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Indian Journal of Animal Nutrition

Cashew Nut Meal as a Feed Ingredient for Livestock – A Review

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ABSTRACT

Traditional protein sources, such as soybean meal and groundnut cake, have seen rising costs, prompting the exploration of alternative, cost-effective feed ingredients for cattle feed. Cashew Nut Meal (CNM), a byproduct of cashew nut processing, presents a promising alternative due to its favorable nutritional profile and potential economic benefits. This review aims to explore the feasibility of utilizing CNM as a protein source in livestock diets, focusing on its nutritional value, economic viability, and challenges associated with its use. The paper discusses the role of agricultural by-products in enhancing livestock nutrition and promoting sustainable farming practices. Future research is recommended to focus on comprehensive nutritional profiling of CNM, the mitigation of anti nutritional factors, and the development of innovative processing methods to improve its bioavailability. Additionally, establishing quality control standards and conducting life cycle assessments can ensure the sustainability of CNM as a feed ingredient. The support of CNM effectively, livestock producers, especially in cashew-growing regions, can reduce feed costs, improve feed efficiency, and contribute to more sustainable and profitable livestock farming practices.

KEYWORDS: Cashew nut meal, Novel Feed Resource, Protein Supplement.

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INTRODUCTION

Traditionally, protein sources like soybean meal and groundnut cake have been the cornerstone of cattle feed in India. However, the rising costs of these conventional feed ingredients have driven the need to explore; alternative, more cost-effective protein sources (Ojediran et al., 2024). Agricultural by-products utilization as animal feeds is the need of the hour to meet the demand of livestock nutrition and indirectly, of the food requirements of a rapidly increasing human population (Shamsi et al., 2012). The objective of this review is to provide an in-depth analysis of CNM as a source of feed ingredient in livestock, exploring its nutritional benefits, economic viability, potential challenges while considering the broader implications for sustainable livestock farming in developing countries.

Source of Cashew Nut Meal

Cashew nut meal (CNM), a byproduct of cashew kernel processing (*Anacardium occidentale*), has gained recognition as a valuable feed ingredient and functional food component due to its rich nutrient profile. Comprising protein, fats, fiber, vitamins, and minerals, it serves as a nutritionally beneficial option. Discarded cashew nuts deemed unfit for human consumption have a protein content ranging from 18–27% of dry matter and an oil content of 36–51% of dry matter (Lebas et al., 2012). The cashew processing industry, particularly prominent in regions such as Kerala, Goa, Karnataka, and Andhra Pradesh, has emerged as a promising source of protein for dairy cattle (Ojediran et al., 2024). India, being one of the world's leading cashew producers, offers an opportunity to utilize CNM as an underexplored resource, potentially transforming it into an economically viable and locally available feed alternative for dairy farmers. Research indicates that CNM can effectively replace soybean meal (SBM) in compounded ruminant feed formulations, with inclusion levels of up to 30% (w/w) showing no negative impact on rumen fermentation or digestibility (Rashmi et al., 2024).

COMPOSITION NUTRITIONAL PROFILE OF CNM

The nutritional composition of CNM typically includes: Crude protein: 25 –30%, Ether extract (Crude fat): 10–15%, Crude fiber: 5–10%, Ash content: 3–5% metabolizable energy approximately 3000–3500 kcal/kg (Rico et al., 2016). Many authors reported that the cashew oil meal had (Akande et al., 2015, Aletor et al., 2007 and Lima et al., 2004) dry matter content of 93.7 per cent, 36.6 per cent crude protein, 0.9 per cent crude fibre, 16.9 per cent crude fat, 5.1 per cent total ash, 2240 kcal/kg of gross energy. Calcium (1.5 %), Phosphorous (12 %), Potassium (2.0 %), Sodium (1.1%), Magnesium (3.2 %), Manganese (11mg/kg), Zinc (25 mg/kg) and Iron (139 mg/kg) per kg of dry matter content. Blomhoff et al., (2006) reported in his study that, CNM contained high protein (21%), carbohydrate (22%), fat (47 %), vitamins (thiamine) and also rich in manganese, potassium, copper, iron, magnesium, zinc and selenium.

1. Crude Protein

CNM is rich in protein, typically ranging from 20 to 25% of its dry weight. The amino acid profile includes essential amino acids, particularly lysine, tryptophan which are often limited in plant-based diets (Diarra et al., 2014). Hence it is particularly suited for dairy cattle, where protein quality is directly related to milk production efficiency (Rashmi et al., 2024). The protein content in CNM typically ranges from 20% to 25%, depending on the processing method and the specific variety of cashew (Diarra and Usman, 2014). The meal has a good quality protein containing 4.6 per cent lysine, 1.3 per cent tryptophan about 2 per cent cystine and 1.6 per cent methionine, further it contains well-balanced essential amino acids such as lysine, leucine and tryptophan (Piva et al., 1971). This is particularly beneficial as lysine and tryptophan are often limited in plant-based diets (Abdulrahman et al., 2011). The bioavailability of proteins in CNM has been shown to be high, making it a valuable protein source in food processing as well (Pinto et al., 2020). The protein and energetic characteristics of CNM shows that it can be used as an alternative supplementation to low-quality forages for lambs (Costa et al., 2021).

2. Crude Fat

Cashew nut meal (CNM) contains a fat content ranging from 20–30% (Ojediran et al., 2024), with values reported at 21.98 ± 0.01% (Silu et al., 2017), 36.70% (Aremu et al., 2006), and 34.95% (Omusuli et al., 2009). This variability in fat content may stem from differences in processing techniques and durations. The fat is primarily composed of unsaturated fatty acids, serving as an excellent energy source, particularly beneficial for lactating cows in high-production phases (Ojediran et al., 2024). Oleic acid (omega-9) and linoleic acid (omega-6) are the dominant fatty acids in CNM, contributing significantly to its nutritional and functional properties. Oleic acid, a monounsaturated fatty acid (MUFA), is known for its cholesterol-modulating effects, particularly in lowering LDL cholesterol levels, thereby supporting cardiovascular health. Linoleic acid, an essential polyunsaturated fatty acid (PUFA), enhances immune function and promotes inflammatory balance (Adeyeye, 2004). The synergistic combination of omega-9 and omega-6 fatty acids further optimizes lipid metabolism and overall health (Pinto et al., 2020).With a fat profile comparable to olive oil, CNM is particularly heart-healthy and offers antioxidant benefits. Its digestibility and palatability make it suitable for diverse applications, including use as a protein fortifier in food products. Additionally, CNM's energetic properties have been demonstrated as an effective alternative supplementation for low-quality forages, particularly in lamb diets (Costa et al., 2021). This versatile profile highlights CNM's potential as both a feed and functional food component.

3. Crude Fiber and Carbohydrates

Cashew nut meal (CNM) contains moderate levels of dietary fiber, which play a significant role in enhancing gastrointestinal health and supporting a balanced gut microbiota. Its carbohydrate content, primarily comprising starch and sugars, ranges between 20–30%, providing an essential energy source for animal feed applications (Okafor and Aniche, 2013). The fiber content contributes to improved digestive health, while the carbohydrate profile ensures sufficient energy supply (Okafor and Aniche, 2013). Furthermore, CNM's fiber composition offers potential as a prebiotic, promoting the growth of beneficial gut bacteria and supporting gut health in food products (Pinto et al., 2020). The fiber content of cashew nut cake has been reported as 3.44 ± 0.35% (Silue et al., 2017), while fat-free cashew samples exhibit lower fiber levels of 1.42 ± 0.2% (Omosuli et al., 2009). The carbohydrate content, calculated by difference, is 38.30 ± 0.12%,

with reducing sugars at 2.66% and total sugars at 9.94%, as noted by Silue et al. (2017), while Omosuli et al. (2009) reported a carbohydrate content of 25.39%. These nutrient values underscore CNM's versatility as a source of both energy and dietary fiber, making it a valuable ingredient in animal nutrition and functional food formulations.

4. Minerals and Vitamins

Silue et al. (2017) reported that potassium (799.27 ± 0.44) was the most abundant mineral, followed by phosphorus (672.38 ± 0.54), magnesium (266.42 ± 0.32), and chromium (262.75 ± 0.88). The least abundant minerals were sodium (8.96 ± 0.01), zinc (16.32 ± 0.04), iron (33.05 ± 0.13), and calcium (55.48 ± 0.03). Olaofe and Sanni (1988) and Aremu et al. (2005) similarly observed lower levels of iron and zinc. Notably, minerals such as chlorine, manganese, and copper were undetected. Despite this, the mineral values are higher than those reported by Pamplona-Roger (2008) for cashew nut flour, who recorded calcium (45.0 mg), phosphorus (490 mg), magnesium (260 mg), iron (6 mg), potassium (565 mg), and zinc (5.6 mg). Rico et al. (2016) also found slightly different values for cashew kernels, reporting calcium (52.0 mg), sodium (6.6 mg), phosphorus (570.0 mg), potassium (670.0 mg), magnesium (265.0 mg), iron (7.1 mg), and zinc (5.9 mg). The significant levels of magnesium, phosphorus, and potassium in CNM are essential for bone health and cellular function (Abdulrahman et al., 2011). Moreover, B-complex vitamins such as B6, niacin, and thiamine contribute to energy metabolism (Pinto et al., 2020). Phosphorus, being highly abundant, supports bone density and cellular functionality (Diarra and Usman, 2014). Potassium helps regulate blood pressure and maintain fluid balance, while iron facilitates oxygen transport and helps prevent anemia (Okafor and Aniche, 2013). The zinc content in CNM is particularly valuable for immune function and skin health, as zinc is involved in DNA synthesis, wound healing, and immune response. Regular consumption of zinc-rich foods like CNM can significantly enhance these processes (Adeyeye, 2004).

5. Antioxidant Compounds

CNM is a rich source of polyphenolic compounds (479.39 ± 0.00 mg/100 g DM) and contains smaller quantities of flavonoids, such as quercetin and catechins (55.48 ± 0.06 mg/100 g DM), as well as tannins (134.19 ± 0.37 mg/100 g DM). It also demonstrates a notable antioxidant activity (AOA) of 75.11 ± 0.00%. Among the phenolics, gallic acid is a key compound, recognized for its anti-inflammatory and antimicrobial properties (Diarra and Usman, 2014). The flavonoids in CNM, including quercetin and catechins, are powerful antioxidants. Quercetin, in particular, is known for its ability to modulate allergic reactions and support respiratory health, making CNM an appealing ingredient in food formulations designed to combat oxidative stress (Okafor and Aniche, 2013; Goufo and Trindade, 2014). The presence of these flavonoids suggests that CNM can play a role in the management of cardiovascular diseases and oxidative stress, as flavonoids act as biological antioxidants (Mbatchou and Kosoono, 2011). These bioactive compounds offer significant nutritional and therapeutic benefits. As potent antioxidants, they protect cells from damage caused by reactive oxygen species, including singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. Furthermore, their anti-inflammatory, antifungal, and antibiotic properties enhance their value in promoting overall health and well-being (Meddleton et al., 1993; Abalokoka et al., 2014).

6. Anti nutritional components of CNM

CNM, like other nut-based byproducts, contains some antinutritional components that can impact nutrient availability digestibility if not properly managed. The primary antinutritional factors in CNM include phytates, oxalates, tannins and Cyanidic acid, which can limit the bioavailability of certain nutrients, particularly minerals. However, processing techniques such as roasting or soaking can help to reduce the levels of these compounds, making CNM more suitable for use in food / animal feed.

a. Phytates

These compounds can bind to essential minerals such as calcium, iron, magnesium, and zinc, forming insoluble complexes that hinder their absorption in the digestive tract (Diarra and Usman, 2014; Erdman, 1979). Although phytates exhibit antioxidant properties, their high concentrations can significantly reduce the bioavailability of critical minerals, posing a concern, particularly for populations whose diets are deficient in mineral-rich foods (Adeyeye, 2004). Research has shown that processing techniques such as soaking and fermenting CNM can effectively reduce phytate levels, thereby enhancing mineral absorption (Okafor and Aniche, 2013). According to Silue et al. (2017), CNM contains 87.27 ± 0.00

mg/100 g DM of phytates, while Mbatchou et al. (2011) reported a lower concentration of 6.78 mg/ 100 g DM in cashew meal. These findings underscore the variability of phytate content and the potential benefits of processing methods to improve the nutritional quality of CNM.

b. Oxalates

CNM also contains oxalates, which can interfere with calcium absorption by forming calcium oxalate, an insoluble compound that reduces calcium availability. In large amounts, oxalates can contribute to the formation of kidney stones, especially in individuals predisposed to oxalate accumulation (Pinto et al., 2020). While the oxalate content in CNM is generally moderate, and the processing methods such as roasting can to help reduce these levels, making the meal safer for consumption in both human and animal diets (Goufo and Trindade, 2014). As per Salimata et al., (2017) the oxalate content of CNM can range from 73.99 mg to 140 mg per 100g.

c. Tannins

Tannins are phenolic compounds present in CNM that can interfere with protein iron absorption by binding to them forming complexes that are less digestible (Reddy Kumar, 2017). Defatted cashew reject meal contains 1.51 percent tannins. Cashew nut testa, also known as the cashew nut husk, contains around 19.9–22.1 percent tannins (Akande et al., 2015). Tannins also have astringent properties, which can reduce palatability, particularly in animal feed. However, tannins possess antioxidant properties that provide some health benefits, such as reduced oxidative stress (Abdulrahman et al., 2011). Processing methods like boiling or soaking can reduce tannin levels, improving the digestibility / palatability of CNM.

d. Saponins and Trypsin Inhibitors

Saponins and trypsin inhibitors are also found in CNM in smaller amounts. Saponins can reduce nutrient absorption by forming complexes with proteins in the digestive enzymes, while trypsin inhibitors specifically hinder protein digestion by blocking trypsin, a key enzyme involved in protein breakdown. In CNM the total phenolic content in methanol extract seems to be higher than that in the other extracts and quantified as 18.1 mg/g tannic acid (Pradhan et al., 2020). The trypsin inhibitor and phytate were 1.045 mg/g and 0.87 g/100 of CNM respectively and no saponins were detected (Pradhan et al., 2020). The presence of these compounds can reduce the nutritional quality of CNM, particularly for animals with high protein requirements (Diarra and Usman, 2014). However, heat treatment, such as roasting, has been shown to inactivate trypsin inhibitors, making CNM more nutritionally accessible (Okafor and Aniche, 2013).

e. Cyanidic Acid

Cyanogenic glucoside is an organic compound containing sugar moiety, and is capable of yielding cyanide on hydrolysis. About ten cyanogenic glucosides including amygdalin, prunasin, dhurrin, linamarin, and taxiphyllin have been reported in edible plants. Hydrocyanic or prussic acid (HCN) is toxic and rapidly acts as a common poison Silue et al. (2017), reported that the CNM has a cyanidic acid content of 1.07 ±00 mg /100g DM. This low level may be due to the naturally low cyanide content of cashew nuts (Nkafamiya et al., 2015).

7. Feeding recommendations for livestock

a. Ruminants:

i) Dairy cows

Cashew nut meal (CNM) can be incorporated into ruminant diets at levels of up to 30% without negatively impacting growth or health, provided it is processed to reduce antinutritional factors (Diarra Usman, 2014). When included at 24% in the concentrate portion of dairy cow diets, CNM maintains production while reducing milk fat content and short-chain fatty acids. At the same time, it increases the proportion of long-chain fatty acids, thereby enhancing the nutraceutical properties of milk and its associated health benefits (Pimentel et al., 2007).

In total mixed rations (TMR) based on maize silage, CNM has been successfully included at levels as high as 50% of the dietary dry matter (Pimentel et al., 2012a). In a sugarcane-based diet, incorporating 24% CNM reduced milk fat content from 36.8 g/kg to 26.6 g/kg. While in maize silage-based diets, CNM inclusion did not affect dry matter intake (DMI), which remained at 21.3 kg/day, in sugarcane-based diets, it reduced sugarcane DMI from 7.7 to 7.05 kg/day and overall diet DMI from 14 to 13.22 kg/day (Pimentel et al., 2012a; 2007). These variations may be attributed to differences in how CNM is offered, either separately or as part of a mixed diet.

Importantly, CNM inclusion does not alter rumen fermentation parameters, ensuring that it integrates well into ruminant digestion systems (Pimentel et al., 2012b). In semi-arid regions of Northeast Brazil, diets containing 20% CNM for dairy cows were found to reduce the interval between calving and first ovulation, indicating potential reproductive benefits (Brasil, 2003). These findings highlight CNM's versatility and value as a feed ingredient for ruminants across diverse production systems.

ii) Sheep

Cashew nut meal (CNM) can be safely incorporated into sheep diets, provided that the dietary lipid content is maintained below 6–7%, as exceeding this threshold may reduce fiber digestibility, dry matter intake (DMI), and forage digestibility (Silva et al., 2008). CNM inclusion at levels of 13–18% of dietary dry matter (DM) in a concentrate fed at 1.2% of body weight (BW) as a supplement to hay showed no adverse effects on sperm quality in breeding rams (Medeiros, 2005; Oliveira et al., 2014). In adult ewes, CNM included at 12% or 24% of DM in a concentrate diet supplemented with hay yielded varying outcomes depending on the inclusion rate. At 12%, CNM had no detrimental effects, whereas at 24%, it increased the incidence of degenerated oocytes and reduced the proportion of viable oocytes. Based on these findings, it is recommended to limit CNM inclusion in ewe diets to levels below 24% to avoid reproductive challenges (Fernandes et al., 2014).

b. Monogastrics

For pigs and poultry, cashew nut meal (CNM) is generally recommended at inclusion levels of 5–10% of the total diet. Higher levels may negatively impact growth performance due to the fiber content and the presence of antinutritional factors, which can hinder nutrient absorption (Goufo and Trindade, 2014; Okafor and Aniche, 2013).

i) Pigs

Fanimo et al. (2003) evaluated the use of cashew nut rejects as a replacement for soybean meal in weaner pig diets. The study found no significant ($P > 0.05$) differences in weight gain or feed conversion ratios among groups. However, protein efficiency ratio and apparent protein digestibility were highest in pigs fed soybean meal. Pigs receiving CNM diets exhibited higher serum creatinine levels compared to those fed soybean meal, but there were no significant differences in total protein, albumin, globulin, urea, or cholesterol levels across treatment groups. It was concluded that CNM could replace soybean meal in weaner pig diets at up to 10% without adverse effects on growth performance.

ii) Poultry

Sogunle et al. (2009) conducted a trial with 9-week-old Yaafa Brown pullet chicks and found that a diet containing 10% cassava peel meal and 30% CNM improved performance in growing pullets. Freitas et al. (2006) reported that CNM could be included in broiler diets at levels up to 25% without negatively affecting performance. Similarly, Cruz et al. (2015) studied Dekalb Brown laying hens at 27 weeks of age and observed that feed intake and egg weight were unaffected by CNM inclusion. However, higher CNM levels negatively impacted egg production, egg mass, feed conversion, and yolk color. Consequently, the study recommended limiting CNM to 10% in layer diets to maintain optimal performance.

iii) Turkeys

Ogungbenro et al. (2013) tested CNM as part of a 30% CNM-maize offal combination in diets for Nicholas White strain turkeys. The results indicated that this combination could effectively replace maize, improving both performance and nutrient digestibility.

These findings highlight the potential of CNM as a feed ingredient for pigs and poultry, provided inclusion levels are carefully managed to avoid adverse effects on performance and health.

8. Processing Methods

a. Roasting

Heat treatment can significantly reduce antinutritional factors, such as trypsin inhibitors tannins, thereby enhancing protein digestibility overall nutrient availability (Pinto et al., 2020). Roasting CNM before feeding can lead to improved growth performance in livestock.

b. Fermentation

Fermentation is another effective method to reduce phytate content to enhance nutrient bioavailability. Incorporating fermented CNM into animal diets can improve growth rates and feed efficiency (Adeyeye, 2004).

c. Enzyme addition/Supplementation

To counteract the effects of antinutritional factors, enzyme supplementation (such as phytase and protease) can be beneficial, particularly in monogastric diets. These enzymes helps in breakdown of phytates thereby improve nutrient absorption and making CNM more effective in enhancing growth performance (Diarra and Usman, 2014).

9. Economic Considerations in feeding CNM to livestock

a. Lower Feed Costs

One of the most significant advantages of incorporating CNM into livestock diets is the potential for substantial cost savings. CNM is generally less expensive than conventional protein sources such as soybean meal or fish meal. Utilizing CNM can help livestock producers reduce overall feed costs, particularly in regions where cashew nuts are widely grown and processed (Pinto et al., 2020).

Research has shown that replacing traditional protein sources with CNM can yield competitive growth performance. For instance, Diarra Usman (2014) found that up to 30% of the dietary protein in ruminants could be replaced with CNM without adversely affecting animal health or performance. This shift can lead to significant savings in feed expenditures.

According to Balaga et al. (2021), cashew nut kernel meal can be included in the diet of ram lambs at levels up to 20 percent, yielding beneficial effects on their health. Additionally, it does not negatively impact palatability, enhances nutrient digestibility, improves overall performance and leads to cost-effectiveness.

b. Utilization of Byproducts

CNM provides a means to utilize agricultural byproducts that might otherwise be discarded. By integrating CNM into livestock feed, producers can take advantage of this readily available and underutilized resource, promoting sustainability in reducing waste (Goufo and Trindade, 2014). This approach not only helps in cutting costs but also contributes to environmental sustainability in agriculture.

10. Future Perspectives Research Directions of CNM feeding

Future research should prioritize a comprehensive nutritional profiling of CNM to gain a clearer understanding of its macro- and micronutrient composition. Mitigation of antinutritional factors present in CNM, can hinder nutrient absorption and limit its effectiveness as a feed ingredient. Development of innovative processing techniques can significantly enhance the nutritional value of CNM. Establishing quality control standards for CNM is essential to ensure consistent nutritional quality. Conducting life cycle assessments can help to quantify the carbon footprint, resource use and waste generation associated with CNM production and use. Developing economic models to evaluate the cost-effectiveness of using CNM in livestock diets can inform producers about its viability as a protein source. Future research should focus on species-specific feeding programs / trials that optimize the use of CNM for different livestock species.

CONCLUSION

As a byproduct of cashew processing, CNM offers several advantages, including cost-effectiveness, improved feed efficiency and favorable nutrient profile. Appropriate processing methods reduce the antinutritional compounds, enhancing the bioavailability of nutrients and thereby improving the overall effectiveness of CNM in animal diets.

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Effect of Three Different Forms of Zinc on Growth Performance, Digestibility, Blood Profiles and Antioxidant Status of Sahiwal Heifers

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ABSTRACT

The current study was designed to investigate the impact of dietary supplementation of organic and nano zinc on the performance of Sahiwal heifers. A total of twenty young Sahiwal heifers were randomly assigned into four groups having five animals based on body weight and age for a period of 90 days. The control group was given on a basal diet whereas, group T1 was supplemented basal diet with 40mg/kg DM Zn glycinate, group T2 was supplemented basal diet with 40mg/kg DM Zn peptide, and group T3 was supplemented with a basal diet with 20mg/kg DM nano Zn oxide. The dietary requirements of the heifers were adequately met by providing a ration consisting of concentrate, jowar (Sorghum) fodder, and wheat straw in a 50:30:20 ratio on a dry matter (DM) basis. No effect on DMI, Feed conversion ratio (FCR), Feed conversion efficiency (FCE) but ADG was significantly higher ($P<0.05$) in the nano zinc-supplemented group. There was no significant effect ($P>0.05$) on blood parameters except MCHC which was significantly higher in nano zinc supplemented groups**.** Treatment groups showed significantly higher ($P<0.05$) plasma globulin, ALP, glucose and lower bilirubin, PUN whereas, no significant effect ($P>0.05$) was found on albumin, total protein, triglycerides, cholesterol, ALT, AST, and creatinine. No effect on plasma minerals except serum zinc level. SOD, FRAP, and total plasma immunoglobulin concentrations were significantly higher in the treatment groups than in the control group. Finally, it may be concluded that nano Zn supplementation @20ppm and Zn pep @40ppm can be supplemented in heifer's diet for better performance without adversely affecting nutrient digestibility, blood profile, and antioxidant status.

KEYWORDS: Haematology, Heifers, Immune response, Nano zinc, Organic zinc

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INTRODUCTION

The health and wellbeing of animals are significantly impacted by mineral shortages in their feed, even when other nutrients are sufficiently present. The importance of zinc is highlighted by the observation that more than 50% of Indian soils currently exhibit zinc deficiency, with projections indicating an increase to 63% by 2025 (Gupta et al., 2016). The prevalent zinc deficit in feed and fodder crops, due to insufficient zinc levels, has negatively impacted animal health and productivity. Zinc is not retained in the body like other trace minerals, requiring consistent dietary intake to ensure optimal health and function. Zinc (Zn) is employed by the body in multiple functions, including the initiation and modulation of immune responses, antioxidant activities, serving as a cofactor for various enzymes, spermatogenesis, steroidogenesis, vitamin A metabolism, insulin storage and secretion, energy metabolism, protein synthesis, stabilization of macromolecules, regulation of DNA transcription, and cellular division (Miller, 1970; Jackson and Lowe, 1992; Salgueiro et al., 2000; Frassinetti et al., 2006; Valle and Falchuk, 1993; Yatoo et al., 2013; Kaur et al., 2014).

Minerals, whether inorganic or organic, can be provided to address these deficiencies. Administering inorganic mineral supplements to animals may lead to deficiencies in other minerals that are only marginally available. Organic minerals also show better absorption and application than their inorganic alternatives. Nano zinc shows excellent bioavailability.

They can reduce environmental contamination, serve as alternatives to antibiotics for growth promotion, eliminate antibiotic residues in animal products, and offer pollution-free animal products at lower dosages (Schmidt, 2009). Research on zinc supplementation in native cows is limited, although numerous studies have investigated the effects of zinc from different sources on growth performance and health-related traits in various animal categories. This study investigates the impact of organic and nano Zn supplementation on the performance of Sahiwal heifers.

MATERIALS AND METHODS

Twenty Sahiwal heifers were selected from the cattle herd kept at the Livestock Farm Complex (LFC), DUVASU, Mathura and were randomly assigned into four groups (five heifers in each) on body weight and age basis. Experimental trial period was of 90 days. Control group were fed on basal diet without any zinc supplementation, whereas group T1 was supplemented with Zinc glycinate (Zn-Gly) @40 mg Zn/kg DM, group T2 was supplemented with Zinc peptide (Zn-pep) @40 mg Zn/kg DMandgroup T3was supplemented with nano zinc oxide (nZnO) @20 mg Zn/kg DM. Basal diet consisted of wheat straw (particle size- 1.5 to 2.0 cm), chaffed green Jowar fodder and compounded concentrate mixture as per ICAR (2013) requirements. The composition of TMR (on %DM basis) in all treatments is Jowar fodder (30%), wheat straw (20%) and concentrate mixture (50%). Concentrate mixture was prepared by mixing 16 parts oats, 15 parts barley, 18 parts wheat bran, 17 parts gram chunni, 32 parts mustard oil cake and 2 parts mineral mixture. The analyzed chemical composition (%) was found to be in TMR were 70.25, 90.34, 3.12, 15.08, 9.70, 23.35, 48.75, 56.27, 34.24% for DM, OM, EE, CP, ASH, CF, NFE, NDF, ADF in control group, T1 , T2 and T3 group, respectively. Zn concentration was 21.62 mg/kg in control, 61.62 mg/kg in T1, 61.62 mg/kg in T2 and 41.62 mg/kg in T3 TMR, respectively.

Zn-Gly procured from noreal Pvt. Ltd., purity 40%; Zn-pep procured from Alltech Pvt. Ltd., purity 20%; ZnO nano particle sprocured from SRL Pvt. Ltd., Maharastra, minimum assay 99.9%. All heifers were kept in a well-ventilated shed with the appropriate setup for individual feeding and watering, ensuring they did not have access to the diets of other animals. All the animals were dewormed using the drug Fenbendazole and Ivermectin at the dose rate of 10mg/kg bodyweight, prior to the commencement of experiment.

The body weight of the experimental heifers was documented at the commencement of the trial and thereafter at fortnightly intervals. Feed, fodder, and residual ort samples were oven-dried at 60/ °C using a hot air oven and subsequently ground using a Wiley mill to pass through a 1-mm sieve. The processed samples were then analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and total ash, following the standard analytical procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified following the methodologies outlined by Van Soest et al. (1991). The concentrations of Calcium (Ca), Phosphorus (P), Copper (Cu), Iron (Fe), and Zinc (Zn) in feed and fodder samples were analyzed using inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA).

Blood samples were obtained prior to the feeding and watering of heifers at 07:00 hours in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60, and 90 days post-supplementation. Blood samples were tested for hematological, biochemical, and hormonal characteristics. A segment of whole blood samples were used for assessing white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) concentration, hematocrit values, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, and mean platelet volume, utilizing the Celltac-á (MEK-6500K) automated hematology analyzer produced by Nihon Kohden Europe. A segment of whole blood samples was employed to evaluate superoxide dismutase (SOD) activity (Madesh and Balasubramanian, 1998), FRAP activity (Benzie and Strain, 1999), and plasma total immunoglobulin using the zinc turbidity method (Mc Ewan et al., 1970). The remaining blood samples were centrifuged at 3000 rpm for 15 minutes to separate the plasma.

Plasma samples were preserved at “20°C until subsequent examination of liver and kidney function biomarkers (glucose, triglycerides, AST, ALT, ALP, bilirubin, and creatinine) and protein metabolism biomarkers (total protein, albumin, globulin, and PUN). The plasma concentrations of glucose, triglycerides, AST, ALT, ALP, bilirubin, creatinine, total protein, albumin, and PUN were measured using

an automated biochemical analyzer (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd., China) with Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). The plasma globulin concentration was determined by subtracting the albumin level from the total protein content. The minerals were analyzed by inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA).

