing nearly 90% of the commercial supply from its cocoons. Approximately 90% of commercial silk is derived from the cocoons of this domesticated insect (Ijaiya and Eko, 2009). The female moth lays a tiny, black egg from which the caterpillar grows. It grows to a full size of 7.5–10 cm in 4–6 weeks by continuously feeding on mulberry and shear butter leaves (Ijaiya and Eko, 2009). Silkworm is regarded as a very good unconventional source of protein (51.8-55.6%) for pig diets following adequate processing. It is also a strong source of iron (15.9 mg/100 g DM) and zinc (16.8 mg/100 g DM), which is an added benefit (Ijaiya and Eko, 2009).

Table 1. Mean levels of protein, iron, and zinc in different insect species evaluated as pig feed sources (FAO, 2013)

Common name	Scientific name	Crude protein (% DM)	Fe (mg/100 g DM)	Zn (mg/100 g DM)
House cricket	*Acheta domesticus*	62.9	9.1	19.9
Tropical house cricket	*Gryllodessigillatus*	70.0	4.23	13.9
Migratory locust	*Locusta migratoria*	59	13.7	14.8
Desert locust	*Schistocerca gregaria*	76	8.38	18.6
Lesser mealworm	*Alphitobiusdiaperinus*	67.85	21.80	26.80
Yellow mealworm	*Tenebrio molitor*	51.7	5.3	11.8
Silkworm	*Bombyx mori*	51.8	15.9	16.8
Termite	*Macrotermessubhyalinus*	38.8	61.9	9.5
Longhorn grasshopper	*Ruspoliadifferens*	43.7	14.8	14.9
Cornfield grasshopper	*Sphenarium purpurascens*	65.2	18	42

Iron and Zinc Bioavailability from Insect-Based Foods

The percentage of a nutrient that is absorbed from the diet and made accessible for usage by the body is known as nutritional bioavailability. It is influenced by factors like age, sex, nutritional status, and feed quality. The process of bioavailability includes the nutrient's release from food, digestion by enzymes, absorption in the intestines, transfer to the bloodstream or lymphatic system, distribution, and excretion. In vertebrates, iron (Fe) is mainly found as myoglobin and hemoglobin, while in insects, it primarily exists in cytochromes. The absorption efficiency of iron bound to cytochromes is considered comparable to that found in hemoglobin and myoglobin. Insects also store Fe as ferritin and holoferritin, which can hold thousands of Fe atoms, enhancing its bioavailability (FAO, 2013). Phytoferritin in legumes like soy is more bioavailable than Fe from reduced salts, as it protects Fe from anti-nutritional factors like phytates, oxalates, and tannins (Finke, 2002).

Processing of insects into insect meal

Using insect meal to feed pigs can be a sustainable and nutritious choice. Ensuring food safety in insect-based feed requires adherence to hygiene and safety standards comparable to those used for conventional food and feed ingredients.The processing method applied depends on the type of insect, associated safety concerns, and the intended end product. The processing pathway for producing insect meal is illustrated in Figure 1.

1. Selection of Insects

Black soldier fly larvae, grasshoppers, mealworms, locusts, crickets, silkworms and earthworms are among the often-employed insects.

2. Insect Farming

Establish an optimal environment for insect breeding, which includes appropriate containers, temperature, humidity, and ventilation. Offer organic waste or specialized feed that is suitable for the selected insect species (e.g., fruit scraps for black soldier fly larvae). Insects can effectively convert materials such as vegetables, fruits, grains, and animal waste into proteins and lipids.

3. Harvesting

Once the insects attain the required size or age, they are harvested either manually or using automated systems. Sieving is typically used to separate larvae, such as mealworms and smaller mealworms, from their substrate. Crickets and grasshoppers, on the other hand, can be collected manually or by gently shaking them out of their hiding spots inside the rearing system. By depriving the insects of food, some farms also implement a temporary fasting period prior to harvest (Garofalo et al., 2019). This practice encourages the insects to clear their intestine (Finke, 2002), which breeders believe enhances flavor and produces cleaner products with lower microbial levels.

4. Processing

i. **Euthanization:** After harvesting, insects undergo several post-harvest treatments before consumption, the first step is to euthanize them. Typical methods for this process include freezing, immersing in hot or boiling water, and steaming. This step is crucial because it influences the final product's microbial load, nutrient content, color, and flavor, among other critical quality attributes (Larouche et al., 2019). The following euthanasia techniques have been investigated for black soldier fly larvae: blanching, desiccation, freezing, high hydrostatic pressure, grinding, and asphyxiation using vacuum, 100% CO_2, or 100% N_2 (Larouche et al., 2019). In terms of processing time, final moisture content, lipid oxidation, microbiological load, and color stability, blanching—immersion in boiling water for 40 seconds—was the most successful method among them. In order to inactivate vegetative microorganisms without damaging bacterial spores, blanching entails short boiling the food and then quickly chilling it in cold water (Xiao et al., 2014).

ii. **Cleaning:** The euthanasia procedure can be combined with washing or rinsing, as is done with fresh produce, to lessen germ contamination. Research has shown that rinsing smaller mealworms with sterile distilled water that contains antimicrobial agents such as sodium hypochlorite, hydrogen peroxide, or ethanol enhances results (Crippen and Sheffield, 2006).

iii. **Milling:** To obtain a uniform product such as powder or paste, edible insects are often subjected to milling or grinding. This process can be applied to fresh insects, after blanching for wet milling, or once the insects

have been dried for dry milling. Because wet milling creates a liquid-like material that is simpler to process, it is frequently preferred in industrial applications (Dossey, 2015). However, the high moisture content in this method can lead to microbial instability in the resulting product.

iv. **Drying:** Wet milling can be followed by drying to create a powder, which will increase shelf life and yield a stable product (Dossey et al., 2016). Several drying techniques are available for insects, including oven drying, dehydration, freeze drying, microwave drying, and sun drying, while spray drying is commonly used for drying insect paste. This method involves introducing the paste into hot air, allowing each droplet to dry individually, resulting in a quick process that yields uniform products with improved nutritional quality (Dossey et al., 2016). However, due to the limitations of spray drying with high-fat products, drum drying is frequently utilized instead (Dossey et al., 2016).

Type of Mill

The final properties of the powder or paste can be greatly impacted by the type of blender used to produce insect food. For example, one study reported that defatted mealworm meal processed with a jet mill had lower moisture content, higher brightness, more uniform particle size, and greater consumer acceptance compared to powders produced using pin, hammer, or cutter mills (Son et al., 2019).

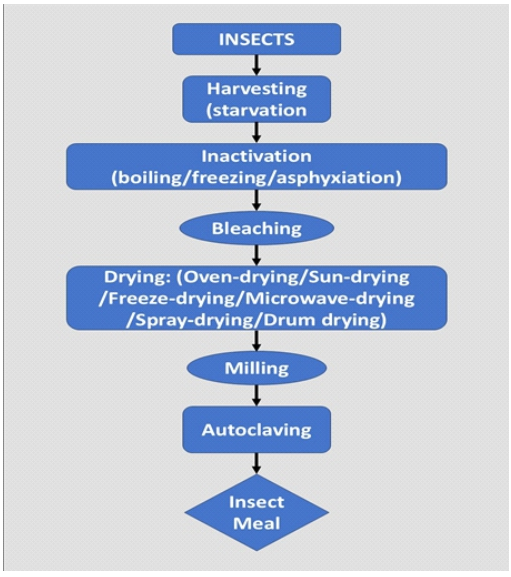

Figure 1. Processing pathway for the production of insect meal (Dossey et al., 2016)

Exploring Insect-Based Products in Swine Research

1. Effect of feeding insect meal on growth performance and nutrient digestibility

Because of its nutritional and functional qualities, Tenebrio molitor (TM) larvae meal has been studied as a possible protein source for pig diets. Jin et al. [19] found that weaned pigs' body weight (BW) and average daily gain (ADG) improved linearly when TM meal was added to their diets at a rate of 0–6% during a five-week period. The gain-to-feed ratio (G:F) and average daily feed intake (ADFI) both showed numerical increases.The study also indicated improved protein and nitrogen utilization, as shown by increased crude protein digestibility, nitrogen retention, and decreased blood urea nitrogen levels.The digestibility of dry matter (DM), gross energy (GE), and nitrogen, as well as growth performance, were not significantly affected by replacing 20% of fishmeal with Tenebrio molitor larvae (Ao et al., 2020). Ji et al. (2016) found that substituting 5% plasma protein with 5% TM larvae powder had no impact on the growth of pigs weaned at 14 days over an eight-week period. Additionally, growing pigs fed diets containing TM larvae had higher standard ileal digestibility (SID) of gross energy, arginine, and cystine than those fed diets containing fish meal (Yoo et al., 2019). Pigs given Tenebrio molitor larvae had better standardized ileal digestibility (SID) of gross energy, arginine, and cystine than pigs fed fish meal diets (Yoo et al., 2019).

In comparison to diets made with meat, chicken, or fish meal, diets containing TM larvae also tended to improve the SID of dry matter, crude protein, and amino acids (sustainable protein source for weanling pigs (Larouche et al., 2019; Yoo et al., 2019). In a similar vein, Chia et al. (2019) assessed the substitution of black soldier fly larval meal (BSFLM) for fish meal (FM) in the meals of 40 hybrid developing pigs. The animals were divided into five groups: one control (0% BSFLM, 100% FM) and four treatment groups where FM was replaced at 25%, 50%, 75%, and 100%. Their results indicated that BSFLM can successfully replace FM in developing pig diets because there were no discernible differences in average daily feed intake (ADFI), average daily gain (ADG), body weight gain (BWG), or feed conversion ratio (FCR). In finishing pigs, however, Chia et al. (2021) observed that increase in the inclusion of BSFLM (9–14%), replacing 50–100% of FM, improved final body

weight, ADG, feed conversion ratio, and carcass weight. At different dietary proportions across three feeding phases, with or without amino acid supplementation, it was also determined that feeding dried black soldier fly prepupae meal to early-weaned pigs as a substitute (0, 50, or 100%) for dried plasma meal was a workable approach. However, full replacement of dried plasma did not perform as well as the control, suggesting potential refinements like cuticle removal may be needed for better suitability (Newton et al., 2005). Yu et al. (2019) investigated how the growth performance of finisher pigs was affected by feeding *Hermetiaillucens* larvae at 0, 4, and 8% (HI0, HI4, and HI8 groups, respectively). According to the findings, pigs given the HI4 diet had a lower ($P < 0.05$) feed-to-gain ratio and a significantly greater ($P < 0.05$) final body weight and average daily gain than the HI0 and HI8 groups.

2. Effect of insect meal on blood biochemical parameters

Hematological indicators including red and white blood cell counts were not significantly affected when growing pigs were fed black soldier fly larvae meal (BSFLM) at different amounts (0–100%) in place of fish meal Chia et al. (2019). However, pigs receiving diets where fish meal was substituted at 75% and 100% exhibited elevated neutrophil levels.Platelet counts were lower in pigs fed 25%, 75%, and 100% diets than in those fed 0% and 50%. Furthermore, blood cholesterol levels were unaffected by the addition of BSFLM to the diet. As the inclusion level rose throughout phase II (14–35 days), Jin et al. (2016) found that introducing dried mealworm (*Tenebrio molitor*) at 0%, 1.5%, 3.0%, 4.5%, and 6.0% decreased blood urea nitrogen and enhanced insulin-like growth factor. Another study found that replacing fish meal with dried mealworm at 50% and 100% in weanling pig diets had no effect on blood parameters, including total RBCs, WBCs, lymphocytes, and monocytes, throughout the trial (Jin et al., 2016). Overall, these studies indicate that insect meals do not adversely affect pig health and can be incorporated into their diets.

1. Effect of insect-based proteins on immune responses in pigs

According to Ko et al. (2020), substituting fish meal with dried mealworm at 50% and 100% in weanling pig diets significantly increased immunoglobulin G (IgG) levels during phase I (0–14 days). They also noted a numerical decrease in pro-inflammatory cytokine IL-6 at the 50% replacement level, with no effects on IL-1β or TNF-α production during phases I and II (15-28 days). Yu et al. (2019) observed that incorporating *Hermetiaillucens* larvae at 4% and 8% in the diets of finishing pigs led to a significant ($P < 0.05$) reduction in TLR-4 expression and the pro-inflammatory cytokine IFN-γ. Additionally, the anti-inflammatory cytokine IL-10 rose at the 4% inclusion level, and genes linked to the intestinal barrier, such as ZO-1, occludin, and mucin-1, showed increased expression. 6% mealworm supplementation did not significantly alter blood IgG concentrations in young piglets (Jin et al., 2016). Overall, these studies suggest that insect meal does not negatively affect immune cells and may have immunomodulatory effects in pigs.

2. Effect of insect meal on gut health

Yu et al. (2019) found that supplementing finishing pigs' diets with *Hermetiaillucens* larvae at 4% and 8% levels improved gut health. Beneficial bacterial communities, such as *Lactobacillus, Pseudobutyrivibrio, Roseburia*, and *Faecalibacterium*, significantly increased ($P < 0.05$) at the 4% inclusion level, while Streptococcus decreased. Furthermore, toxic protein fermentation products such total amines, cadaverine, tryptamine, phenol, p-cresol, and skatole dramatically decreased ($P < 0.05$), but colonic metabolites such as total short-chain fatty acids, butyrate, and isobutyrate rose significantly (Yu et al, 2019; Ramos-Elorduy et al., 2002). Supplementing with insect meal does not adversely affect intestinal integrity or nutrient absorption, according to another research (Ko et al., 2020). Ji et al. (2016) noted that replacing 5% plasma protein with 5% mealworm powder reduced diarrhoea incidences during weeks 2 to 4. Furthermore, the liver and gastrocnemius muscle of pigs fed insect diets showed no indications of oxidative stress or the activation of stress-sensitive signalling pathways, according to Ringseis et al. (2021).

Challenges in Incorporating Insects as Feed Ingredients

Insects hold considerable potential as feed ingredients for swine diets because of their nutrient profile, digestibility, and functional properties. To properly use them in swine nutrition, a number of issues must be resolved.

First, standardized protocols for insect production—including rearing, processing, and storage—are crucial to ensure a consistent large-scale supply.Establishing these standards will help manage critical food safety hazards for both consumers and livestock. Moreover, adopting novel processing techniques can also improve the quality and utility of insect-based products. As demand for insects rises and production technology improves, reliable mass production for feed applications is expected to become achievable.

Second, the current market price of insects is higher than conventional protein sources like soybean meal (SBM) and fish meal, limiting their competitiveness. Insect producers are generally smaller operations, primarily serving markets for birds and reptiles. To make insects a viable option for swine feed, large-scale production systems need to be implemented to stabilize supply and increase production efficiency, which could lead to lower prices.

Lastly, more study is required to completely comprehend the functional and nutritional advantages of pigs eating insect-based products. Finding the ideal inclusion amounts of insect products for various growth periods while accounting for growth, reproduction, pork quality, and general health is crucial, even though the majority of research has concentrated on growth performance and nutrient digestibility. In addition, the potential functional effects of insect components, such as chitin and other bioactive compounds, warrant thorough investigation.Safety concerns, including the presence of toxic substances, antibiotic resistance, and contamination from pathogens or heavy metals, also need to be thoroughly addressed.

CONCLUSION

Insects represent a promising and sustainable alternative for pig nutrition, offering both high-quality protein and valuable trace minerals such as iron and zinc. Their use not only addresses nutritional challenges but also supports circular economy practices by utilizing organic side streams. However, issues such as nutrient variability, processing methods, and regulatory acceptance remain to be resolved. With continued research and innovation, insects could become an integral part of sustainable pig production in the future.

REFERENCES

Ao, X, Yoo, J.S., Wu, Z.L., Kim, I.H. 2020. Can dried mealworm (*Tenebrio molitor*) larvae replace fish meal in weaned pigs? Annual Review of Entomology. 239:104103.

Arango Gutiérrez, G.P., Vergara Ruiz, R.A. and Mejía Vélez, H. 2004. Compositional, microbiological and protein digestibility analysis of the larva meal of *Hermetiailluscens* L.(Diptera: Stratiomyiidae) at Angelópolis-Antioquia, Colombia. RevistaFacultad Nacional de Agronomía Medellín. 57(2):2491-2500.

Biasato, I., Renna, M., Gai, F., Dabbou, S., Meneguz, M., Perona, G., Martinez, S., Lajusticia, A.C.B., Bergagna, S., Sardi, L. and Capucchio, M.T. 2019. Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. Annual Review of Entomology.10:1–11.

Chia, S.Y., Tanga, C.M., Osuga,I.M., Alaru, A.O., Mwangi, D.M., Githinji, M., Dubois, T, Ekesi, S., Van Loon, J.J.A. and Dicke, M. 2021. Black soldier fly larval meal in feed enhances growth performance, carcass yield and meat quality of finishing pigs. Annual Revew of Entomology.7(4):433–447.

Chia, S.Y., Tanga, C.M., Osuga, I.M., Alaru, A.O., Mwangi, D.M., Githinji, M., Subramanian, S., Fiaboe, K.K., Ekesi, S., van Loon, J.J.A. and Dicke, M. 2019. Effect of dietary replacement of fishmeal by insect meal on growth performance, blood profiles and economics of growing pigs in Kenya. Annual Review of Entomology. 9(10):705.

Crippen, T.L. and Sheffield, C. 2006. External surface disinfection of the lesser mealworm (Coleoptera: Tenebrionidae). Annual Review of Entomology. 43(5):916–923.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. and DalleZotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal. 10(12):1923-1930.

DiGiacomo, K. and Leury, B.J. 2019. Insect meal: a future source of protein feed for pigs? Annual Review of Entomology.13(12):3022–3030.

Dominguez, J., Aira, M. and Gomez-Brandon, M. 2010. Verming composting: earthworm enhances the work of microbes. In Microbes at Work, ed. Heidelberg: Springer, p. 93–114.

Dossey, A.T. 2015. Insect products and methods of manufacture and use thereof. U.S. Patent Application No. 14/537,960.

Dossey, A.T., Tatum, J.T. and McGill, W.L. 2016. Modern insect-based food industry: current status, insect processing technology, and recommendations moving forward. In Insects as Sustainable Food Ingredients: Production, Processing and Food Applications, p. 113–152.

FAO. 2013. Edible insects: future prospects for food and feed security. Forestry Paper 171.

Finke, M.D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Annual Review of Entomology. 21:269–285.

Garofalo C, Milanovic V, Cardinali F, Aquilanti L, Clementi F, Osimani A. 2019. Current knowledge on the microbiota of edible insects intended for human consumption: a state-of-the-art review. Annual Review of Entomology. 125:108527.

Henry, M., Gasco, L., Piccolo, G. and Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology. 203:1-22.

Hong, J. and Kim, Y.Y. 2022. Insect as feed ingredients for pigs. Annual Review of Entomology. 35(2):347.

Ijaiya, A.T. and Eko, E.O. 2009. Effect of replacing dietary fish meal with silkworm (*Anapheinfracta*) caterpillar meal on performance, carcass characteristics and haematological parameters of finishing broiler chicken. Annual Review of Entomology. 8:850–720.

Ji, Y.J., Liu, H.N., Kong, X.F., Blachier, F., Geng, M.M., Liu, Y.Y. and Yin, Y.L. 2016. Use of insect powder as a source of dietary protein in early-weaned piglets. Annual Review of Entomology. 94(3):111–116.

Jin, X.H., Heo, P.S., Hong, J.S., Kim, N.J. and Kim, Y.Y. 2016. Supplementation of dried mealworm (*Tenebrio molitor* larva) on growth performance, nutrient digestibility and blood profiles in weaning pigs. Annual Review of Entomology. 29(7):979.

Khatun, R., Azmal, S.A., Sarker, M.S.K., Rashid, M.A., Hussain, M.A. and Miah, M.Y. 2005. Effect of silkworm pupae on the growth and egg production performance of Rhode Island Red (RIR) pure line. Annual Review of Entomology. 4:718–720.

Khusro, M., Andrew, N.R. and Nicholas, A. 2012. Insects as poultry feed: a scoping study for poultry production systems in Australia. Annual Review of Entomology. 68(3):435–446.

Ko, H., Kim, Y. and Kim, J. 2020. The produced mealworm meal through organic wastes as a sustainable protein source for weanling pigs. Annual Review of Entomology. 62(3):365.

Larouche, J., Deschamps, M.H., Saucier, L., Lebeuf, Y., Doyen, A. and Vandenberg, G.W. 2019. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetiaillucens*) larvae. Annual Review of Entomology. 9:182.

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R.A.P and Paengkoum P. 2022. Nutritional composition of black soldier fly larvae (*Hermetiaillucens* L.) and its potential uses as alternative protein sources in animal diets: A review. Insects. 13(9):831.

Makkar, H.P., Tran, G., Heuzé, V. and Ankers, P. 2014. State-of-the-art on use of insects as animal feed. Annual Review of Entomology. 197:1–33.

Newton, L., Sheppard, C., Watson, D., Burtle, G. and Dove, R. 2005. Using the black soldier fly, *Hermetiaillucens*, as a value-added tool for the management of swine manure. Annual Review of Entomology. 17:18.

Oonincx, D.G., Van Broekhoven, S., Van Huis, A. and Van Loon, J.J. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PloS one. 10(12): p.e0144601.

Ramos-Elorduy, .J, González, E.A., Hernández, A.R. and Pino, J.M. 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler

chickens. Annual Review of Entomology. 95(1):214–220.

Ringseis, R., Peter, L., Gessner, D.K., Meyer, S., Most, E. and Eder, K. 2021. Effect of *Tenebrio molitor* larvae meal on the antioxidant status and stress response pathways in tissues of growing pigs. Annual Review of Entomology. 75(4):237–250.

Son, Y.J., Lee, J.C., Hwang, I.K., Nho, C.W. and Kim, S.H. 2019. Physicochemical properties of mealworm (*Tenebrio molitor*) powders manufactured by different industrial processes. Annual Review of Entomology. 116:108514.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. and De Smet, S. 2017. Nutritional composition of black soldier fly (*Hermetiaillucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture. 97(8):2594-2600.

Xiao, H.W., Bai, J.W, Sun, D.W. and Gao, Z.J. 2014. The application of superheated steam impingement blanching (SSIB) in agricultural products processing – a review. Annual Review of Entomology. 132:39–47.

Yoo, J.S., Cho, K.H., Hong, J.S., Jang, H.S., Chung, Y.H., Kwon, G.T., Shin, D.G. and Kim, Y.Y. 2019. Nutrient ileal digestibility evaluation of dried mealworm (*Tenebrio molitor*) larvae compared to three animal protein by-products in growing pigs. Annual Review of Entomology. 32(3):387.

Yu, M., Li, Z., Chen, W., Rong, T., Wang, G. and Ma, X. 2019. *Hermetiaillucens* larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. Annual Review of Entomology. 10:1–16.

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Dietary Supplementation of Urea Molasses Mineral Block for Enhancing Productive Performance of Dairy Cows

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ABSTRACT

A study was undertaken to assess the effect of supplemental urea molasses mineral block (UMMB) in crossbred cows fed paddy straw-based diet to assess its efficacy on productive performance of lactating cows and enhanced income by farm women in rural areas. In an organized dairy farm, fourteen crossbred cows were randomly divided into two groups (T1 and T2) consisting of seven animals each based on body weight (455±10.55 kg) and milk yield (7.20±0.78 kg). Animals were fed homemade concentrate mixture and paddy straw (40:60). An additional supplementation of block at the rate 300 g/d/animals was offered in animals in T2. Feed intake, milk yield, milk composition was recorded at fortnight intervals in a 3-month lactation trial. Daily dry matter intake through concentrate and roughage was similar. The milk yield and 4% fat corrected milk yield were higher by 16.8% and 20.2%, respectively in supplemented group of animals. The milk constituents *viz.* fat, solid not fat and total solid were also increased ($P<0.05$) through UMMB supplementation. The blood biochemical profile except glucose and protein were not influenced by feeding block. The supplemental urea molasses mineral block fed to cows resulted improvement in net return over feed cost and feed efficiency by 35.7% and 12.5%, respectively than control group of animals. It was concluded that the supplementation of urea molasses mineral block in paddy straw-based diet economically improved the milk production, milk composition and feed efficiency of lactating crossbred cows in coastal belt of Odisha.

KEYWORDS: Concentrate, Cow, Fat, Milk, Urea molasses mineral block.

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INTRODUCTION

Dairy cows are mainly dependent upon poor quality roughage like and paddy straw-based diet resulting low milk yield, longer inter calving period with various reproductive problems (Brar et al., 2022). Farm women play a major role in dairy farming activities and usually feed homemade concentrate mixture devoid of mineral mixture which affect the productivity of the animals and hence the economic return. Supplementation of critical nutrients through dietary sources can improve the utilization of poor-quality roughages (Sahoo et al., 2017).

The supplementation of a rich source of protein, energy, and micronutrients, i.e., urea molasses mineral block (UMMB) can show promising results in improving the nutrient utilization and the productivity of lactating animals (Sahoo et al., 2009, Reshi et al., 2022). An on-farm trial was conducted to assess the effect of UMMB on a paddy straw based diet in lactating crossbred cows on productive performance and feed efficiency of cows.

MATERIALS AND METHODS

Animals and experimental design

The present study was conducted at farmers' field in the adopted village of ICAR- Central Institute for Women in Agriculture at Chamarpada of Cuttack district in coastal belt of Odisha. Twenty milch cows (*Bos indicus*) in an organized dairy farm were

randomly divided into two groups (T1; control and T2; treatment) of seven each based on body weight (455±10.55 kg), days in milk (95±9.75), and milk yield (7.2±0.78 kg). Animals were fed on homemade concentrate mixture comprising maize (40%), rice bran (25%), pulse chunni (20%) and locally available ground nut cake (15%). Animals in T2 were additionally supplemented with urea molasses mineral block of 300 g along with concentrate mixture and paddy straw was provided *ad libitum* to the animals throughout the day during the entire experimental period. The animals were housed in a well-ventilated, clean, and concrete shed having individual feeding arrangements. Animals were dewormed before the start of the experiment and managed as per the existing practices by the farmers ensuring individual feeding in their existing housing arrangements satisfying the nutrient requirement (ICAR, 2013).

Preparation of urea molasses mineral block

The urea molasses mineral blocks (UMMB) were prepared at ICAR-Central Institute for Women in Agriculture using hot process containing molasses- 33%, urea- 08%, ground nut cake -15%, rice bran -25%, cement- 3%, lime-1%, mineral mixture-10%, and common salt-5% each. Each block of 2 kg weight was allowed to settle in a specially designed mould (Iron frame designed by Indian Veterinary Research Institute, Izatnagar) by manual pressure followed by air drying under the sunlight.

Lactation and digestibility trial

A lactation trial of 3 months duration was conducted in which intake of concentrate mixture, paddy straw and UMMB by individual animals were recorded at fortnight intervals. The daily milk yield was recorded, and milk samples were collected at fortnightly intervals for constituent analysis at the Milk Analysis Centre of the Odisha State Dairy Cooperative Society (OMFED).

Collection of biological samples and chemical analyses

A 6-day digestibility trial preceded by 3 days adaptation period was conducted at the end of the experiment. Feeds offered, residues left, and faeces voided were quantified. The representative samples offered, residues and faeces were analyzed for proximate composition as per AOAC (2005), milk fat by Gerber's method (IS:1224, Part I, 1970), milk protein by ISI (1970), SNF by modified Richmond's formula and 4% FCM yield using Gaines'(1928) formula. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed according to Van Soest et al. (1991). **T**he perceived attitude of farm women involved in UMMB supplementation for feed intake, health of animals was collected. The experimental data were subjected to analysis of variance for a randomized block design as per SPSS (17.0 version) and the means were assessed by least significance difference.

RESULTS AND DISCUSSION

Feed Intake and nutrient utilization

The chemical composition of UMMB, concentrate mixture and straw offered to dairy cows is shown in Table 1. The level of crude protein (CP) and other nutrients in the total diet was sufficient to meet the nutrient requirement of cows as per ICAR (2013).The CP content of UMMB, concentrate mixture and paddy straw was 35.5%, 18.5% and 4.5%, respectively. Similar nutrient composition was also reported by Meel et al. (2015) and Mengistu and Waseyehon (2017). The dry matter (DM) intake through concentrate and roughage were not changed (P>0.05) due to supplementation of UMMB (Table 2). However, the total DM intake was more (P>0.05) in UMMB supplemented group (11.5 kg/d) than the control (11.1 kg/d). The digestibility of nutrients *viz.* organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and total carbohydrate was higher in UMMB supplemented animals resulting better availability of nutrients to rumen microbes and host animals providing a conducive environment of rumen of buffaloes with optimum production of volatile fatty acids, resulting better nutrient utilization (Sihag et al., 2004). In consistent with present findings, the digestibility of nutrients was increased by UMMB supplementation in cows. The fibrous feeds are fermented in the rumen and broken down to particle sizes that can facilitate the flow and increased rates of rumen fermentation, mediated through a larger population of microflora and increased cellulolytic activity improving digestibility (Sudhakar et al*.,* 2002; Mengistu and Waseyehon, 2017). The nutrient intake through CP, DCP and TDN was found to be higher in UMMB supplemented group of animals resulting in improved nutritional value of diet being attributed to better nutrient utilization efficiency in supplemented group of animals (Meel et al., 2015).

Table 1. Chemical composition (% DM basis) of feed stuff used during the on farm trial

Attributes	UMMB	Concentrate	Paddy straw
DM	82.5	90.2	88.5
OM	72.0	87.0	90.7
CP	35.5	18.5	4.5
EE	3.4	3.5	2.1
NDF	11.5	38.5	72.3
ADF	9.5	19.5	56.3

Table 2. Effect of UMMB supplementation on nutrient utilization in lactating cows

Particulars	Dietary treatments		SEM	P value
	T1	T2		
Digestibility of nutrients (%)				
DM	56.5	58.8	0.75	0.08
OM*	57.4[a]	60.8[b]	0.87	0.05
CP*	58.5[a]	61.6[b]	1.13	0.05
EE*	60.5[a]	63.5[b]	0.85	0.05
NDF*	54.2[a]	57.1	0.86	0.05
ADF	51.2	52.8	1.03	0.15
Feed (DM) Intake (kg/d)				
Concentrate	4.2	4.4	0.19	0.28
Roughage	6.9	6.8	0.53	0.33
UMMB	-	0.31	-	-
Total	11.1	11.5	0.60	0.28
Nutrient Intake(kg/d)				
CP*	0.97[a]	1.12[b]	0.25	0.05
DCP*	0.56[a]	0.81[b]	0.22	0.04
TDN*	5.16[a]	5.95[b]	0.15	0.05
Nutritive value (%)				
CP*	8.72[a]	9.74[b]	0.18	0.05
DCP*	5.04[a]	7.04[b]	0.19	0.04
TDN*	46.45[a]	51.71[b]	0.25	0.05

[*a, b] Means with different superscripts differ significantly (P<0.05)

Feed intake, Milk yield and feed efficiency in lactation trial

Average daily DM intake through concentrate (kg/d) was found to be higher (P>0.05) although values were similar (Table 3). Average consumption of UMMB during the experimental period was 300 g/day/animal. The DM intake in control and UMMB supplemented group of animals was found to be similar. The supplemental molasses in UMMB might have improved the palatability of feed resulting more feed intake in terms of quality and quantity with subsequent increase in milk yield (Sahoo et al., 2009).

Table 3. Effect of UMMB supplementation on milk production and feed efficiency in cows

Particulars	Dietary treatments		SEM	P value
	T1	T2		
DM intake (kg/d)				
Concentrate	4.15	4.35	0.19	0.38
Roughage	6.65	6.55	0.58	0.23
UMMB	-	0.30	-	-

Total	10.80	11.20	0.65	0.28
Lactation Performance				
Milk yield (kg/d)*	7.05[a]	8.15[b]	0.15	0.05
4% FCM yield (kg/d)*	7.89[a]	9.42[b]	0.19	0.04
DM intake /Kg FCM yield*	1.38[b]	1.17[a]	0.25	0.05
Milk composition (%)				
Fat*	4.81[a]	5.03[b]	0.15	0.04
Protein*	3.08[a]	3.3[b]	0.12	0.05
SNF	8.59	8.73	0.22	0.10
Total solid*	13.41[a]	13.77[b]	0.27	0.05
Economics of feeding				
Total feed cost (Rs./d)	170	180	0.18	0.14
Total Income (Rs./d)*	320[a]	395[b]	0.17	0.05
Net return over feed cost (Rs./d)*	150[a]	215[b]	0.16	0.04
Feed cost/kg FCM yield (Rs.)*	21.5[b]	19.1[a]	0.21	0.05

[a,b] Means with different superscripts differ significantly (P<0.05)

The values in Table 3 revealed that the milk yield and 4% FCM yield was augmented by 15.7% and 19.5%, respectively following UMMB supplementation in cows. The dry matter intake coupled with supply of balanced diet through UMMB resulted in significant increase (P<0.05) in milk yield and FCM yield in UMMB supplemented group with the advancement of lactation trial. In consistent with the present findings, milk production was found to be higher (P<0.05) in CB cows (35.97%) and in buffaloes (33.8%) with higher (P<0.05) DM intake without any adverse effect on body weight and health of animals (Misra et al., 2006, Tripathi et al., 2006; Singh et al., 2010). The UMMB might have been the contributing factor for the observed rise in level of milk constituents and milk yield in animals which was supported by various findings. The FCM yield was consistently increased in UMMB supplemented group of animals unlike the control group. The milk constituents *viz.* fat, SNF and TS were also significantly increased (P<0.05) by UMMB supplementation (Avila et al., 2006; Sahoo et al., 2009; Upreti et al., 2010). The Supplementation of UMMB licks increased milk yield and milk fat percent in crossbred cows during dry season feeding in rain-fed agro-ecosystem in India which may be associated with creating an efficient rumen ecosystem which favours the higher cellulolytic fibre utilization by the microbes in the presence of the optimum urea ammonia provided by UMMB (Mengistu and Waseyehon, 2017). The FCM yield was highest in 3rd fortnight (Fig. 1) and gradually declined with the advance of lactation trial resulting in a rise in the level of milk constituents. However, the declining slope in group T2 was lower which indicates more consistency in milk yield of cows supplemented with UMMB. Higher supply of nutrients in the form of protein, energy, and minerals to animals in T2 was attributed to better productive performance of cows. UMMB provides synchronous supply of nitrogen and energy facilitating microbial protein synthesis which is in turn digested by animals using digestive enzymes and amino acids liberated helps in tissue protein synthesis used in maintenance and production by the animals. If urea is fed to dairy animals, it is inefficiently used, therefore, slow usage of urea is practicable with a simultaneous supply of carbohydrate sources like molasses which provide readily fermentable carbohydrates and thereby increases rumen microflora (Sahoo et al., 2009; Reshi et al., 2022). Molasses along with minerals increase the palatability of UMMBs by their pleasant smell and sweet taste (Upadhyay et al., 2018; Reshi et al., 2022). The economics of feeding supplemental UMMB revealed that total income was improved by 23% along with higher net return over feed cost by 43.2% in T2 than T1. The feeding cost per unit milk production was more in UMMB supplemented group. But the feed efficiency (feed intake/ unit FCM yield) was improved by 12.7% indicating better feed efficiency through UMMB supplementation. Several research workers also reported that UMMB supplementation with local diet to cows economically raised (P<0.01) milk production which agreed with our results (Sudhakar et al., 2002; Singh and Singh, 2003; Tripathi et al., 2006). The improvements in net return per day due to UMMB supplementation were 24 and 45% for the rural subsistence-oriented production system and the peri-urban dairy farming, respectively (Upreti et al., 2010; Meel et al., 2015).

Table 4. Effect of UMMB supplementation on blood biochemicals in cows

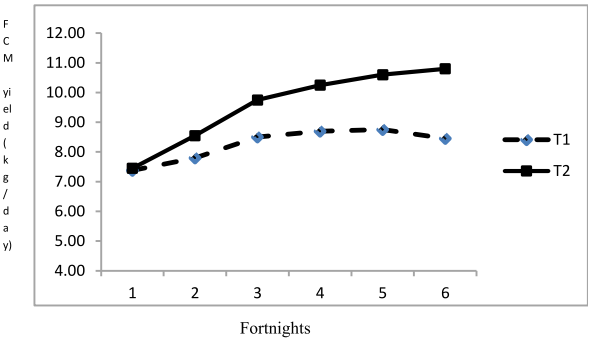
Particulars	Dietary treatments		SEM	P value
	T1	T2		
Blood glucose (mg/dL) *	52.45[a]	58.74[b]	0.25	0.04
Total protein (g/dL)*	6.25[a]	7.13[b]	0.18	0.05
Albumin (g/dL)*	3.05[a]	3.50[b]	0.16	0.05
Globulin (g/dL)*	3.21[a]	3.71[b]	0.21	0.05
Blood urea Nitrogen	30.75	37.15	0.55	0.12
Cholesterol	215	224	10.31	0.68
ALP (g/dL)	132	145	3.13	0.19
ALT (g/dL)	70	87	7.25	0.19
AST (g/dL)	135	155	9.35	0.25

[a, b] Means with different superscripts differ significantly (P<0.05)

The results of biochemical analysis of serum samples (Table 4) revealed significant increase in albumin, globulin, and total serum protein level (P<0.05) in UMMB supplemented group. Blood urea nitrogen level was higher (P>0.05) without any significant change and was within the normal range indicating that UMMB supplementation has not any harmful effect on liver. Although, blood urea nitrogen was higher in treatment groups, no symptoms of urea toxicity were noticed during the study period and all animals were found to be sound in health. The blood glucose level increased in UMMB supplemented group when compared with non-supplemented group which was attributed to supplementation of non-protein nitrogen and energy in the form of urea and molasses in UMMB (Sahoo et al., 2009; Kour et al., 2022). The non-protein nitrogen and energy on the fermentation in the rumen in UMMB supplemented group might have improved the production of the fatty acids in the rumen and would subsequently influence blood glucose metabolism (Kerketta et al., 2017). Biochemical and enzyme profile were not altered by UMMB supplementation. In consistent with the present findings, feeding of UMMB did not have any adverse effect on haematological and biochemical parameters in buffaloes (Sihag et al., 2009).

Almost all the participating farm women associated with the demonstration of UMMB in the lactation trial responded that by supplementing UMMB, cows consumed more concentrate mixture , maintained good health and productivity and these effects were sustained even after reduction of concentrate mixture allowance during summer months when green fodder was scarce. All the farmers readily accepted the practice of using UMMB supplementation and were willing to continue in future, if these were available in the local market.

Fig. 1. Milk production of cows at fortnight intervals in lactation trial



CONCLUSION

Based on the above findings, it can be concluded that supplementation of 300 g urea molasses mineral block per day per cow in paddy straw-based diet economically improved milk production by 17-20% in crossbred cows in coastal belt of Odisha. Supplementation of UMMB to animals fed low quality fodder during summer months may be an appropriate alternative to other supplements (commercial feed) especially when these are short in supply and get expensive. UMMB feeding also demonstrated the potential of increasing viability of livestock production, increasing dry season milk supplies and enhancing household income. The present findings demonstrated that UMMB technology is a cost-effective approach to maximize the utilization of locally available feed resources for better animal productivity in peri urban dairy farming of Odisha.

REFERENCES

AOAC. *Official Methods of Analysis.* 18th Edn. Association of Official Analytical Chemists. Maryland, U.S.A. 2005.

Avila, H.F. 2006. Production and utilization of urea molasses mineral block. FAO/Ag/AGP/doc./Publicat./VIET95, pp. 199-204.

Brar, P.S., Phand, S., Singh, J., Singh, A. and Das, S. 2022. Nutritional and Health Management of Dairy Animals. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana & National Institute of Agricultural Extension Management, Hyderabad, India, P. 1-101.

Gaines, W.C. 1928. The energy basis of measuring milk yields in dairy cows. University of Illinois Agricultural Experimental Station Bulletin: 308.

ICAR. 2013. Nutritional Requirements of Cattle. In Book Series: *Nutritional Requirements of Livestock and Poultry*, 2nd Edn. Indian Council of Agricultural Research, New Delhi, India.

Indian Standards Institute. 1970. IS 1224 (Part I). Fat determination of milk by Gerbers's method, Indian Standards Institute, Mamak Bhavan, New Delhi.

Kerketta, N., Victor, V.M., Chandraker, A.K. and Jogd, S.V. 2017. Effect of urea molasses mineral block as feed supplement on body weight gain and haemato-biochemical parameters of working bullocks. International Journal of Agriculture Innovations and Research. 5(6): 917-920.

Kour, S., Khan A., Pathak A.K., Pande, N., Chakraborty, D. and Kour, S. 2022. Study on the effect of UMMB supplementation on performance of buffaloes of Gujjar tribe of Jammu. Journal of Animal Research. 12: 135-146.

Kumar, S., Singh, S.V., Pandey, P., Lone, S.A. and Upadhyay, R.C. 2016. Effect of molasses feeding on biochemical and hormonal parameters in Sahiwal and Karan Fries heifers. Journal of Animal Research. 6: 995-999.

Meel, A., Sharma, V., Sharma, A. and Kaushik, P. 2015. Effect of feeding urea mineral molasses block on milk production traits and economics in jersey crossbred cows. International Journal of Scientific Research. 2: 368-369.

Mengistu, G. and Hassen, W. 2017. Review on: supplementary feeding of urea molasses multi-nutrient blocks to ruminant animals for improving productivity. International Journal of Animal Husbandry and Veterinary Science. 2(6): 43-49.

Mirza, I.H., Anjum, M.I., Khan, A.G. and Azim, A. 2004. Comparative evaluation of cotton seed meal and sunflower seed meal in urea molasses blocks versus commercial concentrate as supplement to basal ration of wheat straw with stall-fed buffalo calves. Asian-Australasian Journal of Animal Sciences. 17(2): 193-198.

Rafiq, K., Mostofa, M., Awal, M.A. and Hossain, M.M. 2000. Effect of medicated block licks on the performance of indigenous dairy cows of Bangladesh. Asian-Australasian Journal of Animal Sciences. 13(6): 774-780.

Reshi, P.A., Tabasum, T., Ganai, A.M., Ahmad, H.A., Sheikh, G.G., Beigh, Y.A. and Haq, S.A. 2022. Use of urea based multinutrient blocks for enhanced performance of dairy cattle-A Review. SKUAST Journal of Research. 24(1): 12-23.

Sahoo, B., Kumar, A., Panda, A.K., Das, L., Maradana, U., Sarangi, D.N. and Srivastava, S.K. 2021. Role of mineral mixture supplementation in enhancing productivity and profitability of peri-urban dairy farming. Indian Journal of Animal Nutrition.38: 41-47.

Sahoo, B., Kumar, R., Garg, A.K., Mohanta, R.K., Agarwal, A. and Sharma, A.K. 2017. Effect of supplementing area specific mineral mixture on productive performance of crossbred cows. Indian Journal of Animal Nutrition. 34(4): 414-419.

Sahoo, B., Vishwanath, Bhushan, C., Kwatra, J. and Agarwal, A. 2009. Effect of Urea Molasses Mineral Block Supplementation on milk production of cows (*Bos indicus*) in mid Hills of Uttarakhand. Indian Journal of Animal Nutrition and Feed Technology. 9(2): 171-178.

Sankar, V., Singh, P., Verma, A.K., Patil, A.K. and Kumar, D. 2020. Influence of Urea Molasses

Mineral Blocks having bentonite as binder on haemato-biochemical and serum mineral profile of crossbred calves. Journal of Animal Research. 10(4): 651-658.

Sihag, Z.S., Chahal, S.M. and Punia, B.S. 2004. Effect of feeding urea molasses mineral blocks on volatile fatty acids and pH in the rumen of Buffaloes. Indian Journal of Animal Sciences. 74: 890- 892.

Sihag, Z.S., Punia, B.S., Berwal, R.S. and Sihag, S. 2009. Influence of feeding urea molasses mineral blocks (UMMB) on blood parameters in buffaloes. Indian Journal of Animal Nutrition. 26(1): 56-60.

Singh, P.R. and Singh, M. 2003. Effect of UMMB supplementation on milk production in buffaloes and cows. Indian Journal of Animal Nutrition. 20: 1-5.

Singh, R., Kumar, S. and Brar, P.S. 2010. Evaluation of urea molasses multi-nutrient blocks enriched with area specific mineral mixture in buffaloes. Indian Journal of Animal Sciences. 80 (6): 561–564.

SPSS. 1997. SPSS Base Applications Guide Version 7.5., SPSS, USA.

Sudhakar, K., Reddy, G.V.K. and Krishna, N. 2002. Effect of supplementation of urea molasses mineral blocks (UMMB) on quantity and quality of milk production in Murrah buffaloes. Indian Journal of Animal Nutrition. 19: 301-305.

Tiwari, S.P., Singh, U.B. and Mehra, U.R. 1990. Urea molasses mineral blocks as a feed supplement: Effect on growth and nutrient utilization in buffalo calves. Animal Feed Science and Technology. 29: 333-341.

Tripathi, N.K., Pathak, S.K. and Sharma, V.K. 2006. An on farm evaluation of Urea-molasses-mineral- brick feeding for the milk production of cattle and buffaloes. Animal Nutrition and Feed Technology. 6: 251-255.

Upadhyay, N., Tiwari, M.R., Pandey, L.N., Karki, T.B., Acharya, R., Gairhe, S. and Acharya, Y. 2018. Economic analysis of the urea molasses mineral block feeding to lactating cattle of Nepal. Nepalese Journal of Agricultural Sciences. 16: 50-59.

Upreti, C.R., Shrestha, B.K. and Ghimire, B. 2010. Effect of UMMB supplementation during winter on milk production and its composition and infertility in dairy cattle In Hill management production system. Nepal Journal of Science and Technology. 11: 71-78.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583–3597.

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Nutrient Digestibility and Haemato-Biochemical Profile of Malabari Kids Fed Dairy Based Starter Rations

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ABSTRACT

An experiment was conducted to study the impact of dairy based starter ration on nutrient digestibility and haemato-biochemical profile of pre-weaned Malabari goat kids. Malabari kids (n=18) of fourteen days old of either sex, were selected and were raised under an intensive management system for the period of four months. The experimental kids were randomly assigned to three treatment groups (G1, G2 and G3). Milk was offered for three months of age for G1 group and for 45 days of age for both G2 and G3 groups. The kids in G1 were offered conventional starter while dairy based starters were offered to kids in G2 (5% Skimmed milk powder and 15% whey powder) and G3 (10% Skimmed milk powder and 10% whey powder). The daily milk consumption, feed intake and faecal score of kids were recorded. A digestibility trial was conducted for five days duration towards the end of feeding trial. Blood samples were collected at sixth and sixteenth week of the feeding trial. The total DMI of kids were similar ($p > 0.05$) among groups. The kids in G3 had higher ($p < 0.01$) starter DMI than the kids in G2 and G1. The digestibility coefficient of nutrients was found to be similar ($p > 0.05$) among groups. Similar ($p > 0.05$) fortnightly mean faecal condition score was observed in experimental kids maintained on three different dietary regimes. The haemato-biochemical parameters of kids did not vary significantly among groups. Hence, it can be concluded that early weaning and feeding dairy based starter ration did not have any adverse impacts on the health and nutrient digestibility of the kids.

KEYWORDS: Biochemical profile, Dairy, Digestibility, Faecal condition score, Starter

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INTRODUCTION

In India, goat industry is characterized by small-scale operations that play a vital role in the rural economy. Goat farming contributes to the livelihoods of many families, enhancing economic stability and food security in rural societies (Singh et al., 2023). Kid rearing is fundamental to the productivity and profitability of a goat herd. The growth of goat kids during the pre-weaning and weaning periods is crucial for maximizing the future growth, production and lifetime profitability of dairy does (Morand-Fehr et al., 2002). The productivity and profitability of the herd are significantly influenced by nutrient digestibility of feed (Solaiman and Shoemaker, 2009) and hemato-biochemical profile (Nayak et al., 2021) of goats. Improved feed digestibility in goat kids ensures better feed efficiency and higher weight gain, which are critical for growth and development. Similarly, a balanced biochemical profile serves as an indicator for good nutritional status, contributing to healthier herds and better economic outcomes for goat producers (El-Tarabany et al., 2018). By focusing on improving these aspects through effective management practices, producers can enhance growth rates, reduce costs, and ultimately increase the sustainability of their goat farming operations. Despite its importance, there is notable few published research on the effect of rearing goat kids by providing dairy based starter rations on nutrient digestibility and blood biochemical parameters. This gap in knowledge limits the resources available to producers seeking to optimize productivity and health outcomes for their herds.

By investigating these aspects, the study aims to address this gap by providing a comprehensive analysis of how different feeding regimes affect the digestibility and haemato- biochemical parameters of Malabari kids.

MATERIALS AND METHODS

Malabari kids (n=18) of fourteen days old of either sex, were selected and were raised under an intensive management system for the period of four months. All the kids were ear tagged and dewormed before the start of the experiment. The experimental kids were randomly assigned to three treatment groups (G1, G2 and G3). The G1 kids were fed with milk up to three months of age and kids in G2 and G3 were weaned off milk by 45 days of age. All the kids were provided with starter ration (CP-23%; TDN-70%) and hybrid Napier fodder from 14 days of age. The kids in G1 were offered conventional starter while dairy based starters were offered to kids in G2 (5% Skimmed milk powder and 15% whey powder) and G3 (10% Skimmed milk powder and 10% whey powder). The ingredient composition of experimental rations was listed in the Table 1. The daily milk consumption, feed intake and faecal score of kids were recorded.

Table 1. The ingredient composition (%) of rations fed to experimental kids

Ingredients	T1 (control)	T2	T3
Maize	26	16	16
Corn gluten fibre	19	6.5	5.5
Gingelly oilcake	8	8	7
Coconut oilcake	5	5	5
Soybean meal	10	10	9
Alfa-alfa meal	12	12	11
Wheat Bran	14	15.5	16.5
Black gram husk	4	5	8
Dried whey	-	15	10
Skimmed milk powder	-	5	10
Mineral Mixture	1.5	1.5	1.5
Salt	0.5	0.5	0.5
Total	100	100	100

A digestibility trial for five days duration was carried out using all the experimental kids towards the end of feeding trial by total collection method. All the faecal pellets voided by each animal were collected quantitatively uncontaminated with urine, feed or dirt, at 8 A.M. every day during the digestion trial. The faecal pellets collected were weighed individually and stored in double lined polythene bags in deep freezer (-20 ºC). The moisture and the crude protein (CP) were estimated (AOAC, 2016) using the fresh samples. Balance samples were dried at 65ºC for 48 h and finely ground for analysis of crude fibre (CF), ether extract (EE), total ash (TA), neutral detergent fibre (NDF) and acid detergent fibre (ADF) using standard procedures. Representative samples of kid starter and green fodder offered and leftover during the trial were collected for proximate analysis (AOAC, 2016) and fibre fractionization (Van Soest et al., 1991).

Blood samples were collected from all the kids at sixth and sixteenth week of the feeding trial. Whole blood was used to determine the haemoglobin (cyan methaemoglobin method) and serum samples were used to determine serum total protein (Jong and Vegeter, 1950), albumin (Bromocresol green method), blood urea nitrogen (BUN) (modified Berthelot method), serum creatinine (AOAC, 2016), glucose (GOD-PAP methodology), calcium (AOAC, 2016) and phosphorus (Bernhart and Wreath, 1955) by semiautomatic analyser using standard kits supplied by M/s. Agappe Diagnostics Limited, Ernakulam, Kerala.

The data on feed intake, faecal score, digestibility and haemato-biochemical parameters were statistically analysed as per Snedecor and Cochran (1994) using software statistical package for the social sciences (SPSS) version 20.0 by one way ANOVA method.

RESULTS AND DISCUSSION

Chemical composition of milk, feed and fodder

Earlier studies also reported similar composition of Malabari goat milk (Sudharsan et al., 2017; Sasikala, 2018 and Mishra et al., 2022). The CP content in Malabari goat milk was comparable to that of other indigenous goat breeds but slightly higher than the cow milk (Park et al., 2007). Conversely, the CP content of Jamnapari goat milk reported in the work of Mohsin et al. (2019) was 5.8 per cent, which was higher than the values observed in the present study. The composition of goat milk varies depending on genetic, physiological, nutritional and environmental factors. The mineral composition of goat milk is particularly vital for bone health (Park et al., 2007). Furthermore, Albenzio et al., 2016 emphasized the bioavailability of these minerals in goat milk is higher than in bovine milk. Although goat milk provides significant nutritional advantages, its unrestricted use in goat kids can lead to challenges such as dependency on milk, delayed rumen development, and economic inefficiencies (Jiao et al., 2017).

Table 2. Composition (%) of milk fed to experimental kids maintained on three dietary treatments

Components	Percentage
Total solids	13.90 ± 0.26
Crude protein	3.89 ± 0.14
Fat	3.78 ± 0.09
SNF	10.20 ± 0.27
Total ash	0.82 ± 0.02

The formulated kid staters *viz.,* G1, G2 and G3 contained 91.6 ± 0.26, 91.9 ± 0.32 and 91.73 ± 0.2 per cent dry matter, 23.45 ± 0.05, 23.5 ± 0.04 and 23.58 ± 0.03 per cent CP, 8.45 ± 0.1, 8.57 ± 0.1 and 8.22 ± 0.08 per cent CF, 3.42 ± 0.05, 3.39 ± 0.07 and 3.41 ± 0.06 per cent EE, 8.26 ± 0.15, 8.63 ± 0.18 and 8.57 ± 0.19 per cent TA, 56.37 ± 0.15, 55.93 ± 0.17 and 56.22 ± 0.23 Nitrogen free extract, 35.92 ± 0.33, 30.48 ± 0.59 and 30.62 ± 0.13 per cent NDF, 9.96 ± 0.16, 7.87 ± 0.15 and 8.83 ± 0.17 per cent ADF, 1.45 ± 0.02, 1.55 ± 0.02 and 1.53 ± 0.01 per cent calcium, 0.61 ± 0.01, 0.68 ± 0.02 and 0.69 ± 0.03 per cent phosphorus. The composition of starter obtained were consistent with the findings of Jasmine (2015) and Sasikala (2018). The CP per cent of SMP and dried whey used in the starter ration of kids was found to be 35.08 ± 0.06 and 7.09 ± 0.04, respectively and Patel *et al.* (2005) also obtained similar CP (35.5 per cent) values for spray dried SMP. Contrary to the present findings, Banavara *et al.* (2003) reported that CP content of sweetened whey powder ranges from 8.5 to14 per cent.

The fodder used for feeding experimental kids was hybrid napier which encompasses 23.49 ± 1.08 per cent dry matter, 16.01 ± 0.15 per cent crude protein, 30.33 ± 0.06 per cent crude fibre, 1.61 ± 0.11 per cent ether extract, 10.2 ± 0.06 per cent total ash, 41.85 ± 0.14 per cent NFE, 58.6 ± 0.16 per cent NDF, 15.03 ± 0.24 per cent ADF, 0.60 ± 0.02 per cent calcium and 0.35 ± 0.02 per cent phosphorus. Madesh *et al.* (2019) reported that the dry matter and crude protein level of hybrid napier harvested at 30 days of age were 16.68 and 14 per cent respectively. The higher CP level of fodder in the present study could be explained by various factors such as season, soil fertility, fertilization and stage of harvest (Ball et al., 2001; Collins et al., 2017).

Dry matter intake

Abdominal growth appears to be the initial factor governing the maximal feed intake of pre-ruminant animals during the first few days of life. After that, the maximum intake begins to be effectively controlled by energy needs of life (maintenance and growth) (Ternouth et al., 1985; Bas, 1988). It was evident from the earlier studies that the feed intake of animal varies with varied protein (Makun et al., 2016; Santos et al., 2014; Ullah et al., 2012) and energy sources (Adeloye, 2021). However, in the present study the total dry matter intake (DMI) of experimental kids (kg/animal) maintained on three different starter rations was similar ($p>0.05$) during the entire trial period. The average DMI and average daily DMI per 100 kg body weight of kids fed on three experimental rations was comparable ($p>0.05$) among groups. The results obtained in the present study were consistent with the findings of Babu et al. (2009), Rahman et al. (2016) and Salehi et al. (2022). On contrary, incorporation of dairy products (Schingoethe, 1976; Morrill and Dayton, 1974) resulted in improved palatability and thereby increased DMI in calves. However, earlier

studies (Supriyati, 2012 and Kamalahasan, 2018) revealed that whole milk fed group had higher ($p < 0.05$) DMI than those fed with dairy based milk replacers.

Total intake of starter (DM) by experimental kids (kg/animal) during the entire trial period was 6.72 ± 1.01 in G1, 8.95 ± 0.48 in G2 and 9.57 ± 0.36 in G3. The data indicated that starter DMI was significantly higher in G2 and G3 groups compared to G1 group. This variation could be attributed to the differences in diet composition and the duration of milk feeding. The kids in G1 were fed milk for an extended period (up to three months), while G2 and G3 offered with milk only upto 45 days of age. Young ruminants prioritize liquid feed sources such as milk, leading to reduced intake of solid feeds. The reliance on milk as a primary energy source likely influenced the DMI of the kids during the present study. Earlier studies (Kertz et al., 1979; Terre et al., 2007; Raeth-Knight et al., 2009 and Alvarez-Rodriguez et al., 2010) also emphasized an inverse relationship between milk and solid feed intake and concluded that limiting milk intake fostered intake of starter and hay, facilitating weaning at about 7 to 8 weeks of age. Furthermore, the starter rations of G2 and G3 included dried whey and SMP, which are known to enhance palatability and nutrient density. This likely contributed to higher starter DMI in these groups compared to G1, which relied on conventional kid starter rations.

Moreover, the role of dietary composition extends beyond mere intake levels; it significantly influences gastrointestinal development and nutrient absorption in pre-ruminant animals. For instance, a study indicated that ad libitum feeding of dairy milk replacers during the early weeks followed by a gradual reduction in intake, could enhance growth performance without adversely affecting rumen development. This suggests that while protein sources are crucial for immediate feed consumption, the timing and method of introducing solid feeds also play a pivotal role in shaping long-term health outcomes. Furthermore, optimizing starter rations not only aids in maximizing DMI but may also promote better ruminal fermentation processes, leading to improved overall energy efficiency in young livestock. Thus, a comprehensive approach to nutrition that balances both immediate intake and developmental needs is essential for enhancing the productivity of dairy calves and kids alike.

Table 4. Feed intake of experimental kids maintained on three dietary regimes, on DM basis

Parameters	Dietary Treatments			p value
	G1	G2	G3	
Total milk consumed (kg/ animal)	4.15[a] ± 0.22	1.95[b] ± 0.15	1.69[b] ± 0.13	0.00
Total starter intake (kg/ animal)	6.72[b] ± 1.01	8.95[a] ± 0.48	9.57[a] ± 0.36	0.02
Total fodder intake (kg/ animal)	3.4 ± 0.08	3.45 ± 0.15	3.4 ± 0.12	0.95
Total DMI (kg/animal)	14.05 ± 1.21	14.02 ± 0.55	14.37 ± 0.33	0.94
Average daily DMI (kg/animal)	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.94
Average daily DMI (kg) per 100 kg body weight	2.77 ± 0.10	2.54 ± 0.10	2.78 ± 0.14	0.30

[1]Mean values of six replicates with SE

Growth performance

Kids weaned early (45 days) and raised on dairy based rations (G2 and G3) showed similar growth characteristics (Fig. 1) with G1, emphasizing nutritional adequacy of dairy based starter rations and physiological adaptability of kids to those rations. Besides, it has been proposed that whey protein (Moller et al., 2001) is high in essential amino acids particularly leucine which could promote adequate muscle growth in early weaned kids. As stated in Meena et al. (2022), sirohi kids weaned at 60 days had a higher body weight at 120 days than those who were weaned later or earlier. The study also found that, weaning at 60 days did not impair growth when feeding and management were adequate. According to Vickery et al. (2023), gradual weaning produced inconsistent weight gain

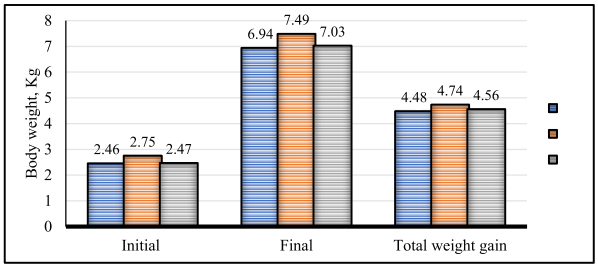

Fig. 1. Initial body weight, final body weight and Total weight gain of experimental kids maintained on three dietary treatments

results. However, they also observed that controlling the post-weaning diet (creep/solid feed) had a significant impact on later body weight, which implies that variations between weaning age groups tend to decrease if post-weaning nutrition is adequate.

Faecal condition score

Similar ($p > 0.05$) fortnightly mean faecal condition score (FCS) was observed in experimental kids maintained on three different dietary regimes (Fig. 2), agreeing the earlier findings (Lammers et al., 1998; Huuskonen, 2017). During the initial few weeks of the feeding trial, the average FCS was found to be greater, which then gradually declined over the weeks as also reported earlier (Kamalahasan, 2018). Yasumatsuya et al. (2012) also observed similar ($p > 0.05$) mean FCS among calves in three treatment groups fed with milk replacers having SMP, whey and its combination.

On contrary to the present findings, the incidence of diarrhoea was lower ($p < 0.05$) in whole milk fed calves than the calves fed with casein based milk replacer (Shukla et al., 2017). Similarly, Mahjoubi et al. (2017) noticed that lambs fed SMP based milk replacer at higher levels (20 per cent of body weight) had better faecal score than those lambs maintained either on whole milk or on SMP based milk replacer at a lower level (10 per cent of body weight).

Digestibility of nutrients

The inclusion of SMP and dried whey did not affect ($p > 0.05$) the digestibility (%) of nutrients (Table 5) among groups. The digestible crude protein of ration in G1, G2 and G3 groups were found to be 17.15, 16.85, 17.08 and the total digestible nutrient of ration were 68.75 in G1, 68.46 in G2 and 68.43 in G3, respectively. The results of the present study were consistent with the findings of Hill et al. (2016); Rahman et al. (2016); Chai et al. (2017); Kamalahasan (2018) and Araujo et al. (2020). In the present study weaning off milk at 45 days of age has no adverse effect on digestibility of kids as observed earlier (Tao et al., 2018 and Chai et al., 2017). On contrary, Sampelayo et al. (1990) and Suresh (2022) reported that the digestibility of nutrients was significantly higher in whole milk fed calves than in calves offered dairy based (SMP and whey) milk replacer. However, Gibney and Walker (1977) reported that the apparent digestibility of nitrogen and fat was significantly higher in lambs fed with SMP based milk replacer than those fed with soy protein based milk replacer. Similarly, Hasanpour et al. (2023) reported that use of 4.5 per cent whey powder in the starter ration of fattening lambs resulted in higher ($p < 0.05$) apparent digestibility of DM and CP over lambs fed with conventional starter ration and rations containing 1.5 and 3 per cent whey powder.

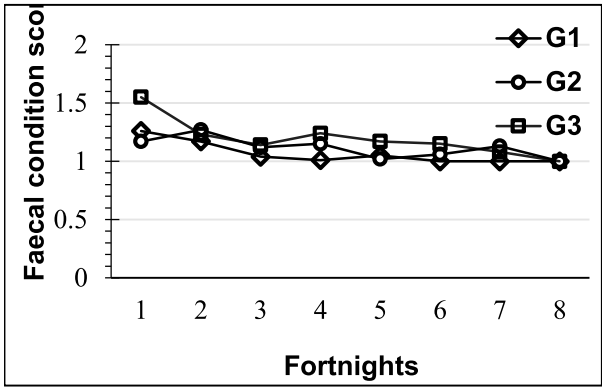

Fig. 2. Fortnightly average faecal condition score of experimental kids maintained on three dietary treatments

Table 5. The digestibility coefficient[1] (%) of nutrients of three experimental rations fed to Malabari kids

Parameters	Dietary Treatments			p value
	G1	G2	G3	
Dry matter	72.15 ± 0.59	71.55 ± 1.52	71.79 ± 0.87	0.92
Crude protein	80.84 ± 0.52	79.32 ± 0.88	80.16 ± 0.76	0.37
Crude fibre	58.77 ± 1.46	60.81 ± 2.76	61.01 ± 1.64	0.70
Ether extract	76.20 ± 1.21	79.84 ± 1.45	79.94 ± 1.17	0.10
Nitrogen free extract	73.87 ± 0.74	72.76 ± 1.57	72.52 ± 1.03	0.69
Neutral detergent fibre	71.16 ± 0.85	69.87 ± 1.98	69.97 ± 1.21	0.78
Acid detergent fibre	51.85 ± 1.52	49.60 ± 2.82	50.55 ± 2.10	0.77
Digestible crude protein	17.15 ± 0.18	16.85 ± 0.18	17.08 ± 0.12	0.39
Total digestible nutrient	68.75 ± 0.54	68.46 ± 1.42	68.43 ± 0.79	0.97

[1]Mean values of six replicates with SE

Hemato-biochemical parameters

The results indicated that different feeding regimes had no influence ($p>0.05$) on hemato-biochemical parameters (Table) of kids and values remained within the normal range documented for the species (Kaneko et al., 2008). The present results were comparable with the findings of Ortigues-Marty et al. (2003), Lee et al. (2009), Yasumatsuya et al. (2012), Huang et al. (2015) and Benak et al. (2021). However, the glucose values decreased over time irrespective of treatment groups. Pre-ruminants primarily use glucose as their energy source. Because of their under developed rumen epithelium, utilization of volatile fatty acids is limited (Vi et al., 2004). A drop in blood glucose with increase in age might be attributed to the shift in energy source from glucose to volatile fatty acids, as the rumen becomes functional (Hammon et al., 2002). The BUN levels of kids at sixteenth week were higher than those documented at sixth week. Urea is not only a waste product of nitrogen metabolism but also an important precursor for rumen microbial protein synthesis and plays crucial role in the development of forestomach in ruminants (Harmeyer and Martens, 1980). The urea nitrogen values in goats increases linearly with the increase in age during the first 12 months (Mbassa and Poulsen, 1992). This occurs because kids in early life are solely dependent on dam's milk. Milk proteins are highly digestible and efficiently utilized by the body, resulting in less protein breakdown and, consequently, lower urea production. As age advances starter rations and roughages having comparatively lower protein quality becomes the part of the ration, leading to increased protein catabolism and higher blood urea levels (Owens et al., 1998 and Blome et al., 2003).

Table 6. Hemato-biochemical parameters[1] of experimental kids maintained on three dietary treatments (sixth and sixteenth week)

Parameters	weeks	Dietary Treatments			p value
		G1	G2	G3	
Haemoglobin (g/dL)	6	10.90 ± 0.58	11.80 ± 0.82	11.43 ± 0.85	0.71
	16	11.88 ± 0.23	12.43 ± 0.41	11.63 ± 0.35	0.27
Serum glucose (mg/dL)	6	61.05 ± 1.51	62.13 ± 0.48	61.07 ± 0.99	0.73
	16	59.13 ± 3.19	58.98 ± 3.61	58.15 ± 6.12	0.97
Serum total proteins (g/dL)	6	6.16 ± 0.23	5.75 ± 0.10	6.05 ± 0.26	0.37
	16	6.12 ± 0.35	6.42 ± 0.31	6.15 ± 0.18	0.73
Serum albumin (g/dL)	6	3.76 ± 0.14	3.75 ± 0.06	3.64 ± 0.18	0.80
	16	3.77 ± 0.11	3.69 ± 0.07	3.47 ± 0.09	0.08
Serum calcium (mg/dL)	6	12.15 ± 0.25	12.22 ± 0.21	12.09 ± 0.20	0.92
	16	12.52 ± 0.26	12.26 ± 0.31	12.51 ± 0.14	0.71
Serum phosphorus (mg/dL)	6	8.00 ± 0.13	8.23 ± 0.08	8.18 ± 0.11	0.33
	16	8.08 ± 0.29	8.56 ± 0.21	8.16 ± 0.40	0.52
Blood urea nitrogen (mg/dL)	6	8.78 ± 0.98	9.10 ± 0.82	8.78 ± 0.44	0.94
	16	13.98 ± 0.78	13.98 ± 1.17	13.33 ± 0.60	0.84
Serum creatinine (mg/dL)	6	0.71 ± 0.06	0.72 ± 0.03	0.70 ± 0.02	0.97
	16	0.64 ± 0.01	0.65 ± 0.01	0.69 ± 0.02	0.13

[1]Mean values of six replicates with SE

CONCLUSION

On summarising the overall results of the study, it is evident that different starter rations did not significantly affect the overall digestibility of nutrients among the groups. Additionally, the hemato-biochemical parameters remained within normal ranges across all treatment groups, suggesting that these feeding strategies did not adversely impact the health or nutritional status of the kids. Furthermore, weaning off milk at 45 days can help in reducing the dependency on prolonged milk feeding while maintaining optimal health, thereby improving the economic efficiency of goat farming operations. However, further studies are warranted to explore the long-term effects of these dietary regimes on productivity and profitability in commercial settings.

REFERENCES

Adeloye, A. A. 2021. The influence of varying ratio of cowpea husk and maize milling waste on the feed intake, protein and energy utilization by the goat. Nigerian Journal of Animal Production. 21:108-112.

Albenzio, M., Santillo, A., Caroprese, M., Marino, R., Muscio, A. and Sevi, A. 2016. Goat milk consumption: Beneficial effects and production aspects. Small Ruminant Research. 134: 33-41.

Álvarez-Rodríguez, J., Sanz, A., Ripoll-Bosch, R. and Joy, M. 2010. Do alfalfa grazing and lactation length affect the digestive tract fill of light lambs? Small Ruminant Research. 94(1-3): 109-116.

AOAC [Association of Official Analytical Chemists]. 2016. Official Methods of Analysis of AOAC International. (20th Edn.). Association of Official Analytical Chemists, Washington D.C. USA. 1885p.

Araujo, A.R., Muir, J.P., de Vasconcelos, A.M., Pompeu, R.C.F.F., Guedes, L.F., Costa, C.S., de Sousa Carneiro, M.S., Campos, W.É. and Rogério, M.C.P. 2020. Consumption, apparent digestibility and nutrient balance of diets with bovine milk whey for goats. Semina: Agriculture Sciences. 41(5): 1719-1728.

Babu, L. K., Pandey, H., Patra, R. C. and Sahoo, A. 2009. Hemato biochemical changes, disease incidence and live weight gain in individual versus group reared calves fed on different levels of milk and skimmed milk. Animal Science Journal. 80(2): 149–156.

Ball, D.M., Collins, M., Lacefield, G.D., Martin, N.P., Mertens, D.A., Olson, K.E., Putnam, D.H., Undersander, D.J. and Wolf, M.W. 2001. Understanding forage quality. American Farm Bureau Federation Publication. 1(01): 1-15.

Banavara, D.S., Anupama, D. and Rankin, S.A. 2003. Studies on physicochemical and functional properties of commercial sweet whey powders. Journal of dairy Science. 86(12):3866-3875.

Benak, S., Bobic, T., Steiner, Z., Ronta, M., Novoselec, J., Đidara, M., Mijić, P. and Gantner, V. 2021. The effect of different starters on performance and physiological characteristics of early-weaned female Holstein calves. Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka. 71(3): 187-193.

Blome, R.M., Drackley, J.K., McKeith, F.K., Hutjens, M.F. and McCoy, G.C. 2003. Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. Journal of Animal Science. 81(6): 1641-1655.

Chai, J.M., Ma, T., Wang, H.C., Qi, M.L., Tu, Y., Diao, Q.Y. and Zhang, N.F. 2017. Effect of early weaning age on growth performance, nutrient digestibility, and serum parameters of lambs. Animal Production Science. 57(1): 110-115.

Collins, M., Newman, Y.C., Nelson, C.J., Barnes, R.F. and Moore, K.J. 2017. Forage quality. Forages.1: 269-286.

El-Tarabany, M.S., El-Tarabany, A.A. and Roushdy, E.M. 2018. Impact of lactation stage on milk composition and blood biochemical and hematological parameters of dairy Baladi goats. Saudi Journal of biological Sciences.25(8): 1632-1638.

Gibney, M. and Walker, D. 1977. Milk replacers for pre ruminant lambs: protein and fat interactions. Australian Journal of Agricultural Research. 28(4): 703-712.

Hammon, H.M., Schiessler, G., Nussbaum, A. and Blum, J.W. 2002. Feed intake patterns, growth performance, and metabolic and endocrine traits in calves fed unlimited amounts of colostrum and milk by automate, starting in the neonatal period. Journal of Dairy Science. 85(12): 3352-3362.

Harmeyer, J. and Martens, H. 1980. Aspects of urea metabolism in ruminants with reference to the goat. Journal of Dairy Science. 63(10): 1707-1728.

Hasanpour, M., Chashnidel, Y., Teimoury Yansary, A. and Ansari Pirsarei, Z. 2023. The effect of different levels of whey powder on growth performances traits, apparent digestibility of nutrients, carcass quality, and serum parameters of fattening male crossed Zell lambs. Journal of Ruminant Research. 11(3): 1-18.

Hill, T.M., Quigley, J.D., Bateman II, H.G., Suarez-Mena, F.X., Dennis, T.S. and Schlotterbeck, R.L. 2016. Effect of milk replacer program on calf performance and digestion of nutrients in dairy calves to 4 months of age. Journal of dairy Science. 99(10): 8103-8110.

Huang, K., Tu, Y., Si, B., Xu, G., Guo, J., Guo, F., Yang, C. and Diao, Q. 2015. Effects of protein sources for milk replacers on growth performance and serum biochemical indexes of suckling calves. Animal Nutrition. 1(4): 349-355.

Huuskonen, A. 2017. Effects of skim milk and whey-based milk replacers on feed intake and growth of dairy calves. Journal of Applied Animal Research. 45(1): 480-484.

Jasmine, 2015. Effect of incorporation of spent rosemary (*Rosmarinus officinalis* l.) Leaf meal as an ingredient in kid starter. M.V.Sc. thesis, Kerala Veterinary and Animal Sciences University, Pookode. 27p.

Jiao, J., Zhou, C., Guan, L.L., McSweeney, C.S., Tang, S., Wang, M. and Tan, Z., 2017. Shifts in host mucosal innate immune function are associated with ruminal microbial succession in supplemental feeding and grazing goats at different ages. Frontiers in microbiology. 8: 1655p.

Jong, H.H. and Vegeter, J.J. 1950. Determination of serum proteins with the biuret test. Pharm. Weekbl. 85: 755-764.

Kamalahasan, K. 2018. Evaluation of performance of crossbred calves under different feeding systems. M.V.Sc. thesis, Kerala Veterinary and Animal Sciences University, Pookode. 27p.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. eds., 2008. Clinical biochemistry of domestic animals. Academic press.

Kertz, A. F., L. R. Prewitt, and J. P. Everett J. R. 1979. An early weaning calf program: Summarization and review. Journal of Dairy Science. 62(11): 1835-1843.

Lammers, B.P., Heinrichs, A.J. and Aydin, A. 1998. The effect of whey protein concentrate or dried skim milk in milk replacer on calf performance and blood metabolites. Journal of Dairy Science. 81(7): 1940-1945.

Lee, H.J., Khan, M.A., Lee, W.S., Yang, S.H., Kim, S.B., Ki, K.S., Kim, H.S., Ha, J.K. and Choi, Y.J. 2009. Influence of equalizing the gross composition of milk replacer to that of whole milk on the performance of Holstein calves. Journal of Animal Science. 87(3): 1129–1137.

Madesh, M., Kishore, K.R., Kumar, D.S. and Anitha, A. 2019. In sacco degradability kinetics of Cumbu Napier (COBN-5) fodder at two different stages of harvest. Indian Journal of Animal Nutrition. 36: 423-427.

Mahjoubi, E., Hossein Yazdi, M., Afsarian, O. and Vonnahme, K.A. 2017. Evaluation of an accelerated growth program for pre-weaned Shall lambs. Livestock Science. 198: 72–75.

Makun, H.J., Daudu, O.M., Otaru, S.M., Barje, P.P. and Odegbile, O.E., 2016. Effect of supplementation with different protein sources on the feed intake and growth in red sokoto and sahelian goats. Journal of Animal Production Research. 28(1): 263-274.

Mbassa, G.K. and Poulsen, J.S.D. 1992. The comparative haematology of cross-bred and indigenous East African goats of Tanzania and breeds reared in Denmark. Veterinary research communications. 16: 221-229.

Meena, A.K., Uddin, A., Bhateshwar, V. and Muwal, H. 2022. Effect of weaning age on growth performance of sirohi kids. Indian Journal of Small Ruminants. 28(1): 204-206.

Mishra, S., Sonawane, M., Lohar, P. and Sonawane, S. 2022. Comparative study on nutritive properties and composition of milk of Sangamneri Goat, Nimari Cow and Surti Buffalo. IOSR Journal of Agriculture and Veterinary Science. 15(1):17-30.

Mohsin, A.Z., Sukor, R., Selamat, J., Hussin, A.S.M. and Ismail, I.H. 2019. Chemical and mineral composition of raw goat milk as affected by breed varieties available in Malaysia. International Journal of Food Properties. 22(1): 815–824.

Moller, P. L., Tvede, M. and Jensen, S. K. 2001. Nutritional adequacy of whey-based starter diets in early-weaned goat kids: Effects on growth performance and metabolic adaptation. Journal of Animal Nutrition. 55: 285-294.

Morand-Fehr, P., Richard, A., Tessier, J. and Hervieu, J., 2002. Effects of decoquinate on the growth and milk performance of young female goats. Small Ruminant Research. 45(2):109-114.

Morrill, J.L. and Dayton, A.D. 1974. Effect of whey on calf starter palatability. International Journal of Dairy Science. 57(4): 430-433.

Nayak, S., Rath, P.K., Panda, S.K., Mishra, B.P., Mishra, R. and Biswal, S.S. 2021. Etiopathological and hematobiochemical profiles in goats with gastrointestinal disorders. Veterinary World. 14(7):1760.

Ortigues-Marty, I., Hocquette, J.-F., Bertrand, G., Martineau, C., Vermorel, M. and Toullec, R. 2003. The incorporation of solubilized wheat proteins in milk replacers for veal calves: effects on growth performance and muscle oxidative capacity. Reproduction Nutrition Development. 43(1): 57–76.

Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. 1998. Acidosis in cattle: a review. Journal of Animal Science. 76(1): 275-286.

Park, Y. W., Juárez, M., Ramos, M. and Haenlein, G. F. W. 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research. 68(1-2): 88-113.

Patel, K., Chen, X. and Kar, S. 2005. The Temperature Uniformity During Air Drying of a Colloidal Liquid Droplet. Drying Technology. 23(12): 2337–2367.

Raeth-Knight, M., Chester-Jones, H., Hayes, S., Linn, J., Larson, R., Ziegler, D., Ziegler, B. and Broadwater, N. 2009. Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. Journal of Dairy Science. 92(2):799–809.

Rahman, M., Talukder, M., Ali, M.Y. and Akter, M.S. 2016. Effect of milk replacer on kid performance among small holder farmers. Asian Journal of Medical and Biological Research. 2(2): 357–360.

Salehi, M.Y., Nikkhah, A., Khabbazan, M.H., Darabi, B.H., Sadeghi, S. and Moshiri, B. 2022. Effects of Freely Accessed Whey on Performance and Metabolism of Growing Murciano-Granadina Goats. Iranian Journal of Applied Animal Science. 12(3): 555-560.

Sampelayo, M.S., Hernandez-Clua, O.D., Naranjo, J.A., Gil, F. and Boza, J. 1990. Utilization of goat milk vs. milk replacer for Granadina goat kids. Small Ruminant Research. 3(1): 37-46.

Santos, A.B., Pereira, M.L.A., Silva, H.G.O., Pedreira, M.S., Carvalho, G.G.P., Ribeiro, L.S.O., Almeida, P.J.P., Pereira, T.C.J. and Moreira, J.V., 2014. Nitrogen metabolism in lactating goats fed with diets containing different protein sources. Asian-Australasian Journal of Animal Sciences. 27(5): 658.

Sasikala, N. 2018. Comparison of different milk replacers on growth performance of Malabari kids. M.V.Sc. thesis, Kerala Veterinary and Animal Sciences University, Pookode, 27p.

Schingoethe, D.J., Ludens, F., Tucker, W.L. and Dash, S.K. 1976. Evaluation of dried whey in concentrate mixtures for lactating dairy cows. Journal of Dairy Science. 59(8): 1466-1470.

Shukla, M., Lunagariya, P.M., Shah, S.V., Pandya, P.R., Pandya, S.S. and Divekar, B.S. 2017. Effect of feeding milk replacer on dry matter intake and feed efficiency in Holstein x Kankrej crossbred calves. Indian Journal of Veterinary Sciences and Biotechnology. 12(3): 105-111

Singh, M.K., Singh, S.K. and Chauhan, M.S. 2023. Exploring potential of goat based dairy farming in India and way forward. The Indian Journal of Animal Sciences. 93(3):243-250.

Snedecor, G. W. and Cochran, W. G. 1994. Statistical Methods (8th Edn.). The Iowa State University Press, Ames, 503p.

Solaiman, S. and Shoemaker, C. 2009. Intake, digestibility, rumen metabolism and growth performance of goat kids raised under different production systems. Tropical and Subtropical Agroecosystems. 11(1):219-223.

Sudharsan, M., Kannan, A., Ks, A., Davis, J. and Radha, K. 2017. Impact of lactation stage on milk composition of Attappady black and Malabari goats. The Pharma Innovation Journal.10: 37-41.

Supriyati, T. 2012. Pre-weaning growth performance of Etawah Crossbred goats fed milk replacer. Journal of Animal Nutrition. 19:189–93.

Suresh, D. 2022. Assessment of performance of pre-ruminant crossbred calves maintained on different feeding strategies. M.V.Sc. thesis, Kerala Veterinary and Animal Sciences University, Pookode, 31p.