The collected data were analysed using a mixed model for repeated measures in the Statistical Package for the Social Sciences (SPSS for Windows, V21.0; SPSS Inc., Chicago, IL, USA). The Tukey Honestly Significant Difference test was employed on treatment means, revealing a statistically significant variation across the samples. The difference was deemed significant at $P<0.05$.

RESULTS AND DISCUSSION

There was no significant effect on dry matter intake (kg/day or % BW)in allgroups. Average daily gain was significantly higher ($P<0.05$) for nano zinc group followed by zinc peptide group. No significant effect ($P>0.05$) of organic and nano zinc supplementation was observed on average body weight, FCR, FCE in heifers (Table 1).Seifdavati et al. (2018) documented substantial enhancements in final weight and weight increase in calves using nano-zinc oxide supplementation. Anil et al. (2020) discovered that nano-zinc supplementation resulted in markedly greater body weight increase in calves relative to control groups. Uniyal et al. (2017) noted markedly elevated average daily gain (ADG) in guinea pigs supplemented with nanoparticles relative to those administered inorganic and organic zinc. This indicates that nano zinc oxide may demonstrate superior bioavailability and absorption relative to its organic equivalents, perhaps leading to enhanced growth performance. Zinc has an established mechanism for regulating the transcription of the growth hormone gene and affects the somatotrophic axis in multiple ways (MacDonald, 2000). Zinc strengthens the function of pituitary hormones through the action of growth hormone (GH) (Henkin et al., 1976).

Zinc deficiency has been shown to inhibit GH production from the pituitary (Root et al., 1979). Zinc significantly influences growth due to its cellular interactions with insulin-like growth factor-binding protein 3 (IGFBP-3), growth hormone (GH), and insulin-like growth factor 1 (IGF-1). IGF-1 concentrations influence the secretion of GH from the pituitary gland via a negative feedback mechanism (Guo et al., 2020).

Table 1. Effect of organic and nano zinc supplementation on dry matter intake and growth performance in heifers

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
Initial BW (kg/day)	141.6	141.2	141.6	141.3	10.6
Final BW (kg/day)	164.8	166.2	170.8	174.5	12.23
Avg BW (kg/day)	150.51	152.55	153.67	157.11	11.39
DMI (kg/day)	3.74	3.52	3.85	3.94	0.177
ADG (g/day)	276.19^{c}	297.61^{c}	347.61^{b}	395.23^{a}	23.13
FCR %	9.62	10.26	9.76	9.56	0.491
FCE %	0.115	0.260	0.133	0.127	0.029

Values bearing different superscripts (a,b,c) differ significantly ($P<0.05$)

Table 2. Effect of organic and nano zincsupplementation on nutrient digestibility (%) of heifers

Parameters	Group				SEM
	Control	T1	T2	T3	
Initial BW(Kg)	164.8	166.2	170.8	174.5	12.234
Final BW (Kg)	167.9	169.1	174.1	180.5	12.254
BW change (kg/day)	3.1^{ab}	2.9^{b}	3.3^{ab}	6.0^{a}	0.377
Apparent Digestibility Coefficients (%)					
DM	69.80	70.03	67.61	68.56	1.006
OM	75.22	75.41	73.42	74.20	0.825
CP	71.69	71.91	69.63	70.52	0.943
EE	81.88	82.02	80.56	81.13	0.603
NDF	63.27	63.56	60.61	61.76	1.223
ADF	49.67	50.06	46.02	47.60	1.676

Values bearing different superscripts (a,b,c) differ significantly ($P<0.01$)

The digestibility coefficients for DM, OM, CP, EE, NDF, and ADF (Table 2) were similar across all treatment groups, indicating that the incorporation of various forms of zinc in the diet did not affect nutrient digestibility. Most studies indicate that Zn supplementation, ranging from 20 to 135 mg/kg DM, did not affect the digestibility of DM and CP (Bedi, 1976; Khan, 1978; Kumar et al., 2002; James et al., 2023), as well as NDF and ADF (Salama et al., 2003) in ruminants. The lack of impact on nutrient digestibility with Zn supplementation may be attributed to the fulfilment of Zn requirements for rumen microbes by the basal diet. The outcomes of this research correlate with those of Mallaki et al. (2015) and Maan and Sihag (2014), which demonstrated that dietary zinc supplementation did not significantly affect dry matter (DM) digestibility in Zandi lambs and goat, respectively.

Likewise, Swain et al. (2018) and Satyanarayana et al. (2017) found no significant effect of various zinc forms on ether extract (EE) digestibility in goats and buffalo heifers, respectively. Jadhav et al. (2008), Singh et al. (2024), and Mandal et al. (2007) found no significant differences in DM digestibility between zinc-supplemented groups and controls in male Murrah buffalo calves, Barbari goats, and crossbred cattle, respectively. Uniyal et al. (2017) reported a significant increase in total weight gain among guinea pigs receiving nanoparticle supplementation. Pati et al. (2024) reported a significant ($P<0.05$) increase in average daily gain in Bengal goat kids supplemented with nano zinc.

Table 3. Effect of organic and nano zinc supplementation on haematological parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
RBC (x 10^6/µL)	7.23	7.15	6.91	7.43	0.103
Hb (g/dl)	9.48	8.76	8.70	9.21	0.134
Platelets (x10^9 / L)	216.85	190.35	212.40	234.15	6.856
WBC (x10^9 / L)	9.92	9.03	9.71	10.54	0.278
HCT (%)	27.71	26.34	25.85	27.04	0.374
MCV (fL)	38.59	39.52	38.03	37.22	0.488
MCH (pg)	12.64	12.94	12.62	12.39	0.158
MCHC (g/dL)	33.15^a	33.09^a	33.30^{ab}	33.70^b	0.074
MPV (fL)	5.34	5.21	5.19	5.29	0.029

Values bearing different superscripts (a,b) differ significantly (P<0.01)

The concentrations of hematological indices (Table 3) remained within the normal range for heifers following supplementation with organic and nano zinc, suggesting that the supplementation did not adversely affect the physiological status of the heifers, which indicates good health. The MCHC concentration was statistically greater (P<0.05) in the nano zinc group compared to other groups, likely due to zinc's significant role in hemoglobin formation, which is essential for improving Hb concentration up to a certain level, as suggested by Ott et al. (1965). The mean values for RBC, Platelets, WBC, HCT, MCV, MCH, and MPV in the supplemental group did not exhibit any significant effects (P>0.05). Similar studies conducted by Anil (2020) on crossbred calves fed ZnSO4 and nano ZnO, as well as Deori et al. (2024) and Singh et al. (2024) on goats supplemented with nano Zn, indicated no effect on hematological parameters. Ahuja et al. (2022), Pawar et al. (2023), and Gami et al. (2024) reported comparable haematological parameters in lactating Kankrej cows.

Table 4. Effect of organic and nano zinc supplementation on blood biochemical parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
Glucose (mg/dl)	50.11	54.06	56.40	58.80	1.653
Triglyceride (mg/dl)	32.62	33.42	33.74	33.10	0.231
Cholesterol (mg/dl)	200.7	197.5	197.7	198.1	1.326
Total protein (g/L)	6.49	6.93	6.94	6.73	0.10
Albumin (g/L)	3.16	3.15	2.99	3.07	0.123
Globulin (g/L)	3.33^b	3.82^{ab}	3.95^a	3.66^b	0.087

Values bearing different superscripts (a,b) differ significantly (P<0.05)

Plasma glucose, cholesterol, and triglyceride levels served as biomarkers for energy and lipid metabolism, whereas plasma total protein, albumin, and globulin were utilized as biomarkers for protein metabolism (Table 4). The plasma glucose level was significantly elevated (P<0.05). Increased serum glucose levels may result from changes in the molar proportion of volatile fatty acids (VFAs) in the rumen, specifically an increase in propionate production (Aliarabi and Chhabra, 2006). No differences were noted in triglyceride and cholesterol levels (Elamin et al., 2015; Kamdev et al., 2016). All biomarkers of protein metabolism are within normal physiological limits, and no significant differences (P>0.05) were observed between supplemented and unsupplemented heifers, except for plasma globulin levels, which were significantly higher (P<0.05) in the organic zinc supplemented groups. The findings align with those of Kudìlková et al. (2016), who similarly observed elevated levels of ã-globulin in the group supplemented with organic zinc. Ramulu et al. (2015) demonstrated that zinc supplementation enhances globulin levels in buffalo calves in a dose-dependent manner, specifically at concentrations ranging from 80 to 140 ppm.

In this research, we observed significantly reduced plasma bilirubin and plasma urea nitrogen levels (Table 5) in the supplemented groups, with the highest values recorded in the control groups, consistent with the findings of Kolaskar et al. (2021) regarding BUN. No adverse effects of supplementation on liver function were observed, as indicated by comparable plasma levels of ALT and AST in both the control and zinc-supplemented groups. The plasma ALP content in the nano Zn group was markedly higher than in all other groups.

Table 5. Effect of organic and nano zincsupplementation on liver, kidney function and antioxidant status parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
ALT (IU/L)	22.24	21.73	21.52	22.10	0.119
AST (IU/L)	22.10	22.38	22.39	22.73	0.133
ALP (IU/L)	148.6^{b}	142.5^{a}	143.5^{ab}	149.3^{b}	0.838
Bilirubin (mg/dl)	0.98^{a}	0.89^{b}	0.97^{a}	0.88^{b}	0.015
Creatinine (mg/dl)	0.91	0.90	0.90	0.92	0.003
PUN (mg/dl)	9.40^{b}	9.34^{b}	9.23^{b}	8.97^{a}	0.072
SOD (µmol MTT formazan/mg Hb)	0.29^{a}	0.40^{b}	0.41^{b}	0.45^{b}	0.013
FRAP (µmol/L)	1192.3^{a}	1229.3^{a}	1298.1^{ab}	1370.3^{b}	17.534
TIg (mg/ml)	13.56^{a}	16.24^{b}	18.19^{c}	18.35^{c}	0.334

Values bearing different superscripts (a,b) differ significantly (P<0.01)

The observed difference between the treatment and control groups can be attributed to the reduced activity of ALP in the control group, rather than an increase in activity within the treatment groups. Song et al. (2021) and Dhruw (2017) observed significantly elevated serum ALP levels in nano zinc supplemented groups compared to the control group in goats. SOD, FRAP, and total plasma immunoglobulin concentrations increased across all groups over time, with treatment groups exhibiting higher levels than the control group, particularly in the nano group. The outcomes correlate with those of Kumar et al.(2021), Nagalakshmi et al. (2016; 2017), and Alimohamady et al. (2019). Zinc serves as an essential cofactor for copper/zinc superoxide dismutase (Cu/Zn-SOD) and additionally enhances its expression (Kumar et al. 2013). Zinc is crucial for both humoral and cell-mediated immune responses (Gruber et al., 2013). Nano zinc enhances the immune function in animals.

Table 6. Effect of organic and nano zinc supplementation on plasma mineral profile

Parameters	Treatment groups				SEM	P value		
	Control	T1	T2	T3		Treatment (T)	Period (P)	T×P
Ca (mg/dl)	8.64	9.01	9.16	8.94	0.196	0.56	0.89	0.99
P (mg/dl)	4.11	4.51	4.78	4.47	0.117	0.12	0.07	0.96
Fe (mg/L)	2.60	2.64	2.57	2.63	0.04	0.79	0.89	0.61
Cu (mg/L)	1.14	1.12	1.22	1.10	0.029	0.98	0.49	0.19
Zn (mg/L)	1.40^{a}	1.94^{b}	2.14^{b}	2.27^{b}	0.078	<0.001	0.63	0.03

Values bearing different superscripts (a,b) differ significantly (P<0.01)

Plasma levels of Ca, Fe, Cu, and P demonstrated that dietary supplementation in heifers had no significant impact (Table 6). Additional researches demonstrated that zinc supplementation did not significantly influence calcium and phosphorus levels (Mahima et al., 2015), nor iron and copper levels (Smerchek et al., 2023). Serum zinc concentrations were significantly elevated (P<0.05) in this trial following supplementation compared to the control group. The application of nZn has demonstrated superior outcomes relative to traditional Zn sources and exhibits less toxicity (Wang et al., 2006; Sahoo et al., 2014b). Uniyal et al. (2017) noted that serum zinc levels were considerably elevated in groups supplemented with nano-zinc compared to other zinc sources. Kala (2013) also observed a positive association between dietary nano Zn and blood Zn levels.

CONCLUSIONS

Nano Zn supplementation@20ppm and Zn pep @40ppm can be supplemented in heifer's diet for better performance without adversely affecting nutrient digestibility, blood profile and antioxidant status.

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Impact of Herbal Feed Supplements and Sodium Sulphate on the Nutrient Utilization and Growth Performance of Indigenous Dairy Cattle Calves

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ABSTRACT

The present study is aimed to evaluate the effects of herbal feed additives and sodium sulphate on nutrient utilization and growth performance in growing Sahiwal cattle calves. A total of twenty calves were randomly assigned to four groups: The control group (C) received no feed additives, while Group T1 was supplemented with herbal feed additives, *Foeniculum vulgare* seeds, and *Terminalia chebula* fruits, @ 1% of DMI (1:1 ratio both). Group T2 was provided with Na_2SO_4 at 0.5% of DMI, and Group T3 received both herbal feed additives and sodium sulfate at the same rates, respectively. The basal diet offered to experimental groups consists of 50% concentrate and 50% roughage. The experiment lasted 90 days; during which daily dry matter intake and fortnightly body weight changes were recorded. A digestion trial was conducted over six days at the end of the study to assess nutrient digestibility. The results indicated no significant differences in body weight, average daily gain, feed conversion ratio, or dry matter intake among the groups throughout the study. The initial and final body weights were similar across all groups, and the average daily gain remained consistent. Nutrient digestibility, assessed during the digestion trial, showed higher mean values for various nutrients in supplemented groups; however, these differences were not statistically significant. In conclusion, supplementing herbal feed additives and sodium sulphate did not significantly impact the calves' growth performance or nutrient digestibility.

KEYWORDS: Calves, Cattle, Growth, Herbal additives, Sodium sulphate.

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INTRODUCTION

Livestock rearing plays a pivotal role in rural areas of the country, significantly contributing to the national economy. It serves as a source of income for households dependent on agriculture and those without land ownership. Moreover, livestock provides essential proteins like milk, eggs, and meat. Most livestock populations thrive on low-quality roughage, such as crop wastes like wheat straw, which have lower digestibility due to lignocellulose.

In recent years, herbal feed additives in livestock nutrition have garnered significant attention due to their potential to enhance nutrient digestibility and growth performance. Traditional feed additives, while effective, often raise concerns regarding antibiotic resistance, environmental impact, and consumer health. As a sustainable alternative, herbal feed additives offer a promising solution to these challenges. Herbal feed additives are increasingly used in ruminant nutrition due to their numerous benefits over conventional chemical additives. A mixture of rumen modifiers (RM-7; neem seed cake, mahua seed cake, Fennel seed seed, harad, fruit pulp of bahera, fruit pulp of amla and ajwain seed) in 2:2:2:1:1:1:1 proportion at 5, 10, 15 and 20% with 0.06% sodium sulphate of substrate did not affect total gas production but there was significant reduction in methane production upto 10% level (Lakhani et al., 2019). Bakshi et al. (2022) also reported that herbal feed additive supplementation reduces methane emissions. Derived from natural plant sources, these additives are safer for animals and humans, as they do not leave harmful residues in animal products such as milk and meat. This reduces the risk of antibiotic resistance and other health issues associated with chemical additives. The active principles in herbal feed additives are plant secondary metabolites (PSMs) and plant bioactive compounds.

PSMs such as tannins, saponins, and essential oils significantly influence ruminant dry matter intake (DMI), nutrient digestibility, and growth performance as well as methane emission. These naturally occurring compounds can have varying effects on DMI, with some PSMs decreasing intake due to their bitter taste or astringent properties, making the feed less palatable for animals. Conversely, certain PSMs can enhance the palatability of feed, thereby increasing DMI (Ebrahim and Negussie, 2020). The complex role of PSMs in modifying feed intake underlines their dualistic nature, where the specific impact is contingent on the type and concentration of the metabolites. Among these, Fennel seeds (*Foeniculum vulgare*) and Harad (*Terminalia Chebula*) have shown varying results in improving nutrient digestibility, growth performance, and overall health in cattle.

When it comes to nutrient digestibility, PSMs primarily exert their effects within the rumen. Tannins, for instance, can bind to proteins and reduce their degradation, leading to increased microbial protein synthesis. However, high tannin levels can inhibit microbial activity, ultimately lowering the overall digestibility of nutrients (Kamra et al., 2012). This dichotomous impact highlights the complexity of tannins' role in ruminant nutrition. Recent research has provided valuable insights into the effects of dietary supplements containing PSMs on dry matter intake (DMI) and nutrient digestibility. Studies such as those by Moosavi-Zadeh et al. (2023) and Singh et al. (2023) demonstrate that supplements like fennel seed powder (FSP) can enhance feed intake across various livestock species, whereas other studies, like those by Gunun et al. (2022), suggest a limited impact on DM intake but potential effects on nutrient utilization at higher supplement levels.

Growth performance, another critical parameter, is also influenced by PSMs. Singh et al. (2023) observed significant improvements in body weight and average daily gain in goats supplemented with FSP. Similarly, Pawar et al. (2024) reported higher body weights in Kankrej calves supplemented with FSP, though not statistically significant. Conversely, Santos et al. (2022) found that higher levels of dietary quebracho extract in growing lambs reduced both DMI and average daily gain, emphasizing the dose-dependent effects of PSMs on growth performance.

Therefore, in the present study, a combination of herbal feed additive and sulfate was tested in an *in vivo* trial for their effect on nutrient utilization and the performance of calves.

MATERIALS AND METHODS

Ethics Approval

Animal care procedures were approved (approval number IAEC/24/1/58) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

Twenty growing Sahiwal cattle calves (16 male and 4 female calves) of about 9 to 16 months were selected from the herd maintained at Livestock Farm Complex (LFC), DUVASU Mathura (Uttar Pradesh), India. Calves were divided into four groups (5 animals each) in a randomized block design. The control group (Group C) received no feed additives. Group T1 was supplemented with herbal feed additives (*Foeniculum vulgare* seed and *Terminalia chebula* fruit) at 1% (1:1 ratio) of dry matter intake (DMI). Group T2 diet included sodium sulphate (Na_2SO_4) at 0.5% of DMI. Group T3 received both herbal feed additives at 1% of DMI and sodium sulphate at 0.5% of DMI. The basal diet contains 50% concentrate and 50% roughage. The composition of the diet and chemical composition of the diet is given in Tables 1 and 2, respectively. The experiment was continued for 90 days where all the calves were managed under similar conditions.

Table 1. Composition (% DM basis) of diet fed to calves during feeding trial

	Ingredients	Groups			
		C	T1	T2	T3
Concentrate	Oats	18	18	18	18
	Barley grain	15	15	15	15
	Wheat bran	18	18	18	18
	Gram chunni	15	15	15	15
	Mustard oil cake	32	32	32	32
	Mineral mixture*	2	2	2	2
Roughages	Jowar fodder	20	20	20	20
	Wheat straw	30	30	30	30

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate

The experimental calves were monitored daily for DMI and fortnightly for growth performance and feed efficiency measures. The feeds offered to the animals and the residue left was recorded daily to find out the total DMI of the experimental calves. Intake of DM was calculated as the difference between the amount of DM offered and the amount of DM residue left. The body weight of experimental animals was recorded at the start of the experiment followed by fortnight intervals.

Table 2. Chemical composition (% DM basis) of feeds and fodders fed during the experimental period

Attributes %	Concentrate	Jowar fodder	Wheat straw
Dry matter	91.50	17.50	91.10
Organic matter	92.10	88.80	86.70
Ether extract	4.50	4.10	1.20
Crude protein	20.68	10.20	3.53
Total ash	7.40	11.20	13.20
Nitrogen free extract	59.22	47.36	43.47
Crude fiber	13.7	32.5	40.23

To compare the efficiency of nutrient utilization in growing calves, a digestion trial for a period of 6 days was conducted at the end of the study. Calves were weighed before the start and at the end of the digestion trial. A weighted amount of feed and fodders was offered during the digestion trial. Representative samples of the feed offered and residue left was collected and analyzed for chemical composition. Faeces voided during 24 hours were collected and measured daily for 6 days. About 1% of thoroughly mixed total faecal matter (as such basis) was taken for chemical analysis. Additionally, for N estimation, approximately 0.1% of the total faecal sample was collected daily for 6 days and stored in glass containers having 25% sulphuric acid solution. The digestibility coefficient of nutrients was calculated from the nutrient intake and nutrient outgo in faeces during the digestion trial.

All statistical analyses were performed as per the standard method by using the SPSS computer package (SPSS Version 20.0, SPSS Inc, Chicago, USA). The data obtained were statistically analyzed by using one-way ANOVA procedures.

RESULTS AND DISCUSSIONS

Growth Performance

The effect of herbal feed additives and sulphate on the fortnightly BW change (Kg) and fortnight BW gain (Kg) of cattle calves is summarized in Tables 3 and 4, respectively. The initial BWs in the C, T1, T2, and T3 groups were 133.84 Kg, 128.48 Kg, 133.04 Kg, and 130.00 Kg, respectively. By the end of the study, the BWs in the C, T1, T2, and T3 groups were 166.12 Kg, 158.58 Kg, 162.84 Kg, and 160.68 Kg, respectively. The overall BW (kg) was found similar in all the experimental groups. Statistical analysis of data showed that variation between the groups for mean BW change was not significant (P>0.05). Whereas, the initial fortnight BW gains in the C, T1, T2, and T3 groups were 5.08 Kg, 4.68 Kg, 4.26 Kg, and 4.51 Kg, respectively. By the end of the feeding trial, the fortnightly BW gain for the C, T1, T2, and T3 groups were 6.65 Kg, 6.05 Kg, 6.32 Kg, and 6.18 Kg, respectively. Statistical analysis revealed no significant differences in the fortnightly BW gain among the groups (P>0.05). During the 1st fortnight, the DMI in the control, T1, T2, and T3 groups was 3.66 Kg/day, 3.59 Kg/day, 3.36 Kg/day, and 3.49 Kg/day, respectively, while, by the 6th fortnight, the DMI in the control, T1, T2, and T3 groups was 4.85 Kg/day, 4.58 Kg/day, 4.69 Kg/day, and 4.48 Kg/day, respectively (Table 5). Statistical analysis indicated no significant differences in fortnightly DMI among the groups (P>0.05), whereas the mean DMI per 100 Kg BW across all fortnights for these groups was 2.73, 2.86, 2.69, and 2.75 Kg per 100 Kg BW, respectively (P=0.096). All groups experienced slight fluctuations in their DMI per 100 Kg BW values, but these fluctuations were not statistically significant.

Table 3. Effect of herbal feed additive and sulphate on Fortnight BW change (Kg) of growing calves

Fortnight	Group				SEM	p Value
	C	T1	T2	T3		
Initial	134	128	133	130	7.22	0.994
1	139	133	137	135	7.35	0.994
2	144	138	142	140	7.52	0.994
3	149	143	147	144	7.76	0.994
4	155	148	152	150	7.92	0.993
5	159	153	157	155	8.11	0.993
6	166	159	163	161	8.37	0.992
Mean	149	143	147	145	2.99	0.888

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, p value >0.05: non-significant

Table 4. Effect of herbal feed additive and sulphate on fortnight BW gain (Kg) of growing calves

Fortnight	Group				SEM	p Value
	C	T1	T2	T3		
1	5.08	4.68	4.26	4.51	0.26	0.759
2	5.18	4.57	4.34	4.97	0.28	0.755
3	5.05	5.10	4.90	4.69	0.33	0.976
4	5.55	5.00	5.00	5.22	0.31	0.925
5	4.79	4.72	5.00	4.87	0.32	0.993
6	6.65	6.05	6.32	6.18	0.47	0.977
Mean	5.38	5.02	4.97	5.07	0.14	0.743

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, p value >0.05: non-significant

Table 5. Effect of herbal feed additive and sulphate on DMI (Kg) of growing calves

Fortnight	Group				SEM	p Value
	C	T1	T2	T3		
1	3.66	3.59	3.36	3.49	0.14	0.909
2	3.67	3.78	3.44	3.76	0.17	0.902
3	3.88	3.98	3.79	4.00	0.19	0.980
4	4.25	4.15	4.10	4.18	0.16	0.991
5	3.90	3.76	3.78	3.56	0.16	0.924
6	4.85	4.58	4.69	4.48	0.21	0.946
Mean	4.04	3.98	3.86	3.91	0.08	0.865

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, P>0.05: non-significant

The effect of herbal feed additives and sulphate on the ADG (Kg) and FCR of cattle calves is summarized in Tables 6 and 7, respectively. During the study, no significant (P>0.05) differences were observed in the ADG among the groups. In the 1st fortnight, the ADG in control, T1, T2, and T3 groups was 0.34 Kg/day, 0.31 Kg/day, 0.28 Kg/day, and 0.30 Kg/day, respectively, and by the 6th fortnight, the ADG for the control, T1, T2, and T3 groups was 0.44 Kg/day, 0.40 Kg/day, 0.42 Kg/day, and 0.41 Kg/day, respectively. The mean FCR across all fortnights for the control, T1, T2, and T3 groups was 12.05, 12.23, 12.01, and 12.21, respectively, with a P value of 0.989. The FCR values remained consistent across all dietary treatments, indicating no substantial impact from the supplements.

Table 6. Effect of herbal feed additive and sulphate on ADG (Kg) of growing calves

Fortnight	Group				SEM	p Value
	C	T1	T2	T3		
1	0.338	0.312	0.284	0.300	0.02	0.761
2	0.345	0.305	0.289	0.331	0.02	0.757
3	0.337	0.340	0.327	0.312	0.02	0.976
4	0.370	0.333	0.333	0.348	0.02	0.924
5	0.320	0.315	0.334	0.325	0.02	0.993
6	0.443	0.403	0.421	0.412	0.03	0.978
Mean	0.359	0.335	0.331	0.338	0.01	0.744

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, p value >0.05: non-significant

Table 7: Effect of herbal feed additive and sulphate on FCR of growing calves

Fortnight	Groups				SEM	p Value
	C	T1	T2	T3		
1	11.11	11.84	11.97	12.08	0.49	0.912
2	10.88	12.54	12.06	11.90	0.50	0.726
3	12.08	12.17	11.73	13.04	0.53	0.863
4	12.57	12.89	12.65	12.48	0.78	0.998
5	13.52	12.18	11.77	11.73	0.80	0.866
6	12.12	11.74	11.88	12.04	0.84	0.999
Mean	12.05	12.23	12.01	12.21	0.27	0.989

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, p value >0.05: non-significant

Similarly, Gunun et al. (2022) investigated the impact of *Terminalia chebula* meal (TCM) supplementation at different levels (0, 8, 16, and 24 g/kg of total DMI) on goats and reported that TCM did not significantly affect roughage, concentrate, and total DM intake ($p > 0.05$). In the same manner, Inamdar et al. (2015) also observed that supplementing mahua seed cake with or without harad seed pulp in buffaloes did not significantly affect DMI. Consistently, Uniyal et al. (2020) supplemented goats' diet with sulfur at levels of 0.08% and 0.16% of dry matter intake and observed no significant differences in DMI, BW gain, and FCR among the groups. The supplementation of amla fruit powder (*Emblica officinalis*) @ 0.75% enhanced the overall performance of broilers (Gaur et al., 2023). These findings support the results of the present study, indicating that the herbal feed additives and sulphate supplementation did not significantly influence DMI or growth performance in cattle calves. The non-significant differences in growth performance parameters in this study may be due to the lower dosages of herbal feed additives and sulphate used, which were likely insufficient to elicit

significant responses. Further research with varied dosages and combinations is needed for a better understanding of their potential effects.

Nutrient Utilization

The mean apparent nutrient digestibility across a 6-day digestion trial is shown in the Table 8. The ADG of the experimental calves during the digestion trial in the control, T1, T2, and T3 groups was 0.45, 0.48, 0.47, and 0.47 Kg/day, respectively. Although the mean digestibility of different nutrients DM, OM, CP, EE, CF, NDF and ADF was higher in the calves supplemented with various treatments, the results were not statistically significant (P>0.05) across the treatment groups. Similarly, Gunun et al. (2022) investigated the impact of *Terminalia chebula* meal (TCM) supplementation at different levels (0, 8, 16, and 24 g/kg of total DMI) on goats and found that TCM did not significantly affect digestibility. Likewise, Kumar et al. (2022) supplemented buffalo calves' diets with a mixture of eucalyptus and poplar leaf meal and found no significant impact on digestibility coefficients. Additionally, Inamdar et al. (2015) observed that supplementing mahua seed cake with or without harad seed pulp in buffaloes did not significantly affect nutrient digestibility. Moreover, Bostami et al. (2021) evaluated the effects of seeds and leaves from traditional medicinal plants (*Emblica officinalis, Terminalia bellirica,* and *Terminalia chebula*) feed additives on crossbred post-weaned bull calves. The treatments did not significantly affect the total tract digestibility of different nutrients. Consistently, Uniyal et al. (2020) reported no significant effect of sulfur supplementation on digestibility coefficients for DM, OM, CP, EE, NDF, and ADF in goats. These findings align with our study, indicating that the herbal feed additives and sulphate supplementation at present dose level did not significantly influence nutrient digestibility in cattle calves.