Tao, H., Guo, F., Tu, Y., Si, B.W., Xing, Y.-C., Huang, D.J. and Diao, Q.Y. 2018. Effect of weaning age on growth performance, feed efficiency, nutrient digestibility and blood-biochemical parameters in Droughtmaster crossbred beef calves. Asian-Australasian Journal of Animal Sciences. 31(6): 864–872.

Terre, M., Devant, M. and Bach, A. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livestock Science. 110(1-2): 82–88.

Ullah, M.B., Islam, K.M.S., Akbar, M.A., Khaleduzzaman, A.B.M. and Debi, M.R. 2009. Effect of levels and sources of protein on the performance and nutrient digestibility in black Bengal goat. Bangladesh Journal of Animal Science. 38(1-2): 53-60.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of dairy science. 74(10): 3583-3597.

Vi, R.B., McLeod, K.R., Klotz, J.L. and Heitmann, R.N. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre and postweaning ruminant. Journal of Dairy Science. 87: 55-65.

Vickery, H.M., Neal, R.A., Stergiadis, S., Meagher, R.K. 2023. Gradually weaning goat kids may improve weight gains while reducing weaning stress and increasing creep feed intakes. Frontiers in Veterinary Science. 10:1200849.

Yasumatsuya, K., Kasai, K., Yamanaka, K., Sakase, M., Nishino, O., Akaike, M., Mandokoro, K., Nagase, T. and Kume, S. 2012. Effects of feeding whey protein on growth rate and mucosal IgA induction in Japanese Black calves. Livestock Science. 143(2-3): 210-213.

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In-Vitro, *In-Situ* and *In-Vivo* Evaluation of *Alfalfa* Residue Based Total Mixed Ration Feed Blocks for Sheep

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ABSTRACT

This study explored the potential of Alfalfa (*Medicago sativa*) residue (AR) as a protein rich concentrate for growing lambs by conducting *In-Vitro*, *In-Situ* and *In-Vivo* methods. The AR was high in crude protein (49.25%) and low in crude fiber (0.91%), with Ca: P ratio of 5.67:1.47. *In-Situ* protein degradability was very slow (RDP:UDP ratio was 10.49:89.51) and the Metabolizable energy (ME) of 4.3 MJ/kg was found. An *In-Vivo* feeding trial was conducted with 18 male lambs (3-4 months old) divided into three groups: T1 (Control) received Total Mixed Ration feed block (TMRfb) containing groundnut haulms and compounded feed mixture (50:50); T2 replaced 25% of crude protein (CP) with AR, and T3 replaced 50%. All rations were iso-nitrogenous. Results showed that DMI (% of body weight) in T2 (4.28) and T3 (4.19) had higher than T1 (4.04), but not varied significantly. Feed efficiency was similar across groups (5.41 to 5.81). Average daily gain (ADG, g/d) was higher in T2 (123.69) compared to T3 (111.29) and T1 (106.9). Nutrient digestibility and nitrogen balance were comparable among groups. Therefore, Alfalfa residue can effectively replace up to 50% of crude protein in lamb diets for its growth.

KEYWORDS: Alfalfa residue, *In-Situ*, *In-Vitro*, lambs, Total Mixed Ration feed block

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INTRODUCTION

Feeding small ruminants in tropical regions largely depends on natural pastures and low-quality crop residues, which are often deficient in crude protein, energy and minerals. This often identified as a major factor limiting production efficiency. To address this issue, residues after the extraction of active medicinal components from legumes and other sources could serve as alternative protein sources for livestock.

Alfalfa (*Medicago sativa*), known as "the queen of forages" valued for its high biomass yield, quality forage, and palatability (Hawkins and Yu, 2018). It can be harvested after 55-80 days of sowing, producing 80-100 tons of green fodder per hectare annually under proper agronomic practices. Alfalfa contains 16-25% crude protein with 72% digestibility, 20-30% fibre, and various vitamins and minerals (Lei et al., 2017). Alfalfa forage is mechanically processed to produce chemical components with medicinal characteristics, leaving behind a residue known as residue called as Alfalfa residue (AR) that can be used as a protein concentrate in animal feeding (Hojilla-Evangelista et al., 2017).

In view of the limited information on utilization of AR based TMR blocks in this region the present research was carried out for its nutritive evaluation by *in-vitro*, *in-situ* and *in-vivo* methods for small ruminant feeding.

MATERIALS AND METHODS

Procurement of ingredients and nutrient analysis

The dry and pelleted form of Alfalfa residue (AR) was procured from a commercial firm (Kabini Enterprises, #77/4, Agasavalli, Honnali road, Kalluru, Shivamogga, Karnataka, India -577204). AR pellets (3-4mm) were stored in a clean and dry area at room temperature. About 600kg of properly

sundried groundnut haulms was procured from local area and grinded to particle size of 6-8mm using mechanical shredder. The CFM was prepared using maize, soybean meal, de-oiled rice bran, sunflower cake, molasses, mineral mixture, salt and urea at 45, 15, 10, 18, 8, 2, 1 and 1 per cent, respectively (Table 1).

All the dried samples were ground in a willey mill (Thomas scientific, wiley® model 4 mills) to pass through 1mm screen and analyzed for proximate composition (AOAC, 2016); fiber fractions (Van Soest et al., 1991); ME and kinetics of gas production as per Rumen *in vitro* incubation and gas production (RIVIGP) of Menke and Steingass (1988). The samples were also subjected to rumen i*n situ* incubation (Singh et al., 1995), the rumen degradable and undegradable fractions (Dry matter (DM) and CP) (Orskov and McDonald, 1979), the mineral content (Ca and P) was estimated by gravimetric method as per AOAC (2005). The AR was checked for qualitative estimation for urea by spot test as per method of Garg et al. (2013).

Preparation of TMR Feed blocks containing Alfalfa residue

The total mixed ration (TMR) feed block was formulated (Table 1) to contain around 18% CP. Premixing of CFM with molasses was made before mixing with groundnut haulms using 150kg capacity horizontal mixer for 5 minutes. The ratio of groundnut haulm and CFM was kept at ratio of 50:50 in all the treatment groups. Feed block was prepared by compressing this mix at 2800-3000 psi for 15-20 seconds using Feed Block Making Machine (HITECH HYDRAULICS, New Delhi, India. model no: DGH 04-3060-H-17) with a capacity to produce 25 to 30 blocks of 1-1.3kg per hour.

Table 1. Composition of TMR feed blocks (% as such basis) used in feeding trial

Ingredients	TMRfb-1	TMRfb-2	TMRfb-3
Groundnut haulms	50	50	50
Maize grain	22.5	23	31
Soybean mea	l7.5	2.5	1
Deoiled Rice Bran	5	7	1
Sunflower cake	9	6.5	1
Molasses	4	4	4
Mineral mixture	1	1	1
3Salt	0.5	0.5	0.5
Urea	0.5	0.5	0.5
Alfalfa residue	0	5	10
Total	100	100	100

TMRfb: Total mixed ration feed block
TMRfb-1: prepared by using groundnut haulms and CFM
TMRfb-2: prepared by replacing 25% of CP of control diet with AR
TMRfb-3: prepared by replacing 50% of CP of control diet with AR

Physical characteristics of TMR Feed Blocks

The bulk density (kg/m^3) of the feed block was calculated by measuring the weight and its dimension in terms of length, width, and height (in cm) of the six TMRfb in each group.

Kinetics of gas production

Air equilibrated samples (200±10 mg) were incubated in 100 ml calibrated glass syringes in triplicate with 30ml mixed rumen suspension along with blanks and roughage and concentrate standards (Menke and Steingass, 1988). Cumulative gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72 and 96 hours of incubation. Data on gas production were fitted to the exponential equation $P = a + b (1-e^{-ct})$, where, P (ml) is gas production at time t; a (ml) is initial gas production; b (ml) is the potential gas production at 96 hours; and c is the rate (ml/h) of gas production (Orskov and McDonald, 1979). The metabolizable energy content and kinetics of gas production for AR, groundnut haulms and TMRfb was calculated.

In situ incubation

The *In-Situ* technique was used to assess the protein degradability of AR in the rumen of cannulated Deoni breed bull that was used for *in-vitro* gas test. Incubation times were set at 0.1, 2, 4, 8, 16, 24, 36, 48, 72 and 96 hours. Residual DM and CP were estimated according to AOAC (2005).

The soluble or rapidly degradable fraction at 0.1h (*a*), insoluble but potentially degradable fraction (*b*), residual component at 96h of incubation (*c*), the rate of degradation (*kd*, /h=0.693/$t_{1/2}$), Potential degradability [$Y=a+b(1-e^{-kd\times t})$], Effective degradability [$P=a+b(Kd/Kd+Kp)$] where *Kp* is the rate of passage, taken as 0.056/h and rumen undegradable fractions (100-*P*) of AR for DM, OM and CP were obtained after fitting data to the exponential model of nonlinear regression (Orskov and McDonald, 1979).

Feeding cum metabolic trial

Eighteen male lambs (non-descriptive types) of about 3-4 months age (an average body weight of 11.05 kg) were selected and randomly allotted into 3 groups (T1, T2 and T3) containing six each. T1: fed with TMRfb-1; prepared by using groundnut haulms and CFM; T2: fed with TMRfb-2: prepared by replacing 25% of CP of control diet with AR; T3: fed with TMRfb-3: prepared by replacing 50% of CP of control diet with AR.

The feeding trial involved two-weeks of adjustment period, followed by a twelve-week feeding trial and 5 days of metabolic trial. The lamb's diet was formulated to target a daily weight gain of 125 g, based on crude protein (CP%) and total digestible nutrients (TDN%) as per ICAR (2013) guidelines. Permission for using animals for the experiment was obtained from Institutional Animal Ethics Committee (IAEC) constituted as per Article No.13 of the CCSEA rules laid down by Government of India.

TMR feed blocks (TMRfb) were provided twice daily at 9:00 am and 4:00 pm and clean water was available throughout the day. Daily feed intake was calculated by making the difference between offered and left feed. All the lambs were weighed every week by using digital platform balance and growth was recorded in terms of ADG (g/d). Metabolic trial was conducted at the end of feeding trial by shifting into individual metabolic cage to estimate digestibility and nutrient balance.

Statistical analysis

Data on DMI, nutrient intake, ADG, digestibility coefficients and Nitrogen, Calcium, and Phosphorus balance were analyzed using one-way ANOVA with GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, CA, USA). Rumen *In-Vitro* gas production and *In-Situ* degradation kinetics were analyzed *via* non-linear regression using GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, CA, USA) to assess gas kinetics with an exponential decay equation model.

RESULTS AND DISCUSSION

Chemical composition of AR, groundnut haulms and experimental diets

The AR had a crude protein (CP) content of 49.25% (Table 2), consistent with findings by Madhekar and Mungikar (2009). The crude fibre (CF) content of AR was 0.91%, similar to Gawel and Grzelak (2014). The ether extract content of AR in this study was 0.41%, lower than the 1.83% reported by Homolka et al. (2012) for Alfalfa hay, due to the defatting process applied to AR production. Calcium and Phosphorus ratio of AR was 5.7:1.4. Groundnut haulms used as roughage source had more CP and compared to legume straws (Chopade et al., 2010 and Patil et al., 2024). The TMRfb were formulated to be iso-nitrogenous to evaluate AR inclusion, with CP of 18.15, 18.13 and 18.08 per cent, respectively.

Table 2. Chemical composition (% on DMB) of AR, groundnut haulms and TMRfb

Parameter	AR	GH	TMRfb-1	TMRfb-2	TMRfb-3
DM	95.8	93.5	90.8	90.9	90.9
OM	80.64	86.18	84.07	83.39	83.15
CP	49.25	16.63	18.15	18.13	18.08
CF	0.91	23.52	14.1	14.04	12.70
EE	0.41	2.56	3.960	3.50	2.74
NFE	24.66	37.3	41.84	41.89	44.17
TA	18.07	13.82	9.93	10.54	10.73
AIA	2.02	1.96	2.9	3.1	3.2
NDF	11.15	44.26	30.51	30.3	27.1
ADF	3.68	30.5	19.89	19.66	17.26
ADL	0.04	8.1	4.77	4.6	4.25
HC	7.46	13.76	10.62	10.64	9.84
C	3.64	22.4	15.12	15.05	13.01
Ca	5.67	0.75	0.48	0.75	0.97
P	1.42	0.52	0.43	0.47	0.65

Physical characteristics of TMRfb

The Average weight (Kg) and density (kg/m^3) of TMRfb was ranged from 1.06 to 1.10 and 686.6 to 747.9 (Table 3). Singh et al. (2016) reported a density of 562.6 kg/m^3 for feed blocks prepared with wheat straw and CFM (50:50) and Samanta et al. (2003) noted that feed blocks made from natural grass hay and CFM (60:40) had a density of 550-600 kg/m^3. In the present study, the particle size of groundnut haulm and CFM was about 6-8mm and 2-3mm, respectively. Feed blocks were prepared by applying pressure of 2800-3000 psi with dwelling time of 15-20 seconds and it has produced a durable feed block of 1-1.1kg.

Table 3. Physical characteristics of TMRfb used in feeding trial

TMRfb	Weight	Length	Width	Height	Density
	kg	cm	cm	cm	kg/m^3
TMRfb-1	1.06	16.2	15.4	6.2	686.6 (19.44kg/ft^3)
TMRfb-2	1.10	15.7	15.3	6.1	747.9 (21.18 kg/ft^3)
TMRfb-3	1.06	15.9	15.3	6.2	701.5 (19.86 kg/ft^3)

Rumen In vitro incubation for gas production (RIVIGP)

The RIVIGP values (RIVIGP^{24}, *a, D, k,* $t_{1/2}$ and RIVIGP^{96}) of AR, groundnut haulm and TMRfb used in the feeding trial are presented in Table 4. Babiker et al. (2017) reported ME value of 7.1 MJ/kg DM in Alfalfa hay. The rate and extent of gas production in AR was lower in present study. The lower ME (4.3 MJ/kgDM) and RIVGP^{24} ml/200mg DM (15.17) in AR was may be due to processing methods used in its production. By application of pressure and maceration about 55–60% of the juice from fresh plant will be removed (Arlabosse et al., 2011) and along with this application of temperature (Koschuh et al., 2004 and Damborg et al., 2020) alters plant structure and impaired the accessibility of nutrients to rumen microbes during *in vitro* analysis for its gas production for ME estimation. Inclusion of AR in TMRfb2 and TMRfb3 reduced gas production (RIVGP^{24}ml/200mg DM) and ME (MJ/kgDM) by 7 and 17 per cent and 6.86 and 13.73 per cent, respectively in comparison with TMRfb1.

Table 4: Kinetics of gas production of AR, GH and feed blocks

Feed sample	ME (MJ/kg DM)	RIVGP^{24} ml/200mg DM	RIVGP^{96}ml/200mg DM	*a*	*D*	*k*	$t_{1/2}$
AR	4.3	15.17	24.87	1.39	26.21	0.04	19.62
GH	8.0	37.78	47.69	0.62	45.99	0.09	7.69
TMRfb1	9.10	49.07	56.69	6.56	52.28	0.08	9.56
TMRfb2	8.75	46.96	56.26	2.93	51.61	0.06	10.38
TMRfb3	8.40	44.30	50.40	1.96	47.35	0.061	0.62

RIVGP^{24}- Rumen *in vitro* Gas Production at 24 hours; RIVGP^{96}- Rumen *in vitro* Gas Production at 96 hours; a-Rapidly produced gas (ml/200mg DM); D- potential gas production (ml/200mg DM); *K* (h^{-1}) – Rate of production per hour; *t1/2* – time at which half of the gas is produced

Kinetics of degradability

The protein degradability of AR was determined as per Orskov and McDonald (1979) method of *in-situ* degradability (Table 5). The degradability percent for DM, OM and CP were 25.57, 15.97, 58.46, 0.000001, 41.54 and 25.57; 17.50, 21.87, 60.63, 0.0012, 39.37 and 18.34; and 9.82, 27.17, 63.01, 0.001, 36.99 and 10.49, respectively.

Lu and Jorgensen (1997) reported 35.3 per cent protein degradability for AR. In this study the ratio of RDP:UDP of AR was 10:90. The lower protein degradability of AR was due to processing methods applied during its production (Lu et al., 1988, Koschuh et al., 2004 and Damborg et al., 2020). The ADIN of AR was only about 1.21 per cent (or 7.56 per cent of total CP), indicating that its UDP (90% of CP) is further digested enzymatically and not lost through dung.

Table 5. *In situ* rumen degradability of DM, OM and CP (*per cent*) of AR

Variables	*DM*	*OM*	*CP*
a	25.57	17.50	9.82
b	15.97	21.87	27.17
Degradability at 24h	29.67	23.52	17.34
c	58.46	60.63	63.01
$k_d (h^{-1})$	0.000001	0.001215	0.001034
Y	41.54	39.37	36.99
P^1	25.57	18.34	10.49 (RDP)
Rumen undegradable Dm[2]	74.43	81.66	89.51 (UDP)
ADIN (%)	1.21		

a = soluble or rapidly degradable fraction; *b* = insoluble but potentially degradable fraction; *c*=Residual dry matter at 96 h of incubation; k_d=0.693/$t_{1/2}$, degradation rate (h^{-1}); [a, b and k_d are based on nonlinear regression using the exponential model]; *Y* is fraction degradation at time t, $Y=a+b\ (1-e^{-kd*t})$; [1]effective degradability, $P= a+b(K_d/K_d+K_p)$ [K_p is the rate of passage, taken as 0.056/h] ; [2](100-*P*).

Average nutrient intake and nutrient density

The average daily dry matter intake (DMI) of TMR feed blocks ranged from 614.20 g/day for T1 to 666.70 g/day for T2 and DMI (% BW) from 4.04 (T1) to 4.28 per cent (T2) but difference was non-significant (P≥0.05).

Nutritive value of diet in terms of DCP (%), TDN (%) and ME (MJ/kg), along with intake of DCP (g/d), TDN (g/d), and ME (MJ/d), are given in Table 6. There were no significant differences in CP, DCP and TDN intake across groups. The average crude protein intake (CPI) was 111.48 g/d, T1; 120.87 for T2; and 114.84 g/d, for T3. CPI differences were also non-significant (P≥0.05) and the all lambs were provided sufficient protein (109.09 g/d) to achieve ADG of 125g/d as stipulated by ICAR (2013) feeding standard. In the entire feeding trial, an additional intake of about 2-5g of CP was found. Whereas, the intake of DCP and energy was lower than the ICAR (2013) standard requirements for an ADG of 125 g/day. Pavan et al. (2024) reported a lower DMI of 3.64% for sorghum-based TMRfb, likely due to its higher roughage portion (60:40) and NDF intake. Rani et al. (2016) found no significant difference in dry matter intake when replacing soybean meal with Alfalfa residue pellets at 20% inclusion in calf rations, indicating similar palatability to conventional protein sources.

Table 6. Average intake of nutrients in lambs during feeding trial

Parameter	T1	T2	T3	SEM	P
Average body weight (kg)	15.81	16.15	15.78	0.33	0.81
DMI (g/d)	614.20	666.70	635.18	18.14	0.53
% body weight	4.04	4.28	4.19	0.08	0.54
CP (%)	18.15	18.13	18.08	0.01	0.001
DCP (%)	9.43	10.01	9.60	0.30	0.75
TDN (%)	60.47	58.69	56.92	0.75	0.17
ME (MJ/kg)[#]	9.10	8.75	8.40	-	-
CP intake (g/d)	111.48 (109.09)	120.87 (109.98)	114.84 (109.01)	3.29	0.53
DCP intake (g/d)	57.91 (73.10)	66.71 (73.67)	60.95 (73.05)	2.50	0.36
TDN intake (g/d)	371.41 (431.13)	391.29 (435.63)	361.54 (430.73)	8.88	0.37
ME intake (MJ/d)[#]	5.58 (6.52)	5.83 (6.59)	5.33 (6.52)	0.20	0.51

P<0.01, P≤0.05, Means bearing different superscripts between the columns differ significantly and P≥0.05 is non-significant. Values in parenthesis are the requirements as stipulated by the ICAR (2013) for the average body weight of lambs with ADG of 125 g/d.

Digestion cum metabolic trial: Nutrient digestibility

The intake of NDF and ADF (Table 7) in all the groups was adequate and at levels required for optimum fermentation in rumen (Van Soest et al., 1991; NASEM, 2021).

The digestibility of DM, OM, CP, CF, NDF and ADF between the treatment groups had no significant difference and the values ranged from 63.81 (T3) to 69.23 (T1); 70.27 (T1) to 71.89(T2); 51.95 (T1) to 55.19 (T2); 50.05 (T3) to 54.21 (T2); 59.36 (T3) to 62.93 (T2); and 52.79 (T3) to 59.96 (T1) respectively. No significant difference (P≥0.05) for nutrient digestibility was observed between the treatment groups. Nutrient digestibility between the treatment group showed no significant difference in a study conducted by Rani et al. (2016) where soybean meal was replaced Alfalfa residue pellets at 20% level. The digestibility values of CF, EE, NDF, and ADF were similar to the values reported in the present study.

Table 7. Mean intake of nutrients and digestibility (%) during metabolism trial

Parameter		T1	T2	T3	SEM	P-value
	g/d	823.37	891.18	848.67	27.91	0.68
DM	% Body weight	4.06	4.20	4.14	0.14	0.82
	Digestibility	69.23	66.00	63.81	0.99	0.08
	g/d	669.54	739.66	697.71	22.77	0.62
OM	% Body weight	3.36	3.46	3.41	0.08	0.84
	Digestibility	70.27	71.89	70.33	1.67	0.54
	g/d	137.87	159.71	144.24	6.05	0.47
CP	% Body weight	0.70	0.75	0.71	0.02	0.53
	Digestibility	51.95	55.19	53.07	1.18	0.75
	g/d	113.85	126.48	107.24	4.35	0.19
CF	% Body weight	0.58	0.59	0.52	0.02	0.28
	Digestibility	53.09	54.21	50.05	1.16	0.32
	g/d	237.84	260.70	223.00	9.18	0.27
NDF	% Body weight	1.20	1.22	1.09	0.03	0.24
	Digestibility	62.60	62.93	59.36	1.76	0.27
	g/d	151.98	155.16	136.53	5.93	0.41
ADF	% Body weight	0.76	0.73	0.67	0.03	0.49
	Digestibility	59.96	55.52	52.79	1.77	0.68

P<0.01, P≤0.05, Means bearing different superscripts between the columns differ significantly and P≥0.05 is non-significant.

Growth performance, feed efficiency and balance of nutrients

The total body weight gain (kg) for 12 weeks of feeding trial was 8.59, 10.39 and 9.35 for T1, T2 and T3, respectively (Table 8). The respective average daily gain (ADG, g/d) was 106.90, 123.69 and 111.29. Feed efficiency was not significantly different (P≥0.05) among the treatment groups. T2 had the best feed efficiency (5.41), followed by T3 (5.71) and T1 (5.81).

The diet for the experimental lambs was formulated to target a daily gain of 125 g (ICAR, 2013) based on CP (%) and TDN (%). While the T2 group achieved this target, the ADG in T1 and T3 was 14 to 19 g lower, though the difference was non-significant, indicating uniform nutrient intake across all groups using TMRfb. Pavan et al. (2024) reported ADG of 85.2 g/d for sorghum-based TMRfb fed lambs. Whereas, in this study, the quality of TMRFb was much superior (TDN, DCP and digestibility) hence higher gain and efficiency was observed. In the present experiment, the positive nitrogen balance was observed in all the treatment which is suggesting nutritional adequacy with respect to energy and protein supply for maintenance and growth. The higher nitrogen retention in T2 was attributed to greater crude protein digestibility compared to the other groups. There was a significant difference (P<0.001) in calcium intake more in T3 and T2 due to presence of AR in these TMRfb (Table 2). Although higher retention in T3 and T2, but of non-significant. Similar trend was noticed with regard to phosphorus metabolism (Table 8).

Table 8. Growth performance, feed efficiency and balance of nutrients during the feeding trial

Parameter	T1	T2	T3	SEM	P
Initial body wt. (kg)	11.32	10.96	11.11	0.23	0.83
Final body wt. (kg)	20.30	21.35	20.46	0.53	0.71
Total weight gain (kg)	8.98	10.39	9.35	0.57	0.61
ADG (g)	106.90	123.69	111.29	6.81	0.61
Feed efficiency	5.81	5.41	5.71	0.36	0.57
Nitrogen balance					
Nitrogen intake (g)	22.99	25.72	23.38	1.93	0.49
Nitrogen outgo (dung + urine) (g)	9.94	8.96	8.88	0.66	0.17
Absorbed nitrogen (g)	18.10	21.99	19.90	0.94	0.38
Retained nitrogen (g)	13.05	16.76	14.49	0.92	0.28
N retained/ N intake (%)	55.84	64.94	60.95	4.63	0.09
N retained/ N absorbed (%)	69.79	76.08	71.73	1.55	0.17
Calcium balanceCalcium intake (g)	3.92^{c}	6.66^{b}	8.29^{a}	0.48	0.001
Calcium excretion in dung	1.75^{c}	2.75^{b}	3.71^{a}	0.22	0.001
Calcium excretion in urine	0.54^{b}	0.70^{ab}	0.81^{a}	0.05	0.04
Ca retained/ Ca intake (%)	40.63	47.93	44.53	2.40	0.51
Phosphorus balance					
Phosphorus intake (g)	3.34^{b}	4.01^{b}	5.44^{a}	0.28	0.001
Phosphorus excretion in dung	1.42^{b}	1.85^{ab}	2.12^{a}	0.10	0.01
Phosphorus excretion in urine	0.24	0.20	0.20	0.01	0.41
P retained/ P intake (%)	49.72	48.72	56.33	1.77	0.13

$P<0.01$, $P\leq0.05$, Means bearing different superscripts between the columns differ significantly and $P\geq0.05$ is non-significant

CONCLUSION

The AR is found to have higher per cent of CP (49.25) and Ca:P ratio (5.7:1.4) which would be one of the better sources of nutrient for the small ruminants. However, the rumen degradability found to be lower indicating good bypass protein value because of processing methods during extraction of compounds from Alfalfa forage. Replacement of AR up to 50 per cent of protein requirement did not affect the intake of nutrient, gain and digestibility in small ruminants.

REFERENCES

AOAC. 2016. Official methods of analysis of the Association of Official Analytical Chemists, 20th Edn., AOAC Inc., Washington DC.

Arlabosse, P. and Blanc, M., 2011, October. Thermo-mechanical fractionation of green biomass: optimisation of the processing conditions according to the water potential of the tissue. In European Drying Conference. pp.26-28.

Babiker, E.E., Juhaimi, F.A., Ghafoor, K. and Abdoun, K.A., 2017. Comparative study on feeding value of Moringa leaves as a partial replacement for Alfalfa hay in ewes and goats. Livestock Science. 195: 21-26.

Chopade, S., Kalbande, V.H., Shelke, S.K. and Dandage, S.D. 2010. Growth performance and economics of urea treated soybean straw based pelleted complete ration in kids. Indian Journal of Animal Nutrition. 27: 138-141.

Damborg, V.K., Jensen, S.K., Weisbjerg, M.R., Adamsen, A.P. and Stodkilde, L. 2020. Screw-pressed fractions from green forages as animal feed: Chemical composition and mass balances. Animal Feed Science and Technology. 261: 114401.

Garg, M.R., Sherasia, P.L. and Bhanderi, B.M., 2013. Quality control manual for cattle feed plants. Animal Nutrition Group, National Dairy Development Board, Anand-388. 1: 8.

Gaweł, E. and Grzelak, M. 2014. Protein from Alfalfa in animals supplement diet. Journal of Food, Agriculture and Environment. 12 (2): 314-319.

Hawkins, C. and Yu, L.X. 2018. Recent progress in Alfalfa (*Medicago Sativa L.*) genomics and genomic selection. Crop Journal. 6(6): 565-575.

Hojilla-Evangelista, M.P., Selling, G.W., Hatfield, R. and Digman, M. 2017. Extraction, composition, and functional properties of dried AR (*Medicago sativa L.*) leaf protein. Journal of the Science of Food and Agriculture. 97(3): 882-888.

Homolka, P., Koukolova, V., Podsednicek, M. and Hlavackova, A. 2012. Nutritive value of red clover and lucerne forages for ruminants estimated by in vitro and in vivo digestibility methods. Czech Journal of Animal Science. 57 (10): 454-568.

ICAR. 2013. Nutritional Requirements of Sheep, Goat and Rabbit, 2nd Edn., Indian Council of Agricultural Research, New Delhi, pp.7-8.

Koschuh, W., Povoden, G., Thang, V.H., Kromus, S., Kulbe, K.D., Novalin, S. and Krotscheck, C. 2004. Production of leaf protein concentrate from ryegrass (Lolium perenne x multiflorum) and Alfalfa (*Medicago Sativa*) Comparison between heat coagulation/centrifugation and ultrafiltration. Desalination. 163 (1-3): 253-259.

Lei, Y., Hannoufa, A. and Yu, P. 2017. The use of gene modification and advanced molecular structure analyses towards improving Alfalfa forage. International Journal of Molecular Science. 18 (2): 298.

Lu, C.D. and Jorgensen, N.A. 1997. LNR Protein Concentrate as a Less Ruminally Degradable Protein Source for High-Producing Dairy Cows. Journal of Agricultural and Marine Sciences. 2:31-35.

Lu, C.D., Jorgensen, N.A. and Satter, L.D., 1988. Site and extent of nutrient digestion in lactating dairy cows fed LNR protein concentrate or soybean meal. Journal of Dairy Science. 71 (3):697-704.

Madhekar, R.D. and Mungikar, A.M. 2009. Chemical composition of Alfalfa (*Medicago sativa* L.) leaf meal and leaf protein concentrate. Journal of Phytological Research. 22: 95-98.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development. 28: 7-55

NASEM, 2021. National Academies of Sciences, Engineering and Medicine, 2021. Nutrient Requirements of Dairy Cattle, 8th Edn. National Academy Press, Washington, DC, The National Academies Press.

Orskov, E.R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science. 92 (2): 499-503.

Patil, P.V., Gendley, M.K. Dubey, M. Dhok, A.P. and Prusti. S. 2024. Effect of Feeding Gram Straw based Complete Feed Pellets on Rumen Fermentation and Haemato-biochemical Parameters in Goats. Indian Journal of Animal Nutrition. 41 (2): 280-287.

Pavan B.L., V. Nagabhushana, G.U. Manju, K.S. Giridhar, N. Suma and B.U. Umesh. Effect of Feeding Sorghum Hay, Maize Stover and Areca Sheath Based Total Mixed Ration Feed Block (TMRfb) on Nutrient Intake, Digestibility and Cost Economics in Growing Lambs. Indian Journal of Animal Nutrition. 2024. 42 (1): 54-60.

Rani, K.J., Ally, K., Seethal, C.C., Surej, J.B. and Rajkumar, G. 2016. Effect of dietary incorporation of nutraceutical residue on dry matter intake and nutrient digestibility in crossbred calves. Journal of Veterinary and Animal Science. 47 (2): 78-81.

Samanta, A.K., Singh, K.K., Das, M.M., Maity, S.B. and Kundu, S.S. 2003. Effect of complete feed block on nutrient utilisation and rumen fermentation in

Barbari goats. Small Ruminant Research. 48 (2): 95-102.

Singh, C.K., Robinson P.H. and Mc Niven, M.A. 1995. Evaluation of raw and roasted lupin seeds as protein supplement for lactating cows. Animal Feed Science and Technology. 52: 63-76.

Singh, P.K., Chandramoni, Kumar, K. and Kumar, S. 2016. Effect of feeding wheat and rice straw based complete feed blocks on nutrients utilization, blood biochemical and growth performance in crossbred calves. Indian Journal of Animal Science. 86 (7): 771-776.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74 (10): 3583-3597.

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Assessment of Milk Replacer Supplementation on Growth Performance and Mortality in Pre-weaned Lambs under Field Conditions in Guntur District of Andhra Pradesh

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ABSTRACT

Lamb mortality and poor growth rates are the major constraint in sheep farming of Andhra Pradesh where Nellore and Macharla lambs often fail to reach their genetic growth potential due to inadequate maternal milk and limited feed resources. To address this, nutritional interventions such as milk replacer feeding have emerged as practical strategies to supplement maternal milk, improve survivability, and enhance growth in lambs. With this perspective, Krishi Vigyan Kendra, Guntur, under the Indian Council of Agricultural Research – Agricultural Technology Application Research Institute (ICAR–ATARI), Zone X sponsored Impactful Interventions programme, conducted a field study to evaluate the effect of milk replacer supplementation on pre-weaning lamb performance. A total of 84 lambs aged 2-3 weeks from 12 flocks with an initial body weight of 4 ± 0.5 kg divided into two groups: Control group (CON) without supplementation and milk replacer (MR) group supplemented with formulated milk replacer procured from the ICAR–National Institute of Animal Nutrition and Physiology (ICAR–NIANP). The MR group lambs were fed with 50-70 grams per day per lamb from 2-14 weeks of age along with maternal milk and ad libitum green fodder. The trail conducted for 14 weeks with regular recording of body weights and mortality. Results revealed that MR lambs attained average body weight of 18.2 kg compared to 13.8 kg in CON group by the end of 16 weeks of age. The MR group recorded significantly (P=0.028) higher average daily gain (141 g/day) compared to CON group (93 g/day), while the mortality was markedly reduced to 7.14% versus 21.4% in non-supplemented lambs. The economic analysis revealed 33 % higher net return and cost benefit ratio of 1.39 in MR group. These findings demonstrate that milk replacer supplementation during the pre-weaning period is a farmer-friendly, cost-effective strategy to enhance lamb survival, growth, and overall productivity in smallholder sheep farming systems.

KEYWORDS: Growth performance, Milk replacer, Mortality, Pre-weaning lambs, Sheep farming

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INTRODUCTION

Andhra Pradesh ranks first in India with a sheep population of 25.6 million, playing a vital role in meat production, food security, and rural livelihoods. Sheep farming is one of the most important livelihood options for resource-poor farmers in the state. However, lamb mortality and poor growth performance remain a major constraint to productivity and profitability. Earlier studies indicate that approximately 10–35% of lambs die by six months of age under different agro-climatic conditions (Yapi et al., 1990; Green and Morgan, 1993), with neonatal lambs being most vulnerable (Gama et al., 1991). In India, Mandal et al. (2007) reported an overall lamb mortality rate of 12.6% from birth to one year of age, with pre- and post-weaning mortality rates averaging 6.6% and 6.0%, respectively.

Major cause of lamb mortality includes pneumonia, digestive disorders, starvation, and parasitism, while perinatal deaths often occur due to dystocia, cold stress, and mismothering (Green and

Morgan, 1993). Additionally, mortality rates are influenced by environmental conditions (Wilson and Murayi, 1988), flock management practices, and even sire progeny groups (Dalton et al., 1980).

From an economic perspective, lamb mortality significantly reduces flock replacement rates and meat production potential, thereby directly lowering farm profitability (Huffman et al., 1985). Fast growing breeds such as Nellore and Macharla with genetic growth potential and feed efficiency pre-weaning survival is critical under limited feed resource situations. Beyond mortality, compromised growth performance poses another major challenge. Many lambs fail to achieve their genetic growth potential due to inadequate maternal milk, limited grazing resources and rising feed cost. Together these factors restrict the nutrient availability during the critical preweaning period, consequently leading to poor growth trajectories reducing flock productivity and profitability.

Globally milk replacers are primarily intended to serve as an alternative nutritional strategy for orphaned or early weaned calves, kids/lambs, widely adopted by western countries. However, several studies have reported that even under such controlled and intensive management systems, complete replacement of dam`s milk with artificial milk replacer adversely affected lamb performance (Grosskopf et al. 2017; Menghwar et al., 2018). These findings indicate that while milk replacers can be a valuable managemental tool, their exclusive use as substitute for dam`s milk may not support optimum growth and health. Under Indian conditions where flocks are largely maintained in extensive and semi-intensive systems such complete replacement could further aggravate nutritional and economic constraints.

Therefore, Nutritional interventions during the pre-weaning stage through partial supplementation may offer practical balance between growth performance and economic returns. Milk replacer supplementation has the potential to support optimum nutrition, reduce dependence on maternal milk, and improve overall survivability. With this perspective, under the ICAR-ATARI, Zone X sponsored Impactful Interventions programme, Krishi Vigyan Kendra, Guntur conducted field trials to assess the effect of milk replacer supplementation on lamb performance under farmer-managed conditions.

MATERIALS AND METHODS

Location of study

This present field study was carried out in Guntur district of Andhra Pradesh under the impactful interventions (Veterinary) of ICAR-ATARI Zone-X, Hyderabad and executed by Krishi Vigyan Kendra (KVK), Sri Venkateswara Veterinary University, Lam, Guntur. The study was conducted in three adopted villages of KVK – Venigandla (Pedakakani Mandal), Jonnalagadda (Tadikonda Mandal) and Kantepudi (Sattenapalli Mandal) during the period July 2024 to October 2024. The region is characterised by semi-arid tropical climate characterised by hot summer and mild winter and moderate humidity with average rain fall of 800-900 mm with predominance of extensive grazing and well-established sheep rearing.

Beneficiary selection and enrolment of lambs

For this study, Shephards having more than 50 breeding sheep and the flock with minimum eight lambs of 2-3 weeks age (Nellore brown/Macharla) were identified through local animal husbandry assistants and survey analysis of KVK, Guntur. Based on these criteria a total of eighty-four lambs from 12 beneficiaries with an average body weight of 4 ± 0.5 kg were enrolled for undertaking the trial. Each beneficiary was considered as one experimental block to minimise the managemental bias arising due to individual variations in grazing practice, feed source and housing management. With in the flock the enrolled lambs were randomly divided into two experimental groups: Control (CON) with no supplementation raising solely on dam`s milk and natural grazing (n=42), Milk replacer (MR) group, (n=42) where lambs were allowed to suckle the dam and additionally bottle/pail fed with diluted milk replacer (1 in 3 parts) ratio using lukewarm water along with access to natural grazing. All the lams were reared under extensive field conditions with little or no shelter provision. The flocks including the dam and enrolled lambs were allowed 8-12 hrs of daily grazing with little or no concentrate supplementation.

Procurement and milk replacer feeding

A total of 200 kg milk replacer, designed for pre-weaned lambs by ICAR- National Institute of Animal Nutrition and Physiology (ICAR–NIANP), Bengaluru, was procured in 2 kg packs through KVK, Guntur. The milk replacer composition was in accordance with the formulation described by

Bhatt et al., 2009 with Crude protein 22-24%, Crude Fat 8-10% and Crude fiber 2-3%. Milk replacer was provided to 44 lambs aged two weeks with initial body weight of 4±0.5 kg was allotted to MR group and were fed with following daily feeding regimen: 2-4 weeks- 50 g (150 ml), 4-6 weeks- 70 g (200 ml), 6-10 weeks - 50 g (150 ml) and 10-14 weeks- 30 g (100 ml). The lambs were weaned at 14 weeks of age and body weights were recorded till 16 weeks of age. A total of 2 packs (4 kg) were distributed per lambs as per the feeding schedule given by Kumar et al. (2021) with minor modifications to suit to local conditions. The milk replacer was reconstituted with warm water and shepherds were instructed to feed the lambs twice daily from 2-6 weeks and then once afterward.

Health management

At the time of enrolment, the lambs were examined for the health status and were dewormed with piperazine adipate @50 mg/kg body weight. The shepherds were instructed to report the deviations in feeding or health status of lambs so as to provide veterinary aid. Mortality of enrolled lambs was recorded and possible causes were assessed based on housing, feeding weather and other managemental details collected from the shepherds.

Data collection and cost economics

The trial was conducted for a period of 14 weeks. Body weight changes of individual lambs were recorded at fortnight intervals using platform electronic weighing balance with 0.2 kg sensitivity. Mortality data were tracked and documented throughout the study period. The cost economics of supplementary strategy with milk replacer was calculated by considering the cost of milk replacer Rs (290/- per kg), mortality rate, body weight gain and kg live weight cost of lambs (Rs.420/) as per the prevailing market price.

Statistical analysis

As the study was conducted under practical shepherd manged flocks maintained in extensive field conditions, inherent variations in lamb age, dam parity, grazing intensity and managemental variations were unavoidable. These variations were minimised by distributing the enrolled lambs uniformly into treatment groups for performance comparison. The collected data was consolidated to represent the field level performance trends rather than rigorous statistical analysis. Mean body weights, average daily gains were subjected to *t*-test, while mortality % was compiled and presented to assess the efficacy of milk replacer feeding on performance and profitability. Statistical significance of collected data was evaluated at 95% level of confidence ($P \leq 0.05$).

RESULTS AND DISCUSSION

The growth performance of lambs fed with and without milk replacer are presented in Table 1. The initial body weights of lambs did not differ significantly between the two groups indicating the uniformity in enrolment of experimental lambs at the start of trial. By the end of 16 weeks, lambs in MR group attained significantly (P=0.021) higher body weight of 18.2 kg over 13.7 kg in control. The MR lambs gained 50% extra body weight over CON group (9.14 kg vs 13.8 kg) during the 14 weeks period (Fig.1). Similarly, the ADG were also found significantly (P= 0.028) higher in MR group (141 vs 93 g/day). These results indicate a positive growth response to milk replacer supplementation during pre-weaning and early post weaning period of lambs.

These observations can be better understood in the context of early life nutrition, which is critical factor for lamb growth survival (Danso et al., 2014). Dam`s milk is the

Table 1. Growth performance and mortality of lambs fed with and without milk replacer

Parameter	Control	Treatment	SEM	P Value
Initial Bwt (kg)	4.56	4.43	0.321	0.914
Final Bwt (kg)	13.7	18.2	0.842	0.021
Body weight gain (kg)	9.14	13.8	0.94	0.009
ADG (g/day)	93	141	32.7	0.028
Mortality (No.s)	8 (42)	3 (42)	-	-
Mortality %	21.4	7.14	-	-

primary source of nutrient for growth, but supplementary feeding along with suckling provides the additional nutrients essential for rapid weight gain, particularly in lambs nursed by ewes with limited feed resources. In the current study all the dams of enrolled lambs were maintained under extensive grazing and could not meet the DM requirements (4% of BW) of lactating ewe with 30-35 kg, as recommended by ICAR, 2012. This nutritional limitation likely contributed to lower weight gains in control lambs, despite their genetic growth potential. Supplementary feeding with milk replacer in MR group in addition to natural suckling helped lambs to express their growth potential, as reflected in higher body weight gains. Similar improvement in weight gains was reported earlier (Lakshmi and Murthy, 2017) in Mandya lambs fed with cow milk as a supplementary feeding source in addition to dam`s milk. Danso et al. (2014) also observed higher growth rates and accelerated rumen and organ development in early weaned Suffolk ram lambs fed with milk replacer and pellets. Further supplementation of milk replacer has also been reported to advance sexual maturity and semen quality in Malpura ram lambs (Kumar et al., 2021).

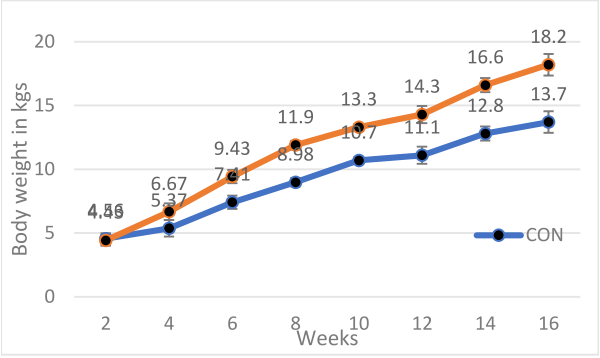

Fig 1: Fortnightly body weights of lambs fed with and without milk replacer

The improved growth in MR lambs was accompanied by reduced mortality rates - only 7.14% (3 out of 42) compared to 21.4% (8 out of 42) in control group - indicating the role of nutritional interventions in improving lamb survival. Similar findings were reported by McManus et al. (2014), where inclusion of bovine milk and multi mixture in suckling lamb diets significantly decreased lamb mortality. The lamb mortality of current study coincides with continuous rainfall during August 2024, primarily attributed to higher humidity, poor housing facilities and diarrhoea incidence.

Alongside the growth performance the economic viability of the intervention was also evaluated (Table 2.) Although the MR group incurred additional cost of Rs. 45,240/- towards milk replacer supplementation (4 kg per lamb at Rs.290/- per kg), the overall income from sale of MR lambs (Rs. 2, 98,116/-) was substantially higher than that of control group (Rs.1, 89,882) resulting in 33% net return. The additional benefit obtained through milk replacer supplantation was Rs. 1,615/- per lamb, with a benefit cost ratio of 1.39. Menghwar et al. (2018) reported a much lower net return when lambs maintained solely on milk replace due to poor growth rate and increased diarrhoea incidence. In contrast, the higher economic returns in present study can be attributed to supplementary rather than complete replacement feeding. Consistent with these findings, Bhatt et al. (2022) reported 20% higher net returns in Malpura lambs raised on milk replacer as compared to control lambs.

Table 2. Cost economics of milk replacer feeding in lambs

	CON	MR
		Rs. 45.240/-
Cost towards milk replacer	Nil	4 kg per lamb @290 per kg
Mortality	9	3
lambs survived	33	39
Avg wt of lamb at 16 wk (Kgs)	13.7	18.2
Income from sale of lambs @420/- per kg live weight	Rs. 1,89,882/-	Rs. 2,98,116/-
		Rs. 2,52,876/-
Net return from enrolled lambs	Rs. 1,89,882/-	
Net return per lamb	Rs. 5,754/-	Rs. 6,484/-
Economic benefit of MR feeding		
MR group (39 lambs)		Rs. 62,994/-
MR group (per lamb)		Rs. 1,615/-
Benefit cost ratio (BCR)		62,994/45,240 = 1.39

While these findings strongly suggest the advantages of milk replacer feeding supplementation, the level of supplementation also plays a crucial role in long term outcomes. Although higher level milk replacer (4% of BW) enhances short term weight gains, Huang et al. (2023) observed reduced starter intake a key factor stimulating the rumen development and microbial colonisation leading to adverse long-term effect on lamb performance. Therefore, moderate supplementation is desirable in balance growth and rumen development. In this study MR lambs were fed with 50-70 g/day of milk replacer along with natural suckling resulting in optimum performance suggesting this as suitable supplementary dose.

Supplementary feeding with milk replacer as demonstrated here, represents more practical and sustainable approach resulting in higher weight gains, improved survival rates and enhanced flock profitability. Collectively, these findings suggest that milk replacer supplementation during the pre-weaning and early post weaning stages is an effective and economically viable intervention to reduce lamb mortality and promote sustainable productivity in small holder and semi-intensive small ruminant production systems.

CONCLUSION

Milk replacer supplementation in young lambs significantly improved the performance in terms of average daily gain and reduced the mortality under real farming conditions. This study conducted by Krishi Vigyan Kendra, SVVU, Guntur under impactful interventions, successfully introduced cost effective strategy for improving the survivability and growth potential in resource limited sheep farming systems of Andhra Pradesh.

REFERENCES

Bhatt, R.S., Sarkar, S., Sahoo, A. and Sankhyan, S.K. 2022. Growth performance, rumen fermentation and economic analysis of Malpura lambs raised on milk replacer at different weaning age under semiarid conditions. Journal of Animal Physiology and Animal Nutrition. 106(2): 250–257.

Bhatt, R.S., Tripathi, M.K., Verma, D.L. and Karim, S.A. 2009. Effect of different feeding regimes on pre-weaning growth, rumen fermentation and its influence on post-weaning performance of lambs. Journal of Animal Physiology and Animal Nutrition. 93(5): 568–576.

Dalton, D, C., Knight, T. W. and Johnson, D, L. 1980. Lamb survival in sheep breeds on New Zealand hill country. New Zealand Journal of Agricultural Research. 23: 167-173.

Danso, A.S., Morel, P.C., Kenyon, P.R. and Blair, H.T. 2014. Effect of early life diet on lamb growth and organ development. Proceedings of the New Zealand Society of Animal Production. 74: 205–208.

Gama, L.T., Dickerson, G.E., Young, L.D. and Leymaster, K.A. 1991. Genetic and phenotypic variation in sources of preweaning lamb mortality. Journal Animal Science. 69:2744-2753.

Green, L.E. and Morgan, K.L. 1993. Mortality in early born, housed lambs in south-west England. Preventive Veterinary Medicine. 17(3–4): 251–261.

Grosskopf, R.K., Grosskopf, H.M., Boito, J.P., Bottari, N.B., Machado, G., Biazus, A.H., Schetinger, M.R., Morsch, V.M., Tonin, A.A., Paiano, D. and Balzan, A. 2017. Natural or replacer sources of milk in lambs during feeding adaptation: influences on performance, metabolism of protein and lipid and oxidative/antioxidant status. Journal of Animal Physiology and Animal Nutrition. 101(2): 243–250.

Huang, Y., Wang, G., Zhang, Q., Chen, Z., Li, C., Wang, W., Zhang, X., Wang, X., Zhang, D., Cui, P. and Ma, Z., 2023. Effects of milk replacer feeding level on growth performance, rumen development and the ruminal bacterial community in lambs. Frontiers in Microbiology. 13:1069964.

Huffman, E.M., Kirk, J.H. and Pappaioanou, M. 1985. Factors associated with neonatal lamb mortality. Theriogenology. 24(2):163-171.

Kumar, D., Bhatt, R.S., Balaganur, K., De, K., Mahla, A.S. and Sahoo, A. 2021. Milk replacer and linseed supplementation promotes puberty and semen quality in growing male lambs. Small Ruminant Research. 202: 106457.

Lakshmi, R. K. S. and Murthy, U. K. 2017. Growth performance of Mandya lambs fed with cow milk as a sole source and also supplementary feed source. Indian veterinary Journal. 94(08):20-22

Mandal, A., Prasad, H., Kumar, A., Roy, R. and Sharma, N. 2007. Factors associated with lamb mortalities in Muzaffarnagari sheep. Small Ruminant Research. 71(1–3): 273–279.

McManus, C.M., Gomes, E.F., Paim, T.P., Louvandini, H., Dallago, B., Borges, B.O., Zorzan, A. and Lima, P.D. 2014. Effect of supplementary milk feeding on growth and survival of Santa Inês lambs. Ciência Animal Brasileira. 15: 451–457.

Menghwar, D.R., Baloch, M.H. and Qureshi, U. 2018. Rearing of Kachhi lambs by using milk replacer. Journal of Livestock Science. 9: 1–4.

Wilson, R.T. and Murayi, T. 1988. Productivity of the Small East African goat and its crosses with the Anglo-Nubian and the Alpine in Rwanda. Tropical Animal Health and Production. 20(4):219-228.

Yapi, C.V., Boylan, W.J. and Robinson, R.A. 1990. Factors associated with causes of preweaning lamb mortality. Preventive Veterinary Medicine. 10(1–2): 145–152.

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Exploration of Mineral and Biochemical Status of Dairy Cattle in Pir Panjal Range of North-Western Himalayas, Kashmir

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ABSTRACT

Mineral deficiency is an area specific problem with marked effect on productivity and can only be corrected by suitable supplementation of specific minerals. This requires careful assessment of the mineral status of livestock in specific regions that depends upon the mineral content of available feedstuffs offered to them. The present study was conducted to find out the mineral profile of some important feedstuffs and plasma of dairy cattle along with their metabolic parameters in different milk yield groups (yielding milk >10 kg/day, 5-10 kg/day, upto 5 kg/day and dry pregnant) under farmers' field condition in Shopian district of Kashmir division. A total of 201 blood plasma samples were collected from dairy cattle, selected randomly from 6 different tehsils of two veterinary blocks (Shopian and Keegam) of the district. Also, samples of feeds and fodders most commonly offered to cattle by the dairy farmers were collected separately, pooled tehsil wise and analysed. The chemical composition of the feedstuffs were within prescribed normal ranges, but available fodders contained low P, Mg, Cu and Zn while all the concentrate feed resources were adequate in all the analysed macro- and micro-minerals. All plasma macro-minerals and only Fe among micro-minerals were above the critical levels, while all plasma biochemical profiles were within normal reference ranges except total protein in all groups of dairy cattle throughout the district. Overall deficiency of Ca, Cu, Mn and Zn exists among 41.56, 80.00, 89.50 and 89.67%, respectively in blood plasma of dairy cattle in the district with higher deficiency prevalence figures for dry pregnant compared to lactating animals. It is concluded that dietary strategy through formulation and supplementation of area specific mineral mixtures to different classes of dairy animals for overcoming the mineral deficiencies could be a suitable approach for cost effective enhancement of milk productivity in the region.

KEYWORDS: Biochemistry, Blood plasma, Dairy cattle, Feedstuffs, Jammu & Kashmir, Mineral status

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INTRODUCTION

Continued ingestion of feeds that are deficient or excessively high in minerals induce biochemical or physiological dysfunctions and nutritional disorders that ultimately affects animal performances like milk production in dairy animals (Fadlalla, 2022). The process of milk production requires the mobilization of minerals, fat and protein from body reserves to cope up with the high nutrient demands during milk production (Alemu et al., 2023). Assessment of blood constituents are used as indicators of the nutritional status and metabolic health of cows, and these constituents can be monitored through analysis of the blood mineral and biochemical profiles (Puppel and Kuczyńska, 2016). Such analysis is generally performed to estimate the prevalence and risk levels of specific metabolic disorders in the herd (Calamari et al., 2016).

Mineral elements are essential to satisfy the normal physiological requirements of dairy animal, as they play crucial role in health, reproduction, and production of animal (Pal et al., 2024). The level of milk production in dairy cattle is known to influence their overall metabolic balance and nutrient requirements (Gross, 2022). Many environmental

and biological conditions like lactation stages, milk yield, parity etc. affect mineral concentration in blood (Spears et al., 2022), but the results are inconsistent probably due to differences in breed, production system, regional feeding practices, environment factors, and physiological conditions. Healthy cows can have different milk production levels even when they are fed the same diet and managed under the similar conditions (Kim et al., 2017); however, it is unknown to what extent the blood mineral and biochemical levels differ among them. Establishing further knowledge in this context may help to evaluate the physiological state of dairy cows for overcoming nutrient deficiencies (if any) to improve the productivity. In this regard, the present study aimed to determine the plasma mineral and biochemical concentrations of different milk yield groups of dairy cattle and most available feeds and fodders fed to them in district Shopian of Jammu and Kashmir.

MATERIALS AND METHODS

The study area

The study was carried out in one of the Southern districts of Kashmir Division, the district Shopian situated in the foothills of Himalayan Pir-Panjal range on the ancient imperial road "*The Mughal Road*" which connects Kashmir valley with Rajouri and Poonch districts of Jammu. The district's cattle population stands at 0.70 lakh, comprising 6.20% of Kashmir Division's total cattle and 36.85% of the district's overall livestock population. The district has mostly hilly terrain, enjoys predominantly dry temperate climate, and is located at a latitude of 330 72' N and a longitude of 74° 53 E with an average elevation of 2057m above mean sea level. The district has vast area under apple orchards and play important role in fruit industry in the region, thus commonly called "*the apple bowl of Kashmir*". The district is administratively divided into two veterinary blocks *viz*. Shopian and Keller.

Sampling

One-time sampling was conducted during the study to establish baseline values for key nutritional contents of available feedstuffs, plasma biochemical parameters, and mineral profile in dairy cattle. A total of 201 blood samples were collected from dairy animals selected randomly in which 165 were collected from the Shopian block and 36 from the Keller block based on breedable cattle population. The number of blood samples collected from tehsils of Shopian, Imam Sahib, Herman, Zainapora and Chitragam were 28, 36, 41, 30, 30, respectively in block Shopian, and 36 from Keegam in block Keller. The samples were divided into four groups based on the milk yield of respective cattle as animals yielding milk >10 kg/day, 5-10 kg/day, upto 5 kg/day and dry pregnant. Moreover, samples of feeds and fodders commonly offered to the cattle by dairy farmers were collected separately, pooled for various villages of each tehsil to get the uniform representative sample for the respective feed/fodder.

Sample processing and analysis

About 10-15 mL of blood was collected aseptically in capped collection vials containing anti-coagulant ethylene diamine tetra acetic acid (EDTA @1.5 mg/mL blood) from jugular vein of dairy animals and transported and stored under frozen conditions. The plasma samples with equal volume of concentrated nitric acid were kept for overnight in digestion tubes followed by low heat (70-80 °C) digestion with di-acid mixture (70% Perchloric acid : conc. Nitric acid in 1: 3 ratio). The final content was filtered through Whatman's filter paper No.1 (Kolmer et al., 1951). Plasma concentrations of calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) were estimated using the flame mode, while cobalt (Co) and manganese (Mn) were estimated using the graphite furnace mode of Atomic Absorption Spectrophotometer (GBC SensAA, Sr. no. A7156, GBC Scientific Equipment, Inc, Australia). Phosphorus was determined colorimetrically in blood plasma (Fiske and Subbarow 1925) using Autozyme Phosphorus kit (ACCUREX Biomedical Pvt. Ltd.). Sodium (Na), potassium (K) and chlorine (Cl) were estimated in blood plasma using flame photometer (Systronics, Mediflame 127). Also, the glucose level was estimated at the time of blood collection by using SD-Codefree Blood Glucose Meter (SD Biosensor Healthcare Pvt. Ltd., Gurgaon, Haryana, India). Another set of stored plasma samples were analysed for contents of biochemicals like cholesterol (Chol), triglycerides (TG), total proteins (TP), albumin (Alb), and urea-N (PUN) through standard methods using commercial diagnostic kits (Diasys Diagnostics Private Limited, India) on Photometer 5010V5^{+} semi-auto biochem analyzer (Robert Riele INC, Berlin, Germany).

The collected feed/fodder samples were dried at 80°C, pooled tehsil wise, ground, labelled and properly stored in airtight polythene bags for

laboratory analysis. The samples were analyzed for proximate composition including dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) following standard procedures of AOAC (2019). Also, the feed/fodder samples were subjected to digestion with di-acid mixture (Trolson, 1969), diluted with triple glass distilled water to prepare extractable aliquots, analysed for estimation of macro- and micro- minerals using the same procedures as done for blood plasma.

Statistical analysis

The data on mineral contents were subjected to statistical analysis for mean, standard error and test of significance (one-way ANOVA). The statistical software program SPSS (2011) was used for analysis of the data. These tests were two sided and were referenced for *P* value for their significance. Any *P* value less than 0.05 ($P<0.05$) was taken to be statistically significant.

RESULT AND DISCUSSION

Composition of available feeds/fodders

The chemical composition (% DMB) of the most common feeds and fodders fed to dairy cattle in all surveyed tehsils of the district (Table 1) were in normal ranges as prescribed for Indian feeds and fodders (ICAR, 2013), with little variations which might be due to difference in herbage plant species, cultivar differences, soil/geographical area, climatic conditions, irrigation and fertilizer uses (Garg et al., 2005). The composite fodder samples analyzed contained low P, Cu and Zn, while the Ca, Fe and Co concentrations were higher than the critical levels as prescribed by McDowell and Conrad (1977) and NRC (1984). The soils of hilly region like Jammu and Kashmir, Himachal, Uttarkhand and North-Eastern states are acidic in reaction due to leaching of the bases under the influence of high precipitation. High levels of Ca in soil is essential to reduce its acidity and may increase Ca concentration in fodders grown in it (Adams and Hartzog, 1960). Cu deficiency in fodders under the present study may be attributed to the typical soil condition that might be restricting its accumulation and availability in plants (Underwood, 1977). The concentrate rations contained adequate levels of all the nutrients and minerals with low Zn content in home-made concentrate mixture probably due to less Zn content in constituent ingredients and little or no use of mineral mixture in its formulation by the dairy farmers of district Shopian. These results were in close agreement with the reports of feeds and fodders from other districts of Kashmir (Bhat et al., 2011; Sheikh et al., 2019); however, the values for CP and NSC for maize stover analysed in the present study were less than those reported by Bhat et al. (2021).

Table 1. Composition (on %DM basis) of most available feeds and fodders fed to dairy cattle in district Shopian, Kashmir

Attribute	Critical level*	Home-made concentrate (n=17)	Commercial compound feed (n=95)	Maize stover (n=116)	Orchard grass hay (n= 115)
Proximate composition					
Dry matter	-	91.14±0.39	90.83±0.18	86.97±0.15	92.52±0.14
Organic matter	-	91.58±0.28	90.36±0.16	89.33±0.29	86.23±0.36
Crude protein	-	14.35±0.48	15.61±0.14	3.70±0.02	8.77±0.05
Ether extract	-	2.35±0.08	2.27±0.01	0.78±0.01	2.56±0.07
Mineral composition					
Calcium	<0.30 %	1.25±0.05	1.49±0.02	0.38±0.00	0.45±0.01
Phosphorous	<0.25 %	0.33±0.01	1.00±0.01	0.23±0.01	0.23±0.00
Magnesium	<0.20 %	0.35±0.01	0.59±0.01	0.15±0.01	0.20±0.01
Sodium	<0.08 %	0.31±0.01	0.60±0.01	0.45±0.01	0.04±0.00
Potassium	<0.25 %	0.99±0.03	1.07±0.03	0.70±0.02	0.23±0.01
Chlorine	<0.10 %	0.20±0.01	0.24±0.00	0.78±0.01	0.49±0.02
Copper	<8.00 ppm	9.63±0.62	14.76±0.75	4.82±0.11	4.14±0.14
Zinc	<30.0 ppm	26.40±1.59	76.62±1.79	12.32±0.94	20.72±0.87
Iron	<50.0 ppm	229.03±6.85	734.58±11.06	371.23±10.07	537.11±6.97
Cobalt	<0.10 ppm	0.11±0.00	1.01±0.02	0.19±0.01	0.33±0.01
Manganese	<40.0 ppm	46.73±1.97	59.19±1.88	40.04±1.51	45.04±1.12

***** McDowell and Conrad (1977), NRC (1984)

Plasma macro- mineral profile of dairy cattle

The macro-mineral levels in blood plasma of dairy cattle are given in Table 2 with the prevalence of their deficiencies in Table 3. All the estimated plasma minerals contents were significantly (P<0.01) influenced by milk yield. The overall average of all the plasma macro-minerals were found above the critical levels throughout the district. The normal range for Ca, P, Mg, Na, K and Cl in dairy cattle has been reported to be 9 to 12, 4 to 7, 1.7 to 2.5 mg/dL, and 134 to 144, 4 to 5.9, 92 to 99 mEq/L, respectively (Radostits et al., 2000).

Table 3. Prevalence (%) of plasma mineral deficiency profile in different milk yield groups of dairy cattle in district Shopian, Kashmir

Plasma mineral	>10 kg/day	5 - 10 kg/day	Upto 5 kg/day	Dry pregnant	Pooled district mean	P value
Macro-mineral deficiency prevalence						
Calcium	24.00^{A}±0.84 (12/50)	38.89^{B}±0.64 (14/36)	55.00^{C}±0.68 (22/40)	57.14^{C}±1.62 (16/28)	41.56±0.89 (64/154)	0.001
Phosphorous	3.57^{AB}±2.78 (1/28)	0.00^{A}±0.00 (0/30)	7.14^{B}±3.69 (2/28)	3.57^{AB}±3.33 (1/28)	3.51±1.18 (4/114)	0.000
Chlorine	0.00^{A}±0.00 (0/39)	3.03^{B}±2.78 (1/33)	0.00^{A}±0.00 (0/36)	3.70^{B}±4.17 (1/27)	1.48±1.92 (2/134)	0.00
Macro-mineral deficiency prevalence						
Copper	81.48^{B}±0.57 (44/54)	85.42^{BC}±0.84 (41/48)	87.27^{C}±0.59 (48/55)	53.57^{A}±0.77 (15/28)	80.00±0.44 (148/185)	<0.001
Manganese	97.10^{B}±0.17 (67/69)	83.67^{AB}±0.56 (41/49)	79.63^{A}±0.85 (43/54)	100.00^{C}±0.00 (28/28)	89.50±0.27 (179/201)	<0.001
Zinc	81.48^{A}±0.81 (44/54)	89.58^{AB}±0.68 (43/48)	94.44^{B}±0.36 (51/54)	96.43^{B}±0.29 (27/28)	89.67±0.29 (165/184)	0.000

The means across the rows with different upper case superscript differ significantly among the milk yield groups

The mean plasma Ca concentration in dairy cattle of all the tehsils of district Shopian was within the normal range with higher values (P<0.01) in animals yielding milk >10 kg/day compared to other groups of dairy animals which might be due to extra care regarding feeding and/or supplementation of Ca rich mineral mixtures to high yielding cows. Moreover, high-yielding cows mobilize large amounts of Ca from their body reserves (Djokovic et al., 2014). The overall prevalence of hypocalcaemia in the district was 41.56% with higher values (P<0.01) in dry pregnant and animals yielding upto 5 kg/day of milk. Workers have reported varying percentage prevalence of hypocalcemia in cattle; however, the results of present study corroborates well with the reports from various agro-climatic zones of North-West Himalayan region of Jammu division (Singh et al., 2016).

The mean plasma P concentration was higher (P<0.05) in animals yielding milk >10 kg and 5-10 kg per day compared to those yielding upto 5 kg/day and dry pregnant animals; however, the values were within the normal range for all groups of dairy animals in all tehsils of the district. Some studies have reported significantly higher level of P in crossbred cows (Siddique, 2011), while other have outlined the level below the normal range (Singh et al., 2016). The differences observed in the level of blood P in various studies could be due to difference in their dietary level, breed, season and other factors like sample preparation, temperature, duration of sample collection and plasma preparation time (McDowell, 2003). Prevalence of hypophosphataemia in dairy cattle was very less in the district (3.51%) with higher values in low milk yielding and dry pregnant animals. Goklaney et al. (2019) also reported higher prevalence of P deficiency in pregnant goats followed by dry and lactating goats. In contrast, Singh et al. (2016) reported widespread P deficiency among crossbred cattle of hilly areas of Jammu division.

Plasma Mg concentration was higher (P<0.01) in dry pregnant and least in animals yielding milk >10 kg/day but within the normal range, with no prevalence of hypomagnesaemia in any animal group of dairy cattle throughout the district. Mg deficiency is rare under normal conditions as it is

generally present in appreciable quantity in fodders (Greene et al., 1983). In the present study also, Mg was in adequate amounts in the feed and fodder samples (except maize stover) which might be responsible for normal Mg levels in the animals under study. In contrast, Singh et al. (2016) reported 3.50% incidence of hypomagnesamia in crossbred cows with higher prevalence in Kathua followed by Jammu district.

The plasma Na, K and Cl levels in all the milk yield groups of dairy cattle throughout the district Shopian were found to be higher than normal ranges with significant (P<0.01) differences among the groups. The average plasma concentrations of these minerals were lowest in dry pregnant animals compared to the other groups with overall prevalence of 1.48% for Cl deficiency only. The content of these minerals in the feed and fodder samples collected from the study areas were also higher which could be related to their high levels in blood plasma of livestock in the district. Further, high level of these minerals in blood plasma might be due to feeding of salt to the dairy cattle (at 100-150 g of salt/cow/day) as a routine practice in this region. Comparatively higher prevalence of Na and K deficiency among cattle from various agro-climatic zones of North-West Himalayan region of Jammu division was reported by Singh et al. (2016).

Plasma micro- mineral profile of dairy cattle

The results of plasma micro-mineral levels in dairy cattle of district Shopian, Kashmir are presented in Table 2 with the prevalence of their deficiencies in Table 3. The overall average of all the micro-minerals were below the critical levels except for Fe. Among micro-minerals, average plasma Cu, Co, Fe and Mn concentrations were higher (P<0.01) in less milk yielding animals compared to those yielding milk >10 kg/day, and the opposite was true for Zn concentration.

Table 2. Macro- and micro- mineral profile in blood plasma of different milk yield groups of dairy cattle in district Shopian, Kashmir

Plasma mineral	Critical level*	>10 kg/day	5 - 10 kg/day	Upto 5 kg/day	Dry pregnant	Pooled district mean	P value
Macro- mineral profile							
Calcium	< 8.00 mg/dL	9.05^{B}±0.19 (n = 50)	8.34^{A}±0.25 (n = 36)	8.07^{A}±0.23 (n = 40)	7.69^{A}±0.18 (n = 28)	8.38±0.12 (n=154)	<0.000
Phosphorous	<3.50 mg/dL	6.02^{B} ±0.23 (n = 28)	5.79^{B} ±0.17 (n = 30)	4.83^{A} ±0.17 (n = 28)	4.45^{A} ±0.10 (n = 29)	5.28±0.10 (n = 114)	<0.000
Magnesium	< 1.20 mg/dL	3.15^{A}±0.24 (n = 38)	3.43^{A}±0.24 (n = 39)	3.89^{A}±0.23 (n = 43)	5.03^{B}±0.35 (n = 33)	3.84±0.14 (n = 153)	<0.000
Sodium	< 132 mEq/L	161.6^{AB}±0.30 (n = 39)	162.4^{BC}±0.50 (n = 34)	163.4^{C}±0.49 (n = 39)	160.3^{A}±0.50 (n = 30)	162.01±0.23 (n = 143)	<0.000
Potassium	< 3.00 meq/L	6.12^{B}±0.26 (n = 42)	6.15^{B}±0.19 (n = 39)	6.51^{B}±0.24 (n = 45)	4.99^{A}±0.16 (n = 30)	6.02±0.12 (n = 156)	<0.000
Chlorine	< 80.0 mEq/L	98.60^{AB}±1.05 (n = 39)	100.03^{AB}±1.26 (n = 32)	102.37^{B}±0.77 (n = 36)	96.42^{A}±1.38 (n = 27)	99.51±0.57 (n = 134)	0.003
Chlorine	< 80.0 mEq/L	98.60^{AB}±1.05 (n = 39)	100.03^{AB}±1.26 (n = 32)	102.37^{B}±0.77 (n = 36)	96.42^{A}±1.38 (n = 27)	99.51±0.57 (n = 134)	0.003
Macro- mineral profile							
Copper	< 0.65 µg/dL	0.56^{A}±0.01 (n=54)	0.59^{A}±0.01 (n=48)	0.59^{A}±0.01 (n=55)	0.64^{B}±0.01 (n=28)	0.59±0.00 (n=185)	<0.000
Iron	< 100 µg/dL	593.95^{AB}±35.17 (n=29)	648.67^{AB}±35.73 (n=25)	720.74^{B}±17.10 (n=31)	539.30^{A}±24.84 (n=28)	627.30±20.13 (n=113)	0.007
Cobalt	< 0.10 µg/dL	0.01^{A}±0.00 (n=55)	0.02^{B}±0.00 (n=51)	0.02^{B}±0.00 (n=58)	0.01^{A}±0.00 (n=28)	0.02±0.00 (n=192)	<0.000
Manganese	< 2.00 µg/dL	0.13^{A}±0.00 (n=70)	0.16^{B}±0.01 (n=49)	0.16^{B}±0.00 (n=54)	0.12^{A}±0.00 (n=28)	0.15±0.00 (n=201)	<0.000
Zinc	< 0.50 µg/dL	0.44^{C}±0.01 (n=54)	0.42^{BC}±0.01 (n=48)	0.41^{AB}±0.01 (n=54)	0.38^{A}±0.01 (n=28)	0.42±0.00 (n=184)	<0.000

The means across the rows with different upper case superscript differ significantly among the milk yield groups*
McDowell (1987)

The overall mean plasma Cu level in the district was below the critical level probably due to lesser content of Cu in fodder resources, low availability of Cu from feeds because of high Fe which interferes in Cu bioavailability in the body (Bremner and Price, 1985), and drainage through milk. Adelstein and Vallee (1962) also reported lower serum Cu levels in lactating animals. Among the milk yield groups, the mean plasma Cu level was higher (P<0.01) in dry pregnant than lactating cattle and was near the normal critical range. This might be attributed to higher progesterone level and/or to the increased fetal demands and utilization of maternal Cu for development of fetal nervous system (Elnageeb and Abdelatif, 2010). Moreover, pregnancy is usually associated with an increase in plasma Cu levels in the form of ceruloplasmin due to increase in oestrogen levels during late pregnancy (Howell et al., 1968). Prevalence of hypocupraemia was recorded in 80.0% dairy cattle in the district with higher (P<0.01) value in lactating animals compared to dry animals. The finding corroborates with the report of Kumar et al. (2008) for livestock of Shivalik hill zone of Himachal Pradesh.

The mean plasma Fe concentration was much above the critical level in all the groups of animals throughout the district with higher (P<0.01) values in lactating compared to dry pregnant animals. Kaneko et al. (1999) reported that elevated Fe level in plasma could be due to refractory anaemia, haemolytic iron overload and liver disease. Thus, elevated level of plasma Fe recorded in present study could be either due to widely prevalent anaemia among the animals or it could also be due to excessive level of Fe in the available feed resources as soil of hilly areas have higher levels of Fe content. Fe deficiency is rarely observed in adult cattle because it is quite abundant in all feeds (Hidiroglou, 1979). These results are in agreement with the findings of other workers who also reported either negligible or no Fe deficiencies in adult cattle in different parts of the country (Singh et al., 2016).

The overall mean value for plasma Co in the district was below the critical level with higher (P<0.01) values in low milk yielding animals. No adequate literature is available to infer the effect of milk yield on plasma Co concentration in dairy animals. Shekher et al. (2017) reported that plasma Co concentration in crossbred cattle varied from 0.01 to 0.27µg/dL in different districts of Bihar against their critical levels of 0.05 to 0.07 ppm as suggested by McDowell (2003). Likewise, mean plasma Mn concentration was higher in low milk yielding animals with the overall mean value of the district below the critical level. This might be due to the antagonistic effect of other minerals like Ca, P and Fe on Mn concentration in animal body as reported by Furll et al. (2004). Overall prevalence of Mn deficiency in the district was 89.50% with the highest value for dry pregnant compared to lactating animals in which least incidence was in low yielding animals.

Average plasma Zn concentration was higher (P<0.01) in high (>10 kg/d) milk yielding animals with the overall mean concentration below the critical level for the district which might be due to its lesser content in feeds and fodders available to the dairy animals and/or increased intake of Fe in diet which might have interfered in normal absorption of Zn (McDowell, 2003). The results of the present study are in accordance with the reports of Hamid et al. (1997) who reported higher levels of Zn in serum of high yielding animals than in the lower yielding animals. Ghedalia et al. (1996) also reported higher and consistent apparent absorption of Zn in lactating ruminants than non-lactating ones. Overall prevalence of Zn deficiency in the district was 89.67% with lowest (P<0.01) values in high milk yielding animals. Masters and Fels (1980) reported decreased serum Zn level in desert ewes during late gestation as a result of hemo dilution.

Blood metabolic profile of dairy cattle

Serum biochemical parameters are the important indicators of the metabolic activities in lactating animals (Paiano et al., 2020). The important indicators of energy profile in ruminants are glucose, cholesterol and triglycerides (Pechova and Pavlata, 2005). All energy parameters in dairy cattle of the district Shopian were within the normal ranges with difference observed among the different milk yield groups for plasma cholesterol (P<0.01) and triglycerides (P<0.05) only (Table 4). The average blood glucose level was numerically (P>0.05) lower in lactating animals, which might be due to high demand of glucose during lactation for milk sugar synthesis. Blood glucose level remained within normal reference range before calving but declined drastically after parturition (Yousuf et al., 2016). Average plasma cholesterol and triglycerides concentrations were higher in animals yielding milk >5 kg/day compared to dry pregnant animals though within the normal reference range (Kaneko et al., 1999) probably due to high energy demand for milk synthesis than supplied by the offered diet

(Cavestany et al., 2005). These results are in accordance with the findings of Naser et al. (2014) who reported significantly higher levels of triglycerides in dairy animals during late stage of lactation.

Overall plasma protein parameters (Alb, Glb, and PUN) in dairy cattle of Shopian district (Table 4) were within the normal ranges except TP which was marginally below the reference range quoted by Kaneko et al. (1999) indicating that protein deficiency was nominally prevalent among dairy cattle in the district. Livestock in the district were mainly offered fodder like maize stover that has a low protein value, which might be the reason for low plasma TP in dairy cattle of the district. Singh et al. (2016) also reported that the cattle from subtropical and intermediate zones of Jammu division were having significantly lower levels of plasma proteins. Mean plasma TP and Alb concentrations were higher ($P<0.01$) in dry pregnant animals, while PUN was higher ($P<0.05$) in animals yielding milk >10 kg/day. In contrast to the results of the present study, Yousuf et al. (2016) reported significantly higher levels of serum TP in cows after two months of parturition. Naser et al. (2014) reported lower (25.85±8.91 mg/dL) levels of serum urea nitrogen in early stage lactation as compared to mid stage and late stage of lactation (29.85±9.6 g/dL).

Table 4. Plasma metabolic profile of different milk yield groups of dairy cattle in district Shopian, Kashmir

Parameter	Reference value*	>10 kg/day	5 - 10 kg	Up to 5 kg	Dry pregnant	Pooled district mean	P value
			Energy profile				
Glucose	45-75 mg/dL	55.03±1.55 (n=31)	55.08±1.24 (n=36)	57.06±1.08 (n=44)	57.71±1.18 (n=55)	56.54±0.63 (n=166)	0.379
Cholesterol	65-220 mg/dL	126.18^{B}±2.17 (n=58)	122.72^{B}±3.01 (n=47)	121.13^{AB}±2.4 2 (n=35)	112.27^{A}±3.30 (n=32)	121.26±1.39 (n=172)	0.006
Triglycerides	0-14 mg/dL	6.49^{B}±0.25 (n=60)	6.41^{B}±0.30 (n=46)	5.76^{A}±0.32 (n=38)	5.98^{A}±0.28 (n=33)	6.23±0.14 (n=179)	0.039
			Protein profile				
Total proteins	5.7-8.1 mg/dL	5.16^{A}±0.13 (n=33)	5.51^{AB}±0.10 (n=38)	5.78^{B}±0.14 (n=45)	5.94^{B}±0.15 (n=49)	5.64±0.70 (n=165)	0.001
Albumin	2.1-3.6 mg/dL	2.92^{A}±0.09 (n=36)	2.99^{AB}±0.09 (n=39)	3.18^{AB}±0.08 (n=46)	3.30^{B}±0.07 (n=49)	3.12±0.04 (n=170)	0.005
Globulin	2.8-5.0 mg/dL	2.58±0.19 (n=36)	2.74±0.19 (n=37)	3.16±0.21 (n=45)	3.19±0.21 (n=42)	2.94±0.10 (n=160)	0.101
Plasma urea nitrogen	6-27 mg/dL	16.61^{B}±0.65 (n=51)	15.15^{AB}±0.84 (n=26)	14.56^{AB}±0.88 (n=49)	12.83^{A}±0.88 (n=34)	14.95±0.42 (n=160)	0.013

The means across the rows with different upper case superscript differ significantly among the milk yield groups
* Kaneko et al. (1999)

CONCLUSION

Plasma analysis revealed deficiency of Ca, Cu, Mn and Zn among 41.56, 80.00, 89.50 and 89.67%, respectively of dairy cattle in district Shopian of Kashmir with higher deficiency prevalence figures for dry pregnant compared to lactating animals. The Mg, Na, K, Fe and Co levels in plasma of these animals were adequate. All the metabolic profile parameters were within the normal physiological ranges for dairy cattle except total protein which was marginally low throughout the district. Supplementing concentrate rations, legume or cultivated green fodders and tree foliages which are good sources of the nutrients and minerals could be a suitable approach. However, to overcome the deficiency, area specific mineral mixtures need to be framed and supplemented for enhancing the milk productivity cost effectively.

REFERENCES

Adams, F. and Hartzog, D.L. 1960. The nature of yields responses of Florunner peanuts to lime. Peanut Science. 7: 120-123.

Adelstein and Vallee, B.L. 1962. Copper. In: Mineral Metabolism. Comar Bronner (Ed). Academic press New York and London part 2-B pp. 623.

Alemu, T.W., Santschi, D.E., Cue, R.I. and Duggavathi, R. 2023. Reproductive performance of lactating dairy cows with

elevated milk β-hydroxybutyrate levels during first 6 weeks of lactation. Journal of Dairy Science. 106(7): 5165-5181.

AOAC. 2019. Official methods of analysis. Association of Official Analytical Chemists, 21st edn. Gaithersburg, USA

Bhat, M.S., Shaheen, M., Zaman, R. and Muhee, A. 2011. Mineral inter-relationship among soil, forage and dairy cattle in Kashmir, India. Veterinary World. 4(12): 550-553.

Bhat, M.A., Ganai, A.M., Sheikh, G.G., Beigh, Y.A., Farooq, J., Sheikh, B.A. and Reshi, P.A. 2021. Nutritional status of feeds and fodders fed to dairy cattle in South Kashmir. The Pharma Innovation Journal. 10(3): 225-228.

Bremner, I. and Price, J. 1985. Trace Elements in Man and Animals (Eds. Mills, C.F., Bremner, I. and Chesters, J.K.), CAB Publications, UK, pp. 374-376.

Calamari, L., Ferrari, A., Minuti, A. and Trevisi, E. 2016. Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on fourier transform mid-infrared spectroscopy: preliminary results. BMC Veterinary Research. 12: 4.

Cavestany, D., Blanc, J.E., Kulcsar, M., Uriarte, G., Chilibroste, P., Meikle, A., Febel, H., Ferraris, A. and Krall, E. 2005. Studies of the transition cow under a pasture-based milk production system: metabolic profiles. Journal of Veterinary Medicine Series A. 52: 1-7.

Djokovic, R.D., Kurcubic, V.S. and Ilic, Z.Z. 2014. Blood serum levels of macro-and micronutrients in transition and full lactation cows. Bulgarian Journal of Agriculture Science. 20(3) 715-720.

Elnageeb, M.A. and Abdelatif, A.M. 2010. The mineral profile in Desert ewes (*Ovis aries*): effects of pregnancy, lactation and dietary supplementation. American-Eurasian Journal of Agricultural & Environmental Sciences. 7(1):18-30.

Fadlalla, M.T.I. 2022. The Interactions of some minerals elements in health and reproductive performance of dairy cows. New Advances in the Dairy Industry (Edn: Qureshi, M.S.), IntechOpen

Fiske, C.H. and Subbarow, Y. 1925. The colorimetric method for the determination of phosphorous. Journal of Biological Chemistry. 66: 375-378.

Furll, M., Sattler, T. and Anke, M. 2004. Secondary manganese deficiency as a herd problem in cattle-a case report. Tierärztliche Praxis Ausgabe G: Grosstiere-Nutztiere. 32: 126-132.

Garg, M.R., Bhanderi, B.M. and Sherasia, P.L. 2005. Assessment of adequacy of macro and micromineral content of feedstuffs for dairy animals in semi-arid zone of Rajasthan. Animal Nutrition and Feed Technology. 5: 9-20.

Ghedalia, B., Miron, J. and Yosef, E. 1996. Apparent digestibility of minerals by lactating cows from a total mixed ration supplemented with poultry litter. Journal of Dairy Science. 79: 454-456.

Goklaney, D., Ahuja, A. and Dhuria, R.K. 2019. Status of macro and micro mineral deficiency in goats in arid zone of Rajasthan. International Journal of Livestock Research. 9(7): 227-234.

Greene, L.W., Fontenot, J.P. and Webb, K.E.J. 1983. Site of magnesium and other macro mineral absorption in steers fed high levels of potassium. Journal of Animal Science. 57: 503-510.

Gross, J.J. 2022. Limiting factors for milk production in dairy cows: perspectives from physiology and nutrition, Journal of Animal Science. 100(3): skac044.

Hamid, T.M. 1997. Zinc and copper levels in the plasma of Nubian goat as affected by physiological status. Thesis submitted in partial fulfilment of the requirement of master's degree in Tropical Animal Production. Faculty of Animal Production University of Khartoum.

Hidiroglou, M. 1979. Trace element deficiencies and fertility in ruminants- A review. Journal of Dairy Science. 62: 1195-1206.

Howell, J.M., Edington, N. and Ewbank, R. 1968. Observation on copper and ceruloplasmin levels in the blood of pregnant ewes and lambs. Research in Veterinary Science. 9:160-164.

ICAR. 2013. Chemical composition of feeds and fodders. Indian Council of Agricultural Research, New Delhi- 110012.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. 1999. Clinical Biochemistry of Domestic Animals. Harcourt Brace and Co. Asia P/C Ltd., Singapore 238884.

Kim, N.Y., Kim, S.J., Jang, S.Y., Oh, M.R., Tang, Y.J., Seong, H.J., Yun, Y.S. and Moon, S.H. 2017. Behavioral characteristics of Hanwoo (*Bos taurus coreanae*) steers at different growth stages and seasons. Asian-Australasian Journal of Animal Sciences. 30(10): 1486-1494.

Kolmer, J.A., Spanbling, E.H. and Robinson, H.W. 1951. Approved Laboratory Techniques. Appleton Century Crafts, New York, USA.

Kumar, R., Sharma, K.B., Sharma, M. and Sharma, R. 2008. Mineral status of livestock of Shivalik hill zone of Himachal Pradesh. Animal Nutrition and Feed Technology. 8: 253-257

Masters, D.G. and Fels, H.E. 1980. Effect of Zn supplementation on reproductive performance of grazing Merion ewes. Biological Trace Element Research. 7: 89.

McDowell, L.R. 1987. Assessment of mineral status of grazing ruminants. World Review of Animal Production. 33: 19-31.

McDowell, L.R. 2003. Minerals in Animal and Human Nutrition. 2nd edn. Elsevier Science B. V. Amsterdam, The Netherlands. Pp. 33–92.

McDowell, L.R. and Conrad, J.H. 1977. Trace mineral nutrition in Latin America. World Animal Review. 24: 24.

Naser, E.M.A., Mohamad, G.E. and Elsayed, H.K. 2014. Effect of lactation stages on some blood serum biochemical parameters and milk composition in dairy cows. Assiut Veterinary Medical Journal. 60(142): 83-88.

NRC. 1984. Nutrient requirements of domestic animals. National Academy of Sciences-National Research Council. Washington, DC.

Paiano, R.B., Birgel, D.B., Bonilla, J. and Birgel, E.H.J. 2020. Evaluation of biochemical profile of dairy cows with metabolic diseases in tropical conditions. Reproduction in Domestic Animals. 55(9): 1219-1228.

Pal, R.P., Mani, V., Tariq, H., Sarkar, S., Sharma, A. and Gupta, D. 2024. Newer trace elements for ruminants. In: Mahesh, M.S. and Yata, V.K. (eds) Feed Additives and Supplements for Ruminants. Springer, Singapore. pp 87-118

Pechova, A. and Pavlata, L. 2005. Use of metabolic profiles of dairy cows in the control diet. In: Nutrition of cattle in terms of production and preventive medicine (ISBN 80-86542-08-4), pp. 102- 111.

Puppel, K. and Kuczyńska, B. 2016. Metabolic profiles of cow's blood; a review. Journal of the Science of Food and Agriculture. 96: 4321-4328.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. 2000. Veterinary Medicine. 9th edn. W B Saunders Harcourt Publishers Ltd.

Sheikh, F.A., Ganai, A.M., Mir, N.A., Sheikh, G.G., Ahmad, H.A. and Beigh, Y.A. 2019. Chemical composition and nutritional profile of feed and fodders fed to cattle of Anantnag district of Kashmir valley during winter season. Veterinary Research International. 7(2): 104-109.

Shekhar, P., Kumar, P., Dimri, U. and Sharma, M. 2017. Micro mineral status of crossbred cattle under different physiological stages in Eastern India and their interrelation in soil and fodder. International Journal of Livestock Research. 7(1).

Siddique, N. 2011. Clinico-biochemical indicators of mineral deficiencies in farm animals. MVSc dissertations. GADVASU, Ludhiana, India.

Singh, R., Singh, V. and Baigh, S.A. 2016. Haemato-biochemical and mineral status of crossbred cattle from various agro-climatic zones of North-West Himalayan region of Jammu division. Indian Journal of Animal Sciences. 86(10): 1125–1131

Spears, J.W., Brandao, V.L.N. and Heldt, J. 2022. Invited review: assessing trace mineral status in ruminants, and factors that affect measurements of trace mineral status. Applied Animal Science. 38(3): 252-267.

SPSS. 2011. Statistical Package for Social Sciences (Version 20.0), Software products, Marketing Department, IBM SPSS Inc., New York, USA

Trolson, J.E. 1969. Outline for *in vitro* digestion of forage samples. Research Station Swist Current, Saskatchewam, Canada.

Underwood, E.J. 1977. Trace Elements in Human and Animal Nutrition, 4th edn. Academic Press, New York, USA.

Yousuf, M., Alam, M.R., Shaika, A.H., Alfaruk, M.S., Saifuddin, A.K.M., Ahasan, A.S.M.L., Islam, K. and Islam, S.K.M.A. 2016. Nutritional status of high yielding crossbred cow around parturition. Journal of Advanced Veterinary and Animal Research. 3(1): 68-74.

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Growth Performance and Nutrient Utilization in Transition Sahiwal Calves Supplemented with Chromium, Zinc and Magnesium

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ABSTRACT

Minerals are essential for growth and reproduction and are involved in a large number of digestive, physiological and biosynthetic processes within the body. The aim of this study was to determine the effect of dietary supplementation of chromium (Cr), zinc (Zn) and magnesium (Mg) on the growth performance and nutrients utilization of calves during transition period. A total of 24 Sahiwal calves were randomly allocated into four groups (having six calves in each group) and fed for a transition period of 120 days (15 days of calf age to 135 days of calf age). Experimental calves either received a basal (devoid of supplemental Cr, Zn and Mg as control group) or supplemented with 0.15 mg Cr picolinate/kg $W^{0.75}$, 80 mg Zn/calf/day and 1.5 g Mg/calf/day. Experimental calves were monitored daily for feed intake and fortnightly for growth performance. At the end of the study, a digestion trial with 7 days collection period was conducted to study the effect of different treatments on nutrients utilization and mineral absorption. Results indicated that dietary Cr, Zn and Mg supplementation did not exert any effect on growth performance, dry matter intake (DMI), feed intake to gain ratio and apparent nutrient digestibility. However, the absorption of Cr, Zn and Mg was significantly ($P<0.05$) greater in its respective supplemented group. In conclusion, dietary supplementation of Cr, Zn, or Mg in Sahiwal calves during transition period did not influence growth performance, feed intake or nutrient digestibility but selectively enhanced mineral absorption.

KEYWORDS: Calves, Growth performance, Nutrients utilization, Mineral absorption, Transition period

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INTRODUCTION

Effective calf management is a critical yet often underdeveloped skill among dairy farmers. Deficiencies in feeding and husbandry practices, as noted by Thakur et al. (2025), can lead to significant future losses in production and reproduction. Research indicates that chromium (Cr) supplemented calf exhibits improved average daily gain (ADG) and feed efficiency. Studies, such as those reviewed by Windeyer et al. (2014), report that calves receiving organic Cr as Cr-yeast show significantly higher body weight gains and improved skeletal growth compared to non-supplemented controls. Improved glucose availability fuels immune cell activity, leading to lower morbidity rates and less growth disruption from disease, as noted by Calder et al. (2007). Zinc (Zn) is the second most abundant trace element in the animal body, but it cannot be stored in the animal's body (Zalewski et al., 2005) and requires regular dietary intake to meet its physiological needs. Zn influences various biological functions and is also a cofactor for more than 300 metalloenzymes (Chasapis et al., 2012). The magnesium (Mg) is an essential macro-mineral fundamental to optimal animal performance, influencing productivity, health, and metabolic efficiency across species. Mg is indispensable for glycolysis, oxidative phosphorylation and all energy-dependent processes, directly linking it to

growth and milk production. Mg is a cofactor for enzymes in the Krebs cycle and for protein synthesis machinery (aminoacyl-tRNA synthetase) (Schonewille, 2012). Adequate Mg is directly linked to improved milk yield and component quality (NRC, 2001). Mg supports efficient rumen fermentation by stabilizing rumen pH and optimizing microbial protein synthesis. This leads to improved digestibility of forages and better ADG.

In the past, numerous studies in farm animals have been conducted with organic and inorganic sources of mineral supplementation for studying performance in calves. Studies with supplementation of Cr, Zn and Mg in Sahiwal calves are limited. Considering these facts, this study was designed to study the effects of Cr, Zn and Mg on the growth performance and nutrients utilization in young Sahiwal calves.

MATERIALS AND METHODS

Ethics approval, animal feeding and experimental design

All animal care and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of DUVASU, Mathura (Approval Number: IAEC/24/1/24). The study was conducted in strict compliance with the standards established by the IAEC, which is constituted according to Article 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules, Government of India.

A total of 24 Sahiwal calves were randomly assigned into four groups (6 calves per group) on a basis of BW (28.12±0.90 kg) and age (15.0 days). 1) Control group: received a basal diet with no supplemental Cr, Zn or Mg; 2) Cr group: received the basal diet supplemented with 0.15 mg Cr/kg metabolic body weight ($BW^{0.75}$) provided as Cr-picolinate [$Cr(C_6H_4NO_2)_3$; molecular weight: 99.13 g/mol; minimum purity: 96%; Research Lab, Fine Chemicals Industries, Mumbai]; 3) Zn group: received the basal diet supplemented with 80 mg Zn/calf/day, provided as Zn-sulfate ($ZnSO_4$; molecular weight: 161.47 g/mol; minimum purity: 98%) and 4) Mg group: received the basal diet supplemented with 1.5 g Mg/calf/day, provided as Mg-sulfate ($MgSO_4$; molecular weight: 120.37 g/mol; purity: 98%). The experimental period continued for 120 days. The nutrient requirements for all the experimental calves were met according to the guidelines of the ICAR (2013). The nutrient composition of Calf starter and fodders offered, residue left and faeces voided during digestion trial was analysed for proximate analysis (AOAC, 2005), fibre fraction (Van Soest et al., 1991) and mineral content (5800 ICP-OES, Agilent, CA, USA). Milk and calf starter were offered @ 10% and 1% of the BW respectively. Green fodder and wheat straw were available *ad libitum*. The nutrient composition of basal diet (consisted of calf starter, green fodder and wheat straw and offered as TMR) fed during the animal feeding trial is presented in Table1.

Table 1. Composition of feedstuffs and milk fed during experimental period

Items	TMR
Ingredient composition	
Maize grain	17.5
Soybean meal	12.5
Mustard oil cake	6.5
Wheat bran	7.0
Gram husk	5.0
Mineral mixture	1.0
Salt	0.5
Wheat straw	20
Berseem fodder	30
Analyzed composition (g/kg DM or as mentioned)	
DM (% as fed)	71.01 (ranges between 70-73)
Total ash	10.09
AIA	3.24
EE	3.36
CP	17.18
CF	20.66
NFE	48.71
NDF	46.84

ADF	29.96
ADL	4.28
Ca	1.14
P	0.49
Na	0.82
K	12.44
Mg	0.90
Cu, mg/kg DM	8.80
Zn, mg/kg DM	28.21
Fe, mg/kg DM	272.36
Mn, mg/kg DM	51.21
Cr, mg/kg DM	0.28

Observation recording and laboratory analysis

The BW of experimental calves was recorded at the start and then fortnightly using a computerized scale (Leotronic Scales, India). Weighing occurred on two consecutive mornings at 06:00 h before feeding; the two-day average gave BW and ADG. A 7-day digestion trial was conducted to assessed nutrient utilization and mineral absorption. Feed, residue and faecal samples were dried at 60 °C to constant weight and ground through a 1-mm sieve for further analysis of DM, ash, ether extract and crude protein by AOAC (2005) methods; neutral and acid detergent fiber and lignin by following methodology of Van Soest et al. (1991). Mineral content in feed, residue and faecal samples were determined by using ICP-OES (5800, Agilent).

Statistical analysis

The data were collected and statistically analyzed by one-way analysis of variance (ANOVA) as per procedures suggested by Snedecor and Cochran (1994) by using SPSS (IBM SPSS Statistics V 20.0 USA). Animal tested within group was considered as a random effect, while group or treatment (Cr, Zn and Mg) was considered as the fixed effect. When the treatment was significant at $P<0.05$, means were compared by applying the probability of difference option of the least squares means statement. The animals were the experimental unit. Results were reported as means with SEM. Homogenous subsets were separated by Tukey's test.

RESULTS AND DISCUSSION

Growth performance

Dietary Cr, Zn and Mg supplementation did not exert any effect on growth performance (Table 2). Pandey et al. (2024) observed no improvement in growth with nano Cu or nano Zn supplementation in dairy calves. Consistent with these findings, Zaboli et al. (2013) found that ADG in goat kids was unaffected by Zn supplementation from different sources and levels, which aligns with the results of the present study. Similar to our findings, Khare et al. (2023) observed no significant ($P > 0.05$) influence of Cr on growth traits, while similar findings were reported in periparturient Murrah buffaloes (Deka et al., 2014) and calves (Mousavi et al., 2018). However, contrasting evidence exists. However, Kumar et al. (2021) reported significant improvements ($P<0.05$) in body weight and ADG in buffalo calves supplemented with Cr-chloride or Cr-picolinate. Kargar et al. (2018) further reported greater overall weight gain in Cr supplemented Holstein calves, although feed efficiency was unaffected. In contrast, other studies have demonstrated growth promoting effects of Zn. Umrao et al. (2025) who observed significantly higher ADG in the nano Zn supplemented Sahiwal heifers. Chang et al. (2020) observed increased ADG in newborn calves supplemented with Zn-methionine during the first two weeks of life, while Seifdavati et al. (2018) and Anil et al. (2019) reported higher body weight gain and ADG in calves receiving nano Zn supplementation. These variable responses across studies suggest that the growth effects of Zn supplementation depend on factors such as Zn source, dosage, physiological stage and baseline dietary Zn status. Work on the effect of supplementation of Mg in animals is very limited. The findings of the present study are consistent with those of Hernández-Calva et al. (2013), who reported no differences in ADG of feedlot lambs following Mg supplementation. In contrast, Aina (2013) observed a significant improvement in growth rate in West African dwarf goats when Mg was supplemented at a level of 0.50 kg/100 kg of DM. The discrepancy in growth performance in the findings of different studies may be a consequence of different sources and levels of Cr, Zn and Mg used, differing ages of animals used in the study, differences in sources and dose levels of the supplemental mineral, differences in study period, different genetics in various breeds, etc.

Table 2. Effect of Cr, Zn and Mg supplementation on growth performance during entire trial

Attributes	Group				P value
	Control	Cr	Zn	Mg	
Fortnightly BW gain (kg)	4.17±0.16	4.24±0.31	4.59±0.23	4.52±0.15	0.839
ADG (g/day)	277.78±10.39	282.50±20.40	305.76±15.41	301.44±10.28	0.837
DMI (kg/day)	1.23±0.04	1.28±0.03	1.38±0.08	1.36±0.04	0.729
DMI (kg/100 kg BW)	2.20±0.07	2.15±0.06	2.25±0.11	2.27±0.06	0.374
Feed intake to gain ratio	4.72±0.29	6.44±0.41	5.01±0.37	5.79±0.29	0.574

Feed intake

Dietary Cr, Zn and Mg supplementation did not exert any effect on feed intake. In the present study, no effects of supplementation of Cr, Zn and Mg were noticed on DMI (Table 2). The outcome aligns with numerous reports in ruminants, where organic Cr supplementation failed to alter DMI in dairy cows under thermoneutral conditions (Hayirli et al., 2001). Wang et al. (2023) reported increased DMI on Cr supplementation in cows. Mousavi et al. (2019) observed that Cr-methionine increased feed intake and DMI in dairy calves reared under hot summer conditions. This divergent response is theorised to be indirect. By enhancing insulin sensitivity, Cr may improve glucose metabolism and mitigate the catabolic effects of cortisol, thereby helping to maintain normal feeding behaviour under duress (Bernhard et al., 2012). Therefore, the absence of a DMI response in the current experiment may directly reflect the controlled, low-stress experimental environment, which did not provoke the metabolic dysfunction that Cr supplementation is intended to ameliorate. The absence of a response of Zn supplementation in the present study aligns with Umrao et al. (2025) who observed no significant effect on DMI in the nano Zn supplemented group in Sahiwal heifers. In opposite, Oconitrillo et al. (2024) reported that compared to control, Zn supplementation decreased the DMI throughout the trial in lactating cattle. Furthermore, the form of Zn is paramount. Organic complexes, such as Zn-methionine or Zn-proteinate, are often reported to be more bioavailable than inorganic salts like Zn oxide or sulfate, leading to more consistent improvements in intake and performance (Spears, 1996). This collective evidence strongly suggests that when animals are fed a well-formulated diet that meets or exceeds established Zn requirements (NRC, 2007), additional Zn provides no further stimulus for voluntary consumption. The results of this investigation demonstrated that Mg supplementation did not influence DMI in the studied animals. This outcome is consistent with a substantial body of ruminant research, where Mg supplementation, typically aimed at preventing hypomagnesemic tetany, has not been associated with increased voluntary feed consumption under non-deficient conditions. For instance, studies in feedlot cattle offered Mg oxide reported no effect on DMI (Colombo et al., 2021), and similar null findings have been documented in dairy cows when basal dietary Mg met established requirements (NRC, 2001). This suggests that Mg, unlike certain other minerals, does not function as a direct intake stimulant when adequate levels are present.

In the present study, statistical analysis of data revealed that variations between the groups for feed intake to gain ratio was non-significant. Results are in agreement with those obtained by Mousavi et al. (2018), who did not find any significant difference in gain: feed ratio in calves following dietary supplementation of different levels and sources of Cr. On contrary, Seifalinasab et al. (2022) observed that in Cr treated groups had lower daily feed intake as well as feed to gain ratio in the Cr-methionine (3 ppm) group than the control group finishing lamb. The present study was in agreement with Umrao et al. (2025) who observed no significant effect on FCR and FCE in the nano Zn supplemented Sahiwal heifers. On contrary, Liu et al. (2023) supplementary Zn of 80 mg/day from Zn-proteinate decreased the feed intake to gain ratio in calves during the whole experimental period of days 1 to 28. The findings align with present research indicating that Mg supplementation did not significantly affect feed conversion in feedlot lambs (Hernandez-Calva et al., 2013) and feedlot cattle (Colombo et al. 2022). In contrast, Orishchuk et al. (2025) found that an Mg based feed additive improved the feed conversion coefficient while reducing feed intake in laying hens. Likewise, Hashemi et al. (2012) reported a significantly lower FCR in ram lambs supplemented with MgO and sodium carbonate compared to the control group.

Nutrient utilization

The mean nutrient digestibility recorded over the 7-day metabolism trial indicated that Cr, Zn and Mg supplementation had no significant effect on apparent nutrient digestibility (Table 3). These findings are in agreement with Zade et al. (2014), who reported a non-significant effect on apparent nutrient digestibility in periparturient Murrah buffaloes supplemented with 0.5, 1.0, and 1.5 mg inorganic Cr/kg DM. Similarly, Kumar et al. (2013, 2015) also observed no differences in nutrient utilization when Murrah buffalo calves exposed to summer and winter conditions were fed diets supplemented with varying levels of inorganic Cr during the summer season. The present results are also consistent with the findings of Khare et al. (2023), who reported that dietary Cr supplementation did not influence nutrient digestibility in calves during transition period. In line with this, Wang et al. (2023**)** noted that the apparent digestibility of DM, organic matter, crude protein and ether extract remained unaffected by Cr supplementation in cows. In the present study, no effects of supplementation of Zn on nutrient digestibility were observed. Several studies have reported that Zn supplementation has little or no effect on nutrient digestibility in ruminants. The dietary supplementation of either nano Zn alone or in combination did not exert any effect on apparent nutrient digestibility in young cattle calves (Pandey et al., 2024). Similarly, Singh et al. (2018) further demonstrated that supplementation with either micro-ZnO or nano-ZnO at 60 ppm resulted in similar nutrient intake and digestibility coefficients in pre-ruminant lambs. In contrast, Abbi et al. (2024) observed that Zn supplementation significantly improved nutrient digestibility coefficients, with notable increases in NDF (P = 0.004) and ADF (P = 0.02) digestibility, along with a tendency toward higher DM digestibility. In the present study, no effects of supplementation of Mg on nutrient digestibility were observed. Limited work has been conducted to determine the effect of Mg supplementation on nutrient digestibility in animals. On contrary, Orishchuk et al. (2025) reported that supplementation with a Mg based feed additive improved nutrient digestibility in laying hens whereas. Accordingly, Xiong et al. (2024) observed that Mg supplemented feedlot lambs exhibited higher total tract nutrient digestibility compared to controls. This apparent disagreement in nutrient digestibility among different experiments could be due to the chemical form of Cr, Zn and Mg used or the minerals level was too low to affect the nutrient digestibility in the present study.

Table 3. Effect of Cr, Zn and Mg supplementation on nutrient utilization (%)

Nutrient	Group				Pooled SEM	P value
	Control	Cr	Zn	Mg		
DM	61.41	61.95	62.60	61.59	0.13	1.000
OM	69.11	69.60	70.46	69.39	0.15	0.685
CP	74.12	71.24	73.16	71.87	0.32	0.443
EE	77.29	78.28	81.22	80.43	0.46	0.675
CF	53.94	55.22	54.93	53.53	0.20	0.589
NFE	71.08	73.67	72.54	71.73	0.28	0.289
NDF	51.24	52.46	52.18	50.85	0.19	0.675
ADF	33.31	34.10	33.92	33.05	0.12	0.958
Ca Absorption (%)	38.19	38.82	39.09	38.48	0.20	0.759
P Absorption (%)	48.94	50.29	48.38	50.41	0.50	0.586
Na Absorption (%)	87.38	90.32	88.49	89.25	0.62	0.576
K Absorption (%)	84.17	85.32	86.01	85.78	0.41	0.384
Mg Absorption (%)	24.92^{a}	28.62^{a}	30.21^{ab}	33.09^{b}	0.48	0.037
Cu Absorption (%)	10.40	10.39	10.23	10.79	0.12	0.493
Zn Absorption (%)	23.74^{a}	23.65^{a}	34.06^{b}	24.14^{a}	2.56	0.043
Fe Absorption (%)	28.38	29.99	28.93	27.39	0.57	0.475
Mn Absorption (%)	4.03	4.02	3.19	3.29	0.23	0.392
Cr Absorption (%)	2.44^{a}	6.42^{b}	2.04^{ab}	2.08^{a}	0.91	0.028

Values within a row with different superscript letters differ significantly (P<0.05).

Mineral absorption

However, treatment showed a significant (P<0.05) effect on the intake, faecal excretion and absorption of Cr, Zn and Mg (Table 3). The treatments showed a significant (P<0.05) effect on the intake, faecal excretion and absorption of Mg. Also, the findings of the present study revealed a significant (P<0.05) effect of Zn supplementation on its intake, faecal excretion and absorption. Intake, faecal excretion and absorption of Zn were significantly (P<0.05) higher in Zn group compared to the control, Cr and Mg groups. Similarly, intake, faecal excretion and absorption of Cr were significantly (P<0.05) higher in the calves supplemented with Cr compared to the calves of the control, Zn and Mg groups. In the present study, the absorption of Cr, Zn and Mg was significantly (P<0.05) higher in the Cr, Zn and Mg supplemented groups respectively as compared to the calves of the other groups, indicating minimal interaction between Cr and other studied minerals. These findings are consistent with studies on Cr-chloride supplemented heifers showed higher trace mineral intake (Biswas et al., 2006), and apparent Cr absorption is known to vary by source, ranging from 4-10% for inorganic Cr to 10–25% for natural sources such as brewer's yeast (Underwood, 1977). Some contrasting observations exist, including increased Zn, Cu, and Fe intake in CrCl3-supplemented heifers (Biswas et al., 2006) and decreased Zn absorption (Underwood, 1977). Overall, the findings indicate that Cr supplementation effectively enhances Cr status without consistently affecting the metabolism of other trace minerals. In the present study, Zn absorption was higher in the Zn supplemented group while its supplementation had no effect on the absorption of other minerals. These results are consistent with Pandey et al. (2024), who reported higher absorption and bioavailability of Zn in the 30 ppm nano-Zn and combination (10 ppm nano-Cu and 30 ppm nano-Zn) groups, with no interaction between other minerals. Similarly, Liu et al. (2021) found that Zn-methionine added to feed and Zn sulphate in drinking water significantly P<0.05) increased its absorption and Zn concentrations in the liver and jejunum of piglets. As similar to the findings of the present study, Garg et al. (2008) also reported no significant differences in Ca and P balance or retention among lambs supplemented with Zn-methionine complexes, ZnSO4, or control diets. The present findings in agreement with Ramirez et al. (1998) who observed that the Mg absorption in Holstein steers enhanced by increasing dietary Mg level. Various factors played a crucial role in Mg absorption. Mg is more soluble at lower rumen pH, enhancing its availability for absorption (Martens et al., 2018). In the present study, dose dependent absorption of Cr, Zn and Mg in respective groups with no effect on the absorption of other studied minerals is indicative of no interaction among Cr, Zn and Mg with dietary minerals.

CONCLUSION

In present study, it would be rational to conclude that dietary supplementation with Cr, Zn, or Mg during the transition period did not result in significant alterations in growth performance, DMI, feed efficiency or apparent nutrient digestibility in Sahiwal calves. The lack of response in productive parameters indicates that the basal diet was likely adequate to meet the nutritional requirements for growth during the study period. Nevertheless, dietary inclusion of these minerals significantly enhanced their respective absorption, demonstrating improved mineral bioavailability. These findings suggest that while Cr, Zn and Mg supplementation may not directly influence growth under normal conditions, they could play a supportive role in maintaining mineral status. Further investigations are required to assess their potential benefits under conditions of nutritional deficiency, physiological stress or enhanced production demands in transition calves.

REFERENCES

Abbi, R., Singh, P., Sharma, A. and Kumar, R. 2024. Effect of zinc supplementation on nutrient digestibility and fiber utilization in small ruminants. Animal Nutrition and Feed Technology. 24(1): 45-54.

Aina, A. B. J. 2013. Effect of dietary magnesium supplementation on serum magnesium concentration and performance of female West African dwarf (*Fouta djallon*) goats. Indian Journal of Animal Sciences. 67: 10-19.

Anil, T. S. V., Seshaiah, V. C., Ashalatha, P. and Sudhakar, K. 2019. Effect of dietary nano zinc oxide supplementation on growth performance in crossbred calves. International Journal of Current Microbiology and Applied Sciences. 8(12): 1852-1856.

AOAC. 2005. Official methods of analysis. 18th Edn. Association of Official Analytical Chemists, Washington, D.C, USA.

Bernhard, B. C., Burdick, N. C., Rounds, W., Rathmann, R. J., Carroll, J. A., Finck, D. N., Jennings, M. A., Young, T. R. and Johnson, B. J. 2012. Effects of chromium supplementation on glucose metabolism and stress response in cattle. Journal of Animal Science. 90(11): 3851-3861.

Biswas, P., Haldar, S., Pakhira, M. C., Ghosh, T. K. and Biswas, C. 2006. Efficiency of nutrient utilization and reproductive performances of pre pubertal anestrous dairy heifers supplemented with inorganic and organic chromium compounds. Journal of the Science of Food and Agriculture. 86: 804-815.

Calder, P. C., Dimitriadis, G. and Newsholme, P. 2007. Glucose metabolism in lymphoid and inflammatory cells and tissues. Current Opinion in Clinical Nutrition and Metabolic Care. 10(4): 531-540.

Chang, M. N., Wei, J. Y., Hao, L. Y., Ma, F. T., Li, H. Y. and Zhao, S. G. 2020. Effects of different types of zinc supplement on growth, incidence of diarrhea, immune function and rectal microbiota of newborn dairy calves. Journal of Dairy Science. 103: 6100-6113.

Chasapis, C. T., Loutsidou, A. C., Spiliopoulou, C. A. and Stefanidou, M. E. 2012. Zinc and human health, an update. Archives of Toxicology. 86(4): 521-534.

Colombo, E. A., Cooke, R. F., Araújo, A. C., Harvey, K. M., Pohler, G. N. and Brandão, A. P. 2021. Supplementing a blend of magnesium oxide to feedlot cattle: effects on ruminal physiological and productive responses. Journal of Animal Science. 99(12): 1-10.

Colombo, E. A., Cooke, R. F., Brandão, A. P. and Bohnert, D. W. 2022. Effects of dietary magnesium supplementation on performance, feed efficiency and carcass characteristics of feedlot cattle. Translational Animal Science. 6(1): 8-12.

Deka, R. S., Mani, V., Kumar, M., Zade, S. S., Upadhaya, R. C. and Kaur, H. 2014. Effect of additional chromium supplementation on health status, metabolic responses and performance traits in periparturient Murrah buffaloes (*Bubalus bubalis*). Biological Trace Element Research. 163(2): 132–143.

Garg, A. K., Mudgal, V. and Dass, R. S. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Animal Feed Science and Technology. 144(1): 82-96.

Hashemi, M., Khorvash, M., Ghorbani, G.R. and Nikkhah, A. 2012. Effects of magnesium oxide and sodium carbonate supplementation on growth performance and feed conversion ratio in ram lambs. Small Ruminant Research. 106: 137-142.

Hayirli, A., Bremmer, D. R., Bertics, S. J., Socha, M. T. and Grummer, R. R. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. Journal of Dairy Science. 84(6): 1218-1230.

Hernandez-Calva, L. M., Zinn, R. A., Ramirez, J. E. and Salinas-Chavira, J. 2013. Influence of dietary magnesium level on growth performance and feed efficiency of feedlot lambs. Journal of Animal Science. 91(1): 152-158.

ICAR. 2013. Nutrient requirements of dairy cattle. Indian Council of Agricultural Research, New Delhi, India.

Kargar, S., Mousavi, F. and Karimi-Dehkordi, S. 2018. Effects of chromium supplementation on weight gain, feeding behaviour, health and metabolic criteria of environmentally heat-loaded Holstein dairy calves from birth to weaning. Archives of Animal Nutrition. 72: 443-457.

Khare, S., Kumar, M., Kumar, V., Kushwaha, R., Vaswani, S., Kumar, A., Yadav, R.S., Singh, S.K., Singh, Y. and Shukla, P.K. 2023. Dietary chromium picolinate supplementation improves glucose utilization in transition calf by ameliorating insulin response. Biological Trace Element Research. 201(6): 2795-2810.

Kumar, D., Yadav, S. P., Chandra, G., Sahu, D. S. and Maurya, P. S. 2021. Influence of different sources of chromium supplementation on growth, immunity and liver function of buffalo calves. Indian Journal of Animal Nutrition. 38: 2-12.

Kumar, M., Kaur, H., Tyagi, A. K., Kewalramani, N. J., Mani, V., Deka, R. S., Sharma, V. K., Chandra, G. and Dang, A. K. 2013. Effect of feeding inorganic chromium on growth performance, endocrine variables and energy metabolites in winter-exposed buffalo calves (*Bubalus bubalis*). Biological Trace Element Research. 155(3): 352-360.

Kumar, M., Kaur, H., Tyagi, A., Deka, R. S., Mani, V., Tyagi, A. K. and Chandra, G. 2015. Effect of feeding inorganic chromium on growth performance, endocrine variables and energy metabolites in winter-exposed buffalo calves (*Bubalus bubalis*). Biological Trace Element Research. 167(1): 18–27.

Liu, F. F., Azad, M. A., Li, Z. H. and Li, J. 2021. Zinc supplementation forms influenced zinc absorption and accumulation in piglets. Animals. 11: 36-46.

Liu, Y., Zhang, H., Wang, J., Li, S. and Zhao, Y. 2023. Effects of zinc proteinate supplementation on growth performance and feed efficiency of calves during early growth period. Animal Feed Science and Technology. 302: 115-701.

Martens, H., Leonhard-Marek, S., Röntgen, M. and Stumpff, F. 2018. Magnesium homeostasis in cattle: absorption and excretion. Journal of Animal Science. 96(1): 1-14.

Mousavi, F., Karimi-Dehkordi, S., Kargar, S. and Ghaffari, M. H. 2018. Growth performance, feeding behavior, health status and blood metabolites of environmentally heat-loaded Holstein dairy calves fed diets supplemented with chromium. Journal of Dairy Science. 101(11): 9876-9887.

Mousavi, F., Karimi-Dehkordi, S., Kargar, S. and Khosravi-Bakhtiari, M. 2019. Effects of dietary chromium supplementation on calf performance, metabolic hormones, oxidative status and susceptibility to diarrhea and pneumonia. Animal Feed Science and Technology. 248: 95-105.

NRC. 2001. Nutrient requirements of dairy cattle. 7th Rev. Edn. National Academy Press, Washington, D.C., USA.

NRC. 2007. Nutrient requirements of small ruminants. National Academy Press, Washington, D.C., USA.

Oconitrillo, M., Wickramasinghe, J., Omale, S., Beitz, D. and Appuhamy, R. 2024. Effects of elevating zinc supplementation on the health and production parameters of high-producing dairy cows. Animals. 14(3): 395-402.

Orishchuk, O. S., Tsap, S. V., Mylostyvyi, R. V., Chernenko, O. I., Chernenko, O. M. 2025. Assessment of a magnesium-based feed additive on productivity and feed conversion efficiency in laying hens. Poultry Science. 104(2): 102-145.

Pandey, P., Kumar, M., Kumar, V., Kushwaha, R., Vaswani, S., Kumar, A., Singh, A., Shukla, P. K. and Prasad, S. 2024. Dietary supplementation of copper and zinc nanoparticles in young dairy calves: effects on growth performance and nutrient utilization. Indian Journal of Animal Nutrition. 41(2): 136-163.

Ramirez, J. E., Zinn, R. A. and Alvarez, E. 1998. Effects of elevated dietary magnesium on growth performance and feed efficiency in Holstein steers. Journal of Animal Science. 76: 165-170.

Schonewille, J. T. 2012. Magnesium in dairy cow nutrition: an update. Animal Feed Science and Technology. 176(4): 1-11.

Seifalinasab, M., Mousaie, A. and Doomary, H. 2022. Effects of chromium supplementation on haematology in finishing lambs. Tropical Animal Health and Production. 54: 112-121.

Seifdavati, J., Ara, M. J., Seyfzadeh, S., Benamar, H. A., Gheshlagh, F. M. A., Seyedsharifi, R. and Vahedi, V. 2018. Effect of zinc oxide nanoparticles on growth performance, blood metabolites and serum enzymes in Holstein suckling calves. Iranian Journal of Animal Science Research. 10: 23-33.

Singh, K. K., Maity, S. B. and Maity, A. 2018. Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs. Indian Journal of Animal Research. 88(7): 805-807.

Snedecor, G. W. and Cochran, W. G. 1994. Statistical methods. 8th Edn. Iowa State University Press, Ames, Iowa, USA.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Animal Feed Science and Technology. 58: 151-163.

Thakur, N., Kasrija, R. and Singh, P. 2025. Advancing calf care: development and standardization of knowledge test for calf management and feeding practices. Archives of Current Research International. 25(8): 206-211.

Umrao, P., Kumar, V., Kumar, M., Kushwaha, R., Vaswani, S., Kumar, A., Yadav, R. D. and Gupta, M. 2025. Effects of nano zinc supplementation on growth performance and hematological indices in Sahiwal heifers. Journal of Animal Physiology and Animal Nutrition. 109: 1123-1134.

Underwood, E. J. 1977. Chromium. In: Trace elements in human and animal nutrition. 4th Edn. Academic Press, New York, USA.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

Wang, M., Yang, J., Shen, Y., Chen, P., Li, Y., Cao, Y., Li, Q., Xu, H., Gao, Y. and Li, J. 2023. Effects of chromium propionate supplementation on lactation performance, nutrient digestibility, rumen fermentation patterns and antioxidant status in Holstein cows under heat stress. Animal Feed Science and Technology. 305: 115-165.

Windeyer, M. C., Leslie, K. E., Godden, S. M., Hodgins, D. C., Lissemore, K. D. and LeBlanc, S. J. 2014. Factors associated with morbidity, mortality and growth of dairy heifer calves up to 3 months of age. Preventive Veterinary Medicine. 113: 231-240.

Xiong, F., Lv, L., Liu, Y., Chen, H. and Yang, H. 2024. Supplementation of feedlot lambs with magnesium oxide and sodium bicarbonate: effects on performance and total tract nutrient digestibility. Animal Feed Science and Technology. 311: 115-151.

Zaboli, K., Aliarabi, H., Bahari, A. A. and Abbas, A. K. R. 2013. Role of dietary nano zinc oxide on growth performance and blood levels of minerals in Iranian Angora goat kids. Journal of Pharmaceutical and Health Sciences. 2(1): 19-26.

Zade, S. S., Mani, V., Deka, R. S., Kumar, M., Kaur, H., Kewalramani, N. J. and Tyagi, A. K. 2014. Energy metabolites, lipid variables and lactation performance of periparturient Murrah buffaloes fed diet supplemented with inorganic chromium. Biological Trace Element Research. 159: 115-127.

Zalewski, P. D., Ai, Q. T., Dion, G., Lata, J., Chiara, M. and Richard, E. R. 2005. Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. Pharmacology and Therapeutics. 105: 127-149.

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Replacement of Shell Grit with Stone Grit and Its Effect on Serum, Carcass and Tibia Bone Characteristics in Egg Type Japanese Quails

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ABSTRACT

A trial was conducted for a period of 46 weeks (6-52 weeks) to evaluate the effect of replacing the shell grit with stone grit in the feed of egg type Japanese quails on serum, carcass and bone characteristics. A total of 360 numbers of five week old female Japanese quails were randomly distributed to 5 dietary treatments with 6 replicates of 12 birds each in a completely randomized design. The calcium in the ration was supplied by replacing shell grit at two different levels (50 and 100 %) with stone grit of two particle sizes (2 mm and 4 mm) and the non replacement (100 % shell grit) group was taken as control. At the end of the 52 weeks, 60 birds were slaughtered to study the carcass and bone parameters. Both right and left tibia bones were collected, defatted and dried. Left tibia bones were measured for length and weight and analyzed for total ash, calcium and phosphorous contents. Right tibia bones were tested for bone breaking strength. Serum samples were analyzed for calcium, phosphorous and alkaline phosphatase. The carcass characteristics such as dressing percentage, eviscerated carcass weight, weights of giblet, liver, heart, gizzard as well as intestinal weight and length, levels of serum calcium, phosphorous and alkaline phosphatase did not vary significantly. The particle size or level of replacement of stone grit did not alter the tibia bone parameters such as tibia weight, length, diameter, Seed or index, Robusticity index and bone breaking strength. From this study, it can be concluded that the stone grit is efficient to supply adequate calcium for growth of bone and muscles as that of shell grit and hence the shell grit can be completely replaced with stone grit of size 4 mm in the feed of egg type Japanese quails without compromising the bone quality and carcass yield.

KEYWORDS: Calcium, Carcass characteristics, Particle size, Stone grit, Tibia bone

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INTRODUCTION

Studies on alternative feed sources have primarily focused on energy and protein rich ingredients. However, limited attention has been given to local alternative sources for major mineral nutrients such as calcium and phosphorus. The main sources of calcium in poultry diets include shell grit, bone meal, limestone, and dicalcium phosphate. Calcium and phosphorus, which are classified as macro-minerals, are essential for birds, as they are key components of bones and play a crucial role in muscle growth. Riberio et al. (2016) experimented in 48 weeks old Japanese quails by feeding with two levels of calcium (29 and 38 g/kg) and two available P levels (1.5 and 3.0 g/kg) for 63 days and found that the supplementation of 38 g Ca/kg (998 mg Ca/bird/day) and 3.0 g avP/kg (78 mg avP/bird/day) increased the marketable egg production and improved the egg quality of Japanese quails at end of the egg production phase.

Coarse limestone particles provide soluble calcium later during the dark period to supply the eggshell formation and reduce the desynchronization between the calcium supply and demand to produce the eggshell. Gloux et al. (2020) concluded that feeding of coarse limestone particles reduced the calcium mobilization from bones which lead to increased bone breaking strength of laying

hens. Sinclair-Black (2019) also observed that feeding coarse limestone (1.5 mm) increased blood ionizable calcium concentration in laying hens overnight when more calcium is needed for eggshell formation, in comparison to a fine limestone (0.2 mm)

The most common coarse calcium source used in layer quail diets is shell grit, which contains 36–38% calcium. However, its particle size is irregular and often contaminated with sand and silica. The approximate market price of shell grit ranges from Rs. 8 to 10 per kg. Moreover, collecting shell grit from seashores has become increasingly laborious and expensive. Hence, there is an urgent need to identify an efficient alternative calcium source to replace shell grit. One such alternative is **stone grit,** derived from metamorphosed limestone rocks composed of crystalline forms of calcium carbonate, with a calcium content (38%) equivalent to that of shell grit. It is available in uniform particle sizes with consistent geometric mean diameters and can be used to replace shell grit in layer quail diets. Additionally, stone grit is more affordable (Rs. 4–5 per kg), which may help reduce production costs.

However, there is a lack of research on the use of stone grit in egg type Japanese quails, creating a knowledge gap regarding its impact on serum, carcass and bone characteristics. Determining the ideal particle size and optimum inclusion level of stone grit could help in enhancing the profitability of quail farming. The objective of this study was to evaluate the effects of replacing shell grit with coarse stone grit of two particle sizes (2 mm and 4 mm) at 50% and 100% inclusion levels on serum, carcass and bone characteristics in egg type Japanese quails.

MATERIALS AND METHODS

Location and climate

The biological experiment was conducted in Veterinary College and Research Institute, Namakkal, Tamil Nadu. The temperature humidity index (TSI) during the study period was between 77.52 and 83.07(Anon, 2025).

Ethical and bio-safety committee approval

Biological trial was approved by the Institutional Animal Ethics Committee (IEAC) of Veterinary College and Research Institute, Namakkal (No.16/VCRI/NKL/2024).

Experimental birds

A 52 week biological trial was conducted by using 360 numbers of five week old "*TANUVAS Namakkal Gold Quail,"* developed for egg production were wing banded, weighed, and randomly assigned to five treatment groups with six replicates of 12 birds each in a completely randomized design. All birds were reared in cage system and fed an isocaloric and isonitrogenous diet with 3% calcium and 0.40% non-phytate phosphorus. The dietary calcium of 3% was provided to dietary treatments as follows.T1 -100 % shell grit (control),T2 -100 % stone grit with 2 mm size, T3 -100 % stone grit with 4 mm size, T4 -50 % shell grit plus 50 % stone grit with 2 mm size, T5 -50 % shell grit plus 50 % stone grit with 4 mm size. Calcium was supplied by replacing shell grit with stone grit (2 mm and 4 mm) at 50% and 100% levels. Maize and soya based experimental diets were identical in nutrient composition, differing only in calcium source, level, and particle size. The in vitro solubility of 2 mm and 4 mm stone grit was estimated as 61.9 and 54.2 per cent respectively (Premkumar et al., 2024)

Blood collection and serum separation

At the end of experimental period, three ml of blood was collected (jugular vein) from twelve birds in each treatment group during slaughter. Blood samples were collected in test tubes and allowed to clot by keeping in slant position and then centrifuged for 10 minutes at 2000 rpm in a centrifuge to separate clear supernatant serum. The serum samples were stored in the deep freezer at -20^{0}C until further analysis.

Serum calcium, phosphorous and alkaline phosphatase estimation

The pooled serum samples for each replicate were analyzed in M/S Biosystems A50 India auto analyzer available in the Clinical Laboratory facility located in the Clinical complex of Veterinary College and Research Institute, Namakkal. The serum calcium, phosphorous and alkaline phosphatase were estimated by using the specific commercial readymade kits purchased from Dhaksha Diagnostics, Salem, India. The values generated in the auto analyser for serum calcium and phosphorous were measured as mg/dL and the serum alkaline phosphatase was measured as U/L.

Carcass characteristics assessment

At the end of 52 weeks of experimental period, two birds having the body weight nearer to the mean body weight were selected from each replicate in all the treatment groups and feed was withdrawn 8

hours prior to slaughter and potable water was provided *adlibitum*. Birds were weighed before slaughter and humanely sacrificed (Genchev and Mihaylo, 2008). Blood was collected for serum separation and stored frozen. Carcasses were manually defeathered, eviscerated, and organs (heart, liver, gizzard without contents, and intestine) were weighed. Pre-slaughter, post-bleeding, defeathered, and eviscerated weights, along with organ and giblet weights, were recorded and expressed as percentage of live weight. Intestinal length was measured with a measuring tape, and both left and right tibia were collected and stored at −20°C for further analysis.

Bone quality assessment

1. Collection of tibia bone

At the end of the 52weeks, both left and right tibia bones were dissected from the slaughtered birds and their adhering muscles along with connective tissue were removed manually. The collected bones were processed by dipping in 10 per cent sodium hydroxide solution for five minutes to remove the adhering fine and soft tissues. De-fattening of dried bones was done by dipping in petroleum ether for overnight following the procedure of AOAC, (2023) and then the bones were dried in a hot air oven at 100°C for 12 hours.

2. Tibia weight and length measurement

Bone weight was measured using a digital electronic scale to the nearest 0.001g in fresh and fat-free dried tibia bone. The length of the tibial bone was measured in digial vernier caliper (Mitutayo Make) from the proximal end to the distal end of the bone. The circumference was measured at their mid points.

3. Estimation of tibial ash, calcium and phosphorous

Tibial ash is the non combustible portion representing the total mineral matter content of the bone. The determination of crude ash was performed as per the standard procedure (Reference IS 14827:2000) in the Animal Feed Analytical and Quality Assurance Laboratory, VCRI, Namakkal. The dry defatted tibia bones were weighed and burnt into ash in a muffle furnace at 600°±30° C for 12 hours as per the procedure described by Panda et al. (2006) and the percentage of total ash was calculated and then cooled in a dessicator to room temperature. The percentage of total ash was calculated by the following formula.

$$\text{Total ash per cent} = \frac{\text{Weight of ash in the crucible (g)}}{\text{Initial weight of the bone sample (g)}} \times 100$$

4. Estimation of tibial calcium

Tibial calcium per cent was estimated from the tibial ash by the titration method as defined by AOAC (2023) official method 927.02 (Dry ash method for calcium estimation in animal feed). The calcium in the sample was precipitated as calcium oxalate by using ammonium oxalate in acidic medium. The precipitated calcium oxalate was filtered out, washed with ammonium hydroxide to free ammonium oxalate from the precipitate and dissolved in hot sulphuric acid and the liberated oxalic acid was estimated by permanganometric titration.

1ml of 0.1N $KMNO_4$ = 0.002 g of calcium

5. Estimation of tibial phosphorous

Tibial phosphorous per cent was estimated from the tibial ash by the standard calorimetric method as defined by AOAC (2023) official method 965.17 (Dry ash method for phosphorous estimation in animal feed). Phosphorous in the sample is converted into phosphomolybdo vanadate complex by adding the ammonium molybdo vanadate reagent. The intensity of colour of the phosphomolybdo vanadate complex was measured at 400 nm in UV visible spectrophotometer.

6. Bone mineral density

The seedor index (g/cm) was calculated by dividing the tibial bone dry weight by its length, as an indicative of bone mineral density (Seedor et al., 1991). Robusticity index and Seedor index were calculated by the following equations (Mohammed et al., 2021).

Seedor index = Weight of the tibia in g / Length of tibia in cm × 100

Robusticity index = Tibia length in cm /cube root of bone weight in g.

Statistical Methodology

The biological trial data collected on carcass, bone and serum parameters were statistically analyzed by one way ANOVA method in SPSS 20th version and the mean of different experimental groups were tested for statistical significance by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of particle size and replacement level of stone grit on serum profile in egg type Japanese quails

1. Serum calcium

The mean serum calcium (mg/dL) of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 1. The calcium source, particle size and replacement levels of stone grit in this study did not significantly (P≥0.05) affect the serum calcium of 52 week old Japanese quails. Similar non significant serum calcium levels with respect to particle size of calcium source were reported in layers, (Nisar 2015; Saki et al., 2018; Lee et al., 2021) and in broilers (Hu et al., 2022). The findings of this study are consistent with those of Novack et al. (2023) who replaced limestone with industrial egg residue in broilers or with cuttlefish bone in the diet of Japanese quails (Bugdayci et al., 2020).

In contrast, Ertas et al. (2006) found highest plasma calcium level in Japanese quails when limestone was replaced with macunim shell at 75 per cent (28.2 mg/dl) as compared to the control group (23.5 mg/dl). Similarly, replacement of limestone with 30 percent coated calcium (Dongare et al., 2024) significantly (P<0.05) increased the serum calcium level, whereas, replacement of limestone with eggshell at 100 per cent (Karim and Abdulla, 2024) in broiler diet resulted significantly (P<0.01) lower serum calcium level as compared to the control group (8.78 vs 13.32 mg/dL).

The absence of significant differences in serum calcium levels in the present study suggests that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent level in the diet of Japanese quail does not impair calcium homeostasis. These results imply that as long as the overall dietary calcium content is adequate, serum calcium levels can be maintained within optimal physiological limits, regardless of calcium source or particle size.

Table 1. Mean (±S.E.) of serum calcium, phosphorous and alkaline phosphatase of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Serum profile parameters	T1	T2	T3	T4	T5	F-Value	P-Value
Serum calcium (mg/dL)	12.60 ± 0.67	11.37 ± 1.33	10.40 ± 0.37	9.95 ± 0.40	11.85 ± 0.96	1.68 NS	0.187
Serum phosphorus (mg/dL)	7.32 ± 0.55	6.73 ± 0.64	5.18 ± 0.50	6.20 ± 0.20	6.53 ± 0.72	2.06 NS	0.117
Serum alkaline phosphatase (U/L)	742.7 ± 82.5	631.6 ± 99.3	535.6 ± 36.3	764.7 ± 57.1	584.8 ± 59.6	1.99NS	0.128

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.
*(P<0.05) ** (P0.01) NS (P>0.05)
T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit
mg/dL- miiligram per deciliter, U/L – Units per litre, mm- millimeter
S.E – Standard error

2. Serum phosphorous

The mean serum phosphorus levels (mg/dL) of egg-type Japanese quails fed diets with different sizes and replacement levels of stone grit are presented in Table 1. The results of this study revealed that serum phosphorus was not significantly (P≥0.05) influenced by the calcium source, particle size, or level of replacement of stone grit in 52-week-old Japanese quails. These findings are consistent with those of Bugdayci et al. (2020), who reported no significant changes in serum phosphorus levels in 8-week-old Japanese quails when limestone was replaced with cuttlefish bone at 0, 50, and 100 per cent levels. A similar observation was made by Karim and Abdulla (2024), who found no significant differences when limestone was replaced with eggshell at 50 and 100 per cent levels in broiler diets. Likewise, non-significant variations in serum phosphorus concentrations with respect to calcium particle size have been reported in layers (Rao and Raju, 2004; Gultepe et al., 2021) and broilers (Hu et al., 2022). In contrast, Souvik-Mondal et al. (2011) recorded significantly higher (P<0.05) plasma phosphorus levels in 3-week-old Japanese quails fed diets containing tricalcium phosphate and dicalcium phosphate compared to other calcium sources. Similarly, Dongare et al. (2024) observed a significant increase in serum phosphorus (P<0.01) in broilers fed diets with 30 per cent coated calcium (5.57 mg/dL) compared to the control (4.90 mg/dL). The results of the present study indicate that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent levels does not alter phosphorus metabolism, provided that the dietary calcium-to-phosphorus ratio is adequately maintained.

3. Serum alkaline phosphatase

The mean serum alkaline phosphatase (U/L) levels of egg-type Japanese quails fed diets with different particle sizes and replacement levels of

stone grit are presented in Table 1. Serum alkaline phosphatase activity was not significantly (P≥0.05) influenced by the calcium source, particle size, or level of replacement of stone grit in 52-week-old Japanese quails. These findings are consistent with those of Hu et al. (2022), who reported no significant effects of calcium level or limestone particle size on alkaline phosphatase activity in broilers.

Physiologically, elevated alkaline phosphatase activity is associated with osteoblastic activity, which is more pronounced during periods of growth and bone remodelling. The relatively lower enzyme levels observed in the present study may be attributed to the advanced age of the birds (52 weeks), aligning with the observations of Brenes et al. (2003), who reported a decline in alkaline phosphatase activity with age. Nevertheless, the numerically higher alkaline phosphatase values observed in the control group (T1) and the 50 per cent 2 mm stone grit replacement group (T4) may reflect ongoing bone remodelling processes. The absence of significant differences in serum alkaline phosphatase levels among treatments indicates that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent levels in the diet of Japanese quails maintains phosphorus homeostasis, provided that the available phosphorus concentration is maintained at 0.40 per cent in both control and treatment diets.

Effect of particle size and replacement level of stone grit on carcass characteristics of egg type Japanese quails

1. Dressing per cent

The mean dressing per cent of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit at 52 weeks of age is presented in Table 2. The results revealed that dressing per cent of Japanese quails fed with different sizes and replacement levels of stone grit at 52 weeks of age did not differ significantly (P>0.05). Similar non significant effects on dressing per cent was observed in Japanese quails supplemented with calcium propionate at the rate of 6g/kg in the diet (Bonos et al., 2010) and in broilers fed with granite grit (Garipoglu et al., 2006), marble powder (Demirel et al., 2017) and various calcium sources like oyster shell, eggshell, and calcium carbonate powder (Rezvani et al., 2019). In this study, grit particle size (2 mm vs. 4 mm) also had no significant impact, consistent with findings by Moghaddam et al. (2016), who observed no difference in dressing percentage when broilers were fed insoluble grit of 2, 3, and 4 mm sizes.

However, contrasting results were noted by Maranan et al. (2021), who found a linear decline in dressing percentage with increasing dietary eggshell levels. Moreover, Hakami et al. (2022) also found significantly higher dressing per cent in broilers fed marine mineral complex (CeltiCal) at 0.4 per cent level as a partial substitute for limestone in broilers compared to the control. As the dressing per cent is indicative of adequate flesh and fat cover of the carcass, it can be inferred that replacement of shell grit with stone grit in the feed supplied sufficient calcium ions for uniform growth of body tissues, organs, skin and feathers irrespective of the particle size.

2. Ready to cook weight

The mean ready to cook weight of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The results revealed that ready to cook weight of Japanese quails fed with different sizes and replacement levels of stone grit at 52 weeks of age did not differ significantly (P>0.05).

These results are in accordance with the findings of Mendonca et al. (2022) who conducted a trial in European quails fed with five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal or oyster shell meal) from day old to 35 days of age and found no significant difference (P>0.05) in absolute and relative weight of carcass. Similarly, Novack et al. (2023) reported no significant differences in carcass yield percentages in broilers by replacing limestone with industrial egg residue at 0, 35, 70, and 100 per cent.

In contrast, Idachaba (2013) reported significant differences in dressed body weights of broilers fed diet with different levels of stone grit in rice offal, and by Usaro and Christopher (2022), who reported improved dressed weights in broilers fed various levels of insoluble granite grit in a brewer's spent grain-based diet. The improved dressed weights were attributed to enhanced nutrient utilization due to the inclusion of grit in the diet. However, in the present study, inclusion of stone grit either 2 or 4 mm at both 50 and 100 per cent level in the diet did not exhibit additional advantage in terms of more dressed weight as compared with control diet.

3. Eviscerated carcass yield

The mean eviscerated carcass yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The results revealed that eviscerated carcass yield of Japanese quails fed with varying stone grit sizes and replacement levels at 52 weeks of age did not

differ significantly (P>0.05).

These findings align with Moghaddam et al. (2005), Garipoglu et al. (2006), and Eser et al. (2018), who also reported no significant effects on carcass yield following the inclusion of zeolite or granite grit in broiler diets. Likewise, Mendonca et al. *(*2022) found no significant differences in carcass yield when European quails were fed five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal) from day old to 35 days of age. However, contrasting results were reported by Dongare et al. (2024) and El-Ganainy et al. (2024) who reported significantly higher carcass yield in broilers supplemented with coated calcium and nano hydroxyapatite, respectively. These results suggest that while certain advanced calcium forms may influence carcass yield, traditional sources like shell grit and stone grit do not significantly impact eviscerated carcass yield in Japanese quail.

4. Giblet yield

The mean giblet yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The giblet yield (per cent of live body weight) was not significantly (P≥0.05) influenced by both particle size and level of replacement of stone grit in 52 weeks old Japanese quails. This finding is consistent with Gongruttananun (2011), who reported no significant differences in giblet weights when ground eggshell replaced limestone in breeder male diets. In contrast, Dongare et al. (2024) and El-Ganainy et al. (2024) observed significantly higher giblet weights in broilers fed coated calcium and nano-hydroxyapatite, respectively. Overall, the present study suggests that giblet yield in Japanese quails is not influenced by the calcium source or its particle size.

5. Liver yield

The mean liver yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The liver yield (per cent of live body weight) was not significantly (P≥0.05) influenced by either the particle size or the replacement level of stone grit at 52 week old Japanese quails. These results are consistent with the findings of Moghaddam et al. (2016) who fed different sizes of stone grits; Demirel et al. (2017) who fed different levels of marble powder and Eser et al. (2018) who fed granite grit in broilers and found no statistical difference in liver yield among the treatment groups. The current findings are further supported by previous studies conducted by Hakami et al. (2022) in broilers, Mendonca et al. (2022) in European quails and Rezende et al. (2024) in Japanese quails, all of which confirmed that the source of calcium did not influence the relative weight of the liver. Similarly, Pacheco *et al.* (2022) and Lima et al. (2024) observed that the particle size of limestone in layer diets did not significantly affect relative liver weights. The present results revealed that relative liver weight is independent of the particle size of stone grit and its inclusion level.

Table 2. Mean (±S.E.) carcass characteristics of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Carcass Characteristics	T1	T2	T3	T4	T5	F-Value	P-Value
Pre slaughter weight (g)	264.7±4.8	260.0±7.6	263.3±3.9	260.4±3.2	257.7±3.3	0.33 NS	0.855
Ready-to-cook body weight(g)	171.05±4.77	170.46±5.3	170.80±4.24	169.04±2.58	166.80±2.96	0.18 NS	0.945
Eviscerated carcass yield (%)	59.18±0.80	60.46±0.92	59.59±0.91	59.52±0.66	59.75±0.61	0.36 NS	0.837
Dressing percentage	64.53±0.88	65.61±0.95	64.79±0.90	64.93±0.68	64.70±0.60	0.27 NS	0.898
Liver yield (%)	2.68±0.17	2.43±0.15	2.54±0.12	2.65±0.21	2.39±0.15	0.56 NS	0.694
Gizzard yield (%)	1.85±0.09	1.90±0.14	1.85±0.05	1.87±0.07	1.82±0.09	0.09 NS	0.984
Heart yield (%)	0.810±0.04	0.815±0.05	0.810±0.04	0.900±0.04	0.753±0.03	1.75 NS	0.151
Giblet yield (%)	5.35±0.14	5.15±0.22	5.20±0.12	5.41±0.24	4.96±0.18	0.92 NS	0.456
Relative intestinal weight (%)	4.64±0.12	4.85±0.24	4.57±0.19	4.55±0.14	4.61±0.16	0.43 NS	0.795
Relative intestinal length (cm)	29.55±0.63	29.54±1.24	28.06±0.82	27.97±1.11	28.28±0.90	0.69NS	0.603

Each value is the mean of 12 observations. Values in each row having common superscript do not differ significantly * (P<0.05) ** (P<0.01) NS (P>0.05)

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

S.E – Standard error, mm- millimeter, cm- centimeter, g- grams

6. Heart yield

The mean heart yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The heart yield (per cent of live body weight) was not significantly influenced by either particle size or level of replacement of stone grit in 52 week old Japanese quails. These results are supported by Mendonca et al. (2022) who reported no significant difference ($P>0.05$) in absolute and relative heart weight when non sexed European quails were fed five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal) from day old to 35 days of age. Similarly, Hakami et al. (2022), Demirel et al. (2017), and Moghaddam et al. (2016), who also reported no significant effect of various calcium sources or grit types on heart weight in broilers. In contrast, Bonos et al. (2010) found that calcium propionate significantly reduced heart weight, while Dongare et al. (2024) reported increased heart weight in broilers with inclusion of coated calcium.

Since heart size is indirectly indicative of cardiac output capacity, birds with larger heart may better meet high metabolic demands. In this study, the absence of significant differences in heart weight suggests that variations in calcium source and particle size did not alter cardiac output capacity in Japanese quails

7. Gizzard yield

The mean gizzard yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The gizzard yield (per cent of live body weight) was not significantly ($P\geq0.05$) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quail. These results are consistent with studies by Pacheco et al. (2022), who found no significant effect of calcium particle size on gizzard yield in layers. Similarly, Rezvani et al. (2019), Hakami et al. (2022) reported no significant changes in gizzard weight with various calcium sources in broilers. Moreover, no significant differences in relative and absolute gizzard weights were found when European quails fed calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal (Mendonca et al., 2022) and when Japanese quails fed with 0.5 percent calcareous seaweed types 1 and 2 as partial replacements for limestone (Rezende et al., 2024). In contrast, Aroujo et al. (2011) reported that coarse limestone (1.00 mm) significantly ($P<0.01$) increased gizzard yield compared to fine limestone (0.60 mm) in layers.

Gizzard weight reflects muscle development and the thickness of the muscle tunic. Grit size affects gizzard stimulation, as harder and larger particles stay longer in the gizzard compared to softer or smaller particles, promoting greater muscular development (Gionfriddo and Best, 1999). Eser et al. (2018) reported that inclusion of insoluble grit in broiler diets increased relative gizzard weight and reduced abdominal fat, suggesting improved digestive function and leaner body composition. However, in the present study, feeding stone grit (2 mm or 4 mm) did not significantly increase gizzard weight, indicating that the soluble stone grit used was not as effective in grinding feed particles as insoluble grits, and therefore were insufficient to induce notable changes in gizzard development.

8. Relative intestinal weight

The mean relative intestinal weight of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 2. The relative intestine weight (per cent of live body weight) was not significantly ($P\geq0.05$) influenced by either the particle size or replacement level of stone grit in 52 week old Japanese quails.

The present findings align with Rezvani et al. (2019), who reported no significant differences in intestinal weight of broilers fed various calcium sources, including calcium carbonate, oyster shell, and eggshell powder. Similar results were observed in layers by Pacheco et al. (2022) and Lima et al. (2024) who found no effect of fine or coarse limestone particles on relative intestinal weight. In contrast, El-Ganainy et al. (2024) reported higher intestinal weight in broilers fed 100 per cent dicalcium phosphate, and Hakami et al. (2022) noted lower intestinal weights in broilers not supplemented with marine calcium source. Overall, the current study suggests that calcium source and grit particle size do not significantly affect intestinal weight in Japanese quails.

9. Relative intestinal length

The mean relative intestinal length of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 2. The relative intestine length (per cent of live body weight) was not significantly ($P\geq0.05$) influenced by both particle size and level of replacement of stone grit in 52 week old Japanese quails.

These findings are consistent with Aroujo et al. (2011) and Pacheco et al. (2022) who also found no significant impact of calcium level or particle size on intestinal length in layers. In contrast, Garipoglu et al. (2006) reported increased gut length in broilers fed granite grit, and El-Ganainy et al. (2024) found longer intestines in broilers fed dicalcium phosphate compared to nano-hydroxyapatite. Overall, the present study suggests that calcium source and grit particle size do not influence intestinal length in egg type Japanese quails.

Effect of particle size and replacement level of stone grit on bone characteristics of egg type Japanese quail

1. Tibia weight, length and diameter

The mean tibia weight, length and diameter of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 3. The tibia dry weight, length, and diameter were not significantly (P≥0.05) influenced by either the particle size or the level of stone grit replacement in 52 week old Japanese quails.

These results are consistent with studies by Codeiro et al. (2017), Lee et al. (2021) and Pacheco et al. (2022) who found no significant effect of calcium source or particle size on tibia parameters in laying hens. Conversely, De witt et al. (2009) found that large limestone particles (2-3.8 mm) in the diet of laying hens resulted in significantly decreased tibia length (P=0.0317) and tibia weight (P=0.0265) at 37 weeks of age when compared with small (0-1.0 mm) and medium (1.0-2.0 mm) limestone particles. Limestone particle size also affected the absolute femur and tibia weight at an earlier age (4 weeks) where pullets fed coarse limestone which had significantly heavier femur (P=0.011) and tibia (P=0.008) than pullets fed fine or mixture of fine and coarse limestone (Khanal et al., 2020). A similar trend was observed by Poudel et al. (2022) who found significantly higher tibia length in 15:85 FL: CL under conventional feeding and 0:100, 35:65 FL: CL groups under split feeding systems. Hakami et al. (2022) substituted limestone with marine calcium sources at different levels in broiler diets and found no significant differences in tibia and femur length or width, although tibia weight was significantly higher in marine calcium supplemented groups compared to the control. A comparable result was reported by Karim and Abdulla (2024) who replaced limestone with eggshell at 50 and 100 per cent levels in broiler diets, while tibial length was not significantly different among the groups, tibial weight was significantly higher (P=0.008) in the group fed 100 per cent eggshell compared to the other two groups. Overall, the results suggest that stone grit can effectively replace shell grit up to 100 per cent without negatively impacting tibia growth in egg type Japanese quails at 52 weeks of age.

2. Tibia ash

The mean tibia ash (per cent) on dry matter basis of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 3. The tibia ash (percent) was not significantly (P≥0.05) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quails. These findings align with studies by Bueno et al. (2016), Codeiro et al. (2017) and Lima et al. (2024) who reported no significant impact of calcium particle size or source on tibia ash in older hens and broiler breeders.

Contrary observations of higher tibia ash by feeding large sized calcium source were observed in broilers (Manangi and Coon, 2007), layers (De witt et al., 2009; Xavier et al., 2015; Mannangi et al., 2018) and layer chicks (Khanal et al., 2020). But, Pacheco et al. (2022) found that fine grained limestone resulted in better mineral matter in the tibia when 3.8 per cent of dietary calcium is provided. In contrast to the present findings, Hakami et al. (2022) and Dongare et al. (2024) found significantly higher tibia ash in broilers when limestone was partially replaced by marine calcium source or with coated calcium compared to the control group. Maranan et al. (2021) also found a linear increase in tibia ash content when limestone was replaced with eggshell powder (0, 50, 75, and 100 per cent) in broilers. These findings suggest that both limestone and eggshell can contribute to bone mineralization in varying proportions depending on inclusion levels. The results of this study inferred that stone grit can be included in the diet of egg type Japanese quails with replacement of shell grit either at 50 or 100 per cent level without compromising the bone development and mineralization at the age of 52 weeks.

3. Tibia calcium

The mean tibia calcium content (per cent) on dry matter basis of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 3. Tibia calcium percent

was not significantly (P≥0.05) influenced by either the particle size or the level of replacement of stone grit in 52 week old Japanese quails. The particle size, calcium source and replacement levels in this study did not significantly influence the tibia calcium per cent.

These results agree with Bueno et al. (2016), who found no effect of limestone particle size on tibia calcium in aged broiler breeders. Similarly, Moura et al. (2020) also reported no significant changes in tibial calcium when limestone was replaced with quail eggshell powder in Japanese quails. In contrast, Molnar et al. (2017) reported lower tibia calcium in hens fed 100 per cent coarse limestone under conventional feeding. Higher tibia calcium was also observed in broilers and layers fed coarse calcium sources (Bassi et al., 2022 and Pacheco et al., 2022). Maranan et al. (2021) noted a quadratic response in broilers, with tibia calcium increasing up to 50 per cent eggshell replacement and then declining. Similarly, Karim and Abdulla (2024) found significantly higher tibial calcium in birds fed 0 per cent eggshell, followed by those on 100 per cent eggshell diets. From this present study, it can be inferred that partial or full replacement of shell grit with stone grit did not affect tibia calcium levels in Japanese quails at 52 weeks, indicating that stone grit is a viable alternative calcium source for maintaining bone mineralization.

4. Tibia Phosphorous

The mean tibia phosphorous per cent of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit are presented in Table 3. Tibia phosphorous (percent) was not significantly (P≥0.05) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quails. The particle size, calcium source and replacement levels in the study did not significantly influence the tibia phosphorous per cent. These results align with studies by Bueno et al. (2016) and Wang et al. (2014) who found that calcium source and particle size did not significantly impact tibia phosphorus in broiler breeders and laying ducks, respectively.

However, Bassi et al. (2022) reported lower tibia phosphorus in broilers fed coarse oyster shell meal with a high calcium/ phosphorous ratio, while Jafari-Arvari et al. (2024) observed higher phosphorus in broilers fed coarse limestone. Similarly, Karim and Abdulla (2024) found significantly higher (P=0.001) tibial phosphorus content in birds fed diets with 0 per cent eggshell, followed by 100 per cent eggshell in broiler diets. Ajani et al. (2024) also noted significant variation in tibia phosphorus based on the inclusion of bone dust in layer diets. These findings suggest that different calcium sources can contribute to bone mineralization in varying proportions depending on their inclusion levels.

Phosphorus content in the tibia is a strong indicator of bone resorption status. The tibia serves as a responsive calcium reservoir for eggshell formation, particularly during the dark period when feed intake is minimal. Inadequate dietary calcium can create a competition between maintaining structural bone strength and supporting eggshell formation, leading to mobilization of calcium from both structural and medullary bone to the uterus (Clunies et al., 1992). During this process, phosphorus is also released due to breakdown of hydroxyapatite, potentially resulting in phosphatemia if bone resorption becomes excessive. The lack of variation in tibia phosphorus content among treatments in the present study clearly indicates that bone remodeling in response to calcium demands for eggshell production occurred efficiently, without compromising bone mineral status, regardless of calcium source, particle size, or inclusion level.

5. Tibial mineral density

The mean Seedor index and Robusticity index of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 3. The Seedor index and Robusticity index were not significantly (P≥0.05) influenced by calcium source, particle size and level of replacement of stone grit in 52 week old Japanese quails. These findings align with Rezende et al. (2024) and Moura et al. (2020) who also reported no significant changes in bone density indices when replacing limestone with calcareous algae or quail eggshell powder in the diet of broilers or Japanese quails. Similar non-significant effects of particle size on Seedor index were reported by Wang et al. (2014), Codeiro et al. (2017) and Pacheco et al. (2022).

However, Lee et al. (2021) found that hens fed eggshell fine particles and oyster shell had significantly higher tibia bone mineral density than those fed limestone, cockle shell, and eggshell coarse particles over a 7 week experimental period (P<0.001). Conversely, Eusebio-Balcazar et al.

(2018) found that feeding a blend of coarse and fine limestone (average particle size 0.875 mm) to Lohmann Brown and Bovans White pullets improved bone mineralization and bone mineral density (P=0.034) compared to feeding only fine limestone (0.431 mm). Additionally, other researchers have observed that feeding finer calcium sources led to significantly higher bone mineral density in layer chicks (Khanal et al., 2020) and layers (Lima et al*.,* 2024). The lack of variation in the bone density indices among the treatments in this study clearly inferred that remodeling of bones in response to the calcium demand for eggshell production takes place promptly without compromising the bone integrity irrespective of the calcium sources particle size and inclusion level.

6. Tibia bone breaking strength

The mean tibia bone breaking strength of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit are presented in Table 3. The calcium source, particle size and replacement levels of stone grit in this study did not significantly (P≥0.05) affect the tibia bone breaking strength of 52 week old Japanese quails. Similarly, inclusion of different sizes of limestone particles in the diet did not significantly affect (P>0.05) tibial breaking strength in 82 week old post moulting broiler breeders (Bueno et al*.,* 2016). Likewise, Lee et al. (2021) and Mendonca et al. 2022 found that tibia breaking strength did not differ among the dietary groups fed with different calcium sources in laying hens and European quails (P>0.05). Wang et al. (2014) fed laying ducks with limestone of two particle sizes (<0.1 mm; 0.85 to 2 mm), and Lima et al. (2024) who fed layers with limestone of two particle sizes (fine 0.222 mm and coarse with 3.332 mm) and found no significant changes in the tibia breaking strength.

In contrast, Leao et al. (2020) found higher bone tensile strength in the European quails fed diets containing 0.684 per cent calcium derived from charru mussel shell meal than from calcitic limestone, calcium carbonate, macunim shell meal and oyster shell meal. On deviation with the present results, Xavier et al. (2015) and Karim and Abdulla (2024) found that increasing levels of coarser limestone or eggshell resulted in a linear increasing effect on the bone breaking strength of the tibia of laying hens and broilers. Whereas, feeding finer calcium sources resulted in significantly greater bone breaking strength in layer chicks (Khanal et al., 2020) and layers (Poudel et al., 2022). The non variation in the bone breaking strength among the treatments in this study clearly inferred that remodeling of bones in response to calcium demand for eggshell formation takes place promptly without altering the morphometric characteristics of the bone irrespective of calcium sources, particle sizes and inclusion levels.

Effect of particle size and replacement level of stone grit on cost economics of egg type Japanese quail

The mean feed cost per kg egg mass or dozen egg, cost of production per kg egg mass or dozen egg was highest in T5 and lowest in T3 and intermediate in other treatment groups, however the differences were not statistically significant (P≥0.05). The benefit- cost ratio was highest in T3 and lowest in T5 and intermediate in other treatment groups, however the differences were not statistically significant (p≥0.05). The higher benefit- cost ratio in T3 may be due to better feed efficiency and livability over the other treatment groups. In a related study, Inoti (2020) conducted an experiment on laying hens using two types of limestone, one sourced from the Athi River near Nairobi (AR) and the other from Ukunda in the coastal region of Kenya (UKC). These sources differed in particle size distribution and *in-vitro* solubility. The study found that the feed cost was lower in birds that consumed UKC limestone compared to those fed AR limestone for producing one kg of egg mass, highlighting the role of calcium source. In contrast, Moura et al. (2020) substituted limestone with quail eggshell powder at 0, 25, 50, 75 and 100 per cent in Japanese quails and found better economic efficiency index (P>0.05) at 25 per cent and 50 per cent level as compared to other treatment groups. However, Islam and Nishibori (2021) reported lowest production cost in hens fed 8 per cent eggshell, followed by 8 per cent limestone, 8 per cent oyster shell, 4 per cent eggshell, 4 per cent limestone and 4 per cent oyster shell, highlighting the role of calcium source. The replacement of shell grit with 2 or 4 mm stone grit at 100 and 50 per cent levels in the diet of Japanese quails did not significantly (P≥0.05) improve the economical parameters such as feed cost per kg egg mass, production cost per kg egg mass, feed cost per dozen egg, production cost per dozen egg, production cost per egg and benefit-cost ratio when compared to control group. This may be due to the insignificant differences in feed efficiency and livability among all treatment groups throughout experimental period of 6 to 52 weeks of age.

Table 3.Mean (±S.E.) tibia bone characteristics of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit

Tibia bone characteristics	T1	T2	T3	T4	T5	F-Value	P-Value
Ready-to-cook body weight(g)	171.05±4.77	170.46±5.3	170.80±4.24	169.04±2.58	166.80±2.96	0.18 NS	0.945
Weight (dry) (g)	0.621± 0.02	10.697 ±0.034	0.612 ± 0.032	0.666 ± 0.049	0.662 ± 0.045	0.86 NS	0.495
Length (cm)	5.41 ± 0.11	5.50 ± 0.05	5.30 ± 0.14	5.39 ± 0.04	5.34 ± 0.07	0.79 NS	0.539
Diameter (cm)	0.283 ± 0.005	0.287 ± 0.006	0.282 ± 0.008	0.277 ± 0.003	0.279 ± 0.002	0.55 NS	0.702
Total ash (%)	56.51 ± 1.05	59.91 ± 0.82	59.22 ± 1.39	58.98 ± 1.19	60.47 ± 1.51	1.54 NS	0.221
Calcium (%)	21.21 ± 0.88	22.70 ± 0.51	22.08 ± 0.77	22.07 ± 0.56	21.72 ± 1.04	0.51 NS	0.728
Phosphorus (%)	10.62 ± 0.20	11.27 ± 0.12	10.96 ± 0.26	10.93 ± 0.22	11.29 ± 0.24	1.74 NS	0.172
Seedor index (g/cm)	11.53 ± 0.44	12.64 ± 0.57	11.58 ± 0.56	12.31 ± 0.80	12.35 ± 0.71	0.64 NS	0.634
Robusticity index	6.36 ± 0.13	6.24 ± 0.08	6.28 ± 0.17	6.23 ± 0.10	6.17± 0.09	0.33 NS	0.853
Breaking strength (N)	16.35 ± 0.68	16.69 ± 0.97	17.50 ± 0.83	18.63 ± 0.78	18.55 ± 0.80	1.62 NS	0.183

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.

*(P<0.05) ** (P<0.01) NS (P>0.05) S.E – Standard error, mm- millimeter, cm- centimeter, g/cm- gram per centimeter, N- Newton

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

Table 4. Mean (± S.E.) cost economic parameters of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Cost economic parameters	T1	T2	T3	T4	T5	F-Value	P-Value
Feed cost / kg egg mass (Rs.)	129.55 ± 2.77	133.21 ± 3.30	124.72 ± 4.68	130.36 ± 6.79	134.44 ± 4.36	0.68 NS	0.614
Cost of production / kg egg mass (Rs.)	139.24 ± 2.99	142.92 ± 3.67	133.97 ± 5.02	140.35 ± 7.59	144.52 ± 4.75	0.64 NS	0.636
Feed cost /dozen egg (Rs)	18.35 ± 0.38	18.56 ± 0.29	17.84 ± 0.74	18.57 ± 0.85	18.93 ± 0.59	0.44 NS	0.779
Cost of production / dozen egg (Rs.)	19.72 ± 0.41	19.92 ± 0.33	19.17 ± 0.80	20.00 ± 0.96	20.35 ± 0.64	0.425 NS	0.789
Cost of production per egg (Rs)	1.644 ± 0.03	1.660 ± 0.03	1.597 ± 0.07	1.667 ± 0.08	1.696 ± 0.05	0.425 NS	0.789
Benefit- cost ratio	1.455 ± 0.02	1.449 ± 0.04	1.538 ± 0.07	1.449 ± 0.07	1.406 ± 0.05	0.91NS	0.472

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.

*(P<0.05) ** (P<0.01) NS (P>0.05) S.E – Standard error, mm- millimeter, Rs-Indian Rupee

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

CONCLUSION

Replacing shell grit with stone grit of varying particle sizes and levels did not significantly affect carcass traits (live weight, dressing per cent, organ yields, intestinal measures) or tibia bone characteristics (weight, length, ash content, calcium per cent, phosphorous per cent and breaking strength). Serum calcium, phosphorous, alkaline phosphatase levels were also unaffected. The economic parameters such as feed cost per kg egg mass or dozen egg, cost of production per kg egg mass or dozen egg were not significantly influenced by the source, particle size of calcium in the diet. It can be concluded that the stone grit is efficient to supply adequate calcium for growth of bone and muscles as that of shell grit and hence the shell grit can be completely replaced with stone grit of size 4 mm in the feed of egg type Japanese quails without compromising the bone quality and carcass yield.

REFERENCES

Ajani, O. W., Oladele, O. A., Adedotun, W. G., Temitope O. O. and Opeolouwa, O. E. 2024. Performance, egg quality characteristics, serum parameters, blood minerals and bone mineralisation of laying chickens fed bone dust as calcium source. Bulgarian Journal of Animal Husbandry. 61(1): 19-30.

Anonymous. 2025. Experimental Agro-metreological Advisory Service. Veterinary College and Research Institute, Namakkal, Tamil Nadu.

AOAC. 2023. Official Methods of Analysis, Association of Official Analytical Chemists, 22nd Edn, Washington, D.C, USA.

Araujo, J. A. D., Silva, J. H. V. D., Costa, F. G. P., Sousa, J. M. B. D.,Givisiez, P. E. N. and Sakomura, N. K. 2011. Effect of the levels of calcium and particle size of limestone on laying hens. Revista Brasileira de Zootecnia, 40: 997-1005.

Bassi, L. S., Durau, J. F.,Zavelinski, V. A. B., Krabbe, E. L., Surek D. and Maiorka, A. 2022. Particle size of oyster shell meal and calcium: phosphorus ratios in broiler diets. Ciencia Rural Santa Maria, 52(10), e20210524.

Bonos, E. M., Christaki, E. V. and Florou-Paneri, P. C. 2010. Effect of dietary supplementation of mannan oligosaccharides and acidifier calcium propionate on the performance and carcass quality of Japanese quail (*Coturnix japonica*). International Journal of Poultry. Science. 9(3): 264-272.