Table 8. Effect of herbal feed additive and sulphate on nutrient digestibility of growing calves

Attribute	Groups				SEM	P Value
	C	T1	T2	T3		
Initial B.Wt. (Kg)	165	161	160	160	8.57	0.997
Final B.Wt. (Kg)	168	164	163	163	8.62	0.998
ADG (Kg)	0.45	0.48	0.47	0.47	0.04	0.995
Nutrient Digestibility						
DM %	57.3	56.9	57.7	58.3	0.45	0.768
OM%	59.8	60.6	61.3	61.7	0.47	0.527
CP%	71.8	72.3	71.6	73.5	0.76	0.839
EE%	81.6	80.9	81.7	80.7	1.07	0.864
CF%	49.2	48.5	47.7	48.2	0.62	0.875
NDF%	53.1	51.3	50.6	52.3	0.60	0.502
ADF%	41.8	40.9	41.1	43.1	0.67	0.696

C: No feed additives, T1: Supplemented with herbal feed additives, T2: Supplemented with sulphate, T3: Supplemented with herbal feed additives and sulphate, p value >0.05: non-significant

CONCLUSION

In conclusion, the dietary supplementation of herbal feed additive (Fennel seed+*Terminalia chebula* in 1:1 ratio) @ 1% DMI) and sodium sulphate @ 0.5% of DMI has no significant (P>0.05) effect on BW, ADG, FCR, intake and digestibility of nutrients, indicating no impact of either herbal feed additive and sulfate supplementation on growth performance and nutrient digestibility. Further research with varied dosages is needed to better understand their potential effects.

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Influence of Dietary Supplementation of Peppermint (*Mentha piperita*) and Lemongrass (*Cymbopogon citratus*) Essential Oils on Health Biomarkers in Crossbred Calves

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ABSTRACT

This study evaluated the effects of dietary supplementation with Peppermint (*Mentha piperita*) essential oil (MPEO) and Lemongrass (*Cymbopogon citratus*) essential oil (CCEO) on the hematological profile, serum biochemical parameters, and blood antioxidant status in crossbred calves. Eighteen crossbred calves (Sahiwal × Holstein Friesian), aged between 15-90 days, were allocated into three groups: T0 (control), T1 (MPEO at 0.2% of calf starter), and T2 (CCEO at 0.2% of calf starter). The study was conducted over 90 days, with blood samples collected at monthly intervals: 0, 30th, 60th and 90th day for study of haemato-biochemical parameters while at 45th and 90th day for antioxidant parameters. Hematological parameters showed a significant ($P<0.05$) increase in total erythrocyte count (TEC) and packed cell volume (PCV) in the T2 group, while eosinophil counts were significantly ($P<0.05$) reduced in both T1 and T2 groups of calves. No significant treatment effects were observed on total leukocyte count (TLC), differential leukocyte counts, or platelet counts. Biochemical analysis revealed significant reductions in serum urea ($P<0.01$) levels and alanine aminotransferase ($P<0.05$) in T1 and T2 group of calves. Lipid profile results revealed significant ($P<0.05$) reductions in triglycerides, total cholesterol ($P<0.01$), LDL, and VLDL in *Cymbopogon citratus* essential oil group and an increase in HDL cholesterol in both *Mentha piperita* essential oil (MPEO) and *Cymbopogon citratus* essential oil groups. As regards antioxidant parameters, lipid peroxidase (LPO) was significantly ($P<0.05$) reduced, and superoxide dismutase (SOD) activity was significantly ($P<0.01$) increased in the *Cymbopogon citratus* essential oil group of calves. The results suggest that *Cymbopogon citratus* essential oil supplementation significantly improved hematological parameters, lipid metabolism, and antioxidant status, with potential benefits for calf health and performance.

KEYWORDS: Antioxidant status, Crossbred calves, Essential oils, Hemato-biochemical parameters

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INTRODUCTION

The growing global concern over antibiotic resistance has emerged as a critical public health threat. In response, regulatory authorities have imposed restriction on the use of antibiotics in livestock production systems (Coimbra et al., 2022). As a result, there is growing scientific interest in identifying natural alternatives to antibiotics, such as essential oils (EOs), prebiotics, and other phytobiotics (Nehme et al., 2021). Essential oils are plant-derived compounds, exhibit antimicrobial, antioxidant, and anti-inflammatory properties, are promising candidates for enhancing livestock health without contributing to the development of antimicrobial resistance (Wells, 2024).

Mentha piperita (peppermint) has been widely utilized in traditional herbal medicine due to its broad range of therapeutic properties. It is particularly noted for its immunomodulatory effects and its potential to help prevent secondary infections. The essential oil of *Mentha piperita* (MPEO), which is abundant in menthol and menthone, has been reported to support digestive health and positively influence various blood biochemical parameters (Brahmi et al., 2017).

Cymbopogon citratus, commonly known as lemongrass, is widely recognized for its medicinal value, primarily attributed to its rich array of health-promoting phytochemicals. These compounds contribute to improved gut health and enhanced nutrient absorption. Phenolic compounds in *Cymbopogon citratus* EO play a significant role in boosting antioxidant capacity by acting as effective reducing agents capable of neutralizing pro-oxidants, free radicals, and metal ions (Ali et al., 2021).

In light of the recognized health-promoting properties of *Mentha piperita* and *Cymbopogon citratus* essential oils, this study was conducted to evaluate the effects of dietary supplementation with *Mentha piperita* essential oil (MPEO) and *Cymbopogon citratus* essential oil (CCEO) on hematological profile, serum biochemical parameters, and blood antioxidant status in crossbred calves.

MATERIALS AND METHODS

The present study was carried out at the Instructional Dairy Farm, Nagla, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India.

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of G.B. Pant University of Agriculture and Technology, established in accordance with Article 13 of the CPCSEA regulations set forth by the Government of India (Registration No. 330/GO/Re/SL/01/CPCSEA, dated 03/01/2001).

Eighteen crossbred (Sahiwal × Holstein Friesian) calves, aged between two weeks and three months, were selected and randomly allocated into three treatment groups based on uniformity in age and body weight, with six calves in each group. The trial was conducted over a period of 90 days.

Prior to the initiation of the study, the animal shed was cleaned, disinfected and all calves were kept in well-ventilated pens to ensure optimal hygiene and comfort. All animals were fed a basal diet comprising milk and calf starter, adjusted according to individual age and body weight, while green fodder was provided ad libitum. Essential oils, obtained from Empirical Aromatics LLP, Gautam Budh Nagar, Uttar Pradesh were supplemented in the treatment groups with calf starter diet.

The treatment groups included: T0, which received only the basal diet (control); T1, which received the basal diet supplemented with *Mentha piperita* essential oil (MPEO) at 0.2% of the calf starter; and T2, which received the basal diet supplemented with *Cymbopogon citratus* essential oil (CCEO) at 0.2% of the calf starter.

Blood samples were collected at four time points during the experimental period: on day 0 (at the beginning of the study), and subsequently at monthly intervals on 30^{th}, 60^{th} and 90^{th} day for haemato-biochemical studies and on 45^{th} and 90^{th} day for study of antioxidant parameters.

Hematological parameters viz. hemoglobin (Hb) was measured using Sahli's method. Total erythrocyte count (TEC) and total leukocyte count (TLC) were assessed using the hemocytometer technique (Jain, 1986). Packed cell volume (PCV) was determined by the capillary tube method with a microhematocrit centrifuge. Differential leukocyte counts were performed manually through microscopic examination of stained blood smears. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulas as described by Coles (1986). Platelet counts were also determined using the hemocytometer method.

Serum biochemical parameters viz. glucose, triglycerides, urea, total protein, albumin, globulin, and liver enzymes i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using commercial diagnostic kits procured from ERBA Mannheim, in a UV-VIS spectrophotometer (Tietz, 1998).

Statistical analysis

The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS software version 21, following the methodology described by Snedecor and Cochran (1994). Where significant differences were observed, Duncan's multiple range test was employed for post hoc comparisons. Statistical significance was considered at the levels of $p< 0.05$ and $p< 0.01$.

RESULTS AND DISCUSSION

Feed intake and growth performance of calves

Supplementation with MPEO and CCEO at 0.2% did not significantly affect feed intake or dry matter intake (DMI) in crossbred calves (Table 1). These findings are consistent with Bhat et al. (2019), who

reported no significant effect of *Mentha piperita* on DMI in sheep. Similarly, Soares et al. (2023) and Del Valle et al. (2024) found no impact of CCEO supplementation on DMI or nutrient intake in *Bos indicus* Nellore steers and sheep, respectively. However, Liu et al. (2020) reported increased DMI in calves with essential oil supplementation.

Moreover, both MPEO and CCEO supplementation at 0.2% significantly increased body weight in calves compared to the control. This aligns with findings by Al-Azzawi and Rasheed (2021), who observed increased body weight in lambs supplemented with MPEO, and Okoruwa and Edoror (2021), who found similar effects with CCEO in goats. Tiwari et al. (2018) in broilers and Fahad and Al-Wazeer (2021) in lambs also reported positive effects of CCEO on body weight. Additionally, Orzuna-Orzuna et al. (2022) and Devi et al. (2018) documented weight increase in EO-supplemented calves and poultry respectively. Singh et al. (2018) also suggested improved rumen fermentation and feed efficiency in buffalo with lemongrass EO supplementation which supported the present findings. These suggest improved body weight may be attributed to enhanced feed efficiency and nutrient absorption.

Table 1. Effect of supplementation of *Mentha piperita, Cymbopogon citratus* EOs on feed intake and growth performance parameters of CB calves

Parameters	T0	T1	T2
FI (kg/d)	6.56±0.04	6.60±0.05	6.52±0.05
DMI (kg/d)	2.33±0.01	2.34±0.01	2.30±0.01
DMI (kg/100kg BW)	2.04±0.04	2.06±0.07	2.02±0.02
DMI (kg/$BW^{0.75}$)	0.065±0.001	0.066±0.001	0.064±0.001
Body weight (kg)*	53.63±2.13^{b}	55.92±2.41^{a}	56.61±2.56^{a}

Means bearing different superscripts vary significantly (*P<0.05)

Effect on hematology of calves

Table 2 depicted the results regarding hemoglobin concentration which indicated no significant treatment effect, although hemoglobin levels were observed to be higher, the difference was not statistically significant (P>0.05). These findings contrast with those of Witkowska et al. (2019) and Al-Janabi et al. (2023), who reported an increase in hemoglobin levels with supplementation of MPEO and CCEO, respectively. However, a significant (P<0.01) day effect was observed, with hemoglobin concentration showing a consistent increase from 0 to 90 day of the trial. This upward trend in hemoglobin levels may be attributed to the natural progression of the calves' growth. As calves age, their digestive systems mature, improving the absorption of essential nutrients like iron, which plays a critical role in hemoglobin production. In the early stages, calves primarily rely on colostrum and milk, which contain limited iron. As there is transition to solid food and their gut microbiome develops, iron absorption improves, leading to a steady rise in hemoglobin levels.

The study's findings demonstrated that there was significant (P<0.05) treatment effect on total erythrocyte count (TEC) with higher values in CCEO supplemented (T2) group. This result aligns with Al-Kassie (2010), who observed no significant change in erythrocyte count with peppermint extract supplementation, and with Al-Janabi et al. (2023), who reported a similar significant increase (Pd"0.05) with lemongrass supplementation in lambs. The observed increase in erythrocyte count in the CCEO group may be attributed to improved nutrient absorption and utilization, as suggested by Ademuyiwa and Grace (2015). CCEO is believed to enhance erythropoiesis by improving nutrient bioavailability, thus supporting the rise in total erythrocyte count. Essential oils from *Cymbopogon citratus are* rich in polyphenolic compounds, known for their antioxidant properties, which can reduce oxidative stress an important factor in erythrocyte production. Antioxidants lower lipid peroxide levels in erythrocyte membranes, enhancing membrane integrity and reducing susceptibility to hemolysis.

The treatment and day effect were non-significant for total leukocyte count (TLC), although the T2 group exhibited a higher TLC value, the difference was not statistically significant. These findings are consistent with Al-Kassie (2010), who also reported a non-significant impact of MPEO supplementation on leukocyte count. However, contrasting results have been documented; Al-Janabi et al. (2023) observed a significant reduction in white blood cell count with lemongrass supplementation in lambs, while Arab Ameri et al. (2015) reported a significant increase in TLC following MPEO supplementation. Such variations may be attributed to differences in species, dosage levels, or physiological conditions. The supplementation with MPEO and CCEO appears to support immune resilience without significantly altering leukocyte counts, suggesting a stabilizing effect on immune homeostasis.

This study revealed a significant ($P<0.05$) increase in packed cell volume (PCV) in the T2 group supplemented with CCEO at 0.2%. These findings align with Alagbe and Oluwafemi (2019), who reported a similar rise in PCV with CCEO supplementation. In contrast, Witkowska et al. (2019) observed increased PCV with MPEO instead. Essential oils like CCEO and MPEO are believed to support immune function by preserving cellular integrity, thereby enhancing nutrient absorption and promoting healthy blood cell formation (Arab Ameri et al., 2015). An elevated PCV indicates a higher proportion of red blood cells, improving oxygen transport and supporting growth, feed efficiency, and overall health in young animals. PCV can decline under stress due to protein denaturation and cell damage (Puvadolpirod and Thaxton, 2000).

The study showed that neutrophil percentage increased in the CCEO-supplemented group, but the difference was not statistically significant. This aligns with Rahman et al. (2021), who reported a similar non-significant effect with peppermint extract supplementation. Lymphocyte percentage also remained unaffected by treatment. These findings are supported by Rahman et al. (2021), Srivastava (2021), and Karageorgou et al. (2023), who observed no significant changes in lymphocyte counts with supplementation of MPEO, CCEO, or plant bioactive compounds. Monocyte levels were unaffected by supplementation, which is in agreement with Witkowska et al. (2019) and Al-Janabi et al. (2023),who found no significant changes with MPEO or lemongrass supplementation. There was non-significant day effect on neutrophil, lymphocyte and monocyte count.

A significant ($P<0.05$) reduction in eosinophil percentage was observed in both MPEO and CCEO groups compared to the control, consistent with Al-Janabi et al. (2023), who reported similar findings with lemongrass in lambs. This decrease may result from the anti-inflammatory and immunomodulatory properties of bioactive compounds like menthol and citral, which help reduce eosinophil activity by improving gut health and modulating immune responses (Nehme et al., 2021; Orzuna-Orzuna et al., 2022). The day and treatment*day effect were non-significant for eosinophil count.

Platelet counts remained stable across treatments revealed by non-significant treatment and day effect. This is supported by Patra et al. (2019) and Rahman et al. (2021), who reported no significant impact of menthol-rich compounds or peppermint extract. However, Palhares et al. (2021) noted a decrease in platelet count with essential oil blends in heifers. In healthy calves, where physiological balance is maintained, additional supplementation may have minimal effect on platelet levels.

The study revealed that supplementation with MPEO, and CCEO had no significant impact on MCV, MCH, and MCHC levels in crossbred calves. The day effect was also non-significant for the erythrocytic indices. These findings are consistent with Patra et al. (2019), who reported no significant effects of menthol-rich compound supplementation in growing sheep. Al-Janabi et al. (2023) also reported no significant differences in MCHC across treatment groups ($P\leq0.05$), suggesting that lemongrass had no measurable effect on these parameters.

Table 2. Effect of supplementation of *Mentha piperita,Cymbopogon citratus* EOs on hematological parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
Haemoglobin	9.92	10.13	10.16	0.09	0.12	0.00	0.50
TEC*	5.78^{b}	5.88^{ab}	5.93^{a}	0.04	0.04	0.38	1.00
TLC	9.64	9.91	10.30	0.21	0.09	0.08	0.83
PCV*	28.59^{b}	28.97^{ab}	29.59^{a}	0.26	0.03	0.36	0.93
Neutrophil	29.56	30.44	30.62	0.35	0.08	0.47	0.92
Lymphocyte	61.62	62.31	61.87	0.44	0.54	0.57	1.00
Eosinophil*	3.96^{a}	3.78^{b}	3.73^{b}	0.07	0.03	0.68	0.33
Monocytes	3.34	3.53	3.45	0.14	0.64	0.54	1.00
Platelets	3.67	3.71	3.61	0.09	0.74	0.99	1.00
MCV	49.25	49.33	49.94	0.53	0.60	0.88	1.00
MCH	17.16	17.20	17.24	0.17	0.94	0.31	1.00
MCHC	34.70	34.91	34.44	0.35	0.65	0.10	0.99

Means bearing different superscripts vary significantly (*P<0.05, **P<0.01)

Effect on biochemical parameters

The effect of supplementation of MPEO and CCEO on serum biochemical parameters of crossbred calves have been presented in Table 3. The results indicated that supplementing calves' diets with 0.2% MPEO and CCEO did not lead to significant changes in serum glucose levels throughout the duration of the study. These findings align with those of Coelho et al. (2023) in calves and Patra et al. (2023) in lambs, who also found no significant impact of peppermint on glucose levels. Al-Janabi et al. (2023) reported comparable results, noting non-significant alterations in glucose levels with lemongrass supplementation in small ruminants.

The treatment had no significant effect on serum total protein levels, consistent with findings by Chiofalo et al. (2012) and Dorantes-Iturbide et al. (2022) who also observed similar results in sheep. Similarly, supplementation with MPEO, CCEO and MOS did not significantly influence serum albumin levels, in line with Bhat et al. (2019) and Al-Janabi et al. (2023). Garg et al. (2025) observed similar non-significant effect on calves with herbal supplements. The lack of change may reflect the stable nature of albumin, regulated by liver function and nutritional status. CCEO supplementation showed a non-significant increase in globulin levels, consistent with Arab Ameri et al. (2015) and Khalifah et al. (2021), who reported similar trends with MPEO and a dose-dependent effect of CCEO. No significant effect was observed on the albumin-to-globulin (A:G) ratio, aligning with Akbari et al. (2016) and Khattab et al. (2017).

The present study revealed that supplementation with CCEO at 0.2% (T2 group) resulted in the lowest serum triglyceride and total cholesterol levels, consistent with Kumar et al. (2011), Alagawany et al. (2021), and Al-Janabi et al. (2023), who reported similar reductions with lemongrass supplementation. No significant effect on lipid profiles was noted with *Mentha piperita* or menthol-rich supplements, as observed by Hosoda et al. (2005) and Patra et al. (2019). A significant (P<0.05) increase in HDL-cholesterol levels was observed in both MPEO and CCEO-supplemented groups, possibly due to the bioactive compounds such as flavonoids and alkaloids influencing lipoprotein metabolism, as noted by Donnelly et al. (2005). Additionally, CCEO at 0.2% significantly reduced LDL and VLDL levels, supporting findings by Alagawany et al. (2021).

The reduction in triglycerides and cholesterol with CCEO may be due to bioactive compounds like citral and geraniol, which inhibit HMG-CoA reductase and lipase activity. Its antioxidant and hormonal effects may further support lipid stability. The increase in HDL and reduction in LDL suggest improved lipid balance, consistent with Kris-Etherton and Yu (1997), and may be partly attributed to PUFAs, known to modulate lipids (Grundy and Denke, 1990).

The results showed a significant ($P<0.01$) reduction in serum urea levels in both MPEO and CCEO-supplemented groups compared to the control, aligning with findings by Ghanima et al. (2021), El-Essawy et al. (2021), Orzuna-Orzuna et al. (2022), and Dorantes-Iturbide et al. (2022). This reduction may reflect improved nitrogen utilization due to enhanced gut health and protein digestion.

The treatment had no significant effect on AST levels, though a numerical reduction was noted in MPEO (T1) and CCEO (T2) groups, consistent with Rekiel et al. (2007). A significant reduction in ALT levels was observed with both EOs, indicating improved liver function, aligning with Uchida et al. (2017), AL-Azzami and Mohammed (2023), Al-Janabi et al. (2023), and Rahman et al. (2024). This hepatoprotective effect is likely due to antioxidant compounds like citral, menthol, and neral. In contrast, Hosoda et al. (2005) reported no significant changes, suggesting variability based on dosage or animal factors. Overall, the decrease in ALT with EOs supplementation suggests a hepatoprotective effect likely driven by antioxidant-mediated protection of liver cells.

Table 3. Effect of supplementation of *Menthapiperita,Cymbopogoncitratus* EOs on serum biochemical parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
Glucose	64.25	65.96	65.37	1.23	0.61	0.19	0.96
Total protein	7.71	8.16	8.39	0.38	0.45	0.98	0.99
Albumin	4.33	4.58	4.36	0.15	0.43	0.28	0.48
Globulin	4.22	4.36	4.54	0.09	0.06	0.65	0.88
AG ratio	1.03	1.06	0.97	0.04	0.23	0.43	0.59
Urea**	23.39^{a}	21.80^{b}	21.11^{b}	0.45	0.00	0.98	0.95
AST	90.61	87.37	86.85	1.62	0.21	0.44	0.15
ALT*	31.30^{a}	29.89^{b}	29.83^{b}	0.45	0.04	0.56	0.69
Triglyceride*	30.83^{a}	30.61^{a}	29.63^{b}	0.32	0.03	0.91	0.98
Total	82.41^{a}	81.94^{a}	80.69^{b}	0.24	0.00	0.64	0.33
HDL*	41.78^{b}	42.27^{a}	42.44^{a}	0.16	0.01	0.18	0.97
LDL*	33.71^{a}	33.54^{a}	32.70^{b}	0.28	0.04	0.98	0.96
VLDL*	6.16^{a}	6.12^{a}	5.93^{b}	0.06	0.03	0.91	0.98

Means bearing different superscripts vary significantly (*$P<0.05$, **$P<0.01$)

Effect on antioxidant parameters

The present study showed a significant ($P<0.05$) reduction in lipid peroxidase (LPO) levels with CCEO supplementation at 0.2%, indicating its effectiveness in alleviating oxidative stress in crossbred calves (Table 4). This aligns with findings by Ojo et al. (2006) and Franz et al. (2010). Strong antioxidant properties of *Cymbopogon citratus* essential oil is due to its high polyphenol and flavonoid content which scavenge free radicals and inhibit lipid peroxidation, thereby lower lipid peroxidase levels,

key marker of oxidative damage. Similar antioxidant effects were noted in studies by Bharti et al. (2013), Su et al. (2018), and Zeeshan et al. (2023), further supporting the present results. The reduction in LPO levels observed in CCEO group is likely due to the radical-scavenging action of the phytochemicals, which effectively neutralize reactive oxygen species (ROS) and enhance oxidative stability.

The study showed a significant (P<0.01) increase in superoxide dismutase (SOD) activity in crossbred calves supplemented with 0.2% CCEO, indicating enhanced antioxidant defense. This aligns with findings by Su et al. (2018) and Zeeshan et al. (2023), who also reported elevated SOD levels with antioxidant supplementation. The improvement is attributed to the presence of polyphenols and flavonoids in lemongrass oil, which effectively scavenge free radicals and reduce oxidative stress, as noted by Ojo et al. (2006) and Franz et al. (2010). The elevated SOD activity reflects the efficacy of CCEO in strengthening the oxidative defense system and promoting cellular health in calves.

Table 4. Effect of supplementation of *Mentha piperita, Cymbopogon citratus* EOs on antioxidant parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
LPO*	11.17^{a}	10.58^{ab}	9.45^{b}	0.44	0.04	0.19	0.89
SOD**	21.33^{b}	22.35^{b}	27.16^{a}	1.03	0.00	0.11	0.95

Means bearing different superscripts vary significantly (*P<0.05, **P<0.01)

Cost evaluation

Calf starter, priced at ¹ 30/kg, was supplemented with MPEO (¹ 1350/kg) and CCEO (¹ 1200/kg) in the treatment groups (Table 5). Despite the higher cost, supplementation led to additional body weight gains of approximately 2.5 kg with MPEO and 3.5 kg with CCEO, along with improved hematological and antioxidant profiles in calves.

Table 5. Effect of supplementation of *Mentha piperita, Cymbopogon citratus* EOs on cost of feed of CB calves

Parameters	Amount (kg)/calf	Cost (□)/calf
Calf starter @ (□ 30/kg)	55.10	1653.00
Calf starter + MPEO @ (1350 □/kg)	55.10 + 0.11	1801.50
Calf starter + CCEO @ (1200 □/kg)	55.10 + 0.11	1785.00

CONCLUSION

The supplementation of CCEO at 0.2% improved hematological health and possible anti-inflammatory action. In terms of lipid profile CCEO contributed to elevated HDL levels and reduced triglycerides, total cholesterol, LDL and VLDL reflecting a hypolipidemic effect. Enhanced antioxidant defense was also evident through significantly lower lipid peroxidation (LPO) levels and increased superoxide dismutase (SOD) activity by 0.2% CCEO. Therefore, CCEO supplementation at 0.2%, can improve physiological resilience and metabolic health in calves, supporting its use as effective natural feed additive.

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A Field Perspective on Supplementation of Specific Critical Minerals in Crossbred Cattle with Reproductive Disorders

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ABSTRACT

A field experiment was conducted in two adopted villages of Chittoor district of Andhra Pradesh to study the effect of supplementation of deficient minerals in crossbred cattle with reproductive inefficiency. Crossbred cattle (40) with different reproductive problems were selected from two adopted villages. The Area Specific Mineral Mixture (ASMM) prepared by the department of Animal Nutrition, SVVU, Tirupati, having specific deficient minerals (Ca, P, Cu and Zn) was supplemented (40g) to selected animals for 90 days. Blood samples were collected from animals before and after supplementation of ASMM and analysed for different minerals (Ca, P, Cu and Zn) and hormones (estrogen and progesterone). Plasma Ca content of repeat breeding cows (8.03 Vs 9.45 mg %) and anoestrous heifers (7.68 Vs 8.86 mg %) indicated significant ($P < 0.05$) improvement. The plasma P (4.10 Vs 5.49 mg %) and Cu (0.52 Vs 0.88 ppm) content in anoestrous heifers significantly ($P < 0.05$) elevated. While Zn content (ppm) in plasma improved significantly ($P < 0.05$) in anoestrous cows, repeat breeder cows as well as anoestrous heifers. The plasma estrogen content of repeat breeding cows (12.68 Vs 16.42 pg/ml) and anoestrous heifers (13.38 Vs 17.19 pg/ml) as well as progesterone content of repeat breeding cows (1.68 Vs 2.31 ng/ml) and anoestrous heifers (0.88 Vs 1.73 ng/ml) elevated ($P < 0.05$) in crossbred animals. Among the animals under study, 61.5% of anoestrous cows, 62.5% of repeat breeder cows and 63.6% of anoestrous heifers responded to supplementation. It can be concluded that, supplementation of specific deficient minerals in the form of ASMM resulted in improved mineral status and hormonal profile of animals thereby improving the reproductive efficiency (62.5%) in crossbred cattle.

KEYWORDS: Area specific mineral mixture, Crossbred cattle, Hormones, Plasma minerals.

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INTRODUCTION

Mineral imbalances are often observed in large number of Indian livestock because, they are mainly maintained on crop residues based rations or grazing without access to mineral supplement (Mc Dowell et al., 1993; Sahooo et al., 2017). Complication of mineral deficiency and metabolic diseases in all categories of dairy livestock have been reported by many scientists due to lower content and low bioavailability of some essential mineral in different feedstuffs. More than 90 percent of mineral deficiencies exist at subclinical level in livestock (Underwood and Suttle, 1999). Normal production and reproductive behavior of animals is associated with hormonal and nutritional status of the animal. Dietary mineral deficiency results in failure of the mineral homeostasis mechanism affecting the productive and reproductive potential of the animal. Some minerals act directly on the gonads, while others act through hypophyseal-pituitary-gonadal axis (Prasad et al., 2007). Deficiency or imbalance of single or multiple minerals results in enzymatic dysfunction and hormonal imbalance associated with fertility of animals (Maurice, 2003), resulting in poor productive performance (Sahooo et al., 2017). In field condition repeat breeding is a major problem in dairy animals (Rohit et al., 2017), results in economic loss (Kavani et al., 2005). Garg and Bhandari. (2005) reported low animal productivity and impaired reproductive behavior due to mineral deficiency and can be combated by supplementation of specific deficient minerals in the ration (Pal et al., 2020).

Present experiment was conducted to study the effect of supplementation of specific deficient minerals in the form of area specific mineral mixture (ASMM) in crossbred cattle showing reproductive disorders under field conditions in Chittoor district of Andhra Pradesh State.

MATERIALS AND METHODS

A field study was conducted in Chittoor district of Andhra Pradesh State, which is situated between 12-37" to 14-8" of Northern latitude and 78-33" to 79-55" of Eastern longitude. Maximum temperature ranges from 36º to 38ºC and in eastern parts it touches 46ºC. Minimum temperature in western parts varies from 12º to 14ºC and in eastern parts it is 16º to 18ºC with average rainfall of 918.1mm. Two villages (viz. village-1 –Pudipatla and village-2 -N. V. Palle) with similar animal husbandry practices were selected from two divisions in Chittoor district for conducting the study (during December to March). A preliminary study was conducted in these villages regarding feeding regimen, mineral status of the feedstuffs and serum mineral profile of animals in order to identify the prevailing mineral deficiencies. Then mineral mixture was prepared with specific minerals and supplemented to the selected crossbred cattle with deficiencies.

Feeding regimen

The crossbred cattle were maintained under semi-intensive feeding system with limited grazing resources, supplemented with paddy straw (*Oryza sativa*), groundnut straw (*Arachis hypogaea*) and sugarcane (*Saccharum officinarum*) tops either alone or in combination. Rice bran (*Oryza sativa*) and groundnut (*Arachis hypogaea* L) cake were common concentrate feeds offered. However, supplementation of concentrates and mineral mixture could be hardly found in case of unproductive animals.

Feeds and Fodders

Samples of green fodder, dry roughages, and individual concentrate ingredients actually fed to the cross bred cattle were collected from all the respondents in the study area. The representative samples of feeds/fodder collected from different farmers, were dried at 80^0C for 24 hours in forced draft oven and subsequently ground to 1mm sieve and were stored in moisture free plastic bags for further analysis.

The feed and fodder samples (0.5g) were digested in microwave sample digester (CEM Mars X-press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through Whatman filter paper no. 1. Macro minerals, Calcium, Sodium, Potassium and magnesium as well as micro minerals Copper, Zinc, Manganese, and Iron were estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta-PM-A-6287). In case of Ca and Mg, the samples were diluted with 0.1% lanthanum chloride before estimation. Phosphorus and proximate principles were analyzed by AOAC (1995) procedures.

Animals

Twenty crossbred cattle (JBX and HFX) with different reproductive disorders, based on the history (with parity of 3-5, milk production of 6-8 L/day in previous lactation and without any incidence of metabolic disorders) and rectal palpation were selected from each village. The crossbred cows that were not showing any signs of estrous since last six months after calving, did not exhibit estrus, had no palpable corpus luteum or follicle of 10 mm diameter on ovarian surface and < 1 ng progesterone level in plasma were termed as anoestrous cows. Cows that were not conceived after three inseminations were considered as repeat breeders. Heifers which did not come to estrus even once after attaining the age of two years or more/did not obtain the specified body weight at >2 years of age were considered as anestrous heifers. The animals having clear watery secretion, with no anatomical defect in reproductive organs, confirmed through normal calving history and per-rectal examination. These crossbred cattle were having deficiency of one or more minerals as exhibited from the level of minerals in their plasma of these animals analysed prior to the start of study.

Feeding regimen

Farmers in this region were having sparse grazing resources hence semi-intensive feeding system is being adopted. The animals after grazing were supplemented with paddy straw, groundnut straw and sugarcane tops either one or in combination. Rice bran and groundnut cake were the common concentrate feeds offered. However supplementation of concentrates could be hardly found in case of unproductive animals. There was no practice of mineral supplementation.

Mineral mixture with specific minerals

The specific mineral mixture containing di-calcium phosphate 22g, calcium carbonate 6.84g, zinc sulphate 0.72g, copper sulphate 0.44g and common salt 10.g, was prepared based on the deficiencies identified in the feed samples and plasma of animals. The mineral mixture was supplemented at the rate of 40g / animal / day along with concentrates (rice bran and GNC mixture), for a period of 90 days. Selection of supplemental minerals was based on incidence of deficiency prevailing in the animals under investigation (Devasena, 2008).

Collection of blood samples

Blood samples from each animal were collected, before supplementation and at 15 day interval after supplementation of ASMM during 90 days. Blood samples collected by puncturing the jugular vein in heparinized vials aseptically, centrifuged at 3000 rpm for 20 minutes and the plasma was stored at -20^{0}C for subsequent analysis.

Mineral analysis

Plasma samples (2 ml) were digested in microwave sample digester (CEM Mars X press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through Whatman filter paper no-1. Ca, Cu, Zn, Mn, and Fe were estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta- PM-A-6287). Phosphorus was analysed as per the method of Fiske and Subba Rao (1925).

Hormonal analysis

Estradiol 17 - â and Progesterone content in plasma samples were estimated by using kits. Radio immune assay kits were procured from "Immuno Tech", France (antibody coated tubes along with tracer, calibrators and other required material were provided for estimation of the hormones). Plasma samples were processed as per the prescribed procedure specified in the kits. Processed samples were counted in a multi well Gamma Counter (Cobra II, Packard Gamma Counter, USA) at National Institute of Animal Nutrition and Physiology (NIANP), Bangalore.

Table 1. Macro and micro mineral profile of feedstuffs offered to animals

Feed Stuff	Macro minerals (%)					Micro minerals (mg/Kg)			
	Ca	P	Na	K	Mg	Cu	Zn	Mn	Fe
Critical Value[1]	<0.3	<0.25	<0.06	<0.8	<0.2	<8.0	<30.0	<40.0	<50.0
Paddy straw	0.25±0.06	0.20±0.02	0.09±0.00	0.96±0.02	0.43±0.05	7.1±0.60	25.2±2.10	150±8.6	414±6.3
Groundnut straw	0.89±0.02	0.31±0.01	0.09±0.00	0.89±0.05	0.94±0.03	5.2±0.40	16.0±0.50	68.0±0.80	537±5.3
Sugar Cane tops	0.24±0.02	0.09±0.03	0.09±0.00	1.07±0.04	0.44±0.02	2.2±0.30	19.2±2.6	47.8±2.4	118±5.1
Local grass	0.27±0.02	0.28±0.02	0.16±0.00	0.98±0.03	0.71±0.01	6.1±0.1	43.6±1.3	43.4±0.9	355±2.1
Groundnut Cake	0.30±0.03	0.71±0.01	0.12±0.00	0.83±0.03	0.55±0.02	14.8±0.40	44.9±6.70	51.4±5.8	236±8.5
Rice bran	0.24±0.04	1.23±0.06	0.10±0.00	1.25±0.06	0.56±0.02	4.22±0.30	24.1±8.3	75.8±6.9	263±38.9

[1] McDowell et al., (1993).

Reproductive performance

The crossbred cattle under experiment were examined throughout the study period (90 days of supplementation) as well as three months after insemination for changes in body condition and reproductive status. The observations on general body condition, exhibition of estrous symptoms and date of insemination and pregnancy confirmation by per rectal palpation were recorded individually.

Statistical analysis

The data were analysed by paired't' test, as per the standard statistical procedures (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Preliminary study

A preliminary study was conducted in the elected villages regarding feeding regimen, mineral status of the feedstuffs and serum mineral profile of animals to identify the deficiencies. Then mineral mixture was prepared with specific minerals and supplemented to the selected crossbred cattle with deficiencies.

Feeds and Fodders

Macro minerals Ca and P concentration in feedstuffs fed in the area under study indicated deficiency (Table 1) except the groundnut cake for calcium and rice bran for phosphorus. Whereas Na, K and Mg concentrations in the feeds were found to be more than the critical levels as indicated. Among the micro minerals, Cu concentration was below the critical level (<8.0 ppm) in both roughages and concentrates. Whereas Zn was below the critical level (<30.0 ppm) in roughages, but found to be higher in concentrate feeds except ground nut cake. The Mn concentration was more than the critical level (<40.0 ppm) in all feeds except in rice bran. It was observed that Fe concentration was exceptionally high (236 to 537 ppm), in all feedstuffs compared to its critical level (<50.0ppm). The results are in accordance with the reports of Tiwary et al. (2007). Most of the feed ingredients available for feeding livestock are deficient in one or other mineral (Gupta et al., 2017).

A survey work in various states, conducted by NDDB indicated that Zn, Cu, S, Mn, and Co were deficient in the ration of dairy livestock (Bhanderi et al., 2006).

Table 2. Plasma mineral profile of crossbred cattle

	Macro Minerals (mg/100 ml)					Micro Minerals (ppm)			
	Ca	P	Na	K	Mg	Cu	Zn	Mn	Fe
Critical Value[1]	<8.0[1]	<4.5[1]	-	-	<1.0[1]	<0.65[1]	<0.8[1]	<0.02[1]	<0.1[1]
Mean	8.25±	4.68±	162±	23.6±	1.99±	0.59±	0.68±	0.12±	1.59±
	0.49	0.52	46	6.3	0.39	0.04	0.04	0.04	0.36
% animals showing deficiency	57	53	-	-	-	69	62	39	-

[1]McDowell, (1985)

The results presented in table 2 indicated that, crossbred cattle in the area under study were showing deficiency regarding Ca (57%), P (53%), Cu (69%) and Zn (62%). Whereas the K, Na, Mg, Mn and Fe profiles of serum were well above the critical levels.

Supplementation study

Based on the mineral concentration in feeds and fodders and serum profile of the crossbred cattle reared by the farmers in the selected area the deficiencies were identified. Accordingly mineral mixture was prepared, which was supplemented to the crossbred cattle with reproductive disorders selected for a period of 90 days. During this period the animals were continuously under close examination both physically and serologically and the data related was recorded.

General condition of animals

The improvement in general health and reproductive efficiency of animals under study during the supplementation period (90 days) was regularly monitored and recorded at every fortnight. Among the animals supplemented with area specific mineral

mixture, 45% showed improvement in feed intake within 30 days. Improvement in general health condition, glossy skin, improved body score were observed after 45days and appearance of estrous symptoms started after 45 to 60 days of supplementation in 55-60% of animals. Phosphorus is associated with energy metabolism and trace elements like Cu and Zn act as co-factors, activates enzymes and influence biochemical functions, resulting in improved appetite and digestibility, thereby improving the general body condition. The present results confirm the reports of Prasad et al. (2007) regarding improvement in general health condition of the cows after area specific mineral mixture supplementation.

Table 3. Plasma mineral profile of animals with reproductive disorders supplemented area specific mineral mixture

Condition of animal	Macro Minerals (%)				Micro Minerals (ppm)			
	Ca		P		Cu		Zn	
Critical value	<8.0		<4.5		<0.65		<0.8	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Anoestrous cows	8.63	8.98	5.03	5.86	0.66	0.78	0.74^{b}	1.06^{a}
(n =13) *	±0.26	±0.42	±0.32	±0.20	±0.04	±0.18	±0.19	±0.21
Repeat breeding cows	8.03	9.45^{a}	4.88	5.23	0.73	0.83	0.78^{b}	0.91^{a}
(n =16)*	±0.36	±0.28	±0.23	±0.28	±0.11	±0.26	±0.11	±0.20
Anoestrous heifers	7.68^{b}	8.86^{a}	4.10^{b}	5.49^{a}	0.52^{b}	0.88^{a}	0.61^{b}	0.82^{a}
(n=11) *	±0.19	±0.26	±0.33	±0.36	±0.22	±0.31	±0.23	±0.19

*Means bearing different superscripts in a row differs significantly ($P < 0.05$)

Macro mineral status

Supplementation of mineral mixture improved ($P< 0.05$) plasma Ca concentration (mg of repeat breeding cows (8.03 Vs 9.45 mg %) and anoestrous heifers (7.68 Vs 8.86 mg %). Plasma P (mg %) showed significant ($P<0.05$) increase after supplementation in anoestrous heifers (4.10 Vs 5.49 mg %), but this improvement was non significant in anoestrous and repeat breeding cows (Table 3). Reduced fertility and reduced or delayed conceptions are the prime signs of phosphorus deficiency and this can be overcome with proper phosphorus supplementation whereas, moderate deficiency may lead to repeat breeding condition and poor conception rate (Rohit et al., 2017). Altered Ca and P ratio has a blocking action on the pituitary and consequently on gonadal function affecting reproductive efficiency (Maurice, 2003). Supplementation of ASMM increased plasma Ca and P levels, which are involved in maintaining normal metabolic process and normal reproductive physiology (Mc Dowell et al., 1993), in turn resulted in improvement of reproductive performance as evidenced in the present study. Similar trend was observed by Samanta et al. (2005), Devasena et al., 2010; Pal et al., 2020.

Micro mineral status

The plasma Cu was influenced by mineral supplementation as indicated by elevation ($P<0.05$) in Cu content of plasma (0.52 Vs 0.88 ppm) in the anoestrous heifers group under study. Similar to the present investigation, Devasena et al. (2010) and Samanta et al. (2005) also reported improvement in plasma trace mineral levels in anoestrous animals after their supplementation. Cu has a significant role in maintaining the optimum fertility as Cu behaves in a regular way to be used as an indicator for FSH, LH and estrogen activity (Desai et al., 1982). Copper deficiency might have effect on reproduction probably through an interaction between Cu and estrogen (Hidiroglou, 1979). Role of Cu in ovarian steroidogenesis through Cu - super oxide dismutase

enzyme activity was reported by Olson et al. (1999), as evidenced by improved reproductive performance in the present study.

While Zn content (ppm) in plasma improved significantly ($P< 0.05$) in all the three categories viz. anoestrous cows, repeat breeder cows as well as anoestrous heifers. Samanta et al. (2005) also reported improvement in plasma Zn levels in anoestrous animals after supplementation. A reduction of Zn level might interfere with prostaglandins receptor medicated phase and cause quaintly the luteolytic process which in turn causes some of the reproductive pathology (Carlson et al., 1982). Deficiency of Zn affects all phases of reproductive processes from estrous to parturition and lactation (Mc Dowell et al., 1993). Optimum serum level of Zn is essential to maintain the activity of FSH and LH (Apagar, 1985), prostaglandins bind Zn and facilitate its transport and enzymatic action (super oxide dismutase) involving reproductive functions was reported by Olsen et al. (1999) as indicated by the present results.

Table 4. Plasma hormonal profile of animals with different reproductive disorders supplemented with area specific mineral mixture

Condition of animal	Estradiol -17 β (pg/ml)		Progesterone (ng/ml)	
	Initial	Final	Initial	Final
Anoestrous cows(n = 13)	15.32±1.2	17.68±2.6	1.03±0.24	1.73±0.25
Repeat breeding cows (n =16)	12.68^{b}±2.1	16.42^{a}±2.8	1.68^{b}±0.18	2.31^{a}±0.20
Anoestrous heifers (n = 11) *	13.38^{b}±1.9	17.19^{a}±1.3	0.88^{b}±0.13	1.73^{a}±0.18

*Means bearing different superscripts in a row differs significantly ($P < 0.05$)

Hormones

Plasma hormonal profile of crossbred cattle with different reproductive disorders, supplemented specific deficient minerals has been presented in table 4. There was an increase ($P<0.05$) in plasma estradiol (pg/ml) content in repeat breeding cows (12.68 Vs 16.42 pg/ml) and anoestrous heifers (13.38 Vs 17.19 pg/ml). Whereas progesterone content (ng/ml) of progesterone content of repeat breeding cows (1.68 Vs 2.31 ng/ml) and anoestrous heifers (0.88 Vs 1.73 ng/ml) indicated significant ($P < 0.05$) elevation in plasma of the crossbred animals under study. Present observations were similar to the reported values of Prasad et al. (1989) in repeat breeding and anoestrous cows, observed during different stages of estrous cycle. While Sampath et al. (2006) reported slightly higher values of estradiol and progesterone in anoestrous cows, repeat breeding cows and anoestrous heifers, as compared to the present study. The trend observed in the present study indicated that, because of the improvement in estrous and conception rate, the profile of both the hormones increased after supplementation, as compared to prior to supplementation.

Table 5. Reproductive performance of Animals with different reproductive disorders supplemented area specific mineral mixture

Reproductive disorder	Total (n=40)	%
Anoestrous cows	8/13	61.5
Repeat breeder cows	10/16	62.5
Anoestrous heifers	7/11	63.6
Total responded	25/40	62.5
Not responded	15/40	37.5

n = number of animals

Reproductive performance

The anoestrous cows (61.5%), of repeat breeding cows (62.5%) and anoestrous heifers (63.6%) improved in terms of their reproductive performance and on an average 62.5% animals under study responded to supplementation of specific deficient minerals (Table 5). The results are in accordance with the reported results of Sahoo et al. (2017) who reported 64% conception rate with sign of first heat occurred earlier (16.3 days), post partum estrus time reduction (15.8 days), service period reduced (15.8 days) due to supplementation of mineral mixture in crossbred cattle. Mineral mixture supplementation resulted in improved reproductive efficiency in terms of shorter first post-partum estrus and higher conception rate (Gupta et al., 2017; Jadoun, et al., 2023 and Pal et al., 2020). Signs of phosphorus deficiency and this can be overcome with proper phosphorus supplementation whereas, moderate deficiency may lead to repeat breeding condition and poor conception rate. Feeding of mineral mixture could improve their reproductive cyclicity with mark display of estrus symptoms (Devasena et al., 2010 and Rohit et al., 2017).

CONCLUSIONS

It can be concluded that, supplementation of specific deficient minerals in the form of area specific mineral mixture can improve reproductive efficiency in crossbred cattle and there by economic condition of the farmers. Long term studies are required to work out the cost benefit ratio in terms of improved reproduction and subsequent improvement in milk production.

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Effect of Inorganic and Nano Selenium Supplementation on Growth Performance and Nutrient Utilization in Growing Hariana Heifers

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ABSTRACT

The present study was conducted to see the effect of inorganic selenium (ISe) and nano selenium (NSe) supplementation on growth performance and nutrient utilization in Hariana heifers. A total of 18 Hariana heifers were selected and allocated into three groups having six heifers in each group and fed treatment diet for 90 days. In present study, control (Con) group was not supplemented with any extra amount of Se other than present in the basal diet, T1 group was supplemented with inorganic Se @ 0.3 mg/kg of dry matter(DM) offered, while T2 group was supplemented with nano Se @ 0.3 mg/kg of DM offered. Basal diet offered to experimental groups containing 50% concentrate, 35% green jowar fodder and 15% wheat straw. DM was offered to all experimental group at about 3.5% of the body weight of animals. All groups of animals were fed with basal diet having same levels of nutrients. Body weight (BW) and dry matter intake (DMI) were recorded fortnightly. DMI (kg/day), DMI (kg/100kg BW),total digestible nutrients(TDN) intake (g/kg $W^{0.75}$) and digestible crude protein (DCP) intake (g/kg $W^{0.75}$) remained similar in all experimental groups. Nutrient digestibility and digestible nutrient intake were not impacted by supplementation of different levels of Ise and NSe supplementation to all treatment groups. Average fortnight body weight gain, average daily gain (ADG), metabolic body weight gain was similar in all groups. Feed conversion ratio (FCR) and feed conversion efficiency (FCE) were not significantly different between treatment and control group. So, it can be finally concluded that supplementation of Se either from inorganic or by nano source did not exert any adverse effect on growth performance and nutrient utilization.

KEYWORDS: Growth Performance, Heifers, Nano Selenium, Nutrient Utilization

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INTRODUCTION

India has a large livestock resource, which plays an important role in improving the social and economic conditions of the rural population (Sultana et al., 2015). Nutrition has a strong influence on livestock productivity. Balanced and high-quality nutrition is critical for optimizing livestock production. Trace mineral research has previously received less attention, and recommendations on these mineral requirements are primarily based on previous research (NRC, 1985). These minerals have recently received attention due to the recognition that they have both a direct and indirect effect on ruminant performance. Deficiency of these minerals in the diet can reduce animal production by 20-30% (Mohanta and Garg, 2014). Se occurs naturally in both inorganic and organic forms. Se supplements could increase live weight gains, wool production, growth rate, and improve the efficiency of the antioxidant system, enhance the disease resistance, and nutritional quality in animal (Mahima et al., 2006). Less bioavailability and more excretion are the major environmental issue related to the inorganic mineral sources (Pandey et al., 2024).

Nowadays, nanoparticles (NPs) production by controlling size and morphology using physical, chemical, or biological methods is now considered an important research direction in biotechnology (Malyugina et al., 2021). According to Singh (2018), they possess important chemical and physical properties like colour, strength, infusibility, solubility, and a high surface-to-volume ratio.Among all the probable approaches, use of nanotechnology to produce nano minerals is a novel and potential

alternate to other conventional used sources. (Singh et al., 2024).Inorganic or organic forms of Se are commonly used as supplement but nano form gains attention recently (Yaghmaie et al., 2017; Kachuee et al., 2019).Recently, elemental NSe has attracted a wide spread attention to its high bioavailability and low toxicity (Zhang et al., 2008). Both the dietary concentration and source of Se have been demonstrated to affect antioxidant system and Se status (Petrera et al., 2009).

MATERIALS AND METHODS

Eighteen growing Hariana heifers were selected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura. All heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. The animals shed was washed daily and thoroughly cleaned to remove faeces and dirt. All the animals were maintained under clean and hygienic conditions. Animals in Con group were fed with basal diet i.e., wheat straw, chaffed green jowar fodder and compounded concentrate mixture as per NRC (2005) requirements. The chemical composition of experimental diet and dietary components (on DM basis) are presented in Table 1.

Concentrate, green fodder and wheat straw were fed in the ratio of 50:35:15, respectively in Con, ISe(T1) and NSe (T2) groups. Ingredients and chemical composition of the basal diet fed during the experimental period is depicted in Table 2. In Con group, no supplementation of Se was there, T1 group was supplemented with inorganic sodium selenite (SS) @ 0.3mg/kg DMI, while T2 group were supplemented Se as nano selenium oxide @ 0.3 mg/kg DMI. Animals were fed with basal diet i.e.,concentrate mixture, green fodder (Jowar) and wheat straw as per NRC(2005) requirements.Supplementation of Iseand NSe to treatment group was done in the form of premix separately. ISe (Na_2SeO_3)and NSe (SeO_2) powder premix was made by mixing with fine grinded barley flour. Deworming of all the animals was done before the start of the experiment.

Table 1. Ingredient and chemical composition (% DM basis) of experimental diet fed to heifer

Item	Concentrate	Jowar fodder	Wheat straw
Dry matter %	91.50	17.50	91.10
Organic matter %	92.10	88.80	86.70
Ether extract %	4.50	4.10	1.20
Crude protein %	20.68	10.20	3.53
Total ash %	7.40	11.20	13.20
Nitrogen free extract %	59.22	47.36	43.47
Crude fibre%	11.7	26.9	40.23
Neutral detergent fibre%	44.80	51.06	80.50
Acid detergent fibre%	13.45	46.15	53.74
Acid detergent lignin %	0.50	4.56	3.60
Cellulose %	16.90	34.70	50.30
Ca %	1.28	1.37	1.07
P %	0.61	0.67	0.54
Cu mg/kg	6.61	9.98	8.5
Zn mg/kg	14.87	45.07	16.27
Se mg/kg	0.21	0.18	0.04

The experimental heifers were monitored daily for DMI and fortnightly for growth performance and feed efficiency measures. The feeds offered to the animals and residue left were recorded on daily basis to find out the total DMI of the experimental heifers. Intake of DM was calculated as the difference between the amount of DM offered and amount of DM ort. Body weight of experimental animals was recorded at start of experiment followed by fortnight intervals. The experimental heifers were weighed before feeding and watering.

Fortnightly weight gain was calculated by increase in body weight in one fortnight and ADG (kg/day) was calculated by dividing the fortnightly weight gain with number of days (15). Feed to gain ratio or FCR was calculated by the amount of DMI (kg) required for unit (per kg) weight gain by animals during the trial period. FCE was calculated as the ratio between ADG (kg) and DMI (kg) by animals during the trial period. The representative samples of feeds and fodders offered and orts left were dried in a hot air oven at 60°C till a constant weight was attained and then ground in a Wiley mill to pass a 1-mm sieve. The samples were analyzed for DM, CP, Ether Extract (EE), and Total ash (TA) following standard procedures (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fibre (ADF) were determined according to the procedures described by Van Soest et al. 1991.

Table 2. Chemical composition (% DM basis) of total mixed ration (TMR) fed to heifer during feeding trial

Ingredients (%)	Groups		
	Con	ISe	NSe
Oats	18	18	18
Barley grain	15	15	15
Wheat bran	18	18	18
Gram chunni	15	15	15
Mustard oil cake	32	32	32
Mineral mixture*	2	2	2
Jowar fodder	35	35	35
Wheat straw	15	15	15
Nutrients	Chemical composition (%)		
DM	76.55	76.55	76.55
OM	85.9	85.9	85.9
EE	2.52	2.52	2.52
CP	12.36	12.36	12.36
ASH	8.59	8.59	8.59
CF	23.57	23.57	23.57
NFE	50.83	50.83	50.83
NDF	56.76	56.76	56.76
ADF	32.48	32.48	32.48
Ca	1.86	1.86	1.86
P	0.91	0.91	0.91
Cu mg/kg	15.45	15.45	15.45
Zn mg/kg	38.08	38.08	38.08
Se mg/kg	0.21	0.37^{a}	0.40^{b}

To compare the efficiency of nutrient utilisation in growing heifers, a digestion trial for a period of 7 days was conducted at the end of the study. Heifers were weighed before start and at the end of digestion trial. Weighed amount of feed and fodders was offered during digestion trial. Representative samples of the feed offered and residue left were collected and analysed for chemical composition. Faeces voided during 24 hours were collected and measured daily for 7 days.

Results were reported as means with SEM. Differences between the treatments mean were considered significant at $P<0.05$. Homogenous subsets were separated by Tukey's test.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the effect of ISe and NSe supplementation on performance of growing heifers. In this experiment, eighteen Hariana heifers were selected and divided into three groups, each group contain six animals. In Con group, no supplementation of Se, T1 group was supplemented with ISe (Na_2SeO_3@ 0.3 mg/kg DMI), while T2 group was supplemented with NSe(SeO_2 @ 0.3 mg/kg DMI). The observations on effect of nano Se supplementation nutrient intake, growth performance and digestibility of nutrients were recorded.

Effect on Growth Performance

Effect of ISe and NSe supplementation on growth performance are depicted in Table 3. As the age of heifers advanced fortnightly BW change also increased. BW gain of heifers in 3 different groups measured at fortnightly intervals and found no effects of treatment on the fortnightly BW change. There was also no significant ($P>0.05$) effect of treatment on the fortnightly BW gain during the experimental period. There was no significant difference in BW observed on supplementation of different levels of ISe and NSe in diet of heifers though overall body weight increases over time but better body weight gain was observed in ISe and NSe supplemented group. The total Se content of the diet should not exceed 0.3 mg/kg, and the total desired supplement should not exceed 3 mg/head/day (Saha et al., 2016).

Table 3. Effect of inorganic and nano Se supplementation on DMI and growth performance

Attributes	Groups			SEM
	Con	ISe	NSe	
DMI (kg/day)	3.62	3.63	3.73	0.15
DMI	2.40	2.43	2.49	0.02
ADG (g/day)	332.28	346.31	353.87	5.91
FCR	11.08	9.89	9.28	0.44
FCE	0.11	0.12	0.13	0.01

Daily DMI (kg/d) in Con, ISe and NSe group during the experimental feeding was recorded and statistical analysis of data revealed that variation between the groups for DMI was not significantly different ($P>0.05$) between the groups and the mean daily DMI in the Con, Ise and NSe groups were 3.62, 3.63 and 3.73 kg/day. The percentage DMI (kg/100 kg BW) in experimental animals in Con, ISe and NSe groups during different fortnights of experimental feeding was also found in a similar pattern in all the experimental groups and the mean daily DMI in the Con, ISe and NSe groups were 2.40, 2.43 and 2.49 kg/100 BW.

The ADG (g/day) was calculated by dividing the fortnightly weight gain by the number of days in a fortnight i.e. 15. Statistical analysis of ADG in treatment groups shows no significant effect of treatment. The mean ADG of the experimental heifers during 90 days study in Con, ISe and NSe groups were 332.28, 346.31 and 353.87g/day, respectively. Similarly, it was found that Se NPs supplementation appears to have no impact on growth performance in Holstein suckling calves (Jamali et al., 2022).On the contrary findings reported by conducted an experiment by Kojouri et al. (2020) in small ruminants showed that Se NPs had positive

effects on newborn lambs' weight gain patterns. The weight gain pattern of newborn lambs was observed throughout all sampling periods. The treated group showed a noticeable increase in weight gain, while the Con group showed a slight increase in weight gain. Also, in a study by Zommara et al. (2020), the ADG of the organic Se (OSe) and NSe supplemented groups increased by 27.13 and 25.83% when compared to the Con group when fed 0.3 mg Se/kg DM as OSe and NSe in the second and third groups, respectively. As similar to the attributes of BW, there was no effect of ISe and NSeon the metabolic BW change. The fortnightly metabolic BW change of the experimental heifers was recorded during 90 days of study and found no significant (P<0.05) effect of treatment on fortnightly metabolic BW gain in different treatment groups.

The FCR during the experimental period was recorded and over-all mean value found 11.08, 9.89 and 9.28 in Con, ISe and NSe groups, respectively. FCR were not significantly different (P>0.05) between groups. The FCE was recorded and found0.11, 0.13 and 0.12 in control, ISe and NSe groups, respectively. FCE were not significantly different (P>0.05) between groups. Just like the current study Alimohamady et al. (2013) randomly assigned 30, 4-5 month-old lambs to treatments including basal diet, SS and Se yeast. There were no significant differences in ADG, average daily feed intake, or feed/gain ratio. Shi et al. (2018) in a study on taihang goats reported that the final BW was increased in bucks supplemented with Se compared to the Con, and ADG in NSe and Se yeast was greater than in SS or Con bucks.

Effect on Nutrient Utilization

Effect of ISe and NSe supplementation on nutrients utilization are depicted in Table 4. DM digestibility was 59.39, 60.58 and 60.69% in Con, ISe and NSe group respectively whereas, the organic matter (OM) digestibility was 62.33, 63.59 and 64.99%, respectively. The statistical analysis of data on DM and OM digestibility percent revealed that difference between the groups were not significantly different (P>0.05). CP digestibility percent of Con, ISe and NSe diets were 68.37, 69.78 and 70.37, respectively.

The CF digestibility in Con, ISe and NSe was 51.38, 53.55 and 55.29%, respectively. The EE digestibility percent in Con, ISe and NSe were 81.54, 81.56 and 82.46%, respectively.