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. and Bravo, C. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Animal Feed Science Technology. 110(1-4): 201-219.

Bueno, I. J. M., Surek, D., Rocha, C., Schramm, V. G., Muramatsu, K., Dahlke, F. and Maiorka, A. 2016. Effects of different limestone particle sizes in the diet of broiler breeders postmolting on their performance, egg quality, incubation results, and pre-starter performance of their progeny. Poultry Science. 95: 860-866.

Bugdayci, K. E., Oguz, M. N., Oguz, F. K.,Uyguralp, I. C. and Akinci, U. Y. 2020. Effects of Mediterranean cuttlefish (*Sepia officinalis*) bone as an alternative calcium source on egg production, egg quality and some blood parameters in laying quails. Fresenius Environmental Bulletein. 29(4): 2683-2690.

Cordeiro, C. N., Bastos-Leite, S. C., Vasconcelos, F. C., Goulart, C. C., Sousa, A. M. and Costa, A. C. 2017. Chelated minerals and limestone particle sizes on performance and bone quality of brown-egg layers. Brazilian Journal of Poultry Science. 19: 35-42.

Clunies, M., Parks, D. and Leeson, S. 1992. Calcium and Phosphorus Metabolism and Eggshell Thickness in Laying Hens Producing Thick or Thin Shells. Poultry Science. 71: 490-498.

De Witt, F. H., Kuleile, N. P., Van der Merwe, H. J. and Fair, M. D. 2009. Effect of limestone particle size on bone quality characteristics of hens at end-of-lay. South African Journal of Animal Science. 39(supplement1): 41-44.

Demirel, R., Baran, M. S., Bilal T. and Cevrim, U. 2007. Effects of different calcium levels on broiler performance and tibia bone parameters. Medcyna Wet. 63(4): 432-434.

Dongare, B. S., Kulkarni, R. C., Vasanthi, B., Awandkar, S. P., Gaikwad, N. Z.,Gadegaonkar, G. M. and More, A. 2024. Effect of coated calcium feeding on growth performance, carcass traits, immunity, blood biochemistry and tibial bone morphometry in commercial broiler chicken. Tropical Animal Health and Production. 56(8): 1-13.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics.. **11**(1): 1 - 42.

El-Ganainy, H. Y., Hermes, I. H., Hanafy, A. M., El-Shafei, M. and Saber, H. S.2024. Impacts of nano-hydroxyapatite replacement for dicalcium phosphate on growth performance, carcass traits and immune organs of broiler chicks. Egyptian Poultry Science Journal. 44(4): 377-391.

Ertas, O. N., Ciftci, M., Guler, T. and Dalkilic, B. 2006. The use of possibility of mussel shell supplementation as calcium source in Japanese quails raised under heat stress conditions: the effect of mussel shell on egg yield and some blood parameters. Sagilik Billimleri Dergisi. 20(1): 15-20.

Eser, H., Yalcin, S.,Onbasilar, I.,Burcak, E. and Yalcin, S. 2018. Effects of grit supplementation to diets containing maize and barley as cereal grains on performance and slaughter characteristics in broilers. Journal of the Faculty of Veterinary Medicine, Kafkas University. 25(5): 683-688.

Eusebio-Balcazar, P. E., Purdum, S., Hanford, K. and Beck, M. M. 2018. Limestone particle size fed to pullets influences subsequent bone integrity of hens. Poultry Science. 97(5): 1471-1483.

Garipoglu, A. V., Erener, G. and Ocak, N. 2006. Voluntary intake of insoluble granite-grit offered in free choice by broilers:its effect on their digestive tract traits and performances. Asian-Australian Journal of Animal Sciences. 19(4): 549-553.

Genchev, A. and Mihaylov, R. 2008. Slaughter analysis protocol in experiments using Japanese quails (*Coturnix Japonica*). Trakia Journal of Sciences. 6(4): 66-71.

Gionfriddo, J. P. and Best, L. B. 1999. Grit use by birds. In Current ornithology, Springer.

Gloux, A., Le Roy, N.,Ezagal, J., Meme, N.,Hennequet-Antier, C., PikettyM. L. and Duclos, M. J. 2020. Possible roles of parathyroid hormone, 1, 25 $(OH)_2$ D_3, and fibroblast growth factor 23 on genes controlling calcium metabolism across different tissues of the laying hen. Domestic Animal Endocrinology. 72; 106407.

Gongruttananun, N. 2011. Effects of using eggshell waste as a calcium source in the diet of Rhode Island Red roosters on semen quality, gonadal development, plasma calcium and bone status. Agriculture and Natural Resources. 45(3): 413-421.

Gultepe, E. E., Iqbal, A.,Uyarlar, C.,Cetingul, I. S.,Ozcinar, U. and Bayram, I. 2021. Effects of partial replacement of conventional limestone with dietary micro-calciumcarbonate on performance, egg quality, hematology, and calcium metabolism of laying hens during peak production. Turkish Journal of Veterinary and Animal Sciences. 45(5): 851-862.

Hakami, Z., Al Sulaiman, A. R., Alharthi, A. S., Casserly, R., Bouwhuis, M. A. and Abudabos, A. M. 2022. Growth performance, carcass and meat quality, bone strength, and immune response of broilers fed low-calcium diets supplemented with marine mineral complex and phytase. Poultry science. 101(6): 101849.

Hu, Y. X., Bikker, P.,Duijster, M., Hendriks, W. H., Van Baal, J. and Van Krimpen, M. M. 2020. Coarse limestone does not alleviate the negative effect of a low Ca/P ratio diet on characteristics of tibia strength and growth performance in broilers. Poultry science. 99(10): 4978-4989.

Idachaba, C. U., Abeke, F. O., Olugbemi, T. S. and Ademu, L. A. 2013. Effect of graded levels of stone grit on the utilization of rice offal based diets by broiler chickens. International Journal of Applied Research and Technology, 2(1): 125-131.

Inoti, D. K., Mbugua, P. N., Gachuiri, C. K. and Maina, J. G. 2020. Physical and chemical characteristics of limestone for use in layer feeds in Kenya. Indian Journal of Animal Nutrition. **37**(3): 242-246.

Islam, M. A., and Nishibori, M. 2021. Use of extruded eggshell as a calcium source substituting limestone or oyster shell in the diet of laying hens. Veterinary Medicine and Science. **7**: 1948-1958.

Jafari Arvari, A. R., Mirzaie Goudarzi, S., Abdollahi, M. R. and Sadeghi, M. 2024. A comparative study on the effect of limestone particle size on performance, ileal digestibility of calcium and phosphorus, and

bone characteristics in broilers and pullets. British Poultry Science. 65(1): 52-61.

Karim, K. K. and Abdulla, N. R. 2024. Effect of using different levels of egg shell as calcium sources in broiler diet on growth performance, blood parameters, and bone characteristics, Kufa Journal For Agricultural Sciences. 16(2): 41-54.

Khanal, T., Bedecarrats, G. Y., Widowski, T. M. and Kiarie, E. G. 2020. Rearing cage type and dietary limestone particle size: II, effects on egg production, eggshell, and bone quality in Lohmann selected Leghorn-Lite hens. Poultry science. 99(11): 5763-5770.

Leao, A. P. A., Lana, S. R. V., Lana, G. Q., De Barros Junior, R. F., Mendonca, D. D. S., Oliveira, T. J. D. 2020. Digestibility and bioavailability of organic calcium sources for European quail. Semina: Ciencias Agrarias, Londrina. 41(6): 3275-3284.

Lee, W. D., Kothari, D., Niu, K. M., Lim, J. M., Park, J. Ko, D. H. and Kim, S. K. 2021. Superiority of coarse eggshell as a calcium source over limestone, cockle shell, oyster shell, and fine eggshell in old laying hens. Scientific Reports. 11(1): 13225.

Lima, D. F., Bastos-Leite, S. C., Angelim, A. M., Evangelista, A. B., Cordeiro, C. N., Freitas, E. R. and Silveira, R. M. F. 2024. Calcium levels and limestone granulometries in the diet of light layers in their second production cycle. Brazilian Journal of Poultry Science. 26(3): 1-8.

Manangi, M. K. and Coon, C. N. 2007. The effect of calcium carbonate particle size and solubility on the utilization of phosphorus from phytase for broilers. International Journal of Poultry Science. 6: 85-90.

Manangi, M. K., Maharjan, P. and Coon, C. N. 2018. Calcium particle size effects on plasma, excreta, and urinary Ca and P changes in broiler breeder hens. Poultry Science. 97: 2798-2806.

Maranan, K. R. A., Bueno, C. M.,Adiova, C. B. and Recuenco, M. C. 2021. Effect of increasing levels of eggshell powder on the production performance, carcass characteristics, and bone properties of broiler chicken. Philippine Journal of Veterinary and Animal Sciences. 47(2): 1-15.

Mendonça, D. D. S., Lana, S. R. V., Lana, G. R. Q., Leao, A. P. A., Barros, R. F. D., Lima, L. A. D. A. and Silva, W. A. D. 2021. Different calcium sources on the productive performance and bone quality of meat quail. Ciencia Rural. 52(6). e 20210446.

Molnar, A., Maertens, L., Ampe, B., Buyse, J., Zoons, J. and Delezie, E. 2017. Supplementation of fine and coarse limestone in different ratios in a split feeding system: Effects on performance, egg quality, and bone strength in old laying hens. Poultry science. 96(6): 1659-1671.

Moghaddam, H. N., Rezaei, M. and Abadi, A. H. 2005. Effect of natural zeolite on performance, and tibia ash of broiler chicks. Proceedings of the 15th European Symposium on poultry nutrition, Balatonfured, Hungary, 25-29 September, pp. 200-202.

Moghaddam, H. N., Rajabiyan, A. A.,Ebrahimnezhad, Y. and Teli, A. A. S. 2016. The effects of different sizes of insoluble grit on growth performance and carcass traits in broiler chickens. Journal of BioScience and Biotechnology. 5(1): 87-91.

Mohammed, A. A., Zaki, R. S.,Negm, E. A., Mahmoud, M. A. and Cheng, H. W. 2021. Effects of dietary supplementation of a probiotic (*Bacillus subtilis*) on bone mass and meat quality of broiler chickens. Poultry Science. 100(3): 100906.

Moura, G. R. S., Reis, R. D. S., Mendonça, M. D. O., Salgado, H. R., Abreu, K. D. S., Madella, G. D. S. and Lima, M. B. D. 2020. Substitution of limestone for eggshell powder in the diet of Japanese laying quails. Rev. Bras. Prod. Anim. 21: 1-13.

Nisar, F. 2015. Effect of particle size of dietary limestone on egg production and egg shell quality in spent layers. M.Sc. (Hons.) Thesis, Department of Animal Nutrition, University of Agriculture, Faisalabad, Pakistan.

Novack, C., Boiago, M. M.,Zampar, A.,Barreta, M., Oliveira, R.,Roscamp, E. and Silva, A. S. 2023. Industrial egg residue as a calcium source in broiler feed: digestibility and growth performance. Anais da Academia Brasileira de Ciencias. 95(2), e20201688.

Pacheco, D. B., Bastos-Leite, S. C., Oliveira, J. V. A., Farias, M. R. S., Sena, T. L., Abreu, C. G.,

Freitas, E. R. and Cordeiro, C. N. 2022. Different calcium levels and two limestone granulometries in the diet of laying hens: Performance and bone characteristics. Brazilian Journal of Poultry Science. 24(2): 1-8.

Panda, A. K., Rama Rao, S. V., Raju, M. V. L. N. and Sharma, S. R. 2006. Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico - lipid profile of broiler chickens. The Journal of Poultry Science. 43(3): 235-240.

Poudel, I., McDaniel, C. D., Schilling, M. W.,Pflugrath, D. and Adhikari, P. A. 2022. Role of conventional and split feeding of various limestone particle size ratios on the performance and egg quality of Hy-Line® W-36 hens in the late production phase. Animal Feed Science and Technology. 283: 115153.

Premkumar, K., Kannan, D., Vasanthakumar, P., Rajendran, K., Ramakrishnan, S. and Serma Saravana Pandian, A. 2024. Assessment of In vitro Solubility of Stone Grit-a Major Calcium Source in Poultry Feed. Indian Journal of Animal Nutrition. 41(3): 480-489.

Rama Rao, S. V., Raju, M. V. L. N., Reddy,M. R., Pavani, P.,Shyam Sunder, G. and Sharma, R. P. 2003. Dietary calcium and non-phytin phosphorus interaction on growth, bone mineralization and mineral retention in broiler starter chicks. Asian-Australasian Journal of Animal Science. 16(5): 719-725.

Rezende, E. B., Valentim, J. K., Garcia, R. G., De Castro Burbarelli, M. F., Komiyama, C. M., Serpa, F. C. and Felix, G. A. 2024. Calcareous seaweed in the diet of growing Japanese quail. Animal Science Papers and Reports. 42(1): 65-80.

Rezvani, M. R., Moradi, A. and Izadi, M. 2019. Ileal digestibility and bone retention of calcium in diets containing eggshell, oyster shell or inorganic calcium carbonate in broiler chickens. Poultry Science. 7(1): 7-13.

Ribeiro, C. L. N., Barreto, S. L. T., Reis, R. S., Muniz, J. C. L., Viana, G. S., Ribeiro, V., and DeGroot, A. A. 2016. The effect of calcium and available phosphorus levels on performance, egg quality and bone characteristics of Japanese quails at end of the egg-production phase. Revista Brasileira de Ciencia Avícola, 18: 33-40.

Saki, A., Rahmani, A. and Yousefi, A. 2019. Calcium particle size and feeding time influence egg shell quality in laying hens. *Acta Scientiarum*, Animal Sciences. 41,e42926.

Seedor, J. G., Quarruccio, H. A. and Thompson, D. D. 1991. The bisphosphonate alendronate inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research. 6: 339 - 346.

Sinclair-Black, 2019. The effect of limestone particle size, phytase inclusion, and time post oviposition on ionized blood calcium levels in commercial laying hens. M.Sc (Agri) Thesis, Faculty of Natural and Agricultural Sciences Department of Animal and Wildlife Sciences University of Pretoria, South Africa.

Souvik-Mondal, S. M., Lalzarzova, V. and Gautam Samanta, G. S. 2011. Feeding of different calcium sources on the carcass characteristics, plasma and bone major mineral status of Japanese quail. Indian Journal of Animal Health. 50(2): 31-37.

Usoro, O. O. and Christopher, G. I. 2022. Efficacy of grit and enzyme supplementation in brewers'spent grains-based diet for broilers. International Journal of Agriculture and Rural Development. 25(1): 6216-6224.

Wang, S., Chen, W., Zhang, H. X., Ruan, D. and Lin, Y. C. 2014. Influence of particle size and calcium source on production performance, egg quality, and bone parameters in laying ducks. Poultry Science. 93(10): 2560-2566.

Xavier, R. P. D. S., Freitas, E. R., Braz, N. M., Farias, N. N. P., Lima, R. C., Watanabe, P. H. and Peixoto, M. S. M. 2015. Limestone particle sizes and lighting regimens on egg and bone quality of laying hens. Pesquisa Agropecuaria Brasileira. 50(8): 718-725.

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Effect of Supplementation of Pomegranate (*Punica Granatum*) Peel Powder and Multienzyme on Performance of Broiler Chickens

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ABSTRACT

The goal of the current study was to determine the effect of supplementation of pomegranate (*Punica granatum*) peel powder (PPP) and multienzyme to broiler chickens' diets on growth performance, carcass characteristics, nutrient digestibility, nitrogen balance and haemato-biochemical parameters. One hundred sixty, day-old Ven cobb 430 broiler chicks were randomly assigned to four treatment groups (T1 to T4), each consisting of four replicates of ten chicks. The feeding trial was carried out using a broiler starter (1–21 days) and finisher (22–35 days) ration. Birds were offered basal feed as per the BIS (2007). The control group (T1) received only a non-supplemented basal diet, while groups T2, T3 and T_4 received 0.5% PPP, 0.05% multienzyme and 0.5% PPP + 0.05% multi-enzyme in their basal feed, respectively. After the feeding study, one bird per replicate from each treatment was randomly selected for a 5-day metabolism trial to assess nitrogen balance and Metabolizable nutrients. At the end of the experiment, blood samples were collected for blood and serum analysis, and one bird per replicate was sacrificed to evaluate carcass traits and meat quality parameters. The supplementation of PPP and multi-enzyme alone and in combination significantly ($P<0.01$) improved body weight, body weight gain, feed conversion ratio, performance index, protein efficiency ratio, dry matter, ether extract and crude protein metabolisability and nitrogen balance. Significant ($P<0.05$) improvement was observed on feed consumption. However, dietary inclusion did not affect dressed weight, eviscerated weight, weight of heart, liver, gizzard, giblet, intestine length of broiler birds. Therefore, on the basis of results obtained, the present study revealed that the inclusion of PPP @ 0.5% and multienzyme @ 0.05% enhanced the overall performance of broilers.

KEYWORDS: Broilers, Carcass, Growth Performance, Multienzyme, Pomegranate peel powder.

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INTRODUCTION

Poultry is one of the fastest growing sectors globally with 35 per cent of the global meat production in 2020, chicken meat showed the largest growth in absolute and relative terms since 2000 (104 per cent or 61 million tonnes) and was the most produced type of meat in 2020 (FAO, 2022). Poultry is one of the agricultural sectors in India that is expanding the fastest. India ranked third in the world for producing eggs, sixth for producing chicken meat and fifth for producing poultry (FAO, 2022). The growth of poultry meat production has increased by 6.86% over previous year i.e. 2021 (Basic Animal Husbandry Statistics, DAHD 2022).

Chicken meat has emerged as one of the cheapest sources of excellent quality protein. Because of worries about antibiotic residues in tissue and the development of bacterial resistance, the use of antibiotics as growth promoters in chicken has been outlawed. These concerns prompted a recent examination into various feed additives as potential replacements for feed antibiotics (Ramteke et al., 2022). Feed price constitutes around 80 per cent of the total production cost (National Action Plan for Egg and Poultry, 2022).

In recent years, broiler production has shown a growing interest in using phytobiotics as natural growth-promoting feed additives (Ramteke et al., 2022). Pomegranate Peel (PP) is a by-product that is

rich in many high molecular weight polyphenol, such as flavonoids (flavanols as catechin, epicatechin and gallocatechin as well as anthocyanins), phenolic acids (gallic, ellagic, caffeic, citric, tartaric, malic and ascorbic), hydrolyzable tannins (ellagitannins, punicalin, punicalagin, pedunculagin and gallotannins) and condensed tannins (Kandylis and Kokkinomagoulos, 2020). Besides, pomegranate and its by-product showed antimicrobial, anticarcinogenic, antioxidant, anti-inflammatory and growth promoting effects (Reddy et al., 2007; Ghasemi-Sadabadi et al., 2021 and Elnaggar et al., 2022). Poultry growth performance metrics are significantly impacted by peel powder, pomegranate powder (PPP), pomegranate peel extract (PPE) and pomegranate peels pomace.

Enzymes are mineral, vitamin and amino acid-based biological catalysts. They do not alter, but they do cause biochemical reactions. Non-Starch Polysaccharides (NSPs) such as cellulose, lignin, arabinoxylan (wheat and oat) and phytase are present in the majority of feed ingredients used in poultry diets at varying amounts (Parsippany, 2008). Supplementation of broiler feeds with multienzyme preparations resulted in improved body weight gain (BWG) and feed efficiency (Daskiran et al., 2004). Enzyme supplementation in feed plays a crucial role in enhancing nutrient availability and mitigating the negative effects of anti-nutritional factors present in feed ingredients. Therefore, the present study was conducted on broiler chickens to observe the effect of supplementation of PPP and multienzyme on growth performance, nutrient digestibility, nitrogen balance, hemato-biochemical parameters and economics of broiler chickens production

MATERIALS AND METHODS

Experimental birds and diet

A total of 160 day-old, unsexed, healthy Ven Cobb-430Y broiler chicks were procured and vaccinated against Ranikhet disease (day 7) and IBD (day 14). Chicks were individually weighed and randomly allotted to four treatment groups (40 chicks each), with four replicates of 10 chicks per group. ISO-certified basal starter and finisher feeds were procured as per BIS (2007) guidelines. Feed supplements (PPP and a commercial multienzyme from Adelbert Vegyszerek Pvt. Ltd., Kanpur) were procured locally and incorporated into the basal diets. Four different treatment diets were prepared for the feeding of broilers under different dietary groups. The treatment groups were as follows: T1 (control) received the basal diet; T2 received the basal diet supplemented with 0.5% PPP; T3 received the basal diet supplemented with 0.05% multienzyme; and T4 received the basal diet supplemented with both 0.5% PPP and 0.05% multienzyme. The proximate nutrient composition of basal feed and PPP has been presented in Table 1.

Feeding trial

A 35-day feeding trial was conducted from October 20 to November 23, 2024, during which broiler starter diets were fed up to 21 days and finisher diets up to 35 days as per BIS (2007) and treatment protocols. Feed and water were provided ad libitum, and daily group-wise feed intake was recorded. Following the feeding trial, a 5-day metabolism trial was conducted using one chick per replicate from each treatment to assess nutrient digestibility and nitrogen balance. One broiler per replicate was sacrificed at the end of the experiment for carcass evaluation.

Table 1. Proximate nutrient composition (%DM basis) of basal feeds and pomegranate peel powder.

Proximate Nutrient(Per cent)	Broiler starter	Broiler finisher	Pomegranate (*Punica granatum*) peel powder
Dry Matter	90.58	90.62	91.60
Crude protein	22.48	20.25	7.61
Ether extract	3.49	3.90	1.28
Crude Fiber	4.73	4.26	19.39
Total ash	4.46	4.37	5.46
Nitrogen Free extract	64.84	67.22	66.26
ME (kcal/kg)	3055	3091	

Table 2. Ingredient composition of the diet

Ingredient	Starter	Finisher
Maize	58.0	62.2
Soybean meal	34.2	30.0
Oil	3.0	3.0
Stone grit	1.60	1.85
DCP	1.90	1.65
Salt	0.40	0.40
DL- Methionine	0.19	0.16
Lysine	0.14	0.12
Vitamin and mineral premix	0.5	0.5

Table 3. Composition of Multienzyme

Sr. No.	Enzyme	International Unit per Kg
1.	Amylase	33,42,000 IU
2.	Protease	12,25,000 HTU
3.	Cellulase	6,85,000 IU
4.	Beta-Glucanase	4,36,000 IU
5.	Xylanase	9,75,000 IU
6.	Pectinase	2,69,000 IU
7.	Phytase	1,40,000 FTU

Experimental starter feed, finisher feed and PPP were analyzed for proximate principles as per standard procedures (AOAC, 2005).

Performance Parameters

Daily feed intake and body weight were recorded, and weekly body weight gain was calculated. Feed conversion ratio, performance index, and protein efficiency ratio were also computed. After the feeding trial, a 5-day metabolism trial was conducted using one bird per replicate per treatment to evaluate dry matter digestibility and nitrogen retention. Nutrient metabolizability was assessed using the total collection method, with daily excreta collection. Feed and dried excreta samples were analyzed for proximate principles (AOAC, 2005), and nitrogen content was determined by the Kjeldahl method using an automatic nitrogen analyzer.

Carcass parameters

Broilers were humanely sacrificed following standard procedures (Panda, 1995), processed, and eviscerated for carcass evaluation, with individual weights of giblets recorded. Breast meat pH (Trout et al., 1992), water-holding capacity, and drip loss were measured using standard methods. Haematological parameters (haemoglobin, RBC, WBC, and PCV) were determined by conventional techniques, while serum biochemical parameters were analyzed using an Automatic Biochem Analyzer of Schiapparelli biosystems, INC, using standard kits.

Statistical analysis

The experimental data were subjected to statistical analysis (SPSS Ver. 24.0) using one way analysis of variance as described by Snedecor and Cochran (2004) to test for significant variation between treatment groups. Probabilities values of less than 0.05 ($P<0.05$) were considered significant. Comparison of mean values was carried out by Duncan's Multiple Range Test (Duncan, 1955). The results were interpreted and expressed as means ± pooled SEM.

RESULTS AND DISCUSSIONS

Growth performance

Cumulative feed consumption was significantly ($P < 0.05$) higher in the control group than in the treatment groups. These findings align with earlier reports showing significant effects of PPP supplementation on broiler feed intake (Hafeez et al., 2023; Ghosh et al., 2020) and increased feed intake with multienzyme supplementation (Attia et al., 2020).

Overall body weight gain was significantly

higher (P < 0.01) in broilers fed diets supplemented with PPP and multienzyme. These findings are consistent with earlier studies reporting improved body weight gain with PPP supplementation (Baqer and Ibrahim, 2022; Kamel et al., 2021), herbal additives combined with multienzymes (Singh et al., 2024; Tanwar et al., 2021), multienzyme alone (Attia et al., 2020), and herb–multienzyme combinations (Gaur, 2022).

The overall mean feed conversion ratio was found to be significant (P<0.05) on supplementation of PPP and multienzyme. The results of the present study are in agreement with Ahmed and Yang (2017) who observed improved (P<0.05) overall FCR of broiler supplemented with *Punica granatum* L. byproduct at level of 0.5% and 1% with basal feed. Similar findings were observed by Baqer and Ibrahim (2022) with 50 ml/literaquous extract of PPP.

Table 4. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on performance of broiler chickens

Attributes	Treatments				P value
	T1	T2	T3	T4	
Feed consumption (g/bird) (0-5 wk)	3017.08b±53.89	2772.00a±92.80	2837.00ab±37.97	2744.00a±81.94	<0.05
Body weight gain (g/b)	1550.62a±29.26	1632.30b±6.30	1762.30c±25.102	1715.86c±21.09	<0.01
Feed conversion ratio	1.95b±0.05	1.70a±0.06	1.61a ±0.027	1.60a±0.06	<0.05
Performance index	798.71a±35.28	964.57b±34.02	1095.41b±20.32	1077.64a±51.11	<0.01
Protein efficiency ratio	2.43a±0.06	2.72b±0.11	2.93b±0.02	2.90b±0.08	<0.01
Metabolizability of DM (%)	80.85a±0.22	82.46b±0.13	83.65c±0.05	83.45c±0.08	<0.01
Metabolizability of EE (%)	86.50a±0.41	86.91a±0.18	89.16b±0.20	88.72b±0.16	<0.01
Nitrogen retention (g/bird)	2.21±0.12	2.66±0.10	2.62±0.08	2.69±0.03	0.99

a, b, c- Means superscripted with different letters within a column differ significantly from each other.

** P<0.01, *P<0.05, NS= Non-significant

Table 5: Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on offals weight (g) of broilers in different treatment groups

Attributes	Treatments				P value
	T1	T2	T3	T4	
Heart (g)	9.94±0.24	10.01±0.46	9.84±0.21	10.38±0.39	0.081
Liver (g)	36.24±0.58	37.35±1.31	35.57±1.01	34.84±0.06	0.075
Gizzard (g)	27.84±0.60	26.44±1.32	26.84±1.43	24.01±1.18	0.655
Giblet (g)	74.53±1.08	74.30±1.81	73.50±1.03	70.18±1.22	0.823
Drumstick (g)	162.75±4.785	166.50±4.941	179.25±3.816	171.00±7.188	0.688
Spleen (g)	1.80±0.039	1.84±0.090	1.75±0.043	1.81±0.183	0.105
Intestinal length (cm)	168.05±3.434	164.91±3.279	165.38±1.083	166.58±3.359	0.531

Table 6. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on drumstick weight (g), spleen weight (g) and intestine length (cm)

Attributes	Treatments				P value
	T1	T2	T3	T4	
pH	6.08±0.04	6.10±0.07	6.05±0.06	6.05±0.09	0.656
Water holding capacity (%)	32.51±0.28	36.14±2.20	36.42±1.05	32.49±0.75	0.068
Drip loss (breast) %	9.48b±0.22	8.00b±0.32	7.27a±0.78	8.20b±0.27	<0.05

Mean with different superscript differ significantly within a column

Significant (P<0.01) improved effect of supplementation of PPP and multienzyme was found on cumulative performance index in broiler chickens. The improved performance index observed with multienzyme supplementation agrees with Nizamuddin et al. (2013). Similar significant enhancements in performance index with combined herb and multienzyme supplementation have been reported by Sharma (2022), Gaur (2022), Chaudhary (2022) and Singh (2022).

Statistical analysis of the data revealed a highly significant effect (P<0.01) on the cumulative protein efficiency ratio, which was significantly higher in the treatment groups compared to the control group. Several studies have reported significant improvements in protein efficiency ratio (PER) with combined supplementation of herbs and multienzymes in broilers (Chaudhary, 2022; Singh, 2022; Sharma, 2022; Gaur, 2022). However, Gosai et al. (2023) reported a non-significant effect on overall PER with varying levels of PPP supplementation.

A highly significant (P<0.01) effect of PPP and multienzyme supplementation was observed on the metabolizability of dry matter, crude protein, and ether extract. Nitrogen balance was significantly higher (P < 0.01) in all treatment groups compared to the control. These findings are consistent with earlier reports showing improved nutrient digestibility and nitrogen utilization with herb and multienzyme supplementation (Chaudhary, 2022; Gaur, 2022; Sharma, 2022; Rezvani et al., 2018) and with multienzyme supplementation alone (Attia et al., 2020).

Supplementation of PPP and multienzyme showed no significant effect on dressed weight, eviscerated weight, organ weights, giblet weight, drumstick and spleen weight, or intestinal length of broilers, while a significant difference (P < 0.05) was observed in meat drip loss. The lowest drip loss was recorded in the T3 group. Similar observations have been reported by El-Rayes et al. (2023) and Singh (2022).

The statistical analysis of data showed no significant effect of supplementation of PPP and multienzymeon Hb (g/dl), RBC ($\times 10^6$/µl), WBC ($\times 10^3$/µl) and PCV (%).The results of the study are supported by Gosai et al. (2023) who observe non-significant (P>0.05) difference in Hb, RBC and WBC with supplementation of 0.25%, 0.5% and 1% levels of PPP on broilers. Contrary to this, Elnaggar et al. (2022) showed that Hb, RBC, WBC and PCV were significantly increased (P=0.001) with 0.25%, 0.5%, 1% and 1.5% levels of PPP supplementation on broilers. Similarly, Kamel et al. (2021) observed significant (P<0.01) results with supplementation of 0, 3, 6 and 9% level of PPP on Japanese quail.Regarding multienzyme, the results are in agreement with Mohammadigheisar et al. (2018) who recorded non-significant (P>0.05) effects on blood cells of chicken fed diet supplemented with 0.05% and 0.10% multienzyme.

All serum biochemical parameters remained within normal physiological ranges, and PPP and multienzyme supplementation showed no significant differences (P > 0.05) among treatment groups. Similar findings were reported by El-Rayes et al. (2023) for AST and ALT activities, and by Nizamuddin et al. (2013), Kumar et al. (2013), and Omojola et al. (2014) for enzyme supplementation, although El-Rayes et al. (2023) observed increased plasma protein fractions with higher PPP levels.

Table 7. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on haematological parameters of broilers in different treatment groups

Group	T1	T2	T3	T4
Hb (g/dl)	19.25±0.323	2.48±0.048	25.19±0.190	32.65±0.132
RBC (106/µl)	9.38±0.315	2.58±0.165	25.52±0.278	32.10±0.599
WBC (103/µl)	9.12±0.125	2.50±0.173	25.28±0.149	32.35±0.622
PCV (%)	9.75±0.144	2.62±0.131	25.15±0.155	32.30±0.178

Table 8. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on serum parameters at 35th day of age of experimental broilers in different treatment group

Group	T1	T2	T3	T4
Glucose (mg/dl)	215.49±0.41	215.95±0.33	215.96±0.63	216.26±0.99
Creatinine (mg/dl)	0.22±0.03	0.22±0.01	0.20±0.03	0.18±0.00
Albumin (g/dl)	1.43±0.13	1.20±0.05	1.22±0.05	1.44±0.44
Globulin (g/dl)	1.99±0.05	2.07±0.09	2.18±0.10	1.92±0.16
Total protein (g/dl)	3.42±0.07	3.27±0.09	3.39±0.05	3.36±0.09
Urea (mg/dl)	4.52±0.22	4.60±0.29	5.15±0.26	4.18±0.55S
GOT/AST (U/L)	253.02±1.61	255.92±0.89	253.00±1.43	255.40±1.02
SGPT/ALT (U/L)	17.88±0.14	17.98±0.26	17.15±0.45	17.62±0.33
Calcium (mg/dl)	8.73±0.90	9.52±0.37	9.45±0.23	9.00±0.14

CONCLUSION

From the finding of the present study, it can be concluded that supplementation with pomegranate (*Punica granatum*) peel powder @ 0.5% and multienzyme @ 0.05% alone and in combination causes improvement in live body weight, weight gain, feed conversion ratio and performance index as compared to that of control group of broilers. Thus, pomegranate (*Punica granatum*) peel powder and multienzyme in the rations of broiler chicks may be useful for the safe, economical and efficient production of broiler and this formulation of ration could be used as an alternative to commercial growth promoters.

REFERENCES

Ahmed, S.T. and Yang, C.J. 2017. Effects of dietary *Punica granatum L*. by-products on performance, immunity, intestinal and faecal microbiology and odorous gas emissions from excreta in broilers. The Journal of Poultry Science. 54(2):157-166.

AOAC. 2005. Official methods of analysis of AOAC International, 18th Edn Gaithersburg, MD: AOAC International; 2005.

Attia, Y.A., Al-Harthi, M.A. and El-Shafey, A.S. 2020. Influence of Different Time and Frequency of Multienzyme Application on the Efficiency of Broiler Chicken Rearing and Some Selected Metabolic Indicators. Italian Journal of Animals Science. 10(3):450.

Baqer, H.Q. and Ibrahim, B.M. 2022. Effect of adding pomegranate (*Punica granatum*) and consumption and production index of broiler chickens. Journal of Kerbala for Agricultural Sciences. 9(3):1-16.

BIS. 2007. Nutrient Requirements of Poultry. Bureau of Indian Standards, New Delhi, India.

Chaudhary, G. 2022. Effect of supplementation of neem (*Azadirachta indica*) leaves powder and multienzyme on performance of broiler chickens. M.V.Sc. Thesis Rajasthan University Veterinary and Animal Sciences, Bikaner, Rajasthan.

Daskiran, M., Teeter, R.G., Fodge, D. and Hsiao, H.Y. 2004. An evaluation of endo-β-D-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in β-mannan content. Poultry Science. 83(4):662-668.

Duncan., D. B. 1955. Multiple range and multiple F tests. Biometrics. 11(1):1-42.

Elnaggar, A.S., Elsebai, A., Eltahawy, W. and Elattar, F. 2022. Effect of dietary inclusion of pomergranate peel powder (*Punica granatum*) on Growth performance and some physiological parameters of broiler chicks. Journal of Agricultural and Environmental Sciences. 21(2):19-44.

El-Rayes, T.K., Badawy, G.E. and El-Morsy, W.A. 2023. Effect of pomegranate peel powder supplementation on growth performance, carcass characteristics and blood metabolites of Japanese quails. Journal of Sustainable Agricultural and Environmental Sciences. 2(3):52-58.

FAO 2022. World Food and Agriculture – Statistical Yearbook 2022. Rome.

Gaur, K. 2022. Effect of Dietary supplementation of amla fruit powder (*Emblica officinalis*) and multienzyme on performance of broiler

chickens. Indian Journal of Animal Nutrition. 40 (2): 181-188.

Ghasemi-Sadabadi, M., Ebrahimnezhad, Y., Maheri-Sis, N., ShaddelTeli, A., GhiasiGhalehkandi, J. and Veldkamp, T. 2021. Supplementation of pomegranate processing waste and waste soybean cooking oil as an alternative feed resource with vitamin E in broiler nutrition: effects on productive performance, meat quality and meat fatty acid composition. Archives of Animal Nutrition. 75(5):355-375.

Ghosh, S., Chatterjee, P.N., Maity, A., Mukherjee, J., Batabyal, S. and Chatterjee, J.K. 2020. Effect of supplementing pomegranate peel infusion on body growth, feed efficiency, biochemical metabolites and antioxidant status of broiler chicken. Tropical Animal Health and Production. 52:3899-3905.

Gosai, A.S., Pawar, M.M., Patil, S.S., Ankuya, K.J., Srivastava, A.K. and Ashwar, B.K. 2023. Effect of pomegranate (*Punica granatum*) peel powder supplementation on performance, carcass characteristics and haemato-biochemical parameters of broiler chickens. The Indian Journal of Animal Sciences. 93(5):481-486.

Hafeez, A., Piral, Q., Naz, S., Almutairi, M.H., Alrefaei, A.F., Ayasan, T., Khan, R.U. and Losacco, C. 2023. Ameliorative effect of pomegranate peel powder on the growth indices, oocysts shedding and intestinal health of broilers under an experimentally induced coccidiosis condition. Animals. 13(24): 3790-3799.

Kamel, E.R., Shafik, B.M., Mamdouh, M., Elrafaay, S. and Abdelfattah, F.A.I. 2021. Response of two strains of growing Japanese quail (*Coturnix Coturnix japonica*) to diet containing pomegranate peel powder. Tropical Animal Health and Production. 53:1-11.

Kandylis, P. and Kokkinomagoulos, E. 2020. Food applications and potential health benefits of pomegranate and its derivatives. Foods. 9(2):122-142.

Kumar, M., Sharma, R.K., Chaudhari, M. and Jakhar, A. 2013. Effect of Indian gooseberry and multi-enzyme supplementation on the performance of broilers during hot weather. Haryana Vet. 52:66-68.

Mohammadigheisar, M., Kim, H.S. and Kim, I.H. 2018. Effect of inclusion of lysolecithin or multi-enzyme in low energy diet of broiler chickens. Journal of Applied Animal Research. 46(1):1198-1201.

Omojola, A. B., Otunla, T. A., Olusola, O. O., Adebiyi, O. A. and Ologhobo, A. D. 2014. Performance and carcass characteristics of broiler chicken fed soybean and sesame/soybean-based diets supplemented with or without microbial phytase. American Journal of Experimental Agriculture. 4(12): 1637-1648.

Panda P.C. 1995. Slaughtering techniques. In "Textbook on egg and poultry technology". Vikas Publications, New Delhi, India.

Parsippany, N.J. 2008. Non-starch polysaccharide enzymes for poultry. Proceedings of the 6th Mid Atlantic Nutrition conference, University of Maryland.

Ramteke, R., Gendley, M.K., Dubey, M., Dutta, G.K. and Jain, A. 2022. Effect of turmeric and coriander powder as phytobiotic feed supplement in poultry production. Just Agriculture. 2(8):1-13.

Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I. and Ferreira, D. 2007. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum L*. Planta Medica. 53(5):461-467.

Rezvani, M.R., Sayadpour, N. and Saemi, F. 2018. The effect of adding pomegranate seed extract to fat containing diet on nutrient digestibility, intestinal microflora and growth performance of broilers. Iranian Journal of Veterinary Research. 9(4):310-317.

Singh, D. 2022. Effect of garlic powder (*Allium sativum*) and multienzyme supplementation on performance of broiler chickens. M.V.Sc. Thesis Rajasthan University Veterinary and Animal Sciences, Bikaner, Rajasthan.

Sharma, J., Karnani, M., Choudhary, S. and Manju. 2022. Effect of supplementation of rosemary (*Rosemarinus officinalis*) leaves and multienzyme on growth performance and nutrient utilization of broiler chicken. Indian Journal of Animal Nutrition. 39:73-80.

Singh, V., Manju, Karnani, M. and Choudhary, S. 2004. Effect of supplementation of *Coriandrum sativum* seed powder and multienzyme on performance of broiler chickens. Indian Journal of Animal Nutrition. 41 (3): 490-497.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. Publ. Oxford and IBH publishing Co. New Delhi, 4:445-446.

Tanwar, R., Sharma, V.,Karnani, M., Choudhary, S., and Manju. 2021. Effects of supplementation of Aloe vera (*Aloe barbadensis*) and Tulsi (*Ocimum sanctum*) as feed additives on performance of broiler chickens. Indian Journal of Animal Nutrition. 38 (3): 304-309.

Trout E.S., Hunt, MC., Jhonson, D.E., Clans, J.R., Castner, C.L. and Kropf, D.H. 1992. Characteristics of low fat ground beef, containing texture modifying ingredients. Journal of Food science. 57(1): 19-24.

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Effect of Feeding Neem and Papaya Leaf Meal as Feed Additive on Performance, Immune response, Antioxidant status and Serum biochemical parameters in Broilers

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ABSTRACT

The present study was conducted to evaluate the impact on performance, immune response, antioxidant level, and serum biochemical components of broiler production by supplementing Neem Leaf Meal and Papaya Leaf Meal, individually as well as combined. 220 day-old VenCobb-400 commercial broiler chicks were divided randomly into 11 treatment groups with four replicates per treatment, keeping five chicks per replicate. In treatment groups, besides basal diet, neem leaf meal, as well as Papaya leaf meal, at two different levels, individually (0.5 and 1.0 % diet) and three different combinations (1:1, 1:2, and 2:1 part NLM:PLM @ 1.0 and 2.0 % diet), were supplemented. Body weight, weight gain, feed consumption, and feed conversion ratios (FCR), as well as performance index (PI), were recorded weekly up to 42 days. Humoral immunity against Newcastle disease virus was evaluated on 30th and 42nd day post-feeding trial through hemagglutination inhibition (HI) test. At the end of trial, on 42nd day, two birds per replicate were sacrificed to estimate antioxidant property in liver and breast muscles. Biochemical estimations were done on serum samples at completion stage. The results revealed that 1:1 ratio of neem, as well as Papaya leaf meal (at 1.0 %), and 1:2 ratio (at 2.0 % diet level) of NLM:PLM supplement, individually, possessed a significant (P<.05) impact on their performance. Their humoral immunity, as well as antioxidant levels, were found to be significantly (P<.05) high. Biochemically, serum levels of total triglycerides, total, LDL-cholesterol, and HDL-cholesterol were found to reduce and increase, significantly (P<.05), due to supplementation.

KEYWORDS: Antioxidant status, Broilers, Immune response, Neem Leaf Meal, Papaya Leaf Meal, Serum biochemical.

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INTRODUCTION

The ever-growing rate of chicken production has traditionally utilized antibiotic growth promoters. In this manner, the overuse of antibiotics has been associated with antimicrobial resistance, antibiotics within chicken products and a rising level of public health concerns. This has led to restrictions on antibiotics by the government, necessitating to find a suitable alternative that is safe and reliable (Soren et al., 2025). In this respect, phytogenic feed supplements are identified as capable alternative to antibiotics in poultry nutrition. They are obtained from natural herbs and leaves; contain bioactive compounds such as phenolic compounds, flavonoids, saponins and tannins, which exhibit antimicrobial, antioxidant and immunomodulatory activities in poultry diets (Obianwuna et al., 2024). Some recent findings have revealed that phytogenic supplements significantly enhance growth performance by modulating the intestinal microbial population, stimulating the activities of enzymes and alleviating oxidative stress in the body, consequently increasing nutritional utilization and immune abilities without selective antimicrobial resistance (Singh et al., 2019 and Mounika et al., 2025). Likewise, poly-herbal feed additives have shown a great potential as natural alternations to synthetic growth promoters in broilers (Rao, 2021).

Neem leaves (*Azadirachta indica*) contain biological active compounds like azadirachtin, nimbin, and salannin, which possess anti-bacterial, anti-viral, anti-fungal, hepatoprotective, and

immunostimulatory actions that ultimately improve the health of the intestinal tracts and immune and efficacy of feeds in poultry (Islam et al., 2025). Papaya leaves (*Carica papaya*) are endowed with proteolytic enzymes like papain and chymopapain, which increase the digestion of proteins and make amino acids more available, while the phenolic constituents of papaya leaves possess powerful antioxidant actions that protect tissues against oxidative injury (Okonkwo et al., 2021). The combined supplementation of neem and papaya leaf meal will, therefore, have a synergistic effect. Therefore, the current study aims to explore their effect on the immune, antioxidant value and the serum biochemical constituents of the broiler chickens.

MATERIAL AND METHODS

Birds, Housing and Experimental Design

The present experiment was conducted in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) in year 2020. Total of 220, day old VenCobb-400 broiler chicks were randomly selected for the experiment and were allotted eleven dietary treatments. Each treatment consisted of four replicates with five chicks in each replicate. Experimental diets were formulated in mash form as per VenCobb-400 broiler chickens (Venkys, 2017) and supplemental ingredient i.e. neem and papaya leaf meal was added. The ingredients were procured in one lot for whole experiment. They were grinded and screened properly to get uniform particle size. A phase feeding regimen was followed, comprising three phases: pre-starter (0-14 days), starter (15-28 days), and finisher (29-42 days), consisted of eleven isonitrogenous and isocaloric dietary treatments, by adjusting basal ingredients; therefore, the observed effects were attributed to the bioactive phytochemicals of neem and papaya leaf meal rather than differences in nutrient composition.

Preparation of Leaf meal

Neem and Papaya leaf were airdried; powdered separately and meal was stored in air-tight bags. No phytochemical analysis was conducted in this study, however the presence of phytochemicals was referred from existing literature. The proximate composition of neem leaf meal (NLM) and papaya leaf meal (PLM) on dry matter (DM %) basis is presented in Table 1. Composition of experimental broiler diets used in the study is given in Table 2 and Table 3.

Table 1. Proximate Composition of NLM and PLM (on % DM Basis)

Nutrient Composition (%)	NLM	PLM
Moisture^{+}	8.27	11.73
Crude Protein^{+}	19.25	24.79
Crude Fibre^{+}	15.78	7.64
Ether Extract^{+}	5.02	1.70
Ash^{+}	6.50	8.94

$^{+}$Calculated values

Treatment-wise dietary supplementation of neem and papaya leaf meal was applied as follows:

T0: Control (basal diet) as per commercial chick feed specifications

T1: Basal diet + Neem leaf meal (NLM) at 0.5% of diet

T2: Basal diet + Neem leaf meal (NLM) at 1.0% of diet

T3: Basal diet + Papaya leaf meal (PLM) at 0.5% of diet

T_4: Basal diet + Papaya leaf meal (PLM) at 1.0% of diet

T5: Basal diet + 1:1 combination of NLM and PLM at 1.0% of diet

T6: Basal diet + 1:1 combination of NLM and PLM at 2.0% of diet

T7: Basal diet + 1:2 combination of NLM and PLM at 1.0% of diet

T8: Basal diet + 1:2 combination of NLM and PLM at 2.0% of diet

T9: Basal diet + 2:1 combination of NLM and PLM at 1.0% of diet

T10: Basal diet + 2:1 combination of NLM and PLM at 2.0% of diet

Table 2. Ingredient composition of Broiler experimental diets

Feed Ingredients	Experimental Diets (%)		
	Pre-starter (0 - 14 days)	Starter (15 - 28 days)	Finisher (29 - 42 days)
Maize	55.21	57.33	59.27
Soybean meal	38.83	35.10	31.52
Calcite/LSP	0.62	0.66	0.69
DCP	1.65	1.64	1.63
Vegetable oil	2.28	3.91	5.60
Methionine	0.22	0.22	0.21
Lysine	0.28	0.28	0.22
Soda Bicarb	0.10	0.10	0.10
Salt	0.24	0.22	0.20
Trace Mineral Premix[1]	0.06	0.05	0.05
Vitamin Premix[2]	0.06	0.05	0.05
Threonine	0.075	0.05	0.02
Maduramicin	0.00	0.00	0.05
Robimidine	0.033	0.033	0.00
Choline Chloride 60 %	0.10	0.12	0.15
Liver tonic	0.05	0.05	0.05
Emulsifier	0.05	0.05	0.05
Toxin binder	0.10	0.10	0.10
Supplemental ingredient	+	+	+
TOTAL	99.99	99.99	99.99

[1]Trace mineral provided per kg diet: Manganese, 120mg; Zinc, 80mg; Iron, 25mg; Copper, 10mg; Iodine, 1mg; and Selenium, 0.1mg.

[2]Vitamin premix provided per kg diet: Vitamin A, 20000IU; Vitamin D3, 3000IU; Vitamin E,10mg; Vitamin K,2mg; Riboflavin, 25mg; VitaminB1,1mg; Vitamin B6, 2mg; Vitamin B12,40mcg and Niacin, 15 mg.

[+] Varying level of NLM and PLM supplement.

Table 3. Nutrient composition (%) of basal diet during different phases (on % DM Basis)

Nutrient Composition (%)	PHASE		
	Pre-starter (0 - 14 days)	Starter (15 - 28 days)	Finisher (29 - 42 days)
Moisture[++]	11.00	10.70	10.64
Crude Protein[++]	23.57	21.81	20.11
Crude Fibre[++]	4.72	6.33	6.47
Ether Extract[++]	2.18	3.75	4.65
Ash[++]	5.61	6.04	5.23
Metabolisable energy (kcal/kg)[+]	3000	3100	3200
Calcium[++]	1.00	0.90	0.85
Available phosphorus[++]	0.50	0.45	0.40
Lysine[+]	1.35	1.20	1.05
Methionine[+]	0.55	0.50	0.45
Methionine + cysteine (%)[+]	0.88	0.80	0.74
Threonine (%)[+]	0.77	0.70	0.65
Sodium (%)[+]	0.16	0.16	0.16
Chloride (%)[+]	0.18	0.18	0.18

[+]Calculated values [++]Analysed values

Broiler trial

Experimental chicks were reared under controlled conditions in a battery brooder system. Experiment was conducted under tropical climatic conditions (temperatures ranging from 28°C to 34°C and relative humidity between 65-80%), while brooding temperature was maintained at 33-35°C during the first week period under standard management practices.

Birds were weighed individually at weekly intervals up to six weeks of age to know the body weight gain of broilers and weekly feed intake was recorded on a replicate basis by measuring the feed offered and residues at the end of each week. The FCR was calculated using the formula: FCR = Feed consumption (g) / Body weight gain (g). The performance index (PI) was calculated according to Bird (1995) as: PI (%) = Body weight gain (g) × Feed efficiency ratio.

Broilers were vaccinated on 7thday of age with F_1 strain, 14thday with infectious bursal disease and 21st of age with Lasota strain of Newcastle disease. Humoral immunity towards Newcastle disease was measured on day 30th and 42nd by the Haemagglutination inhibition test according to the method of O.I.E. (2005). At the end of trial (42nd day), birds were sacrificed, parts from breast muscle and liver tissue samples (two from each replicate) was taken and analyzed for degree of lipid oxidation. After storage at -20°C for 30 days lipid oxidation was determined as thiobarbituric acid reactive substances (TBARS) according to the extraction method described by Witte et al. (1970). TBARS was expressed as mg of malondialdehyde (MDA)/kg of tissue. Serum biochemical parameters was determined: total protein (g/L), total cholesterol (mg/dL), high density lipoprotein (HDL) cholesterol (mg/dL), low- density lipoprotein (LDL) cholesterol (mg/dL) and triglycerides (mg/dL) by spectrophotometrically using commercial diagnostic kits.

Ethical approval

The experiment on animals including all procedures of this study was approved by Institutional Animal Ethics Committee, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.).

Statistical analysis

The experimental data were analysed using analysis of variance (ANOVA) under a completely randomized design (CRD) following the procedure described by Snedecor and Cochran (1994). Differences among the dietary treatments were tested for significance by using Duncan's Multiple Range Test (1955) to determine statistical significance.

RESULTS AND DISCUSSION

Average Body Weight Gain, Feed Intake, Feed Conversion Ratio and Performance Index

Addition of neem and papaya leaf meal had a significant effect on growth performance of broiler chickens (Table 4). Weight gain was significantly ($P < 0.05$) higher in broiler chickens fed neem and papaya leaf meal than the control during the pre-starter (0-14 days), starter (15-28 days), and finisher (29-42 days) phases. Among the supplemented diets, weight gain was highest in broiler chickens fed 1.0% neem and papaya leaf meal in a 1:1 ratio (T5), followed by T7 (1:2 ratio @ 1.0% concentration) and T8 (1:2 ratio @ 2.0% concentration), and these diets were significantly ($P < 0.05$) better than the control. The improved growth performance may be due to its antimicrobial and growth-stimulating properties (Paul et al., 2020) of neem leaf meal and due to proteolytic enzymes papain and chymopapain in papaya leaf meal (Rahman et al., 2023). Thus, it supports protein digestion and enhance the release of free amino acids required to increase growth of broilers results in growth promoting effect. The feed intake was significantly ($P < 0.05$) different between the treatments, with the highest feed intake recorded for the treatment where the ratio of neem to papaya leaf meal was 1:2 ratio of 1.0% (T7) (Table 04). Higher feed intake in the supplemented groups may be attributed to neem leaf meal's appetite-stimulating, antibacterial and hepatoprotective properties, along with its carminative action, which together improve digestive efficiency (Deka et al., 2019; Ampode et al., 2021). Higher feed intake has also been reported with papaya leaf supplementation in poultry, likely due to improved palatability of the diet (Jimenez et al., 2024). The feed conversion ratio was also improved significantly ($P < 0.05$) in the supplemented groups, with the best FCR being recorded in T5, indicating better feed utilization. The improved feed efficiency can be attributed to the better utilization of nutrients, reduction of pathogenic gut flora and increased digestive activity. The performance index also demonstrated a similar trend, with T5 recording the highest performance index values ($P<0.05$), attributing to the combined effect of increased body weight gain and better feed conversion efficiency.

Table 4. Growth performance of broilers during pre-starter, starter and finisher phases under different dietary treatments

Treat.	Body weight gain (g/bird)			Feed intake (g/bird)			FCR			Performance Index		
	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher
T0	393.70^{c}	1211.95^{e}	2277.70^{f}	524.60	1897.60^{ab}	4111.10^{bcd}	1.34^{a}	1.68^{a}	2.08^{a}	295.48^{c}	488.03^{e}	513.35^{e}
T1	399.90^{c}	1252.90^{de}	2342.95^{ef}	525.20	1904.40^{ab}	4121.45^{ab}	1.32^{a}	1.62^{ab}	2.03^{ab}	304.99^{c}	527.65^{de}	536.21^{de}
T2	397.10^{c}	1272.80^{de}	2358.45^{ef}	515.30	1882.00^{b}	4073.23^{e}	1.30^{a}	1.57^{b}	2.02^{ab}	306.35^{c}	564.26^{d}	537.98^{de}
T3	400.50^{c}	1286.15^{cd}	2409.90^{de}	519.53	1900.90^{ab}	4116.93^{abc}	1.30^{a}	1.56^{bc}	1.97^{bc}	309.54^{c}	568.65^{d}	570.04^{bcd}
T4	406.30^{bc}	1297.15^{cd}	2445.05^{d}	519.65	1893.93^{ab}	4108.70^{bcde}	1.28^{ab}	1.54^{bc}	1.93^{c}	318.01^{bc}	577.87^{cd}	595.04^{b}
T5	444.45^{a}	1456.70^{a}	2668.20^{a}	512.60	1900.53^{ab}	4142.03^{ab}	1.16^{c}	1.38^{d}	1.85^{d}	388.73^{a}	739.58^{a}	655.24^{a}
T6	404.70^{bc}	1342.50^{bc}	2485.70^{cd}	512.10	1887.85^{ab}	4132.35^{ab}	1.27^{ab}	1.47^{cd}	1.97^{bc}	320.02^{bc}	639.44^{bc}	582.50^{bc}
T7	436.60^{a}	1400.70^{ab}	2570.70^{b}	523.15	1909.00^{a}	4151.50^{a}	1.20^{bc}	1.44^{d}	1.92^{cd}	364.45^{ab}	670.72^{b}	610.52^{b}
T8	430.90^{ab}	1373.90^{b}	2538.25^{bc}	515.05	1897.98^{ab}	4113.43^{bcd}	1.20^{bc}	1.47^{cd}	1.91^{cd}	360.95^{ab}	643.49^{bc}	612.12^{b}
T9	396.40^{c}	1244.00^{de}	2325.55^{ef}	522.55	1889.55^{ab}	4077.33^{de}	1.32^{a}	1.61^{ab}	2.03^{ab}	300.86^{c}	525.89^{de}	535.28^{de}
T1	0395.05^{c}	1249.93^{de}	2344.43^{ef}	521.60	1888.65^{ab}	4082.25^{cde}	1.32^{a}	1.60^{ab}	2.01^{ab}	299.41^{c}	534.81^{de}	546.39^{cde}
SEM	3.66	12.29	19.01	1.49	2.12	4.80	0.01	0.02	0.01	6.23	12.56	7.21
P-value	0.017	0.009	0.006	0.081	0.042	0.010	0.021	0.016	0.009	0.014	0.008	0.011

Means of column with different superscript differ significantly (P<0.05)

Humoral immune response of broilers (30th and 42nd day)

Results indicate that humoral immune response was significantly improved (P<0.05) by dietary supplementation of neem and papaya leaf meal individually and in combination, when compared with the control. However, maximum and significantly (P<0.05) higher antibody titre (log10 value of HI titre) was observed at 30th and 42nd day of experiment i.e. in T5 and T8 group, respectively (Table 5). Neem and papaya leaves could play a major role in stimulating the humoral immune response because of its significant role in killing or slowing the growth of many pathogenic microorganisms like bacteria, virus, fungi etc. Our results are in agreement with findings that neem leaf supplementation enhances humoral immune response against Newcastle disease virus, likely due to its immunomodulatory effects on B lymphocytes (Ikpendu et al., 2023). This could be attributed to the immunomodulatory effects of neem on B- lymphocyte which differentiate into memory cells and plasma cells. The plasma cells secrete antibodies against antigen and are capable of producing about 2,000 molecules of antibodies per second while the memory cells have the ability to rapidly differentiate into antibody- producing plasma cells when they encounter the same antigen in another infection (Richard and Tracey, 2001). Papaya leaf supplementation has been shown to increase relative weights of lymphoid organs such as the bursa of Fabricius and spleen, indicating enhanced immune function in broiler chickens (Nabhan et al., 2025). Papaya extract also has antibacterial and antifungal properties that may enhance immune activity (Alhodieb et al., 2025). Also factors like saponin present in papaya leaf meal may produce an immunomodulatory effect in the birds (Ojiako et al., 2019).

Table 5. Antibody titre (Log10 value of HI titre) against Newcastle disease virus in broiler under different treatment groups

Treat.	30th Day	42nd Day
T0	2.15^{e}	2.43^{f}
T1	2.34^{d}	2.55^{de}
T2	2.46^{c}	2.74^{bc}
T3	2.18^{e}	2.45^{ef}
T4	2.37^{cd}	2.55^{de}
T5	2.77^{a}	2.96^{a}
T6	2.44^{cd}	2.63^{cd}
T7	2.43^{cd}	2.72^{bc}
T8	2.72^{a}	2.94^{a}
T9	2.56^{b}	2.93^{a}
T10	2.60^{b}	2.80^{b}
SEM	0.03	0.04
P-Value	0.012	0.007

Means of column with different superscript differ significantly (P<0.05)

Antioxidant status

Significantly (P<0.05) better antioxidant status in terms of TBARS values (mg Malondialdehyde/ kg) at 30 days of storage in breast and liver tissue were observed in broilers fed on basal diets supplemented with neem and papaya leaf meal in 1:1 combination at 1.0% of diet (T5) as compared to control (Table 6). Recent research has also shown that supplementation with papaya leaf components enhances antioxidant capacity in quails, marked by increased total antioxidant capacity and enzyme activities alongside reduced oxidative stress markers (El-Rayes et al., 2024). Papaya leaves are a rich source of antioxidant nutrients, including carotenoids, vitamin C, B-complex vitamins (such as pantothenic acid) and essential minerals, which can contribute to improved antioxidant status (Koul et al., 2022) and metabolic health in broiler chickens. Likewise, recent studies have reported that ethanolic extracts of neem contain potent antioxidant compounds such as terpenoids, limonoids, quercetin and phytosterols, which shows strong antioxidant activity and protection against lipid oxidation–induced stress (Zahid et al., 2025).

Table 6. Thiobarbituric acid reactive substances (mg Malondialdehyde (MDA) kg^{-1}) in liver tissue and breast muscle of broilers in different treatment groups

Treatment	Fresh		After 30 days storage	
	Liver tissue	Breast muscle	Liver tissue	Breast muscle
T0	0.98^{a}	0.40^{a}	1.66^{a}	1.29^{a}
T1	0.92^{b}	0.39^{a}	1.67^{a}	1.12^{b}
T2	0.86^{cd}	0.37^{ab}	1.46^{c}	0.95^{c}
T3	0.85^{cd}	0.39^{a}	1.56^{b}	0.93^{cd}
T4	0.86^{cd}	0.39^{ab}	1.56^{b}	0.94^{c}
T5	0.77^{e}	0.32^{c}	1.23^{f}	0.72^{f}
T6	0.88^{bc}	0.39^{a}	1.47^{c}	0.93^{cd}
T7	0.85^{cd}	0.37^{ab}	1.41^{cd}	0.86^{de}
T8	0.78^{e}	0.35^{b}	1.31^{e}	0.79^{ef}
T9	0.82^{de}	0.37^{ab}	1.37^{de}	0.84^{e}
T10	0.82^{de}	0.37^{ab}	1.34^{de}	0.83^{e}
SEM	0.01	0.01	0.02	0.02
P-value	0.009	0.015	0.011	0.008

Means of column with different superscript differ significantly (P<0.05)

Serum biochemical parameters

Significantly lowered (P<0.05) serum concentration of total triglycerides (mg/dL), total cholesterol (mg/dL) and LDL cholesterol (mg/dL) and significantly higher (P<0.05) HDL cholesterol (mg/dL) were observed in broilers fed on T5 (neem and papaya leaf meal in 1:1 combination at 1.0% of diet) compared to those assigned control diet (T0). However, the concentration of total protein in serum of broilers was significantly (P>0.05) not affected by the supplementation and was statistically similar (P>0.05) in all the experimental groups (Table 7).

Findings of the current study are in agreement with Almamury (2024) neem leaf powder supplementation (2.5-3.5 g/kg) in broiler diets led to significantly (P<0.05) higher HDL and lower LDL cholesterol levels compared to control birds, suggesting improved lipid metabolism with neem inclusion. Possible reason could be that neem leaf meal exerts inhibitory effects on cholesterol biosynthesis via modulation of HMG-CoA reductase activity, thereby reducing serum cholesterol levels (Asghar et al., 2023). Also, papaya leaf supplementation has been shown to exert hypocholesterolemic effects, significantly reducing serum cholesterol in broiler chickens fed papaya leaf powder (Oloruntola et al., 2024). Some phytochemicals such as saponin and phenolic compounds present in papaya leaf meal have been reported to exert a hypocholesterolemic effect and reduces gut cholesterol uptake through intralumenal physicochemical interaction. This may be responsible for the decrease in cholesterol and LDL level in papaya leaf meal inclusive diets. This equally infers that neem and papaya leaf meal should be used to produce poultry products with desirable reduction in cholesterol level.

Table 7. Serum biochemical parameters of broilers in different treatment groups.

Treatment	Total Protein (g/L)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	LDL(mg/dL)	HDL (mg/dL)
T0	2.50	68.74^{a}	141.60^{a}	71.64^{a}	26.36^{d}
T1	2.29	55.72^{bcd}	136.01^{abc}	58.68^{abc}	28.88^{bc}
T2	2.40	55.13^{cd}	124.95^{e}	59.04^{abc}	27.21^{cd}
T3	2.38	42.12^{f}	129.23^{cde}	50.24^{bc}	29.09^{bc}
T4	2.65	54.40^{d}	129.56^{cde}	53.46^{bc}	29.24^{bc}
T5	2.48	44.94^{e}	124.05^{e}	46.58^{c}	32.02^{a}
T6	2.70	41.70^{f}	133.30^{bcd}	51.22^{bc}	30.22^{ab}
T7	2.33	56.07^{bc}	129.57^{cde}	66.74^{a}	28.16^{bcd}
T8	2.50	54.98^{cd}	126.31^{de}	62.66^{ab}	27.75^{cd}
T9	2.22	56.71^{b}	127.68^{de}	62.00^{ab}	26.59^{d}
T10	2.51	54.67^{cd}	138.66^{ab}	63.30^{ab}	28.17^{bcd}
SEM	0.03	1.61	1.28	1.79	0.37
P-value	0.063	0.012	0.018	0.021	0.009

Means of column with different superscript differ significantly (P<0.05)

CONCLUSION

The present study shows that supplementation of broiler diet with a 1:1 blend of neem and papaya leaf meals at 1.0% of the total diet has the potential to enhance several key performance indicators significantly. The birds improved significantly in terms of general performance, indicated by increased growth rate and feed conversion ratio. In addition, the immune response was positively modulated, implying the supplementation enhances the birds' resistance to infection and health maintenance capabilities. The antioxidant status was also significantly improved, implying an intensified defense against oxidative stress during development. Lastly, serum biochemical parameters were within optimal ranges, affirming that the dietary intervention does not compromise metabolic health. These results together validate the promise of neem and papaya leaf meal as an effective and natural alternative to antibiotics for enhancing strong growth and better health in broiler production.

REFERENCES

Alhodieb, F. S., Farid, M., Sabir, M., Nisa, S., Sarwar, S. and Abbas, S. 2025. Exploring the bioactive compounds of *Carica papaya* leaves: Phytol's role in combatting antibiotic-resistant bacteria. Frontiers in Cellular and Infection Microbiology. 15.Article 1564787.

Almamury, A. 2024. Effects of dietary supplementation of neem leaf powder (*Azadirachta indica*) on growth performance, carcass characteristics and serum biochemical parameters of broilers. European Journal of Ecology, Biology and Agriculture. 1(5): 3–15.

Ampode, K. M. B. and Asimpen, S. M. 2021. Neem (*Azadirachta indica*) leaf powder as phytogenic feed additive improves the production performance and immune organ indices of broiler chickens. Journal of Animal Health and Production. 9(4):362–370.

Asghar, H. A., Syed, Q. A., Shukat, R. and Israr, B. 2023. Exploring the prophylactic potential of *Azadirachta indica* leaf extract against dyslipidemia. Journal of Ethnopharmacology. 303. 116008.

Bird, H. R. 1995. Performance index as a measure of broiler efficiency. Poultry Science.

Deka, J., Mahanta, J. D., Kalita, K. P., Choudhury, D., Ahmed, H. F. and Tamuly, S. 2019. Effect of dietary supplementation of neem (*Azadirachta indica*) leaf powder on the performance of commercial broiler chicken. Journal of Entomology and Zoology Studies. 7(3): 658–663.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1–42.

El-Rayes, T. K., El Basuini, M. F. M., Fouad, W., Ahmed, S. A. I., Ahmad, E. A. M. andDawood, M. A. O. 2024. Comparison of different papaya forms on growth performance, immune response, antioxidative capacity and caecal microbiota of Japanese quails. Scientific African. 20: e02033.

Ikpendu, C. N., Ndiana, L. A., Ezeifeka, G. O., Ukwueze, J. I. and Ukwuoma, C. C. 2023. Immunomodulatory effects of aqueous extracts of *Azadirachta indica* and *Piper guineense* on Newcastle disease vaccination in cockerels. Journal of Veterinary and Applied Sciences. 4(2): 151–154.

Koul, B., Singh, S., Sharma, A. and Kaul, S. 2022. *Carica papaya* L.: A tropical plant with nutritional, antioxidant and therapeutic potential beyond the fruit. Diversity. 14(8): 683.

Nabhan, M. N., Yunianto, V. D., Mulyono, M. and Krismiyanto, L. 2025. The effect of encapsulated Japanese papaya leaf extract supplementation in diets on protein digestibility, immunity, and performance of broiler chickens. Journal of Advanced Veterinary Research. 15(6): 790–794.

O.I.E. 2005. *Manual of diagnostic tests and vaccines for terrestrial animals*. World Organisation for Animal Health.

Obianwuna, U. E., Chang, X., Oleforuh-Okoleh, V. U., Onu, P. N., Zhang, H., Qiu, K., and Wu, S. 2024. Phytobiotics in poultry: Revolutionizing broiler chicken nutrition with plant-derived gut health enhancers. Journal of Animal Science and Biotechnology. 15, Article 169.

Ojiako, C. M., Okoye, E. I., Oli, A. N., Ike, C. J., Esimone, C. O. and Attama, A. A. 2019. Preliminary studies on the formulation of immune-stimulating complexes using saponin from *Carica papaya* leaves. Heliyon 5(6): e01962.

Okonkwo, J. C., Ezekwe, V. N., Onunkwo, D. N. and Okonkwo, I. F. 2021. Haematological characteristics of broiler birds administered neem and pawpaw leaf extracts. International Journal of Environmental and Agriculture Research. 7(7): 55–61.

Oloruntola, O. D., Ayodele, S. O., Daramola, O. T., Ayedun, E. S., Adeyeye, S. A., Oloruntola, D. A., Akinjoye, D. J. and Osowe, C. O. 2024. Effect of *Carica papaya* leaf and seed meal composite mix on haemato-biochemical parameters of broiler chicks. Nigerian Journal of Animal Production. 566–569.

Paul, T. K., Hasan, M. M., Haque, M. A., Talukder, S., Sarker, Y. A., Sikder, M. H., Khan, M. A. H. N. A., Sakib, M. N. and Kumar, A. 2020. Dietary supplementation of neem (*Azadirachta indica*) leaf extracts improved growth performance and reduced production cost in broilers. Veterinary World. 13(6):1050–1055.

Rahman, M., Monira, K., Sultana Queen, N. and Hasan, M. S. 2023. Effect of papaya leaf extract on growth performance, carcass characteristics, meat quality and serum biochemistry of broiler chickens. Meat Research. 2.

Rao, H. 2021. Effect of Herbal Feed Additives on Performance, Immunity, Serum Parameters and E. coli Counts of Broilers under Heat Stress. Indian Journal of Animal Nutrition. 38(1).

Richard, A. and Tracey, L. 2001. *Immunobiology: The immune system in health and disease*. Garland Science.

Singh, V. V., Singh, V. K., Tewari, D., Gautam, S., Singh, V. B. and Singh, P. 2019. Performance of broilers fed diets supplemented with a phytogenic mixture as an alternative to antibiotic growth promoters. Indian Journal of Animal Nutrition, 36(1).

Snedecor, G. W. and Cochran, W. G. 1994. *Statistical methods* (8th Edn.). Iowa State University Press.

Soren, S., Ghosh, R., Mukherjee, J., Mandal, S., Mandal, S., Samanta, I. and Mandal, G. P. 2025. Effect of dietary synbiotic as a replacement for antibiotics on growth performance, gut health and immune response in broiler chickens. Indian Journal of Animal Nutrition. 42(2): 251–260.

Venkys. 2017. *VenCobb-400 broiler nutrition specifications*. Venkateshwara Hatcheries Pvt. Ltd.

Witte, V. C., Krause, G. F. and Bailey, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science. 35: 582–585.

Zahid, A., Islam, J., Iqbal, J., Marvi, M., Arif, F., Ali, Q., Ahmad, S.and Malik, A. 2025. Chemical fingerprint of *Azadirachta indica*: Unraveling the bioactive profile and therapeutic potential. Bulletin of Biological and Allied Sciences Research. (1): 98.

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Zinc Oxide Nanoparticles: A Comprehensive Study of Its Effects on Growth Performance and Intestinal Barrier Functions in Broiler Chickens

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ABSTRACT

Nano-trace elements have gained significant attention as a promising, emerging technology in the animal feed industry in recent years. Their minute size allows for enhanced absorption and efficiency compared to conventional zinc counterparts. The present study aimed to investigate the effects of Zinc oxide nanoparticles (ZnONPs) on the growth and intestinal barrier function of broiler chicks. A total of 150 one-day-old straight-run broiler chicks were randomly allotted to 5 dietary treatment groups are as follows. T1 (Control group) - 80 mg/kg of inorganic zinc (ZnO); T2 - Zn-Met group (60 mg/kg organic zinc- zinc methionine) T3, T4 and T5 - ZnONPs group (ZnONPs at doses of 60 mg/kg, 40 mg/kg and 20 mg/kg respectively. The chicks were reared for 35 days under standard conditions. Improved feed conversion ratio (FCR) was recorded in birds fed 20 and 40 mg/kg ZnONPs, among all the treatment groups. The different treatment groups did not affect the feed intake. Compared with the control group, the villus length (VL) and crypt depth (CD) in the duodenum and jejunum were increased in the 20 mg/kg ZnONPs group. In the ileum, CD was significantly higher in all studied doses of ZnONPs. Villi length to crypt depth ratio (VH/CD) was higher in ZnONPs groups in the duodenum, at 20 mg/kg ZnONPs in the ileum and in the jejunum. Dietary inclusion of ZnONPs showed no significant effects on mRNA expression of the Zona Occludens-1 (ZO-1) gene, while 20 mg/kg ZnONPs significantly upregulated the mucin-2 and claudin-3 genes in the ileal mucosa. The findings of the present study suggest that ZnONPs at a lower dose (20 mg/kg) promote growth and intestinal health.

KEYWORDS: Broiler, Growth and Intestinal Health, ZnONPs

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INTRODUCTION

Trace minerals are essential feed additives in the broiler diet to ensure better productivity and health. In poultry feeds, zinc (Zn) is the most commonly added trace mineral (Abd El-Ghany et al., 2021). Zinc acts as an important co-factor for numerous metalloenzymes, which have effects on the metabolism of carbohydrates, proteins, lipids and nucleic acids (NRC, 2001; Mcdonald et al., 2010) as well as for transcription factors and hormones. In order to meet the zinc requirement of animals, inorganic Zn is added 20 to 30 folds higher than the usual requirement due to its low bioavailability in the body (Bratz et al., 2013; Yusof et al., 2023). Furthermore, higher levels of Zn supplementation may disrupt the mineral balance (Hazra et al., 2025) and may increase the cost of production (Mahmoud et al., 2020).

As a result, the research aiming at more bioavailable minerals that facilitate efficient utilization by poultry while minimizing its environmental excretion is gaining more importance worldwide (Rama Rao et al., 2025). One dietary tactic to minimize excessive trace mineral supplementation is replacing the inorganic sources with organic or nano zinc sources (El-Katcha et al., 2017). However, the usage of organic Zn is restricted due to its high cost. Therefore, by promoting the bioavailability of inorganic zinc, ZnONPs open an opportunity for augmenting the accessibility of this essential mineral (Qu et al., 2023).

In the form of nanoparticles, zinc has greater bioavailability even at a lower concentration than the inorganic and organic sources, having a better therapeutic impact on animals (Lail et al., 2023;

Hafez et al., 2020). It has been reported in several studies that nano forms of zinc surpass conventional zinc sources, even at lower doses, concerning growth (Zhao et al., 2014; Fathi et al., 2016; Fawaz et al., 2021) and intestinal barrier function (Hafez et al., 2017; Fawaz et al., 2021) of broiler chicks. Due to the limited availability of information regarding the optimal levels of ZnONPs in broiler chicken diets, this study aims to explore the effects of various levels of ZnONPs supplementation on growth performance, intestinal morphology and barrier function.

MATERIALS AND METHOD

Synthesis and characterization of ZnONPs

The ZnONPs were synthesized using the planetary ball milling technique (Mozhiarasi et al., 2023). Briefly, 5 g of food-grade Zinc oxide (Kesari Scientific Chemicals, India) was added to the zirconium jar (50 ml) along with 50 balls of 5 mm diameter. The ball mill was operated at a speed of 250 RPM for 9 hours. The particle size and shape of the ZnONPs used in this study were analyzed using High-Resolution Transmission Electron Microscopy (HR-TEM), as shown in figure 1. Results indicated that the particle size of the ZnONPs ranged from 36.28 to 47.48 nm and exhibited nearly hexagonal-shaped nanoparticles. X-Ray Diffraction (XRD) analysis confirms the existence of the hexagonal wurtzite structure of ZnONPs (Figure 2).

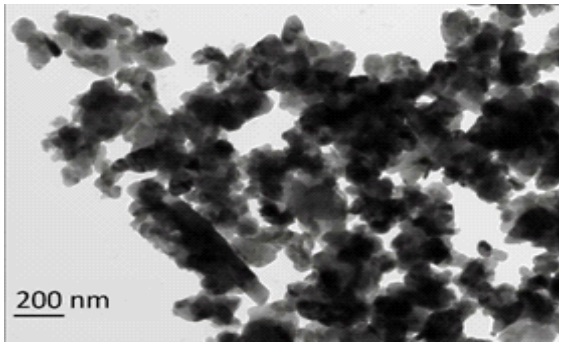


Fig 1. Transmission electron microscopic image of ZnONPs

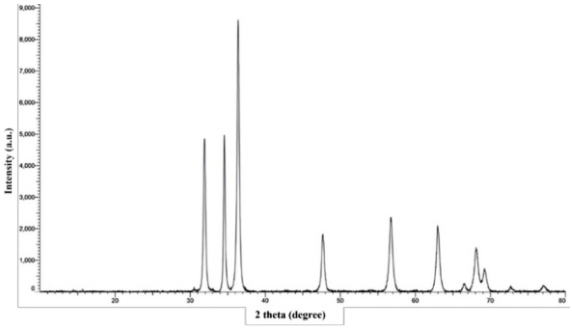


Fig 2. X-Ray Diffraction analysis of ZnONPs

Experimental design and bird management

The feeding trial was conducted at the environmentally controlled poultry shed of Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 07. The experimental procedures were approved by the Institutional Animal Ethical Committee (Lr.No. 508/DFBS/IAEC/2022 dated on 05.05.2022). A total of 150 one-day-old broiler straight run chicks (Vencobb 400) were equally distributed into five dietary treatments randomly, with three replicates containing ten chicks in each pen replicate (each pen measures 6 × 3.5 ft). The birds were reared in a deep litter system up to 5 weeks of age. The experimental birds had *ad libitum* access to water and feed, and the light program was 23 hours of light per day until the experiment termination. Temperature was maintained at 34 ± 1 ºC during the first week and it was gradually decreased by 3 ºC during the second week to reach 31 ± 1 ºC; thereafter, the average temperature maintained was 28 ± 1 ºC. The relative humidity was 60-70%. All the chicks were vaccinated against Newcastle disease on the 7th day (*RDV- F strain)* by intra ocular route and on the 21st day (*Lasota strain)* by drinking water and against infectious bursal disease on the 14th day (*Georgia strain)* by intra ocular route.

Experimental diets

The dietary treatments composed of different zinc sources per kg of maize soybean meal-based basal diets (Table 1) as follow: T1 (Control group) - 80 mg/kg of inorganic zinc (ZnO) at 100 % of BIS, 2007 requirement; T2 - Zn-Met group (60 mg/kg organic zinc (zinc methionine) at 75 % of the requirement); T3, T4 and T5 - ZnONPs group (ZnONPs at doses of 60 mg/kg (75 % of the requirement), 40 mg/kg (50 % of the requirement) and 20 mg/kg (25 % of the requirement) respectively). To prepare experimental diets and increased precision during mixing the ZnONPs in the basal diet, graded levels of ZnONPs were added into the premix which contained other trace elements as per the requirements and then the premix blend was thoroughly mixed with 5 kg of basal diet. The basal diets for broiler pre-starter (0 – 7 days), starter (8 - 21 days) and finisher (22 - 35 days) were formulated according to Bureau of Indian Standards (BIS, 2007) specifications.

Table 1 Composition of basal diets

Ingredients (g/Kg)	Pre - Starter	Starter	Finisher
Maize	495.60	503.50	542.50
Soya bean meal	425.20	401.00	350.20
Palm Oil	38.00	54.00	65.00
Dicalcium Phosphate	18.00	18.40	18.10
Calcite	12.20	12.20	13.00
Salt	3.00	3.00	3.00
Choline chloride	1.50	1.50	1.50
Methionine	1.50	1.40	1.20
Threonine	1.00	1.00	1.50
Trace Mineral Mix*	1.00	1.00	1.00
Vitamin Premix**	0.50	0.50	0.50
Liver tonic	0.40	0.40	0.40
Antioxidant	0.10	0.10	0.10
Probiotic	0.50	0.50	0.50
Toxin binder	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50
Total	1000.00	1000.00	1000.00
Nutrients (%)			
ME, kcal/kg	3002.00	3103.00	3198.00
CP, %	23.05	22.08	20.02
Calorie/protein ratio	130.23	140.53	159.74
Calcium, %	1.00	1.00	1.00
Available Phosphorus, %	0.45	0.45	0.45
Lysine, %	1.56	1.48	1.31
Methionine, %	0.52	0.50	0.45

Trace mineral mix* - provided per kg of diet: Copper 12 mg, Iodine 1.6 mg, Iron 80 mg, Selenium 0.3 mg and Manganese 100 mg. Vitamin Premix** – provided per kilogram of diet: Vitamin A 10000 IU, Vitamin D_3 3000 IU, Vitamin E 40 IU, Vitamin K_3 1.5 mg, Vitamin B_{12} 0.01 mg, Thiamine 2.5 mg, Choline 500 mg, Biotin 0.15 mg, Folic acid 1 mg, Niacin 40 mg, Pantothenic acid 15 mg, Pyridoxine 5.5 mg and Riboflavin 6.5 mg. CP – Crude Protein; ME- Metabolizable Energy; Kcal/kg – Kilo Calorie per Kilo gram.

All the experimental diets were chemically analyzed for their proximate composition as per AOAC (2012) methods (Table 2).

Table 2. Proximate composition (% DM) of experimental diet (Mean# ± SD)

Parameter	Pre-starter feed	Starter feed	Finisher feed
Crude Protein	23.14 ± 0.05	22.17 ± 0.58	20.19 ± 0.04
Crude Fibre	3.05 ± 0.18	3.89 ± 0.05	3.93 ± 0.10
Ether Extract	7.24 ± 0.14	7.38 ± 0.16	8.00 ± 0.01
Total Ash	6.16 ± 0.06	6.12 ± 0.12	5.819 ± 0.07
Nitrogen Free Extract*	60.40 ± 0.17	60.42 ± 0.19	62.32 ± 0.19

#Each value represents the mean of three observations; *Calculated by the difference

Measurements

Performance study

On the first day of the trial, the initial body weight of all chicks was recorded. The individual body weight of chicks and replicate pen-wise feed intake were recorded at weekly intervals throughout the experimental period (0 – 35 days) and the weekly FCR was calculated by dividing the respective feed intake (FI) by weight gain for each replicate pen. mRNA expression of ileal tight

junction protein genes by quantitative real-time PCR.

To evaluate the gene expression of ileal tight junction proteins (ZO-1, Mucin-2 and Claudin-3), ileal tissues were collected from six birds per treatment on the day of slaughter. Total RNA from the ileum tissues were isolated using Trizol method (Rio et al., 2010). The concentration and purity of RNA were determined using spectrometry in a Nanodrop (Thermo Scientific NanoDrop™ One Microvolume UV-Vis Spectrophotometer). The iScript™cDNA synthesis kit (BIO-RAD) was used to synthesize cDNA from RNA as per the manufacturer's instructions. Real-time PCR analysis was carried out using a BIO-RAD, CFX Opus 96 Real-Time PCR system using iTaq Universal SYBR Green Supermix (BIO-RAD). The specific primers and thermal cyclic conditions used in Real-Time PCR are listed in table 3 and 4, respectively. The relative mRNA expressions were analyzed by 2^{-Ct} method (Livak and Schmittgen, 2001) and normalized to the level of 16s rRNA as a housekeeping gene.

Table 3. The sense and antisense primer sequences of genes used for Real-Time PCR study

Gene	Orientation	Primers sequences (5' - 3')	Product size (bp)	Reference
Mucin-2	Forward	TTCATGATGCCTGCTCTTGTC	93	
	Reverse	CCGTAGCCTTGGTACATTCTTGT		
ZO-1	Forward	GCCTGAATCAAACCCAGCAA	197	
	Reverse	TATGCGGCGGTAAGGATGAT		
Claudin-3	Forward	GAAGGGCTGTGGATGAACTG	221	
	Reverse	GAGACGATGGTGATCTTGGC		
16 S	Forward	GTAACGCAAGCGATCNCG	130	Xie et al., (2020)
	Reverse	AACCGCGACGCTTTCCAA		

Table 4. Thermal cyclic conditions used for Real-Time PCR study

Thermal cyclic conditions	Genes (ZO –1, Mucin – 2 & Claudin – 3)
Initial denaturation temperature	95 °C for 3 minutes
Final denaturation temperature	95 °C for 30 seconds
Annealing temperature	60 °C for 30 seconds
Extension temperature	72 °C for 45 seconds
Number of Cycles	40
Melting curve	65 °C – 95 °C

Histomorphometry parameters of the small intestine

On day 35, six birds were slaughtered per treatment and samples were collected from the midpoint of the duodenum, jejunum and ileum, fixed in 10 % of buffered formalin, dehydrated manually using ascending grades of alcohol, cleared using xylol, embedded in paraffin wax, cut to 3 µm thick, stained with hematoxylin and eosin (Uni et al., 1998). Histological indices measured using an image analyzer software (Magvision) included villus length (from the top of villi to the junction of the villus and crypt), crypt depth (defined as the depth of the invagination between adjacent villi) and villus height to crypt depth (VH/ CD) ratio.

Statistical Analysis

The experimental data were analyzed by one-way ANOVA using SPSS v.20.0 statistical package and means of different treatments were computed using Duncan's multiple range test (Duncan, 1995). The obtained results were expressed as mean ± standard deviation and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Growth Performance

The results for body weight gain (BWG), feed intake, and FCR are shown in Table 5.

Table 5. Effect of dietary addition of zinc oxide nanoparticles (ZnONPs) on broiler growth performances

Age/Phase	Parameter	Control	Zn-met 60 mg/kg	ZnONPs (mg/kg)			Pooled SEM	p value
	BWG	108.5	111.7	114.8	114.2	111.5	2.77	0.21
I wk	FI	147.6	154.4	152.0	153.1	144.7	4.03	0.16
	FCR	1.36^{bc}	1.38^{c}	1.32^{ab}	1.34^{bc}	1.30^{a}	0.01	0.09
	BWG	260.4	273.0	274.4	267.7	274.5	8.39	0.17
II wk	FI	527.8	516.7	516.4	537.3	526.3	15.40	0.64
	FCR	2.03	1.89	1.88	2.01	1.92	0.07	0.24
	BWG	434.9	434.3	440.0	444.3	433.9	12.92	0.85
III wk	FI	603.9	630.6	628.0	621.4	599.0	21.65	0.51
	FCR	1.39	1.45	1.43	1.40	1.38	0.04	0.74
	BWG	586.1^{ab}	593.0^{ab}	564.8^{a}	596.5^{ab}	610.7^{b}	12.06	0.20
IV wk	FI	888.8	913.4	919.2	898.3	910.1	22.40	0.67
	FCR	1.52	1.54	1.63	1.51	1.49	0.08	0.80
	BWG	475.5	475.2	508.5	510.3	521.2	17.85	0.41
V wk	FI	908.7	927.2	937.3	933.8	950.6	36.29	0.83
	FCR	1.91	1.95	1.84	1.83	1.82	0.03	0.88
	BWG	1856.0^{a}	1889.6^{ab}	1904.8^{ab}	1972.5^{b}	1945.1^{ab}	48.20	0.13
0 – V wk	FI	3076.8	3142.8	3143.9	3152.3	3130.9	56.55	0.87
	FCR	1.66^{b}	1.66^{b}	1.65^{b}	1.60^{a}	1.61^{a}	0.01	0.02

Values bearing different superscript differed significantly; NS = non-significant (P > 0.05)

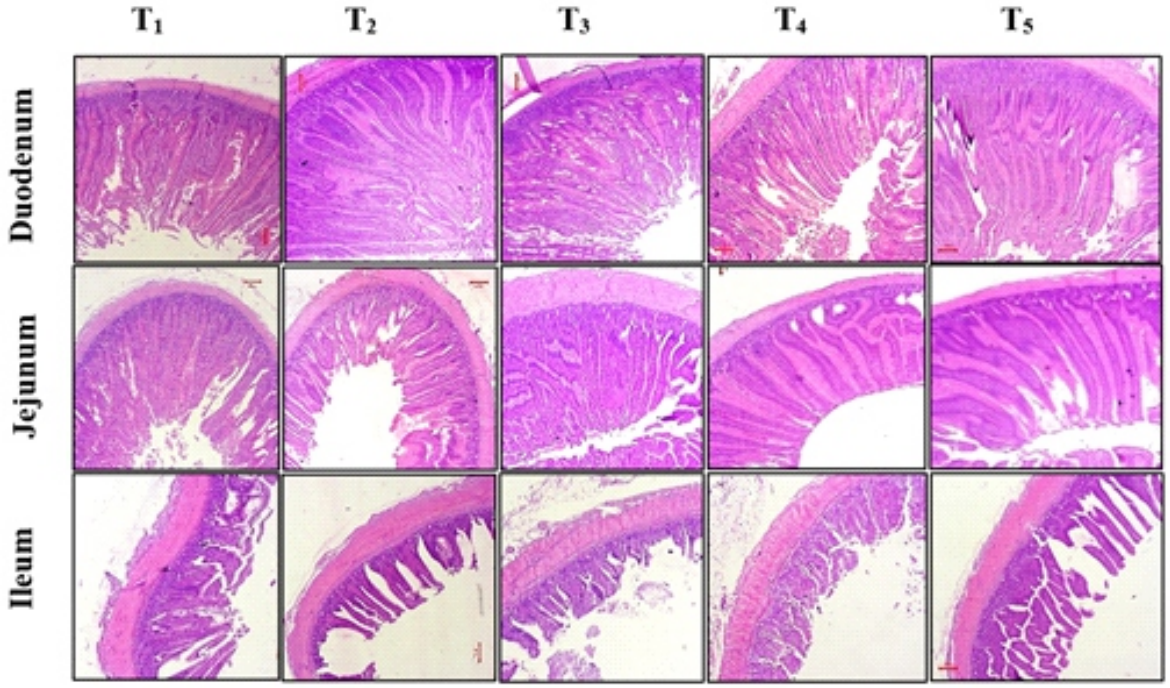
The body weight gain of the broilers was significantly (P 0.05) higher with better FCR fed with 40 mg/kg ZnONPs, followed by 20 mg/kg ZnONPs and 60 mg/kg ZnONPs, compared to the birds fed with the basal diet alone. In the overall trial, among the treatment groups, birds fed with 20 mg/kg ZnONPs exhibited better FCR values of 1.61 than other treatment groups. The different dietary treatments did not affect the feed intake of the broilers. Several reports stated positive effects of dietary supplementation of ZnONPs on broiler growth performances (Hafez et al., 2017; Zhao et al., 2014; Fathi et al., 2016; Ibrahim et al., 2017). However, Bami et al., 2018, Hazra et al., 2025 and Asheer et al., 2018 reported a non-significant result on BWG. Profitability of broiler farming mainly depends upon the enhanced FCR and growth rate (Bhalsing et al., 2026). In this study, birds fed with the 40mg/kg diet ZnONPs differed significantly ($p < 0.05$) in overall BWG compared with the control group. However, overall BWG did not differ between different levels of ZnONPs and Zn-met group. The results implied that the replacement of ZnO with Zn-met or ZnONPs did not affect average feed intake in broilers. However, FCR of 20 mg/kg of ZnONPs group has significant ($p< 0.05$) effects compared to conventional Zn at 1 week of age and overall period. This displays that the lower level (20mg/kg) of ZnONPs is equally efficient to conventional zinc sources at higher levels. The results show that replacement of conventional Zn sources with ZnONPs had no negative effects on broiler growth. A low FCR value implies better feed efficiency, thus demonstrating the efficiency of ZnO NPs in supplying sufficient Zn in the broilers even at lower dosages (Yusof et al., 2023). Likewise, Hafez et al., 2017 found that optimal levels of ZnONPs (40 and 80 mg/kg food) might improve feed conversion rate and increase body weight gain when compared to the control group (40 mg/kg diet); however, feed intake of broiler was not significant among all groups.

Intestinal health status

Histomorphometry parameters of the small intestine

Supplementation of zinc in different forms did not show any pathological alterations (Figure 3).

Fig 3. Effects of ZnONPs on histomorphometry of the small intestine of 35- day old broiler chicks. T1 – 80 mg/kg ZnO. T2 – 60 mg/kg Zn-methionine; T3 - 60 mg/kg ZnONPs; T4 - 40 mg/kg ZnONPs; T5 - 20 mg/kg



kg Zn-methionine; T3 - 60 mg/kg ZnONPs; T4 - 40 mg/kg ZnONPs; T5 - 20 mg/kg ZnONPs, showing significantly increased villi length in duodenum and jejunum

Interestingly, the villus length, crypt depth and villi height-to-crypt depth ratio in all segments of the small intestine is increased in a dose-dependent manner with decreasing levels of dietary ZnONPs (Table 6). Compared with control and Zn-Met groups, supplementation of the lowest level of ZnONPs in broiler diet at 20 mg/kg increased ($p< 0.05$) the villus length and crypt depth in the duodenum, while in the jejunum, in addition to 20 mg/kg, villus length and crypt depth was increased ($p < 0.05$) in 40 mg/kg ZnONPs. In the ileum, no significant ($p> 0.05$) changes in the villi length were observed among broiler chicks fed on different sources of zinc, while crypt depth was significantly ($p< 0.05$) higher in all studied doses of ZnONPs in the feed. Villi length to crypt depth ratio was higher at 20,40 and 60 mg/kg of ZnONPs in the duodenum, at 20 mg/kg ZnONPs in the ileum and in the jejunum, it didn't vary ($p > 0.05$) among all groups. Gut health is vital for poultry well-being and efficient production. Zinc is reported to be vital for developing intestinal cells, normal intestinal barrier function, absorptive capacity and regeneration of damaged gut epithelium (Barzegar et al., 2021). The highest VL, CD, and VH/CD in the duodenum, jejunum, and ileum were observed in 20 mg/kg of ZnONPs supplemented groups, suggesting the improvement of mucosal barrier functional capacity (El-Katcha et al., 2017). In addition, the increase in crypt depth of broiler chicken supplemented with different levels of ZnONPs could provide more surface area for nutrient absorption by increasing the proliferation of enterocytes and intestinal mucin secretion because mucin-producing goblet cells are present primarily in the crypts (Tsirtsikos et al., 2012) and the increased VL and VH/CD are appropriate indicators of mucosal integrity and intestinal function (El-Katcha et al. 2017; Lei et al. 2014). Also, several previous studies revealed the improvement of villi length or crypt depth or V/C in the jejunum (Barzegar et al. 2021) and all parts of the intestine (Ali et al., 2017; Hafez et al., 2017; Fawaz et al., 2021) with lower levels (10 – 40 mg/kg) of dietary ZnONPs, but Zhang et al., 2022 reported that supplementation with 80 mg/kg ZnONPs increased the villus width and height in the jejunum.

Table 6. Effect of supplementation of ZnONPs on histo-morphometric parameters of small intestine of 35-day-old broiler chicks (Mean# ± SD)

Position	Indicator	T1	T2	T3	T4	T5	Pvalue
Duodenum	Villi length, *µm*	554.30a ± 134.3	614.46ab ± 110.77	655.02b ± 132.53	681.40b ± 149.91	847.20c ± 138.32	0.00
	Crypt depth, *µm*	38.50a ± 15.31	41.37a ± 8.76	42.07ab ± 8.79	43.93ab ± 11.12	45.53b ± 9.73	0.08
	VH/CD	15.08a ± 4.21	15.70a ± 3.24	18.16b ± 6.29	18.63b ± 4.25	19.01b ± 5.04	0.00
Jejunum	Villi length, *µm*	548.41a ± 77.02	544.42a ± 141.51	591.54ab ± 71.91	616.40bc ± 115.19	650.27c ± 94.61	0.00
	Crypt depth, *µm*	39.15a ± 12.29	39.47a ± 7.42	43.44ab ± 9.67	44.73b ± 6.75	44.82b ± 9.92	0.01
	VH/CD	13.83 ± 3.11	13.47 ± 3.17	14.24 ± 3.12	14.37 ± 3.11	14.66 ± 3.13	0.55
Ileum	Villi length, *µm*	338.82± 80.36	346.79 ± 55.48	369.48 ± 97.35	349.07 ± 84.78	372.43 ± 103.82	0.40
	Crypt depth, *µm*	32.85a ± 7.22	33.30a ± 5.80	36.83b ± 7.71	37.73b ± 6.78	39.69b ± 8.00	0.00
	VH/CD	8.98a ± 2.30	9.23a± 2.13	9.65ab± 3.32	9.94ab± 3.27	10.72b ± 2.81	0.10

#Each value represents the mean of thirty-six observations; V/C – Villi length to crypt depth ratio; Means bearing different superscripts in a row differ significantly with ($p < 0.05$); [T1 - 80 mg/kg ZnO; T2 - Zn met-60 mg/kg; T3 - ZnONPs- 60 mg/kg; T4 - ZnONPs - 40 mg/kg; T5 - ZnONPs - 20 mg/kg]

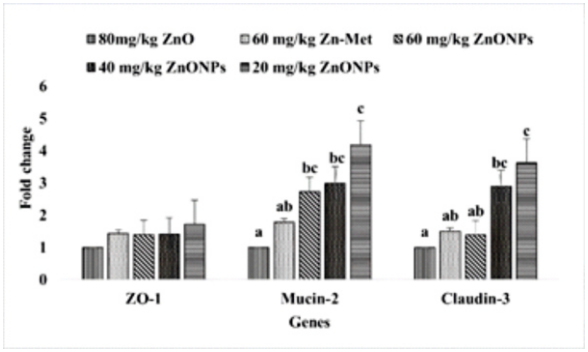


Fig 4. Effects of ZnONPs on mRNA expression of ileal tight junction protein of 35- day old broiler chicks (Mean# ± SD)

In the present study, dietary supplementation of ZnONPs in broiler chicks did not affect the mRNA expression of ZO-1 ($p>0.05$), but numerically, there was an upregulation in the 20 mg/kg ZnONPs, suggesting that nano zinc supplementation had not negatively affected the barrier function. Conversely, mucin-2 and claudin-3 genes were significantly ($p<0.05$) upregulated (4.18 and 3.63-fold respectively) in the 20 mg/kg diet of ZnONPs group compared with other groups (Figure. 4). Therefore, upregulation of these tight junction protein genes might strengthen the epithelial barriers by increasing intestinal integrity and restricting paracellular permeability, thus serving as a front line of defense against invading pathogens into the body (Bobíková et al., 2016). Accordingly, Zhang et al. (2022) reported that compared to negative control group, mucin-2 mRNA expression was higher in the 160 mg/kg ZnONPs group. Contrary to our result, several reports show addition of ZnONPs in the diet of broiler chicks increased the expression of the ZO-1 gene in the jejunum (Zhang et al., 2022; Barzegar et al., 2021; Fatholahi et al., 2021) and did not affect the Mucin-2 gene expression (Zhang et al., 2012, Jose et al., 2018). Taken together, the protective effects of ZnONPs supplementation on gut health might be attributable to the enhancement in the mRNA expression of genes related to ileal tight junction protein and small intestinal morphology of broiler chicks.

CONCLUSION

ZnONPs exhibited superior effects compared to conventional zinc sources in terms of enhancing growth and intestinal barrier functions. These findings validate the potential of dietary supplementation with nano forms of zinc as a novel feed additive, capable of replacing both inorganic and organic forms of zinc. The results suggest that an optimal level of 20 mg/kg of ZnONPs in the

broiler chick diet could be effective. Furthermore, additional research is warranted to unravel the underlying mechanisms of ZnONPs and their effects on diverse biological processes. It is imperative to address regulatory considerations and conduct thorough safety assessments to safeguard the well-being of broiler chickens.

REFERENCES

Abd El-Ghany, W.A., Shaalan, M. and Salem, H. M. 2021. Nanoparticles applications in poultry production: an updated review. World's Poultry Science Journal. 77(4): 1001-1025.

Ali, S., Masood, S., Zaneb, H., Faseeth-ur-Rehman, H., Masood, S., Khan, M., Tahir, S.K. and Rehman, H. 2017. Supplementation of zinc oxide nanoparticles has beneficial effects on intestinal morphology in broiler chicken. Pakistan Veterinary Journal. 37(3): 335-339.

AOAC, 2012. Official Methods of Analysis. 19th Edn. Association of Official Analytical Chemist. Int., Arlington, VA.

Asheer, M., Manwar, S. J., Gole, M. A., Sirsat, S., Wade, M. R., Khose, K. K. and Sajid Ali, S. 2018.Effect of dietary nano zinc oxide supplementation on performance and zinc bioavailability in broilers. Indian Journal of Poultry Science. 53(1): 70-75.

Bhalsing, J., Nagalakshmi, D., Venkateshwarlu, M. and Rama Rao, S.V. 2026. Effect of Dietary Supplementation of Lauric Acid on Performance and Carcass Characteristics in Commercial Broiler Chicken. Indian Journal of Animal Nutrition. 42(3): 403-411.

Bami, M.K., Afsharmanesh, M., Salarmoini, M. and Tavakoli, H. 2018. Effect of zinc oxide nanoparticles and Bacillus coagulans as probiotic on growth, histomorphology of intestine, and immune parameters in broiler chickens. Comparative Clinical Pathology. 27: 399-406.

Barzegar M., Zaghari, M., Zhandi, M. and Sadeghi, M. 2021. Effects of zinc dosage and particle size on gut morphology, tight junctions and TNF-α expression in broiler breeder hens. Journal of Animal Physiology and Animal Nutrition. 106(4): 772-782.

BIS, 2007. Bureau of Indian Standards, Indian Standards for Poultry Feeds - Specifications. 5th Edn. New Delhi.

Bobíková, K., Levkuta, M., Husáková, E., Levkutová, M., Kissová, V. and Ivanišinová, O. 2016. Effect of glycine-zinc complex on mucin and iga expression, secretory iga concentration and lengths of intestinal villi in chickens. Journal of Comparative Pathology. 154:81.

Bratz, K., Golz, G. and Riedel, C. 2013. Inhibitory effects of high dosage zinc oxide dietary supplementation on Camphylobacter coli excretion in weaned piglets. Journal of Applied Microbiology. 115 (5): 1194-1202.

Duncan, D..B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.

El-Katcha, M., Soltan, M.A. and El-Badry, M. 2017. Effect of Dietary Replacement of Inorganic Zinc by Organic or Nanoparticles Sources on Growth Performance, Immune Response and Intestinal Histopathology of Broiler Chicken. Alexandria Journal for Veterinary Sciences. 55(2): 129-145.

Fathi, M., Abdullah, M., Haydari and Tanha, T. 2016. Effects of zinc oxide nanoparticles on antioxidant status, serum enzymes activities, biochemical parameters and performance in broiler chickens. Journal of Livestock Science and Technology. 4(2): 7-13.

Fatholahi, A., Khalaji, S., Hosseini, F. and Abbasi, M. 2021. "Nano-Bio zinc synthesized by Bacillus subtilis modulates broiler performance, intestinal morphology and expression of tight junction's proteins. Livestock Science. 251:104660.

Fawaz R., Ibrahim, R.S., Ali, N.M., Mahmoud, U.T. and Amen, O. 2021. Effect of Dietary Zinc Oxide Nanoparticles on Growth Performance and Clostridium perfringens Infection in Broiler Chickens. Bulgarian Journal of Veterinary Medicine. 1-14.

Hafez A., Hegazi, S.M., Bakr, A.A. and Shishtawy, H.E. 2017. Effect of zinc oxide nanoparticles on growth performance and absorptive capacity of the intestinal villi in broiler chickens. Life Science Journal. 14: 67-72.

Hafez, A., Nassef, E., Fahmy, M., Elsabagh, M., Bakr, A. and Hegazi, E. 2020. Impact of dietary nano-zinc oxide on immune response and antioxidant defense of broiler chickens. Environmental Science and Pollution Research. 27: 19108-19114.

Ibrahim, D., Ali, H.A., and El-Mandrawy, S.A. 2017. Effects of different zinc sources on performance, bio distribution of minerals and expression of genes related to metabolism of broiler chickens. Zagazig Veterinary Journal. 45(3): 292-304.

Jose N, Elangovan A.V., Awachat, V.B., Shet, D., Ghosh, J. and David, C.G. 2018. Response of in ovo administration of zinc on egg hatchability and immune response of commercial broiler chicken. Journal of Animal Physiology and Animal Nutrition. 102(2): 591-595.

Lail, N.U., Sattar, A., Omer, M.O., Hafeez, M.A., Khalid, A.R., Mahmood, S., Shabbir, M.A., Ahmed, W., Aleem, M.T., Alouffi, A. and Almutairi, M.M. 2023. Biosynthesis and characterization of zinc oxide nanoparticles using Nigella sativa against coccidiosis in commercial poultry. Scientific Reports. 13(1): 6568.

Lei X.J., Ru, Y.J. and Zhang, H.F. 2014. Effect of Bacillus amyloliquefaciens based direct-fed microbials and antibiotic on performance, nutrient digestibility, cecal microflora, and intestinal morphology in broiler chickens. Journal of Applied Poultry Research 23(3): 486–493.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25(4): 402-408.

Mahmoud, U.T., Abdel-Mohsein, H.S., Mahmoud, M.A., Amen, O.A., Hassan, R.I., Abd-El-Malek, A.M., Rageb, S.M., Waly, H.S., Othman, A.A. and Osman, M.A. 2020. Effect of zinc oxide nanoparticles on broilers' performance and health status. Tropical Animal Health and Production. 52(4): 2043-2054.

McDonald, P., Edwards, R.A., and Greenhalagh, J.F.D. 2010. Animal Nutrition. New York.

Mozhiarasi, V., Karunakaran, R., Raja, P. and Radhakrishnan, L. 2023. Effects of Zinc Oxide Nanoparticles Supplementation on Growth Performance, Meat Quality and Serum Biochemical Parameters in Broiler Chicks. Biological Trace Element Research. 1-16.

National Research Council, Nutrient requirements of dairy cattle: 2001. National Academies Press.

Hazra, P., Kumar, P., Soren, S., Samanta, I. and Mandal, G.P. 2025. Performance of Broiler Chickens Supplemented with Zinc oxide Nano Particles. Indian Journal of Animal Nutrition. 42(1): 119-127.

Qu, J., Zuo, X., Xu, Q., Li, M., Zou, L., Tao, R., Liu, X., Wang, X., Wang, J., Wen, L. and Li, R. 2023. Effect of Two Particle Sizes of Nano Zinc Oxide on Growth Performance, Immune Function, Digestive Tract Morphology, and Intestinal Microbiota Composition in Broilers. Animals. 13(9): 1454.

Rama Rao, S. V., Raju, M. V. L. N., Nagalakshmi, D., Anusha Savaram, Sai Pavan, S. Prakash, B., Srilatha, T., Paul, S. S. and Kannan, A. 2025. Organic Trace Minerals at Lower Concentrations Can Replace Inorganic Trace Mineral Premix in Broiler Chicken Diet. Indian Journal of Animal Nutrition 42 (2): 292-299.

Rio, D.C., Ares, M., Hannon, G.J. and Nilsen, T.W. 2010. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harbor Protocols. 2010(6).

Tsirtsikos P., Fegeros, K., Kominakis, A., Balaskas C. and Mountzouris, K. 2012. Modulation of intestinal mucin composition and mucosal morphology by dietary phytogenic inclusion level in broilers. Animal. 6:1049– 1057.

Xie, Z., Zhao, Q., Wang, H., Wen, L., Li, W., Zhang, X., Lin, W., Li, H., Xie Q. and Wang, Y. 2020. Effects of antibacterial peptide combinations on growth performance, intestinal health, and immune function of broiler chickens. Poultry Science. 99(12): 6481-6492.

Uni ZEHAVA, Ganot SAHAR and Sklan DAVID. 1998. Post hatch development of mucosal function in the broiler small intestine. Poultry Science. 77(1): 75-82.

Yusof, H.M., Mohamad, R., Zaidan, U.H., Arshad M.A. and Samsudin, A.A. 2023. Effects of dietary zinc oxide nanoparticles supplementation on broiler growth performance, zinc retention, liver health status, and gastrointestinal microbial load. Journal of Trace Elements and Minerals. 4: 100072.

Zhang, J., Yu, C., Li, Z., Li, J., Chen, Y., Wang, T. and Wang, C. 2022. Effects of zinc oxide nanoparticles on growth, intestinal barrier, oxidative status and mineral deposition in 21-day-old broiler chicks. Biological Trace Element Research. 200(4): 1826-1834.

Zhao, C.Y., Tan, S.X., Xiao, X.Y., Qiu, X.S., Pan, J.Q. and Tang, Z.X. 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. Biological Trace Element Research. 160: 361-367.

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Comparative Study of Proximate Composition, Minerals, Vitamins and Fatty Acid Contents in *Labeo rohita* (Hamilton, 1822) Reared In Freshwater and Treated Wastewater

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ABSTRACT

The study was conducted to investigate the variation in proximate composition, mineral contents, vitamin contents and fatty acid profile of *Labeo rohita* reared in Freshwater (FW) and wastewater (WW) of different weight ranges. The samples were collected from different location of West Bengal and were categorized based on body weight as 1-500(g) as BW1 and 501-2000 (g) as BW2 for both (FW and WW) reared system and they were grouped into 4 treatments viz., TR1(BW1FW), TR2(BW1WW), TR3(BW2FW) and TR4(BW2WW). The protein content was significantly higher (P<0.05) in TR4(BW2WW) reared in WW. Sodium content was significantly higher (P<0.05) in TR1(BW1FW), reared in Freshwater whereas Potassium content significantly higher (P<0.05) in TR4(BW2WW). Iron and Zinc content in rohu was significantly higher (P<0.05) in TR2 and TR3. All the fat-soluble vitamins content (A,D,E &K) were significantly higher (P<0.05) in TR1(BW1FW) reared in Freshwater. The SFA content in rohu sample was significantly (P<0.05) higher in TR2 whereas palmitic acid was significantly higher (P<0.05) in TR1 and TR2.MUFA and oleic acid content in rohu is significantly higher (P<0.05) higher in TR4. PUFA, α linoleic acid, EPA and DHA are significantly higher (P<0.05) TR4 having weight range (501-2000g) reared in WW. The protein, PUFA, α linoleic acid, EPA, DHA, MUFA, Oleic acid and potassium is higher in rohu of weight range (501- 2000g) reared in WW (TR4(BW2WW). Sodium and Vitamin A,D ,E and K is higher in rohu of weight range (1-500g) reared in FW (TR1(BW1FW).

KEYWORDS: Fatty acids, Mineral, Proximate composition, Rohu, Vitamin

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INTRODUCTION

The fisheries and aquaculture sectors are vital in ensuring global food and nutritional security. As per FAO (2022), the global production of aquatic animals was a staggering 178 million tonnes in 2020, with aquaculture contributing significant 87.5 million tones. The present practice in India is bispecies aquaculture, in which 80% to 90% of the population is shared by rohu (Giri, 2024). Globally, more than 1 billion people rely on fish for consumption and livelihood (FAO, 2016). Fish are excellent sources of essential nutrients like protein, amino acids and fatty acids (Zula and Desta, 2021). It plays a major role in the creation of growth hormones of the body (Holecek, 2020). The protein, fat and moisture content range between (15-30), (0-25) and (50-80) % respectively (Chakma et al., 2020). Apart from that minerals play a pivotal role in maintaining acid-base and water balance, formation of teeth structure and bones and accelerating metabolic reactions in the human body (Zhang et al., 2020). In catla the protein, zinc and selenium were in WW reared catla compared to FW as reported earlier (Paul et al., 2018). Fish consumption plays an important role in preventing coronary artery disease in humans, lowering the risk of breast cancer, asthma, inflammatory bowel disease, rheumatoid arthritis etc (Ullah et al., 2022).

Fish is a source of macro minerals (Ca, Mg, Na, K, P, Cl, S) and micro minerals (Fe, Zn, Mn, Cu,I, Co, Ni, F, V, Cr and etc.) minerals. The analysis of chemical composition of fish gives assessment of food composition of fish, its physiological condition and can serve as a guide for any future feed for fish in captivity (Dempson et al., 2004). Hence, this study was undertaken to find out the differences in chemical composition of fish reared in fresh and wastewater conditions.

MATERIALS AND METHODS

The samples were collected from different places of West Bengal viz., Rahara fish farm of ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga (Odisha), Malancha, Berhampur, Barasat, Mudiyali Fishery co-operative, Pandua, Bongaon, Nazat; from each location 20 nos. of rohu samples were collected from each Freshwater (FW) and Wastewater (WW) rearing systems. Weight of the fish samples were noted down at the time of collection which was divided into BW1 (1-500g) and BW2 (501-2000g). As per the weight ranges BW1 (1-500g) and BW 2 (501-200g) and water sources Freshwater (FW) and Wastewater (WW); grouped to four treatments viz., TR1(BW1FW), TR2(BW1WW), TR3 (BW2 FW) TR4 (BW2WW). The collected fish samples were immediately stored in an ice container and after brought into the laboratory prepared for evisceration and the head was removed. The representative portion of the edible part was taken and homogenized in a mixer for further analysis. The sampling procedure and sample preparation for analysis were followed as per the methodology of the Outreach Project on Nutrient Profiling of ICAR (Sankar et al., 2010). The sample preparation for vitamin analysis was done as per (Sankar et al., 2010). 10 g of Fish tissue was grinded with anhydrous sodium sulphate and extracted oil using 2:1 chloroform: methanol after adding BHA. Finally, the non-saponifiable matter was filtered through 0.45µ syringe filter and stored under refrigerator. Vitamins were quantified by injecting 5µl of prepared sample in High pressure liquid chromatography (HPLC). The HPLC consisting of a quaternary gradient pump, programmable variable wavelength, UV detector was used for the analysis. The wavelength used for eluting different vitamins is as follows. 265nm for vitamin D, 325nm for vitamin A, 291nm for vitamin E and 250nm for vitamin K. The vitamin content in the unknown sample was determined from the linear graph drawn for the standard.

Proximate composition of fish tissue was analyzed as per (AOAC, 2005). The mineral (Na, K, Fe, Mn, Zn and Se) assay was done as per (AOAC, 2005; Paul et al., 2014). Fatty acid profile was analyzed as per standard protocol of Gas Chromatography. Pooled samples were extracted as per (Folch et al., 1957) using chloroform: methanol in 2: 1 ratio (v/v) as solvent system that contained 0.01% butylated hydroxyl anisole as an antioxidant. Fatty Acid Methyl Esters were prepared by the transmethylation with boron trifluoride in methanol from lipids fraction according to (Metcalfe et al., 1966). The FAMEs were quantified by injecting 1 µL (50:1 split ratio) into a Gas Chromatograph/GC (Perkin Elmer, CLARUS 480). The oven temperature was programmed from an initial temperature at 30^0C rising to 140^0C (hold time 4 minute) and up to 200^0C. Nitrogen gas was used as a carrier gas. The injection port and the flame ionization detector were maintained at 260^0C and 300^0C. GC operating software "Total Chrom" was followed. Identification of individual fatty acids was done by comparison of retention time to those of standards (SUPELCO, Cat No. 47885-U) and quantification by comparing with respective areas as per the method of Paul et al., (2015b). Data were subjected to statistical analysis (Snedecor and Cochran, 1994) by one way ANOVA, and the least significant difference was used for comparison of the mean values.

RESULTS AND DISCUSSION

The proximate composition, mineral concentration and vitamin content of rohu of different weight ranges of WW and FW are presented in Table 1. The moisture and protein content ranged from 76.96-79.09 and 13.59-16.52 respectively. The crude protein content of rohu was significantly higher ($P<0.05$) higher in TR1 and TR2. Moisture and lipid content of rohu did not differ significantly among the treatments.

The moisture content of IMC ranged from 76 to 77(%) as reported by (Ullah et al., 2022) was in agreement with our result. The moisture content of the rohu in the present study was also within the ranged reported by (Paul et al., 2016; Shakir et al., 2013). The moisture content of the fish in the present study was higher than earlier reports in carps (Jabeen and Chaudhary, 2011) However, Sankar and Ramachandran, 2001 reported the 77-81% moisture content in IMC, which was higher than our findings.

In the present study protein content in the rohu muscles ranged from 13.59 to 16.52, was in the range of protein levels for carp (FAO, 2008). The protein content of rohu as reported in our study was in agreement with earlier reports (Paul et al., 2016; Shekhar et al., 2004). (Hossain et al., 2015) reported protein content in *Puntius gonionotus* is 16.7% which is in agreement with our results

Table 1. Nutrient composition, mineral concentration and vitamin content of rohu of different weight ranges collected from freshwater (FW) and wastewater (WW).

Particulars	TR1 (BW1FW)	TR2 (BW1WW)	TR3(BW2FW)	TR4 (BW2WW)
Nutrient	Composition	(% WW basis)		
Moisture ^{NS}	76.96 ±1.11	79.09±0.33	76.54±0.56	76.69±0.57
Crude Protein	13.59^{a} ±0.15	14.76^{b}±0.21	15.79^{bc}±0.66	16.52^{c}±0.62
Crude lipid	1.80 ±0.26	1.93±0.30	2.04±0.2	2.16±0.23
Ash	3.35^{b} ±0.34	3.38^{b}±0.07	2.33^{a}±0.23	2.57^{a}±0.14
Mineral	content (ppm)			
Sodium (Na)	189.54^{b}±7.94	214.94^{c}±2.94	164.73^{a}±5.78	189.44^{b}±4.77
Potassium (K)	262.73^{b} ±2.60	268.61^{bc} ±2.53	241.77^{a}±5.92	278.93^{c} ±3.96
Iron (Fe)	2.59^{c} ±0.55	1.34^{b}±0.22	1.73^{b}±0.22	1.2^{a}±0.07
Manganese (Mn)	0.43±0.23	0.55±0.02	0.45±0.07	0.53±0.03
Zinc (Zn)	3.66^{c} ±0.34	1.9^{b} ±0.2	1.53^{b} ±0.07	1.30^{a}±0.04
Selenium (Se)	0.80^{c}±0.18	0.54^{ab}±0.06	0.76^{bc}±0.15	0.44^{a}±0.09
Fat soluble Vitamin	Content			
Vitamin A (I.U/100g)	19.63^{c} ±1.18	11.73^{b}±1.74	4.20^{a}±0.59	7.67^{a}±1.62
Vitamin D(I.U/100g)	208.40^{c}±4.17	30.80^{a}±1.68	31.60^{a}±1.02	62.40^{b}±1.24
Vitamin E(I.U/100g)	0.88^{b}±0.21	0.16^{a}±0.18	0.54^{bc}±0.16	0.7^{c}±0.18
Vitamin K (µg/100g)	1.45^{c}±0.48	0.16^{a}±0.08	0.41^{bc}±0.03	0.99^{c}±0.17

Data are expressed as Mean ±SE. Values bearing different superscripts in a row differ significantly (P<0.05)

Felts et al (1996) stated that 16.60-19.59% of protein content was found in IMC. The data stated were higher than our findings. In the present study the fat content of FW and WW reared rohu for both the weight ranges varied from 1.80-2.16%. Paul *et al*. reported lipid content in rohuas 1.30-2.94% and Shakir et al.(2013) reported 1.0- 2.71 % lipid in IMC which is in agreement with our present study. The crude lipid content of the present study was below the range as reported (Shekhar et al., 2004) fat content of rohu was 1.2-1.5%. However, Hossain et al. (1999) have shown higher crude lipid content than our findings.

Fish muscles and bones behave as an excellent source of dispensable minerals and about 65% of minerals are stocked in the skeleton, particularly vertebra (Njinkoue et al., 2016). Generally, the ash content of the fish samples indicates its potential as a source of minerals such as zinc, magnesium, iron, potassium and sodium (Bolawa et al., 2011). The ash content in the present study was ranged from 2.33-3.38 which is in agreement with the findings of (Paul et al., 2016 and Paul et al., 2015a). Jabeen and Chaudhary (2011) found a higher content of ash in *L. rohita* than the values obtained in our study. Whereas Shakir et al. (2013) reported a lower ash content in IMC than our findings. Sodium and potassium content of rohu was significantly higher (P<0.05) in TR1 and TR4 as given in Table 1. Iron and zinc content of rohu was significantly higher (P<0.05) higher in TR2 and TR3. Selenium content was significantly higher (P<0.05) in Tr1.

The higher concentration of potassium and lower concentration of sodium found in the present study making it an excellent source of these minerals to utilize for the improvement of public health, particularly in the prevention of the cardiovascular disease (Perez et al., 2014). The current findings was also in corroboration with Paul et al. (2016) who recorded higher concentration of potassium and lower sodium level in rohu.

Iron plays an important role in oxidation-reduction reaction and electron transport associated with cellular respiration (Paul and Mukhopadhyay, 2001). Iron is important for the formation of haemoglobin, necessary to form red blood cells (Oksuz et al., 2011). The level of Fe in rohu in the

present study was comparable to magur and singhi (Paul et al., 2015a) and Indian Major Carp (Paul et al., 2016) but higher than freshwater small indigenous fishes (Jabeen et al*.,* 2015 and Hossain et al., 2015).

Manganese is mainly used as co-factor for the enzymes kinase, peptidase, arginase, succinic decarboxylase. It has also been implicated in oxidative phosphorylation. It is responsible for normal functioning of brain and proper metabolism of lipid and carbohydrate (Chanda et al., 2015). The Mn content in the studied fish was similar to the values reported by (Paul et al., 2015a).

Zinc has a structural role in nucleoproteins and involved in prostaglandin metabolism (Lall, 2002). The Zn contents in the current study were under the recommended maximum limits of 50mg/kg in fish (WHO, 1985). The concentration of zinc in rohu was in comparison with our earlier findings (Paul et al., 2016). The concentration of Zn in the studied fish was lower than the values as reported earlier (Jabeen et al., 2015).

Selenium has shown to decrease the toxicity of methyl mercury and cadmium (Watanabe et al., 1997). It is implicated in the metabolism of tocopherol compounds (Chanda et al.,2015). Lower levels of selenium have been correlated with increased risk of cancer and renal disease (Chanda et al., 2015). Minerals are essential building blocks for many enzymes and metabolic processes as well as contributing to fish growth and a deficiency of these critical nutrients causes lower productivity and disease (Marichamy et al., 2012).

Vitamin A ranged from 4.20-19.63 (I.U/100g) in FW reared rohu and ranged varied from 7.67-11.73 in WW reared rohu for both the weight ranges. Vitamin A, D, E and K was significantly higher in TR1 having weight range (1-500g) reared in Freshwater. Fish acts as a good source of fat-soluble vitamins viz., A, D, E and K. Vitamin A also play a major role in immunity, growth, oxidation resistance, glucose and lipid metabolism, erythropoiesis and in the regulation of iron metabolism (NRC, 2011). Most of the vitamin A in fish is concentrated in the eyes and viscera. This distribution makes the cleaning practice extremely important for the retention of vitamin A. Cleaning practice depends on the fish species, size of the fish and the person cleaning the fish. This distribution makes the cleaning practice extremely important for the retention of vitamin A. Cleaning practices depend on the fish species, size of fish and the person cleaning the fish (Roos et al., 2002). Fat soluble vitamin content in fish flesh is affected by the level of fat (Ozyurt et al., 2009). Vitamin A content of rohu in the present study was found to be higher than salmon, mackerel and dogfish (Dias et al., 2003), rainbow trout 74.33 IU/100g (Stancheva et al., 2010); common carp 75.06 IU/100g and European catfish 21.0 IU/100g (Ozyurt et al., 2009).

Vitamin D content varies between 0.5 and 30 mg/100 g fish muscle in various species (Mattila et al., 1995). Deficiency of vitamin D leads to rickets, osteomalacia, low bone mineral density and thereby osteoporosis. The form of vitamin D found in fish is vitamin D_3 (Cholecalciferol). Vitamin D content in the present study was lower than earlier reports (Hansen et al., 1998).

In the present study vitamin E content ranged from 0.16-0.88 IU/100g, was comparable with common carp (0.46 mg/100g) and European catfish (0.80mg/100g) but lower than pike perch (0.94 mg/100g) (Ozyurt et al., 2009). Earlier studies have shown that vitamin E protects highly unsaturated fatty acids such as DHA and EPA from attack and oxidation by free radicals, reduce lipid peroxidation and help accumulation of polyunsaturated fatty acids (Lebold et al., 2011).

Vitamin K belongs to the lipid soluble vitamins which naturally occur as phylloquinone (vitamin K_1) and menaquinone (vitamin K_2). Our body needs vitamin K for modification in post translational stages of certain proteins required for blood coagulation and in metabolic pathways in bone and other tissues (Halver, 2002).

Fatty acid profile of different weight ranges of rohu reared in FW and WW is presented in Table 2. Perusal of Table 2 reveals that saturated fatty acid (SFA) in rohu is significantly ($P<0.05$) higher in TR 2 followed by TR 1. The predominant fatty acid among SFA; palmitic acid significantly higher ($P<0.05$) higher in TR1 and TT2 in rohu sample. The other fatty acid (stearic acid) is significantly higher in TR 4.SFA is higher in rohu reared in wastewater of weight range (1-500 g).

Table 2. Fatty acid profile (% of total fatty acid) of rohu different weight ranges collected from freshwater (FW) and wastewater (WW)

Particulars	TR1 (BW1FW)	TR2 (BW1WW)	TR3(BW2FW)	TR4 (BW2WW)
Lauric acid	0.03^{a}±0.005	0.13^{b}±0.05	0.16^{b} ±0.0.02	1.24^{c}±0.12
Tridecanoic acid	0.11^{c}±0.01	20.06^{b}±0.05	0.26^{d}±0.045	0.02^{a}±0.01
Myristic acid	1.82^{a}±0.19	2.53^{b}±0.49	1.99^{a} ±0.035	ND
Pentadecanoic acid	1.23^{b}±0.18	50.70^{a}±0.055	ND	ND
Palmitic acid	68.03^{c}±1.79	70.42^{c} ±1.48	57.44^{b}±2.26	34.27^{a}±1.91
Heptadecanoic acid	0.71^{a}±0.09	0.92^{ab} ±0.10	1.97^{bc} ±0.07	2.04^{c} ±0.93
Stearic acid	1.22^{a}±1.06	4.01^{b}±0.03	4.81^{b}±0.50	9.68^{c}±0.44
Arachidic acid	1.70^{b}±1.44	0.16^{a}±0.005	0.27^{a}±0.01	0.38^{a}±0.02
Heneicosanoic acid	1.72^{b} ±0.44	1.10^{a}±0.10	3.36^{c} ±0.04	3.14^{c} ±0.18
Σ SFA	76.49^{c}±1.82	81.85^{d}±0.63	70.69^{b}±2.63	51.14^{a}±0.07
Myristoleic acid	0.44^{b}±0.38	0.06^{a}±0.005	0.05^{a}±0.01	0.02^{a}±0.01
Pentadecanoic acid	1.41^{b}±0.07	ND	0.02^{a}±0.01	0.04^{a}±0.005
Palmitoleic acid	1.62^{b}±0.08	2.82^{c}±0.32	ND	0.77^{a} ±0.09
Heptadecanoic acid	0.22^{a}±0.19	0.26^{a}±0.18	0.52^{b} ±0.04	0.98^{c} ±0.04
Oleic acid	5.97^{b}±0.12	0.05^{a}±0.01	10.28^{b}±0.74	27.82^{c}±3.31
Eicosanoic acid	ND	0.88^{b}±0.06	0.31^{a}±0.03	0.91^{b}±0.08
Erucic acid	1.57^{a}±0.07	2.97^{b}±0.37	ND	1.25^{a}±0.09
ΣMUFA	11.22^{b}±0.47	7.06^{a}±0.54	11.32^{b}±0.90	29.35^{c}±3.16
Linoleic acid	4.04^{b}±0.20	6.64^{c}±0.12	8.07^{d}±0.61	0.12^{a}±0.005
α-Linolenic acid	3.30^{a}±0.18^{b}	2.73^{a}±0.13	7.16^{b}±0.84	10.32^{c}±0.70
ϒ-Linolenic acid	0.69 ±0.40	0.89 ±0.53	0.37 ±0.03	0.45 ±0.07
EPA	1.29^{b}±0.14	0.14^{a}±0.005	0.99^{a}±0.03	3.58^{c}±1.57
DHA	0.81^{b}±0.26	0.84^{b}±0.08	0.47^{a}±0.07	4.42^{c}±0.17
ΣPUFA	13.01^{a}±2.33	12.24^{a}±0.46	18.72^{b}±1.73	20.78^{b}±1.05
EPA+DHA	2.1^{a}±0.93	0.99^{a}±0.09	1.46^{a}±0.1	8.0^{b}±1.70

Data are expressed as Mean ±SE. Values bearing different superscripts in a row differ significantly (P<0.05). EPA: Eicosapentanoic acid, DHA: Docosahexanoic acid, SFA: Saturated Fatty acid, MUFA: Monounsaturated fatty acid and PUFA: Polyunsaturated fatty acid

Fatty acid composition of aquatic animals is influenced by intrinsic variables like species, sex, age and size and extrinsic factors such as diet, salinity, temperature, geographical regions and the general rearing conditions (Rahman et al., 1995). Fatty acids in fishes are derived from two main sources, viz., biosynthesis and diet (Kamler et al., 2001). The chain length varies from C14-C24 of varying degrees of unsaturation, from saturated to polyunsaturated (Swapna et al., 2010). The fish lipid is a rich source of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which is beneficial for human improvement in several types of disease (Panchal and Brown, 2021). In this study the PUFA content was lesser than the SFA and MUFA. Other researchers have also shown the freshwater fish have lower PUFAs (Paul et al., 2015b) because freshwater fishes feed largely on vegetation and plant materials (Vlieg and Body, 1988). Palmitic acid content among the SFA was maximum in the studied fish which is in agreement with earlier reports (Paul et al., 2015b). The monounsaturated fatty acid(MUFA) and oleic acid is significantly (P<0.05) higher in TR4 having weight range (5001-2000g) reared in wastewater. These data confirmed earlier observations of (Gutierrezand de Silva, 1993). The MUFA contains 31-39% of the total fatty acids in the major carps reported by (Sankar and Ramachandran, 2001) which was higher than our findings. Perusal of Table 2 reveals that the Polyunsaturated fatty acid (PUFA), α linolenic acid, EPA and DHA is significantly (P<0.05) higher in TR 4 having weight range (5001-2000g) reared in wastewater. (Ward and Singh, 2005) reported that α-Linolenic acid is the most abundant fatty acids among PUFA which was in comparable with our findings. In the present findings rohu contained

good amount of long chain PUFA which was in agreement with (Memon et al., 2011). In present study, linoleic acid range was 0.12-8.07, similar to result obtained by (Swapna et al., 2010). The maximum amount of PUFA was estimated to be 18.72-20.78 in WW reared rohu which was similar to result reported by (Jakhar et al., 2012; Swapna et al.,2010). Fish muscles contain higher levels of ω-3 PUFAs which are known to be anti-atherosclerotic, anti-thrombotic and anti-arrhythmic (Givens et al., 2006). DHA and EPA had been reported to have preventive effects on human coronary artery disease.

CONCLUSION

The aim of this paper was to investigate the proximate composition, mineral concentration, vitamin and fatty acids composition of rohu reared in freshwater and wastewater. The variation in nutrient composition was observed in fish reared in freshwater and wastewater environment. Natural and artificial diet, aquatic environment, habitat, physiology and morphology of fish that influence the nutrient composition of fish. Results of the presently reported study indicated that the protein, PUFA, α linoleic acid, EPA, DHA, MUFA, Oleic acid and potassium is higher in rohu of weight range (501- 2000g) reared in wastewater. Sodium and Vitamin A,D,E and K is higher in rohu of weight range (1-500g) reared in freshwater.

REFERENCES

AOAC. 2005. Official Methods of Analysis.18th Edn. Association of Official Analytical Chemists. Washington, D.C, USA.

Bolawa, O.E., Gbenle, G.O., Ayodele, S.O., Adewusi, O.R., Mosuro, A.O. and Apata, O.S. 2011. Proximate composition properties of different fish species obtained from lagos, Nigeria. International Journal of Food Safety. 13: 342-344.

Chakma, S., Saha, S., Hossain, N., Rahman, M.A., Akter, M., Hoque, M.S., Ullah, M.R., Mali, S.K. and Shahriar, A. 2020. Effect of frozen storage on the biochemical, microbial and sensory attributes of Skipjack Tuna (*Katsuwonus pelamis*) fish loins. Journal of Applied Biology and Biotechnology. 8 (4): 58–64.

Chanda, S., Paul, B.N. and Ghosh, K. 2015. Dietary Essentiality of Trace Minerals in Aquaculture: an overview. Agricultural Review. 36(2): 100-112.

Dempson, I.B., Schwarz, C.J., Shears, M. and Furey, G. 2004. Comparative proximate body composition of Atlantic salmon with emphasis on parr from fluvial and lacustrine habitats. Journal of Fish Biology. 64: 1257-1271.

Dias, M.G., Sanchez, M.V., Bartolo, H. and Oliveira, L. 2003. Vitamin content of fish and fish products consumed in Portugal. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2(4):510–513.

FAO. 2022. The state of world fisheries and Aquaculture 2022. Towards blue transformation. Rome: FAO.doi:10.4060/cc0461 en.

FAO. 2016. State of the World's Fisheries and Aquaculture. Rome: FAO.

FAO. FAO Fisheries and Aquaculture- Chemical elements of fish. 2008; http://www.fao.org/fishery/topics/14820/en.

Felts, R.A., Rajts, F. and Akhtaruzzaman, M. 1996. Small indigenous fish species culture in Bangladesh. Technical brief.IFADEP Sub-project- 2. Development of Inland Fisheries. 41 pp.

Folch, J., Lees, M. and Slonae-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226: 497-509.

Giri, S.S. 2024. Sustainable aquaculture practices in South Asia: a comparative analysis of feed formulation and utilization. Animal Frontiers: 14, pp 6-16.

Givens, D.I., Kliem, K.E. and Gibbs, R.A. 2006. The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. Meat Science. 74: 209-218.

Gutierrez, L.E. and da Silva, R.C.M. 1993. Fatty acid composition of commercially important fish from Brazil. Scientia agricola Piracicaba. 50(3): 478-483.

Halver, J.E. 2002. The vitamins. In: *Fish Nutrition* (J.E. Halver and R.W.Hardy, Eds.) 3rd Edn., Academic Press, New York. pp 62-132.

Hansen, M., Thilsted, S.H., Sandstrom, B., Kongsbak, K., Larsen, T., Jensen, M. and Sorensen, S.S. 1998. Calcium absorption

from small soft-boned fish. Journal of Trace Elements in Medicine and Biology. 12: 148–54.

Hossain, Md., Afroz, N., Haque, H.Z. Md and Begum, M. 2015. Evaluation of nutritional properties of small indigenous fishes species in Bangladesh. International Journal of Biosciences. 6: 102-109.

Hossain, M.A., Afsana, K. and Azad, Shah A.K.M. 1999. Nutritional value of some small indigenous fish species (SIS) of Bangladesh. Bangladesh Journal of Fisheries Research. 3: 77-85.

Jabeen, F., Aasma, N., Hossain, S.M., Chaudhry, A.S., Irfan, M., Shakeel, M., Shabbir, S., Yaqub, S., Ahmad, S. and Shaheen, T. 2015. Chemical and mineral composition of *Cyprinus carpio, Labeo rohita* and *Wallago attu* inhabiting river Indus in Mianwali district. International Journal of Biosciences. 6(5): 333-342.

Jabeen, F. and Chaudhary, A.S. 2011. Chemical compositions and fatty acid profiles of three freshwater fish species. Food Chemistry. 125: 991-996.

Jakhar, J.K., Pal, A.K., Reddy, A.D., Sahu, N.P., Venkateshwarlu, G. and Vardia, H.K. 2012. Fatty acid composition of some selected Indian fishes. African Journal of Basic and Applied Science. 4(5): 155-160.

Kamler, E.B., Krasicka, S. and Rakusa-Suszczewski. 2001. Comparison of lipid content and fatty acid composition in muscle and liver of two Notothenioid fishes from Admiralty Bay (Antartica): An eco-physiological perspective. Polar Biology. 24: 735-743.

Lall, S.P. 2002. The minerals. In: Fish nutrition, 3rd Edn. J.E. Halver and R.W. Hardy (Eds). Academic Press, San Diego, CA. pp: 259-308.

Lebold, K.M., Jump, D.B., Miller, G.W., Wright, C.L., Labut, E.M., Barton, C.L. and Traber, M.G. 2011. Vitamin E deficiency decreases long-chain PUFA in zebrafish (*Danio rerio*). Journal of Nutrition. 141(12): 2113.

Marichamy, G., Badhul Haq, M.A., Vignesh, R., Sedhuraman, V. and Nazar, A. 2012. Assessment of Proximate and mineral composition of twenty edible fishes of Parangipettai coastal waters. International Journal of Pharma and Bioscience. 3:54–64.

Mattila, P., Piironen, V., Uusi-Rauva, E. and Koivistoinen, P. 1995. Cholecalciferol and 25-Hydroxycholecalciferol contents in fish and fish products. Journal of Food Composition and Analysis. 8: 232–243.

Memon, N.N., Farah, N.L., Bhanger, M.I. and Balouch, A. 2011. Changes in a fatty acid composition in muscle of three farmed carp species (*Labeo rohita, Corrhinus mrigala, Catla catla*) raised under the same conditions. Food Chemistry. 126:405-410.

Metcalfe, L.D., Schimtz, A.A. and Pelka, J.R. 1966. The rapid preparation of fatty acid esters from lipid for gas chromatographic analysis. Journal of Analytical Chemist. 38: 514-515.

National Research Council. *Nutrient requirements of fish and shrimp.* National Academies Press; 2011.

Njinkoue, J. M., Gouado, I., Tchoumbougnang, F., Ngueguim, J. Y., Ndinteh, D. T., Fomogne-Fodjo, C. Y., and Schweigert, F. J. 2016. Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian coast: *Pseudotolithus typus* (Bleeker, 1863) and *Pseudotolithus elongatus* (Bowdich, 1825). NFS Journal. 4: 27–31.

Oksuz A, Ozyılmaz, A. and Kuver, S. 2011. Fatty acid composition and mineral content of *Upeneus moluccensis* and *Mullus surmuletus.* Turkish Journal of Fisheries and Aquatic Science. 11: 69-75.

Ozyurt, G., Polat, A. and Loker, G.B. 2009. Vitamin and mineral content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*) and European catfish (*Siluris glanis*). Turkish Journal of Veterinary & Animal Sciences. 33: 351-356.

Panchal, S.K. and Brown, L. 2021. Addressing the insufficient availability of EPA and DHA to meet current and future nutritional demands. Nutrients. 13 (8): 2855.

Paul, B.N., Singh, P., Bhowmick, S., Mandal, R.N., Adhikari,S., Pandey, B.K., Das, A. and Chakrabarti, P.P. 2018. Proximate, mineral and fatty acid composition of *Catla catla* reared in Freshwater and Wastewater environment. Indian Journal of Animal Nutrition. 35(4):463-468.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S,S. 2016. Proximate, mineral and vitamin contents of Indian Major Carp. Indian Journal of Animal Nutrition. 33(1): 102-107.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2015a. Proximate and Mineral composition of Magur (*Clarias batrachus*) and Singhi (*Heteropneustes fossilis*). Indian Journal of Animal Nutrition. 32: 454-457.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S. and Giri, S.S. 2015b. Fatty acid profile of Indian Major Carp. Indian Journal of Animal Nutrition. 32:221-226.

Paul, B.N., Chanda, S., Das, S., Singh, P., Pandey, B.K. and Giri, S.S. 2014. Mineral assay in atomic absorption spectroscopy. Beats of Natural Science. 1: 1-l7.

Paul, B.N. and Mukhopadhyay, P.K. 2001. Importance of trace minerals in aquaculture nutrition. Fishing Chimes. 21: 34-36.

Perez, V., Ellen, T. and Chang, V. 2014. Sodium-to-potassium ratio and blood pressure, hypertension, and related factors. Advances in Nutrition. 5: 712–741.

Rahman, A.S., The, S.H., Osman, H. and Daud, N.M. 1995. Fatty acid composition of some Malaysian freshwater fish. 54: 45-49.

Roos, N., Leth, T., Jakobsen, J. and Thilsted, S.H. 2002. High vitamin A content in some small indigenous fish species in Bangladesh: perspectives for food-based strategies to reduce vitamin A deficiency. International Journal of Food Sciences and Nutrition. 53: 425–437.

Sankar, T.V., Susheela, M., Anandan, R., Asha, K.K. and Mohanty, B.P. 2010. Annual Report. Nutrient Profiling of Fish. Central Institute of Fisheries Technology, Indian Council of Agricultural Research. Cochin, Kerala, India. p 1-61.

Sankar, T.V. and Ramachandran, A. 2001. Changes in biochemical composition in Indian Major Carps in relation to size. Fishery Technology. 38: 22-27.

Shakir, H.A., Qazi, J.I., Chaudhary, A.S,, Hussain, A. and Ali, S. 2013. Nutritional comparison of three fish species co-cultured in an earthen pond. Biologia (Pakistan). 59, 353–356.

Shekhar, C., Rao, A.P. and Abidi, A.B. 2004. Changes in muscle biochemical composition of *Labeo rohita* (Ham.) in relation to season. Indian Journal of Fisheries. 51: 319-323.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. 8th Edn. Oxford and IBH Publishing Co.Pvt. Ltd., New Delhi.

Stancheva, M., Dobreva, D., Merdzhanova, A. and Galunska, B. 2010. Vitamin content and fatty acids composition of rainbow trout (*Oncorhynchus mykiss*). Plovdiv University, Paisii, Hilendarski – Bulgaria, Scientific papers. 37(5):117–124

Swapna, H.C., Kumar, R.A., Bhaskar, N. and Sachindra, N.M. 2010. Lipid classes and fatty acid profile of selected Indian freshwater fishes. Journal of Food Science and Technology. 47(4): 394-400.

Ullah, M.R., Roy, N., Chakma, S. and Rahman, M.A. 2022. Biochemical composition of some catfishes from a coastal river of Bangladesh in relation to a biometric indicator. Archives of Agriculture and Environmental Science. 7 (2): 185–191.

Vlieg, P. and Body, D.B. 1988. Lipid contents and fatty acid composition of some New Zealand freshwater foinfish and marine finfish, shellfish and roes. New Zealand Journal of Marine and freshwater Research. 22: 151-162.

Ward, O.P. and Singh, A. 2005. Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry. 40: 3627-3652.

Watanabe, T., Kiron, V. and Satoh, S. 1997. Trace minerals in fish nutrition. Aquaculture. 151:185-207.

WHO. (World Health Organization). Guidelines for drinking water quality 1, 130. 1985; Recommendation WHO, Geneva. ISBN: 978 92 4 154761 1.

Zhang, X., Ning, X., He, X., Sun, X., Yu, X., Cheng, Y., Yu, R. and Wu, Y., 2020. Fatty acid composition analyses of commercially important fish species from the Pearl River Estuary, China. PLoS One. 15 (1): e0228276.

Zula, A.T. and Desta, D.T. 2021. Fatty acid related health lipids index of raw and fried Nile tilapia (*Oreochromis niloticus*) fish muscle. Journal of Food Quality. 5(9): 6676528.

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Assessment of Manganese and Phyto-Additives (Turmeric) Supplementation in Low Input Technology (LIT) Layer Bird Diets on Egg Quality Parameters and Hatchability

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ABSTRACT

A study was conducted to evaluate the effect of supplementation of manganese and turmeric in a class of Low Input Technology (LIT) layer birds' diet namely Him-Smridhi (HS) under a complete randomized design. For this purpose, 44-week-old HS birds were divided into 4 treatment groups viz. T0 (BF), T1 (TP0.5%), T2 (Mn50mg/kg diet), T3 (TP 0.5% & Mn 50mg/kg diet) . Each treatment group had three replicates, with ten birds (9 female, 1 male) per replicate (T0)BF served as control group and was given standard feed, T1 was supplemented with 0.5 per cent turmeric powder w/w, treatment T2 offered with feed additional manganese 50mg/Kg and treatment T3 was supplemented with additional manganese (50mg/Kg) + (0.5%)) turmeric powder w/w. Fertility per cent, per cent total egg set hatched and per cent fertile egg set hatched were calculated during trial period (44^{th} to 53^{rd} week). Significant ($p<0.05$) effect of turmeric supplementation was observed on average albumen height and average haugh unit and reduction in egg yolk cholesterol level. Significant ($p<0.05$) higher albumen manganese, eggshell calcium and eggshell manganese was recorded with additional Mn supplementation affecting improve eggshell strength. Mn and turmeric powder supplementation showed no effect on hatchability in LIT bird Him-samridhi, but numerically 2.98 per cent higher hatchability with supplementation of turmeric powder alone was exhibited.

KEYWORDS: Egg yolk Cholesterol, Hatchability, Manganese, Turmeric.

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INTRODUCTION

Poultry is one of the fastest growing sectors in the Indian agricultural industry according to Indian Chamber of Food and Agriculture. Egg production increased from 78.48 billion in 2014-15 to 129.60 billion Nos. in 2021-22. Egg production in the country is growing at the rate (CAGR) of 7.4 per cent per annum (PIB,2024). The prevalence of protein deficiency in rural areas, especially among pregnant and lactating mothers and growing children, is very high. This problem can be addressed through the introduction of Low Input Technology Birds (LIT Birds) in rural backyard poultry initiatives. Him-samridhi is crossbreed of native chicken from the North Indian state (Himachal Pradesh) with a popular Dahlem Red (DR) poultry breed (Sharma et al., 2021). Poor eggshell quality is a major problem in the poultry industry, responsible for decreased hatchability, increased egg mortality, decreased protection against penetrating pathogens such as *Salmonella* spp. Eggs with shell defects contribute around 6-10 per cent of total egg produced, which negatively impacts the profitability of egg production (Nys, 2001). In past studies, dietary effect of macro minerals and vitamins on eggshell quality were under focus, however recent experiments showed positive results of using micro-minerals in layer feed.

Manganese (Mn) and Zinc (Zn) along with feed additive shows positive affect on eggshell quality. Manganese and Zinc act as co-factors of certain

metallo-enzymes (Mn- Glycosyltransferase, Zn-Carbonic anhydrase) which helps in carbonate and mucopolysaccharides synthesis. Mn involved in the biosynthesis of proteoglycans keratan and dermatan sulphate which are responsible for egg shell structure and strength. (Arias et al., 1993; Nys et al., 2001). Thus, Mn may affect the mechanical strength of eggshell.

Phyto-additives are substances of plant origin added to animal diets at recommended levels with the aim of improving animal performance. Essential oils, herbs, and spices, all serve as sources for bioactive ingredients, e.g., phenols and flavonoids. Besides enhancing performance, phyto-additives also have antioxidant property, the effects of which are associated with essential oils (EOs) and their components. Turmeric (*Curcuma longa*) is a perennial herb belonging to the family of *Zingiberaceae*, distributed throughout tropical and subtropical regions of the world (Beevers C.S et al., 2011). The active ingredients are tetrahydrocurcuminoids, curcumin, demethoxycurcumin and bisdemethoxycutcumin (Osawa et al., 1995; AL-Sultan, 2003). Curcuminoid is primary active principle present in turmeric and is responsible for various biological actions *i.e.* hypocholesteremic, hepatoprotective, hypotensive, antiviral, antifungal, antibacterial anti-inflammatory, antifertility, and antioxidant etc.

Various factors affecting hatchability are genetics, age, nutrition, egg selection and handling of fertile eggs, position of egg during incubation, temperature and humidity during incubation and metabolism of chick embryo. Both quality and quantity of feed is important for optimum reproductive performance *i.e.* production of good quality ova in females and sperm in males. Fertile egg contains all essential nutrients for development of embryo till hatching. Owing to the role of manganese in eggshell formation and synthesis of steroid hormones, the present study was thus undertaken to evaluate the effect of additional supplementation of manganese and turmeric on hatchability, physical and chemical parameters of eggs laid in LIT bird (Him-Smridhi).

MATERIALS AND METHODS

The experiment was conducted in University Poultry Farm, Department of Animal Genetics and Breeding, DGCN COVAS, CSKHPKV, Palampur, Himachal Pradesh, India. The experiment encompassed 120 old LIT layer birds (Him-samridhi) of age ranging from 44th to 53rd week. At the beginning of the experiment, all birds were dewormed and randomly distributed into four experimental groups: T0 (BF), T1 (TP0.5%), T2 (Mn50mg/kg diet), T3 (TP 0.5% & Mn 50mg/kg diet). The birds were kept under deep litter system during experimental period for 10 weeks. Each treatment group had three replicates, with ten birds (9 female, 1 male) per replicate. T0 served as control group and was given standard feed as per ICAR 2013 standards (basal diet), T1 supplemented with (0.5 %) turmeric powder w/w, T2 offered feed with additional calculated Mn (50mg/Kg) and T3 with additional calculated Mn (50 mg/Kg) + 0.5 % turmeric powder w/w. The experimental diets (Table 1) were formulated to meet nutritional requirements for energy, protein, minerals, and vitamins in accordance with the ICAR 2013 standards. Inorganic manganese sulphate and turmeric powder were purchased locally in Palampur, Himachal Pradesh, India. Routine activities involved feeding of treatment feed (120gm/bird/day), watering (*ad libitum*), collection of eggs (4 times a day) and shifting to hatchery (A.P. Poultry Equipment). The eggs were collected for 8-10 day and stored in egg storage room at temperature ranging between 13-15°C and relative humidity ranging from 75 - 80 per cent. After every 8 days, collected eggs were set in the egg setter after its fumigation. Eggs were evaluated for fertility after 18 days and unfertile eggs were removed, and fertile eggs were transferred to hatcher room for 3 days.

The estimation of proximate principles of feed ingredients (Table 1) utilized in formulating experimental diets was done by employing standard AOAC (2005) techniques. Metabolizable energy was calculated using the equation suggested by Lodhi et al. (1976).

Table 1. Ingredients and Chemical composition of experimental diets (% D.M.B)

Ingredients (%)	T0	T1	T2	T3
Soy flake	20.1	20.1	20.1	20.1
Maize	60.25	60.25	60.25	60.25
DORB	5	5	5	5
FM	5	5	5	5
MnSO4	0	0	0.0167	0.0167
Limestone	6.65	6.65	6.65	6.65
DCP	3	3	3	3
Salt	0.2	0.2	0.2	0.2
Premix	0.5	0.5	0.5	0.5
Turmeric powder	0	0.5	0	0.5
Chemical Composition (%)				
Crude Protein	16.59	16.59	16.59	16.59
Crude Fibre	3.91	3.91	3.91	3.91
Ether extract	0.98	0.98	0.98	0.98
LA	1.34	1.34	1.34	1.34
Ca (%)	3.39	3.39	3.39	3.39
P (%)	1.33	1.33	1.33	1.33
Manganese (mg/kg)	64	64	114	114
ME (KCal/Kg)	2569	2569	2569	2569
E/P	154.85	154.85	154.85	154.85

Premix was prepared by mixing following in 500gm maize flour. Traymix =20gm (Vitamin A-82,500 IU, B2-50mg, D3-12,000 IU, K-10mg/gm). VENTRIBEEPLUS 20g (vitamin B1-25 mg, B6-35 mg, B12-250 µg, E-225 mg, Pantothenate -225 mg, Niacinamide -300 mg, Folic acid-20 mg/5 g). E-care Se fort 10 gm (Vitamin E- 0.20g, Se-0.04 mg/g). Toxin binder-10 gm. Trace minerals – 100gm (Manganese sulphate=20gms, Ferrous sulphate =30gms, Zinc oxide=13gms, Copper sulphate=3.7gms, Potassium iodide=2.5gms, Dicalcium phosphate=30.8gms), Choline chloride=10gms, Methionine=14gms, Lysine=15gms.

* DORB: De-oiled rice bran, FM: Fish meal, DCP: Dicalcium phosphate

Digestion of the samples for mineral estimation was done using QLAB microwave digestion system: Questron Technologies Corp. (Canada). Mineral estimation of eggshell (Ca, Mn) was done using microwave assisted digestion method. The eggshells from respective treatment eggs were collected and grinded into homogenized powder form. Eggshell powder kept in hot air oven at 98°C for 12 hours. Digestion of eggshell powder, egg yolk and whole egg (Mn) samples was done by microwave assisted acid digestion in which 0.2 gm of homogenized sample was taken in digestion tube (eVHP TFM liner). The egg yolk and albumen were separated after boiling the eggs in water for 10-15minutes. Egg yolk samples and albumin were kept in hot air oven for 48-72hrs at 60°C and thereafter homogenized with a mechanical mixer. Acids used were nitric acid: perchloric acid: hydrogen peroxide in ratio 7: 1.5: 1.5 respectively. With an atomic absorption spectrophotometer, the final filtrate was used for analysis to determine the presence of Ca and Mn. Estimation of calcium and manganese was done by Atomic Absorption Spectrometer (AAS) (LABINDIA AA8000) and estimation of phosphorous was done spectrophotometrically (Spectronic 200 spectrophotometer) by method proposed by Parks and Dunn (1963).

External egg parameters such as egg weight, egg length, egg width, shape index, shell thickness and percentage shell were recorded. Eggs were weighed using electronic balance to an accuracy of 0.01 g. (Danwer scales India)**.** The length of the eggs was measured using digital vernier calipers (Aerospace Inc.). The width of the eggs was measured using digital vernier calipers (Aerospace Inc.). Shape index was calculated as the ratio of egg width to egg length × 100. Shell thickness was measured using a micrometer. Percentage shell was measured by taking the weight of eggshell to egg weight × 100. Shell weight was measured after washing the interior egg membrane and after its drying at 60°C for 48 h Venglovska et al. (2014).

Internal egg parameters such as yolk width, yolk height, yolk weight, albumen width, albumen height, albumen weight, Yolk: Albumen ratio and haugh unit were recorded during trial period (44th to 53rd week). Yolk width was measured using a digital vernier caliper (Aerospace Inc.). Yolk height was recorded using the vernier caliper (Aerospace Inc.). Yolk weight is measured by separating the yolk from albumen of egg. Albumen width was recorded using the digital vernier caliper (Aerospace Inc.). Albumin height was measured using spherometer in millimeters. Albumen weight was calculated by subtracting the shell and yolk weights from the egg weight. Yolk: Albumen ratio was calculated using weight of yolk/ weight of albumen. Haugh unit= 100 log (H + 5.57 – 1.37 $W^{0.37}$), Where H is albumen height (mm), and W is egg weight (g).

Egg hatchability records such as fertility per cent, per cent total egg set hatched (TESH), per cent fertile egg set hatched (FESH) and per cent infertile egg (IE) on total egg set were recorded during trial period (44th to 53rd week).

Fertility per cent = Number of fertile eggs / Total eggs set ×100

TESH = Number of eggs hatched / Total eggs set × 100

FESH = Number of eggs hatched / Fertile egg set × 100

IE (Total egg set) = Total infertile number/ Total egg set × 100

Total cholesterol in egg yolk was measured according to Zaltkis et al. (1953) with minor modifications as reported by Rajkumar et al. (2004) using a cholesterol test kit (Agappe).

Statistical analysis

ANOVA (analysis of variance) utilizing the complete randomized design (CRD) was performed on all recorded and computed data as explained by Snedecor and Cochran (1968). A 5 per cent level of significance was used to evaluate the outcomes.

RESULTS AND DISCUSSION

The egg production was not affected by the supplementation of Mn and turmeric powder. Average value of feed conversion ratio (FCR) was found to be 1.65, 1.74, 1.73 and 1.65 in control T0 and treatment groups T1, T2 and T3 respectively. The result obtained revealed that the average value of average feed conversion ratio (FCR) exhibited non- significant differences among control and treatment groups (Table 2). The effect of supplementing additional Mn^{50mg} along with $TP^{0.5\%}$ on the egg quality parameters is presented in Table 3. Results obtained implied that by the end of the trial, egg weight, egg width, egg length, shape index, yolk width, albumen width, yolk: albumen ratio, shell thickness, per cent shell exhibited non-significant ($p>0.05$) difference in treatment groups compared to BF on weekly basis. However, Haugh Unit (HU) and albumin height exhibited significant ($P<0.05$) difference in treatment groups compared to control, BF.

Table 2. Overall Egg production & FCR

Parameters	T0	T1	T2	T3
Total Egg Produced	937±2.27	884±3.50	897±4.60	927±3.10
Avg. Egg Wt (gm)	52.13±1.21	51.41±0.45	51.10±1.12	52.80±1.05
Total Egg Mass Produced (gm)	48845.81±0.045	45446.44±0.042	45836.70±0.035	48945.60±0.036
Total Feed Intake (gm)	80750.0±0.053	79200.00±0.036	79410.00±0.065	79850.00±0.043
FCR	1.65±0.03	1.74±0.01	1.73±0.02	1.63±0.03

FCR = Total feed consumed (kg) ÷ Total egg mass produced (kg)
Total egg mass produced (kg) = Numbers of eggs produced * Avg. Egg Wt
*Mean bearing different subscript within row differ significantly ($P<0.05$) from each other.

HU is a measure of the quality of eggs and higher value of HU enhances the grade of the egg. Interestingly HU value was significantly ($p<0.01$) higher in $TP^{0.5\%}$ whereas albumin height was recorded to be higher in Mn^{50mg} and in combination with $TP^{0.5\%}$. In our study, the results obtained reinforced the earlier findings implying that turmeric and Mn help to maintain freshness of egg components such as albumen and yolk. These findings are consistent with those of park et al. (2012b), where supplementation of layer diet with turmeric (Zadeh et al., 2022, Park et al., 2012, & Devi et al., 2023) improved the HU significantly. Improvement in HU due to turmeric powder

consumption need further study for examination of variations in mineral and moisture levels in the albumen. It is well documented with previous studies that turmeric contains curcumin which prevents lipid peroxidation and Mn as a cofactor of superoxide dismutase prevents oxidation activity and maintains freshness of egg. Increased HU may be attributed to the antioxidant properties of curcumin present in turmeric powder. In our study, no effect on HU was recorded with Mn^{50mg} supplementation but many of the authors have reported higher HU with Mn^{50mg} supplementation. (Kim et al., 2022, Gumus et al., 2017).

The average albumen height in BF, $TP^{0.5\%}$, Mn^{50mg}, $Mn^{50}TP^{0.5}$ during trial period (44th to 53rd week) was 6.76, 6.8, 7.23 and 6.98 mm respectively and significant ($P<0.05$) results were obtained in Mn^{50mg}, $Mn^{50}TP^{0.5}$ compared to BF. Supplementing turmeric alone did not influence the albumin height, where as additional Mn^{50mg} supplementation alone and in combination with $TP^{0.5\%}$ influenced albumin height. Increase in albumin height was also observed by other workers owing to Mn supplementation. (Rubio Zapata NK 2016, Attia et al., 2018, Zadeh et al., 2022).Contrary to our findings, Park et al. (2012) found improvement in albumen height due to turmeric powder consumption and attributed it to the antioxidant properties of curcumin present in turmeric powder. Gumus et al. (2017) and Laganá et al. (2011) also reported similar results in turmeric supplemented groups.

Overall, results for average egg weight over a period exhibited non-significant ($p>0.05$) difference, although numerically highest value of egg weight was exhibited by $Mn^{50}TP^{0.5}$ (52.82 gm) and lowest value of egg weight was exhibited by Mn^{50mg} (51.11 gm). Our findings are in agreement to those of Xiao et al. (2014) and Zarghi et al. (2023), that supplementing layer diet with Mn didn't affect egg weight. As Sazzad et al. (1994) concluded that layers fed diet enriched with Mn conferring to the minimal manganese requirements do not impact egg weight & egg production. Further, Kujero et al. (2021) and Attia et al. (2018) concluded that layers fed diet enriched with turmeric didn't exhibit significant egg weight. This might be linked to the relatively low levels or concentrations of essential compounds such as protein and fats, along with the volatility of secondary compounds like volatile oils in turmeric powder. These factors could potentially be necessary to improve egg weight. However, results of the experiment though revealed no impact of Mn and TP fed alone, but their combination led to 3.23 per cent increase in average egg weight. The higher egg weight in Mn and TP containing diet may be due to the fact that Mn is required for growth and development and also for normal functioning of enzymes, hormones, and carbohydrate metabolism. (McDowell LR 1992). Similarly in one of the studies, compared with the control diet, the levels of turmeric powder supplementation significantly improved nutrient digestibility ($p < 0.001$). (Mohamed A Fawaz et al., 2023). It is thus apparent that the combination of Mn^{50mg} and $TP^{0.5\%}$ might be having a synergistic action by increasing the availability of nutrients at the time of digestion and metabolism leading to average increase in egg weight.

Cited literature in previous studies on commercial egg layers attributed the supplementation of turmeric powder to enhance the fertile egg per cent by improving the semen quality because of the antioxidant action of turmeric preventing the lipid membrane from oxidation. Whereas in our study, the results obtained (Table 4) revealed non-significant ($p<0.05$) difference in treatment groups compared to control on fertile eggs at 18 days of candling. Numerically the highest fertile egg per cent at 18 days was recorded in treatment group $TP^{0.5\%}$ (92.55) compared to BF (91). Results of per cent TESH and FESH (Table 3) and per cent IE (total egg set) revealed non-significant ($p>0.05$) difference in treatment groups compared to control.

Table 3. Egg hatchability

		T0	T1	T2	T3
0-18th day	Total egg set (no.)	413	287	345	344
	Infertile eggs (no.)	37	22	36	47
	Infertile eggs %	8.96	7.66	10.43	13.66
	Fertility (%)	91±3.39	92.55±1.56	89.23±3.65	86.21±6.48
18th -21st day	Egg hatched (no.)	339	245	281	272
	TESH (%)	82.13±3.28	85.11±2.26	80.68±4.86	78.96±5.12
	FESH (%)	90.33±4.25	92±3.94	90.38±2.36	91.64±1.55
	Total infertile eggs (no.)	72	41	64	72
	IE (%)	17.43±3.28	14.28±2.26	18.55±4.86	21±5.17

*Mean bearing different subscript within row differ significantly ($P<0.05$) from each other

Cholesterol in egg yolk mg/100g (Table 3) exhibited significant ($p<0.05$) difference in $TP^{0.5\%}$ and $Mn^{50}TP^{0.5}$ compared to BF. Dalal et al. (2018) explained that turmeric powder has the potential to lower cholesterol levels through mechanisms such as enhancing the activity of cholesterol-7-α hydrolase or inhibiting HMG Co-A reductase. Curcumin, a component of turmeric, promotes the conversion of cholesterol into bile acid, facilitating its elimination from the body by inhibiting reabsorption of dietary cholesterol. In our study, the results obtained reinforced the earlier findings implying that turmeric powder reduces the egg yolk cholesterol. (Amir Mosayyeb Zadeh et al ., 2022, Xu et al., 2016 and Li et al., 2023). The anti-atherogenic properties of curcumin contribute to decreased blood cholesterol levels, leading to a reduction in the transfer of cholesterol into eggs. Hence, egg yolk cholesterol level was reduced in turmeric supplemented treatments.

Results for whole egg Mn (ppm) and yolk Mn (ppm) (Table-3) in treatment diets exhibited non-significant ($p>0.05$) difference compared to control. Whereas results for albumen Mn (ppm) and eggshell Ca (Table 3) exhibited significant ($p<0.05$) difference Mn^{50mg} and $Mn^{50}TP^{0.5}$ compared to BF. Results of the experiment revealed enhanced deposition of Mn and Ca in egg albumin and eggshell in treatment groups by additional Mn supplementation. It is well documented with previous studies that manganese plays a role in the activity of glycosyl transferases involved in cartilage muco-polysaccharide synthesis. These mucopolysaccharide act as store house of Ca^{++} ion during eggshell formation in their mammillary lobes. In our study, the results obtained reinforced the earlier findings implying that Mn supplementation improves the Ca^{++} deposition in eggshell.