Table 4. Effect of inorganic and nano Se supplementation on nutrients digestibility percentage coefficient

Attributes	Groups			SEM
	Con	ISe	NSe	
DM	59.39	60.58	60.69	1.42
OM	62.33	63.59	64.99	2.06
CP	68.37	69.78	70.37	1.61
CF	51.38	53.55	55.29	1.24
EE	85.54	81.76	82.46	1.56
NFE	70.41	71.06	72.31	3.08
NDF	56.53	56.33	56.73	1.88
ADF	49.07	50.70	52.53	1.75

P>0.05: Non significant

Digestibility percent of NFE in Con, ISe and NSe were 70.41, 71.06 and 72.31% respectively. The NDF digestibility of control, ISe and NSe were reported as 56.53, 56.33 and 56.73% respectively. ADF digestibility percent were found 49.07, 50.7 and 52.53% in Con, ISe and NSe respectively. All nutrients digestibility coefficient were found similar in Con and treatments groups. similarly in a study conducted by Liu et al. (2024) on forty-eight Holstein dairy cows averaging 720 ± 16.8 kg of body weight,

the addition of NSe increased the digestibility of dietary DM, OM, CP, NDF, and ADF while ether extract digestibility remained unchanged. Also, Ibrahim et al. (2018) studied the effects of ISe, OSe, and NSe particles on the nutritive digestibility, productivity and serum biochemical indices of 32 Ossimi lambs aged 4 months. Results showed higher digestibility of DM, increased digestibility of OM, CP, CF, EE, NFE, DCP, and TDN in lambs fed with the said diets. Digestible DM, OM, EE, NFE, CF, NDF and ADF intake were found similar in Con and treatment groups and shown in Table 5.

Total DMI (kg/d), DCP intake, TDN intake were not significantly different (P>0.05) in all four groups as mentioned in Table 5. The digestibility coefficients of DM, OM, CP, EE, CF, NFE, NDF, and ADF were unaffected by different levels of ISe and NSe supplementation in the diet of Hariana heifers. Digestible nutrient intake i.e.DMI (kg/100 kg BW),DCP(g/kg $W^{0.75}$)andTDN intake (g/kg $W^{0.75}$) intake were similar across treatment groups. In the current study, supplementation with ISe and NSe had no effect on nutrient intake, nutrient digestibility, or BW changes. In contrast to the current study, Liu et al. (2019) investigated the effects of SS on cannulated bulls using a replicated 4 x 4 latin square design with eight ruminally cannulated dairy bulls. The treatments were Con, low SS (LSS), medium SS (MSS), and high SS (HSS), with SS levels of 0, 0.1, 0.3, and 0.5 mg/kg of Se in dietary DM. The digestibility of DM, OM, CP, EE, NDF, and ADF has increased linearly.

Table 5. Effect of inorganic and nano Se supplementation on nutrient intake during digestion trial

Attribute	Groups			SEM
	Con	ISe	NSe	
Initial BW (kg)	154.77	156.41	158.23	11.84
Final BW (kg)	158.81	161.83	163.80	11.81
BW change (kg)	5.03	5.42	5.48	0.24
DM intake (Kg/day)	3.74	3.75	3.73	0.34
DM intake (%BW)	2.25	2.28	2.29	0.05
DCP (g/day)	528.30	491.42	587.81	0.509
DCP (g/kg $W^{0.75}$)	11.30	12.02	12.73	0.726
TDN intake (kg/d)	2.43	2.50	2.54	0.25
TDN (g/kg $W^{0.75}$)	57.4	61.63	62.97	47.15

P>0.05: Non significant

CONCLUSION

From the above study it may be concluded that supplementation of inorganic and nano Se did not have any effect on daily dry matter intake and body weight gain as compared to control group and also, the supplementation of inorganic and nano Se did not have any effect on nutrients digestibility. So, it can be finally concluded that supplementation of Se either inorganic or by nano source did not exert any adverse effect on growth performance and nutrient utilization in Hariana heifers.

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Influence of Probiotic, Prebiotic and Synbiotic Additives on Feed intake and Conversion ratio in Jaffarabadi Buffalo Calves during Early and Late Post-natal Phases

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ABSTRACT

A study was conducted to evaluate the impact of probiotic, prebiotic and synbiotic supplementation on the feed intake and feed conversion ratio of newborn Jaffarabadi buffalo calves (early post-natal stage: 2nd to 13th week and late stage: 14th to 26th week of age). Twenty-four 8-day-old calves were chosen and divided into four groups of six at random: probiotic (T1), prebiotic (T2), synbiotic (T3) and control (C). All calves received restricted suckling plus a basal diet and pelleted concentrate as per ICAR (2013) standards. T1 calves were fed probiotics (*L. sporogenes* and *S. cerevisiae*, 5 g/day), T2 received prebiotics (mannan-oligosaccharides, 5 g/day) and T3 were given a synbiotic mix (2.5 g each of probiotic and prebiotic per day). Average dry matter intake (DMI) as % of body weight varied slightly among groups, with pooled values of 3.20%, 3.28%, 3.13% and 3.36% for C, T1, T2 and T3, respectively. DMI increased by 2.40% (T1) and 5.08% (T3) and decreased by 2.21% (T2) compared to control, though differences were not significant ($p>0.05$). Overall feed conversion ratio (FCR) remained unaffected statistically but showed numerically lower values in supplemented groups, particularly in T2 (5.78) compared to control (6.34). Results suggest potential benefits of supplementation on feed efficiency without significant changes in FCR. Overall fecal coliform count was also reduced in the feces of supplemented buffalo calves than control.

KEYWORDS: FCR. Fecal coliform count, Feed intake, Jaffarabadi calves, Probiotic, Prebiotic, Synbiotic.

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INTRODUCTION

The Jaffarabadi buffalo, indigenous to the Saurashtra region of Gujarat, stands out as the heaviest and one of the most productive Indian breeds. It is renowned for its high milk yield and rich fat content, making it a valuable genetic resource for dairy improvement programs (Jayebhaye et al., 2020). However, the long-term productivity and profitability of such breeds are closely linked to the health and management of their calves. Effective calf management is essential to ensure the survival, growth and future productivity of buffalo herds. Neonatal calf mortality in India ranges from 12.5% to 30%, largely due to inadequate care and nutrition during the early stages of life (Singh et al., 2009). Therefore, implementing sound management practices from birth is critical for reducing mortality and enhancing the lifetime performance of dairy animals. During the neonatal phase, calves are highly susceptible to infections and growth setbacks. Optimal nutrition, particularly the establishment of a healthy gut microbiota, plays a crucial role in improving immunity, digestion and overall development. Scientific evidence highlights that appropriate dietary interventions, such as the use of probiotics, prebiotics and synbiotics, can significantly influence gut health and promote better growth outcomes in young calves (Lucas et al., 2007). Hence, early nutritional strategies that support gastrointestinal health are integral to sustainable and efficient buffalo farming.

The supplementation of probiotics, prebiotics and synbiotics in calf diets has been shown to significantly improve health by promoting a balanced gut

microbiota, enhancing immune response and lowering the risk of gastrointestinal infections (Heyman and Menard, 2002; Markowiak and Œli¿ewska, 2017). Probiotics such as *Saccharomyces cerevisiae* and *Lactobacillus* species aid in digestion and inhibit pathogenic microbes (Dawson et al., 1990; Nocek and Kautz, 2006), while prebiotics like fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) support the proliferation of beneficial bacteria and suppress harmful strains like *E. coli* (Cangiano et al., 2020). Synbiotics, which combine probiotics and prebiotics, offer synergistic effects, further enhancing gut health and growth performance (Alloui et al., 2013). Effective early-life nutrition during this period directly influences growth rate, immune development and long-term productivity. Research in exotic and crossbred calves has demonstrated that dietary supplementation with probiotics, prebiotics and synbiotics significantly improves feed conversion efficiency and overall performance (Ratre et al., 2019).

Keeping in view the importance of topic, a study was conducted to assess the feed intake and feed conversion ratio of Jaffarabadi buffalo calves receiving diets enriched with probiotics, prebiotics and their combination as synbiotics.

MATERIALS AND METHODS

A study was undertaken to evaluate the impact of dietary supplementation with probiotic, prebiotic and their combination (synbiotic) on the feed intake and feed conversion ratio of Jaffarabadi buffalo calves. The trial involved 24 calves (Average body weight and age in days), divided into four equal groups (n=6 per group) and was conducted at the Cattle Breeding Farm, Kamdhenu University, Junagadh, following approval from the Institutional Animal Ethics Committee (Protocol No. KU-JVC-IAEC-LA-99-22). The experimental period spanned from 8 to 182 days of age. Calves were allocated to groups based on birth weight, dam parity, previous and current average milk yield of the dam and calf sex, ensuring equal distribution (3 males and 3 females per group).

Pelleted concentrate was offered to meet protein requirements as per ICAR (2013) feeding standards and mineral mixture @10-15 g/h/ (Table 1). Representative of feed and fodders samples were tested for proximate principles (AOAC, 2023). Dry matter intake (kg/day, % body weight, g/kg Wp ·w u) and feed conversion ratio (kg DMI/kg body weight gain) data of experimental Jaffarabadi buffalo calves were recorded and analyzed across two post-natal phases: early (2^{nd} –13^{th} week) and late (14^{th} –26^{th} week) stages.

Table 1. Schedule for probiotic, prebiotic and synbiotic inclusion in feed

Treatment Groups	No. of animals	Dietary treatment details
Control (C)	6	Restricted suckling milk of their dam + basal diet
Probiotic (T-1)	6	Restricted suckling milk of their dam + basal diet + supplementation of probiotic (*Lactobacillus sporogenes* $5x10^7$ c.f.u./g, *Saccharomyces cerevisiae* $1.5x10^8$ c.f.u./g (in 1:1) @ 5 g/day/calf.
Prebiotic (T-2)	6	Restricted suckling milk of their dam + basal diet +supplementation of prebiotic (mannan-oligosaccharides) @ 5 g/day/calf
Synbiotic (T-3)	6	Restricted suckling milk of their dam + basal diet+ supplementation of synbiotic (*Lactobacillus sporogenes* $5x10^7$ c.f.u./g, *Saccharomyces cerevisiae* $1.5x10^8$ c.f.u./g (in 1:1)@ 2.5g/day/calf + mannan-oligosaccharides @ 2.5g/day/calf)

c.f.u: Colony Forming Units

Statistical analysis

Statistical analysis was performed using ANOVA, following the method of Snedecor and Cochran (1994). Group differences were assessed using Duncan's Multiple Range Test (Duncan, 1955) with SPSS software version 16.0 (SPSS Inc., Chicago, USA). Results are expressed as mean ± standard error, with statistical significance considered at $p \leq 0.05$ and $p \leq 0.01$ levels.

RESULTS AND DISCUSSION

Proximate Composition of Feed Stuff

Among all the feedstuffs, the compound concentrate mixture exhibited the highest crude protein (CP) content, making it the most protein-rich component of the diet. This was followed by groundnut haulms, while green fodders such as green sorghum and Napier grass had relatively lower protein levels. Crude fiber exhibited an inverse pattern, being lowest in the compound concentrate mixture. Additionally, the compound concentrate mixture contained lower levels of ether extract and total ash (Table 2).

Table 2. Proximate composition of seasonal green fodder, dry fodder and compound concentrate mixture (% DM basis)

Nutrients	Green Sorghum	Napier grass	Groundnut Haulms	Compound concentrate mixure
DM	30.02	25.30	91.20	90.00
OM	90.45	91.78	89.70	92.29
CP	06.80	05.58	10.67	20.42
CF	32.02	28.15	32.30	10.14
EE	02.56	02.27	03.25	02.85
NFE	49.07	55.78	43.48	58.88
Total Ash	09.55	08.22	10.30	07.71

Dry Matter Intake (Kg/d)

Dry matter intake (DMI) was measured to assess the nutritional intake of Jaffarabadi buffalo calves. As experimental groups were similar in key traits, milk suckling was not considered in feed intake and efficiency calculations. DMI was monitored weekly during two post-natal phases: early (2^{nd} – 13^{th} week) and late (14^{th} –26^{th} week).

At the initial phase (week 2), DMI was 0.71±0.023, 0.77±0.024, 0.83±0.076 and 0.73±0.031 kg/day in control, T1, T2 and T3 groups, respectively, which increased to 2.14±0.09, 2.17±0.05, 2.13±0.14 and 2.23±0.077 kg/day by week 13. Treatment groups showed higher DMI than control, with T1 showing the highest increase (0.08 kg/day, 5.37%), followed by T3 (0.06 kg/day, 4.28%) and T2 (0.05 kg/day, 3.57%). However, differences were statistically non-significant ($p>0.05$). Overall average DMI during this phase was 1.40±0.06, 1.48±0.04, 1.45±0.08 and 1.46±0.04 kg/day in control, T1, T2 and T3, respectively (Figure 1). The overall mean DMI for the late phase were 3.18±0.09, 3.32±0.05, 3.09±0.20 and 3.50±0.14 kg/day in control, T1, T2 and T3 groups, respectively, difference being insignificant. The group T3 exhibited the highest overall increase in DMI (10.06%), followed by T1 (4.40%) and T2 (-2.83%) when compared with the control (Figure 1).

The pooled mean DMI for both the phases combined were 2.32±0.07, 2.43±0.04, 2.30±0.14 and 2.52±0.08 for control, T1, T2 and T3 groups, respectively. T3 group of calves showed the highest increase (0.20 kg/day, 8.62%) over the control, while T2 showed a slight decrease (-0.02 kg/day, -0.86%). the overall trend indicated a non-significantly ($p>0.05$) increase in intake for the treatment groups.

From weeks 14th to 26th of age, buffalo calves fed with prebiotics showed a reduction in dry matter intake (DMI) compared to those fed with control, probiotic and synbiotic. While all groups generally showed increasing feed intake with age, the prebiotic group had consistently lower DMI values, indicating that prebiotics may slightly suppress appetite or enhance feed efficiency, leading to reduced intake. In contrast, probiotic and synbiotic groups maintained higher DMI, suggesting better palatability or digestive stimulation.

The findings of the current study are consistent with those of Riddell et al. (2010), who found that the inclusion of probiotics in the diet did not significantly affect feed intake ($p>0.05$). Similarly, Uzmay et al. (2011) reported no significant ($p>0.001$) differences in feed intake across different age periods, although calves receiving MOS feeding consumed 19.9% more calf starter as compared to those on the control diet. Dimova et al. (2013) also concluded that there were no significant ($p>0.05$) differences in daily feed intake between the treatment and control groups. Hossain et al. (2012) conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U./g) and found that DM intake (kg/day) did not differ statistically ($p>0.05$), but were numerically higher in treatment groups than control. Singh et al. (2014) in their experiment revealed that no any significant difference ($p>0.05$) found in dry matter intake between groups.

In week 2, the DMI (% of b.wt.) for T1, T2 and T3 were 2.04±0.08, 2.23±0.24 and 1.98±0.09, respectively, slightly higher as compared to 1.93±0.07 for the control. Over 13 weeks, DMI increased to 3.31 in T1, 3.26 in T2 and 3.41 in T3 ($p>0.05$). Overall DMI observations in percent body weight were 2.72±0.10, 2.83±0.03, 2.80±0.05 and 2.81±0.08 in the control, T1, T2 and T3 groups, respectively. During the early post-natal phase, average DMI (% of body weight) increased from 1.93% in week 2 (control) to 3.41% in week 13 (Figure 2).

Initial observations (14th week) of the late post-natal phases were 3.35±0.33, 3.29±0.14, 3.22±0.24 and 3.48±0.19 % in the control, T1, T2 and T3 group of calves, respectively. Final observations (26th week) were 3.91±0.42, 4.04±0.19, 3.71±0.24 and 4.25±0.21 % in the control, T1 T2 and T3 group of calves, respectively. Similar to the early phase, the treatment groups showed some variations in their DMI, with T1 and T2 generally having slightly lower DMI values in comparison to the control group. Overall DMI observations in percent body weight during 14th to 26th weeks of age were estimated to be 3.64±0.10, 3.69±0.03, 3.43±0.22 and 3.87±0.17 in the control, T1, T2 and T3 groups, respectively ($p>0.05$) (Figure 2).

Pooled DMI percent of body weight were 3.20±0.09, 3.28± 0.03, 3.13 ± 0.18 and 3.36± 0.11 of the control, T1, T2 and T3 group of calves respectively ($p>0.05$). The treatment groups had changes of 2.40% (increase) for T1, 2.21% (decrease) for T2 and 5.08% (increase) for T3 in DMI relative to the control.

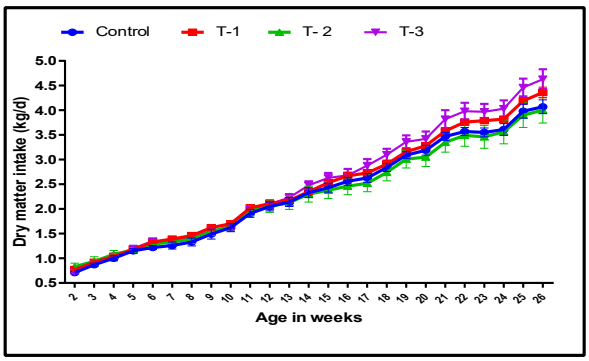

Figure 1. Change in DMI (kg/d) in early and late post-natal phases of experimental Jaffarabadi buffalo calves

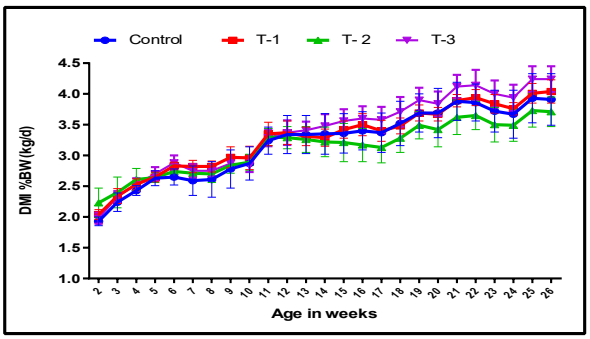

Figure 2. Change in DMI expressed as % of BW in early and late phase of experimental Jaffarabadi buffalo calves

The findings of the current study are consistent with those of Riddell et al. (2010), who found that the inclusion of probiotics in the diet did not significantly affect feed intake ($p>0.05$). Similarly, Uzmay et al. (2011) reported no significant ($p>0.05$) differences in feed intake across different age periods, although calves receiving MOS feeding consumed 19.9% more calf starter as compared to those on the control diet. Dimova et al. (2013) also concluded that there were no significant ($p>0.05$) differences in daily feed intake between the treatment and control groups. Hossain et al. (2012) conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U./g) and found that DM intake (kg/100 kg B.wt.) did not differ statistically ($p>0.05$), but were numerically higher in treatment groups than control. Singh et al. (2014) in their experiment revealed that no any significant difference ($p>0.05$) found in dry matter intake between groups.

The average DMI (g/kg $W^{0.75}$) of experimental Jaffarabadi buffalo calves during the first observation period was 125.43±1.30, 130.71±1.12, 143.21±5.10 and 127.11±2.93 in control, T1, T2 and T3 group, respectively. End day of experiment observations were 124.79±4.75, 122.42±3.24, 113.41±8.05 and 127.06±5.54 in the control, T1, T2 and T3, respectively. Overall mean showed no statistically significant ($p>0.05$) differences between treatments, with the overall mean values being 137.53±2.84 for the control group, 136.30±1.09 for T1, 132.07±5.35 for T2 and 138.38±3.27 for T3 group. There was an increasing trend till 5^{th}/6^{th} weeks of age no definite trend from 7^{th} to 12^{th} week (Figure 3) and, thereafter, from 13^{th} week of age it tended to decline till 26^{th} week of age (Figure 3).

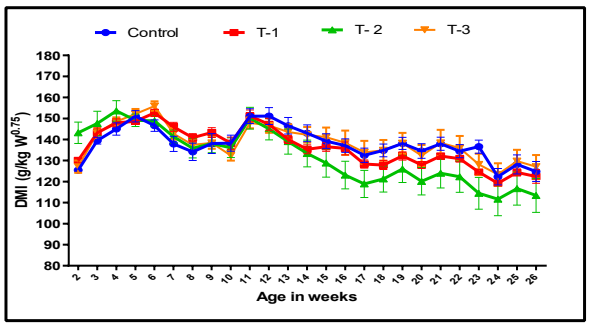

Figure 3. Change in DMI in terms of g/kg $w^{0.75}$ in early and late post-natal phase of Jaffarabadi buffalo calves

These results aligned with the finding of Hossain et al. (2012), who conducted an experiment on Kankrej calves to study the influence of dietary feeding of live yeast (*Saccharomyces cerevisiae*, 5 x 10^9 cells C.F.U. /g) and found that DM intake (g/kg $W^{0.75}$) did not differ statistically ($p>0.05$), but numerically higher in treatment groups than control. Kara et al. (2015) observed the effects of mannan-oligosaccharides (MOS) feeding on Holstein cattle calves and revealed that average daily feed intake (ADFI) was statistically similar ($p>0.05$) in supplemented group but ADFI was numerically higher by 10.97%, in MOS than in control calves.

Feed Conversion Ratio

Initial observations were 2.22±0.05, 2.37±0.04, 2.54±0.20 and 2.17±0.13 in the control, T1, T2 and T3 group of calves, respectively. The weekly values were relatively close to the control group, with slight increases in the treatment groups (Figure 4).

The average feed conversion ratio for the control group was 4.09±0.09 kg, while the treatment groups T1, T2 and T3 had mean of 3.96±0.04, 3.96±0.15 and 3.83±0.08, respectively. These represented differences of 3.17%, 3.17% and 6.35% as compared to the control group ($p>0.05$).

First observations of experimental Jaffarabadi buffalo calves in late post-natal phase were 6.36±0.13, 6.06±0.06, 5.72±0.21 and 5.99±0.13 in the control, T1, T2 and T3 groups, respectively. Overall means of the late Post-natal phase were 8.60±0.10 in the control and the treatment groups T1, T2 and T3, 8.04±0.03, 7.61±0.22 and 8.19±0.16, respectively (Figure 4). The differences between control and treatment groups for the overall period were -6.51% for T1, -11.51% for T2 and -4.77% for T3 group of Jaffarabadi buffalo calves, indicating favourable effect of prebiotic feeding on FCR of the calves.

The treatment groups did not show statistically significant differences when compared with the control group ($p>0.05$). Pooled means were 6.34±0.09, 6.00±0.03, 5.78±0.18 and 6.01±0.11 of the control, T1, T2 and T3 group respectively. Treatment groups T1 and T3 had relatively positive-favourable impact by decrease of 5.36% and 5.20%, whereas treatment T2 (prebiotic feeding) exerted a greater favourable results by lowering the FCR by 8.83%, these differences were not statically significant ($p>0.05$).

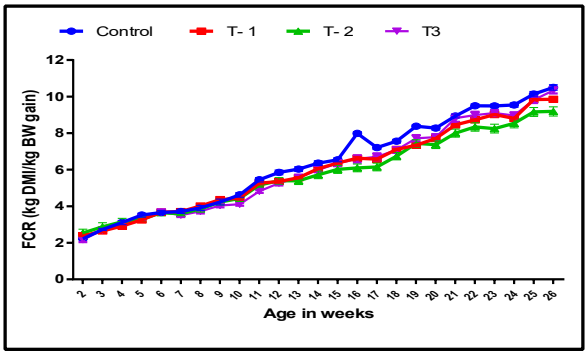

Figure 4. Change in FCR (kg DMI/kg body weight gain) in early and late post-natal of Jaffarabadi buffalo calves

In this study, buffalo calves supplemented with prebiotics, probiotics, or synbiotics showed a reduction in feed conversion ratio (FCR) compared to the control group, indicating enhanced feed efficiency. Prebiotics, in particular, led to the greatest improvement, which may be attributed to better nutrient absorption and more favorable microbial activity. These results differ from the findings of Sri Lekha et al. (2021), who reported a significantly lower FCR in Murrah buffalo calves receiving synbiotic supplementation. Similarly, Liu et al. (2020) found that Holstein calves fed a combination of essential oils and prebiotics had a significantly improved FCR compared to the non-supplemented group, reinforcing the beneficial role of prebiotics in improving feed utilization.

Fecal Sampling and measurements

Fecal samples were collected on days 8th day (Initiation of experiment, 84th day (midpoint of the experiment) and at the end of the experiment (175th day of the experiment). (Samples were collected directly from the rectum using sterile rubber gloves and placed into clean, sterile containers.). All samples were kept at 4°C and processed within a maximum of two hours. For microbial analysis, one gram of each fecal sample was homogenized in 9 ml of 0.1% sterile peptone water. (A series of tenfold dilutions was prepared). Dilutions a 10{ u , 10{ v , and 10{ w were plated using the pour plate method to facilitate colony enumeration. The plates were incubated at 37°C for 48 hours. Colony growth was assessed on Eosin Methylene Blue (EMB) agar (Lab Supply Company, Heliopolis, Cairo, Egypt), with observations made after 24 and 48 hours of incubation. Colonies exhibiting a characteristic green metallic sheen were identified and counted. (Only plates showing 30–300 colonies were considered for enumeration). (The mean colony count from three replicate plates was calculated and expressed as log € colony-forming units (CFU) per gram of feces) to estimate the *E. coli* population.

Table 3. Effect of supplementation on mean coliform count (CFU/g ± SE) in feces of Jaffrabadi buffalo calves

Days	Control	T1 (Probiotic)	T2 (Prebiotic)	T3 (Synbiotic)	P value
8th day	6.88±0.12	6.37±0.07	6.41±0.17	6.60±0.17	0.06
84th day	6.90±0.09^{a}	5.17±0.09^{b}	6.02±0.17^{c}	6.15±0.17^{c}	0.01
175th day	6.93±0.10^{a}	4.62±0.10^{b}	5.52±0.21^{c}	5.68±0.16^{c}	0.01
Overall	6.90±0.04^{a}	5.38±0.17^{b}	5.98±0.08^{c}	6.14±0.08^{c}	0.01

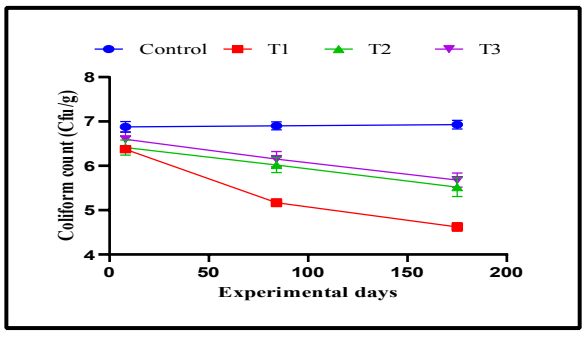

Figure 5. Change in faecal coliform count (Cfu/g) in Jaffarabadi buffalo calves

Coliform counts were measured in four groups, control, probiotic (T1), prebiotic (T2) and synbiotic (T3) across three-time period. On the 8th day (At the beginning), there were no significant differences among groups. By the 84th and 175th days, probiotic supplementation (T1) significantly reduced coliform counts compared to control and other treatments (P≤0.01). Overall, the probiotic group showed the lowest mean coliform levels, followed by prebiotic and synbiotic groups, all significantly lower than the control (P≤0.01).

The findings of this study are consistent with earlier reports. Agazzi et al. (2014) observed a higher lactic acid bacteria to *E. coli* ratio in Holstein calves supplemented with probiotics and a corresponding decrease in diarrheal incidence. Similarly, Omran et al. (2020) reported significantly lower faecal *E. coli* counts in probiotic-fed buffalo calves compared to non-supplemented controls. Ayala-Monter et al. (2019) also demonstrated the beneficial impact of inulin and *Lactobacillus casei* on reducing coliform loads and improving gut health in lambs.

All in all, the results clearly show that adding beneficial microbes to the diet can play an important role in shaping the gut bacteria and improving overall gut health in young ruminants. Among the different approaches, probiotics stood out as the most effective and reliable in reducing harmful coliform bacteria in the calves' faeces. This suggests that probiotic supplementation could be a simple and practical way to help protect buffalo calves from gut-related infections and health problems early in life.

CONCLUSION

DMI increased by 2.40% with supplementation of probiotic and 5.08% with synbiotic supplementation, where as slight reduction of 2.21% was observed with prebiotic feeding compared to the control group. In terms of FCR, prebiotic-fed calves showed the most efficient feed utilization (5.78), followed by equal values in probiotic and synbiotic groups (6.0), all of which performed better than the control group (6.34). Total coliform count was decreased in the treated groups than control. These findings suggest potential improvements in nutrient use efficiency and health with additive supplementation to Jaffrabadi calves for long term enhanced productivity and performance.

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Laboratory Preparation and Quality Evaluation of Cabbage and Cauliflower Waste Silage

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ABSTRACT

The experiment was conducted to evaluate the quality of cabbage and cauliflower waste silage and to study the *in vitro* parameters of cauliflower and cabbage waste silage ensiled with salt, urea and or molasses. Cabbage and cauliflower waste were collected from the local market and after cleaning cut into small pieces and kept in air tight container for 28 days. 5 different types silage were prepared from cabbage and cauliflower waste *viz.* I: Cabbage waste silage with 0.5% salt, 1% urea; II: Cauliflower waste silage with 0.5% salt, 1% urea; III: Cabbage and Cauliflower mixed (1:1) waste silage with 0.5% salt, 1% urea; IV: Cabbage waste silage with 0.5% salt, 1% urea and 5% molasses V: Cauliflower waste silage with 0.5% salt, 1% urea and 5% molasses and VI: Cabbage and Cauliflower mixed (1:1) waste silage with 0.5% salt, 1% urea and 5% molasses. The fermentation characteristics and nutritive values of silage were evaluated using representative fresh silage samples. Based on appearance and smell, all silage samples in this study were classified as high-quality. Similarly, the analysis of organic acid content confirmed that all silages were found to have good quality silage. The IVDMD (%) is significantly differ ($P<0.05$) among various cauliflower waste silage. From this experiment it can be concluded that incorporating 5% molasses into silage prepared from cabbage and cauliflower waste can uphold its high quality, as demonstrated by reduced pH and NH_3-N levels, coupled with increased lactic acid bacteria (LAB) content.