Table 4. Egg quality parameters

Parameters	T0	T1	T2	T3
Egg weight (gm)	52.12±2.11	51.42±3.32	51.11±1.61	52.82±3.55
Egg breadth (mm)	40.94±0.24	40.86±1.26	40.45±3.45	41.32±1.12
Egg length (mm)	55.11 ±0.51	54.66±0.46	54.22±0.92	55.22±1.52
Shape Index (SI)	74.37±1.47	74.66±1.96	74.77±7.17	74.93±1.43
Albumin height (mm)	6.76a±0.26	6.8a±0.38	7.23b±0.74	6.98ab±1.53
Yolk width (mm)	42.52±0.68	42.43±2.07	43.83±4.47	43.13±4.27
Albumen width (mm)	101.65±4.45	97.35±6.25	97.17±6.77	97.02±4.62
Yolk height (mm)	17.57±0.67	17.5±0.6	17.16±0.76	17.65±0.65
Yolk weight (gm)	16.63±0.73	16.6±1.41	6.78±0.68	17.18±0.48
Albumen weight (gm)	27.68±0.98	27.33±2.13	27.4±1.3	27.61±1.61
A:Y ratio	0.60±0.03	0.61±0.03	0.61±0.02	0.63±0.05
Shell thickness (mm)	36.6±1.00	36.06±1.66	34.93±1.83	35.57±2.77
Per cent shell (%)	9.03±0.2	9.09±0.41	8.99±0.35	8.99±0.36
Haugh unit	84.37a±2.53	89.43b±1.53	84.16a±1.96	84.76a±1.36
Whole egg Mn(ppm)	0.02±0.01	0.03±0.01	0.03±0.01	0.05± 0.03
Yolk Mn(ppm)	0.04±0.00	0.03±0.02	0.03±0.02	0.05±0.03
Egg Albumen Mn(ppm)	0.036a±0.000	0.049a±0.007	0.135ab±0.005	0.083b±0.003
Eggshell Ca (ppm)	5.37a±0.3	5.31a±0.9	6.32ab±0.6	8.28b±0.4
Eggshell Mn(ppm)	0.0080a±0.000	20.0086ab±0.000	20.0080a±0.0004	0.0160b±0.0008
Egg Yolk cholesterol (mg/100 gm)	199.77ab ±28.38	164.93b ±14.94	191.52a±23.22	160.88b ±17.98

*Mean bearing different subscript within row differ significantly ($P<0.05$) from each other.

CONCLUSION

It is thus concluded that additional supplementation of Mn @ 50 mg/ Kg feed enhanced the HU significantly whereas turmeric powder supplementation increased the albumin height and lowered the egg yolk cholesterol significantly ($p<0.05$). Higher content of albumen Mn, eggshell Ca & eggshell Mn was recorded with supplementation of additional Mn and turmeric powder improving the eggshell strength. No significant ($p>0.05$) effect of Mn and turmeric powder on hatchability in LIT bird Him-samridhi was recorded, but numerically 2.98 per cent higher hatchability with supplementation of turmeric powder alone was exhibited.

REFERENCES

Amir Mosayyeb Zadeh , Seyyed Ali Mirghelenj, Peyman Hasanlou and Hossein Shakouri Alishah. 2022. Effects of turmeric (*Curcuma longa*) powder supplementation in laying hens' diet on production performance, blood biochemical parameters and egg quality traits. Journal of Animal Physiology and Animal Nutrition (Berl). 107(2):691-702.

-Al-Sultan, S.I. 2003. The Effect of *Curcuma longa* (Turmeric) on Overall Performance of Broiler Chickens. International Journal of Poultry Science. 2: 351-353.

AOAC, 2000. Official methods of analytical chemists (17th Edn.) Washington DC: Association of Official Analytical Chemists.

AOAC, 2005. Official methods of analytical chemists (18th Edn.) Washington DC: Association of Official Analytical Chemists.

Arias, J. L., Fink, D. J., Xiao, S., Heuer, A. H. and Caplan, A. I. 1993. Biomineralization and eggshells: Cell-mediated acellular compartments of mineralized extracellular matrix. International Review of Cytololgy. 145:217–250.

Beevers, C.S. and S. Huang. 2011. Pharmacological and clinical properties of curcumin, Review. Botanics: Targets and Therapy. 1: 5-18.

Devi, D.N., Zuyie R., Vidyarthi, V.K., Nizamuddin, Rutsa, M.C., Longkumer, J., Das, S. and Singh, R. 2023. Laying Performance and Egg Quality Traits of Vanaraja Birds as Influenced by Dietary Supplementation of Turmeric Powder. Biological Forum – An International Journal. 15(6): 297-301.

Kazemizadeh, A., Shahneh, A. Z., Zeinoaldini, S., Yousefi, A. R., Yeganeh, H., Pirsaraei, Z. A., and Akhlaghi, A. 2019a. Effects of dietary curcumin supplementation on seminal quality indices and fertility rate in broiler breeder roosters. British Poultry Science. 60(3): 256–264.

Leach, R. M. and J. R. Gross. 1983. The effect of manganese deficiency upon the ultrastructure of the eggshell. Poultry Science. 62:499–504.

Leach, R. M. and J. R. Gross. 1983. The effect of manganese deficiency upon the ultra structure of the eggshell. Poultry Science. 62:499–504.

Li, C., Gao, J., Guo, S., He, B. and Ma, W. 2023. Effects of curcumin on the egg quality and hepatic lipid metabolism of laying hens. Animals. 14(1): 138.

Lodhi, G.N., Daulat Singh, Icchponani, J.S. 1976.Variation in nutrient content of feeding stuffs rich in protein and reassessment of the chemical method for metabolizable energy estimation for poultry. Journal of Agricultural Science. 86 (2): 293-303.

McDowell, L.R.1992. Minerals in Animal and Human Nutrition. Harcourt Brace Jovanovich, San Diego, 2. Pp. 246-265.

Mohamed A Fawaz , Karl-Heinz Südekum , Hamdy A Hassan and Ahmed A Abdel-Wareth. 2023. Productive, physiological and nutritional responses of laying hens fed different dietary levels of turmeric powder. Journal of Animal Physiology and Animal Nutrition (Berl). 107(1):214-221.

Noetzold, T.L., Vieira, S. L., Favero, A., Horny, R. M., Silva, C. M. and Martins, G. B. 2020. Manganese requirements of broiler breeder hens. Poultry Science. 99:5814–5826.

Nys, Y. 2001. Recent developments in layer nutrition for optimizing shell quality. Pages 42–52 in Proc. 13th European Symposium on Poultry Nutrition. Blankenberge, Belgium.

Osawa,T., Sugiyama,Y., Inayoshi, M. and Kawakisi, S. 1995. Anti-oxidative activity of tetrahydrocurcuminoids. Biotechnology and Biochemistry. 59: 1609-161.

Park, S., Kim, J. M., Kim, E., Kim, H., An, B. and Kang, C. 2012. Effects of dietary turmeric powder on laying performance and egg qualities in laying hens. 한국가금학회지, 39(1), 27–32. https://doi.org/10.5536/kjps.2012.39.1.027

Parks, P.F. and Dunn, D.E. 1963. Evaluation of the molybdovanodate photometric determination of phosphorus in mixed feeds and mineral supplements. Journal Association of Official Analytical Chemists. 46:836-838.

Rajkumar, V., Agnihotri, M.K. and Sharma, N. 2004. Quality and shelf life of vacuum and aerobic packed chevon patties under refrigeration. Asian Australian Journal Animal Science. 17(4):548-553.

Snedecor, G.W. and Cochran, W.G. 1968. Statistical methods. 6th Edn. Oxford and ING Publ. Co. Calcutta.

Tg, T. 1970. How an Eggshell is made. Scientific American. 222(3): 88–95.

Venglovska, K., Gresakova, L., Placha, I., Ryzner, M. and Cobanova, K. 2014.Effects of feed supplementation with manganese from its different sources on performance and egg parameters of laying hens. Czech Journal of Animal Science.59: 147–155

Xie, J., Tian, C., Zhu, Y., Zhang, L., Lu L. and Luo X. 2014. Effects of inorganic and organic manganese supplementation on gonadotropin-releasing hormone-I and follicle-stimulating hormone expression and reproductive performance of broiler breeder hens. Poultry Science. 93:9

Xu, Z., Jiang, G., Wang, X., Haobang, L., Huang, X., Li, C. and Dai, Q. 2016. Effects of turmeric residue on performance, egg quality and the contents of cholesterol and malondialdehyde in yolk of laying hens. Chinese Journal of Animal Nutrition. 28(9): 2795–2801.

Zhang, Y. N., Zhang, H. J., Shu, W., Wang, J. and Qi, G. 2018. Dietary manganese supplementation affects mammillary knobs of eggshell ultrastructure in laying hens. Poultry Science. 97(4): 1253–1262.

Zhu, Y. W., Xie, J. J., Li, W. X., Lu, L., Zhang, L. Y., Ji, C., Lin, X., Liu, H. C., Odle, J. and Luo X. G. 2015. Effects of environmental temperature and dietary manganese on egg production performance, egg quality, and some plasma biochemical traits of broiler breeders. Journal of Animal Science.93:3431–3440.

Zlatkis, A., Zak, B. and Boyle, A.J. 1953. A new method for the direct determination of serum cholesterol. Journal of Laboratory Clinical Medicine. 41: 486.

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Effect of Supplementing Organic and Nano Trace Minerals at Reduced Dietary Levels on Broiler Chicken Performance

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ABSTRACT

An experiment was conducted to evaluate the effect of supplementing organic and nano trace minerals (Mn, Cu and Zn) at reduced dietary levels on the growth performance of broiler birds. 240 day-old commercial broiler chicks (Cobb strain) were assigned in equal numbers to five groups consisting of four replicates each. The basal diet was supplemented with inorganic trace minerals (Sulphateof Mn, Cu and Zn) at 100% of the standard recommendation to serve as control (ITM-100). Four test diets were prepared by supplementing organic trace minerals (glycinate form) at 75 % (OTM-75) and 50 % (OTM-50) of the standard recommendation and nano trace minerals at 50 % (NTM-50) and 25 % (NTM-25) of the standard recommendation. Each diet prepared for pre-starter (1-7 days), starter (8-21 days) and finisher (22-42 days) phases was offered to four replicates. The results revealed that, there was a non-significant difference in cumulative body weight, feed intake, feed conversion ratio, nutrient metabolizability, blood biochemical parameters and tibial mineral deposition in broiler chickens under different test diets when compared to the control (ITM-100) at the end of trial. Survivability remained comparable among all the groups during all the phases. Mineral balance (Mn, Cu and Zn) was significantly ($P< 0.05$) influenced by different sources and higher retention percentage was observed in the organic and nano groups at reduced levels compared to control. It was concluded that similar performance in broilers can be achieved by using organic at 50 % or nano particle trace minerals at 25% of the standard recommendation in broiler chickens.

KEYWORDS: Broiler chicken, Nano and organic forms, Performance, Trace minerals

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INTRODUCTION

Poultry production has advanced considerably through genetic selection, improved management, and optimized nutrition meeting the requirements of birds for metabolizable energy, crude protein, fat, minerals and vitamins. Yet the realization of maximum growth potential remains contingent upon the adequate provision of micro-nutrients. Among these, trace minerals such as zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), selenium (Se) and iodine (I) are indispensable due to their roles as enzyme cofactors, structural components, and regulators of metabolic and immune functions (McDowell, 1992; Favero et al., 2013). Conventionally, trace minerals are included in their inorganic forms (sulphates, oxides, carbonates, chlorides) in broiler diets. The inorganic trace minerals (ITM) are commonly used due to their easy accessibility and low price but their low bioavailability necessitates using it at higher doses in order to meet the requirements (Patra and Lalhriatpuii, 2020).

Over the last three decade, progress in poultry nutrition has led to the development of organic trace minerals (OTM), which enhances bioavailability, reduces antagonistic interactions in the gut resulting in improved poultry performance and overall health and minimizes environmental excretion compared to conventional sources.Organic minerals are the minerals which are chelated with organic compounds like amino acid complexes, proteinates, chelates, polysaccharide complexes and propionates (Byrne and Murphy, 2022). The ITM can be replaced with OTM at lower doses while achieving similar or improved growth performance in broilers (Britanico et al., 2012).

Similarly, nanotrace minerals (NTM) exhibit enhanced bioavailability due to their smaller particle size, larger specific surface area, higher catalytic efficiency, and stronger absorption capacity (Gopi et al., 2017). Studies suggested that supplementation with nano forms of essential trace minerals not only supports optimal growth, skeletal integrity, and immune competence in poultry but also reduces dietary inclusion levels and minimizes mineral excretion, thereby contributing to both productivity and environmental sustainability (Gowtham et al., 2022; Mane et al., 2022) and similar egg production performance in layers (Sachin et al., 2024). Studies have indicated that the dose of NTM can be reduced up to 25 % of the requirement without negatively affecting the production performance of birds (Gowtham et al., 2022; Mane et al., 2022; Aminullah et al., 2023; Aminullah et al., 2024; Sachin et al., 2024). Currently, there are limited comparative studies available on the effects of organic and nanoparticle trace minerals at reduced dietary levels in broiler chickens, particularly on nutrient metabolizability, blood biochemical profile, mineral retention and tibial mineral deposition. Hence, the present study was undertaken to assess the impact of replacing inorganic trace minerals (Mn, Cu, Zn) with organic and nanoforms at reduced dietary levels on the performance of broiler chickens.

MATERIALS AND METHODS

Experimental birds, diets, and management

The experiment was carried out with 240 day-old (Cobb strain) straight-run commercial broiler chicks for a period of 42 days (6 weeks). At the initiation, the day-old broiler chicks were weighed, wing banded and randomly distributed into five treatment groups with 4 replicates of 12 chicks in each replicate. A basal diet excluding Mn, Cu, and Zn salts was prepared as per BIS (2007) specifications. The control diet was prepared with supplementation of inorganic trace minerals (ITM 100) at 100% of recommendation to the basal diet. Test diets were prepared by supplementing organic trace minerals at 75 % (OTM 75) or 50 % (OTM 50) of recommendation or nanoparticles trace minerals at 50 % (NTM 50) or 25 % (NTM 25) of recommendation. Such diets were prepared for pre-starter (1-7 days), starter (8-21 days) and finisher phases (22-42 days) and offered *ad libitum* to the respective groups. The ingredient composition of basal diets prepared for different phases is presented in Table 1. The inorganic, organic, and nano trace minerals used were procured from M/s Quadragen Vet Health Pvt. Ltd., Bengaluru, Karnataka, India. The trace minerals in ITM, OTM, and NTM premixes is presented in Table 2.

Table 1. Feed ingredient and nutrient composition of basal diets prepared for different

Ingredients, Kg	Pre-starter diet (1-7 days)	Starter diet (8-21 days)	Finisher diet (22-42 days)
Yellow maize	54.2	55.1	59.81
Soybean meal	40.3	37.75	32.24
Vegetable oil	2.1	3.75	4.65
Di-calcium phosphate	1.6	1.7	1.5
Limestone	1.0	1.05	1.05
Common salt	0.2	0.15	0.2
DL- Methionine	0.1	0.1	0.15
L-Lysine	0.2	0.1	0.1
Mineral premix[1]	0.1	0.1	0.1
Vitamin premix	0.05	0.05	0.05
Toxin binder	0.1	0.1	0.1
Coccidiostat	0.05	0.05	0.05
Total	100	100	100
Nutrient composition (%)			
ME (kcal/kg)	3012	3120	3206
Crude protein	22.96	21.89	20.06
Calcium	1.06	1.05	0.9
Available phosphorous	0.46	0.48	0.44
Digestible lysine	1.36	1.15	1.03
Digestible methionine	0.44	0.41	0.44

[1]Each kg contain: Calcium 300g, Phosphorus 60g, Magnesium 10g, Cobalt chloride 500mg, Choline chloride 10g, Chromium 0.2g, Iron 40g, Iodine 1g, Selenium 0.35g, excluding Manganese, Copper and Zinc.

Table 2. Composition of trace mineral premixes

Trace mineral (g/kg)	Inorganic trace minerals (ITM)	Organic trace minerals (OTM)	Nano trace minerals (NTM)
Yellow maize	54.2	55.1	59.81
Manganese	100	66.66	100
Copper	15	10	15
Zinc	100	66.66	100

Growth performance

The growth performance of broilers was evaluated in terms of weekly body weight, feed consumption, and feed conversion ratio. Mortality was recorded as it occurred, and the weight of each deceased bird was measured to reduce errors in calculating the feed conversion ratio. The overall mortality observed during the experiment was expressed as a percentage for each corresponding treatment group.

Nutrient metabolizability

A three-day metabolic trial was conducted using 2 birds from each replicate in which excreta from the 39th to 41st day were collected, pooled within the pen, weighed, and dried at 60°C for 72 h. The feed and dried excreta samples were ground to pass through a 1 mm mesh screen and mixed thoroughly before analysis. The dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) contents were determined according to AOAC (2023).

Balance of trace minerals

Balance of trace minerals in the body of broiler chicken was calculated based on their intake and outgo. The feed and excreta samples were processed to prepare soluble ash as per AOAC (2023) method. The trace mineral composition of soluble ash samples were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The mineral retention was estimated using the formula,

Retention (mg/d) = Mineral intake (mg/d) – Mineral outgo (mg/d)

Tibial mineral deposition

At the end of the experiment (42nd day), left tibia samples were collected from 2 birds in each replicate for tibia mineralization study. The procedure involved preparation of mineral extract for estimation of trace minerals (Mn, Cu, Zn) deposition on tibial bone. Mineral extract samples were subsequently analysed for Mn, Cu and Zn using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Statistical analysis

The experimental design employed in this study was a complete randomized design (CRD) with one-way analysis (ANOVA). The data collected for all parameters were statistically analyzed following the methods outlined by Snedecor and Cochran (1994) and significant differences in means if any were tested using Tukey's test at $P<0.05$.

RESULTS AND DISCUSSION

Body weight

The body weight of birds at different weeks of experimental period is presented in Table 3. Although a significant difference ($P<0.05$) observed during first three weeks, the body weights of broilers during last three weeks of age did not differ among groups supplemented with inorganic, organic, or nano trace minerals. These findings are consistent with the reports of Britanico et al. (2012) and Gheisari et al. (2010), who observed similar body weights in broilers receiving reduced levels of organic trace minerals. Likewise, Mane et al. (2022) reported no significant effect at 75–50% organic or 50–25% nano trace minerals inclusion levels. In contrast, Gowtham et al. (2022) documented improved body weight gains in birds supplemented with nano minerals at 50% of the requirement compared with those receiving 100% inorganic or organic supplementation.

Table 3. Weekly body weight of experimental birds under different treatments

Experimental group	Trace mineral form and level	Body weight (g/bird)					
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
ITM 100	Inorganic 100%	154.5 ± 1.37^{ab}	434.4 ± 3.16^{a}	876.1 ± 6.27^{a}	1409 ± 17.64	2094 ± 25.00	2707± 21.95
OTM 75	Organic 75%	154.6 ± 1.73^{ab}	439.0 ± 3.08^{ab}	902.7± 4.75^{b}	1454 ± 19.36	2153 ± 29.15	2761 ±28.60
OTM 50	Organic 50%	156.2 ± 1.57^{b}	445.0 ± 1.65^{b}	920.2± 3.03^{c}	1466 ± 17.49	2133 ± 25.58	2769 ±20.82
NTM 50	Nano 50%	152.9 ± 1.42^{ab}	441.4 ± 2.34^{ab}	878.9± 3.72^{a}	1434 ± 24.33	2102 ± 29.76	2741 ±24.76
NTM 25	Nano 25%	149.7 ± 1.25^{a}	436.7 ± 1.81^{ab}	884.7 ± 3.58^{a}	1436 ± 15.78	2130 ± 23.96	2756± 20.30
	P value	0.029	0.031	<0.001	0.213	0.291	0.273

^{ab}Mean values bearing different superscripts within the column differ significantly (P≤0.05)

Feed consumption

The weekly cumulative feed intake was non-significant across treatments (Table 4). The results are in consistent with Britanico et al. (2012) who reported non-significant differences in feed intake by the supplementation of OTM at 50% of recommendation. Aminullah et al. (2022) found that dietary supplementation of organic and nano forms of Cu with the dose reduced up to 50 and 25% of recommendation, respectively had not affected the feed intake of Giriraja birds compared to group fed inorganic Cu at 100 % of recommendation. Feed intake in the 50% nano Zn, Mn, and Cu group was higher than in the 100% inorganic group, resulting in improved body weight gain (Gowtham et al., 2022). In contrast, Mane et al. (2022) reported comparable feed intake among broilers supplemented with 100% inorganic, 75% organic or nano, and 50% organic or nano trace minerals, suggesting that responses may vary depending on mineral source and inclusion level.

Table 4. Cumulative feed consumption of experimental birds under different treatments

Experimental group	Description of the treatment	Cumulative feed consumption (g/bird)					
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
ITM 100	Inorganic 100%	121.6 ± 4.07	471.3 ± 14.28	1112 ± 16.21	2008 ± 12.33	3126 ± 10.86	4377 ±11.45
OTM 75	Organic 75%	127.3 ± 4.80	488.3 ± 14.37	1159 ± 21.40	2055 ± 15.66	3163 ± 19.59	4385 ±19.77
OTM 50	Organic 50%	125.2 ± 6.58	484.0 ± 15.56	1145 ± 21.35	2048 ± 27.39	3157 ± 20.70	4424 ±18.67
NTM50	Nano 50%	119.0 ± 3.58	472.1 ± 7.58	1114 ± 8.40	2018 ± 14.09	3129 ± 8.81	4360 ±12.33
NTM 25	Nano 25%	124.7 ± 2.75	477.9 ± 8.66	1126 ± 13.24	2052 ± 16.84	3164 ± 12.24	4429 ± 15.50
	P value	0.733	0.839	0.263	0.239	0.145	0.095

Feed conversion ratio

The weekly cumulative feed conversion ratio (FCR) of broiler birds did not differ significantly (P>0.05) among the treatment groups (Table 5). These findings are consistent with Britanico et al. (2012) who reported that supplementation with OTM at 50% of recommended levels had no significant effect on FCR in broilers. Similarly, Aminullah et al. (2022) observed that Cu supplementation in organic and nano forms at 50% and 25% of recommended levels, respectively did not influence cumulative FCR. In contrast, Ahmadi et al. (2013) and Zhao et al. (2014) documented improved FCR in broilers fed diets supplemented with OTM, while Mane et al. (2022) reported comparable values across treatments. This study suggests that lowering dietary mineral supplementation, while using alternative organic or nano sources, can maintain feed efficiency comparable to conventional inorganic supplementation in broilers.

Table 5. Cumulative feed conversion ratio of experimental birds under different treatments

Experimental group	Description of the treatment	Cumulative feed conversion ratio					
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
ITM 100	Inorganic 100%	1.074 ± 0.01	1.201± 0.01	1.333 ± 0.01	1.469 ± 0.01	1.522 ± 0.01	1.641± 0.01
OTM 75	Organic 75%	1.120 ± 0.03	1.231 ± 0.02	1.347 ± 0.02	1.456 ± 0.03	1.498 ± 0.02	1.612± 0.01
OTM 50	Organic 50%	1.093 ± 0.03	1.200 ± 0.02	1.304 ± 0.02	1.437 ± 0.01	1.508 ± 0.01	1.621± 0.02
NTM 50	Nano 50%	1.056 ± 0.01	1.184 ± 0.03	1.332 ± 0.01	1.450 ± 0.01	1.518 ± 0.01	1.615± 0.02
NTM 25	Nano 25%	1.139 ± 0.02	1.210 ± 0.01	1.336 ± 0.02	1.471 ± 0.01	1.515 ± 0.01	1.635± 0.01
	P value	0.287	0.758	0.102	0.338	0.904	0.264

Survivability

No mortality occurred during the 42-day trial, indicating that reduced levels of trace minerals in organic and nano forms did not compromise survivability. These findings are consistent with Baloch et al. (2017),who reported no significant differences in mortality among broilers supplemented with varying levels of OTM (60, 50 and 25%) compared with those receiving inorganic sources. Similarly, Gowtham et al. (2022) concluded that supplementation with organic and nano forms of trace minerals at lower inclusion levels did not affect the survivability of birds. These results confirm that reduced inclusion of organic and nano mineral sources can maintain flock health and survivability comparable to conventional inorganic supplementation.

Nutrient metabolizability

The metabolizability of DM, CP, CF, EE and NFE was unaffected by dietary treatments (Table 6). These results are consistent with the findings of Sarvestani et al. (2016), who reported that supplementation of broiler diets with 100 ppm nano-Cu did not exert a notable effect on nutrient metabolizability. In contrast, Sa'aci et al. (2021) observed a significant improvement ($P< 0.05$) in metabolizability when diets were supplemented with nano-Se (0.10 ppm) and nano-Zn (50 ppm), likely due to their role as essential enzyme cofactors in nutrient metabolism.

Table 6. Metabolizability (%) of nutrients in experimental diets under different treatments

Experimental group	Description of treatment	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract
ITM 100	Inorganic 100%	72.59 ± 2.09	74.86 ± 4.16	70.34 ± 2.04	77.13 ± 1.38	46.13 ± 2.58	79.83 ±1.09
OTM 75	Organic 75%	74.79 ± 0.90	77.05 ± 1.45	73.76 ± 0.41	80.12 ± 0.65	48.29 ± 1.95	80.87± 0.82
OTM 50	Organic 50%	73.42 ± 1.74	75.65± 2.93	75.01 ± 1.68	79.24 ± 0.40	50.55 ± 2.48	78.87± 0.74
NTM 50	Nano 50%	72.85 ± 1.13	74.81 ± 4.05	74.00 ± 1.20	78.85 ± 1.34	48.32 ± 1.98	82.16± 0.84
NTM 25	Nano 25%	72.54 ± 1.26	75.29 ± 2.72	72.83 ± 1.61	76.95 ± 2.56	49.67 ± 1.90	80.67± 0.28
	P value	0.492	0.987	0.311	0.281	0.893	0.496

Mineral balance

Trace mineral intake decreased linearly with reduced supplementation in experimental birds, while excretion was lowest and retention highest in the 25% nano-supplemented group (Table 7). These findings corroborate the reports of Chen et al. (2022) and Cufadar et al. (2020), who demonstrated that inclusion of organic and nano forms of trace minerals in layer diets, even at reduced levels, enhances the retention of key minerals such as Mn, Cu, and Zn. The present results reinforce the superior bioavailability of nano and organic mineral sources compared with conventional inorganic forms, highlighting their potential to improve mineral utilization efficiency in poultry nutrition.

Table 7. Balance of trace minerals in experimental birds under different treatments

Particulars,mg/d	ITM 100	OTM 75	OTM 50	NTM 50	NTM 25	P value
ManganeseIntake	18.65 ± 0.40^{a}	13.46 ± 0.54^{b}	9.48 ± 0.09^{c}	9.66 ± 0.18^{c}	4.96 ± 0.26^{d}	<0.001
Outgo	15.39 ± 0.63^{a}	9.89 ± 0.57^{b}	6.95 ± 0.27^{c}	7.49 ± 0.44^{c}	3.47 ± 0.30^{d}	<0.001
Retention	3.26 ± 0.34^{a}	3.57 ± 0.13^{a}	2.53 ± 0.20^{b}	2.16± 0.26^{bc}	1.49 ± 0.12^{c}	<0.001
Retention(%intake)	17.47 ± 2.02^{a}	26.52 ± 1.50^{ab}	26.68 ± 2.36^{ab}	22.55 ± 3.06^{ab}	30.26 ± 3.06^{b}	0.038
Copper						
Intake	2.79 ± 0.06^{a}	2.01 ± 0.08^{b}	1.42 ± 0.01^{c}	1.45 ± 0.02^{c}	0.74 ± 0.03^{d}	<0.001
Outgo	1.66 ± 0.11^{a}	1.11 ± 0.04^{b}	0.79 ± 0.03^{c}	0.77 ± 0.07^{c}	0.30 ± 0.03^{d}	<0.001
Retention	1.18 ± 0.05^{a}	0.90 ± 0.04^{b}	0.62± 0.03^{cd}	0.67 ± 0.05^{c}	0.43 ± 0.04^{d}	<0.001
Retention (%intake)	40.61 ± 2.84^{a}	44.70 ± 0.82^{ab}	44.20 ± 2.44^{ab}	46.76 ± 4.15^{ab}	58.59 ± 4.67^{b}	0.027
ZincIntake	18.97 ± 0.40^{a}	13.46 ± 0.54^{b}	9.48 ± 0.90^{c}	9.66 ± 0.18^{c}	4.96 ± 0.26^{d}	<0.001
Outgo	14.58 ± 0.91^{a}	9.72 ± 0.15^{b}	6.02 ± 0.23^{c}	7.19 ± 0.32^{c}	3.36 ± 0.13^{d}	<0.001
Retention	4.39 ± 0.64^{a}	3.74± 0.38^{ab}	3.46± 0.17^{ab}	2.47± 0.25^{bc}	1.60 ± 0.18^{c}	<0.001
Retention(%intake)	23.14 ± 3.65^{a}	27.78 ± 1.80^{ab}	36.49 ± 2.05^{b}	25.56 ± 2.74^{ab}	32.25 ± 2.31^{b}	0.020

Blood biochemical profile

The mean blood biochemical profile values viz., serum calcium, phosphorus, total protein, albumin, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid and triglycerides were statistically similar (P>0.05) among groups (Table 8). The current study demonstrated that reducing recommended dietary levels of organic and nano trace minerals had no significant (P>0.05) impact on the blood biochemical parameters. Ghasemi et al. (2020) studied the effects of replacing inorganic trace minerals with organic trace minerals at lower levels (25 and 50%) observed no significant difference in serum glucose, triglycerides, cholesterol, total protein, or albumin levels among the treatment groups (P>0.05) supplemented with Mn, Cu and Zn (40-7-40 mg/kg) in organic and nano forms compared to control.

Table 8. Blood biochemical profile of experimental birds on 42nd day under different treatments

Experimental group	Calcium (mg/dl)	Phosphorus (mg/dl)	Total Protein (g~~m~~/dl)	Albumin (g~~m~~/dl)	Cholesterol (mg/dl)	ALT (U/L)	AST (U/L)	Uricacid (mg/dl)	Triglycerides (mg/dl)
ITM 100	10.92±0.11	8.80 ±0.45	3.27 ±0.10	1.75 ±0.06	110.00±9.82	3.970±1	448.60±38.50	4.45 ±1.04	45.77±5.26
OTM 75	11.03±0.30	9.27 ±0.42	3.67 ±0.16	1.92 ±0.08	117.85±1.06	3.050±0.96	393.60±19.54	3.42 ±0.60	41.57±4.11
OTM 50	10.86±0.12	8.72 ±0.25	3.44 ±0.16	1.82 ±0.08	108.80±7.16	1.375±0.33	385.67±22.82	3.95 ±1.46	38.85±2.03
NTM 50	10.92±0.18	8.75 ±0.23	3.30 ±0.17	1.75 ±0.09	117.42±7.55	2.800±0.64	401.90±23.57	4.47 ±1.01	33.85±3.22
NTM 25	10.84±0.16	8.67 ±0.61	3.07 ±0.06	1.70 ±0.09	103.15±8.51	2.200±0.47	370.12±36.19	3.87 ±0.53	41.75±4.84
P value	0.057	0.068	0.062	0.395	0.608	0.164	0.424	0.937	0.363

Tibial mineral deposition

At 42 days of age, tibial mineral deposition in broilers did not differ (P>0.05) in manganese (Mn), copper (Cu), or zinc (Zn) concentrations among the treatment groups (Table 9). These findings are consistent with previous studies (Zhao et al., 2014; Britanico et al., 2012), which reported that partial substitution of inorganic Zn, Cu, and Mn with organic chelates (up to 50%) did not significantly influence tibial mineral content. Similarly, Underwood and Suttle (1999) observed that reduced supplementation with organic minerals had no measurable effect on tibia weight, length, or mineral composition. Collectively, these results suggest that moderate replacement of inorganic trace minerals with organic sources does not compromise skeletal mineralization in broilers.

Table 9. Tibial mineral content in experimental birds on 42nd day under different treatments

Treatments	Mn (ppm)	Cu (ppm)	Zn (ppm)
ITM 100	10.81 ± 0.43	2.52 ± 0.26	220.55 ± 7.01
OTM 75	11.62 ± 0.52	2.64 ± 0.19	238.76 ± 4.50
OTM 50	11.44 ± 1.16	2.80 ± 0.35	236.96 ± 5.66
NTM 50	12.59 ± 0.70	2.90 ± 0.28	223.77 ± 8.59
NTM 25	11.23 ± 0.51	2.75 ± 0.25	228.73 ± 10.79
P value	0.529	0.878	0.419

CONCLUSION

The results revealed that the dietary supplementation with organic trace minerals at 75% and 50%, as well as nano trace minerals at 50% and 25% of the recommended levels, produced comparable outcomes in growth performance, nutrient metabolizability, and tibial mineral deposition. Importantly, trace minerals retention was significantly ($P<0.05$) enhanced at 50% and 25% inclusion levels. These findings demonstrate that organic and nano trace minerals can effectively substitute inorganic sources at reduced dietary inclusion rates (50% and 25%, respectively) without compromising broiler growth performance. The improved bioavailability of these mineral forms highlights their potential contribution to sustainable poultry nutrition by reducing dependence on conventional inorganic supplementation and minimizing environmental mineral excretion.

REFERENCES

Ahmadi, F., Khah, M. M., Javid, S., Zarneshan, A., Akradi, L. and Salehifar, P. 2013. The effect of dietary silver nanoparticles on performance, immune organs, and lipid serum of broiler chickens during starter period. International Journal of Bioscience. 3(5): 95-100.

Aminullah, N., Prabhu, T. M., Naik, J., Suresh, B. N. and Suma, N. 2022. Effect of reduced dietary copper levels sourced from organic and nanoparticles forms on performance and nutrient utilization in Giriraja birds. Indian Journal of Animal Research. 56(1): 58-64.

Aminullah, N., Prabhu, T. M., Suresh, B. N., Ahmadullah, Z., Sudharshan, S. and Azizi, M.N. 2024. Carcass, Bone and meat quality characteristics of Giriraja chicken fed reduced levels of organic and nano copper supplemented diets. Indian Journal of Animal Nutrition. 40 (3): 337-346.

Aminullah, N., Prabhu, T. M., Suresh, B. N. and Mostamand, A. 2023. Nano copper in poultry nutrition: potential effect and future prospect - a review.Indian Journal of Animal Nutrition. 39(4): 332-347.

AOAC. 2023. Official Methods of Analysis. 22nd Edn. Association of Official Analytical Chemists. Washington D.C., USA.

Baloch, Z., Yasmeen, N., Pasha, T. N., Ahmad, A., Taj, M. K., Khosa, A. N., Marghazani, I. B., Bangulzai, N., Ahmad, I. and Hua, Y. S. 2017. Effect of replacing inorganic with organic trace minerals on growth performance, carcass characteristics, and chemical composition of broiler thigh meat. African Journal of Agricultural Research. 12(18): 1570-1575.

BIS. 2007. Chicken Feeds Specification. IS:1374 (5th revision). Bureau of Indian Standards, Manak Bhavan, New Delhi, India.

Britanico, E. B., Merca, F. E., Angeles, A. A., Acda, S. P. and Luis, E. S. 2012. Effects of supplementing diets with amino acid chelates of copper, zinc, manganese, and iron on the performance of broilers. Philippine Journal of Veterinary and Animal Sciences. 38(1):1-10.

Byrne, L. and Murphy, R. A. 2022. Relative bioavailability of trace minerals in production animal nutrition: A review. Animals. 12(15): 1981-1990.

Chen, X., Ma, X. M., Yang, C. W., Jiang, S. Z., Huang, L. B., Li, Y. and Yang, W. R. 2022. Low level of dietary organic trace elements improve the eggshell strength, trace element utilization, and intestinal function in late-phase laying hens. Frontiers in Veterinary Science. 9: 615-903.

Cufadar, Y., Gocmen, R., Kanbur, G. and Yıldırım, B. 2020. Effects of dietary different levels of nano, organic and inorganic zinc sources on performance, eggshell quality, bone mechanical parameters and mineral contents

of the tibia, liver, serum and excreta in laying hens. Biological Trace Elements Research.193: 241-251.

Favero, A., Vieira, S. L., Angel, C. R., Bess, F., Cemin, H. S. and Ward, T. L. 2013. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. Journal of Applied Poultry Research. 22(1): 80-91.

Ghasemi, H. A., Hajkhodadadi, I., Hafizi, M., Taherpour, K. and Nazaran, M. H. 2020. Effect of advanced chelate technology-based trace minerals on growth performance, mineral digestibility, tibia characteristics, and antioxidant status in broiler chickens. Nutrition Metabolism.17: 1-12.

Gheisari, A. A., Rahimi-Fathkoohi, A., Toghyani, M. and Gheisari, M. M. 2010. Effects of organic chelates of zinc, manganese, and copper in comparison to their inorganic sources on performance of broiler chickens. Journal of Animal and Plant Sciences. 6(2): 630-636.

Gowtham, B., Ramesh, J., Karunakaran, R., Karthikeyan, N. and Raja, P. 2022. Effects of dietary supplementation of different levels of zinc, manganese and copper nanoparticles on growth performance and bioavailability in commercial broilers chicks. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4950.

Gopi, M., Beulah, P., Kumar, R. D., Muthuvel, S. and Govindasamy, P. 2017. Role of nanoparticles in animal and poultry nutrition: modes of action and applications in formulating feed additives and food processing. International Journal of Pharmacology. 13(7): 724-731.

Mane, A. V., Khose, K. K., Nikam, M. G., Dhumal, K. V., Gangane, G. R. and Munde, V. K. 2022. Effect of organic and nanoparticle trace minerals replacing inorganic trace minerals in diets on growth performance, immune response and economics of broilers. Indian Journal of Animal Sciences. 92 (4): 465–470

McDowell, L R. 1992. Minerals in Animal and Human Nutrition. Elsevier Science BV, Amsterdam.

Patra, A. and Lalhriatpuii, M. 2020. Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding: a review. Biological Trace Element Research.197(1): 233-253.

Sa'aci, Z. A., Jiya, E. Z. and Ijaiya, A. T. 2021. Influence of dietary nano zinc and selenium supplementation on growth performance, nutrients digestibility and carcass characteristics of broiler chickens. Nigerian Journal of Animal Science.23(3): 185-198.

Sachin, B. K., Suresh, B. N., Prabhu, T. M., Indresh, H. ~~M~~ C., Suma, N. and Gouri, M. D. 2024. Effect of organic and nano trace minerals at reduced levels in layer diets on egg quality characteristics and its trace mineral content. Indian Journal of Animal Sciences. 94(8): 700-706.

Sarvestani, S. S., Resvani, M., Zamiri, M. J., Shekarforoush, S., Atashi, H. and Mosleh, N. 2016. The effect of nano copper and mannan oligosaccharide supplementation on nutrient digestibility and performance in broiler chickens. Journal of Veterinary Research.71(2): 153- 161.

Snedecor, G. W. and Cochran, W. G. 1994. Statistical Methods. 8th Edn. Iowa State University Press, Ames, Iowa.

Underwood, E. J. and Suttle, N. F. 1999. The Mineral Nutrition of Livestock. 3rd Edn.Wallingford, Oxon: CABI Publishing.

Zhao, C. Y., Tan, S. X., Xiao, X. Y., Qiu, X.S., Pan, J. Q. and Tang, Z. X. 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. Biological Trace Element Research. 160: 361-367.

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Incorporation of Dry Sugar Beet Pulp with and Without Enzymes in Broiler Diets: A Sustainable Alternative for Cost-effective Poultry Production

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ABSTRACT

This research aimed to evaluate the feasibility of incorporating dry sugar beet pulp (DSBP) as a partial replacement for conventional feed ingredients in broiler diets, with a focus on its effects growth metrics, nutrient utilization, blood biochemical profiles, carcass characteristics, and economic efficiency. A total of 216-day-old Vencobb-430 broiler chicks were randomly distributed into six dietary groups, each with three replicates. The experimental design included diets containing 0%, 4%, and 8% DSBP, either with or without exogenous enzyme supplementation. Feeding was structured across three phases: starter, grower, and finisher. Parameters such as feed intake, body weight gain, and feed conversion ratio (FCR) were measured throughout the trial. A metabolic trial was conducted at the end, comprising a 3-day adaptation period followed by a 4-day collection phase. Total of 36 birds were sacrificed to study carcass traits at the end of experiment (42 days). The findings revealed that dietary inclusion of DSBP up to 4%, especially in combination with enzyme supplementation, promoted optimal growth performance, efficient nutrient utilization, and favourable economic returns, without negatively impacting bird health or carcass quality. However, increasing DSBP levels beyond 4% led to decrease feed efficiency and economic benefits, even with enzyme addition. Overall, the results support the use of DSBP at moderate levels of 4 percent as a sustainable and cost-effective feeding strategy in broiler production.

KEYWORDS: Broiler, Dry sugar beet pulp, Enzymes, Feed intake, Sustainable poultry.

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INTRODUCTION

The global population is expected to surge from 7.7 billion in 2019 to nearly 9.7 billion by 2050, putting immense pressure on existing food systems (United Nations, 2019). As a result, there is a growing need to adopt agricultural strategies that are more sustainable and resource-efficient (Galanakis, 2020). Among animal-based proteins, poultry meat stands out for its high efficiency in feed utilization, lower environmental impact, and wide social acceptance (Mottet & Tempio, 2017). In India, the poultry sector has grown significantly, fuelled by factors such as higher income levels, improved infrastructure, and better nutritional and genetic management (Gulati & Juneja, 2023). In the state of Punjab, poultry farming plays a significant role in the rural economy and accounts for approximately 3.08% of the national poultry population (Toor & Goel, 2022). However, the continued reliance on conventional feed ingredients like soybean meal and maize is proving to be financially and environmentally unsustainable (Thirumalaisamy et al., 2016). This calls for the evaluation of cost-effective, nutritionally adequate alternatives that are locally sourced.

Dry sugar beet pulp (DSBP) is one such option offering potential benefits for poultry feeding (Koschayev et al., 2019). It is a fibrous residue left after sugar extraction from beet roots and is notable for its high levels of digestible fiber and residual energy content (Abousekken et al., 2013). The fiber in DSBP is composed largely of pectin, cellulose, and hemicellulose, which contribute to improved gut health and water retention in poultry diets (Aziz-Aliabadi et al., 2021). When included at moderate levels, such fiber can support beneficial microflora and enzymatic activity, thereby improving nutrient utilization and growth performance (Jiménez-

Moreno et al., 2009). In Punjab, sugar beet cultivation spans roughly 13,000 acres, generating around 56,000 metric tons of wet pulp annually. However, much of this resource remains underused (Yadav et al., 2020). Despite its availability, DSBP's rich fiber content—particularly non-starch polysaccharides—can interfere with nutrient absorption. To address this, enzymes such as xylanase, phytase, and cellulase are often supplemented to break down complex fibers and enhance nutrient release (Abdel-Daim et al., 2020). These enzymes also degrade phytates, improving the availability of minerals like phosphorus (Narmuratova et al., 2025). Incorporating enzyme-supplemented DSBP into poultry diets aligns with sustainability goals by lowering feed costs, reducing waste, and supporting animal health. Hence, this study aims to evaluate the nutritional and economic outcomes of including DSBP with or without enzyme additives in broiler diets, focusing on growth, digestibility, and overall feed efficiency.

MATERIALS AND METHODS

A total of 216-day-old Vencobb-430 broiler chicks were employed in this study, randomly assigned to six dietary treatment groups, each comprising three replicates with 12 birds per replicate. The experimental design involved six different dietary regimens:

Table 1. Experimental Diets

T1	Diet containing 0% dry sugar beet pulp.
T2	Diet containing 0% dry sugar beet pulp with enzymes.
T3	Diet containing 4% dry sugar beet pulp.
T4	Diet containing 4% dry sugar beet pulp with enzymes.
T5	Diet containing 8% dry sugar beet pulp.
T6	Diet containing 8% dry sugar beet pulp with enzymes.

All experimental diets were formulated to be isocaloric and isonitrogenous across the three feeding phases, as per the guidelines of ICAR (2013). The enzyme blend included xylanase, cellulase, and phytase, designed to enhance fiber degradation and nutrient release.

Following the formulation of isocaloric and isonitrogenous experimental diets based on ICAR (2013) and supplementation with the enzyme blend, growth performance was evaluated by measuring body weight, body weight gain, feed intake, and feed conversion ratio (FCR) at the end of each phase. Daily feed intake was recorded, and leftover feed was weighed to calculate average feed consumption. To assess nutrient utilization, a metabolic trial was conducted during the finisher phase using total excreta collection. Nutrient digestibility parameters evaluated in the study comprised dry matter, organic matter, crude protein, nitrogen-free extract, and crude fiber. Carcass evaluation was performed at the end of the trial. Three birds per replicate were randomly selected, fasted overnight, weighed, slaughtered, and dressed. Carcass traits such as dressing percentage, eviscerated yield, breast meat yield, abdominal fat, and internal organ weights (liver, gizzard, heart) were recorded.

Statistical analysis

All data were subjected to statistical analysis using the General Linear Model (GLM) procedure within SPSS softaware (Version 22.0, 2013). Differences among treatment means were determined using Tukey's post-hoc test, with statistical significance set at $p < 0.05$. The model evaluated the effects of varying inclusion levels of dry sugar beet pulp (DSBP), both with and without enzyme supplementation, along with their interaction, on growth performance, blood biochemical indices, and carcass characteristics.

RESULTS AND DISCUSSION

Chemical composition of DSBP and experimental diet

The chemical composition of dry sugar beet pulp (DSBP) used in the study revealed 89% dry matter, with moderate crude protein (10.7%), ether extract (1.65%), and a crude fiber content of 17.05 %. It also contained essential minerals such as calcium (0.72%) and phosphorus (0.12%). The experimental diets were formulated to be isocaloric and isonitrogenous across all treatments and feeding phases, ensuring balanced nutrient intake.

Experimental Diets

Table 2. Ingredient composition of Starter, Grower and Finisher feed

Ingredient (%)	T1	T2	T3	T4	T5	T6
STARTER						
SBM	23.5	23.5	23.4	23.4	23.5	23.5
MBM	5	5	5	5	5	5
Maize	59.6	59.6	53.6	53.6	48.9	48.9
DORB	4	4	5.3	5.3	5.2	5.2
RGM	5	5	5	5	5	5
DSBP	0	0	4	4	8	8
DCP	0.3	0.3	0.3	0.3	0.3	0.3
LSP	0.8	0.8	0.7	0.7	0.7	0.7
Oil	1	1	1.9	1.9	2.6	2.6
Additives	0.72	0.72	0.72	0.72	0.72	0.73
Enzyme	0	0.1	0	0.1	0	0.1
GROWER						
SBM	22.1	22.1	21.9	21.9	22	22
MBM	5	5	5	5	5	5
Maize	60.4	60.4	54.5	54.5	50.1	50.1
DORB	4	4	5.3	5.3	5.2	5.2
RGM	5	5	5	5	5	5
DSBP	0	0	4	4	8	8
DCP	0	0	0	0	0	0
LSP	0.84	0.85	0.77	0.78	0.70	0.70
Oil	2.0	2.0	2.9	2.9	3.4	3.4
Additives	0.59	0.59	0.59	0.59	0.58	0.58
Enzymes	0	0.1	0	0.1	0	0.1
FINISHE						
RSBM	16.6	16.6	16.2	16.2	16.4	16.4
MBM	5	5	5	5	5	5
Maize	68.2	68.1	60.1	60.1	56.9	56.9
DORB	2.5	2.5	5.7	5.7	4.5	4.5
RGM	5	5	5	5	5	5
DSBP	0	0	4	4	8	8
DCP	0	0	0	0	0	0
LSP	0.5	0.62	0.55	0.55	0.50	0.50
Oil	1.5	1.5	2.8	2.8	3.1	3.1
Additives	0.56	0.56	0.55	0.55	0.54	0.54
Enzymes	0	0.1	0	0.1	0	0.1

* Additives include: Vitamin A 8, 25, 000 IU; Vitamin D3 1, 20, 000 IU; Riboflavin 500mg; Vitamin K 100mg; Vitamin E 800mg; Thiamin 80mg; Pyridoxine 160mg; Cynacobalamin 800mg; Niacin 1200mg; Calcium pantothenate 800mg; Manganese Sulphate 25g; Zinc Sulphate 25g; Ferrous sulphate 10g; Copper sulphate 500mg; Potassium iodide 100mg; Coccidiostat 55g; Toxin binder 50g; salt 300g.

Growth Performance

The inclusion of dry sugar beet pulp (DSBP) in broiler diets influenced growth performance parameters such as body weight (BW), body weight gain (BWG), feed intake, and feed conversion ratio (FCR) across the three production phases. During the Starter phase (0–14 days), birds receiving diets with 4% DSBP and enzyme supplementation (T4) showed marginally better BWG and FCR compared to other groups, indicating improved nutrient

assimilation when moderate fiber was coupled with exogenous enzymes. However, increasing DSBP levels to 8% (T5 and T6) led to a marginal decline in growth performance and a corresponding rise in FCR, especially in T5, suggesting that high fiber levels may have reduced nutrient digestibility and energy utilization at this early stage. Feed intake remained relatively stable across all groups, with no significant differences observed. However, González-Alvarado et al. (2010) found that during the initial growth phase (days 1 to 10), broilers fed diets containing 3% DSBP showed an improvement in FCR compared to those on the control diet. Moreover, AbouSekken et al. (2013) also reported that enzyme supplementation did not produce a significant effect during the initial phase.

Table 3. Effect of supplementing different levels of DSBP with and without enzyme supplementation on performance of broilers during the starter phase (0-14 day)

Group	DSBP	Enzyme	Initial BW, g	Final BW, g	WG, g	FI, g	FCR
T-I	0	0	42.0	322.8	280.8	403.7	1.43
T-II	0	1	42.8	322.8	280.0	401.0	1.43
T-III	4	0	42.3	321.3	279.0	402.0	1.44
T-IV	4	1	42.7	320.7	277.9	400.5	1.44
T-V	8	0	43.1	318.4	275.2	404.7	1.47
T-VI	8	1	43.4	319.0	275.6	402.7	1.46
Pooled SEM			0.209	0.758	0.938	0.657	0.0067
Main effect							
DSBP	0		42.4	322.8^{c}	280.4^{c}	402.4	1.43^{a}
	4		42.5	321.0^{b}	278.4^{b}	401.3	1.44^{a}
	8		43.2	318.7^{a}	275.4^{a}	403.7	1.46^{b}
SEM			0.268	1.186	1.455	0.553	0.010
Enzyme		0	42.5	320.8	278.3	403.5	1.45
		1	43.0	320.8	277.8	401.4	1.44
SEM			0.250	<0.001	0.250	1.233	0.001
Source of variation							
DSBP			0.174	<0.001	<0.001	0.210<	0.001
Enzyme			0.218	0.966	0.234	0.071	0.175
DSBP×Enzyme			0.814	0.192	0.326	0.891	0.921
Linear			0.095	<0.001	<0.001	0.319	<0.001
Quadratic			0.398	0.411	0.190	0.141	0.064

Figures with different superscripts in a row differ significantly P≤0.05

In the Grower phase (15–21days), the performance trend continued, with birds in T4 maintaining superior weight gain and efficient feed utilization. The control groups (T1 and T2) performed moderately well, while T3 (4% DSBP without enzyme) showed slight reductions in BWG and FCR compared to T4. Broilers fed the highest DSBP level (T5 and T6) experienced further deterioration in performance, reinforcing the potential limitations of high-fiber inclusion during the critical growth window. Kumari et al. (2014) also reported that incorporating sugar beet pulp (SBP) at a 2.5% inclusion level did not negatively influence growth performance, as both final body weight and daily weight gain remained statistically similar to those of the control group. The findings suggest that SBP can be incorporated at moderate levels without compromising growth.

Table 4. Effect of supplementing different levels of DSBP with and without enzyme supplementation on performance of broilers during the grower phase (14-21 days)

Group	DSBP	Enzyme	Initial BW, g	Final BW, g	WG, g	FI, g	FCR
T-I	0	0	322.8	556.6	233.7	373.4	1.59
T-II	0	1	322.8	555.2	232.3	369.8	1.59
T-III	4	0	321.3	551.9	230.6	371.5	1.61
T-IV	4	1	320.7	548.7	228.0	366.0	1.60
T-V	8	0	318.4	546.5	228.1	376.9	1.65
T-VI	8	1	319.0	544.0	225.0	368.3	1.63
Pooled SEM			0.76	2.02	1.30	1.58	0.009
Main effect							
DSBP	0		322.8^{c}	555.9^{c}	233.0^{b}	371.6	1.59^{a}
	4		321.0^{b}	550.3^{b}	229.3^{a}	368.7	1.61^{ab}
	8		318.7^{a}	545.3^{a}	226.6^{a}	372.6	1.64^{b}
SEM			1.19	3.08	1.87	1.15	0.014
Enzyme		0	320.8	551.7	230.8	373.9	1.62
		1	320.8	549.3	228.5	368.0	1.61
SEM			<0.001	1.18	1.18	2.95	0.005
Source of variation							
DSBP			<0.001	<0.001	0.003	0.492	0.046
Enzyme			0.966	0.062	0.075	0.047	0.559
DSBP×Enzyme			0.192	0.811	0.836	0.748	0.930
Linear			<0.001	<0.001	0.001	0.757	0.017
Quadratic			0.411	0.829	0.701	0.259	0.536

Figures with different superscripts in a row differ significantly P≤0.05

During the Finisher phase (22–42 days), birds in T4 again recorded the best growth parameters, reflecting the enzyme's favorable influence on supplementation with regard to mitigating the negative impact of dietary fiber. T6 showed slight improvements over T5, indicating partial recovery of performance with enzyme addition, though still lower than T4. Overall, FCR was significantly affected by both DSBP level and enzyme use, with the best values consistently observed in birds fed 4% DSBP with enzymes. AbouSekken et al. (2013) also reported that enzyme supplementation did not produce a significant effect during the finisher phase.

Table 5. Effect of supplementing different levels of DSBP with and without enzyme supplementation on performance of broilers during the finisher phase (22-42 day)

Group	DSBP	Enzyme	Initial BW, g	Final BW, g	WG, g	FI, g	FCR
T-I	0	0	556.6	1684.5	1127.9	2797.0	2.48
T-II	0	1	555.2	1678.4	1123.2	2763.4	2.46
T-III	4	0	551.9	1671.1	1119.2	2778.4	2.48
T-IV	4	1	548.7	1665.6	1116.8	2760.3	2.47
T-V	8	0	546.5	1672.0	1125.5	2829.3	2.51
T-VI	8	1	544.0	1666.7	1122.7	2808.2	2.50
Pooled SEM			2.023	2.946	1.654	11.014	0.008
Main effect							
DSBP	0		555.9^{c}	1681.4	1125.5	2780.2^{b}	2.47^{a}
	4		550.3^{b}	1668.3	1118.0	2769.3^{a}	2.47^{a}
	8		545.3^{a}	1669.4	1124.1	2818.8^{c}	2.50^{b}
SEM			3.062	4.195	2.302	15.017	0.010
ENZYME		0	551.7	1675.9	1124.2	2801.6^{b}	2.49
		1	549.3	1670.2	1120.9	2777.3^{a}	2.47
SEM			1.20	2.850	1.650	12.150	0.010
Source of variation							
DSBP			<0.001	0.040	0.301	<0.001	0.019
ENZYME			0.062	0.192	0.427	<0.001	0.130
DSBP×ENZYME			0.811	0.997	0.968	0.168	0.948
Linear			<0.001	0.033	0.770	<0.001	0.009
Quadratic			0.829	0.128	0.135	<0.001	0.272

Figures with different superscripts in a row differ significantly P≤0.05

Nutrient Metabolizability

The inclusion of dry sugar beet pulp (DSBP) and enzyme supplementation had a measurable influence on nutrient digestibility in broilers, particularly for fiber and protein components. During the metabolic trial conducted in the finisher phase, broilers fed diets containing 4% DSBP with enzyme supplementation (T4) demonstrated the highest digestibility coefficients for crude protein and crude fiber among all treatment groups. This improvement suggests enhanced microbial activity and fiber breakdown, likely facilitated by the exogenous enzymes which helped liberate nutrients otherwise bound in complex fiber matrices.

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In contrast, birds receiving 8% DSBP without enzyme support (T5) recorded the lowest digestibility values for dry matter, organic matter, and nitrogen-free extract. The high fiber content at this inclusion level may have hindered nutrient absorption by increasing digesta viscosity and reducing the accessibility of digestive enzymes. However, enzyme addition at the same level (T6) led to modest improvements, particularly in crude fiber digestibility, reinforcing the ability of enzyme blends to mitigate the adverse effects of high-fiber diets. Control groups (T1 and T2) and the 4% DSBP without enzyme group (T3) showed intermediate digestibility values, indicating that moderate fiber inclusion without enzyme assistance does not severely compromise nutrient utilization. Statistical analysis revealed a significant improvement in crude protein digestibility at 4% DSBP inclusion ($p = 0.006$) and in crude fiber digestibility with enzyme supplementation ($p = 0.017$), while other parameters showed no significant differences across treatments.

These findings highlight that DSBP, when used at moderate levels in combination with appropriate enzymes, can enhance nutrient digestibility without compromising broiler health or performance. However, higher inclusion rates without enzymatic support may limit feed efficiency due to reduced nutrient availability. Abdel-Daim et al. (2020) also reported a decrease in the digestibility of organic matter and dry matter with an increase in the inclusion of DSBP.

Table 6. Effect of supplementing different levels of DSBP with and without enzyme supplementation on nutrient Metabolizability (%).

Group	Diet	Metabolizability (%)								
	DSBP	ENZYME	DM	OM	EE	CP	CF	NFE	Ca	P
T-I	0	0	66.1	68.5	90.2	61.3	31.8	69.9	43.6	35
T-II	0	1	66.1	68.4	90.9	61.7	32.7	69.5	43.9	34.5
T-III	4	0	65.9	68.2	91.5	63.7	33.7	68.4	45	34.8
T-IV	4	1	66.1	67.9	89.5	65.7	35.1	67.9	44.2	33.9
T-V	8	0	65.2	67.4	90.5	61	30.6	68.3	41.1	34.1
T-VI	8	1	64.7	66.3	90.8	61.3	31	66.3	42.7	34.6
Pooled SEM			0.243	0.331	0.262	0.294	0.486	0.561	0.423	0.152
Main effect										
DSBP	0		66.1	68.5	90.5	61.5[a]	32.2	69.7	43.8	34.7
	4		66	68	90.5	64.7[b]	34.4	68.1	44.6	34.3
	8		64.9	66.9	90.6	61.1[a]	30.8	67.3	41.9	34.4
SEM			0.368	0.473	0.300	0.328	0.752	0.854	0.551	0.058
ENZYME		0	65.7	68	90.7	62	32	68.8	43.2	34.6
		1	65.6	67.5	90.4	62.9	32.9	67.9	43.6	34.3
SEM			0.050	0.217	0.283	0.100	0.017	0.283	0.450	0.050
Source of variation										
DSBP			0.520	0.375	0.990	0.006	0.444	0.181	0.791	0.994
ENZYME			0.901	0.618	0.586	0.324	0.693	0.358	0.915	0.931
DSBP×ENZYME			0.955	0.892	0.163	0.695	0.984	0.763	0.954	0.984
Linear			0.301	0.181	0.919	0.770	0.610	0.070	0.644	0.932
Quadratic			0.635	0.703	0.922	0.002	0.246	0.764	0.618	0.952

Figures with different superscripts in a row differ significantly $P \leq 0.05$

Carcass Traits

The evaluation of carcass traits by the conclusion of the feeding trial revealed that inclusion of dry sugar beet pulp (DSBP) up to 8%, with or without enzymes, did not significantly alter dressing percentage, eviscerated yield, or breast meat yield among the treatment groups ($p > 0.05$). Birds in the T4 group (4% DSBP with enzyme) showed a slight numerical advantage in carcass yield, suggesting a marginal benefit from improved nutrient utilization. Internal organ weights, including those of the liver, gizzard, and heart, remained within normal physiological ranges across all treatments, although a slight increase in gizzard weight was observed in birds receiving higher fiber diets (T5 and T6). Birds in enzyme-supplemented groups exhibited a modest improvement in proventriculus and intestinal weights, likely reflecting enhanced digestive function. These results indicate that DSBP, when used at moderate levels, particularly with enzyme supplementation, does not adversely impact carcass quality and may even support efficient organ function associated with digestion and nutrient absorption. Abdel-Hafeez et al. (2018) reported that fat was numerically lower in the SBP-fed group as compared to birds offered the standard diet. Abdel-Hafeez et al. (2018) also reported that fat was numerically lower in the SBP-fed group when contrasted with birds fed the control diet, regardless of enzyme supplementation. But AbouSekken et al. (2013) revealed that neither SBP inclusion nor enzyme supplementation exerted significant effects on most slaughter performance indicators, including dressing percentage and breast meat production.

Table 7. Effect of supplementing different levels of DSBP with and without enzyme supplementation on carcass parameters of broilers

Group	Diet		Carcass Parameters				
	DSBP	ENZYME	Dressing, %	Eviscerating, %	Abdominal fat %	Heart, %	Gizzard, %
T-I	0	0	57.4	74.5	2.30	0.589	2.79
T-II	0	1	60	76.0	1.86	0.583	2.68
T-III	4	0	58.3	77.4	1.81	0.595	3.00
T-IV	4	1	58.8	77.7	2.05	0.591	3.08
T-V	8	0	58.1	77.7	1.95	0.607	2.88
T-VI	8	1	59.2	76.7	2.13	0.638	2.92
Pooled SEM			0.394	0.521	0.032	0.009	0.063
Main effect							
DSBP	0		58.7	75.2	2.08	0.586	2.74
	4		58.5	77.5	1.93	0.593	3.04
	8		58.8	77.2	2.04	0.623	2.92
SEM			0.088	0.722	0.045	0.011	0.087
ENZYME		0	58.1	76.5	2.02	0.596	2.88
		1	59.2	76.8	2.01	0.604	2.92
SEM			0.550	0.150	0.005	0.004	0.020
Source of variation							
DSBP			0.943	0.113	0.660	0.456	0.114
ENZYME			0.106	0.799	0.951	0.784	0.724
DSBP*ENZYME			0.280	0.567	0.114	0.785	0.616
Linear			0.896	0.099	0.835	0.242	0.199
Quadratic			0.754	0.191	0.379	0.667	0.096

Figures with different superscripts in a row differ significantly $P \leq 0.05$

Economics

The economic evaluation revealed that moderate inclusion of DSBP can be a cost-effective feeding strategy, while excessive levels may lead to reduced profitability. Among all treatments, T-III, which included 4% DSBP without enzyme supplementation, recorded the highest net profit per group at ₹9.54. This suggests that partial replacement of conventional feed ingredients with DSBP at moderate levels can successfully lower

feed costs while maintaining good growth performance and carcass yield. Treatments T-II and T-IV, which included 0% and 4% DSBP with enzyme supplementation, respectively, also showed favorable profits of ₹9.29 and ₹9.32, indicating that enzyme addition does not significantly influence profitability when DSBP is included at optimal levels. Interestingly, even the control group T-I (0% DSBP without enzyme) achieved a respectable profit of ₹9.05, showing that while traditional feed remains efficient, moderate DSBP inclusion offers an economically competitive alternative. However, the profitability declined considerably in treatments T-V and T-VI, where 8% DSBP was included, resulting in net profits of only ₹5.31 and ₹5.27 per group, despite enzyme supplementation. These groups also recorded the highest feed costs per bird and overall group feed cost, while bird weights and income remained similar to other treatments. This indicates that higher DSBP levels, although reducing reliance on traditional feed ingredients, do not translate into better economic returns and may, in fact, increase production costs without improving performance. Therefore, it can be concluded that while DSBP is a promising and economical feed ingredient at inclusion levels up to 4%, its use beyond this threshold is not recommended, even with enzyme support, as it adversely affects overall profitability in broiler production.

Table 8. Effect of supplementing DSBP with and without enzyme supplementation on economics of broiler

Attributes	T-I	T-II	T-III	T-IV	T-V	T-VI
Number of chicks	36	36	36	36	36	36
Total Cost / Chick	134	133	132	132	137	136
Mean Slaughter body weight (grams)	1684	1678	1671	1665	1672	1666
Income from bird (@ Rs. 85/kg live weight)	143	143	142	141	142	142
Net profit per bird (Rs.)	9.01	9.26	9.50	9.30	5.30	5.26

CONCLUSION

The study demonstrated that incorporating dry sugar beet pulp (DSBP) at a 4% dietary level, especially when supplemented with exogenous enzymes, can be a viable approach to reduce feed costs in broiler production without compromising growth performance, nutrient digestibility, or carcass yield. The enzyme-treated DSBP diets enhanced protein and fiber utilization, improved metabolic parameters, and supported gut health. While higher inclusion rates (such as 8%) negatively influenced feed efficiency and economic returns, moderate levels proved nutritionally and economically advantageous. Therefore, DSBP, when strategically used with enzymes, holds promise as a sustainable and cost-effective alternative to conventional feed ingredients in broiler nutrition, particularly for producers aiming to balance performance with profitability in an increasingly resource-limited environment.

REFERENCES

Abdel-Daim, A. S. A., Tawfeek, S. S., El-Nahass, E. S., Hassan, A. H. A. and Youssef, I. M. I. 2020. Effect of feeding potato peels and sugar beet pulp with or without enzyme on nutrient digestibility, intestinal morphology, and meat quality of broiler chickens. Poultry Science Journal. 8(2):189–199.

Abdel-Hafeez, H. M., Saleh, E. S. E., Tawfeek, S. S., Youssef, I. M. I. and Abdel-Daim, A. S. A. 2018. Utilization of potato peels and sugar beet pulp with and without enzyme supplementation in broiler chicken diets: effects on performance, serum biochemical indices and carcass traits. Journal of Animal Physiology and Animal Nutrition. 102(1): 56-66.

AbouSekken, M. S., Shabban, S. A. M. and Deifallah, R. A. 2013. Effect of enzyme supplementation on productive performance of broilers fed diets containing different levels of sugar beet pulp. Egyptian Journal of Nutrition and Feeds. 16(2):319-336.

Aziz-Aliabadi, F., Hassanabadi, A., Golian, A. and Zerehdaran, S. 2021. Optimisation of broilers performance to different dietary levels of fibre and different levels and sources of fat from 0 to 14 days of age. Italian Journal of Animal Science. 20(1): 395–405.

Galanakis, C. M. 2020. The food systems in the era of the coronavirus (COVID-19) pandemic crisis. Foods. 9(4), 523.

Gulati, A., and Juneja, R. 2023. *Poultry Revolution in India: Lessons for smallholder production systems* (No. 225). ZEF Working Paper Series.

ICAR. 2013. *Nutrient Requirements of Animals - Poultry* (ICAR-NIANP) 3rd edition. Krishi Bhawan, New Delhi.

Jiménez-Moreno, E., González-Alvarado, J., De Coca-Sinova, A., Lázaro, R. and Mateos, G. 2009. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. Animal Feed Science and Technology. 154(1–2): 93–101.

Koschayev, I., Boiko, I., Kornienko, S., Tatiyanicheva, O., Sein, O., Zdanovich, S. and Popova, O. (2019, November). Feeding efficiency of dry beet pulp to broiler chickens. In *1st International Symposium Innovations in Life Sciences (ISILS 2019)* (pp. 269-272). Atlantis Press.

Kumari, P., Kumar, K., Kumar, S., Gattani, A. and Kumar, A. 2014. Effect of dietary supplementation of sugar beet, neem leaf, linseed and coriander on haemato-biochemical profile and muscle cholesterol of vanaraja chicken. Indian Journal of Poultry Science. 49(3): 346-349.

Ministry of Fisheries, Animal Husbandry and Dairying. 2019. *20th Livestock Census – 2019: All India Report*. Department of Animal Husbandry and Dairying, Government of India.

Moss, A. F., Chrystal, P. V., Cadogan, D. J., Wilkinson, S. J., Crowley, T. M. and Choct, M. 2021. Precision feeding and precision nutrition: A paradigm shifts in broiler feed formulation. Animal Bioscience. 34(3): 354–364.

Mottet, A. and Tempio, G. 2017. Global poultry production: current state and future outlook and challenges. World's poultry science journal. 73(2): 245-256.

Narmuratova, Zh., Blieva, R., Suleimenova, Zh., Akhmetsadykov, N., and Zhumagalieva, G. 2025. The role of microbial enzymes β-glucanase and α-amylase in optimising feed production for poultry. Scientific Horizons. 28(1): 30–40.

Sun, R. and Hughes, S. 1998. Fractional extraction and physico-chemical characterization of hemicelluloses and cellulose from sugar beet pulp. Carbohydrate Polymers. 36(4):293–299.

Thirumalaisamy, G., Muralidharan, J., Senthilkumar, S., Hema Sayee, R. and Priyadharsini, M. 2016. Cost-effective feeding of poultry. International Journal of Science. Environment and Technology. 5(6): 3997-4005.

Toor, J. S. and Goel, R. 2024. Poultry Farming in India with Special Reference to Punjab: An Overview. Agricultural Science Digest. 44(6).

United Nations. (2019, June 17). *9.7 billion on Earth by 2050, but growth rate slowing, says new UN population report*. United Nations Department of Economic and Social Affairs.

Yadav, P., Singh, P. and Singh, U. 2020**.** Feeding value of ensiled sugar beet pulp in cattle. International Journal of Current Microbiology and Applied Sciences. 9(9): 2480–2487.

Indian J. Anim. Nutr. 2026. 43 (1): 135-142
doi: 10.56093/IJAN.v43i1.16
Indian Journal of Animal Nutrition

Mycotoxin Management in Animal Feed Research Trends: Scientometric Analysis

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ABSTRACT

Mycotoxins are hazardous secondary metabolites produced by fungus that contaminate animal feed and have the potential to seriously impair food safety, animal health, and the global food supply chain. Over the past ten years, there has been an increase in scientific interest in mycotoxin contamination in feed systems. In order to examine global shifts in research trends pertaining to mycotoxin control in animal feed, this study used a scientometric approach. Search on the bibliographic data in the PubMed database was performed and analyzed it using the Biblioshiny interface in the Bibliometrix R package to access collaboration networks, to identify the highly productive authors, to analyze publishing trends, and to assess new research areas. The studies show a rise in scientific publications in recent years, indicating the increasing interest in this type of research around the world. There was also significant international participation in some European countries, along with China and the US being some of the leading providers of research outputs. Based on the findings, aflatoxin contamination, food safety, toxicity evaluation, and methodologies for risk management were the key themes of research. All this together suggests that the research effort related to mycotoxins in animal feed is growing, and more scientific collaboration and interdisciplinary approaches are warranted for addressing the challenges associated with animal health and feed safety.

KEYWORDS: Animal Feed Safety, Contamination of Food, Fungal Feed Security, Mycotoxin Management, Scientometric Analysis

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INTRODUCTION

Mycotoxins that constitute animal feed contamination are not limited to any region, and the differences are subject to climatic conditions, modes of storage, and farming practices(Kos et al., 2023). One of the significant places where scientometric analysis has become an effective method is in measuring trends of the research and some of the major contributors, plus measuring the development of the science research on a certain basis(Gomis et al., 2023). Using the methods of bibliometrics, one can trace the development of research in the field of influences on Mycotoxins and identify leading authors, institutions, and nations that take the lead in the field. In addition, researchers and policymakers can adjust their strategies in the context of effective measures of Mycotoxin control by knowing the publication trend ('–'DallAsta et al., 2023). Also, differences between the regulations used in different countries create challenges for using common safety standards(López-García, 2022). Thus, the need for an in-depth scientometric review emerges to measure the development of the research on mitigating Mycotoxins and come up with trends in research. The purpose of the study is to evaluate the knowledge field of Mycotoxin control in animal feed with the help of scientometric tools. The results of the assessment of the trends in publications, author networks, and thematic regularities will give important answers to questions about the trend in the development of this vital area. By examining diverse research findings and publications on mycotoxin management in animal feed, this study requires a detailed analysis, utilizing scientometric and bibliometric comparisons to present a

comprehensive overview of the field. Through robust citation and productivity metrics, it honors great contributors and prominent authors, celebrating leading innovators. It elaborates to reveal thematic changes and emerging hotspots over the years based on keyword trends and vibrant author collaborations, as well as country-wise contributions to create a global picture of interrelated efforts. In doing so, it provides meaningful guidance from which practical suggestions, future directions, and policy recommendations arise; identifies key gaps in existing studies, and offers key insights relevant to the area; and provides innovative approaches to mycotoxin detection and mitigation for promoting the protection of animal health and food security.

Figure 1. Research Framework

MATERIALS AND METHODS

This bibliometric study seeks to survey the academic literature and resources about "Mycotoxin management in animal feed" between 1979 and March 2025 on growth, collaboration networks, diversity of search terms, and participation in international communities. Data was collected from databases including PubMed, using subject-specific keywords such as “Mycotoxin Prevention and control in Animal feed,” “mycotoxin/aflatoxin management,” and “Animal feed and Livestock food contamination.” Boolean operators and clarifiers were used to ensure that peer-reviewed articles, reviews, and conference papers would be included. The dataset (Fig 1) included 292 documents in 115 journals or sources published between 1979 and early 2025. Data was cleaned and prepared by deduplication, author name, institutional associations, and journal title standardization, and normalization according to keyword spelling and phrasing. The exported data were written in formats available for the analysis in bibliometric software. The bibliometric analysis was performed using the R programming language, with emphasis on the Bibliometrix and Biblioshiny packages (Aria & Cuccurullo, 2017). Main functions consisted of descriptive statistics, co-authorship, network analysis, keywords and co-occurrence mapping, Lotka's Law, and visualization tools such as collaboration, word clouds, and theme clusters. Key bibliometric features for interpretation Analysis of the dataset included the following bibliometric indicators: Annual Scientific Production, Authorship Patterns, Collaboration Index, Keyword Analysis, Average Document Age, and Citation Metrics. Network and thematic mapping were conducted to assess national and institutional collaborations, while keyword co-occurrence networks were examined through multidimensional scaling and Louvain clustering. Shortcomings are that there was no citation data, outdated affiliation data to date, more bias about English language journals, and no incorporation of patents and industry linkages. Ethical issues were addressed; the article considered only public sources of bibliographic data in this study.

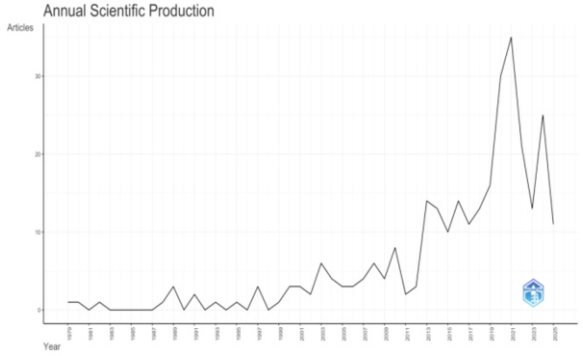

Figure 2. Annual Scientific Publication

RESULTS AND DISCUSSION Year-wise Publication

Figure 2 shows the frequency of publications from the previous years. Published articles appeared in the first few years (1970-1990), and only 0-2 of them were published. Annual growth in output to 3–6 publications from the latter part of the 1990s through the early 2000s further illustrates that the academic gaze on this area is growing. From 2010 onwards, this upward movement indicates a trend of increasing research output and academic engagement. A striking increase on the whole occurred between 2018 and 2021, when the number of publications sharply increased and reached the highest peak of more than 30 articles in a single year, indicating heightened research attention and productivity. After this peak, a slight decline and fluctuation are observed in the subsequent years,

though the output remains comparatively higher than earlier decades.

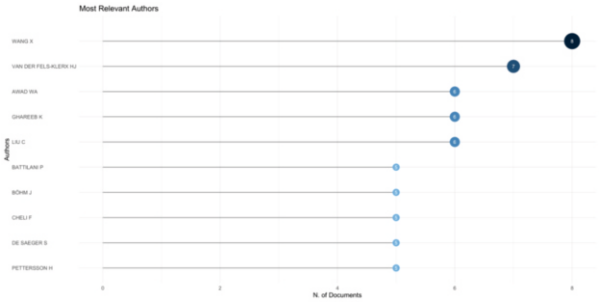

Figure 3. Productive Authors

Most Prolific Authors

It presents the most prolific authors over the paper number of Mycotoxin management research, through a visualization (Figure 3). The author's name of Wang X is reported multiple times in the dataset, but the author of each publication was found to be different researchers (Xiaodan Wang, Xichun Wang, and Xinxin Wang) on DOI verification. As articles were produced by different authors, these articles did not aggregate and thus were not considered as prolific authors (as is suggested for bibliometric data-cleaning procedures to remove ambiguity of author names) (Strotmann & Zhao, 2012). The most prolific author has been Van der Fels-Klerx HJ with seven publications, Awad WA, Ghareeb K, and LIU C have contributed six documents, all contributing positively to findings in this field. Other authors have contributed five documents each which include Battilani P, Böhm J, Cheli F, De Saeger S, and Pettersson H. The quite high number of publications by many authors indicates a vibrant and competitive area of research.

Table 1. List of Most Productive Authors

S.No	Author	Affiliation	No Publications	H-Index
1	Van Der Fels-Klerx HJ	Senior Scientist Wageningen Food Safety Research (WFSR), Wageningen, Netherlands https://orcid.org/0000-0002-7801-394X	7	55
2	Awad WA	Scientists University of Veterinary Medicine, Vienna Austria https://orcid.org/0000-0003-0843-2089	6	40
3	Ghareeb K	Professor and Head, Veterinary Medicine South Valley University, Egypt	6	29
4	Liu C	Researcher, Wageningen Food Safety Research, Wageningen University and Research Wageningen, Netherlands https://orcid.org/0000-0003-0513-9610	6	18
5	Battilani P	Professor Catholic University of the Sacred Heart, Piacenza and Cremona Campuses: Piacenza Italy https://orcid.org/0000-0003-1287-1711	5	63
6	Böhm J	Department of Animal Hygiene, Behaviour and Management, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt	5	-
7	Cheli FProfessor	Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy https://orcid.org/0000-0003-2682-8685	5	-
8	De Saeger S	Professor Center of Excellence in Mycotoxicology & Public Health, Department of Bioanalysis, Ghent University, B-9000 Ghent, Belgium https://orcid.org/0000-0002-6151-5126	5	87
9	Pettersson H	Professor Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden	5	-
10	István Pócsi	Professor, Department of Biotechnology and Microbiology, University of Debrecen, http://orcid.org/0000-0003-2692-6453	5	51

Table 1 shows the most productive authors for researching mycotoxin contamination in animal feed. Van der Fels-Klerx H.J. from Wageningen Food Safety Research, Netherlands, ranks first with seven publications, suggesting this area should be of a lot of interest to researchers on the topic. With six journals each, Awad W.A., Ghareeb K., and Liu C. attentively demonstrate steady scholarly contributions. With five publications individually, Battilani P., Böhm J., Cheli F., De Saeger S., Pettersson H. and Istě Pócsi come next. Since the key contributions are primarily associated with European research institutes, the connections have offered an intriguing reflection that supports their significance in advancing mycotoxin research related to animal feed safety and contamination management.

Author Productivity based on Lotka's Law

The idea that a small number of authors make important contributions to the structure of academic literature while the bulk continue to contribute seldom is supported by Lotka's Law, which is frequently employed in bibliometrics to explain scientific productivity. Pao (1986). The notion of a "core" group of productive writers has been supported by this trend, which has been seen in several scientific fields. The discrepancy between the actual and the expected distributions (as reflected in the gap between the solid and dashed lines) can indicate the existence of specific differences in the dataset, be it discipline specificity, institutional collaboration, or the changing publication habit. Nicholls (1989). Besides, reasons such as publishing trends and publishing online may influence the shape of productivity, thus violating the classic Lotka's paradigm. This distribution is crucial for policymakers, librarians, and academic institutions to appreciate and engage with influential researchers, allowing for suitable resource allocation.

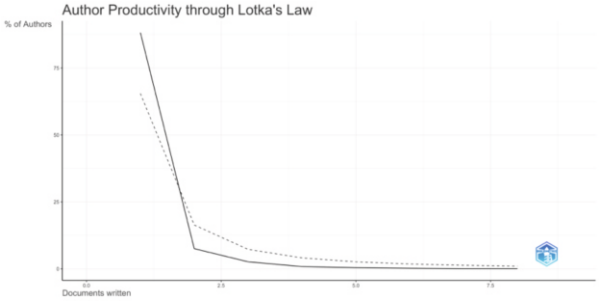

Figure 4. Distribution of Author Productivity Based on Lotka's Law

Geographic Distribution of Corresponding authors

The regional distribution of the main authors in Figure 5 shows the differences in cooperation and effort in research. China takes the lead with about 30 publications (20 SCP, 10 MCP), indicating there is a wider prevalence at home and some light international interest. The USA has an impressive 21 (16 MCP, 5 SCP), indicating strong international cooperation. There are about 10 publications in India (8 MCP, 2 SCP) and 14 in Italy (9 MCP, 5 SCP). It is three of the others: Poland, Austria, and Canada producing nine, of which Canada is a leading MCP supporter. But the Netherlands and Korea are donors that have similar trends.

Trending Topics in Mycotoxin Research

In earlier studies, food handling, ochratoxins, and regulatory standards were common themes (2006-2010). Between 2010 and 2014, however, the emphasis started moving toward consumer product safety and the impact of mycotoxins on health. The literature between the years 2014 and 2018 focused on toxicological processes and exposure of livestock. Toxicity of Aflatoxin B1, analysis of mycotoxin, and risk assessment are the main focus on recent literature (2019–2023), highlighting an emphasis on advanced analytical methods and food chain monitoring.

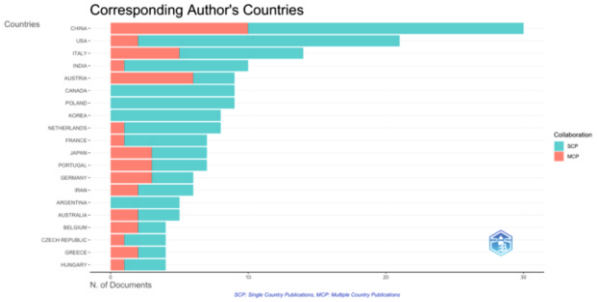

Figure 5. Country-wise distribution of Corresponding Authors

Sankey Visualization of Mycotoxin Research

Related to this, the Sankey graph in Figure 6 demonstrates the correlation between title terms (TI_TM), authors (AU), and authors' countries (AU_CO) in the field of mycotoxin studies. Authors related to title terms including mycotoxin, aflatoxin, zearalenone, contamination, risk, food, and deoxynivalenol appear in most literature associated with major mycotoxin toxicity and risk assessment studies, e.g., Pócsi I., Gyŏri Z., Mahato D.K. In fact, the authors (Wang X. & Liu C.) focus on some detection work conducted as a literature study in

aflatoxin and food contamination. Kumar P. and Kumar A. are published in the food safety risk management literature. Other countries—such as China, Italy, Hungary, and India—have made important contributions in research terms. Mahato D.K. is associated with India; Pócsi I. and Gyęri Z. with Hungary. Due to these countries—Germany, Korea, and the Netherlands—it has the international research component. These columns—author, titles, and nations—set the stage for research dynamics and focus on sectors including food safety risk assessment and mycotoxin contamination as areas of global collaboration.

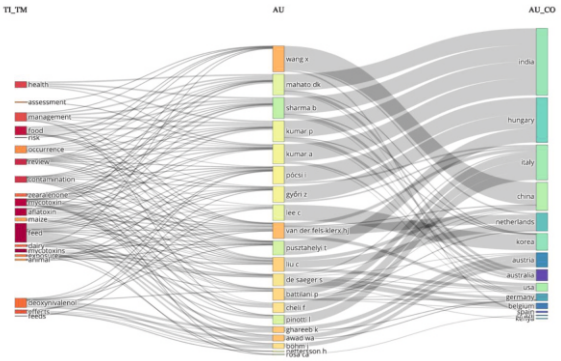

Figure 6. Sankey Visualization of Authors, Titles and Countries

Thematic Map of Mycotoxin Research

The thematic plot in figure 7 classifies research areas concurring to intensity (the level of development) and centrality (the relevance) and requires a basis for how the research data on food contamination, aflatoxins, and Risk Assessment development are organized. The quadrant Motor Themes (top-right) is dominated by highly developed and central topics such as animal feed/microbiology, fungi/metabolism, and food chains that tend to be at the center of interest in the fields of Food Safety, microbiology, and animal nutrition studies. The quadrant of Basic Themes (bottom-right) contains relatively less developed but important areas, like humans, Risk Assessment, food contamination/analysis, male, animal feed, and animals, suggesting that they are fundamentally important in their regard to toxicology and Food Safety. Niche Themes (top-left) possess specific and developed subjects with reduced overall functionality, such as aflatoxin M1/analysis, aflatoxin toxicity analysis, and food contamination prevention & control analysis, which allow highlighting their focused and partial embedding in other themes, e.g., research in China or infant health. The Emerging or Declining Themes quadrant (bottom-left) includes, for example, the topics of immunoassays and Aspergillus flavus, and these might be either new areas of investigation or fields in which the emphasis is diminishing, perhaps because of changes in methods. The thematic map will serve as a strategic tool to identify current research. significance, specialized niches, and future trends in food safety and toxicology, providing guidelines for researchers and policymakers.The scientometric analysis also needs an examination summarizing the status of field of mycotoxin infection in animal feed according to publications, country contribution, and corresponding keywords. This study also shows that there are several research areas and gaps in the literature, alongside a global trend in increasing research output, showing that there is concern about the worldwide impact of mycotoxin contamination of animal health and feed safety (Yilmaz et al., 2025 ; Luo et al., 2021 ; "Eskola et al., 2020). This increased publications reflects a rising awareness regarding economic losses and food safety issues from mycotoxins (Goda et al., 2025). However, in line with existing bibliometric studies focusing on this dominance of those nations, China and the US in general dominate scientific output, denoting heavy commitment to agricultural biotechnology and food-safety research –(Abdul & Pavoni, 2025 ; Altun et al., 2024 ; –Adeniji et al., 2025 ; Wang et al., 2024). The study also detected the presence of aflatoxin contamination levels, toxicity, and its use in food safety and risk assessment based on co-occurrence analysis. As mycotoxins found in the feed products, Aflatoxins are highlighted in highly toxic forms –(Yohannis et al., 2025 ; Okechukwu et al., 2024). This is consistent with previous studies indicating the importance of aflatoxin monitoring for feed and food safety '(Smaoui et al., 2023). Yet a scientometric analysis found only specific keywords associated with regulatory frameworks or policy building — with terms like "regulation" and "governance" appearing infrequently. This points to a lack of attention to coordinating internationally in mycotoxin regulatory and risk management (López-García, 2022 ; Chilenga et al., 2025). This study reflects that regulatory issues in toxin detection and mitigation studies have received less research than laboratory studies. Thematic analysis shows a key emphasis in the discussion of laboratory detection and contamination mechanisms, whereas the emphasis on broad implementation of these techniques and their field validation is diminishing. It also highlights the importance of field-based evaluations for application in agricultural practice. (Kahl et al., 2026 ; Reza et al., 2025). It shows there

are international research partnerships but concentrated in a few countries and institutions. Increased international collaboration could encourage knowledge transfer and the development of consolidated international mycotoxin contamination control efforts. '(Mafe & Büsselberg, 2024). Overall, the scientometric results have indicated a strong emphasis in mycotoxin research on analytical detection methods, toxicological evaluation, and contamination monitoring. Extenuation strategy validation, policy review, and regulatory harmonization are also underrepresented. Additional studies on these issues can improve scientific knowledge in facilitating secure feed application, possibly reducing the risk of mycotoxins(Yeassin et al., 2026).

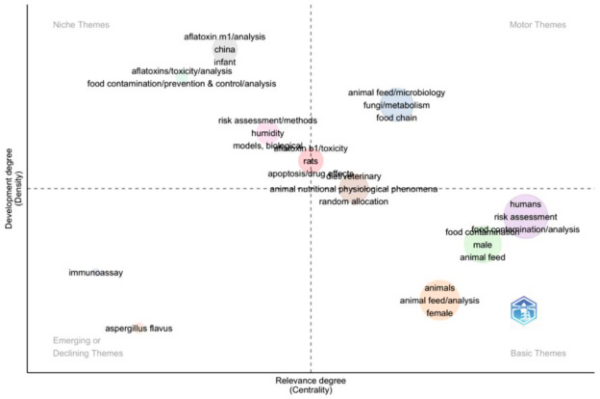

Figure 7. Thematic Mapping of Mycotoxin Research Areas

CONCLUSION

This scientometric study offers a general overview of advancements in the research area of mycotoxins, using a bibliometric and thematic approach. With one eye toward mycotoxin science, the analysis provides evidence of a steady rise in scientific papers throughout the study period, which illustrates how mycotoxin science has gained attention around the world. It has also reflected the authors, contributing countries, and forms of cooperation that have helped to spread this discipline. Based on co-occurring and trend analysis of the keywords, the main study themes are food safety, analytical detection techniques, and aflatoxin contamination and toxicity effects. These are the main issues in the dataset that require scientific exploration. The reviewed literature does not provide much coverage of topics like policy evaluation and regulatory frameworks. The scientometric results may give an organized understanding of the development, protagonists, and thematic trajectories of mycotoxin research and therefore, are a useful reference for further research in this field.

REFERENCES

Abdul, M.-E. and Pavoni, E. 2025. Bacillus cereus in food safety: A bibliometric analysis. Frontiers in Microbiology, 16, 1574802. https://doi.org/10.3389/fmicb.2025.1574802

Adeniji, O. O., Akinmoladun, O. F. and Njobeh, P. B. 2025. Microbial interventions for aflatoxin control in food systems: A 25-year global bibliometric analysis (2000–2024) with implications for food security and smart agriculture. Food Safety and Risk. 12(1): 11. https://doi.org/10.1186/s40550-025-00120-2

Agriopoulou, S., Stamatelopoulou, E. and Varzakas, T. 2020. Advances in Occurrence, Importance, and Mycotoxin Control Strategies: Prevention and Detoxification in Foods. Foods. 9(2): 137. https://doi.org/10.3390/foods9020137

Altun, S. K., Aydemir, M. E. and Alkan, S. 2024. Trends in Aflatoxin M1 Global Research: A Bibliometric Analysis Study. Iranian Journal of Public Health.https://doi.org/10.18502/ijph.v53i1.14694

Awuchi, C. G., Ondari, E. N., Nwozo, S., Odongo, G. A., Eseoghene, I. J., Twinomuhwezi, H., Ogbonna, C. U., Upadhyay, A. K., Adeleye, A. O. and Okpala, C. O. R. 2022. Mycotoxins' Toxicological Mechanisms Involving Humans, Livestock and Their Associated Health Concerns: A Review. Toxins. 14(3), 167. https://doi.org/10.3390/toxins14030167

Cano, P. M., Puel, O. and Oswald, I. P. 2016. Mycotoxins: Fungal Secondary Metabolites with Toxic Properties. In Fungi. CRC Press.

Chilaka, C. A., Obidiegwu, J. E., Chilaka, A. C., Atanda, O. O. and Mally, A. 2022. Mycotoxin Regulatory Status in Africa: A Decade of Weak Institutional Efforts. Toxins. 14(7): 442. https://doi.org/10.3390/toxins14070442

Chilenga, C., Masamba, K., Kasapila, W., Ndhlovu, B., Munkhuwa, V., Rafoneke, L. and Machira, K. 2025. Mycotoxin management in Sub-Saharan Africa: A comprehensive systematic review of policies and strategies in Malawi. Toxicology Reports. 14: 101871. https://doi.org/10.1016/j.toxrep.2024.101871

Dall'Asta, C., De Boevre, M., Dellafiora, L., De Saeger, S., Moretti, A., Pinson-Gadais, L., Ponts, N., Richard-Forget, F. and Susca, A. 2023. Boosting knowledge and harmonisation in the mycotoxin field through sustainable scientific alliances – MYCOBOOST. EFSA Supporting Publications. 20(11). https://doi.org/10.2903/sp.efsa.2023.EN-8420

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S. and Krska, R. 2020. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. Critical Reviews in Food Science and Nutrition. 60(16): 2773–2789. https://doi.org/10.1080/10408398.2019.1658570

Goda, A. A., Shi, J., Xu, J., Liu, X., Zhou, Y., Xiao, L., Abdel-Galil, M., Salem, S. H., Ayad, E. G., Deabes, M., Pooe, O., Donia, M. A. A., Abou-Arab, A. A. K. and Ramzy, S. 2025. Global health and economic impacts of mycotoxins: A comprehensive review. Environmental Sciences Europe. 37(1): 122. https://doi.org/10.1186/s12302-025-01166-x

Gomis, M. K. S., Oladinrin, O. T., Saini, M., Pathirage, C. and Arif, M. 2023. A scientometric analysis of global scientific literature on learning resources in higher education. Heliyon. 9(4): e15438. https://doi.org/10.1016/j.heliyon.2023.e15438

Kahl, G., Chapple, A. C., Scherr, F., Sur, R. and Preuss, T. G. 2026. Field-specific risk management for plant protection products: A "digitalized" way forward. Integrated Environmental Assessment and Management. 22(2): 523–533. https://doi.org/10.1093/inteam/vjaf165

Klingelhöfer, D., Zhu, Y., Braun, M., Bendels, M. H. K., Brüggmann, D. Groneberg, D. A. 2018. Aflatoxin – Publication analysis of a global health threat. Food Control, 89: 280–290.https://doi.org/10.1016/j.foodcont.2018.02.017

Kos, J., Anić, M., Radić, B., Zadravec, M., Janić Hajnal, E. and Pleadin, J. 2023. Climate Change—A Global Threat Resulting in Increasing Mycotoxin Occurrence. Foods. 12(14): 2704. https://doi.org/10.3390/foods12142704

Liu, M., Zhao, L., Gong, G., Zhang, L., Shi, L., Dai, J., Han, Y., Wu, Y., Khalil, M. M. and Sun, L. 2022. Invited review: Remediation strategies for mycotoxin control in feed. Journal of Animal Science and Biotechnology, 13(1): 19. https://doi.org/10.1186/s40104-021-00661-4

Logrieco, A., Battilani, P., Leggieri, M. C., Jiang, Y., Haesaert, G., Lanubile, A., Mahuku, G., Mesterházy, A., Ortega-Beltran, A., Pasti, M., Smeu, I., Torres, A., Xu, J. and Munkvold, G. 2021. Perspectives on Global Mycotoxin Issues and Management From the MycoKey Maize Working Group. Plant Disease, 105(3): 525–537. https://doi.org/10.1094/PDIS-06-20-1322-FE

López-García, R. 2022. Mycotoxin management. In Ensuring Global Food Safety (pp. 213–219). Elsevier. https://doi.org/10.1016/B978-0-12-816011-4.00024-0

Luo, S., Du, H., Kebede, H., Liu, Y. and Xing, F. 2021. Contamination status of major mycotoxins in agricultural product and food stuff in Europe. Food Control, 127. 108120. https://doi.org/10.1016/j.foodcont.2021.108120

Mafe, A. N. and Büsselberg, D. 2024. Mycotoxins in Food: Cancer Risks and Strategies for Control. Foods 13(21): 3502. https://doi.org/10.3390/foods13213502

Nicholls, P. T. 1989. Bibliometric modeling processes and the empirical validity of Lotka's Law. Journal of the American Society for Information Science 40(6): 379–385. https://EconPapers.repec.org/RePEc:bla:jamest:v:40:y:1989:i:6:p:379-385

Okechukwu, V. O., Adelusi, O. A., Kappo, A. P., Njobeh, P. B. and Mamo, M. A. 2024. Aflatoxins: Occurrence, biosynthesis, mechanism of action and effects, conventional/emerging detection techniques. Food Chemistry. 436: 137775. https://doi.org/10.1016/j.foodchem.2023.137775

Onyeaka, H., Anumudu, C., Miri, T. and Ahmad, N. H. 2024. A bibliometric analysis of research trends on the microbiological safety of lowmoisture foods. Food Research. 8(2): 467–488. https://doi.org/10.26656/fr.2017.8(2).277

Pao, M. L. 1986. An empirical examination of Lotka's law. Journal of the American Society for Information Science. 37(1): 26–33. https://doi.org/10.1002/asi.4630370105

Patel, T. K. 2025. Mycotoxins: Challenges and Strategies for Mitigation. In Mycological Inventions for Sustainable Agriculture and Food Production (pp. 377–406). IGI Global Scientific Publishing. https://doi.org/10.4018/979-8-3693-8201-1.ch017

Pitt, J. I. and Miller, J. D. 2017. A Concise History of Mycotoxin Research. Journal of Agricultural and Food Chemistry. 65(33): 7021–7033. https://doi.org/10.1021/acs.jafc.6b04494

Reza, M. N., Lee, K.-H., Karim, M. R., Haque, M. A., Bicamumakuba, E., Dey, P. K., Jang, Y. Y. and Chung, S.-O. 2025. Trends of Soil and Solution Nutrient Sensing for Open Field and Hydroponic Cultivation in Facilitated Smart Agriculture. Sensors. 25(2): 453. https://doi.org/10.3390/s25020453

Smaoui, S., D'Amore, T., Tarapoulouzi, M., Agriopoulou, S. and Varzakas, T. 2023. Aflatoxins Contamination in Feed Commodities: From Occurrence and Toxicity to Recent Advances in Analytical Methods and Detoxification. Microorganisms, 11(10): 2614. https://doi.org/10.3390/microorganisms11102614

Strotmann, A. and Zhao, D. 2012. Author name disambiguation: What difference does it make in author-based citation analysis? Journal of the American Society for Information Science and Technology. 63(9): 1820–1833. https://doi.org/10.1002/asi.22695

Wang, C., Huang, Y., Wang, Y., Zhang, J., Guo, M., Chen, C., Zhang, H., Yue, B., Kong, D., Luo, J. and Yang, M. 2024. Developments and trends in mycotoxin analysis: A review with bibliometric research. Microchemical Journal. 207: 111774.https://doi.org/10.1016/j.microc.2024.111774

Yeassin, R., Chowdhury, P., Das, P., Agyekum, E. B., Farrok, O. and Kumar, P. 2026. Navigating towards efuel: A scientometric insight into the application of membrane reactors. Energy Conversion and Management: X, 30: 101545. https://doi.org/10.1016/j.ecmx.2026.101545

Yilmaz, N., Verheecke-Vaessen, C. and Ezekiel, C. N. 2025. Mycotoxins: An ongoing challenge to food safety and security. PLOS Pathogens. 21(11), e1013672.https://doi.org/ 10.1371/journal.ppat.1013672

Yohannis, E., Urugo, M. M., Teka, T. A., Getachew, P., Tola, Y. B., Forsido, S. F., Kebede, Y. S. and Teferra, T. F. 2025. Aflatoxin Contamination in Agri-Food Systems: A Comprehensive Review of Toxicity, Food Security, Economic Impacts, and Sustainable Mitigation Across the Value Chain. Food Science & Nutrition, 13(10): e71104. https://doi.org/10.1002/fsn3.71104

Zyoud, S. H. 2019. Global scientific trends on aflatoxin research during 1998–2017: A bibliometric and visualized study. Journal of Occupational Medicine and Toxicology. 14(1):27. https://doi.org/10.1186/s12995-019-0248-7

Indian J. Anim. Nutr. 2026. 43 (1): 143-152
doi: 10.56093/IJAN.v43i1.17
Indian Journal of Animal Nutrition

Calcium, Phosphorus and Magnesium, but not Protein alone, Drive Growth in Pre-pubertal Captive Grasscutters (*Thryonomys swinderianus*)

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ABSTRACT

This study examined the interactive effects of dietary crude protein (CP), calcium (Ca), phosphorus (P), and magnesium (Mg) on pre-pubertal growth in captive grasscutters. Fifty weaned grasscutters (6 weeks old; initial mean weight 0.582 kg) were assigned to a 5 × 2 factorial design (diets × sex; n=10/treatment, 1:1 sex ratio). Treatments comprised four pelleted concentrate diets and fresh elephant grass (control, mimicking farmers' practice): Diet 1 (15% CP, 1.2% Ca, 0.6% P, 0.3% Mg); Diet 2 (15% CP, 1.5% Ca, 0.75% P, 0.33% Mg); Diet 3 (18% CP, 1.2% Ca, 0.6% P, 0.3% Mg); Diet 4 (18% CP, 1.5% Ca, 0.75% P, 0.33% Mg); elephant grass (11.37% CP, 0.9% Ca, 0.19% P, 0.13% Mg). Dietary CP influenced feed intake: Diets 1 and 2 (110.4 g/d) > Diets 3 and 4 (103.0 g/d), but elephant grass provoked the highest intake (160.1 g/d). Mineral synergies showed no clear feed intake effects (e.g., Diet 4 = 102.2 g/d vs. Diet 3 = 103.8 g/d; Diet 2 = 109.1 g/d vs. Diet 1 = 111.7 g/d). Water consumption increased with elevated mineral levels: Diets 2 and 4 (114.3 ml/d) > Diets 1 and 3 (106.7 ml/d). At 15% CP, elevated minerals (Diet 2) boosted average daily gain (0.013 kg vs. 0.010 kg), total weight gain (1.540 kg vs. 1.240 kg), and final body weight (2.132 kg vs. 1.808 kg) compared to Diet 1. At 18% CP, minerals had minimal impact (Diet 4 vs. Diet 3: 0.014 kg vs. 0.014 kg daily gain; 1.736 kg vs. 1.693 kg total gain; 2.317 kg vs. 2.275 kg final weight). It is concluded that synergies of elevated Ca, P, and Mg drive growth in CP-restricted diets (≤15%) beyond CP alone, and even at 18% CP, a mineral threshold is required to optimize pre-pubertal growth of captive grasscutter.

KEYWORDS: Pre-pubertal grasscutter, CP-Ca-P-Mg synergy, Pelleted diet, Growth performance

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INTRODUCTION

Grasscutters (Thryonomys swinderianus), the preferred bushmeat in sub-Saharan Africa (Falade et al., 2010), face production limits due to nutritional inadequacies of tropical grasses (*Pennisetum purpureum* and *Panicum maximum*), which are the main diets for captive grasscutters. These diets cause low birth weights, poor growth and reproduction capabilities, and high pre-/post-weaning mortalities (Adu et al., 2017). Past researches have focused on crude protein (CP) effects on growth (Kusi et al., 2012; Okyere et al., 2021) but have overlooked key minerals such as Ca, P, and Mg vital for skeletal structure and metabolism (Kothari et al., 2024). Grasses contain a substantial amount of Ca, but lower levels of P and Mg. Imbalanced Ca:P:Mg ratios impair absorption and utilization, thereby hindering growth (David et al., 2023).

Again, grasscutter farming is increasingly becoming popular in urban areas in Ghana. Urban farming challenges, such as grass scarcity, transport costs, and labor (Annor and Kusi, 2008), necessitate the production of pelleted concentrates for easy accessibility. But how much CP, Ca, P, and Mg should be included in the pelleted concentrates? This study targets optimal levels of these nutrients to boost feed and water intake of pre-pubertal grasscutters to optimise growth via the nutrients' synergies.

MATERIALS AND METHODS

Ethical Approval

This study was performed in line with the 'principles of laboratory animal care' and animal rights standards. Approval was granted by the Ethics Committee of Council for Scientific and Industrial Research (CSIR), Accra, Ghana. (2023-07-02/ CSIR020/2023).

Location and duration of study

The experiment was conducted at the grasscutter unit of the Department of Animal Science Education farm, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Asante Mampong Campus, Ghana. The experiment lasted for 18 weeks (including 2 weeks for adaptation. Table 1 shows mean temperature, humidity, and wind speed data recorded during the experimental period.

Table 1. Mean temperature, humidity and wind speed recorded over the period of study

Period	Temperature (°C)	Humidity (%)	Wind speed (kmph)
November	27.00	70	6.44
December	23.50	79	6.44
January	28.50	60	8.69
February	30.50	55	9.33

Experimental animals

The study used 50 weaned captive grasscutters (6 weeks old; average initial weight 0.583 kg; 25 males, 25 females), balanced by weight and randomly allocated to 5 dietary treatments with 10 animals/treatment and 1:1 sex ratio. They were housed singly in three-tier battery cages (measuring 60×50×40 cm) with each tier holding three cubicles. Crimped wire mesh was used as partitions and protective lining on wooden columns of the cages to prevent gnawing. Pull-out drawers were placed under the cubicles to collect feaces and waste feed.

Plate 1: A three-tier grasscutter cage

Plate 2: Pubertal-aged male grasscutter

Ration

Four diets containing varying levels of CP, Ca, P, and Mg were formulated, compounded, and pelletised. Fresh elephant grass was fed as a control to mimic local grasscutters farmers' practice. The proportions of the individual feed ingredients included in each of the four dietary treatments are detailed in Table 2.

Table 2. Composition of experimental diets

Ingredients	% Composition				
	Control	Diet 1	Diet 2	Diet 3	Diet 4
Maize	0.0	30.0	28.0	24.0	20.9
Rice husk	0.0	16.7	14.4	10.0	10.0
Elephant grass (fresh)	100.0	0.0	0.0	0.0	0.0
Elephant grass (dry)	0.0	8.0	8.0	8.0	8.0
Soy bean mea	l0.0	17.4	18.0	27.1	27.5
Wheat bran	0.0	10.0	10.0	13.0	12.0
Moringa	0.0	3.0	3.0	5.0	5.0
Oyster shell	0.0	0.7	2.0	0.7	2.0
Dicalcium phosphate	0.0	3.0	6.0	3.0	6.0
Magnesium oxide	0.0	0.2	0.6	0.2	0.6
[1]Vit. mineral premix	0.0	1.0	1.0	1.0	1.0
Common salt	0.0	2.0	2.0	2.0	2.0
Cassava flour	0.0	0.0	7.0	6.0	5.0
Total	100.0	100.0	100.0	100.0	100.0

[1]Vitamin mineral premix composition: vit A (800 IU), vit D (3000 IU), vit E (8 IU), vit K (2 mg), vit B1 (1 mg), vit B2 (2.5 mg), vit B12 (5 mg), Niacin (10 mg), Pantothenic acid (5 mg), Antioxidant (6 mg), Folic acid (0.5 mg), Choline (150 mg), Iron (20 mg), Manganese (80 mg), Zinc (50 mg), Cobalt (0.22 mg), Iodine (2 mg) and selenium (0.1 mg).

Feeding and watering

Experimental grasscutters were fed twice daily at 07:00 h and 17:00 h GMT. For weeks 1–8, each animal received 100 g/day (70 g morning, 30 g evening); for weeks 9–16, 150 g/day (100 g morning, 50 g evening). Feed leftovers were collected each morning, weighed, and subtracted from the amount offered to calculate daily feed intake before the next feeding.

Plate 3: Pubertal-aged female grasscutter feeding on pelleted diet

Preparation of feedstuffs

The freshly cut feedstuffs (elephant grass and moringa leaves) were air-dried to about 12% moisture and milled to pass through a 2 mm sieve using a Hammer mill. The remaining ingredients were obtained from university feed stores.

Proximate composition of experimental diets

The proximate compositions of feed ingredients and the diets were analysed using the methods prescribed by the Association of Official Analytic Chemists (AOAC, 2008) at the Nutritional Laboratory of the Kwame Nkrumah University of Science and Technology (KNUST). The metabolizable energy (ME) of the diets was calculated using the formula: ME = 37 x %CP + 81.8 %EE + 35 x %NFE as proposed by Pauzenga (1985). Details are shown in Table 3.

Table 3. Chemical composition of diets

Parameter	Experimental diets				
	Control	Diet 1	Diet 2	Diet 3	Diet 4
Moisture (%)	90.77	8.24	8.98	8.83	8.87
Crude protein (%)	11.37	15.15	15.21	18.25	18.18
Ether Extract (%)	1.32	2.23	2.08	2.59	2.97
Crude Fibre (%)	28.47	9.61	9.83	9.48	9.21

Ash (%)	7.65	9.89	11.62	10.61	11.38
NFE (%)	48.11	49.57	49.43	51.02	51.23
Ca (%)	0.90	1.17	1.48	1.18	1.47
P (%)	0.19	0.58	0.74	0.58	0.73
Mg (%)	0.13	0.32	0.37	0.32	0.36
Calculated Values					
[1]Energy MJ/ kg ME	9.28	10.37	10.31	11.18	11.23
Protein: Energy	1.23	1.46	1.48	1.63	1.62

[1]Estimated using equation by Pauzenga (1985); ME = (37 × percent crude protein) + (81.8 × percent ether extract) + (35 × percent nitrogen free extract).

Experimental design

5 x 2 factorial consisting of five dietary treatments and the sex of the grasscutters (male and female) was used. MINITAB version 18.1 was used for the analysis (Minitab, 2017). The differences in means were separated using Tukey's pair-wise comparisons at 95% confidence level.

Statistical model

The following statistical model was used to explain the effect and the relationship between the diets fed and the final body weight of the grasscutters:

$Y_{ijk}=\alpha+\tau_j+\gamma_k+\epsilon_{ijk}$, where $\epsilon_{ijk}\sim N(0,\sigma^2)$

- Y_{ijk} = the true body weight for the j^th observation from the j^th treatment and kth sex
- i = the observation number within the j^th treatment and kth sex
- j = the type of treatment (Elephant Grass, D1, D2, D3, D4)
- k = the type of sex (Male, Female)
- α = the true mean body weight for the treatment E Grass group (the baseline group)
- τ_j = the true deviation for jth treatment (D1, D2, D3, D4) from the true mean of the baseline group (E Grass)
- γ_k = the true deviation for the kth sex (Female) from the true mean of the baseline group (Male).
- ϵ = the random error associated with the i^th observation from the j^th treatment and kth sex.

RESULTS AND DISCUSSION

Treatment effect on feed intake

Elephant grass-fed grasscutters consumed more feed (160.1 g/animal/d) than those on pelleted diets (106.5 g/animal/d), with Diets 1 and 2 showing higher intake (110.4 g) than Diets 3 and 4 (103 g); intake increased with age (Fig. 1).

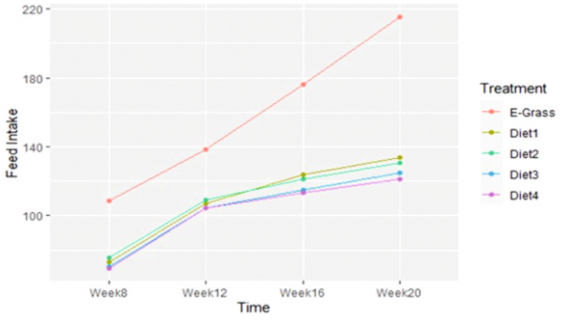

Fig. 1. Effect of dietary compositions on feed intake of pre-pubertal grasscutters

Pre-pubertal grasscutters fed fresh elephant grass consumed substantially more feed than those on pelleted concentrate diets varying in mineral levels. This likely stems from the forage's lower dry matter (DM) content (9.23%) compared to 91.27% DM in pellets. Lower DM feeds promote higher intake rates to achieve satiety, as noted by Karikari and Nyameasem (2009) for grasscutters.

Additionally, animals fed ad libitum adjust intake to meet nutrient needs (Azevêdo et al., 2016). Less nutrient-dense feeds like elephant grass (Singh, 2024) require greater consumption to satisfy metabolic demands compared to the pelleted concentrates. Thus, higher intake of forage reflects compensatory behaviour. Again, feed intake increased with the advancement of age (Fig. 1). A similar observation was made by Suman et al. (2024) on Gaddi goat bucks fed bypass fat.

The interactive effects of dietary crude protein (CP), calcium (Ca), phosphorus (P), and magnesium (Mg) did not influence feed intake in pre-pubertal grasscutters. Information on such interactions remains scarce for farm animals. The variations in Ca, P, and Mg levels typically do not affect feed intake in species like poultry (Al-Ghamdi, 2022), pigs (Yang et al., 2022), and cows (McArt and Oetzel, 2023). This suggests that minerals in general have minimal effect on livestock feed intake, as other studies with zinc show similar

results (Padmaja et al., 2024; Umrao et al., 2025). However, intact sheep fed Mg-deficient diets showed reduced voluntary intake compared to controls (Ammerman et al., 1971). Specifically, complete Mg absence slashed intake by 32%, with a minimum requirement of 8–10 mg/kg body weight needed to restore normal appetite (Ammerman et al., 1971). This underscores the importance of meeting Mg thresholds to sustain feed intake. In the present study, the Mg levels offered evidently met growing grasscutters' minimum needs, explaining the consistent intake across treatments.

Interactive effect of diet and sex on feed intake

Except for grasscutters fed diet 4, feed intake was slightly higher for male animals across the dietary treatments; however, the differences were bridged as the animals aged (Fig. 2).

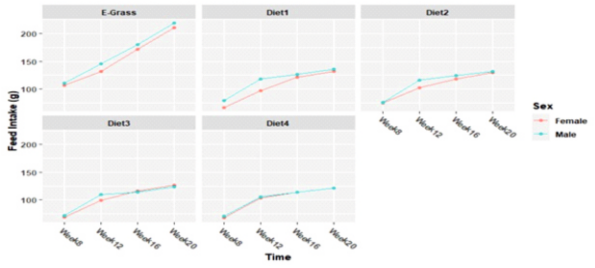

Fig. 2. Effect of age and sex on feed intake

Age significantly influenced feed intake, with consumption rising from weaning (6 weeks) to 24 weeks old. This aligns with increased physiological demands during growth, necessitating higher intake. Grandl et al. (2016) observed similar patterns in growing cows where organic matter intake increased with age, possibly as a result of distention of the rumen. The caecum in the grasscutter mimics the rumen in ruminants, which may account for similar results.

Sex had no significant effect on feed intake, though males consumed slightly more than females early in growth before patterns converged. This corroborates the findings by Hussein and Abd El-Fattah (2020), which indicated a lack of sexual dichotomy in feed intake of growing California rabbits.

Feed wastage

Feed wastage differed greatly between pelleted concentrate diets (diets 1–4) and fresh elephant grass. The average percentage of feed wastage for animals fed pelleted diets was 8.66%, in contrast to the 62.20% recorded for their counterparts fed fresh elephant grass. Males wasted more grass than females, but no sex differences appeared in pelleted diet groups.

Grasscutters exhibit substantial feed wastage, particularly on forage diets, wasting about 70% of forage (Mensah et al., 2001) and 17% of mashed concentrates (Kusi et al., 2012). In this study, however, captive pre-pubertal grasscutters on forage diets wasted approximately 62.2%—slightly less than previously reported. Wastage dropped markedly to 8.7% for those fed pelleted concentrates, a roughly 50% improvement over mashed feeds.

This reduction likely stems from the pelleted form's lower surface area compared to forage or mashed diets, which limits scattering. Farmers should prioritize pelleted diets to cut losses, boost profits, and curb environmental impacts such as vast land for disposal and emission of greenhouse gases like methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) (Liu and Liu, 2018). Sex influenced feed wastage in grasscutters on forage diets but not on pelleted concentrates. Males wasted about 7.6% more fresh forage than females, likely due to their greater aggression (Soro et al., 2014).

Synergistic Effects of Dietary CP, Ca, P, Mg, and Weather on Water Intake in Pre-pubertal Grasscutters

Figure 3 illustrates how daily water intake was influenced by dietary CP and mineral levels and weather. Pelleted concentrate diets with higher mineral levels (diets 2 and 4) increased water intake to 117.5 ml/animal/day, compared to 100 ml/animal/day for lower-mineral diets (diets 1 and 3).

Feed type significantly affected intake: forage-fed grasscutters drank ~20 ml/animal/day, versus ~103 ml/animal/day for pelleted concentrates. Water intake, furthermore, rose with higher ambient temperature and lower relative humidity (RH), averaging 103 ml/animal/day and peaking in February at 30.5°C and 55% RH, whereas the least amount was drunk in December at 23.5°C and 79% RH (Fig. 4).

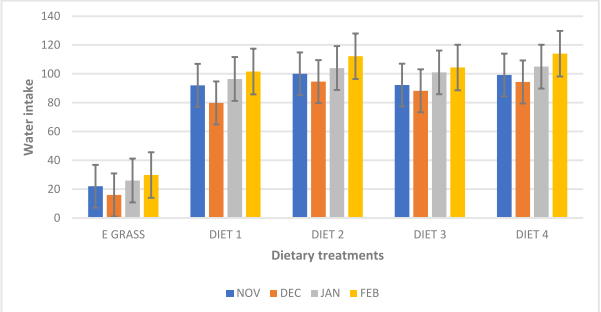

Fig. 3.Water intake dynamics of pre-pubertal grasscutters

Grasscutters fed Diets 2 and 4 (1.5% Ca, 0.75% P, 0.38% Mg) drank more water than those on 1.2% Ca, 0.6% P, and 0.3% Mg (diets 1 and 3), regardless of CP content. The elevated levels of the minerals in diets 2 and 4 might have caused a corresponding increase in water intake. Similar findings were observed in rats (Oyeyipo et al., 2010). Kusi et al (2012) reported an average water intake of 101.93 ml/animal/day, which is similar to that of animals fed diets 1 and 3 but lower than 114.3 ml/animal/day recorded for animals fed diets 2 and 4. The similarities and the variations could be attributed to the levels of Ca, P, and Mg in the diets. This could be ascribed to the role of water to dissolve minerals for possible absorption and excretion of the excess from the blood (Khan et al., 2016; Shkembi and Huppertz, 2021). For instance, inadequate water intake risks Ca supersaturation, which could lead to kidney stones, with over 80% of global cases linked to calcium compounds like oxalate (Khan et al., 2016; Susilo et al., 2021).

Grasscutters fed elephant grass drank approximately 70 ml less (per animal/day) compared to those fed pelleted concentrates. The differences in dietary moisture might have accounted for this. Forage's high moisture provided adequate hydration, reducing drinking needs, while drier pellets demanded more water for digestion and metabolism. Torres et al. (2019) alluded that dietary characteristics such as dry matter and ash could influence the drinking habits of cows, as observed in this study.

The variations observed in water intake during the study period could also be attributed to the prevailing weather conditions. The months with relatively higher temperatures drove intake to support thermoregulation, countering dehydration from increased respiration (Wolf, 2020; Orakpoghenor et al., 2021). Adequate clean water is thus vital for homeostasis, especially in hot sub-Saharan regions like Ghana (Rivera and Anjum, 2023), because hypohydration affects body fluids concentrations, risking hypotonic hypovolemia (Thornton, 2016).

Growth performance

Dietary synergies of CP, Ca, P, and Mg significantly influenced ($P < 0.05$) growth rate, final body weight, and total weight gain. Grasscutters on diets 3 and 4 achieved the highest values, outperforming ($P<0.05$) those on diets 1, 2, and elephant grass (control). The control group performed the poorest ($P<0.05$) across all growth metrics.

Sex × diet interactions also affected body weight measures, with males and females on diets 3 and 4 superior ($P<0.05$) to other groups. Daily weight gain, final body weight, and total weight gain were similar for males on diet 2 and both sexes on diet 3, but higher ($P<0.05$) than diet 1 and control. Within the diets, sexes showed similar performance (Table 4), except males on diet 2 that outperformed their female counterparts.

Table 4. Dietary CP, Ca, P, and Mg synergies on growth performance of captive pre-pubertal grasscutters

Variable	Initial Weight (kg)	Daily weight (kg)	Total weight (kg)	Final Body weight (kg)
Dietary treatments				
Grass	0.585	0.006[d]	0.721[d]	1.301[d]
Diet 1	0.573	0.010[c]	1.240[c]	1.808[c]
Diet 2	0.592	0.013[b]	1.540[b]	2.132[b]
Diet 3	0.582	0.014[a]	1.693[a]	2.275[a]
Diet 4	0.581	0.014[a]	1.736[a]	2.317[a]
SEM	0.007	0.001	0.014	0.014
P-value	0.552	0.001	0.001	0.001
Sex effect				
Male	0.591[a]	0.012[a]	1.402[a]	1.994a
Female	0.574[b]	0.011[b]	1.369[b]	1.939[b]
SEM	0.005	0.001	0.022	0.008
P-value	0.019	0.001	0.012	0.001
Treatment*Sex				
Diet 4*Males	0.594	0.015[a]	1.742[a]	2.336[a]
Diet 4*Females	0.568	0.014[a]	1.731[a]	2.299[a]

Diet 3*Males	0.592	0.014^{ab}	1.690^{ab}	2.281^{ab}
Diet 3*Females	0.572	0.014^{ab}	1.700^{ab}	2.268^{ab}
Diet 2*Males	0.600	0.013^{b}	1.611^{b}	2.211^{b}
Diet 2*Females	0.584	0.012^{c}	1.468^{c}	2.052^{c}
Diet 1*Males	0.580	0.010^{d}	1.248^{d}	1.828^{d}
Diet 1*Females	0.566	0.010^{d}	1.233^{d}	1.788^{d}
Grass*Males	0.590	0.006^{e}	0.721^{e}	1.311^{e}
Grass*Females	0.580	0.006^{e}	0.721^{e}	1.291^{e}
SEM	0.011	0.001	0.020	0.018
P-value	0.961	0.001	0.002	0.001

D 1 = 15% CP; 1.2% Ca; 0.6% P & 0.3% Mg; D 2 = 15% CP; 1.5% Ca; 0.75% P & 0.35% Mg; D 3 = 18% CP; 1.2% Ca; 0.6% P & 0.3% Mg and D 4 = 18% CP; 1.5% Ca; 0.75% P & 0.35% Mg, EG = Elephant Grass F = Female; M = Male; Means in a row with different superscripts are significantly different (P<0.05). SEM = Standard Error of Mean.

Dietary synergies of CP, Ca, P, and Mg remarkably influenced daily body weight gain, final body weight, and total weight gain in pre-pubertal grasscutters. Those fed 18% CP pelleted diets, irrespective of the mineral levels, averagely gained 14 g/day—over double that of elephant grass-fed animals and 2.5 g/day higher than 15% CP fed groups—confirming 18% CP as optimal for promoting growth of captive grasscutters, consistent with prior findings (Kusi et al., 2012; Nyameasem et al., 2019). Protein promotes growth primarily by providing the essential amino acids that serve as the building blocks for tissues in the body (Petkova et al., 2025). The body uses protein to repair and build muscles, bones, skin, and other tissues, supporting overall tissue growth and maintenance (Lopez and Shamim, 2024; Qamar et al., 2025). The growth rate, final body weight, and total body weight gain observed for animals fed diet 4 in this study were higher than those of Kusi et al. (2012). Even though the diets involved in the two separate studies had similar CP levels (18%), they differed in mineral levels. The diet fed by Kusi et al. (2012) had lower levels of Ca (0.51%), P (0.66%), and Mg (0.18%) compared to the levels used in this present study (diet 4) which presupposes that even at 18% CP, a certain threshold of Ca, P, and Mg levels is necessary to optimise growth in pre-pubertal captive grasscutters.

Moreover, the captive pre-pubertal grasscutters fed diets 1 and 2 showed significant protein-mineral-dependent growth patterns. Irrespective of the similar CP levels, grasscutters fed Diet 2 (15% CP with 1.5% Ca, 0.75% P, and 0.38% Mg) achieved markedly higher daily body weight gain, total body weight gain and final body weight compared to their counterparts fed Diet 1 (15% CP with 1.2% Ca, 0.6% P, 0.3% Mg), emphasizing mineral optimization synergies over CP alone in promoting growth at pre-pubertal stage of captive grasscutters. This study reaffirms the role of Ca, P, and Mg as growth promoters via bone mineralization (Michigami and Osono 2019; Wongdee et al., 2019), energy metabolism in the cell (P in ATP/creatine phosphate) (Karger et al., 2024) and homeostasis (Morris and Mohiuddin 2021) to the extent that their deficiencies (hypocalcemia, hypophosphatemia, and hypomagnesemia) in growing animals risk delayed growth and development and may act as predisposal factor to rickets (Miller and Imel, 2022).

Sexual dichotomy did not show any significant variation in daily body weight gain, final body weight, or total body weight gain among animals that received the same diet, except for those fed diet 2, which warrants further investigation. Aside from pre-pubertal grasscutters fed diet 2, the weight gap between males and females at weaning was mitigated by 24 weeks of age. These findings align with earlier reports that sex-based growth variations in some animals diminish when environmental and dietary factors are standardised. Lamptey et al. (2022) reported a lack of sexual dichotomy in the growth performance of New Zealand rabbits when offered similar dietary treatments as observed in this study. This emphasizes the nutritional impact over biological sex on growth outcomes of pre-pubertal grasscutters.

Feed conversion ratio

Grasscutters fed diet 4 exhibited the lowest FCR, which was statistically similar to that of diet 3 but different from diets 1 and 2. In contrast, animals fed elephant grass recorded the highest FCR compared to those offered concentrate pelleted diets (Fig.4).

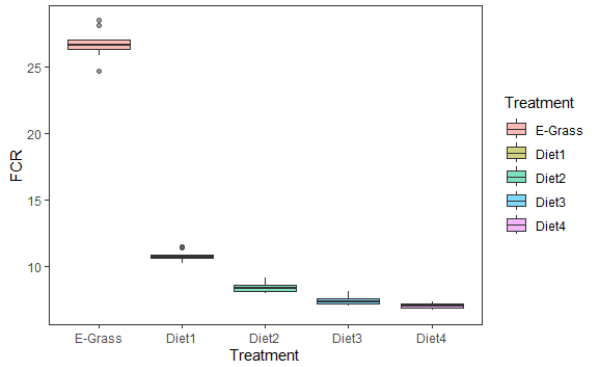

Fig. 4.Effect of dietary treatments on feed conversion ratio

Captive pre-pubertal grasscutters fed 18% CP showed similar feed conversion ratios (FCR), indicating that varying Ca, P, and Mg levels had minimal impact at this protein level. Contrary, they outperformed those on other diets, aligning with Nyameasem et al. (2019), who found higher efficiency of diets with dietary protein of 18% in growing grasscutters. At 15% CP, however, higher minerals (1.5% Ca, 0.75% P, 0.38% Mg) enhanced feed efficiency (lower FRC) as compared to lower levels (1.2% Ca, 0.6% P, 0.3% Mg), suggesting mineral synergy boosts efficiency at moderate protein levels.

Toxicology and Mortality

Two deaths were recorded between the first and eighth week of the experiment: one from animals fed the control diet and the other from those fed diet 1. Post mortem examination conducted showed no sign of adverse effect on any of the organs; nor was there any observed in the bladder or the kidneys. However, traces of blood stains were found in the intestines of the animals during the examination, giving an indication of coccidiosis. Grasscutters are coprophagous (Van Zyl and Delport, 2010); hence, there is a possibility of contracting coccidiosis, as occurs in rabbits. This suggests that the dietary materials and their levels offered posed no health risk to the growing grasscutters studied.

CONCLUSION

Synergies of elevated Ca, P, and Mg drive growth in CP-restricted diets (≤15%) beyond CP alone, and even at 18% CP, a mineral threshold is required to optimize pre-pubertal grasscutter growth. Adopting pelleted diets is vital to reduce feed wastage to maximise profit. Adequate water supply is key when offering pelleted diets with elevated mineral levels to avert possible kidney-related complications.

REFERENCES

Adu, E. K., Asafu-Adjaye, A., Hagan, B. A., and Nyameasem, J. K. 2017. The grasscutter: an untapped resource of Africa's grasslands. Livestock Research for Rural Development. 29(3): 47.

Al-Ghamdi, E.S. 2022. Influence of calcium and nonphytate phosphorus (NPP) on meat-type quail's growth, carcass features, and tibia indices. Journal of Experimental Biology and Agricultural Sciences. 10(3): 668-678.

Ammerman, C. B., Chicco, C. F., Moore, J. E., Van Walleghem, P. A. and Arrington, L. R. 1971. Effect of dietary magnesium on voluntary feed intake and rumen fermentations. Journal of Dairy Science. 54(9): 1288-1293.

Annor, S.Y. and Kusi, C. 2008. Factors influencing the adoption of grasscutter production in the Brong Ahafo region of Ghana. Livestock Research for Rural Development. 20: 9.

AOAC. 2008. Official Methods of Analysis. 17th Edn. Association of Official Analytic Chemists. Gaithersburg, MD. USA.

Azevêdo, J. A. G., Valadares Filho, S. C., Costa e Silva, L. F., Dos Santos, A. B., Rotta, P. P., Rennó, L. N. and Prado, I. N. 2016. Regulation and prediction of dry matter intake. In: Nutrient Requirements of Zebu and Crossbred Cattle. Eds. Valadares Filho, SC, Costa e Silva, L.F, Rotta, P.P, Gionbelli, M.P, Marcondes, M.I, Chizzotti, M.L, Prados, L.F. *BR-CORTE* p. 15-42.

David, L. S., Anwar, M. N., Abdollahi, M. R., Bedford, M. R. and Ravindran, V. 2023. Calcium nutrition of broilers: current perspectives and challenges. Animals. 13(10): 1590.

Falade, L.O., Idahor, K.O. and Ayodele, I.A. 2010. Survey of domestication process of grasscutter (*Thryonomys swinderianus*) in some selected states in Southwestern Nigeria. Journal of Agriculture, Forestry and the Social Sciences. 8(2): 49 – 55.

Grandl, F., Luzi, S.P., Furger, M., Zeit, J.O., Leiber, F., Ortmann, S. and Schwarm, A. 2016. Biological implications of longevity in dairy cows: Changes in feed intake, feeding behaviour and digestion with age. Journal of Dairy Science. 99(5): 3457-3471.

Hussein, A., and Abd El-Fattah, M. G. 2020. Effect of sex and feed frequency on growing California rabbits, carcass characteristics and

meat quality. Egyptian Poultry Science Journal. 40(2): 405-419.

Karger, G., Berger, J. and Dringen, R. 2024. Modulation of cellular levels of adenosine phosphates and creatine phosphate in cultured primary astrocytes. Neurochemical Research. 49(2): 402-414.

Khan, S.R., Pearle, M.S., Robertson, W.G., Gambaro, G., Canales, B.K. and Doizi, S. 2016. Kidney stones. Nature Reviews Disease Primers. 2:16008.

Karikari, P. K. and Nyameasem, J. K. 2009. Productive performance and carcass characteristics of captive grasscutters (*Thryonomys swinderianus*) fed concentrate diets containing varying levels of guinea grass. World Applied Sciences Journal. 6(4): 557-563.

Kothari, M., Wanjari, A., Shaikh, S. M., Tantia, P., Waghmare, B. V., Parepalli, A. and Nelakuditi, M. 2024. A comprehensive review on understanding magnesium disorders: pathophysiology, clinical manifestations, and management strategies. Cureus. 16(9): e68385.

Kusi, C., Tuah, A.K., Annor, S.Y. and Djan-Fordjour, K.T. 2012. Determination of dietary crude protein level required for optimum growth of grasscutters *(Thryonomys swinderianus*) in captivity. Livestock Research for Rural Development. 24: 10

Lamptey, V.K., Hagan, B.H. and Kruenti, F. 2022. The influence of sex and age on growth rate of domestic rabbits (Oryctolagus cuniculus). Journal of Innovative Agriculture 9(2).

Liu, Z. and Liu, Y. 2018. Mitigation of greenhouse gas emissions from animal production. Greenhouse Gases: Science and Technology. 8(4): 627-638.

Lopez, M.J. and Shamim S. M. 2024. "Biochemistry, essential amino acids." In *StatPearls [Internet]*. StatPearls Publishing.

McArt, J. A. and Oetzel, G. R. 2023. Considerations in the diagnosis and treatment of early lactation calcium disturbances. Veterinary Clinics: Food Animal Practice. 39(2): 241-259.

Mensah, G. A., Gnimadi, A. and Houngnibo, G. 2001. Formulation d'un projet de promotion de la filière aulacode au Bénin -Volume III - Projet de Promotion de la Filière Aulacode au Bénin (PPFAB). CBDD/Bénin. 28 p.

Michigami, T. and Ozono, K. 2019. Roles of phosphate in skeleton. Frontiers Endocrinology (Lausanne). doi:10.3389/fendo.2019.00180. PMID: 30972027; PICID: PNC6443631.

Miller, W. L. and Imel, E. A. 2022. Rickets, vitamin D, and Ca/P metabolism. Hormone Research in Paediatrics. 95(6): 579-592.

Minitab. 2017. Statistical Software, Version 18.1. Minitab Incorporation, State College.

Morris, A.L. and Mohiuddin, S.S. 2021. In StatPearls. Treasure Island (FL).

Nyameasem, J. K., Akoloh, M. and Adu, E. K. 2019. Effects of protein content on feed intake and performance of grasscutters fed diets containing forage meal. Animal Production Science. 59(2): 314-318.

Okyere, K., Kyere, C.G., Sarpong, A.A. and Poku Jnr., P.A. 2021. Influence of different dietary protein concentration on growth and reproductive performance of the grasscutter (*Thryonomys swinderianus*). World Journal of Pharmaceutical and Life Sciences. 7(5): 104 – 110.

Orakpoghenor, O., Ogbuagu, N. E., and Sa'Idu, L. 2021. Effect of environmental temperature on water intake in poultry. Advances in Poultry Nutrition Research.

Oyeyipo, I. P., Olatunji, L. A., Akhigbe, R. E., Arokoyo, D. S. and Soladoye, A. O. 2010. Effect of increased dietary calcium on body weight, food and water intake in oral contraceptive treated female rats. Nigerian Journal of Physiological Science. 25(1): 73–79.

Padmaja, D., Ravi, A., Devasena, B., Durga, S. and Suresh, J. 2024. Evaluation of Ayurvedic zinc (Yashada Bhasma) in commercial broilers. Indian Journal of Animal Nutrition. 41(3): 528-540.

Pauzenga, U. 1985. Feeding parent stock. Zootenicia International. 17: 22–24

Petkova, D., Stoyanova, S., Dinkov, G. and Bogdanov, M. G. 2025. Beyond Protein Building Blocks: A Review of Biological Roles and Therapeutic Potential of Free Amino Acids. International Journal of Molecular Sciences. 26(23): 11264.

Qamar, M. N., Nafees, H. and Feroz, S. A. 2025. Role of Protein Nutrition for Maintaining the Health of the Musculoskeletal System. In: Evaluating the Effectiveness of Functional

Ingredients in Sports Nutrition. Eds. Sehar, C. and Hamit, C. IGI Global Scientific Publishing. pp. 177-214.

Rakshit, S., Roy, K. and Datta, I. C. 2021. Hypercalciuria in the house rabbit: an ecological anomaly. Haryana Veterinary. 60(SI): 103-105.

Rivera, J.G.M. and Anjum, F. 2023. Hypovolemia. Statpearls. National Library of Medicine. National Centre for Biotechnology Information.

Shkembi, B. and Huppertz, T. 2021. Calcium absorption from food products: food matrix effects. Nutrients, 14(1): 180.

Singh, G. 2024. Nutrition and feeding management of goats for chevon production. International Journal of Science, Environment and Technology. 13(5): 339.

Soro, D. Okon, A. Kimsé, M. and Fantodji, A. 2014. Using communication to improve grasscutters (*Thryonomys Swinderianus*) reproductive behaviour in Cote D'ivoire. International Journal of Science and Research. 3(6): 2465-2468.

Suman, M., Katoch, S., Verma, N., Thakur, D. and Dinesh, K. 2024. Impact of bypass fat on growth and testicular measurements in Gaddi goat bucks. Indian Journal of Animal Nutrition, 41(3): 385-392.

Susilo, J., Purwanto, B., Doewes, M. and Indarto, D. 2021. Calcium oxalate crystals: Epidemiology, causes, modeling of crystal formation and treatment management. Journal of Pharmaceutical Sciences and Research. 13(2): 118-123.

Thornton, S. N. 2016. Increased hydration can be associated with weight loss. Frontiers in nutrition. 3: 183053.

Torres, R. N. S., Silva, H. M., Donadia, A. B., Menegazzo, L., Xavier, M. L. M., Moura, D. C. and Oliveira, A. S. 2019. Factors affecting drinking water intake and predictive models for lactating dairy cows. Animal Feed Science and Technology. 254: 114194.

Umrao, P., Kumar, V., Kumar, M., Kushwaha, R., Vaswani, S., Kumar, A., Yadav, R.D. and Gupta, M. 2025. Effect of three different forms of zinc on growth performance, digestibility, blood profiles and antioxidant status of Sahiwal heifers. Indian Journal of Animal Nutrition. 42(2): 151-160

Wolf, P., Cappai, M. G. and Kamphues, J. 2020. Water consumption in small mammals (dwarf rabbits, Guinea pigs and chinchillas): New data about possible influencing factors. Research in Veterinary Science. 133: 146-149.

Wongdee, K., Rodrat, M., Teerapornpuntakit, J., Krishnamra, N. and Charoenphandhu, N. 2019. Factors inhibiting intestinal calcium absorption: Hormones and luminal factors that prevent excessive calcium uptake. Journal of Physiology Science. 69: 683-696.

Yang, A., Wang, K., Peng, X., L.F., Wang, Y., Cui, Y. and Si, H. 2022. Effects of different sources of calcium in the diet on growth performance, blood metabolic parameters, and intestinal bacterial community and function of weaned piglets. Frontiers in Nutrition. 9: 885497.

Van Zyl, A. and Delport, J. H. 2010. Digestibility of nutrients and aspects of the digestive physiology of the greater cane rat (*Thryonomys swinderianus)*. African Zoology. 45: 254-264.

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Carbon Quantum Dots Essential Oils Complex as A Substitute for Antibiotic Growth Promoter in Broiler Chicken Diet

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ABSTRACT

Essential oils (EO) are volatile in nature. To maintain their efficacy as antimicrobial compounds, seven EO were complexed with carbon quantum dots (CQEC). The *in vitro* antimicrobial efficacy in terms of minimum inhibitory concentration and minimum bactericidal concentration of the CQEC was either similar or higher to that of antibiotic growth promoter (AGP i.e. BMD) against common pathogens (*Salmonella, E. coli*, and *Clostridia*). To synthesize a complex of CQ-EOs, and assess the antimicrobial activity, and their potential benefits on performance, carcass traits, immune responses, antioxidant variables, and caecal microbiota composition in broiler chicken-fed diets without AGP. Two feeding trials were conducted to study the *in vivo* efficacy of CQEC as an alternative to BMD. In experiment 1, CQEC was tested at 200 and 250g/ton, while in experiment 2, CQEC was included at 100, 250, 500, and 1000g/ton feed. A positive control (PC) with BMD and a negative control (NC) without BMD or CQEC were fed in both experiments. The diets were in mash form, and each diet was fed to 10 replicates having 25 birds in each floor pen. The results indicated that feed efficiency (FE) reduced significantly in the NC group compared to the PC. The regression analysis indicated nonlinear improvement in FE with supplementation of different concentrations of CQEC to the NC diet. Similarly, nonlinear improvement in immune responses (CMI response and HI titres), and activity of superoxide dismutase, reduced lipid peroxidation and caecal colony count of pathogenic bacteria (*E. coli* and *Salmonella*) were observed when CQEC was supplemented to the NC diet. The gut microbiome analysis indicated upregulation of the Bacteroides population in CQEC-supplemented groups compared to the NC group. Based on the results, it is concluded that the broiler performance can be maintained with supplementation of CQEC to the AGP-free NC diet. The improved performance with CQEC supplementation could be due to the reduction in gut pathogens (*E coli* and *Salmonella*), improvement in the Bacteroides population in the gut, and improvement in immune and antioxidant variables in broilers fed the alternative compound (CQEC) to AGP.

KEYWORDS: Antioxidation, Broilers, Carbon quantum, Essential oils, Gut microbiome, Immune responses.,performance.

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INTRODUCTION

The improved chicken strains are reared in large numbers in a closed environment in the commercial farming system, which has forced to use of antimicrobial compounds in diet to realize maximum performance. Over a half-century, antibiotics at sub-therapeutic concentration () were considered as the essential feed additives in broiler diet to attain higher production and for a balanced gut ecosystem (Huyghebaert et al., 2011). One of the major reasons to use antibiotics as growth promoters (AGP) in poultry diets was their cost effectiveness (Fernandez-Rubio et al., 2009). In recent times, there has been increased resistance

from consumers on the use of AGPs in poultry diets. But, the withdrawal of AGPs in poultry diets resulted in an increased incidence of necrotic enteritis () and other gut-related disorders. Therefore, a need was felt to use alternative and safe compounds that mimic the activity of AGP by improving the function of the gastrointestinal tract and sustaining the broiler performance without leaving any residues in poultry meat or causing antimicrobial resistance in the chicken gut microbiota.

Several alternatives have been tested as non-antibiotic growth promoters in broiler meat production like probiotics, prebiotics, microbial enzymes (), herbal products (Diaz-Sanchez et al., 2015), organic acids (Rama Rao et al., 2023) and essential oils (). Among the several alternatives, essential oils (EO) were reported to have positive effects on broiler performance by improving weight gain (Kim et al, 2016), feed efficiency (Pirgozliev et al., 2019), enzyme secretion in the gut (Zeng et al., 2015), and nutrients digestibility (Amad et al., 2011). Therefore, supplementation of EO was reported to replace AGP in the broiler diet (Basmacioğlu-Malayoğlu et al, 2016, Attia, et al., 2019). The blend of essential oils in diets was reported to improve the intestinal microbial balance by a reduction of coliform bacteria and an increase in *Lactobacillus* spp. counts resulted in an improvement in the weight gain of broiler chickens (Cetin et al, 2016).

Essential herbs oils (EO) like thyme, cinnamon, tea tree, eucalyptus, oregano*, citral*, and mentha are known to have several beneficial roles in human health and livestock farming (Gheorghita et al., 2022). However, the beneficial role of EO in poultry production is quite inconsistent (Agung Irawan et al., 2021**).** As these EO are volatile in nature, coating, tagging/binding these volatile substances with safe organic bases like carbon quantum dots makes this complex (carbon quantum essential oil complex – **CQEC**) stable and retains the activity of EOs (Vishal et al., 2021). EO tagged with carbon quantum dots has been explored for their effectiveness as antibacterial and antifungal properties (Vishal et al., 2021). Carbon quantum dots (CQ) consists of oxygenous carbon-based nanomaterials in the size range of 2–10 nm with multiple surface functional groups. The CQ are non-toxicity, biocompatible, high water-solubility, photostability, and tuneable surface capacity. The CQ is doped with EOs to potentiate their antimicrobial properties. Recent literature demonstrated the antimicrobial properties when the CQ complex with curcumin (Chin-Jung Lin et al., 2019*) or* orange juice (Nguyen et al., 2021). The potential benefits of CQ-EO complex (CQEC) in diets devoid of AGP on the performance of broilers are scanty. Therefore, a study was conducted to synthesize a complex of CQ-EOs, assess the antimicrobial activity, and their potential benefits on performance, carcass traits, immune responses, antioxidant variables, and caecal microbiota composition in broiler chicken-fed diets without AGP.

MATERIALS AND METHODS

Synthesis of CQEC involved two stages as reported by Wang et al. (2019B). The first one is a synthesis of carbon quantum (carbon dots) and the second stage involves conjugation of carbon quantum with essential oils.

Synthesis of carbon quantum dots

The method described briefly. Citric acid (2.1 g) and ethylenediamine (670 uL) were dissolved in 20 mL Milli-Q water. Then the mixture was transferred into a Teflon-lined autoclave (125 mL acid digestion vessel no. 4748, Parr, France) and heated at 250 °C for 5 h. The resulting product was cooled to room temperature and dialyzed against Milli-Q water using a cellulose ester dialysis membrane for 3 days (Biotech CE N°131093, pore size 500-1000 Da) to remove unreacted small molecules and dried at 100C for overnight. Then, the dry mass of 200 mg solution was weighed by microbalance (Sartorius, TG 209 F3 Tarsus, Netzsch) and further dissolved with water for evaluation of the biological activity. The yield of carbon quantum dots was transformed from citric acid which was about 60 % as a carbon source. The final solution was stored at room temperature till further use.

Preparation of CQ–essential oil conjugates

Seven essential oils (thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), eucalyptus (*Eucalyptus* globulus), oregano (*Origanum vulgare),* and mentha (*Mentha piperita*)) were used in preparations of CQEC. The CQEC was prepared by doping the EO blends with CQ (Nanobiotics TM,

M/s. Imkuraq Animal Health Private Limited, Hyderabad, India) which was in powder form. The conjugate was prepared by using the CQ as a nanocarrier for essential oils. Briefly, the procedure includes CQ–EO conjugation was performed by a single-step method suggested by Wang et al. (2019B). The typical procedure includes 0.5 ml of EOs and 0.1 ml of synthesized CQ (5 : 1 ratio) dispersed in 100 mL of 0.05 M H_2SO_4. The resulting homogeneous mixture was refluxed at 110 °C for 3 hours. Subsequently, the reaction mixture was cooled to room temperature and 20 mL of cold water (4°C) was added to the mixture. The formed conjugated material was separated using diethyl ether. Two distinct aqueous and organic layers were observed and the aqueous layer containing the CDs–essential oil conjugate was separated and dried at 100°C in a vacuum oven (HLT-VO, Hi-Tech lab Solutions, India). The solid product obtained was stored at room temperature for further use as an alternative to AGP. The active components in CQEC were analysed with Adams (2005) method. The brief procedure includes isolation of essential oils with steam distillation. The extract was diluted in methanol (25µL in 10 mL), and the diluted samples (10 µL) were analyzed by GC (Agilent 7890B, Santa Clara, California, USA), and the results were compared to chromatograms of standard essential oil extracts.

In vitro efficacy of CQEC

The anti-microbial activity of CQEC was evaluated by studying minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against three microorganisms. The MIC and MBC values of CQEC against the *Salmonella typhimurium* strain (ATCC 14028), *Escherichia coli* (ATCC 25922), and *Clostridium perfringens* (ATCC 13124), as a measure of antimicrobial efficacy of the product, were determined by the micro broth dilution method (CLSI Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition). The MIC was determined by incubating 50 µL of the individual test cultures of *E coli* (1 X10^7 CFU/mL) in nutrient broth medium (50 µL) containing (g/l) glucose 1.0, yeast extract 2.5, and tryptone 5.0. with decreasing concentrations of extracted CQEC as well as Bacitracin Methylene Disalicylate (BMD) as a negative control (2000-2 µg/mL) in 96-well flat-bottom microtiter plates for 24 h, where each plate included a positive control as nutrient broth. All the wells were added with (1 X 10^7 CFU/mL test bacterial culture). The lowest concentration of compound without visible growth was designated as the MIC. For MBC determination, approximately 10 uL of the seeded inoculum was drawn from each well, having no visible growth, and placed on a Nutrient agar plate. The lowest concentration that produced 99.90% killing of the test culture was considered the MBC value of the compound.

In Vivo efficacy of CQEC

Two feeding trials were conducted to study the efficacy of CQEC on broiler performance in an open-sided poultry house with litter floor pens having 26 sft floor area (per pen/replicate) at Sri Ramdhoota Poultry Research Farm Pvt Ltd, Kothur, Hyderabad, India. The conditions and standards of rearing animals used in this experiment were approved by the Institute Animal Ethics Committee (ICAR-Directorate of Poultry Research, Hyderabad, India, IAEC/DPR/17/1; 21/10/2017).

Diets and treatments

Maize-soybean-meat and bone meal-based basal diets (BD) were prepared in the form of mash for three different phases i.e., pre-starter (1-14 d), starter (15-28 d), and finisher (29-42 d) phases (Table 1). The CQEC is a complex of CQ and EO as described earlier (Nanobiotics, Imkuraq Animal Health Pvt Ltd, Hyderabad, India) and tested as an alternative to AGP in boiler chicken diet. In experiment 1, the BD was fed without supplementing AGP (BMD) i.e. negative control (NC). Another three diets were prepared by supplementing BMD (500g/ton) i.e. positive control (PC), two doses of CQEC (200 and 250g/ton). The second experiment was conducted to further explore the benefits of lower and higher concentrations of CQEC and also to test the repeatability of the results of experiment 1. In experiment 2, both the PC and NC diets were fed, and also CQEC was supplemented to the NC at 4 different concentrations (100, 250, 500, and 1000g/ton). The lower level of CQEC (100g/ton) was tested in experiment 2 to study the possibility of reducing the dose of the product in the broiler diet, which was not tested in experiment 1. Each diet was randomly allotted and fed *ad libitum* to birds in 10 pens (replicates) at the rate of 25 broiler male chickens per pen from d 1 to 42 d of age.

Table 1. Ingredient and nutrient composition (g/kg) of control diet

Ingredient	Pre starter (1-14d)	Starter (15-28d)	Finisher (29-42d)
Maize	565.5	610.1	661.6
oil-veg	26.1	33	31.360
Soya DOC 45%	336.1	289.4	243.9
Meat cum bone meal	40	40	40
Salt	3.666	3.667	3.665
Sodium bi-carbonate	1.000	1.000	1.000
Dicalcium phosphate	10.440	8.51	5.94
LSP-powder	7.351	5.338	4.349
DL-Methionine	3.259	2.671	2.199
L-Lysine Hcl	2.187	2.034	2.033
L-Threonine	0.659	0.386	0.216
Premix[1]	4.20	4.20	4.20
Nutrient, g/kg			
M.E (MJ/kg)	12.55	12.97	13.18
Protein	225.9	207.7	190.9
Dig. Lysine	12.5	11.2	10.1
Dig.Methionine	6.4	5.6	4.9
Dig TSAA	9.29	8.32	7.50
Calcium	8.80	7.60	6.60
Available Phosphorus	4.20	3.80	3.30
Sodium	1.80	1.80	1.80
dig. Threnine	8.29	7.42	6.69
dig. Leucine	17.34	16.30	156
dig. Iso-leucine	8.42	7.64	6.90
dig. Valine	9.67	8.65	7.87

[1] Supplied per kg of diet: retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg.

[2]calculated concentrations; [3]calculated based on analysed ingredient composition.

Birds and management

In experiments 1 and 2, 1000 and 1500 one-day-old broiler male chicks (Cobb 430, Venkateswara Hatcheries Pvt. Ltd., Hyderabad, India), respectively were evenly distributed into 40 and 60 pens, respectively at the rate of 25 birds in each pen (198 x 122 cm). The floor of each pen was covered with built-up litter at about 8 cm thickness. Built-up litter was used as the bedding material in the house and also mean and bone meal was included (40g/ton) in the BD to create a passive pathogen challenge in experimental animals. The litter and feed samples were analysed for *Clostridium perfringens* and total bacterial count (Smith et al., 1998). Briefly, litter and feed samples were collected aseptically in 0.5 ml sterile test tube to quantify *Clostridia perfringens* and total bacterial count by following the serial dilution method, which was expressed as log 10 values. A ten-fold serial dilution of each sample was made in a sterile normal saline solution. *Clostridia perfringens* counts were determined using Clostridium Agar Base supplemented with *Clostridium perfringens* supplement (Himedia laboratories Pvt. Ltd., Mumbai), whereas the total bacterial count was determined using nutrient agar. The Clostridia plates were incubated anaerobically, while the total bacteria count plates were incubated aerobically for 48 hrs. The colonies were counted and expressed as Log_{10} cfu/g of sample content. *Clostridium perfringens* and total bacterial counts in the litter material were 4.62 and 5.745 log10/g, respectively and the respective counts in a gram of feed were 0.123 and 0.182 log10. The litter was covered with old newspaper to prevent intake of litter material by chicks during the initial 4 days of age after which the

paper was removed. Brooding was done with incandescent bulbs (100 watt/pen) and coal to provide the required temperature (about 35°C in week 1, 32°C in week 2, and 27°C in week 3) in the experimental shed after which the birds were exposed to ambient temperature. The ambient temperature ranged from 24.1+1.66 to 30.5+4.65°C during experiment 1 and 24.1+1.81 to 36.3+2.99°C during experiment 2. Fluorescent bulbs were used to provide light during night-time from 4 to 6 weeks of age.

Performance and slaughter variables

Body weight and feed intake (FI) were recorded at two-week intervals, and body weight gain (BWG) and feed efficiency (FE) were calculated. Feed left in the feeder was placed back in the respective feed drum to calculate the amount of feed consumed by the birds in each pen. All the birds present in each pen were weighed to calculate the BWG. The FE was calculated as FI per unit BWG. At the end of experiment 2 (day 42), one bird representing the mean body weight of the respective pen (replicate) was selected to study carcass traits including ready-to-cook yield (RTC), breast weight, liver weight, and abdominal fat content. The carcass yields were expressed as g/kg live weight of the respective bird.

Caecal Bacterial count

At 42nd d of age, caecal digesta was collected aseptically in 0.5 ml sterile test tubes to quantify *E. coli, Salmonella,* and *Clostridia perfringens* count by following serial dilution method, which was expressed as log 10 values. The caecal digesta samples were collected aseptically and diluted in normal saline (0.1gin 1mL) using a vertex mixer. A ten-fold serial dilution of each sample was made in a sterile normal saline solution. *E. coli* counts were assayed using Eosin Methyline Blue agar (Himedia laboratories Pvt. Ltd. Mumbai) and inoculated plates were incubated for 24 hours. Clostridia were determined using Clostridium Agar Base supplemented with *Clostridium perfringens* supplement (Himedia laboratories Pvt. Ltd., Mumbai) and inoculated plates were incubated anaerobically for 48 hrs. The colonies were counted and expressed as Log10 cfu/g of caecal content (Smith and Macfarlane, 1998).

Immune responses

Immune responses were measured in experiment 2 in terms of HI titer to Newcastle disease vaccine and cell-mediated immune response to phytohemaglutinin-P (PHA-P) inoculation.

HI titre against ND vaccine

The chickens were vaccinated against Newcastle disease (ND) by an ocular route at 5 and 21 d of age with the Lasota strain (ND Lasota Vac-500, Indovax Pvt., Ltd., Hyderabad, India). The humoral immune response was measured as antibody titre against ND vaccine by collecting blood from the brachial vein on the 35th day of age, which was 14 d post inoculation of the vaccine. For this, 2 mL of blood was collected from one bird per replicate and the antibody titres in sera against ND virus were measured (Reynolds and Maraqa, 2000) by haemagglutination test. The antibody titre against the disease was expressed as $\log_2$ values. The reciprocal of the highest dilution where there was complete agglutination was taken as the titre.

Cell mediated immune (CMI) response

The CMI response was assayed by cutaneous basophilic hypersensitivity test *in vivo* by using phytohemaglutinin-P (PHA-P) (TC 226, HiMedia Laboratories Pvt Ltd, Mumbai, India) employing the method as described by Corrier and Deloach (1990). On 35th d of age, one bird from each replicate was selected and the thickness of both right and left wattles was measured by micrometer (p no 7301, Mitutoyo, Japan). 100 µg of PHA-P suspended in 0.10 mL of phosphate buffer saline (PBS) and 0.1 mL of the PBS without PHA-P were injected intradermally into right and left (acted as a control) wattles, respectively. The thickness of both the wattles was measured at 24 h post-injection. The CMI response was calculated as the difference in thickness between right and left wattles due to PHA-P inoculation, which was expressed in relation to the increased thickness due to PBS alone.

Serum anti-oxidant variables

A blood sample (about 2.5 to 3 mL) was collected from the brachial vein of one layer in each replicate at 74 weeks of age. The oxidative parameters like lipid peroxidation (LP) and the activities of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) in serum were measured. About 2.0mL of blood sample from each bird were placed into a centrifuge tube containing citrate buffer (1.5 mL/10 mL blood) for erythrocyte separation and antioxidant enzyme estimation. The blood samples were centrifuged at 500×g for 15 min at 4°C to separate buffy coat (WBC) and form erythrocyte

pellet. The erythrocytes were washed thrice with PBS (pH 7.4). The packed RBC obtained was mixed with an equal volume of PBS and then diluted as per the requirement with distilled water.

The LP was estimated in serum by quantifying malonyl dialdehyde (MDA). The MDA reacts with 2-thiobarbituric acid to form a trimethine-colored substance (pink chromogen), which was extracted into butanol. The color intensity was measured at 548 nm. The LP activity in the erythrocytes was expressed in nmol MDA/mg protein (Placer et al., 1966). The activity of SOD and GSHPx were estimated following the method of Paglia and Valantine (1967).

Caecal microbiome analysis

Sample collection and DNA extraction

On day forty-two, ten chickens each group were chosen at random and executed via jugular vein exsanguination. The DNA/RNA Shield TM Fecal Storage Tube (Zymo Research, CA, USA) was used to aseptically collect the caecal contents, which were then kept at room temperature until DNA extraction. DNA was extracted and library was prepared with LSK-SQK114.96 kit of Oxford Nanopore. Sequencing was performed through Mk1C device (Name, number of the equipment, address of the company) of Oxford Nanopore.

Microbiome analysis

FastQC was used to verify the quality of the Illumina paired-end V3-V4 reads (2 bp × 300 bp) after they had been demultiplexed using the bcl2fastq1 tool. The biome data was extracted in advance for more sophisticated analysis and visual aids. Twelve5,000 sequences were chosen per sample (rarefaction) to equalize sequence counts among samples and serve as a foundation for comparing OTU abundances. With the help of the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010), the microbial composition and diversity in the stitched reads were examined. UCLUST was used to pick Open-reference Operational Taxonomic Units (OTUs) (Edgar 2010). After generating raw files, the abundance was identified and estimated by aligning them against microbial genomes. The raw data was examined and normalized. Different alpha diversity metrics (the diversity within each treatment groups) were estimated based on rarified data to assess different aspects of the groups. The Shannon, Simpson, Chao, and observed OTUs were used to compute alpha diversity. This technique, known as pair-wise analysis, is a beta diversity measure that looks at variations in the overall microbial community structure between samples by taking into account the evolutionary divergence among OTUs. Differental_Abundance_EdgeR was used to find significant group changes over treatment. It can show whether type of bacteria is affected more or less by a treatment.

Statistical analysis

The GraphPad Prism Software, CA, USA was used to examine the relative abundances of bacterial communities. $P < 0.05$ was considered statistically significant. One-way analysis of variance (ANOVA) was used to compare relative abundances at each level of classification (phylum, class, order, family, and genus). Using the Krona tools (Ondov et al., 2011), which provide quantitative phylogenetic data for every sample, Krona charts were created. The QIIME pipeline is used to create PCoA (Caporaso et al., 2010). The performance data were analyzed by considering the pen as an experimental unit, and other (carcass, immune responses, antioxidant variables, caecal bacterial count). The individual bird data were considered as a unit for statistical analysis. The effect of graded inclusion levels CQEC on dependent variables was assessed by ANOVA (experiment 1) regression analysis (experiment 2), i.e., linear ($y = a + bx$), and nonlinear ($y = a+bx+cx^2$, x = the inclusion level of CQEC, y = response in dependant variable). The response of supplementing CQEC to NC diet vis a vis the PC was compared with simple contrast analysis (ANOVA) (SAS Institute, 1994).

RESULTS AND DISCUSSION

MIC and MBC of CQEC

As an antibacterial indicator, the MIC and MBC values of CQEC were determined against *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* strain (ATCC 14028) and *Clostridium perfringens* (ATCC 13124). The MIC values of the CQEC tested ranged from 31.25 to 62.5ug/mL while MBC values were in the range of 62.5 to 125ug/ml for these bacteria while BMD showed similar activity which ranged from 31.25 to 62.5ug/mL, while MBC values ranged between 62.5 to 250ug/ml (Table 2). The analyzed concentrations of thyme, cinnamon, eucalyptus, oregano, and mentha in CQEC (5.21, 4.89, 5.01, 4.18 and 5.41%, respectively) were close to the estimated values (5% each).

Table 2. Determination of MIC and MBC values of carbon quantum dots essential oil complex (CQEC) against *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* strain (ATCC 14028) and *Clostridium perfringens* (ATCC 13124)

Treatment	*Escherichia coli* (ATCC 25922)		*Salmonella typhimurium* strain (ATCC 14028)		*Clostridium perfringens* (ATCC 13124)	
	MIC (ug/ml)	MBC (ug/ml)	MIC (ug/ml)	MBC (ug/ml)	MIC (ug/ml)	MBC (ug/ml)
CQEC	62.5	125	31.25	62.5	62.5	125
BMD	125	250	31.25	32.5	62.5	125

CQEC carbon quantum dots essential oil complex; BMD bacitracin methylene di salicylate; MIC minimum inhibitory concentration; MBC minimum bactericidal concentration

The antimicrobial parameters of CQEC and BMD indicated that the bactericidal (MBC) dose of both compounds was almost double the dose of MIC concentrations. Further, it is also evident that the dose of both CQEC and BMD for antimicrobial effect was almost similar, which means the *in vitro* efficacy of CQEC is similar to that of AGP tested (BMD) in the current study. Since the bacteria tested in the current study are the most common pathogens that prevail in commercial poultry operations, it is expected that CQEC will have a similar response to BMD on these pathogens. The antibacterial concentration of both CQEC and BMD for *Salmonella typhimurium* was lower, while the concentrations of CQEC were similar for both *Escherichia coli* and *Clostridium perfringens*. Higher doses of BMD were required against *E coli* compared to *Clostridium perfringens*. This variation in MIC and MBC values could be attributed to the differences in bacterial strain, variations in virulence factors, or structural differences in the bacterial cell membrane (Abishad et al., 2021). In this study, CQEC exhibited similar antimicrobial properties against *Salmonella typhimurium* and *Clostridium perfringens* and a higher response against *E coli* as compared to the BMD.

Experiment 1

Body weight gain during all the periods (1-2, 1-4, and 1-6 weeks of age) and feed efficiency during 1-4 weeks of age were not affected (P>0.05) by supplementation of either AGP or CQEC compared to those fed the NC diet (Table 3). The FE during the initial 2 weeks (1-2 weeks) was significantly reduced in broilers fed the NC compared to those fed the AGP (PC). Supplementation of CQEC improved the FE compared to those fed NC, and at 250g CQEC the FE was similar to those fed the PC diet. Similarly, at the end of the experiment (1-6 weeks) the FE in NC diet-fed groups was lower than those fed the PC diet. Supplementation of CQEC at 250g/ton significantly improved the FE compared to the NC diet-fed broilers. However, the FE in the latter group was significantly lower than those fed the AGP-supplemented diet.

Table 3. Performance of broiler chicken fed diet containing carbon quantum dots essential oil complex (CQEC) in place of antibiotic growth promoter

Treatment	1-2 weeks		1-4 weeks		1-6 weeks	
	BWG	FE	BWG	FE	BWG	FE
PC	448.0	0.857^{A}	1510	0.690	2574	0.603^{A}
NC	439.4	0.842^{C}	1487	0.681	2526	0.592^{C}
CQEC-200	440.7	0.848^{BC}	1494	0.686	2543	0.595^{BC}
CQEC-250	443.4	0.855^{AB}	1504	0.688	2560	0.598^{B}
P	0.564	0.005	0.654	0.195	0.603	0.001
N	10	10	10	10	10	10
SEM	2.269	0.0018	6.735	0.0016	13.04	0.0010

BWG body weight gain; FI feed intake; AGP antibiotic growth promoter; PC positive control with AGP; NC negative control without AGP/alternatives; CQEC carbon quantum dots essential oil complex 200 and 250g/ton; P probability; N number of replicates; SEM standard error mean

^{ABCD} means having no common superscripts in a column varies significantly (P<0.05)

Maximum and minimum temperature and humidity = 30.9+4.65 & 24.2+1.66°C and 81.4+8.85 & 56.7+18.11%, respectively

Experiment 2

Performance

In general, the BWG and FE in experiment 2 were marginally lower than the experiment 1. The lower performance could be due to higher ambient temperature during Experiment 2 (Maximum and minimum temperature 35.3+2.99 & 24.1+1.81°C, respectively) compared to Experiment 1 (Maximum and minimum temperature 30.9+4.65 & 24.2+1.66°C, respectively). The BWG was not affected (P>0.05) by supplementation of CQEC to the NC diet during the pre-starter phase. However, the BWG during 1-4 and 1-6 weeks and FE during all the phases were improved non-linearly (<0.05) with the level of CQEC in the broiler diet (Table 4). The contrast analysis indicated that the FE in the NC group was significantly (P<0.05) lower than those fed the PC diet. However, supplementation of CQEC at different concentrations improved the FE similar to the PC group during pre-starter phase. The FE in during 1-4 and 1-6 weeks of age in 100 g CQEC groups was similar to the PC group. Supplementation of higher concentration, i.e. 250g CQEC, significantly improved the FE compared to those fed the PC diet. Higher concentrations of CQEC (500 g/kg) also improved the FE during 1-4 weeks of age and further higher concentrations did not improve the FE compared to the PC diet during 1-4 or 1-6 weeks of age.