KEYWORDS: Cabbage waste, Cauliflower waste, Fermentation, *in vitro* quality, Silage

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INTRODUCTION

Globally, each year 2.5 billion ton of food is wasted and in India approximately one third of total production goes wasted or gets spoiled before it is eaten (FSSAI). This massive amount of food and vegetable waste biomass may contribute to the annual emission of about 3.3 billion tons of CO_2 equivalent greenhouse gases from dumping sites, leading to global warming (India Today, 2020). Fruit and vegetable waste is approximately 4.65-5.99 % of total food wastage. The declining fodder production has led to the search for alternative feed resources, providing an effective solution for the efficient disposal of vegetable waste. Cabbage and cauliflower, in particular, generate an average of 30–50% waste in the form of stems, stalks, and leaves (Das et al., 2018).

The cabbage and cauliflower waste can be utilized as silage and presents several benefits. Firstly, it addresses the issue of agricultural waste management, promoting a more sustainable and eco-friendly approach. Secondly, silage can serve as an alternative and cost-effective feed source for livestock, reducing reliance on traditional animal feeds. However, preserving cabbage and cauliflower as animal feed presents challenges due to their high moisture content (>80%) and perishability. Ensiling is a cost-effective preservation technique for these vegetables. High-quality vegetable waste silage (87.8% moisture) can be produced by incorporating wheat straw (29%) and wheat bran (9–20%) as absorbents (Ozkul et al., 2011). Additionally, applying molasses could enhance the vegetable waste ensiling process (Colombatto et al., 2003; Mtengeti et al., 2003). Considering these aspects the present study was carried out to study the quality of cabbage and cauliflower waste silage and *in vitro* evaluation of cauliflower waste silage.

MATERIALS AND METHODS

Fresh cabbage and cauliflower waste were collected from Bareilly Sabji Mandi, India, cleaned, chopped, and stored in airtight containers for 28 days at 25 ± 5°C. 6 silage types were prepared *viz*. I: Cabbage waste silage with 0.5% salt, 1% urea; II: Cauliflower waste silage with 0.5% salt, 1% urea; III: Cabbage and Cauliflower mixed (1:1) waste silage with 0.5% salt, 1% urea; IV: Cabbage waste silage with 0.5% salt, 1% urea and 5% molasses V: Cauliflower waste silage with 0.5% salt, 1% urea and 5% molasses and VI: Cabbage and Cauliflower mixed (1:1) waste silage with 0.5% salt, 1% urea and 5% molasses. The chemical composition and bio-active components of silage materials before ensiling is depicted in Table 1.

The fermentation characteristics and nutritive values of silage were analyzed using representative fresh silage samples. Silage extract was prepared according to Kim et al., 2016. The pH was measured from fresh silage extract with a pH meter right after extraction. The sample was stored at – 20 ^{0} C for further analysis. NH3-N in the silage extract was assessed by the method of Weatherburn. (1967). Lactobacilli MRS agar was used for the isolation and enumeration of LAB. Volatile fatty acids were assessed using Nucon-5765 gas chromatograph.

The proximate principles were estimated by the method recommended by AOAC 2005. NDF and ADF was estimated by Van Soest method. (1991). Calcium and phosphorus were estimated as per the modified method of Talapatra et al. (1940).

Table 1. Formulation of concentrate portion for *in vitro* studies

Ingredients	0% CLS	10% CLS	20% CLS	30% CLS	40% CLS	50% CLS
Maize	37	23	30	27	24	21
Wheat bran	37	35	32	29	26	23
Soya bean meal	23	19	15	11	7	3
CLS	-	10	20	30	40	50
Mineral mixture	02	02	02	02	02	02
Salt	01	01	01	01	01	01
Total	100	100	100	100	100	100
% CP	18.93	19.07	19.04	19.00	18.98	18.95

Where, CLS: cauliflower waste silage

The *in vitro* gas production was run as per the technique of Menke and Steingass,1988. Formulation of concentrate portion with cauliflower waste silage for *in vitro* studies were presented in Table 2.

Table 2. Formulation of concentrate portion for *in vitro* studies

Ingredients	0% CLS	10% CLS	20% CLS	30% CLS	40% CLS	50% CLS
Maize	37	23	30	27	24	21
Wheat bran	37	35	32	29	26	23
Soyabean meal	23	19	15	11	7	3
CLS	-	10	20	30	40	50
Mineral mixture	02	02	02	02	02	02
Salt	01	01	01	01	01	01
Total	100	100	100	100	100	100
% CP	18.93	19.07	19.04	19.00	18.98	18.95

Where, CLS: cauliflower waste silage

The data generated from the study were analysed as per the standard statistical procedure Snedecor and Cochran using SPSS (Version 20.0) software.

RESULTS AND DISCUSSION

Chemical composition of cabbage and cauliflower waste before ensiling

The DM content for cabbage waste, cauliflower waste and mixed waste in the present experiment (Table 3) is higher than those reported by Ozkul et al. (2011) and lower than the values observed by Meneses et al. (2007). The CP content for cabbage waste, cauliflower waste and mixed waste surpasses the results reported by Ozkul et al. (2011). The higher NDF content reported in the present experiment might be attributed to the elevated cell wall content in cauliflower and cabbage, as noted by Wadhwa and Bakshi, (2013) and Meneses et al. (2007). The Ca and P content in cabbage waste, cauliflower waste and mixed waste resemble the findings of Bakshi and Wadhwa (2006). The higher TPC in cabbage waste compared to cauliflower waste might be cabbage waste have a greater potential for antioxidant applications. In the present experiment the TPC for cabbage waste, cauliflower waste and mixed waste is aligned with Das et al. (2024) and lower than the values observed by Oberoi et al. (2007). The slightly higher glucosinolate content observed in cabbage waste compared to cauliflower waste may result from genetic and environmental factors influencing the accumulation of these compounds in different Brassica vegetable (Cartea and Velasco, 2008). The greater flavonoid content found in cauliflower waste as compared to cabbage waste suggests that cauliflower waste may serve as a more abundant source of these beneficial compounds.

Table 3. Chemical composition (%) of cabbage and cauliflower waste before ensiling

Parameters	Cabbage waste	Cauliflower waste	Mixed waste
Dry matter	28.88	22.97	26.21
Organic matter	89.5	85	87.25
Total ash	10.5	15	12.75
Acid insoluble ash	2.54	2.14	2.34
Crude protein	20	24.60	22.30
Ether extract	1.53	2.46	2.00
Neutral detergent fibre	45	36	41
Acid detergent fibre	28	22	25
Acid detergent lignin	9	11	10
Cellulose	19	11	15
Hemicellulose	17	14	15.50
Calcium	2.50	2.25	2.25
Phosphorus	0.72	0.52	0.62
Total phenols (mg of GAE/g)	3.85	3.10	3.48
Condensed tannins	0.37	0.42	0.39
Glucosinolates	0.054	0.030	0.042
Total Flavonoid mgQE/g)	1.63	2.02	1.83

Mixed waste: Cabbage waste + cauliflower waste (1:1)

Physical characteristics of cabbage and cauliflower waste silage

The colour of cabbage waste and cauliflower waste was ranging from greenish-yellow to olive green in this experiment. The smell of cabbage and cauliflower waste, without molasses, was acidic; however, the addition of 5% molasses for ensiling resulted in a sweet and acidic odour. The structure of all the silage samples was firm and easily separable. Upon opening the silage, there was no evidence of mould growth. In all the silage, the greenish colour was retained and the odour and structure were well-preserved, following the approach outlined by Breirem and Ulvesli, (1960). In terms of appearance and smell, all the silage in this experiment demonstrated qualities of good silage.

Chemical composition (%) of cabbage and cauliflower waste silage

The chemical composition of cabbage and cauliflower waste silage are presented in Table 4. In this experiment DM content of silages are notably lower compared to those observed by Alcicek et al. (2000), Meneses et al. (2007) and Ozkul et al. (2011).

Table 4. Chemical composition of cabbage and cauliflower waste silage

Parameters	I	II	III	IV	V	VI
Proximate principles (%)						
Dry matter	19.03	17.32	21.43	20.92	19.96	22.05
Total ash	22	26	32	20	22	28
Organic matter	78	74	68	80	78	72
Acid insoluble ash	4	4	2	3	2	3
Crude protein	21.88	24	23.50	20	22.60	21.03
Ether extract	2.87	3.62	2.63	2.60	3.35	2.44
Cell wall components (%)						
Neutral detergent fibre	35.68	29.40	27.53	36.12	30	28.25
Acid detergent fibre	22.64	18.30	17.46	22.01	17.85	18
Acid detergent lignin	08	06	06	07	05	04
Cellulose	14.64	12.30	11.46	15.01	12.85	14
Hemicellulose	13.04	11,10	10.07	14.11	12.15	10.25
Minerals (%)						
Calcium	2.50	2.25	2.50	2.25	2.50	2.50
Phosphorus	0.65	0.48	0.40	0.68	0.52	0.69
Bioactive components (%)						
Condensed tannin	0.49	0.51	0.33	0.45	0.46	0.31

Chen et al. (2004) also reported higher DM levels in corn silage while supplemented with 3% molasses. The CP content of cauliflower silage surpasses the values reported by Bakshi et al. (2006). The improvement in CP and EE levels in cabbage waste and cauliflower waste silage in this experiment supports the notion that ensilage is a convenient method for preserving the nutritive values of feeds (Kilic, 2005; Khorsed et al., 2006). In this experiment the NDF content is comparable to Bakshi et al. (2006) (31.5%) and it is lower than the values observed by Alcicek et al. (2000) and Meneses et al. (2007).

Fermentation characteristics of silage

The fermentation indices of cabbage and cauliflower waste silage are presented in Table 5. The silage exhibited an acidic pH, with the presence of acetic acid, while no butyric acid was detected. The lower pH levels in molasses-added silage may be attributed to the additional water-soluble carbohydrates from molasses, which facilitated lactic acid production by lactic acid bacteria (LAB) (Colombatto et al., 2003; Mtengeti et al., 2013). Additionally, the reduced NH3-N levels in molasses-treated silages could be due to lower proteolysis. as molasses restricts proteolysis by reducing silage pH (Colombatto et al., 2003; Mtengeti et al., 2013).

Table 5: Fermentation indices of cabbage and cauliflower waste silage

Parameters	I	II	III	IV	V	VI
pH	4.50	4.00	4.50	4.10	3.50	3.88
NH_3-N g/kg DM	2.43	2.24	2.02	1.58	1.20	1.13
LAB Log_{10} cfu/g	8.62	8.84	8.79	8.96	9.00	9.05
Lactic acid (%)	2.64	2.41	2.73	3.20	3.16	3.27
Acetic acid (Mm/L)	3.56	3.30	3.24	3.81	3.56	3.83
Butyric acid (%)	ND	ND	ND	ND	ND	ND

The higher LAB count observed in the latter three silages (IV, V, and VI) in this study was associated with the addition of molasses, which provided water-soluble carbohydrates as nutrients for LAB. This finding is consistent with the results of Chen et al. (2014). The highest lactic acid content was recorded in silage VI, while the lowest was found in cauliflower waste silage without molasses. These values align with the findings of Kinh et al. (2010).

***In vitro* evaluation of cabbage and cauliflower waste silage**

in vitro DM digestibility (IVDMD), total gas and methane production of cabbage and cauliflower waste-based silage was presented in Table 6. The IVDMD (%) is significantly differ among various cauliflower waste silage ($P<0.05$). However non-significant difference was observed in cabbage waste silage. The percent total gas production and methane production in both cabbage and cauliflower waste silage is non-significant ($P>0.05$). The IVDMD (%) is significantly differ ($P<0.05$) among various cauliflower waste silage. The ensiling cauliflower waste resulted in depression in gas production and *in vitro* digestibility of dry matter, which could be because of the very low dry matter content. Similar results also reported by Bakshi et al. (2006). The high gas production observed in cabbage suggests its strong potential for rumen fermentation. The low NDF content in vegetable wastes contributed to the increased in vitro gas production and digestibility. Mekasha et al. (2002) reported comparable in vitro dry matter digestibility (IVDMD) values of 80.4% for cabbage waste. High IVDMD values indicate a lower presence of cell wall constituents, aligning with the low ADF and high NDF content.

Table 6. *in vitro* digestibility and total gas production of cauliflower waste silage

Silages	IVDMD (%)	Total gas (ml/g DM)	Methane (ml/g DM)
0% CBS	71.36	146.34	21.95
10% CBS	69.63	135.34	15.12
20% CBS	63.01	122.91	17.38
30% CBS	62.73	129.40	14.09
40% CBS	60.25	120.37	15.07
50% CBS	58.82	117.16	12.72
SEM	3.22	7.15	3.34
P value	0.091	0.918	0.988
0% CLS	77.03^{b}	130.34	24.12
10% CLS	63.01^{a}	129.16	20.07
20% CLS	62.98^{a}	122.91	17.09
30% CLS	61.36^{a}	126.40	15.72
40% CLS	59.63^{a}	120.37	13.30
50% CLS	57.82^{a}	119.82	12.95
SEM	2.13	6.77	2.32
P value	0.021	0.788	0.870

CBS: Cabbage waste silage; CLS: Cauliflower waste silage
Values bearing different superscripts a, b in a row differ significantly ($P<0.05$)

CONCLUSION

This study suggests that incorporating 5% molasses into cabbage and cauliflower silage significantly improves fermentation stability and nutritional value, making it a viable livestock feed alternative. Future studies should focus on long-term feeding trials to validate its effectiveness in animal diets.

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Quality Parameters and Fermentation Characteristics of Ensiled Water Hyacinth (*Eichhornia crassipes*)

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ABSTRACT

The present experimentwas performed to explore the nutritional potential of wilted Water Hyacinth silage as an unconventional feed resource. A total of seven Water hyacinth silage; six were wilted viz. T1 (Eichhoria78.9%+ rice straw (RS) 16.84%+ molasses 4.2%) T2 (Eichhoria 78.9%+ RS 16.3+ molasses 4.2%+Urea 0.52%); T3 (Eichhoria 78.9%+ RS10.5%+ molasses 4.2%+ wheat bran (WB) 6.3%); T4 (Eichhoria 78.9%+ RS 10.5%+ molasses 4.2%+ moringa 6.3%); T5 (Eichhoria 68.42%+ RS 26.31%+ molasses 4.2%); T6 (Eichhoria 68.42% + RS 26.31%+ molasses 4.2% and Urea 1.05%) and one without wilting T7 (Eichhoria100%) were prepared.The DM and NDF content was (P<0.001) found to be higher in T-5 and T-6. However, T-3 and T-5 treatment WH silage produced with higher (P<0.001) C.P. content.The fibre content NDF, ADF and cellulose levels were lower(P<0.001) in T-3 and T-4 and higher (P<0.001) RS26.31% (T5 and T6). Addition of 10.5% RS +6.3 % WB or 6.3% moringa (T3 and T4) resulted in (P<0.001) increase of CP, DM intake (%BW), digestible DM (%), TDN (%), RFV and RFQ. NGP (ml/g) decreased (P<0.05) in T-3 and T-4, whereas OMD (%) and ME (Kcal/g), NDFD%, PF and MBP levels were augmented (P<0.001). WH silage (T7) was especially poor in quality with unacceptable odor and colour. It may be stated that wilted water hyacinth silage may be enhanced with rice straw (10.5%) or wheat bran (6.3%) or *moring aoleifera* (6.3%) and molasses (4.2%) with the view to use as unconventional feed resource in animal feed.

KEYWORDS: Fermentation Characteristics, Quality parameters, Silage, Water hyacinth

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INTRODUCTION

Water hyacinth (*Eichhorniacrassipes*) is a plant that floats and is found in waters around the globe.The water hyacinth (WH) is recognized for its capacity to puritfy water waste. Due to its rapid growth, it is considered a noxious water weed that can hinder fish farming and power generation (De Groote et al., 2003). Limited research has been carried out to its use as a feedstuff for the livestocks. WH can be fed to fish and pigs (Yang Huazhu et al., 2001) and to ruminants in fresh, dried or ensiled material. Islam et al.(2009) supplemented wilted WH in growing bulls and reported increased protein conversion efficiency and average daily gain (ADG) performance due to increased level of digestible crude protein (DCP) and total digestible nutrients (TDN) content in the diet. However, feed intake is often limited in animals when whole water hyacinth was fed to the animal instead of feeding after processing (Aregheore and Cawa 2000). Reduction in dry matter intake might be due presence of oxalate crystals which can reduce palatability (Franceschi et al., 1980; Bolenz et al., 1990). Processing through different methods viz. physical and chemical treatments, cooking and fermentation can enhance its nutritive value and utilization of water hyacinths in animal feed (Bolenz et al., 1990; Jafari, 2010)

Ensiling is an effective practice of feed preservation that can improve palatability and enhance nutrient utilization in animals. However, ensiling a high-moisture level materials like water hyacinth remains challenging. Earlier, Water hyacinth dried through shredding or screw-pressing following wilting in a shed for 48 hours (Göhl, 1982). However, later studies reported that the procedure of drying can leads to higher degree of nutrient losses while it can be prevented by different methods of

reconstitution and ensiling water hyacinth with low-moisture feedstuffs, leftover feed ingredients and additives such as urea, molasses and others (Simsa et al., 1993; Indulekha et al., 2019). This study's goal was to examine the nutritional potential of ensiled water hyacinth made with different combinations of rice straw, wheat bran, moringaoleifera, urea and molasses.

MATERIALS AND METHODS

Trial area and procurement of material

The study was carried out during the year 2024 at the Department of Animal Nutrition, Guru AngadDev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India (30°562 N, 75°522 E, 247 m above sea level) between November 2021and February 2022. The study was planned to examine the nutritional potential of wilted Water hyacinth silage as an unconventional feed resource. The Water hyacinth was collected from the College of Fisheries Science, GADVASU, Ludhiana, Punjab, India and wilted for 48 hours in the shade.

Preparation of silage

The wilted water hyacinth was chopped (into 2-4 cm) pieces, separately mixed with different feed ingredients and ensiled for 45 days in low-density polypropylene tubes bags. Total weight of each WH silage treatment bag was 95 kg.A total of seven Water hyacinth silage (Table 1); six were wilted viz. T1 (Eichhoria78.9%+ rice straw (RS) 16.84%+ molasses 4.2%) T2 (Eichhoria 78.9%+ RS 16.3+ molasses 4.2%+Urea 0.52%); T3 (Eichhoria 78.9%+ RS10.5%+ molasses 4.2%+ wheat bran (WB) 6.3%); T4 (Eichhoria 78.9%+ RS 10.5%+ molasses 4.2%+ moringa 6.3%); T5 (Eichhoria68.42%+ RS 26.31%+ molasses 4.2%); T6 (Eichhoria 68.42% + RS 26.31%+ molasses 4.2% and Urea 1.05%) and one without wilting T7 (Eichhoria100%) were prepared.

Table 1. Ingredient composition of silage prepared from *Eichhornia* in kgs

Ingredients	Wilted water hyacinth Silage (kg)						
	T -1	T-2	T-3	T-4	T-5	T-6	T-7
Eichhornia	75	75	75	75	65	65	95
Molasses	4	4	4	4	4	4	-
Rice straw	16	15.5	10	10	26	25	-
WB	0	0	6	0	0	0	-
Moringa	0	0	0	6	0	0	-
Urea	0	0.5	0	0	0	1	-
Total (kg)	95	95	95	95	95	95	95

Estimation of nutrient content

Chemical analysis of water hyacinth prior to ensiling and after each treatment method was done by drying samples in hot air oven (Narang Scientific Works, India) at 60 o Celsius in a period of 48 hrs to determine dry matter (DM). All samples were homogenized in a laboratory using Wiley mill to pass a 1-mm sieve and then analysed in respect to crude protein (CP), ether extract (EE), total ash, organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al., 1991) and acid detergent lignin (ADL) under proximate (AOAC, 2005). The method used to determine cellulose was that of Crompton and Maynard (1938).

Estimation of TDN, net energy lactation, relative feed values and relative feed qualities of silage

Parameters such as total digestible nutrient (TDN), net energy for lactation (NE_L, dry matter intake as a percentage of body weight (DMI% BW),Digestible dry matter, (DDM%), relative feed value (RFV) and relative feed quality (RFQ) were calculated by using equations given by Schroeder (2004) to evaluate the nutritive potential of the

different treatment silages.

DMI (% BW) = 120 / (% NDF)

Digestible dry matter (DDM) = 88.9 - (0.779 ×% ADF)

RFV = (% DDM ×% DMI) / 1.29

RFQ = (TDN × intake) / (16.8 + 39.2)

TDN = 87.84 - (0.79×% ADF)

NEl (Mcal/kg) = 0.0245 × TDN - 0.12

Estimation of *in vitro* gas productions and digestibility

The procedure of rumen liquor involved taking rumen fluid in the morning without feeding male buffaloes fitted with rumen fistulas and subjected to diet with 2 kg conventional concentrate mixture, 17 kg green fodder and 3 kg wheat straw. The rumen was sampled in the thermo bottle and immediately moved to the laboratory and thereafter passed through 4 layered muslin cloth. The SRL was preserved in 39 ! and the addition of CO_2 circulation was fixed. This was followed by SRL dilution (1:4 v/v) in the culture media (which contained the macro, micro-mineral solution, medium resazurin, as well as a bicarbonate buffer solution as yourself, according to Menke and Steingass, 1988) and the *in-vitro* assay approach to gas production was adopted which included the measurement of feed digestibility, net gas production (NGP) as well as OM and measuring the NDF digestibility (Van Soest and Robertson, 1988). The amount of gas was figured to derive ME (Menke et al., 1979). A partitioning factor (PF) was determined in accordance with the description of the procedure provided by France et al. (1993).

Statistical analysis

The one-way analysis of variance (ANOVA with full factorial design and fixed factor of treatment) was used to write data concerning chemical constitutions of every variety. All the statistical processes were carried out in SPSS (2012) and posthoc was conducted using Tukey b test and significance was set at $P<0.05$

RESULTS AND DISCUSSION

Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and acid detergent fiber (ADF) contents of silage prepared with various treatments were statistically similar (Table 2). The chemical composition of wilted water hyacinth fell within the range reported in previous studies (Li et al. 2007; Tham et al., 2013). The DM content of Water hyacinth silage in various treatment groups ranged from 17.2 to 25.5% DM, and the silage without it was found to be 7.25% DM. DM content was reported highest in T-5 and T-6, while lowest in T-7. CP and EE content were found ($P<0.001$) higher in T-4 and T-6 silage. These findings have an agreement with previous studies (Liu et al., 2001; Tham et al., 2013; Indulekha et al., 2019) reported the addition of wheat bran with molasses can enhance DM, CP, WSC and their fermentation characteristics. However, ensiling Water hyacinths remain challenging due to their high moisture content. Baldwin et al. (1974) found unsuccessful in achieving good fermentation quality with water hyacinth. Wilting of water hyacinth done in sheds can regulate water activity and their moisture level desired for the ensiling. However, prolonged wilting can leadtoan excess loss of dry matter, nutrients and water-soluble carbohydrates (WSC) which can also reduce acetic acid levels in ensiled fodder and affect silage quality (Liu et al., 2001). Therefore, the findings of present the study can suggest most suitable combination of Water hyacinth silage with feed ingredients and additives that can expedite wilting, prevent nutrient loss and improve its quality. Usually, rice straw usedas a complement of dry matter and to provide absorbent properties, while wheat bran serves as a source of WSC for silage (Malek et al., 2008; Liu et al., 2001).

Results (Table 2) indicate that fibre content NDF, ADF and cellulose in T-4 and T-3 reduced ($P<0.001$) and found to be higher ($P<0.001$) in T-5 and T-6 treatment WH silage. However, NDF, ADF and cellulose content wasalso found lower in T-7 silage and similar to T-3 and T-4, however, DM content in T-7 WH silage was lowest in all silages therefore, results are not completely relatable to the T-3 and T-4 silage treatments. Therefore, the finding suggested that adding dry matter through wheat bran or moringa instead of rice straw can increase CP and WSC content while adding DM through rice straw can increase fibre content (NDF and ADF) in wilted water hyacinth silage. Variation in results from different studies might be due to the different additives, feed ingredients and plant material used for silage. Studies have suggested that ADF and NDF content are reduced with the addition of 10% wheat bran (Li et al., 2007), *moringaolifera* or urea and molasses (Abo-Donia et al., 2022). A lower ADF is inversely related to a higher total digestible nutrients (TDN) value, which indicates higher feed intake and feed utilization in animals.

Table 2. Nutrient composition and feed values of silage prepared from wilted *Eichhornia*

Parameter	T-1	T-2	T-3	T-4	T-5	T-6	T-7	SEM	P value
Nutrient composition, % DM									
Dry matter	21.2^{c}	17.2^{b}	21.4^{b}	21.4^{b}	25.0^{c}	25.5^{c}	7.25^{a}	0.638	<0.001
Crude protein	7.92^{c}	8.20^{c}	6.34^{a}	14.9^{e}	7.29^{b}	13.0^{d}	16.3^{f}	0.119	<0.001
Ether extract	2.00^{b}	1.90^{b}	1.90^{b}	2.37^{c}	1.35^{a}	1.35^{a}	1.60^{b}	0.113	0.001
Neutral detergent fiber	66.3^{d}	61.7^{c}	58.1^{b}	53.9^{a}	69.6^{e}	67.9^{de}	60.4^{bc}	0.770	<0.001
Acid detergent fiber	52.1^{cd}	48.8^{c}	37.8^{b}	37.2^{b}	49.7^{c}	55.5^{d}	30.7^{a}	1.307	<0.001
Acid detergent lignin	6.70^{cd}	7.45^{d}	5.23^{a}	6.03^{bcd}	5.37^{abc}	6.95^{d}	4.10^{a}	0.387	0.004
Cellulose	45.4^{d}	41.6^{c}	32.6^{b}	31.2^{b}	44.4^{cd}	48.5^{e}	26.6^{a}	0.998	<0.001
Hemi-cellulose	14.2^{ab}	12.7^{a}	20.3^{d}	16.7^{bc}	19.9^{cd}	12.1^{a}	29.6^{e}	0.917	<0.001
Ash	18.0^{b}	20.1^{d}	16.2^{a}	15.6^{a}	18.8^{c}	19.0^{e}	16.0^{a}	0.170	<0.001
Organic matter	82.0^{c}	79.8^{a}	83.8^{c}	84.4^{d}	81.2^{b}	81.0^{b}	84.0^{d}	0.170	<0.001
Feed values									
Dry matter intake, % BW	1.80^{ab}	1.94^{c}	2.06^{d}	2.23^{e}	1.72^{a}	1.77^{ab}	2.01^{d}	0.023	<0.001
Digestible dry matter, %	48.3^{ab}	50.7^{b}	59.4^{c}	59.9^{c}	50.1^{b}	45.4^{a}	65.0^{d}	1.018	<0.001
Total digestible nutrients, %	50.4^{ab}	53.5^{b}	61.4^{c}	61.8^{c}	53.0^{b}	48.7^{a}	66.3^{d}	0.915	<0.001
Relative feed value	67.9^{a}	76.5^{b}	95.1^{c}	103^{d}	67.0^{a}	62.4^{a}	100.0^{cd}	2.167	<0.001
Relative feed quality	1.67^{a}	1.86^{b}	2.35^{cd}	2.46^{d}	1.63^{a}	1.54^{a}	2.35^{d}	0.048	<0.001
$NE_{L,}$ M cal/kg	1.14^{ab}	1.19^{b}	1.38^{c}	1.39^{c}	1.18^{b}	1.07^{a}	1.50^{d}	0.022	<0.001

NE_{L}: Net energy lactation; SEM: standard error mean.
Values with different superscripts variedsignificantlyin a row($P<0.05$).

Water hyacinths are characterized by bulbous and elongated petiole structures with relatively low dry matter content, which may affect intake (Parsons and Cuthbertson 2001). Results from the study showed (Table 2) that T-3 and T-4 WH silage found higher dry matter intake (% body weight), dry matter digestibility (DMD%), TDN and net energy for lactation (NE_{L}). Additionally, the relative feed value (RFV) of water hyacinth silage was higher in T-4 treatment, and relative feed quality (RFQ) was higher for treatments T-4 and T-7. RFV and RFQ values are commonly used through in-vitro models. The RFV is a parameter used to predict voluntary feed intake and assess the quality of the feed, using the NDF and ADF content in the feed. Relative feed value and quality of silage was enhanced with the addition of urea and molasses. The supplementation of wheat bran and moringaolifera in silage decreases ADF and NDF and improves the feed value and feed quality. Similarly, other trials (Hundal et al., 2019; 2020; Gursoy et al., 2023; Abo-Donia et al., 2022) have recorded the impact of additives in the values of RFQ and RFV of different usual fodder silages (maize/wheat) and non-conventional fodder silages.

Results pertaining to in-vitro gas production method (Table 3) suggests that net gas production, total organic matter digestibility (TOMD) and metabolizable energy were higher in treatments, T-3 and T-4. The ME can of the WH silages without

wilting were 5.41 MJ/kg, which denote that the addition of wheat bran or moringa to the WH silages along with urea and molasses raised the ME content of the T-3 and T-4 sets. On the same note, the ME of silage was also found to have a similar effect with molasses and urea addition to it (Li et al., 2014; Gursoy et al., 2023). T-4 and T-3 groups were also found to have higher (P<0.001) level of neutral detergent fibre digestibility (NDFD%), partitioning factor (PF) and microbial biomass production (MBP), but lower (P<0.05) level of NGP.Reason of increased NDFD digestibility might be lower NDF and ADF levels in these groups due to addition of wheat bran or moringa in silage. Microbial protein and PF values increases as the gas production decreases, which indicated efficiency of feed shifted towards microbial protein production (Gürsoy et al., 2023).

Table 3. Fermentation Characterstrics of wilted *Eichhornia* silage

Parameter	T-1	T-2	T-3	T-4	T-5	T-6	T-7	SEM	P value
NGP, ml/g	64.6^{bc}	72.5^{c}	55.4^{ab}	51.2^{a}	55.0^{ab}	55.6^{ab}	43.7^{a}	3.327	0.007
NDFD, %	40.3^{c}	40.3^{c}	49.2^{d}	41.6^{c}	27.7^{a}	34.0^{b}	21.4^{a}	0.430	<0.001
TOMD, %	48.6^{b}	53.9^{c}	55.8^{d}	58.2^{e}	47.9^{b}	48.0^{b}	41.9^{a}	0.430	<0.001
PF, mg/ml	7.71^{a}	7.60^{a}	9.77^{bc}	11.5^{c}	8.73^{ab}	8.63^{ab}	9.62^{abc}	0.546	0.006
MBP, mg/g	423^{b}	488^{c}	485^{c}	533^{d}	426^{b}	426^{b}	377^{a}	4.694	<0.001
ME, MJ/kg	4.66^{ab}	4.76^{abc}	5.26^{d}	5.13^{cd}	4.83^{bc}	4.46^{ab}	5.41^{a}	0.110	0.005

Values with different superscripts in a row varied significantly(P<0.05).

The study highlights the potential benefits of using different feed ingredients combinations of additives and dry matter sources to improve the nutritional quality and fermentation characteristics of water hyacinth silage. Proper evaluation of unconventional feed sources is essential before incorporating them into ruminant diets.

CONCLUSION

The livestock and agriculture sectors are essential to social and economic development in rural India. Sustainable livestock farming can be possible with enough fodder availability. One way to increase fodder availability is to utilize unconventional feed for the profitability of livestock husbandry. Therefore study was conducted to know the nutritional potential of ensiled water hyacinth silage to be used as an unconventional feed resource for animals. Results indicate that wheat bran or *moringaolifera* with urea and molasses were the most suitable combinations for Water hyacinth silage preparation, and can improve nutritional composition (DM, CP, EE) nutritive value (DM digestibility, TDN, RFV and RFQ) and fermentation characteristics (NDFD, TOMD and MBP). Supplementing moringa or wheat bran along with rice bran, urea and molasses can improve water hyacinth utilization in animal feed and can assure sustainable fodder availability.

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Nutrient Utilization and Growth Performance of *Jalauni* Lambs Grazed on Three Tier Silvipasture System

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ABSTRACT

Chemical composition of pasture biomass as well as nutrient utilization and growth performance of *Jalauni* lambs were studied under grazing on three tier silvipasture consisting (stocking rate of 2ACU/ha) of grass (*Cenchrusciliaris*), legume (*Stylosanthesseabrana*), shrubs(*Ziziphusxylopyrus, Ziziphusmauritiana, Acacia catechu)* and tree (*Hardiwickiabinata*) during growing (August-October) as wellas post-growing (November-January) periods of pasture in Bundelkhand region along with supplementation (1% ofbody weight) of concentrate mixture. Average dry matter (DM) content of pasture increased with advancement of maturity from 42.89% during growing period to 52.59% in post growing period, with concomitant increase in neutral detergent fiber (NDF) content from 57.67% to 61.36% and reduction in crude protein(CP) content from 11.55% to 8.64%. DM intake of lambs was significantly ($P<0.05$) higher in growing than post growing period. Similarly, digestibility of nutrients namely DM, OM, NDF and CP were higher in growing than post growing period. Intake ($g/W^{0.75}$) of digestible crude protein (DCP) was higher (6.15) in September as compared to December (3.74). Metabolizable energy (ME) intake ($kJ/W^{0.75}$) also followed the same trend.Daily live weight gain (g/d) of *Jalauni* lambs was also significantly ($P<0.05$) higher in growing than post growing period. It was concluded that nutrient intake, nutrient utilization and growth performance of *Jalauni* lambs were significantly ($P<0.05$) affected during post growing period due to deterioration ofnutritive value of available pasture biomass, however, three tier sylvipasture system in the present study could be utilized for rearing of small ruminants for sustainable production.

KEYWORDS: Chemical composition, Growth performance, *Jalauni* lambs, Nutrient utilization, Seasonal variation, Three tier silvipasture

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INTRODUCTION

Silvipasture is the practice of integrating trees, forage, and the grazing of domesticated animals in a mutually beneficial way and is well suited for rearing domesticated animals, particularly small ruminants. Sheep play a significant role in the subsistence economy of farmers in the country. Sheep provides wool, meat and raised generally under grazing on degraded range lands and or offered low quality fibrous feedstuffs like cereal straws and stubbles. The production of meat from sheep play role in the supply of animal protein for human consumption. Small ruminant production in village systems in tropical countries is often characterized by poor growth rates and high mortality (Suresh and Chaudhary, 2015). The productivity of grazing animals can be enhanced by improving the nutrition either through concentrate feeding or by providing additional forage (Salem, 2010).Although the potential of silvipastoral systems in enhancing fodder production is widely known but there is a paucity of information on nutritional aspects of animal grazing freely on such reconstituted silvipasture. The objectives of the present experiment were to assess seasonal variations on intake,nutrient utilization and growth performance of *Jalauni* lambs kept on three tier silvipasture system.

MATERIALS AND METHODS

Twenty *Jalauni* lambs of 9-11 months age (average body weight 25.32± 1.46)were allowed to graze on 1.65 ha (stocking rate of 2 ACU/ha)for 7 hours daily in 6 years old synthesized (3 tier) silvipasture comprising of grass (*Cenchrusciliaris*),

legume (*Stylosanthesseabrana*), shrubs (*Ziziphusxylopyrus, Ziziphusmauritiana, Acaciacatechu)* and tree (*Hardiwickiabinata*), The animals were also supplemented with concentrate mixture (comprising of mustard cake, maize, wheat bran, mineral mixture and common salt; 35: 50: 13: 1: 1)@ 1.0% of their body weight at Central Research Farm, Indian Grassland and Fodder Research Institute, Jhansi during growing (August-October) as well as post growing (November-January) periods. The annual forage production potential of the silvipasture system from ground and above ground vegetations were estimated as per the procedure described by Prajapati, (1980).Body weight of animals were recorded fortnightly. After 50 days of experimental grazing, digestion trial of 6 day duration was conducted in the month of September and December on 6 animals each following lignin as internal marker (Ranjhan,1994). Total faeces voided for 24 hr were collected using faeces collection bags.

Botanical composition of the diet

A direct observation and simulation method was used to determine the botanical composition of the diet consumed by the animals. Samples of the ingested species that were being taken by the animals were hand clipped for three consecutive days. The individual animals were observed and forage samples were collected for the entire grazing period from 9 am to 4 pm (Pepeta et al., 2022).

Analysis of samples and data

The representative samples of feeds and faeces collected during digestion trial were analyzed for dry matter (DM), ash and ether extract according to AOAC (1995). Samples were also analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Goering and Van Soest., 1970).Total nitrogen was determined by micro-kjeldhal method (AOAC, 1995). Mean data were compared over different seasons for statistical differences using Student's t-test (Snedecor and Cochran, 1989)

RESULTS AND DISCUSSION

Chemical composition of silvipasture

Chemical composition of pasture forages in silvipasture system indicated that DM content of different forages varied from 38.25% (*C.ciliaris*) to 47.26% (*A. Catechu*) during growing period and 45.00% (*H.binata*) to 59.53% (*Z.xylopyrus*) in post growing period (Table 1). Similar to present findings, Navaleet al.(2022) also recorded higher DM content (62.50%) in *A. catechu* among different forage species. The total ash contents in all the feeds were similar during both the periods. Similar to the present finding, Dattet al.(2008) also reported an average OM concentration of around 90% in different fodder tree leaves. CP content varied from 15.48% in *A. catechu* to 6.84% in *C.ciliaris* during growing while during post growing period the values range from 13.57% (*A.catechu*) to 3.83% (*C.ciliaris*). Jindal and Satpal,(2020) also reported similar CP and NDF content in different *C.ciliaris* varieties. However, Coelho et al.(2018) reported higher CP content (9.8%) and low lignin content (2.2%) than the present findings which might be due to different stage of harvesting of plant samples.The frequency of harvesting also in general can promote improvement in the quality of the nutritional traits of warm-season grasses.

Table 1.Seasonal variation in chemical composition of pasture vegetation (%DM basis)

Forage species	Period					
	Growing Period					
	DM	CP	NDF	ADF	Lignin	Ash
Z.xylopyrus	46.96	12.96	47.23	35.44	10.58	7.01
Z.mauritiana	41.08	13.35	49.98	35.49	8.88	7.15
A. catechu	47.26	15.48	51.35	39.09	9.55	6.73
H. binata	44.57	10.20	58.87	38.13	10.84	9.08
S. seabrana	39.22	10.52	61.17	45.99	9.40	6.42
C. ciliaris	38.25	6.84	77.47	50.49	6.55	7.74
	Post growing Period					
Z.xylopyrus	59.53	10.74	50.17	40.88	11.17	7.64
Z.mauritiana	45.75	10.91	52.09	39.60	11.98	6.92
A. catechu	50.65	13.57	54.53	43.04	12.32	8.91
H. binata	45.00	8.14	62.97	45.14	13.4	9.69
S. seabrana	58.74	6.48	69.98	49.59	10.69	5.02
C. ciliaris	55.90	3.83	78.44	54.14	7.26	7.25

NDF contents of browse legume component varied from 47.23% in *Z.xylopyrus* to 54.53% in *A.catechu*. Similarly, Hassen et al.(2017)also reported that almost all browse species had a moderate to high crude protein (CP) content (52.4 - 220 g/kg DM), moderate neutral detergent fiber (283 - 552 g/kg DM) and acid detergent fiber (128 -433 g/kg DM) contents.Higher lignin content was observed in *H. Binata* as compared to other legumes in both the seasons and corroborated with the earlier study of Singh et al.(2016). With the advancement in plant maturity from September to December, the average crude protein content of pasture biomass was decreased by 22.51% whereas on the other hand, NDF and lignin content was increased by 6.40% and 19.57%, respectively during post growing period. Similarly, Singh and Todaria,(2012) reported that the CP contents of MPTs foliage declined as the season proceeded from summer to winter, i.e., from younger to mature leaves. This may be attributed to the dilution effect, which happens when nutrients (particularly N) are redistributed to other plant parts at the end of the growth cycle. Navaleet al.(2022).also reported the highest CP content in the spring season leaves (15.35%) and the lowest in winter (10.75%) and CF (20.58–28.94%) and carbohydrate contents (69.77–75.78%) had an opposite trend. Subhalakshmi et al.(2011) reported that the chemical composition of pasture is influenced by season, type of soil, stocking density, type of grazing pasture and climate. The nutritional content of any forage is dependent on its nutrient content such as protein, which is essential for the growth, development and production status of ruminant animals.During growing phase most of the pasture components were in pre-flowering/full bloom stage, during which the nutrient concentration is maximum.The change in nutrient composition could be correlated with stage of maturity.The differences in fiber components between season suggested that less amount of rainfall (Fig. 1) and lower temperature tend to affect the photosynthetic process,caused faster maturation resulting in lower in proximate composition during post growing period and this resulted in higher cell wall contents and lower cell contents than those of growing season (Ravhuhali et al.,2022).

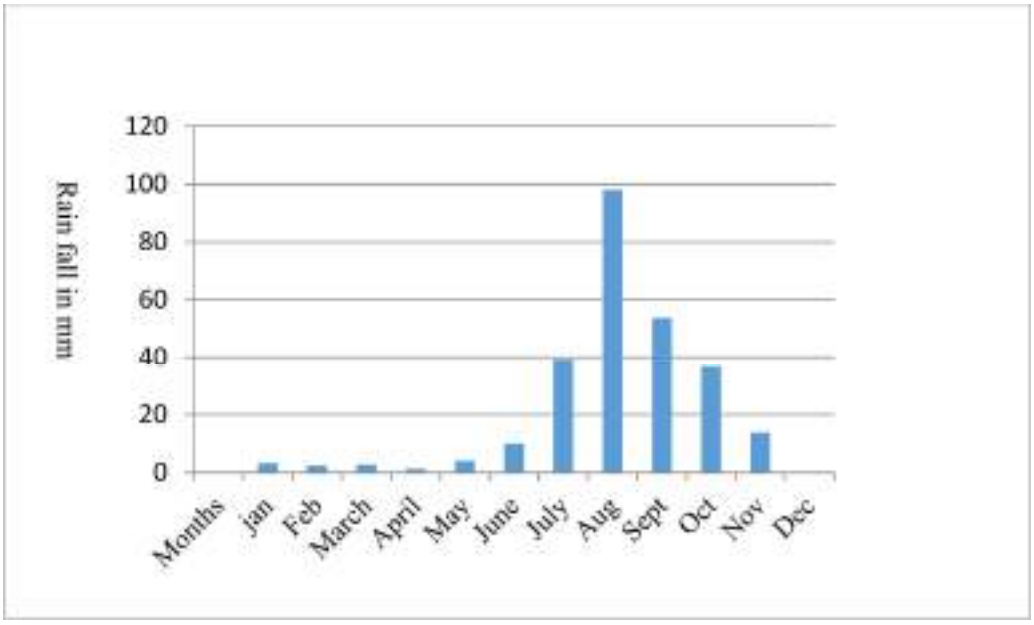

Fig-1.Rainfall pattern during the growing and post growing season

Nutrient intake and digestibility

The forage biomass that was consumed by the animals under grazing were primarily consisted of grass (*C. cilliaris)*, legume (*stylosanthesseabrana)* shrubs (%(*Z. xylopyrus, Z.mauritianaA. catechu* and trees *(H. binata)*.The present finding on dry matter intake from grazing during digestion trial indicated that daily DM intake (kg/100 kg body weight) in sheep was decreased from growing to non growing period of pasture biomass (Table 2). Das et al.(2021) also reported that pasture DM intake by the growing lambs was 3.15% during August to September and reduced to 2.45% during November to January while grazed on *H.binata* based sylvi-pasture system.

Table 2.Nutrient intake in *Jalauni* lambs from grazing in three-tier silvipasture system

Parameters	Growing period	Post growing period	*SEM*	*P Value*
Pasture intake(kg)	0.832	0.802	0.05	0.537
Concentrate intake(kg)	0.283	0.342	0.02	0.003
Total DM intake(kg)	1.114	1.144	0.06	0.585
DMI%BW	3.90^{b}	3.41^{a}	0.12	0.004
Pasture intake, % B W	2.91^{b}	2.39^{a}	0.12	0.003
Pasture intake,% DMI	74.38	71.10	2.03	0.064
DMI g/kg $W^{0.75}$	89.57^{b}	79.93^{a}	4.01	0.043
CPI (g)/kg $W^{0.75}$	10.61^{b}	8.15^{a}	0.49	0.001
DCPI (g)/kg $W^{0.75}$	6.15^{b}	3.74^{a}	0.28	0.000
TDNI (g/kg $W^{0.75}$)	59.19^{b}	45.96^{a}	0.83	0.002
MEI (kJ/ kg $W^{0.75}$)	863.16^{b}	695.26^{a}	13.19	0.015
N intake (g/kgDOMI)	32.34	31.21	0.87	0.230

*Means bearing different superscript in a row differ significantly (P<0.05)

A rapid increase in the herbage fiber, namely the ADF content, over time from the growing to post growing season and the maturity-driven decline in forage quality negatively affects forage DMI (Van Soest, 1994). Xiao et al.(2020) also reported that the change of grazing season (from warm season to cold season) had a negative effect on DMI. This is because there were of higher ADF and NDF concentrations, harder stems, and fewer leaves in pasture biomass in the cold season, therefore, Tibetan sheep had lower DMI in the cold season. Similarly, Askar et al.(2014) also reported that increased forage lignin and consequent decline in digestibility have negative effects on voluntary intake. The grazing season can also indirectly affect DMI. It can reduce the bite weight and thus the DMI. Nitrogen content and cell wall constituents are important factors which determine feed intake. N content has a significant influence on microbial activity in the rumen, and the cell wall content affects the outflow rate of rumen contents (Weston, 2002).Mir et al.(2018) and Amiri et al. (2012) reported that due to increase in fibre and lignin content and decreasein CP content of the feed with maturity directly affects the intake by decreasing the palatability. Similarly, Carvalho et al.(2022) also reported greater DMI during the rainy seasonby the experimental ewes, due to better forage nutritive value relative to other seasons. Pasture intake depends upon digestibility of pasture, rumen fill, metabolic factors, chemical and physical properties of concentrate and stage of growth. DM intake(g) per kg metabolic body weight in *Jalauni* lambs ranged from 89.57 to 79.93, respectively, in different seasons. However, Shinde and Mahanta,(2020) reported lower DM intake (g/ $kgW^{0.75}$) values ranging from 49.6 in monsoon to 43.7 in winter in sheep grazed on range land which might be due to lower biomass availability from such pasture. Similar with the present findings, DM intake levels of 40-90(g/ kg $BW^{0.75}$) have been reported as normal for grazing ruminants (Cordova et al.,1978).

Digestibility of DM, OM,CP and NDF were significantly ($P<0.05$) higher in growing than in post growing period (Table 3). This indicates the impact of seasonality on forage nutritive value and, consequently, nutrient utilization by the lambs.

Table 3.Nutrient utilization and growth performance of *Jalauni* lambs grazed on tree-tier silvipasture system

Apparent digestibility(%)	Growing period	Post growing period	*SEM*	*Pvalue*
DM	61.28^{b}	52.85^{a}	0.96	0.000
OM	64.95^{b}	57.36^{a}	0.69	0.000
CP	58.04^{b}	46.01^{a}	1.45	0.000
NDF	56.49^{b}	51.10^{a}	1.10	0.004
ADF	51.21^{b}	44.53^{a}	1.11	0.000
Cellulose	70.26^{b}	66.28^{a}	1.98	0.078
Nutritive value(%)				
DCP	6.97^{b}	4.72^{a}	0.24	0.000
TDN	65.61^{b}	57.41^{a}	0.92	0.000
ME(Mcal/kg)	2.35^{b}	2.01^{a}	0.09	0.004
Initial body weight(kg)	25.32^{b}	30.99^{a}	0.72	0.000
Final body weight(kg)	30.99^{b}	34.54^{a}	0.71	0.000
Gain in body weight(kg)	5.67^{b}	3.55^{a}	0.09	0.000
Average daily gain (g/d)	61.63^{b}	38.59^{a}	1.16	0.000

*Means bearing different superscript in a row differ significantly ($P<0.05$)

As the season shifts towards dry season, the plants in the pasture grow old increasing the lignin and fibre content and thereby decreasing the digestibility (Mayouf and Arbouche,, 2015). Moreover, encrustation of lignin with cellulose, hemicelluloses and proteins of the cellwall render them inaccessible to microbes thereby decreasing their digestibility. Similarly, Sun and Zhou,(2007) also reported that metabolizable energy content and digestibility of DM, GE,OM, CP, NDF and ADF in sheep grazed on *Leymuschinensis* induced pasture were significantly greater (P<0.05) in spring and summer than in winter and autumn. With the decrease of CP and the increase of ADF and NDF in the diet of grazing lambs during the month of December, DM digestibility was decreased, which indicated a negative relation between the DM digestibility and the maturity of forage. Mertens, (1987) reported that diets with lesser DM digestibility during dry period, can restrict ruminant intake as a result of rumen fill limitations, with consequent negative effects.In the present study the digestibility of organic matter was reduced by 11.7% from growing to non growing period and corroborated with the earlier findings of Carvalho et al.(2022) where it was reported that rainy season diet OMD was 28.8 % higher than during the dry season in sheep indicating the association between organic matter digestibility and season.Greater OM digestibility in diets selected by lambs during the growing season indicates greater energy availability compared to post growing season which was reflected by higher intake of TDN or ME by the grazing lambs during growing season.

DCP intake (g/100 kg body weight) in lambs were in close agreement with the suggested values of ICAR(2013). DCP intake (g/kg $W^{0.75}$) was higher (6.15) in September than in December (3.74), which might be due to decrease in protein content of pasture biomass. Chaturvedi and Sahoo, (2013), however,observed much higher DCP intake in sheep from similar type of ration which might be due to superior quality of supplemented concentrate and roughage fed to the experimental animals.However,Shinde and Mahanta, (2020) reported lower DCP intake in sheep because of inferior quality of the pasture.ARC (1980) states that rumen microbes require 30 g of N from dietary sources per kg of OM apparently degraded for efficient rumen microbial activity and growth. In present study the N intake (g)values per kg DOM intake in lambs during both the periods indicatedits sufficiency for efficient utilization of energy by the rumen microbes and its optimum growth.TDN intake (g/kg$W^{0.75}$) was significantly higher (P<0.05) during growing period as compared to post growing period and sufficient for achieving a live weight gain of 60 g daily (ICAR, 2013).

Nutritive value in terms of DCP and TDN (%) were significantly (P<0.05) lower during post growing season as compared to growing season. Similar DCP and TDN contents were recorded by Das et al. (2021) in small ruminants under grazing on *H. binata* based sylvipasture system with concentrate supplementation. Sun et al.(2014) also recorded reduced energy content from autumn (9.04 MJ/kg) to winter season(7.94 MJ/kg) in cashmere goats grazing on *L. chinensis* pasture. The quality and quantity of grazing biomass is known to decline markedly after rainy season during onset of winter and imposes major constraint for small ruminant production as it was observed in the present study.

Average daily live weight gain showed significant difference for growing and non growing periods .Das et al.(2021) reported a daily gain of 53 g in *Jalauni* lambs grazed on *H. binata* based silvopasture system along with supplementation. Pent et al.(2020) also recorded a daily gain of 63 g in crossbred lambs gazed on black walnut (*Juglansnigra* L.)-based or honey locust (*Gleditisiatriacanthose* L.)-based silvopasture systems. A live weight gain of 20-22 kg with average daily gain (head/day) of 56-61 g and 93-102g in lambs and kids, respectively were recorded on two tier (*Cenchrusciliaris* + *A. excelsa*) and three tier (*C. Ciliaris* + *D. cinerea* + *A. excelsa*) silvopastoral systems with stocking density of 14 animals/ha (Ramana et al., 2000).Rao et al. (2013) however, reported higher body weight gain in sheep grazed on *L. leucocephala* based sylvipasture which might be due to better availability of nutrients to the animals.The average body weight and average daily gain varied (P<0.05) parallel to the level of protein availability as it is evident in different season in the present study.

CONCLUSION

It was concluded that nutrient intake and nutrient utilization of *Jalauni* lambs were affected significantly during post growing period due to deterioration of pasture quality.Results also indicated that three tier sylvipasture system under semiarid situation could be utilized for rearing of small ruminants for sustainable production.

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Effect of Dietary Chromium Supplementation in Transition Calves on Insulin Sensitivity and Biomarkers of Rumen Development

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ABSTRACT

A study of 100 days using calves in transition phase of growth (15 to 115 days) was conducted. 24 Hariana calves were randomly assigned to four groups of six each and fed on diets without supplemental chromium (Cr) as control or 0.05 mg, 0.10 mg, and 0.15 mg Cr/kg $BW^{0.75}$ groups. Treatment had no significant effect on the average daily gain (ADG) and body condition score (BCS). Plasma glucose, insulin, insulin: glucose ratio, and insulin receptor substrate – (IRS-1) as biomarkers of insulin sensitivity while â-hydroxy butyrate (BHBA) and insulin like growth factor -1 (IGF-1) as biomarkers of rumen development were studied on 0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days post-Cr supplementation. Plasma glucose and insulin concentrations decreased ($p < 0.05$) with the advancement of age of calves and their levels were lowest ($p < 0.01$) in the calves supplemented with highest Cr level (0.15 mg of Cr/kg $BW^{0.75}$). The insulin: glucose ratio was higher ($p < 0.05$) in the calves supplemented with Cr. Treatment, period and treatment × period interaction had a significant effect on plasma IRS-1 concentration as its concentration increased with the increase in supplementation level of Cr. Treatment and treatment × period interaction had non significant on plasma BHBA and non-esterified fatty acids (NEFA) levels while period had significant effect on BHBA ($p<0.05$ and NEFA ($p<0.01$) concentrations. However, a significant effect of treatment, period, and treatment × period interaction on plasma IGF-1 concentrations was observed with higher ($p < 0.01$) plasma IGF-1 concentration in 0.15 mg of Cr/kg $BW^{0.75}$ supplemented calves. In conclusion, the dietary supplementation of Cr was beneficial in improving insulin sensitivity and modulating biomarkers of rumen development of calves during the transition period from pre-ruminant to ruminant phase.

KEYWORDS: Biomarkers, Chromium, Rumen development, Transition calf,

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INTRODUCTION

The process of transitioning of dairy calves from their pre-ruminant to the ruminant stage results in various metabolic ramifications (Baldwin et al., 2004). The energy metabolism of dairy calves during this phase experiences major changes with the shift from a pre-ruminant glucose, cholesterol, and â-hydroxybutyric acid (BHBA) dependency to a functional ruminant metabolism with volatile fatty acids (VFAs) dependency in adult animals (Nussio et al., 2003). An antagonistic relationship between glucose and BHBA concentrations is observed during this phase, showing a decrease in glucose and an increase in BHBA concentrations (Khan et al., 2011). BHBA is considered as an indicator of rumen maturation and its VFA utilization capability (Deelen et al., 2016). BHBA also maintains functions involved in cellular signalling to regulate rumen cell growth by activating or inhibiting various signal pathways (Han et al., 2020). A decline in insulin sensitivity during the transition phase is a normal homeorhetic metabolic adaptation that helps in this transformation by making glucose-dependent tissues of young calves into glucose-resistant tissues in adult ruminants (Bauman et al., 2000; Ingvartsen and Andersen, 2000). However, a progressive decrease in insulin sensitivity during this phase predisposes calves towards various metabolic ramifications, such as neonatal calf diarrhea (Pantophlet et al., 2016),

negative energy balance, promoting lipolysis, and other metabolic disorders occurring in later life (Contreras et al., 2017). The promoting effect of BHBA on rumen epithelial proliferation was associated with improved insulin sensitivity (Kato et al., 2011). Studies claimed that an increase in insulin sensitivity or infusion of insulin significantly stimulated cell proliferation in the rumen epithelium (Sakata et al., 1980). Insulin like growth factor 1 (IGF-1) also acts as a growth promoter and regulates the proliferation of many cell types, including the epithelial cells of the rumen (Wang et al., 2017). Calves weaned earlier showed significantly more ruminal epithelial growth and had higher circulating levels of IGF-1 than calves weaned later (Zitnan et al., 2005). During this transitioning, increase in blood non-esterified fatty acid (NEFA) concentration was observed which may reflect the initiation of lipolysis (Stanley et al., 2002).

Several studies and clinical trials with humans and animals have provided confirmation in favour of the beneficial role of chromium (Cr), which has been shown to improve insulin sensitivity more effectively than other nutritional strategies (Wang et al., 2022). Cr as a low-molecular-weight Cr (LMWCr) binding substance enhances insulin sensitivity by potentiating the binding of insulin to its receptor (Wada et al., 1993). LMWCr enhances communication between insulin to its receptors by activating insulin receptor kinase and by increasing phosphorylation rates of insulin binding receptors, thus facilitating the expression of insulin receptor substrate 1 (Wang et al., 2009; Kooshki et al., 2021). Several studies concluded a better glucose clearance during the glucose challenge test, indicating greater insulin sensitivity in Cr supplemented animals (Hayirli et al., 2001; Kumar et al., 2023; Khare et al., 2023). Enhanced insulin action upon Cr supplementation results in variation in plasma NEFA and liver triglyceride concentrations in transition cows (Hayirli et al., 2001). The activity of IGF-1 that has functional homology to insulin receptors and BHBA is increased in Cr supplemented animals (Al-Saiady et al., 2004).

Recent studies (Khare et al., 2023; Kumar et al., 2023) provided evidence that Cr supplementation in calves during the transition period stimulates insulin sensitivity. Various studies have been conducted to fasten the rumen development using nutritional approaches like feeding of milk replacer, calf starter, liquid vs. solid feed etc. (Górka et al., 2009; Górka et al., 2011). Nevertheless, an association study on the role of Cr and age of calves on insulin sensitivity and biomarkers of rumen development has not yet been documented. To develop nutrition strategies for the smooth transitioning of dairy calves by regulating insulin sensitivity will be helpful in controlling various metabolic disorders. We tested the hypothesis that the Cr supplementation may assist in smooth transitioning of young calves from pre-ruminant to ruminant stage by increasing insulin sensitivity and modulating biomarkers of rumen development.

MATERIALS AND METHODS

Ethics approval, animals and experimental design

All animal procedures and protocols were approved by the article number 13 of Institutional Animal Ethic Committee (IAEC) of DUVASU, Mathura (approval number: IAEC/21/22), in compliance with the guidelines set forth by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

24 healthy Hariana calves were selected from the Livestock Farm Complex (LFC), assigned at random to four groups (n=6 in each group), and fed on diets without supplemental Cr (control) or basal diet supplemented with 0.05, 0.10, 0.15 mg Cr per kg $BW^{0.75}$. The daily dose of Cr as Cr-picolinate (Research Lab, Fine Chemicals Industries, Mumbai, India) was calculated per kg $BW^{0.75}$, weighed and encapsulated in 500 mg capacity gelatinized capsules, and fed orally in the morning at 7.00 h to the experimental calves of respective groups. The daily nutrient requirement of the experimental calves was met by feeding milk, calf starter, green maize fodder, and wheat straw (ICAR, 2013). The fresh milk and calf starter were offered at 10% and 1% of BW individually at 8.00 h and 10.00 h, respectively. The calves were dewormed before start of the study and access to fresh water, green maize fodder, and wheat straw was *ad libitum*. The ingredient and nutrient composition of the calf starter, maize fodder, and wheat straw are presented in Table 1.