Table 4. Performance of broiler chicken-fed diets containing graded concentrations of carbon quantum dots essential oil complex (CQEC) in place of antibiotic growth promoter

Treatment	1-2 weeks		1-4 weeks		1-6 weeks	
	BWG	FE	BWG	FE	BWG	FE
PC	345.2	0.821	1329	0.718	2265	0.593^{BC}
NC	332.8	0.814	1320	0.713	2226	0.588^{D}
CQEC-100	342.5	0.819	1322	0.715	2250	0.592^{CD}
CQEC-250	349.1	0.821	1360	0.721	2277	0.599^{A}
CQEC-500	351.3	0.823	1362	0.724	2296	0.595^{ABC}
CQEC-1000	339.7	0.816	1357	0.720	2288	0.597^{AB}
SEM	3.137	0.0009	6.09	0.0006	13.29	0.0062
P values						
Regression						
Linear	0.420	0.319	0.015	0.001	0.140	0.001
Quadratic	0.182	0.002	0.034	0.001	0.250	0.001
Contrast						
PC vs NC	0.263	0.016	0.673	0.020	0.408	0.020
PC vs C-100	0.805	0.593	0.739	0.249	0.745	0.418
PC vs C-250	0.582	0.337	0.107	0.001	0.519	0.018
PC vs C-500	0.721	0.915	0.131	0.017	0.810	0.495
PC vs C-1000	0.617	0.129	0.166	0.091	0.628	0.150

BWG body weight gain; FI feed intake; AGP antibiotic growth promoter; PC positive control with AGP; NC negative control without AGP/alternatives; CQEC carbon quantum dots essential oil complex 100, 250, 500, and 1000g/ton; P probability; N number of replicates; SEM standard error mean

^{ABCD} means having no common superscripts in a column varies significantly (P<0.05)

Maximum and minimum temperature and humidity = 35.3+2.99 & 24.1+1.81°C and 69.1+17.6 & 25.2+15.94%, respectively

The literature on the specific EOs tested in broiler diets was limited, therefore, the current results are compared with related herbal compounds reported in the literature. The improved broiler performance observed in the current study is consistent with the findings of Parade et al. (2019), who found that feeding 1.5% lemongrass leaf powder increased growth and reduced the market age of the broilers to attain the desired slaughter weight. Comparably, feeding 2% lemongrass leaf powder-supplemented diets was reported to enhance weight gain in comparison to the control group (Shaheed, 2021). The authors attributed the antioxidant and antibacterial properties of lemongrass to the higher performance observed in broilers fed lemongrass.

The improved performance observed in the

current study could be attributed to the non-linear reduction in oxidative stress variables (reduced LP and improved SOD activity) and pathogen count (*E coli* and *Salmonella*) in caecum compared with CQEC supplementation to the NC diet.

Slaughter variables

The regression analysis indicated that the slaughter variables (breast meat weight, abdominal fat, and liver weight) were not affected (P>0.05) by supplementation of CQEC to the NC diet (Table 5). Similarly, these carcass variables were not affected by supplementation of AGP (PC) compared to those fed the NC diet. The contrast analysis also indicated that supplementation of CQEC @ 250g/ton significantly improved the RTC yields compared to the PC diet-fed broilers.

Caecal bacterial count

The Clostridia count in the caecum was not affected (P>0.05) by the supplementation of CQEC or AGP to the NC diet (Table 5). The colony counts of salmonella and *E. coli* were reduced non-linearly with the concentration of CQEC in the NC diet. The contrast analysis indicated that Salmonella count was not affected, but *E coli* count reduced significantly with AGP supplementation compared to the NC group. Similarly, the Salmonella count reduced significantly with CQEC supplementation at all levels compared to the PC group.

Table 5. Slaughter variables (g/kg live weight) and caecal bacterial count (log 10/g) in boiler chicken fed graded levels of carbon quantum dots essential oil complex (CQEC) in place of antibiotic growth promoter

Treat	RTC	Breast	Abdfat	Liver	Caecal bacterial count, log 10		
					Salmonella	E coli	Clostridia
PC	761.0	254.7	14.03	22.38	5.317	5.266	5.406
NC	768.4	253.0	14.06	21.44	5.184	5.865	5.312
CQEC-100	761.2	249.3	14.06	21.02	4.856	5.560	5.201
CQEC-250	776.3	257.5	13.44	21.07	4.599	5.208	5.183
CQEC-500	763.5	246.9	14.23	21.61	4.668	5.238	5.331
CQEC-1000	761.7	256.2	13.36	20.40	4.519	5.177	5.262
SEM	2.026	1.892	0.445	0.359	0.0621	0.0608	0.0442
P values							
Regression							
Linear	0.133	0.796	0.725	0.562	0.001	0.001	0.932
Quadratic	0.318	0.893	0.938	0.794	0.001	0.001	0.836
Contrast							
PC vs NC	0.248	0.801	0.982	0.460	0.452	0.002	0.546
PC vs C-100	0.975	0.419	0.482	0.288	0.011	0.121	0.193
PC vs C-250	0.018	0.678	0.715	0.304	0.001	0.753	0.158
PC vs C-500	0.103	0.245	0.897	0.546	0.001	0.878	0.629
PC vs C-1000	0.914	0.818	0.678	0.123	0.001	0.635	0.358

RTC ready to cook yield, Abdfat abdominal fat, AGP antibiotic growth promoter; PC positive control with AGP; NC negative control without AGP/alternatives, CQEC carbon quantum essential oil complex 100, 250, 500, and 1000g/ton; P probability; N number of replicates; SEM standard error mean

^{ABCD} means having no common superscripts in a column varies significantly (P<0.05)

Immune responses

The regression analysis indicates that both CMI response to PHA-P and HI titers against ND vaccination improved non-linearly (P<0.05) in broilers fed graded concentrations of CQEC in the NC diet (Table 6). The contrast analysis indicated significantly lower HI titers in broilers fed the NC diet with or without CQEC supplementation compared to those fed the PC diet. Though the CMI response was not affected by AGP supplementation (PC) compared to those fed the NC diet, the immune response in groups fed 250 or 1000g/ton was significantly higher than those fed the PC, and the immune response at other concentrations (100 and 500 g/ton) was similar to the PC group. The ND titres in broilers fed the highest concentrations of CQEC (1000g/ton) was similar to the PC group.

Serum anti-oxidant variables

The regression analysis indicated that the LP reduced, and SOD activity increased non-linearly with the concentration of CQEC in the NC diet, such affect was not noticed in the activity of GSHPx (Table 6). Similarly, the contrast analysis indicated no significant affect in LP and GSHPx activity with AGP supplementation compared to the NC group, while the activity of SOD was significantly lower in NC compared to the PC group. The LP in groups fed CQEC at 100, 500 or 1000g/ton was significantly lower than those fed the PC diet. The SOD activity in broilers fed CQEC at all concentrations except 1000g/ton (100, 250 and 500g/ton) was similar to those fed the PC diet.

Table 6. Immune responses and serum antioxidant variables in broiler shed fed graded levels of carbon quantum dots essential oil complex (CQEC) in place of antibiotic growth promoter boiler chicken diet

Treat	Immune responses		Serum antioxidant variables		
	CMI, %	ND titre (Log2)	LP, Nano moles MDA	SOD, u/mg of protein	GSHPx, unit/ml
AGP	51.20	8.500	1.884	5.010	303.0
NC	49.00	5.800	1.852	3.340	257.1
CQEC-100	52.00	7.500	1.667	4.548	288.2
CQEC-250	66.80	7.300	1.887	4.233	367.8
CQEC-500	61.20	6.900	1.399	5.213	287.4
CQEC-1000	71.40	8.100	1.558	3.233	331.2
SEM	2.286	0.277	0.040	0.213	11.84
P values					
Regression					
Linear	0.002	0.001	0.010	0.545	0.205
Quadratic	0.010	0.001	0.011	0.012	0.297
Contrast					
PC vs NC	0.763	0.001	0.790	0.018	0.250
PC vs C-100	0.913	0.003	0.073	0.502	0.709
PC vs C-250	0.036	0.001	0.980	0.261	0.107
PC vs C-500	0.174	0.001	0.001	0.768	0.694
PC vs C-1000	0.007	0.224	0.008	0.012	0.478

CMI cell mediated immune response; ND Newcastle disease; LP li[id peroxidation; SOD superoxide dismutase; GSHPx glutathione peroxidase; PC positive control with AGP; NC negative control without AGP/alternatives, CQEC carbon quantum dots essential oil complex 100, 250, 500, and 1000g/ton; P probability; N number of replicates; SEM standard error mean

[ABCD] means having no common superscripts in a column varies significantly (P<0.05)

Both the immune responses improved non-linearly with supplementation of CQEC to NC diet and the response of HI titre was significantly higher at 250g and CMI at 1000g/ton was similar compared to the PC diet fed broilers. Literature demonstrated a strong positive correlation between gut microbiota and immune responses (Wang et al., 2019B). Bacteroides is essential to the intestinal IgA response. Bacteroides-derived IVA induces M2 polarization of macrophages and the expression of IL-10, IL-4, TGF-β, and BAFF to promote IgA response by activating the mTOR/PPAR-γ/STAT3 signaling pathway in the small intestine, independently of the bacterial TLR ligands, which induce IgA responses through the binding of TLRs. The increased population of Bacteroides might have modulated the immunity and has a promising role in gut immunity (Xinkai et al., 2023). Therefore, improving the population of Bacteroides might have improved gut immunity.

Caecal microbiome study

Sequence data analysis

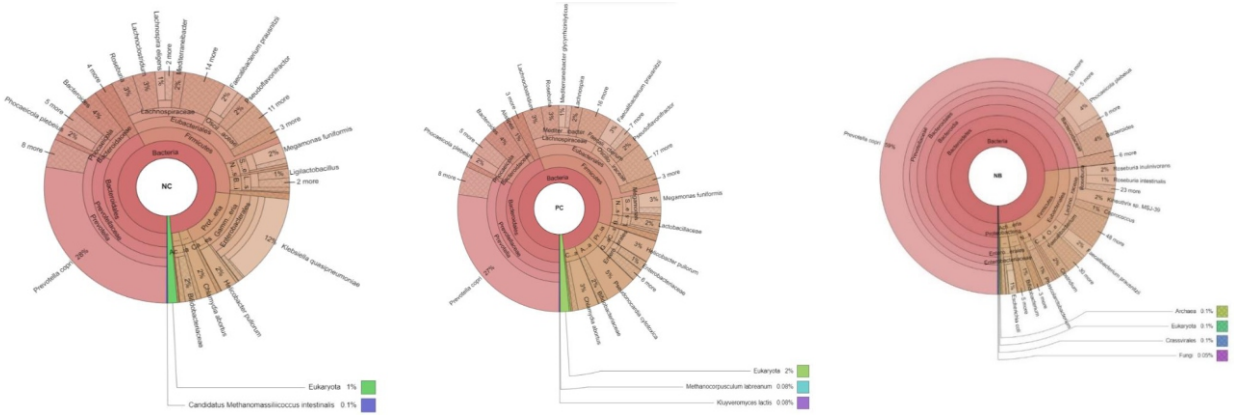
A total of 9.6 million paired-end reads were obtained from 10 pooled cercal samples from each group of birds. After selecting the right reads and removal of chimeric reads, the average clean sequence tags after aligning against microbial genomes were obtained for the NC, PC, and NB250 groups.

At the phylum level, *Firmicutes, Bacteroidetes,* and *Proteobacteria* accounted for more than 96% of the caecal microbiota as normalized % abundance,

in which *Firmicutes* (21%–38%) and *Bacteroidetes* (41%–72%) were the predominant microbes followed by *Proteobacteria* (2%–7%) and *Actinobacteria* (2%–6%) in all the sequences.

Most predominant *Prevotella copri* accounts for 20%–56% of the sequences in the phylum *Bacteroidetes* under class *Bacteroidia*. While *Faecalibacterium prausnitzii* accounts for 1-2% was the most prominent under phylum *Firmicutes*. Furthermore, the *Krona chart* is an interactive plot used to explore the relative abundances and confidences within the complex hierarchies of metagenome classifications. In the present study, NB250 treatment produces an impact on *Bacteroidetes* population which was presented visually in one representative Krona chart (Figure 1).

Figure 1. Krona chart of one representative sample from each group. Bacteroides population has significantly ($p > 0.001$) increased in a carbon quantum dots essential oil complex (CQEC) group a compare to Negative (NC) as well as the positive (PC) control group.



(a) Normal feed group (NC) – without addition- as Negative control

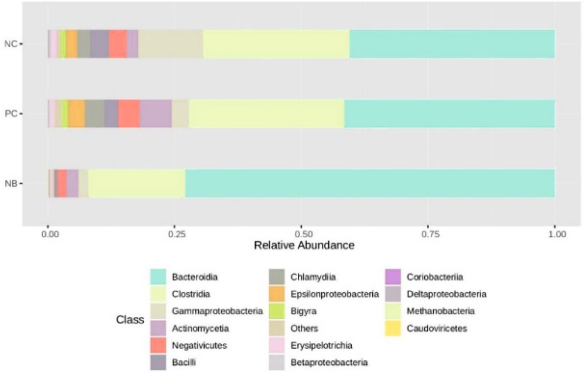
(b) BMD feed group (PC) 500g/TON of feed - as positive control

(c) carbon quantum essential oil complex (CQEC) fed group 250g/TON of feed (NB-250)

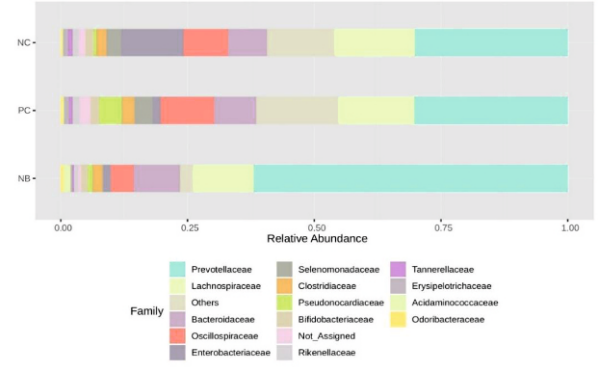
In addition, stacked column plots were generated for the top 16 unique classified organisms identified at phylum, family, genus, and species taxonomic level using relative abundance values for all the samples (Figure 2). Moreover, the *Bacteroides* population was elevated upon supplementation with NB250 in line and with improved body weight and FCR of broiler birds (Table -2 &3).

Figure 2. Stacked Bar plot showing the relative abundance of Phyla and family distribution. carbon quantum dots essential oil complex (CQEC) group a compare to Negative (NC) as well as positive (PC) control group.

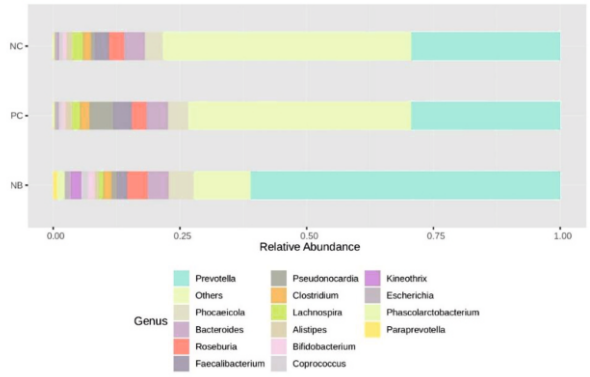
Class level relative abundance plot



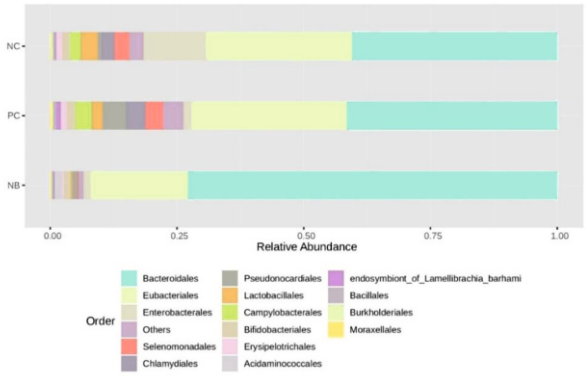
Family-level relative abundance plot



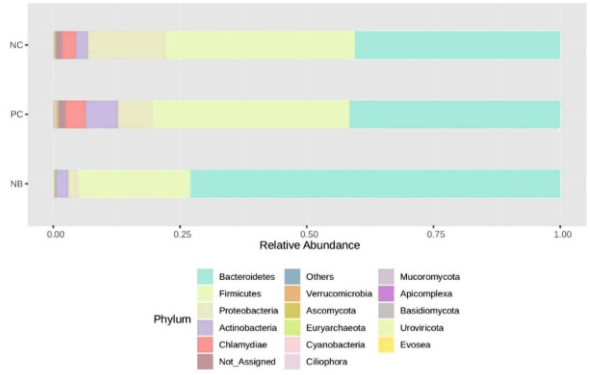
Genus level relative abundance plot



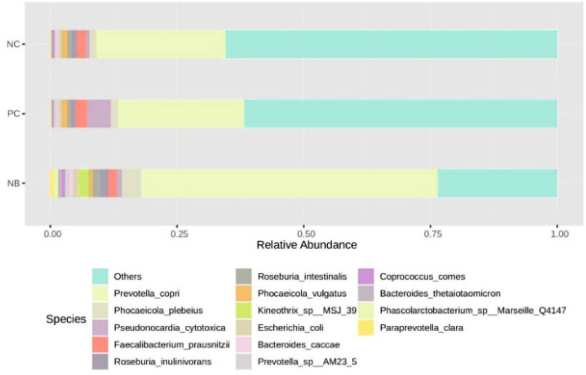
Order level relative abundance plot



Phylum level relative abundance plot



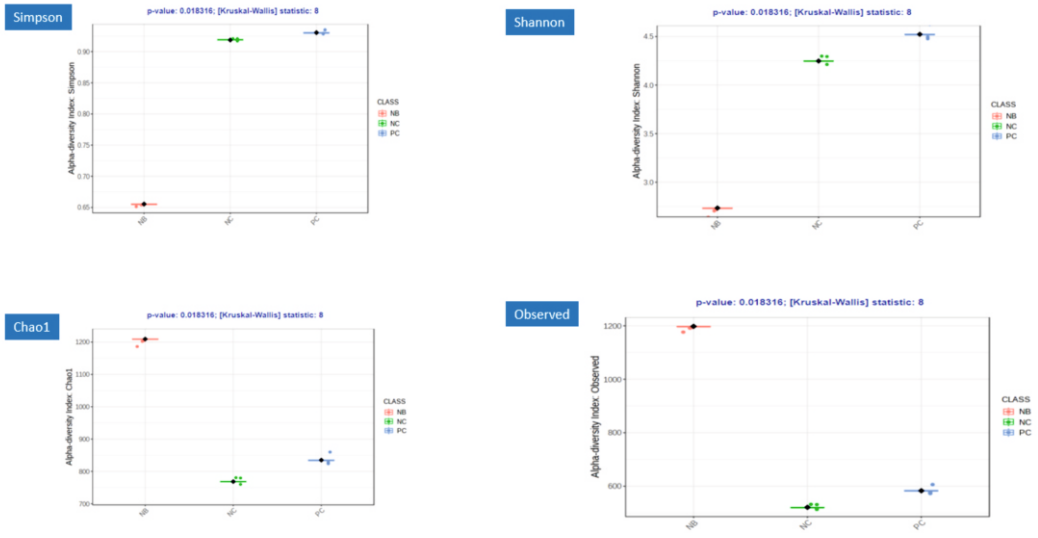
Species level relative abundance plot



Alpha Diversity

Internal sample alpha diversity was estimated through the number of observed species, Simpson, Chao, and Shannon indices (Alpha diversity) Figure 3. NB250 group showed lower 0.66 and 2.5 Alpha diversity index in Simson plot as well as Shannon plot as compared to NC and PC groups values 0.94 - 0.96 and 4.25 - 4.66, respectively (p-value: 0.0183) (Figure 3). While Alpha diversity index was observed with higher values (1200 for NB250) as compared to PC and NC (780-840) for Chao1 and (560 and 590) for Observed, respectively (p-value: 0.0183).

Figure3. Effect of carbon quantum dots essential oil complex (CQEC) supplementation on alpha diversity measures for comparing different statistical parameters (a) Shannon Index, (b) Simpson Index, (c) Chao Index, (d) number of observed species (mean ± standard error of the mean)



The Krona chart (Figure 1) from the current study revealed a favourable relation between the feed efficiency and the *Bacteroides* genus (Tables 3, and 4). It has a similar finding with both cellular immunities as measured by the PHAP test and humoral immunity parameters for the ND vaccination response (Table 6).

The present study's NGS analysis identified a wide range of bacteria in the cecum of broiler chicken and examined the effects of supplementing the essential oil-based carbon quantum dots complex (CQEC). In the current investigation, the impact of CQEC on the caecal microbiome was assessed. The NGS analysis of the broiler chicken cecum revealed the presence of three important phyla in the ceca, namely, *Firmicutes, Bacteroidetes,* and *Proteobacteria* which are in accordance with the literature (Corrigan et al., 2015). The FE and Firmicutes-Bacteroides Ratio (FBR) were higher with supplementation of CQEC at 250 g/ton and are in line with the trend of FE. Essential oils supplementation was reported to improve FBR and performance of chicken (Saravana et al. 2019 and Tesfaye et al., 2023). Similar to our study, the inclusion of certain growth promoters i.e antibiotic growth promoters (Costa et al., 2017), plant extracts (Salaheen et al., 2017), and prebiotics (Choi et al., 2015) were reported to improve the FBR. The effect of polyphenolic compounds from blueberry and blackberry has been shown to improve the performance and FBR in the ceca of broilers (Salaheen et al., 2017).

When compared to the AGP (BMD) group and the NC group, the beta-diversity study showed that the CQEC supplementation resulted in a noticeable shift in the microbiota (Figure 1 and Figure 2). Nonetheless, the beta diversity among the CQEC treatments was statistically significant, suggesting that the microflora is shifted in a way that is similar to the increase in the Bacteroides population. The predominant phylum increased upon CQEC supplementation was *Bacteroides.* These organisms is known to play a vital role in the fermentation of dietary carbohydrates to convert into short-chain fatty acids (SCFA). The increased prevalence of Bacteroides in ceca could indicate a subsequent development in the microbial succession, involving the shift from facultative anaerobes, like Lactobacilli, to stringent anaerobes, like Bacteroides, Ruminococcaceae, and Lachnospiraceae. According to van Der Wielen et al. (2000), an anaerobic gut environment and undigested carbohydrates entering the ceca are necessary for the synthesis of SCFAs, which help for proper function of the gut epithelium and improve nutrient absorption across cellular membranes. The results of this study also showed that the CQEC supplementation changed the caecal microbiota, which is beneficial for gut health. The improvement in the microbiota also leads to an improvement in the FBR, which in turn might have helped to improve the FE. Pie chart showed that the Bacteroidetes population increased with CQEC (250g/ton) supplementation compared to the NC or PC (BMD) group.

To evaluate various facets of the microbial community structure, several alpha diversity metrics (the diversity between groups) were computed using rarified data (Figure 4). Two indices are used to quantify diversity i.e. the Shannon-Wiener and the Simpson. The higher numbers denote less diversity and lower ones suggest more diversity (Harini, 2002). According to Simpson and Shannon analysis, the current study revealed that CQEC had larger diversity of the bacterial community (>1200 species). Alpha diversity, or species diversity within a single community or habitat, is also measured by Chao1. It is employed to calculate the overall number of species present in a community for both known species and uncommon or undiscovered species. The total number of species in a sample, or species richness, is indicated by Chao1. In comparison to both the PC and NC groups, the species diversity of the CQEC group was significantly higher which might provide a better preposition to provide improvement in growth.

The gut microbiota data also indicated a higher abundance of beneficial bacteria in broilers fed CQEC compared to the CD. The caecal microbiota plays a critical role in maintaining gut health and utilization of nutrients left undigested in the small intestine. Several studies have shown the importance of microbes in the digestion of nutrients entering the caeca. It is estimated that 5–10% of the energy from the poultry diet is extracted by caecal fermentation. Improvement in the caecal microflora to extract the undigested nutrients that reach the hindgut provides a great opportunity to improve the productivity of chickens. From our study, it is evident that the prevalence of Bacteroides, which are essential for the fermentation of food substrates improved with the supplementation of CQEC. Bacteroides are crucial for the breakdown of

complex carbohydrates and produce short-chain fatty acids (SCFAs) that are well-suited for the health of the hindgut. Corrigan et al. (2015) demonstrated the improved host's ability to utilize dietary energy with Bacteroides dense ceca. The gut microbiota helps in the fermentation of partially- or non-digestible polysaccharides and produces SCFAs (acetate, propionate, butyrate, and valerate). Liao et al. (2020) found a favorable correlation between the relative abundance of Bacteroides and the caecal SCFA profiles. Though the digestibility of nutrients was not estimated in the current study, the improved feed efficiency observed in our study could also be due to a probable increase in enzyme secretion with CQEC supplementation. The literature reported improved enzyme secretion (Zeng et al., 2015), and nutrient digestibility (Amad et al., 2011) with supplementation of EO in broiler and other monogastric animal diets.

CONCLUSIONS

Based on the results, it is concluded that supplementation of carbon quantum-essential oils (thyme, cinnamon, tea tree, eucalyptus, oregano, citral, and mentha) complex (CQEC) showed potent antibacterial properties comparable to antibiotic growth promoter (BMD) while testing with MIC and MBC methods. Supplementation of CQEC positively affected the bacterial communities in the cecum for improved gut health and enhanced feed efficiency. CQEC improved the population of the beneficial bacteria (Bacteroides), which is known to maintain a balanced microflora in the gut. The increase in the population of the beneficial bacteria is associated with the feed efficiency and meat yields of broilers fed CQEC. The results indicated the possibility of utilizing CQEC as a potent growth promoter in broiler chicken diet in the era of post-AGP ban in poultry and livestock production.

REFERENCES

Abishad, P., Niveditha, P., Unni, V., Vergis, J., Kurkure, N.V., Chaudhari, S., Rawool, D.B. and Barbuddhe, S.B. 2021. In silico molecular docking and in vitro antimicrobial efficacy of phytochemicals against multi-drug-resistant enteroaggregative *Escherichia coli* and non-typhoidal Salmonella spp. Gut Pathology. 13: 46.

Adams Robert. 2005. Identification of essential oil components by gas chromatography / quadrupole mass spectroscopy. 4th Edition, Carol Stream, IL 16. 65-120.

Agung Irawan, Cecep Hidayat, Anuraga Jayanegara and Adi Ratriyanto. 2021. Essential oils as growth-promoting additives on performance, nutrient digestibility, cecal microbes, and serum metabolites of broiler chickens: a meta-analysis. Animal Biosciences. 34: 1499-1513.

Amad, A.A., Männer, K., Wendler, K.R., Neumann, K. and Zentek, J. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poultry Science. 90: 2811-2816.

Attia, Y., Al-Harthi, M. and El-Kelawy, M. 2019. Utilisation of essential oils as a natural growth promoter for broiler chickens. Italian Journal of Animal Science. 18: 1005-1012.

Basmacioğlu-Malayoğlu, H., Ozdemir, P. and Bağriyanik, H.A. 2016. Influence of an organic acid blend and essential oil blend, individually or in combination, on growth performance, carcass parameters, apparent digestibility, intestinal microflora and intestinal morphology of broilers. British Poultry Science. 57: 227-34.

Broom, L.J. 2017. Necrotic enteritis; current knowledge and diet-related mitigation. World's Poultry Science Journal. 73: 281-292.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D. and Costello, E.K. 2010. QIIME allows analysis of high-throughput community sequencing data. Natural Methods. 7**:** 335-336.

Cetin, E., Yibar, A., Yesilbag, D., Cetin, I. and Cengiz, S.S. 2016. The effect of volatile oil mixtures on the performance and ilio-caecal microflora of broiler chickens. British Poultry Science. 57**:** 780-787.

Chin-Jung Lin, Lung Chang, Han-Wei Chu, Han-Jia Lin, Pei-Ching Chang, YL Robert, Wang, Binesh Unnikrishnan, Ju-Yi Mao, Shiow-Yi Chen, Chih-Ching Huang 2019. High Amplification of the Antiviral Activity of Curcumin through Transformation into Carbon Quantum Dots. Small 15: 1902641

Choi, K.Y., Lee, T.K. and Sul W.J. 2015. Metagenomic analysis of chicken gut microbiota for improving metabolism and health of chickens – A review. Asian-Australasian Journal of Animal Sciences. 28: 1217-1225.

CLSI Performance Standards for Antimicrobial Susceptibility Testing, 32th Edn. Available online: https://clsi.org/standards/products/microbiology/d (accessed on 20 April 2022).

Corrier, D.E., Deloach, J.R. 1990. Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. Poultry Science. 69: 403-8.

Corrigan, A., De Leeuw, M., Penaud-Frezet, S., Dimova, D. and Murphy R.A. 2015. Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. Applied Environmental Microbiology. 81: 3460-3470.

Costa, M.C., Bessegatto, J.A., Alfieri, A.A., Weese, J.S., Filho, J.A. and Oba, M.T. 2017. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS One 12: e0171642.

Diaz-Sanchez S, Doris D'souza, Debrabrata Biswas, Irene Hanning. 2015. Botanical alternatives to antibiotics for use in organic poultry production. Poultry Science. 94: 1419–1430

Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26: 2460-2461.

Fernandez-Rubio, C., Ordonez, C., Abad-Gonzalezj, J., Garcia-Gallego, A., Honrubia, M.T. Mallo, J.J. and Balana-Fouce, R. 2009. Butyric acid-based feed additives help protect broiler chickens from *Salmonella Enteritidis* infection. Poultry Science. 88: 943-948

Gheorghita, D., Robu, A., Antoniac, A., Antoniac, I., Ditu, L.M., Raiciu, J Tomescu, Grosu, E. and Saceleanu. A. 2022. In Vitro Antibacterial Activity of Some Plant Essential Oils against Four Different Microbial Strains. Applied Science. 22(12): 9482.

Harini, N, 2002. Opposite trends in response for the Shannon and Simpson indices of landscape diversity. 22(2): 0–186.

Huyghebaert, G., Ducatelle, R. and Vanimmersee, L.F. 2010. An update on alternatives to antimicrobial growth promoters for broilers. Veterinary Journal. 187: 18288.

Huyghebaert, G., Richard Ducatelle and Filip Van Immerseel. 2011. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal. 187: 182-188.

Kiarie, Elijah, Luis F. Romero and Charles M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutrition Research Reviews. 26 (1): 71-88.

Kim, S. J., Lee, K.W., Kang, C.W. and An, B.K. 2016. Growth performance, relative meat and organ weights, cecal microflora, and blood characteristics in broiler chickens fed diets containing different nutrient density with or without essential oils. Asian- Australasian Journal of Animal Sciences. 29: 549-54. https://doi.org/10.5713/ ajas.15.0426.

Liao, X., Shao, Y., Sun, G., Yang, Y., Zhang, L., Guo, Y., Luo, X. and Lu, L. 2020. The relationship among gut microbiota, short-chain fatty acids, and intestinal morphology of growing and healthy broilers. Poultry Science. 99: 5883–5895.

Nguyen, M.H., Le, A.T., Pham, V.D., Pham, H.M., Do, H.T., Le, D.T., Vu, V.D. and Nguyen, T.B. 2021. A Comprehensive Study on the Antibacterial Activities of Carbon Quantum Dots Derived from Orange Juice against Escherichia coli. Applied Sciences. 14: 2509.

Ondov, B.D., Bergman, N.H., Phillippy, A.M. 2011. Interactive metagenomic visualization in a web browser. BMC Bioinformatics. 12: 385.

Paglia, D.E., Valantine, A.M. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidise. Journal of Laboratory Clinical Medicine 70: 158-69.

Parade, A.K., Thombre, B.M., Patil, R.A., Padghan, P.V., Gaikwad, B.S. and Meshram, P.B. 2019. Use of lemongrass (*Cymbopogon citratus*) leaf meal as a natural feed additive on growth performance and economics of broilers. International Journal of Current Microbiology and Applied Sciences. 8: 1842-1849.

Pirgozliev, V., Mansbridge, S.C. and Rose, S.P. 2019. Dietary essential oils improve feed efficiency and hepatic antioxidant content of broiler chickens. Animal. 13: 502-8.

Placer, Z.A, Cushman, L.L. and Johnson, B.C. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Annals of Biochemistry. 16: 359-64.

Rama Rao, S.V., Nagaraja Kumari, K., Raju, M.V.L.N., Prakash, B., Paul, S.S. and Santosh Phani, K.P. 2023. Feed emulsifier improves the performance and nutrient digestibility in broiler chicken fed diets without antibiotic growth promoter. British Poultry Science. 64: 745-750.

Reynolds, D.L., Maraqa, A.D. 2000. Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. Avian Diseases. 44: 138-44.

Salaheen, S., Kim, S.W., Haley, B.J., Van Kessel, J.A. and Biswas, D. 2017. Alternative growth promoters modulate broiler gut microbiome and enhance body weight gain. Frontiers in Microbiology. 8**:** 2088.

Saravana Kumar Marimuthu, Brindhalakshmi Balasubramanian, Ramasamy Selvam and Prashanth D'souza. 2019. Modulation of Chicken Cecal Microbiota by a Phytogenic Feed Additive, Stodi®: A Metagenomic Analysis. Pharmacognosy Research. 11: 201-209.

SAS Institute Inc. 1994. SAS/STAT User's guide: Version 6, fourth ed. SAS Institute Inc., Cary, NC, USA.

Shaheed, M.J. 2021. The effect of added lemongrass leaf powder (*Cymbopogon citratus*) to the diet and drinking water on some productive, carcass and microbial traits of Japanese quail. Annals of the Romanian Society for Cell Biology. 25: 6035-6044.

Smith, E.A. and Macfarlane, G.T. 1998. Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiology Ecology 25: 355–368.

Tesfaye Engida, D., Mihretu Ayele, Hika Waktole, Berhan Tamir, Fikru Regassa and Takele Beyene Tufa. 2023. Effects of Phytogenic Feed Additives on Body Weight Gain and Gut Bacterial Load in Broiler Chickens. World Veterinary Journal. 13(1): 205-213.

Van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, M.A. and Van Knapen, F. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied and Environmental Microbiology. 66: 2536–2540.

Vishal, R., Shubham Shishodia, Prem Kumar Srivastava, Subhadeep Gupta and Amirul Islam Mallick. 2021. Synthesis and characterization of Indian essential oil Carbon Dots for interdisciplinary applications. Applied Nanoscience. 11**:** 1225–1239.

Wang, L., Zhu, L. and Qin, S. 2019[A]. Gut microbiota modulation on intestinal mucosal adaptive immunity. Journal of Immunology Research. 4735040.

Wang, X., Feng, Y., Dong, P. and Huang, J. 2019[B]. A mini review on carbon quantum dots: preparation, properties, and electrocatalytic application. Frontiers in Chemistry. 7**:** 671.

Xinkai Wang, Yifan Hu, Xiaoyan Zhu, Liyuan Cai, Muhammad Zahid Farooq and Xianghua Yan. 2023. *Bacteroides*-derived isovaleric acid enhances mucosal immunity by facilitating intestinal IgA response in broilers. Journal of Animal Science and Biotechnology. 14: 4.

Yang, Y., Iji, P.A. and Choct, M. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World's Poultry Science Journal. 65: 97–114.

Zeng, Z., Zhang, S., Wang, H. and Piao, X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology. 6: 7.

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Effect of Dietary Supplementing Graded Concentrations of Sodium Sulphate on Performance, Carcass Traits, And Antioxidant Variables In Broiler Chicken

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ABSTRACT

A study was conducted to assess the effect of supplementing graded concentrations of sodium sulphate (NaS) at a constant dietary electrolyte balance (DEB) on performance, carcass traits, immune responses, and antioxidant variables in broiler chicken. A total of 1,800 one-day-old broiler male chicks (Cobb 430) were evenly distributed into 72 pens, with 25 birds per pen (198 x 122 cm). A control diet (CD), based on maize-soybean meal, was prepared in mash form for three different phases: pre-starter (1-14 days), starter (15-28 days), and finisher (29-42 days). Sodium chloride (NaCl) was used as the sole source of supplemental Na and Cl in the CD. Five additional diets were formulated by supplementing sodium sulphate (NaS) at five graded concentrations (1.12, 2.25, 3.37, 4.50, and 5.62g/kg) to the CD. The DEB in pre-starter, starter, and finished diets were 215, 192, and 163 mEq/kg, respectively in all the treatment diets in each phase. The levels of NaCl were adjusted to arrive at a constant DEB among the diets in each phase. Body weight gain (BWG) and feed intake (FI) were not affected due to dietary variations. The feed conversion ratio (FCR) in groups fed with 3.37 or 4.50g/kg NaS was significantly lower compared to those fed 5.62 g/kg NaS. The carcass traits (ready to cook yield, breast meat weight, abdominal fat, liver weight and gizzards weight), immune responses (HI response to ND vaccine and CMI response to phytohaemogglutinin-P), and antioxidant variables (activity of glutathione peroxidase, glutathione reductase), respiration rate (panting), and cloacal temperature were not affected by dietary variation in NaS concentration. Based on the results, it can be concluded that NaS can be included in broiler diets up to 4.50g/kg, at constant dietary electrolyte balance, without any adverse effects on performance.

KEYWORDS: Antioxidant status, Broilers, Carcass parameters, Immunity, Production, Sodium sulphate.

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INTRODUCTION

Sodium and chloride are the major extracellular cation and anion, which play a pivotal role in maintaining the proper balance of body fluids and cell function. Sodium (Na) is essential for several physiological processes, including the regulation of acid-base balance, osmotic pressure, and cell permeability (Olanrewaju et al., 2007). Sodium also facilitates the absorption of monosaccharides and amino acids, which are vital for the utilization of proteins and carbohydrates. Therefore, a deficiency of Na can impair these vital processes (Smith et al., 2000; Gal-Garber et al., 2003). Adequate Na intake has been shown to improve the performance of chicken (Watkins et al., 2005). Sodium chloride (NaCl) and sodium bicarbonate (NaHCO3) are commonly used as supplemental Na sources in poultry diets. Commercial poultry diets typically contain NaCl to meet the Na and Cl requirements. Inclusion of NaCl as a sole source of Na in the chicken diet may pose a risk of excess Cl intake. The Cl intake may also get complicated due to variation in concentrations of Cl in drinking water. Therefore, an excess intake of Cl can disrupt the balance of chloride (Cl−) and carbonate ions [CO2(3−)], negatively affect the synthesis of calcium carbonate,

and result in reduced performance (Mushtaq et al., 2007). Excessive intake of Cl can also result in wet feces, excessive water intake, ascites, edema, and weakened eggshell quality. An estimate reported that about 9 g of water is excreted for every 0.25% increase in dietary NaCl (Smith et al., 2000). Increasing the NaCl in the diet will also increase the Cl concentration in the diet, which may have a negative impact on bird physiology and performance, as indicated. Substituting sulfate for chloride as an anion has been considered particularly beneficial during stress periods that disrupt gut homeostasis and increase electrolyte losses (Hooge et al., 1999; Ahmad et al., 2006; Zdunczyk et al., 2012).

Sodium sulphate (NaS), also known as Glauber's salt, has been explored as chlorine-free Na source for poultry (Ahmad and Sarwar, 2005; Wang et al., 2019). Furthermore, dietary sulphate may contribute to the synthesis of several essential compounds, including methionine, cysteine, taurine, and glutathione, which are crucial for growth and antioxidant properties (Battin and Brumaghim, 2009; Del-Vesco et al., 2014). Furthermore, NaS has been observed to moderately stimulate digestive tract mucosa, enhance gastrointestinal motility, and regulate Na+/K+-ATPase activity, thereby improving digestive function (Gal-Garber et al., 2003).

Studies have demonstrated that dietary NaS at 1 g/kg can effectively replace about 18% of the recommended methionine (Rahimi et al., 2005). It is widely accepted that the sulphate requirements of most animals can generally be fulfilled by sulphate-containing amino acids through the oxidation of these amino acids. Conversely, dietary supplementation of inorganic sulphate may partly spare the dietary requirements for methionine or total sulphur amino acids (TSAA). Therefore, an effort was made in the present study to evaluate the potential advantages of incorporating sodium sulphate (NaS) at graded concentrations on the performance of broiler chicken, while maintaining constant dietary electrolyte balance (DEB) and TSAA levels in the diet.

MATERIALS AND METHODS

Diets and treatments

Maize-soybean-meat based control diets (CD) were prepared in the form of mash for three different phases i.e., pre-starter (1-14 d), starter (15-28 d), and finisher (29-42 d) phases (Table 1, 2, and 3, respectively). Feed grade sodium chloride (NaCl) was supplemented as the sole source of supplemental Na and Cl in the CD. Another five diets were prepared by supplementing sodium sulphate (NaS, Prosodium, Garsin, New Delhi, India) at five different concentrations (1.12, 2.25, 3.37, 4.50, and 5.62g/kg) to the CD. The dietary electrolyte balance (DEB) was maintained constant in each phase (about 215, 192, and 163 mEq/kg, respectively in pre-starter, starter, and finisher phases) irrespective of sodium level. Each diet was randomly allotted and fed *ad libitum* to birds in 12 pens (replicates) at the rate of 25 broiler male chickens per pen from d 1 to 42 d of age.

Table 1. Ingredient and nutrient composition (g/kg) of pre-starter (1-14d) diets

Ingredient	NaSO4, g/kg					
	CD	1.12	2.25	3.37	4.50	5.62
Maize	565.4	565.4	566.4	566.4	566.4	566.4
Oil-Veg	30.9	30.9	30.9	30.9	30.9	30.9
Soya Doc 45%	358.3	358.3	358.3	358.3	358.3	358.3
Salt	4.70	4.30	3.90	3.50	3.10	2.70
Dicalcium phosphate	14.5	14.5	14.5	14.5	14.5	14.5
LSP-Powder	12.9	12.9	12.9	12.9	12.9	12.9
DL-Methionine	3.643	3.643	3.643	3.643	3.643	3.643
L-Lysine SO4	3.674	3.674	3.674	3.674	3.674	3.674
L-Threonine	0.973	0.973	0.973	0.973	0.973	0.973
L-Tryptophan	0	0	0	0	0	0
L- Arginine	0.465	0.465	0.465	0.465	0.465	0.465
L-Valine	0.434	0.434	0.434	0.434	0.434	0.434
L-Isoleucine	0.051	0.051	0.051	0.051	0.051	0.051
Premix[#]	4.1	4.1	4.1	4.1	4.1	4.1

Na2SO4	0	1.12	2.25	3.37	4.50	5.62
Nutrient, g/kg						
M E (kcal/kg)	3000	3000	3000	3000	3000	3000
Protein	220.0	220.0	220.0	220.0	220.0	220.0
Dig Lysine	12.70	12.70	12.70	12.70	12.70	12.70
Dig. Methionine	6.65	6.65	6.65	6.65	6.65	6.65
Dig. Meth + Cyst	9.60	9.60	9.60	9.60	9.60	9.60
Calcium	9.50	9.50	9.50	9.50	9.50	9.50
Av. Phosphorus	5.00	5.00	5.00	5.00	5.00	5.00
Sodium	20.07	18.55	17.03	15.51	13.99	12.47
Chloride	32.10	29.78	27.46	25.14	22.82	20.50
Potassium	85.07	85.07	85.07	85.07	85.07	85.07
Sulphur ppm	2522	2634	2747	2859	2972	3084
DEB (meq/Kg)	215	215	215	215	215	215
Dig. Arginine	13.50	13.50	13.50	13.50	13.50	13.50
Dig. Tryptophan	2.41	2.41	2.41	2.41	2.41	2.41
Dig.Threonine	8.50	8.50	8.50	8.50	8.50	8.50
Dig. Iso-Leucine	8.50	8.50	8.50	8.50	8.50	8.50
Dig. Valine	9.60	9.60	9.60	9.60	9.60	9.60

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase

[##] analysed values

Table 2. Ingredient and nutrient composition (g/kg) of Starter (15-28d) diets

Ingredient	NaSO4, g/kg					
	CD	1.12	2.25	3.37	4.50	5.62
Maize	611.9	611.9	611.9	612.9	612.9	612.9
Oil-Veg	38.3	38.3	38.3	38.3	38.3	38.3
Soya Doc 45%	305.7	305.7	305.7	305.7	305.7	305.7
Salt	4.70	4.30	3.90	3.50	3.10	2.70
Dicalcium phosphate	12.1	12.1	12.1	12.1	12.1	12.1
LSP-Powder	13.6	13.6	13.6	13.6	13.6	13.6
DL-Methionine	3.292	3.292	3.292	3.292	3.292	3.292
L-Lysine SO4	4.041	4.041	4.041	4.041	4.041	4.041
L-Threonine	0.965	0.965	0.965	0.965	0.965	0.965
L-Tryptophan	0.067	0.067	0.067	0.067	0.067	0.067
L- Arginine	0.702	0.702	0.702	0.702	0.702	0.702
L-Valine	0.519	0.519	0.519	0.519	0.519	0.519
L-Isoleucine	0.252	0.252	0.252	0.252	0.252	0.252
Premix[#]	4.10	4.10	4.10	4.10	4.10	4.10
Na2SO4	0	1.12	2.25	3.37	4.50	5.62
Nutrient, g/kg						
M E (kcal/kg)	3100	3100	3100	3100	3100	3100
Protein	200.0	20.00	20.00	20.00	20.00	20.00
Dig Lysine	11.60	11.60	11.60	11.60	11.60	11.60
Dig. Methionine	6.08	6.08	6.08	6.08	6.08	6.08
Dig. Meth + Cyst	8.80	8.80	8.80	8.80	8.80	8.80
Calcium	9.00	9.00	9.00	9.00	9.00	9.00
Av. Phosphorus	4.50	4.50	4.50	4.50	4.50	4.50
Sodium	20.00	18.48	16.96	15.44	13.92	12.40
Chloride	32.13	29.81	27.49	25.17	22.85	20.53
Potassium	76.47	76.47	76.47	76.47	76.47	76.47

Sulphur ppm	2386	2498	2611	2723	2836	2948
DEB (meq/Kg)	192	192	192	192	192	192
Dig Arginine	12.30	12.30	12.30	12.30	12.30	12.30
Dig. Tryptophan	2.20	2.20	2.20	2.20	2.20	2.20
Dig .Threonine	7.80	7.80	7.80	7.80	7.80	7.80
Dig. Iso-Leucine	7.80	7.80	7.80	7.80	7.80	7.80
Dig. Valine	8.80	8.80	8.80	8.80	8.80	8.80

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase

[##]analysed values

Table 3. Ingredient and nutrient composition (g/kg) of Finisher (29-42d) diets

Ingredient	NaSO4, g/kg					
	CD	1.12	2.25	3.37	4.50	5.62
Maize	653.4	654.4	654.4	654.4	655.4	655.4
Oil-Veg	43.1	43.1	43.1	43.1	43.1	43.1
Soya Doc 45%	263.6	263.6	263.6	263.6	263.6	263.6
Salt	4.2	3.8	3.4	3.0	2.6	2.2
Dicalcium phosphate	10.7	10.7	10.7	10.7	10.7	10.7
LSP-Powder	13.3	13.3	13.3	13.3	13.3	13.3
DL-Methionine	2.936	2.936	2.936	2.936	2.936	2.936
L-Lysine SO4	1.532	1.532	1.532	1.532	1.532	1.532
L-Threonine	0.804	0.804	0.804	0.804	0.804	0.804
L-Tryptophan	0.191	0.191	0.191	0.191	0.191	0.191
L- Arginine	0.936	0.936	0.936	0.936	0.936	0.936
Phytase 5000	0.100	0.100	0.100	0.100	0.100	0.100
L-Valine	0.509	0.509	0.509	0.509	0.509	0.509
L-Isoleucine	0.254	0.254	0.254	0.254	0.254	0.254
Premix[#]	4.10	4.10	4.10	4.10	4.10	4.10
Na2SO4	0	1.12	2.25	3.37	4.50	5.62
Nutrient, g/kg						
M E (kcal / kg)	3175	3175	3175	3175	3175	3175
Protein	182.5	182.5	182.5	182.5	182.5	182.5
Dig Lysine	9.20	9.20	9.20	9.20	9.20	9.20
Dig. Methionine	5.55	5.55	5.55	5.55	5.55	5.55
Dig. Meth + Cyst	8.10	8.10	8.10	8.10	8.10	8.10
Calcium	8.50	8.50	8.50	8.50	8.50	8.50
Av. Phosphorus	4.20	4.20	4.20	4.20	4.20	4.20
Sodium	18.06	16.54	15.02	13.50	11.98	10.46
Chloride	33.57	31.25	28.93	26.61	24.29	21.97
Potassium	69.72	69.72	69.72	69.72	69.72	69.72
Sulphur ppm	1916	2028	2141	2253	2366	2478
DEB (meq/Kg)	163	163	163	163	163	163
Dig Arginine	11.40	11.40	11.40	11.40	11.40	11.40
Dig. Tryptophan	2010	2010	2010	2010	2010	2010
Dig .Threonine	7.10	7.10	7.10	7.10	7.10	7.10
Dig. Iso-Leucine	7.10	7.10	7.10	7.10	7.10	7.10
Dig. Valine	8.10	8.10	8.10	8.10	8.10	8.10

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, α tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg; phytase

[##]analysed values

Birds and management

A total of 1,800 one-day-old broiler male chicks (Cobb 430, Venkateswara Hatcheries Pvt. Ltd., Hyderabad, India) were evenly distributed into 72 pens at the rate of 25 birds each in litter floor pen (198 x 122 cm). The floor of the pens were covered with fresh paddy husk at about 8 cm thickness. The litter was covered with old newspaper to prevent accidental intake of litter material by chicks during the initial 4 days of age, after which the paper was removed. Brooding was done with incandescent bulbs (100 watt/pen) and coal to provide the required temperature (about 35°C during week 1, and gradually reduced to ambient temperature at day 21 and after which the birds were exposed to ambient temperature. The ambient temperature ranged from 24.7±1.35 to 30.4±2.65°C and the humidity were ranged from 32.7±16.01 to 63.5±8.45% at minimum and maximum, respectively. Fluorescent bulbs were used to provide light during nighttime from 4 to 6 weeks of age.

Performance and slaughter variables

Body weight and feed intake (FI) were recorded at weekly intervals, and body weight gain (BWG) and feed conversion ratio (FCR) was calculated as FI/BWG. Feed left in the feeder was placed back in the respective feed drum to calculate the average amount of feed consumed by the birds in each pen. All the birds present in each pen were weighed to calculate the average BWG. At the end of the experiment, one bird representing the mean body weight of the respective pen (replicate) was selected to study carcass traits, including ready-to-cook yield (RTC), breast meat weight, thigh weight, liver weight, and abdominal fat content. The relative weight of lymphoid organs (bursa, spleen) was recorded and expressed as g per kg live weight**.**

Immune responses

Immune responses were measured in terms of HI titer to Newcastle disease vaccine and cell-mediated immune (CMI) response to phytohemagglutinin-P (PHA-P) inoculation.

HI titre against ND vaccine

The chickens were vaccinated against Newcastle disease (ND) by an ocular route at 5th and 21nd d of age with the Lasota strain (ND Lasota Vac-500, Indovax Pvt., Ltd., Hyderabad, India). The humoral immune response was measured as antibody titre against ND vaccine by collecting blood from the brachial vein on the 35th day of age, which was 14 d post inoculation of the vaccine. For this, 2 mL of blood was collected from one bird per replicate, and the antibody titres in sera against ND virus was measured (Reynolds and Maraqa, 2000) by haemagglutination inhibition test. The antibody titre against the disease was expressed as log2 values. The reciprocal of the highest dilution where there was complete inhibition of agglutination was taken as the titre.

Cell mediated immune (CMI) response

The CMI response was assayed by cutaneous basophilic hypersensitivity test *in vivo* by using phytohemagglutinin-P (PHA-P) (TC 226, HiMedia Laboratories Pvt Ltd, Mumbai, India) employing the method as described by Corrier and Deloach (1990). On 35th d of age, one bird from each replicate was selected and the thickness of both right and left wattles was measured by micrometer (p no 7301, Mitutoyo, Japan). A total of 100 µg of PHA-P suspended in 0.10 mL of phosphate buffer saline (PBS) and 0.1 mL of the PBS without PHA-P were injected intradermal into the right and left (acted as a control) wattles, respectively. The thickness of both the wattles was measured at 24 h post-injection. The CMI response was calculated as the difference in thickness between right and left wattles due to PHA-P inoculation, which was expressed in relation to the increased thickness due to PBS alone.

Anti-oxidant variables

The activities of antioxidant enzymes like glutathione peroxidase (GSHPx), and glutathione reductase (GSHRx) in blood were measured at the end of the study (42d of age). About 2.0 mL of blood sample from each bird was placed into a centrifuge tube containing citrate buffer (1.5 mL/10 mL blood) for erythrocyte separation and antioxidant enzyme estimation. The blood samples were centrifuged at 500 rpm for 15 min at 4°C to separate the buffy coat (WBC) and form erythrocyte pellet. The erythrocytes were washed three times with PBS (pH 7.4). The packed RBC obtained was mixed with an equal volume of PBS and then diluted as per the requirement with distilled water. The activity of GSHPx and GSHRx was estimated following the method of Paglia and Valentine (1967).

Panting rate and cloacal temperature

One bird per pen was selected to count the number of respirations in a minute. The number of respirations was recorded two times in a day i.e. at 1-2 PM and 5-6 PM from all the replicates once in each

week. Simultaneously, rectal temperature was measured from the same bird utilizing a digital thermometer.

Statistical analysis

The performance data were analysed by considering the pen as an experimental unit, and other parameters (carcass traits, immune responses, lymphoid organ weight, panting, and cloacal temperature), the individual bird data were considered as a unit for statistical analysis. The data were statistically analysed by a complete randomized design with One-way Analysis of Variance (ANOVA) (SAS Institute, 1994). The differences among treatment means were compared using Duncan's multiple range test. Further, the response in the dependent variables with change in the concentration of NaS was fitted by the polynomial equation in the form of $Y=a+bx+cx^2$ to know the trend in the dependent variable in relation to the NaS concentration in the diet.

RESULTS AND DISCUSSION

Performance

The inclusion of NaS in broiler chicken diets did not significantly influence BWG or FI (P>0.05) during the pre-starter, starter, finisher phases, and overall production period (Table 4 and 5). However, the FCR, expressed as the ratio of FI/BWG, was significantly improved at 2.25 and 3.37 g/kg NaS inclusion compared to 5.62 g/kg during pre-starter phase. In contrast, no significant differences in FCR were observed among broilers fed diets with 0, 1.12, and 4.50 g/kg NaS.

Table 4. Performance of broiler chicken fed graded concentrations of sodium sulphate (NaS)

NaS	Pre-Starter (1-14 days)			Starter (15-28 days)		
g/kg	BWG, g	FI, g	FI/BWG	BWG, g	FI, g	FI/BWG
CD	474.9	504.5	1.182^{BC}	996.0	1522	1.457^{CD}
1.12	474.1	504.7	1.185^{AB}	986.1	1530	1.479^{AB}
2.25	482.2	509.2	1.173^{D}	984.7	1522	1.473^{AB}
3.37	477.3	505.3	1.178^{CD}	991.0	1525	1.468^{BC}
4.50	476.6	505.2	1.180^{BCD}	1007	1524	1.444^{D}
5.62	469.0	500.2	1.189^{A}	979.6	1527	1.486^{A}
SEM	1.83	2.013	0.0011	4.43	5.87	0.0025
P						
One-way	0.472	0.897	0.001	0.564	0.999	0.001
Regression						
Linear	0.471	0.578	0.290	0.887	0.937	0.501
Quadratic	0.185	0.523	0.001	0.976	0.996	0.688

^{ABCD} means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; BWG body weight gain; FI feed intake; P probability; n: number of replicates; SEM standard error of the mean

Table 5. Performance of broiler chicken fed graded concentrations of sodium sulphate (NaS)

NaS	Finisher (29-42 days)			Cumulative (1-42 days)		
g/kg	BWG, g	FI, g	FI/BWG	BWG, g	FI, g	FI/BWG
CD	977.2	2023	2.079	2448	4049	1.654_{BC}
1.12	945.9	1983	2.106	2406	4017	1.670_{AB}
2.25	960.7	2007	2.093	2428	4038	1.664_{AB}
3.37	995.5	2027	2.041	2464	4057	1.647_{C}
4.50	983.6	2020	2.060	2467	4049	1.642_{C}
5.62	939.9	1977	2.105	2389	4005	1.676^{A}
SEM	12.66	20.46	0.009	12.8	20.72	0.0031
P						
One-way	0.787	0.972	0.235	0.406	0.977	0.001
Regression						
Linear	0.882	0.819	0.765	0.764	0.800	0.878
Quadratic	0.833	0.931	0.538	0.650	0.900	0.256

^{ABC} means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; BWG body weight gain; FI feed intake; P probability; n: number of replicates; SEM standard error of the mean

Significantly lower FCR was observed at 4.50 g/kg NaS inclusion during the starter phase compared to all other inclusion levels, except the control group. The cumulative FCR was significantly ($P<0.05$) reduced with the level of NaS in diet. Broilers fed diets with 3.37 or 4.50 g/kg NaS demonstrated significantly lower FCR compared to those receiving higher concentrations (5.62 g/kg NaS, Table 5). Similar to the current study, Rahimi et al. (2005) also observed an improvement in the FCR of broiler chickens due to the supplementation of NaS in the diet. However, the improved BWG without affecting the FCR at day 42 was reported by Mushtaq et al. (2014) when the broiler diets were supplemented with NaS at 2.6 g/kg.

The findings of this study differ from those of Sharma et al. (2011), who reported improved body weight with the supplementation of NaS in turkey broiler diets. Similarly, Ali et al. (2019) observed the lowest FI in broilers fed NaS supplemented diets compared to other treatments. In their study, NaS (3 g/kg) was supplemented in combination with tyrosine (0.5g/kg), the additive effect probably might have decreased the FI in Arbor Acers male broilers.

The exact metabolic role of NaS in chicken is not very clear, however, based on the literature, the probable beneficial role of NaS supplementation on chicken performance was discussed. There is a limited recent literature on NaS use in poultry diets, majority of work was conducted during 1970s. The beneficial effects of NaS supplementation on feed efficiency might be due to the methionine-sparing effects of S present in NaS. Similar to this hypothesis, the methionine-sparing effect of NaS on broiler performance was reported in the literature (Ross et al., 1972; Harms, 1972; Hinton and Harms, 1972; Rama Rao et al., 2022). Maintenance of optimum blood sulphate concentration (0.01 µc/ml, higher than those achieved with standard diets) and optimum feather development was attributed (Gordon and Sizer, 1955) to the higher growth rate in chicken fed NaS. Similarly, Almquist (1952) reported that NaS was approximately 40% as effective as amino acid sulphate in promoting growth response. Harms (1972) also found that the peak growth rate of broiler chicks was achieved when a corn-soybean basal diet (containing 4.0 g methionine and 3.9 g/kg cystine) was supplemented with a combination of methionine (1 g/kg) and NaS (1 g/kg). In their studies, an addition of 1.6 g/kg NaS was found as the most effective level for stimulating growth in chicks. Improved nutrient digestion in upper digestive tract with NaS supplementation compared to NaHCO3 was reported by Lawlor et al. (2005). The higher beneficial effects of NaS over NaHCO3 was attributed to lower acid binding capacity (96 mEq H+/kg vs. 110.66 mEq H+/kg) of NaS relative to NaHCO3, which likely contributes to the improved performance of broilers. The beneficial effects of NaS may also be attributed to its potential role in conserving sulphur-containing amino acids by facilitating the direct synthesis of taurine and chondroitin sulphate from NaS, thereby preventing the degradation of sulphur amino acids (Youssef, 2002). Additionally, NaS has been shown to maintain dietary electrolyte balance (DEB) within optimal limits in broiler diets (Jarule et al., 2009). In the current study, the DEB was uniformly maintained among the different treatment groups in each pase, which might explain the lack of negative effects on performance with lower sodium levels than recommended by NRC 1994.

The improvement of FI per BWG indicated that higher levels of sulphates in NaS supplemented diets might have induced appetite in birds since better absorption of sulphates is Na dependent active process or because Na involves in cysteine sparing effect as per the literature (Langridge-Smith et al., 1983; Ahearn and Murer, 1984; Florin et al., 1991). This increased presence of Sulfur might have converted to amino acids, which might have improved the feed efficiency. The amino acid-sparing effect was further demonstrated by Sharma et al. (2012) with turkey broilers by evaluating the impact of adding 4 g/kg NaS to a vegetable-based, fishmeal-free ration.

Improved weight gain and feed efficiency were reported by Plavnik and Bornstein (1978) with increasing levels of NaS in diets containing sub-optimal concentrations of total sulphur amino acids. This could explain the improved feed efficiency observed in the current study with the supplementation of NaS in diets.

In the current study, the feed efficiency reduced significantly in groups fed the highest concentration of NaS (5.62 g/kg). The calculated concentrations of S in those diets (3084, 2948 and 2478 ppm, respectively in pre-starter, starter and finisher diets) were nearer to the harmful level (3000 mg/kg) as suggested by NRC (1994). Trials conducted by the University of Guelph (Canada) to evaluate broiler growth in response to sulphate intake revealed a linear decline in weight gain with increased

inorganic sulphate content (1400, 2700, 4000, 5300 mg/kg S at 0.37 and 1.32% Ca levels) in diet and the negative effects were attributed to disruption of anion-cation balance (Pinon et al., 2021).

Carcass parameters

Dietary inclusion of NaS in broiler chicken diets did not have any significant effect (P>0.05) on carcass variables such as ready-to-cook yield (RTC), abdominal fat, breast meat, or the weights of lymphoid organs, with the exception of thigh weight (Table 6). The thigh weight showed a progressive increase (P<0.05) with NaS concentration up to 2.25 g/kg and however, further increases in NaS concentration resulted in a reduction in thigh weight. These results are in line with findings of Borgatti et al. (2004) and Musthaq et al. (2013), who reported higher thigh weights in broilers fed graded concentrations of NaS (1.7, 2.6, 3.5, and 4.4 g/kg) in diets.

Table 6. Slaughter parameters (g/kg live weight) of broilers fed graded concentrations of sodium sulphate (NaS)

NaS	RTC	BW	Abdfat	Liver	Thigh	Bursa	Spleen
g/kg				g/kg live weight			
CD	782.1	257.0	14.98	18.99	214.9^{BC}	0.494	0.899
1.12	778.1	259.0	17.41	18.62	214.4^{BC}	0.315	0.905
2.25	790.5	269.8	15.87	19.45	238.1^{A}	0.450	0.982
3.37	783.5	267.5	11.39	17.61	220.0^{B}	0.526	0.994
4.50	787.3	270.1	14.65	18.13	213.1^{BC}	0.506	0.908
5.62	786.1	273.2	15.21	17.62	201.1^{C}	0.373	0.916
SEM	1.874	2.653	0.631	0.365	2.679	0.029	0.031
P							
One-way	0.498	0.415	0.137	0.639	0.002	0.230	0.922
Regression							
Linear	0.292	0.038	0.377	0.174	0.098	0.945	0.867
Quadratic	0.514	0.105	0.537	0.390	0.002	0.839	0.663

^{ABC} means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; RTC ready to cook yield; BW breast weight; Abdfat abdominal fat; P probability; n: number of replicates; SEM standard error of the mean.

Contrarily, Mushtaq et al. (2007) observed reduced breast and leg meat by increasing Na from 2.0 to 3.0 g/kg. These attributes (breast and thigh meat weights) were negatively affected by heat stress conditions provided in the experiment by Mushtaq et al. (2007), therefore it is obvious that more nutrients particularly energy were utilized for increased activities like panting. Whereas, in the present study there is no change in caracass parameters (except thigh muscle weight), which might be due to the fact that the DEB maintained uniform among the groups, and also optimum ambient temperature prevailed during the study period.

In the current study, there is no change in the weights of bursa and spleen. Studies conducted by Raymond and Karunajeewa 1985, Borges et al. (1999) and Borgatti et al. (2004) also did not observe any combination effects of electrolytes (sodium carbonate, potassium carbonate, and ammonium chloride) on the weights of proventriculus, heart, liver, and pancreas.

Immunity and fitness traits

Inclusion of NaS in broiler chicken diets did not have a significant impact (P>0.05) on the immune responses (CMI and HI titres against ND, Table 7). Similarly, the inclusion of NaS in the diet did not affect panting rate or cloacal temperature in broilers.

Table 7. Immune responses, panting, cloacal temperature, and serum anti-oxidant variables in broilers fed graded concentrations of sodium sulphate (NaS)

NaS	ND titres	CMI	Panting	Cloacal temp	GSHPx	GSHRx
g/kg	Log	2%	no °F			u/L
CD	6.70	52.50	30.92	107.1	366.3	1956
1.12	6.60	54.17	24.83	107.4	372.1	1737
2.25	6.90	63.75	26.83	106.8	381.6	1712
3.37	6.70	61.50	27.92	107.2	332.3	1740
4.50	6.50	51.67	27.92	107.0	363.6	1637
5.62	6.70	56.83	27.58	107.5	392.9	1647
SEM	0.1586	2.198	0.8201	0.1145	6.375	36.63
P						
One-way	0.990	0.531	0.449	0.581	0.115	0.133
Regression						
Linear	0.879	0.793	0.709	0.660	0.659	0.014
Quadratic	0.979	0.466	0.500	0.704	0.330	0.029

[ABC] means having common superscripts in a column do not vary significantly (P<0.05)
NaS sodium sulfate; CD control diet; CMI cell mediated immunity; GSHPx glutathione peroxidase, GSHRx: glutaredoxins; P probability; n: number of replicates; SEM standard error of the mean.

Sulphate undergoes various metabolic processes within living organisms through reduction and oxidation pathways. It is essential for the synthesis of glutathione, which is a component of cysteine and methionine. Sulphate is metabolized into sulphate compounds, which are either assimilated or excreted (Stipanuk, 2004; Nimni et al., 2007; Toledano et al., 2007). As a natural antibiotic, sulphate aids in detoxification by binding with toxins or heavy metals in the liver (Parcell, 2002). However, in the present study, no significant variations were observed in panting rates or cloacal temperatures. Similarly, Ahmad et al. (2006) concluded that maintaining a constant DEB (250 mEq/kg), altering the concentrations of dietary sodium content (through NaHCO3, Na2CO3, and Na2SO4) significantly improved broiler performance during the hot summer months. In the current study, the temperature in the sheds was maintained at optimal comfort levels, allowing the birds' performance to be maximized according to standards, even with lower DEB and sodium (Na) levels than those recommended by the NRC. The National Research Council recommends 2g/kg sodium, chloride, and 3g/kg potassium for the starter phase of broilers, with reduced sodium and chloride levels for the finisher phase. However, under heat stress conditions, these requirements increase, as birds perform better with elevated electrolyte levels while maintaining DEB approximately 250 mEq/kg. Higher electrolyte levels, particularly sodium, effectively promote growth but also lead to increased water consumption, resulting in higher litter moisture during summer (Mushtaq et al., 2013).

In the current study, no variation was observed in the levels of antioxidant enzymes, such as GSHPx and GSHRx, among the different groups of birds fed varying concentrations of NaS. These antioxidant enzymes are vital for protecting cells from oxidative damage by reducing hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively. The lack of change in enzyme levels suggests that the birds were kept within their comfort zone and provided with optimal nutrition. This could be one reason why there is no variation in immunity, organ weights, panting, and temperature among the groups.

CONCLUSION

Based on the data, it can be concluded that supplementation of sodium sulphate at 3.37 or 4.50 g/kg in broiler diets significantly improves feed efficiency. Additionally, an inclusion level of 2.25 g/kg NaS notably enhances the relative weight of the thigh, demonstrating its beneficial effects on growth performance and carcass characteristics in broiler chicken.

REFERENCES

Ahearn, G.A., Murer, H. 1984. Functional roles of Na+ and H+ in SO4 2– transport by rabbit ileal brush border membrane vesicles. Journal of Membrane Biology. 78: 177-186.

Ahmad, T.and Sarwar, M. 2005. Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. Animal Feed Science and Technology. 120: 277e98.

Ahmad, T., Mushtaq, T., Mahr Un, N., Sarwar, M., Hooge, D.M. and Mirza, M.A. 2006. Effect of different non-chloride sodium sources on the performance of heat-stressed broiler chickens. British Poultry Science. 47: 249–256.

Ali, M.N., Shamseldeen, A.E., Mohamed, S., Arafa, A.S. and Sayed, M.A. 2019. Effect of tyrosine, vitamin, and butylated hydroxytoluene without or with sodium sulphate on broiler performance. Egyptian Journal of Poultry Science. 39(IV): 809-824.

Almquist, H.J. 1964. Inorganic sulphate in animal nutrition. Feedstuffs. 36(24): 60.

Almquist, J.J. 1952. Amino acid requirement of chickens and turkeys: A review. Poultry Science. 31: 966-981.

Battin, E.E. and Brumaghim, J.L. 2009. Antioxidant activity of sulphate and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. Cell Biochemistry and Biophysics. 55: 1-23.

Borgatti, L.M.O., Albuquerque, R., Meister, N.C., Souza, L.W.O., Lima, F.R. and Trindade Neto, M.A. 2004. Performance of broilers fed diets with different dietary electrolyte balance under summer conditions. Review Bras. Cienc. Avic. 6: 153-157.

Borges, S.A., Ariki, J., Martins, C.L. and de Moraes, V.M.B. 1999. Potassium chloride supplementation in heat stressed broilers. Rev. Bras. Zootec. 28: 313-319.

Corrier, D.E. and DeLoach, J.R. 1990. Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. Poultry Science. 69: 03–408.

Del-Vesco, A., Gasparino, E., Grieser, D., Zancanela, V., Gasparin, F. and Constantin, J. 2014. Effects of methionine supplementation on the redox state of acute heat stress exposed quails. Journal of Animal Science. 92: 806e15.

Florin, T., Neale, G., Gibson, G.R. and Cummings, J.H. 1991. Metabolism of dietary sulphate: Absorption and excretion in humans. Gut. 32: 766-773.

Gal-Garber, O., Mabjeesh, S.J., Sklan, D. and Uni, Z. 2003. Nutrient transport in the small intestine: Na+,K+-ATPase expression and activity in the small intestine of the chicken as influenced by dietary sodium. Poultry Science. 82: 1127-1133.ISSN 0032-5791

Gordon, R.S. and Sizer, I.W. 1955. Ability of sodium sulfate to stimulate growth of the chicken. Science. 122(3183):1270-1.

Harms, R.H. 1972. Response of poultry to supplemental inorganic sulfate. Proc. Maryland Nutrition Conference. pp 51.

Hinton, C.F. and Harms, R.H. 1972. Evidence for sulfate as an unidentified growth factor in fish solubles. Poultry Science. 51: 701-703.

Hooge, D.M., Cummings, K.R. and McNaughton, J.L. 1999. Evaluation of sodium bicarbonate, chloride, or sulfate with a coccidiostat in corn-soy or corn-soy-meat diets for broiler chickens. Poultry Science. 78: 1300–1306.

Jarulė, V., Gružauskas, R., Racevičiutė Stupelienė, A., Šašytė, V., Semaškaitė, A., Tėvelis, V., Švirmickas, G.J. and Švirmickienė, V. 2009. Influence of sodium bicarbonate and sodium sulphate on electrolyte balance and productivity in broiler chickens. Veterinarija Ir Zootechnika. 47: 3-9.

Koong, L.J., Farcell, C.C. and Nienata, S.A. 1985. Assessment of interrelationship among levels of intake and production, organ size, and fasting heat production in animals. Journal of Nutrition. 115: 1383-1390.

Langridge-Smith, J.E., Sellin, J.H. and Field, M. 1983. Sulphate influx across the rabbit ileal brush border membrane: Sodium and proton dependence, and substrate specificities. Journal of Membrane Biology. 72: 131-139.

Lawlor, P.G., Brendan Lynch, P., Patrick, J. Caffrey, James, J O'Reilly M. and Karen O'Connell. 2005. Measurements of the acid-binding capacity of ingredients used in pig diets. Irish Veterinary Journal. 58: 447-452.

Liu, B., Zhu, J., Zhou, Q. and Yu, D. 2021. Tolerance and safety evaluation of sodium sulfate: A subchronic study in laying hens. Animal Nutrition. 7: 576–586.

Machlin, L.J. and Pearson, P.B. 1956. Studies on utilization of sulfate sulphate for growth of the chicken. Proceeding of the Society for Experimental Biology and Medicine. 93: 204-206.

Mushtaq, M.M.H., Parvin, R. and Kim, J. 2014. Carcass and body organ characteristics of broilers supplemented with dietary sodium and sodium salts under a phase feeding system. Journal of Animal Science Technology. 56: 1-7.

Mushtaq, M.M.H., Pasha, T.N., Mushtaq, T. and Parvin, R. 2013. Electrolytes, dietary electrolyte balance and salts in broilers: an updated review on growth performance, water intake and litter quality. World's Poultry Science Journal. 69: 789-802.

Mushtaq, T., Mirza, M.A., Athar, M., Hooge, D., Ahmad, T. and Ahmad, G. 2007. Dietary sodium and chloride for twenty-nine-to forty-two-day-old broiler chickens at constant electrolyte balance under subtropical summer conditions. Journal of Applied Poultry Research. 16: 161e70.

Mushtaq, M.M.H., Pasha, T.N., Saima, M., Akram, T., Mushtaq, R., Parvin, U., Farooq, S., Mehmood, K.J. and Iqbal, J. Hwangbo. 2013. Growth performance, carcass traits and serum mineral chemistry as affected by dietary sodium and sodium salts fed to broiler chickens reared under phase feeding system. Asian Australasian Journal of Animal Science. 26: 1742-1752.

National Research Council, N.R.C. 1994. Nutrient requirements of poultry. 9th ed. Washington, DC: National Academy Press.

Nimni, M.E., Han, B. and Cordoba, F. 2007. Are we getting enough sulphate in our diet? Nutrients Metabolism. 4: 24-36.

Olanrewaju, H., Thaxton, J., Dozier, III W. and Branton, S. 2007. Electrolyte diets, stress, and acid base balance in broiler chickens. Poultry Science. 86: 1363-71.

Paglia, D.E and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory Clinical Medicine. 70(1): 158-69. PMID: 6066618.

Parcell, C. 2002. Sulphate in human nutrition and applications in medicines. Alternative Medicine and Review. 7**:** 30-31.

Pinon, A., Maurin, J. and Animine. 2021. Looking at Sulphur and Sulphate levels in Poultry diets. Poultry World. No.02.

Plavnik, Y. and Bornstein, S. 1978. The sparing action of inorganic sulphate on sulphur amino acids in practical broiler diets: The replacement of some of the supplementary Methionine in broiler finisher diets. British Poultry Science. Rahimi, G., Yousofi, A., Hashemi, M. 2005. Replacement of sulphate amino acid (methionine) by sodium sulfate in fat containing diet of broiler chickens. Journal of Agriculture Sciences and Natural Resources. 11(4): 107-115.

Rama Rao, S.V., Raju, M.V.L.N., Shyam Sunder Paul, Nagalakshmi Devanaboyina, Srilatha Thota, Prakash Bhukya, and Rajkumar Ullengala. 2022. Enhancement of performance and anti-oxidant variables in broiler chicken fed diets containing sub-optimal methionine level with graded concentrations of sulphur and folic acid. Animal Biosciences. 35**(**5):721-729.

Raymond J. Johnson and Hector Karunajeewa. 1985. The effects of dietary minerals and electrolytes on the growth and physiology of the young chick. The Journal of Nutrition. 115: 1680-1690.

Reynolds, D.L. and Maraqa, A.D. 2000. Protective immunity against Newcastle disease: the role of cell-mediated immunity. Avian Diseases. 44: 145-154.

Ross, E., Damrom, B.L. and Harms, R.H. 1972. The requirement for inorganic sulfate in the diet of chicks for optimum growth and feed efficiency. Poultry Science. 51: 1606-1612.

SAS Institute (1994) SAS User's Guide: Statistics. Version 7.0. SAS Institute, Cary, NC.

Sharma, V., Sharma, R.K. and Singh, D. 2012. Effect of inorganic sulphate supplementation in fish meal free ration on growth performance of turkey broilers. Indian Journal of Poultry Science. 47: 391-394.

Smith, A., Rose, S., Wells, R. and Pirgozliev, V. 2000. Effect of excess dietary sodium, potassium, calcium and phosphorus on excreta moisture of laying hens. British Poultry Science. 41: 598e607.

Soares, J.H., Jr, Nicholson, J,L,, Bossard, E.H. and Thomas, O.P. 1974. Effective levels of sulfate supplementation in broiler diets. Poultry Science. 53**:** 235-240.

Stipanuk, M.H. 2004. Sulphate amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annual Review of Nutrition. 24: 539-577.

Toledano, M.B., Kumar, C., LE Moan, N., Spectorr, D. and Tacnet, F. 2007. The system biology of thiol redox system in *Escherichia coli* and yeast: differential functions in oxidative stress, iron metabolism and DNA synthesis. FEBS Letter. 581: 3598-3607.

Wang, J., Zhang, H.J., Wu, S.G., Qi, G.H. and Xu, L. 2019. Dietary chloride levels affect performance and eggshell quality of laying hens by substitution of sodium sulfate for sodium chloride. Poultry Science. 99: 966e73.

Watkins, S., Fritts, C., Yan, F., Wilson, M. and Waldroup, P. 2005. The interaction of sodium chloride levels in poultry drinking water and the diet of broiler chickens. Journal of Applied Poultry Research. 14: 55e9.

Wei, S.M., Yue, H.Y., Wu, S.G., Zhang, H.J., Wang, J. and Qi, G.H. 2015. Study on the tolerance of Jinghong laying hens to dietary sodium sulfate. Chinese Journal of Animal Nutrition. 27: 2493-2501.

Youssef, S.F. 2002. Physiological, productive, and reproductive studies on the effects of supplemented basal diet with organic and inorganic sulphate on chickens. PhD Thesis. Cairo University.

Zdunczyk, Z., Jankowski, J., Juskiewicz, J. and Kwiecinski, P. 2012. The response of the gastrointestinal tract of broiler chickens to different dietary levels and sources of sodium. Veterinarija Ir Zootechnika. 60: 92–98.

ADVANCES IN NUTRITIONAL INTERVENTIONS TO IMPROVE PRODUCTIVE INDICES IN LIVESTOCK

A Regional Conference of Animal Nutrition Society of India entitled **ADVANCES IN NUTRITIONAL INTERVENTIONS TO IMPROVE PRODUCTIVE INDICES IN LIVESTOCK** was organized on 12th - 13th March, 2026 at College of Veterinary University, jointly organized by SVVU & ANSI. Four retired Animal Nutrition professionals (*Dr.M Parthasarathi, Dr D. Srinivasa Rao, Dr P. Eswar Prasad* and *Dr A Ravi*) stationed at Tirupati were felicitated as a mark of honour from ANSI. On the day one, all the scientific presentations and deliberations were completed by eminent animal nutritionists from the Southern Region and a total of 58 delegates from Karnataka, Tamilnadu, Telengana, Andhra Pradesh have registered out of which 21 are post graduate and PhD scholars. The second day was totally for the farmers especially Women farmers who are directly associated with the livestock for many hours in day to day livestock activities. A panel of experts were nominated for the interactive sessions with the farmers on the 2nd day and it was fruitful. About 60 women farmers have participated in the interactions and the two day conference was concluded with valedictory function in which our Hon'ble Vice Chancellor was the Chief Guest, Director of Extension, Dr K. Shobhamani , Dr Udeybir Singh Chahal, Former President & Secretary ANSI and Dr Shivani Katoch, Secretary, ANSI were the Guests of Honour.

Release of Souvenir

A view of Participating Women

WASTE TO WEALTH

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Dr. DVR Prakash Rao, Promoter Chairman of Prakash Foods & Feed Mills

The impact of Industrial solid wastes emanating from the animal based Industries on the ecology and environment is huge and efforts are made constantly to salvage them. However Prakash Foods & Feed Mills Pvt Ltd., Chennai has taken this challenge head on and proved victorious. They have developed a technology of recycling these solid wastes for manufacture of protein feed supplement containing protein, fat, calcium and phosphorous to replace partially soyabean meal, DCP and fish in poultry and aqua feeds. The promoter Chairman and Managing Director is Dr. D.V.R Prakash Rao has an academic career of par excellence with a PhD in Animal Nutrition. He has been conferred Doctor of Science by three eminent Institutions of India, GADVASU, Ludhiana, NDRI, Karnal and O.UA.T Bhubaneswar for his contribution in Animal Nutrition & Feed Technology. He has developed a technology of manufacturing Animal protein feed supplement from meat and bone offal of the animal based Industries. The capacity for creativity and innovation towards new product development is his biggest strength. He was an International consultant in United Nations Industrial development Organization, Vienna before heading Animal Feeds operations of a British multinational. His academic excellence coupled with business acumen has graduated the Company from a small scale into a large medium scale company. His son Shriram Duvvuri is business graduate from Australia and is the Managing Director of the company. This technology is of paramount importance as public health phenomena, besides being an economic and efficient model for of manufacturing protein feed supplement. This helps to increase the profitability of the farmer without any loss of nutrients in the final product.

Prakash Foods & Feed Mills Pvt Ltd is recycling around 40,000 tons of animal offal per month on moisture basis through their factories located at Kanchipuram, Sholavaram and a third factory is being built at Arani (Tamilnadu) to expand the production capacity. Prakash Foods & Feed Mills has certainly created a new revolution in its domain by creating wealth from wastes.

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