Table 1. Composition of calf starter, maize fodder and wheat straw

Items	Calf starter	Maize fodder	Wheat straw
Ingredients, g/kg			
Soybean meal (solvent extracted)	380		
Maize grain (yellow)	350		
Wheat bran	150		
Gram husk	100		
Mineral and vitamin premix[α]	10		
Salt	5		
Dicalcium phosphate	5		
Analyzed composition (% DM, except for DM)			
Dry matter	90.4	17.9	89.0
Crude protein	23.0	9.8	3.9
Ether extract	40.3	3.3	1.4
Total ash	14.9	4.5	13.3
Neutral detergent fibre	33.6	47.2	78.1
Acid detergent fibre	11.2	27.3	53.3
Acid detergent lignin	3.5	2.9	8.7
Calcium	1.4	0.5	0.4
Phosphorus	0.6	0.2	0.2
Chromium, mg/kg DM	0.32	0.19	0.15

[α]Premix per kg composed of vitamin A: 10,000,000 IU; vitamin E: 80,000 IU; vitamin D: 1,500,000 IU; Fe: 50 g; Zn: 60 g; Mn: 50 g; Co: 0.1 g; Cu: 12 g; Se: 0.15 g; I: 0.5 g

Observation recording, blood sampling, and laboratory analysis

The experimental calves were monitored fortnightly for growth performance and BCS (Anitha et al., 2010). The representative samples of feeds and fodders offered were collected and analysed for their chemical composition. AOAC (2005) procedures were used for dry matter (DM; methods 967.03), ash (method 942.05), ether extract (EE; method 920.39), and crude protein (CP; method 984.13) determination. Amylase treated neutral detergent fibre (aNDFom), acid detergent fibre (aADFom), and acid detergent lignin (ADL) were determined (Mertens, 2002). Peripheral blood samples were collected on 0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days post-Cr supplementation for the determination of variation in biomarker of rumen development by venipuncture of anterior vena cava in heparinized vacuutainer tubes (BD Franklin, USA). The vacuutainer tubes with blood were immediately placed in an ice box and transferred to the laboratory for centrifugation at 3000 × g at 4°C for 30 min for plasma isolation. Plasma samples were stored at 20°C until further analysis of biomarkers, i.e., glucose, insulin, IRS-1, BHBA, NEFA, IGF-1, and Cr level. Glucose in plasma was measured using the "Endpoint Assay Test Kit" (Span Diagnosis Ltd. Surat, Gujrat). Serum insulin, IRS-1, BHBA, and NEFA concentrations were quantified using a "Bovine Specific ELISA Test Kit" (Bioassay Technology Laboratory, China). The mineral analysis in feedstuffs and plasma was carried out by using Agilent Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES-5800, USA).

Statistical analysis

The data were analyzed using the MIXED procedure of SPSS (Version 21.0, Inc., Chicago, IL). Plasma variables determined during IVGTT were analyzed by using one-way ANOVA, while other blood variables were analyzed by using the following model of repeated measures.

$$Yijk = \mu + Ti + Dj + (T \times D)\, ij + eijk$$

Where Yijk is the dependent variable, ì is the overall mean of the population, Ti is the mean effect of the treatment, Dj is the mean effect of period (j=0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days of dietary treatment) of sampling, (T×D)ij is the effect of the interaction between the effect of Cr supplementation and the day or period of sampling, and eijk is the unexplained residual element assumed to be independent and normally distributed. The effects of treatment, period, and treatment by period interaction were considered fixed while experimental calves as a random effect. If the statistical analysis revealed a significant effect ($p < 0.05$), the differences between treatment, period, and treatment by period interaction were then determined by Duncan's post hoc test.

RESULTS AND DISCUSSION

There was no significant effect of treatment, period, and treatment × period interaction on ADG, BCS, and calf starter intake (Table 2).

Table 2. Effect of Cr on growth performance during experimental period

Particulars	Supplemental Cr (mg/kg $BW^{0.75}$)				Pooled SEM	P value		
	0.00	0.05	0.10	0.15		Treatment (T)	Period (P)	T×P
Initial BW (kg)	29.24	30.52	30.67	30.89	1.42	0.554	0.839	0.994
Final BW (kg)	45.88	46.96	48.44	50.45	2.24	0.550	0.878	0.996
Calf starter intake (g/day)	369.50	387.5	392.2	407.5	15.28	0.189	0.277	0.582
ADG (g/day)	184.81	175.28	201.37	217.30	12.23	0.357	0.662	1.000
BCS	2.88	2.88	2.92	2.90	0.060	0.648	0.719	0.772

Calves are born with a functional monogastric stomach that relies on nutrients from milk or milk replacer. During the transition from pre-ruminant to ruminant stage, various physiological and metabolic adaptations take place. The change from functional monogastric to ruminant not only relies on VFA production in the rumen to supply energy but also on well-functioning endocrine and biochemical features such as ruminant specific insulin homeostasis and hepatic gluconeogenesis (Schwarzkopf et al., 2019). In the present study the dietary supplementation of Cr did not exert any significant effect on ADG and BCS. Accordingly, no effect of 400 and 800 µg Cr/kg as Cr-L-methionine supplementation on ADG was noticed in calves (Kegley et al., 2000). A similar body weight gain was observed in winter-exposed buffalo calves receiving diets supplemented with different levels of inorganic Cr (Kumar et al., 2017). Others also did not observe any influence on weight gain in Cr supplemented calves (Swanson et al., 2000; Yari et al., 2010; Mousavi et al., 2019). In contrast to the findings of this study, better weight gain was found in Cr supplemented Holstein calves (Kegley et al., 1997; Ghorbani et al., 2012; Kargar et al., 2018). No effect of 1.0 mg Cr/kg DM from different Cr sources (Cr-picolinate, Cr-polynicotinate, and Cr-yeast) was noticed by Keshri et al. (2019) in Hariana calves. No effect of Cr supplementation on growth and higher BCS was observed in post-partum cows fed diet supplemented with Cr as Cr-methionine (Hayirli et al., 2001).

Insulin sensitivity

The changes in plasma glucose, insulin, insulin: glucose ratio, and IRS-1 from pre-ruminant to ruminant stage were used as biomarkers of insulin sensitivity (Table 3).

Table 3. Effect of Cr on biomarkers of insulin sensitivity and rumen development

Biomarker	Supplemental Cr (mg/kg $BW^{0.75}$)				Pooled SEM	Significance		
	0.00	0.05	0.10	0.15		Treatment (T)	Period (P)	T×P
Biomarkers of insulin sensitivity								
Glucose concentration (mMol/L)	4.34^{b}	4.02^{ab}	3.68^{a}	3.72^{b}	0.15	0.045	0.028	0.049
Insulin concentration (mIU/L)	1.76^{b}	1.73^{a}	1.72^{a}	1.71^{a}	0.02	<0.001	0.016	0.0289
Insulin: glucose ratio	0.41^{a}	0.43^{ab}	0.47^{b}	0.46^{b}	0.13	0.027	0.047	0.898
IRS-1 level (ng/ml)	16.31^{a}	17.75^{a}	22.19^{c}	19.48^{b}	1.31	<0.001	0.006	0.041
Biomarkers of rumen development and energy balance								
BHBA concentration (nMol/ml)	434.32	455.54	487.06	500.67	15.13	0.272	0.031	0.994
IGF-1 level (ng/ml)	198.83^{a}	219.49^{ab}	241.34^{b}	263.29^{b}	13.97	<0.001	0.038	0.039
NEFA concentration (µMol/L)	224.62	259.76	262.18	267.11	5.08	0.073	<0.001	0.899
Cr level (µg/L)	113.37^{a}	209.68^{b}	246.89^{bc}	292.58^{c}	10.10	<0.001	0.493	0.918

As the age of the experimental calves advanced, plasma glucose concentration decreased, with the lowest value ($p < 0.05$) in the calves fed on a diet supplemented with 0.15 mg of Cr/kg $BW^{0.75}$ (Fig. 1). A similar trend of lower ($p < 0.01$) plasma insulin concentration was observed in Cr supplemented calves than in calves of the control group (Fig. 2). The period and treatment x period interaction showed a significant ($p < 0.05$) effect on insulin concentration while interaction of treatment × period had non-significant effect on insulin: glucose ratio and the ratio was higher in Cr supplemented calf compared to non Cr supplemented calves.

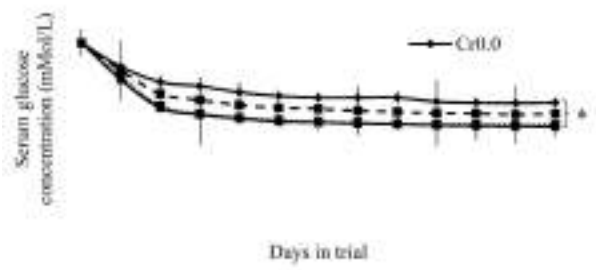

Fig. 1 The effect of Cr supplementation on plasma glucose concentrations in transition calves. * ($p < 0.05$)

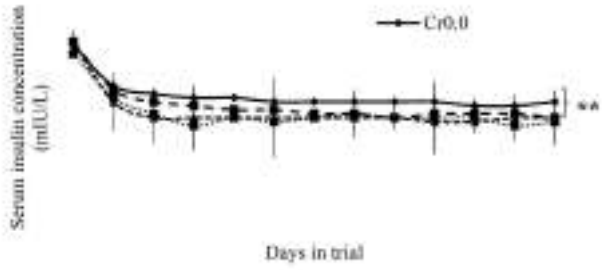

Fig. 2 The effect of Cr supplementation on plasma insulin concentrations in transition calves. ** ($p < 0.001$)

The better insulin sensitivity in this study was in accordance with other reports. The serum glucose concentrations in calves administered with 400 µg supplemental Cr per kg of diet were lower at 5 and 10 min after glucose infusion than in control and in calves supplemented with 800 µg supplemental Cr/kg of diet (Kegley et al., 2000). Similarly, calves supplemented with 400 µg Cr-nicotinic acid complex/kg of feed had higher plasma glucose concentration after 15 min of an IVGTT, and that their serum glucose concentrations declined faster than those of non Cr-supplemented calves (Kegley and Spears, 1995). However, no effect of Cr supplementation on the serum insulin response during glucose challenge was noticed in calves fed on diet supplemented with Cr as Cr-Pic (Bunting et al., 1994). The amount of insulin released was considerably lower in heifers fed a diet supplemented with Cr as compared to the un-Cr-supplemented heifers (Spears et al., 2012). A tendency for a higher glucose clearance rate in calves receiving 0.05 mg of supplemental Cr-Met/kg of body weight was reported while insulin sensitivity remained unaltered (Mousavi et al., 2019). In other reports also the supplementation of Cr improved insulin sensitivity and glucose kinetics following the glucose challenge test (Hayirli et al., 2001; Stahlhut et al., 2006; Yari et al., 2010; Spears et al., 2020) whereas insulin concentrations and insulin: glucose ratio did not differ among heifers supplemented with 0.47, 0.94, and 1.42 mg Cr/kg DM (Spears et al., 2016).

Treatment, period and treatment × period had a significant ($p < 0.05$) effect on plasma IRS-1 concentration (Table 3 and Fig. 1). As the level of Cr supplementation and age of calves advanced, plasma IRS-1 concentration increased due to better insulin sensitivity in Cr supplemented calves. IRS-1 is a substrate of the insulin receptor tyrosine kinase and appears to have a central role in the insulin-stimulated signal transduction pathway (De Meyts, 2016). A study on transition Hariana calves supplemented with Cr-Pic reported better IRS-1 response in treatment than in control group (Kumar et al., 2023). However, work to date in dairy cattle has not attempted to determine the effects of Cr supplementation on IRS-1 response. Turgut et al., (2018), reported that Cr supplementation can increase the expression level of IRS-1 mRNA in skeletal muscles. The results of the study corroborate previous report (Jain et al., 2010), who reported that IRS-1 expression in the liver tissues of type 2 diabetic rats increased after Cr supplementation. In other studies, Cr-Pic supplementation improved glucose disposal rates and IRS-1 expression in skeletal muscles (Wang et al. 2006). In contrast, an inhibitory effect of Cr supplementation was observed on IRS-1 in hepatoma cells (Yurkow and Kim, 1995). Enhanced insulin-mediated tyrosine phosphorylation of IRS-1 after Cr exposure could be one of the explanations for this finding (Chen et al., 2006).

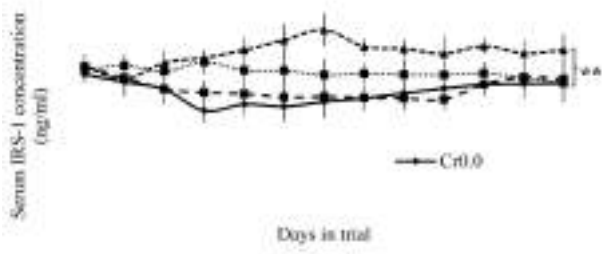

Fig. 3 The effect of Cr supplementation on plasma IRS-1 response in transition calves. ** ($p < 0.001$)

Dynamics of biomarkers of rumen development and energy balance

The changes in plasma BHBA and IGF-1 levels from pre-ruminant to ruminant stage were used as biomarker of rumen development whereas, variation in plasma NEFA concentration were used as biomarker of energy balance (Table 3).

The effect of treatment on plasma BHBA concentration was not significant whereas, plasma BHBA concentration increased ($p < 0.05$) with the advancement of age of the calves. Recent research suggested that circulating BHBA levels may be a meaningful indicator of rumen development and a surrogate measure of rumen function in pre-ruminant calves (Deelen et al., 2016). Before the rumen develops, glucose is used as a primary energy source by the calf. When calves are offered a starter concentrate and fermentation occurs, a large amount of BHBA is produced; afterward, the calf is adapted to this new nutrient as a source of energy (Quigley et al., 1991; Klotz and Heitmann, 2006). The serum BHBA concentration increased after weaning for the early-weaned calves, whereas it remained low in the late-weaned calves and increased after their weaning period (Schwarzkopf et al., 2019) and BHBA concentration was negatively correlated with glucose concentration. Following supplementation with organic Cr for 63 days, no significant changes were detected between treatments in plasma BHBA

in young calves (Earley et al., 2002). However, lower blood BHBA levels in Cr supplemented calves were reported by Ghorbani et al. (2012).

There was a significant effect of treatment ($p < 0.01$), period ($p<0.05$), and treatment × period interaction ($p < 0.05$) on plasma IGF-1 concentrations (Table 3 and Fig. 4). As the level of Cr supplementation and age of calves increased, plasma IGF-1 concentrations also increased (Fig. 4).

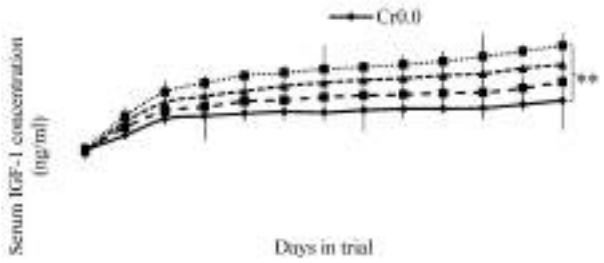

Fig. 4 The effect of Cr supplementation and period on plasma IGF-1 response in transition calves ($p<0.05$)

IGF-1 is an anabolic hormone that plays an important role in cell proliferation (Obradovic et al., 2019; Yoshida and Delafontaine, 2020). IGF-1 can stimulate epithelial cell proliferation and differentiation to enhance ruminal papillae development by regulating IGF-1 binding proteins (Hayashi et al., 2005). Cr is involved in the up-regulation of mRNA IGF-1 expression (Peng et al., 2010). In the present study, as the age of calves and level of Cr supplementation increased, plasma IGF-1 concentration also increased. Increased serum IGF-1 concentrations due to Cr-supplemented diets were shown to play a role in regulating protein and fat metabolism in pigs (Wang et al., 2014). Subiyatno et al. (1996) reported a tendency for increased circulating IGF-1 in response to Cr supplementation periparturient dairy cows. The offspring of Cr-treated male mice showed increased serum IGF-1 serum concentrations (Cheng et al., 2002). Modulation of IGF-1 signalling after Cr supplementation is well-recognised by others also (Peng et al., 2010; Chen et al., 2014; Ullah Khan et al., 2014; Morvaridzadeh et al., 2022). However, Depew et al. (1998) reported that blood IGF-1 concentrations were not affected by Cr-methionine supplementation in young calves. A trend of decrease in insulin and insulin IGF-1 concentrations with advancement of age was observed in calves (Breier et al., 1988; Abdelsamei et al., 2005; Kesser et al. 2017).

Statistical analysis of the data revealed a non significant effect of the treatment and treatment × period interaction while period showed a significant effect ($p < 0.001$) on plasma NEFA concentrations. Mostly, variation in NFEA concentrations was more often observed in animals having a state of negative energy balance (Spears et al., 2012; Leiva et al., 2017). In the present study, plasma NEFA concentration was not significantly different among groups and similar observation was reported with, no significant variation in NEFA concentrations in animals with positive energy balance and supplemented with Cr-amino acid chelates in Holstein cows (Yang et al., 1996) or Cr-Pic in Holstein steers (Besong et al., 2001). However, lower serum NEFA concentrations were noticed in Cr-Pic or chelated Cr supplemented animals (Kitchalong et al., 1995; Subiyatno et al., 1996). Lower serum NEFA concentration was noticed in calves fed milk replacer supplemented with Cr, indicating indirect evidence of enhanced insulin sensitivity in calves fed milk replacer or starter supplemented with Cr (Depew et al., 1998).

Cr supplementation resulted in a significant ($p < 0.01$) increase in plasma Cr concentration (Table 3) while the period and the interaction of treatment × period was not-significant and it was highest in the calves supplemented with 0.15 mg of Cr/kg $BW^{0.75}$. However, the serum concentration of Cu, Zn and Fe in Cr supplemented summer exposed Buffalo calves Kumar et al. (2013) remained unaltered. In opposite to findings of the present study, Cr supplemented heifer have high intake of Zn, Cu, Fe, and Mn in compared to non Cr supplemented cross-bred dairy heifers (Biswas et al., 2006). Kumar et al. (2013), Deka et al. (2015), and Kumar et al. (2023) also observed a dose-dependent increase in serum Cr concentration while the level of other minerals remained comparable in treatment as well as in the control group.

CONCLUSION

The results of the study indicated that the dietary supplementation of Cr may be beneficial in improving insulin sensitivity and modulating biomarkers of rumen development in calves during the transition period from pre-ruminant to ruminant phase.

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Effect of Different Levels of Protein in Total Mixed Ration on Growth Performance, Digestibility and Microbial Protein Production in Vechur Cattle

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ABSTRACT

The effect of different levels of protein in total mixed ration was evaluated based on the growth performance, digestibility, and microbial protein production in Vechur cattle. Fifteen Vechur cattle of six to ten months of age were randomly assigned to one of the three treatments. T1 - Total mixed ration containing 16 % CP and 60% TDN, T2 – Total mixed ration containing 14% CP and 60% TDN, and T3 - Total mixed ration containing 12 % CP and 60% TDN. The results indicated that total dry matter intake (DMI), fortnightly average dry matter intake, total body weight gain, average daily gain (ADG), and feed conversion efficiency (FCE) were similar ($P > 0.05$) among the three treatment groups. No variations were observed for the apparent digestibility of nutrients. The purine derivatives excretion, urinary creatinine excretion, microbial protein production, the duodenal flow of microbial nitrogen, PDC index, and the efficiency of microbial nitrogen production showed no significant ($P > 0.05$) distinction among the dietary treatments. These findings suggest that a total mixed ration containing 12% CP may be adequate to meet the nutritional requirements of growing Vechur cattle without compromising performance and digestibility, thereby offering a potentially cost-effective feeding strategy.

KEYWORDS: Digestibility, Microbial protein, Total mixed ration, Vechur cattle

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INTRODUCTION

Livestock rearing is an integral part of the agriculture system in tropical countries, which have the highest share in the livestock population of the world (FAO 2013). According to 20th livestock census (2019) of India, the total livestock population is 535.78 million of which the cattle population is 192.49 million. India has an indigenous/nondescript cattle population of 142.11 million.

The preservation of indigenous cattle breeds is important because of their unique traits. Vechur cattle are the sole recognized indigenous breed in Kerala and are classified as a critically maintained breed category by the Food and Agriculture Organisation.

The protein requirement of ruminants encompasses the requirement of the rumen microorganisms to maintain optimum growth and proliferation in the rumen and the requirement of the host animals for various physiological functions in the body. In ruminants, 50 to 100% of their total protein requirements are met from ruminal microbial synthesis. More specifically for the ruminant, an adequate protein level in the diet is needed for maximal growth and activity of ruminal microorganisms, thus producing desired microbial crude protein amounts and maximizing ruminal fermentation. Microbial protein (MP) plays a pivotal role in ruminant nutrition because ruminants get most of their protein from microbial cells formed in the rumen as a result of feed digested under anaerobic conditions. This microbial protein provides 60 to 85 % of amino acids (AA) reaching the animal's small intestine (Fujihara and Shem, 2011). The amino acid profile of MP is better than several dietary protein sources. Increasing the efficiency of its production would subsequently improve cattle productivity. Protein supplementation is costly and can result in excess nitrogen (N) excretion (Zhang et al., 2017).

Proper determination of the protein requirement of animals is crucial for maximizing production and minimizing N input in dairy production systems. The reports on the nutrient requirements of Vechur cattle are scanty. They are fed as per the requirements of crossbred cattle. Additionally, there haven't been many studies done on the optimum dietary levels of crude protein in the Total mixed ration (TMR) for feeding Vechur cattle. Hence, the present research project is undertaken to study the effect of different levels of protein in the TMR on growth performance and digestibility of nutrients in Vechur cattle, and to assess the efficiency of rumen microbial protein production.

MATERIALS AND METHODS

This experiment was conducted at the Vechur Cattle Conservation Unit, Centre for Advanced Studies in Animal Genetics and Breeding (CASAGB), Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India, for 120 days in Vechur cattle.

Fifteen Vechur cattle of six to ten months old age (Average body weight 50 kg) were used in this study. The Vechur cattle were randomly assigned into three treatment groups of five animals each in a completely randomized design and were allotted randomly to one of the three dietary treatments T1, T2 and T3. All the experimental animals were fed with total mixed ration (TMR) with a concentrate: roughage ratio of 70:30. Three experimental rations were formulated as follows. T1 - TMR containing 16 % CP and 60 % TDN T2 - TMR containing 14 % CP and 60 % TDN, T3 - TMR containing 12 % CP and 60 % TDN. The paddy straw-based TMRs were prepared in the School of Animal Nutrition and Feed Technology (SANFT), Mannuthy, Thrissur. The animals were maintained under a uniform system of feeding and management throughout the experimental period. The feeding trial will be conducted for a period of four months. All the calves will be fed as per ICAR standards (ICAR, 2013). All the animals were dewormed for controlling endoparasites. All the experimental cattle were housed in the experimental shed with individual feeding and watering facilities. Clean fresh drinking water was offered to all the animals *ad libitum*. Individual data on quantities of feed offered daily were recorded. Weighed quantities of TMR were fed individually to the animals of the three experimental groups based on their requirement, and the leftover feed in the manger was collected manually and weighed, twice a day, in the morning and afternoon at 9 AM and 2 PM, respectively. Samples of the left-over portions of the feed were taken daily for analyzing the moisture content and the daily dry matter intake was calculated. Daily dry matter intake data was recorded during the entire experimental period. Based on the body weight, feed allowances were reviewed fortnightly. Animals were weighed at the beginning of the experiment and thereafter at fortnightly. The ingredient and chemical composition of the total mixed rations used for this study is presented in Table 1.

A digestibility trial for five days duration was carried out towards the end of the feeding trial by total collection method. The feed and dung samples were analyzed for proximate principles following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were measured using the procedures of Van Soest et al. (1991). Additionally, calcium and phosphorus content in both feed and faeces were determined using standard AOAC methods.

Total urine collected during the digestion trial were used for the estimation of the purine derivatives such as allantoin and uric acid and thus for the estimation of microbial protein production (IAEA-TECDOC-945, 1997; Cetinkaya et al., 2006; George, 2012). Urine samples were centrifuged, diluted (1: 10), filtered using (0.22µm) Millipore filter and analyzed (George et al., 2006). Urinary uric acid was determined by the uricase method and creatinine by Modified Jaffe's method using standard kits. Urinary purine derivative excretion is the sum of urinary allantoin and uric acid excretion in mM/L. The microbial Nitrogen (g N/day) from the microbial purine derivatives (X, mMol/day) was calculated as described by Chen and Gomes (1992). The efficiency of microbial nitrogen production was expressed as g N/kg of organic matter digested in the rumen (DOMR) by multiplying digestible OM by 0.65. (Chen and Gomes, 1992).

Statistical analysis of the experimental data was conducted using one-way ANOVA with SPSS 24.0, and means were compared through Duncan's range test, as described by Snedecor and Cochran (1994)

RESULTS AND DISCUSSION

Chemical composition of ration

The chemical composition of the total mixed

rations used for the feeding trial is given in Table 2. Purushothaman (2018) prepared TMR for Vechur heifers containing 15.20 and 15.02 % of CP which was within the range of CP content of the TMR used in the present study. Nair (2020) also used similar levels of protein and energy in TMRs (12, 14, 16 and 18 % CP with 60 % TDN) in the study using lactating crossbred cows.

Yousefinejad et al. (2021) also formulated the ration for Brahman bulls with a total mixed ration containing 12.2 and 14.1 % CP which was similar to the formulation in this study.

Table 1. Ingredient and chemical composition of total mixed ration offered to cattle maintained on three dietary treatments

Ingredient (%)	% composition of total mixed ration		
	T1	T2	T3
Maize	26	24.5	26
Rice Polish	6.5	7.5	8
De-oiled Rice Bran	5.5	5.5	6.5
Alfalfa pellet	15	9	5.5
Black Gram Husk	1.5	6	8
Corn gluten fibre	8	9.5	7
Coconut Oil Cake	3.5	4	5
Paddy Straw	30	30	30
Calcite	1.5	1.5	1.5
Salt	0.5	0.5	0.5
Mineral mixture	2	2	2
Total	100	100	100
Vitamin AB2D3K g/100 kg	20	20	20
Nutrients	% Chemical composition of total mixed ration		
Dry matter	91.88 ±0.18	91.66 ± 0.13	91.50 ± 0.23
Organic matter	90.03 ± 0.16	90.41 ± 0.05	90.57 ± 0.09
Crude protein	16.31 ± 0.31	14.12 ± 0.12	12.55 ± 0.19
Ether Extract	3.40 ± 0.07	3.54 ± 0.09	3.57 ± 0.05
Crude fibre	13.76 ± 0.04	13.55 ± 0.03	13.72 ± 0.04
Total Ash	9.97 ± 0.21	9.59 ± 0.08	9.43 ± 0.10

[1]Values from the second row onwards are expressed on DM basis, an average of six values.

Growth Performance

The average body weight of experimental Vechur cattle maintained on different treatments, documented at fortnightly intervals. Statistical analysis of the data showed that there was no significant (P>0.05) variation in the average body weight of Vechur cattle fed on different dietary treatments. Summarized data on total weight gain and ADG for the Vechur cattle maintained on three treatments are given in Table 2. The ADG of Vechur cattle fed on three dietary treatments T1, T2 and T3 were 350, 340 and 360 g and total weight gain was 41.63, 41.51, and 43.36 kg. Statistical analysis of the data revealed that there was no significant variation (*P*>0.05) in ADG and total weight gain of Vechur

cattle fed on various treatments. In agreement with the results, Ozkaya and Toker (2012) found no significant difference (P>0.05) in body weight of Holstein calves fed with a starter diet containing 22 % CP and 18 % CP. Gowda (2019) also observed no significant difference in the average body weight of crossbred heifer calves fed different TMR. The present ADG values were comparable to Lohakare et al. (2006), who got values in the range of 337 to 367 g in crossbred calves fed different protein levels (100, 75, 125 % of protein requirement). In disagreement with the results of this study, Bhadane et al. (2004) discovered improved body weight gain in goats fed with complete pelleted feed containing 12 % CP, 65.81 % TDN and 14 % CP, 67.44 % TDN of 75.7 and 72.9 g/day. Similarly, Girdhar and Balaraman (2005) also detected better body weight gain in lactating cross bred cows fed on berseem based TMRs containing higher levels of protein and energy (12 % CP and 60 % TDN, 14 % CP and 65 % TDN) than those fed on a lower level of protein and energy (10 % CP and 55 % TDN).

Table 2. Growth performance of Vechur cattle fed on TMR with varying levels of protein in total mixed ration

Parameters	T1	T2	T3	p- value
Initial body weight (kg)	50.17 ± 8.19	50.21 ± 8.54	51.74 ± 6.62	0.987^{ns}
Final body weight (kg)	91.80 ± 15.75	91.72 ±10.57	95.10 ± 8.01	0.974 ^{ns}
Total weight gain (kg)	41.63 ± 7.81	41.51 ± 3.06	43.36 ± 2.84	0.960 ^{ns}
Average daily gain (kg)	0.35 ± 0.07	0.34 ± 0.03	0.36± 0.02	0.953 ^{ns}
Total dry matter intake (kg/animal)	235.31 ± 35.21	237.17 ± 29.08	242.67± 23.23	0.983 ^{ns}
Average daily dry matter intake (kg/animal/day)	1.96 ± 0.29	1.98 ± 0.24	2.02 ± 0.19	0.982 ^{ns}

[1]Mean values are based on five replicates with SE
ns- non-significant

Dry matter intake and Feed conversion ratio (FCR)

Statistically, there was no significant variation (P>0.05) found in the daily DMI of Vechur cattle maintained in the three treatment groups. In accordance with the results in the present study, Lohakare et al. (2006) reported that the dry matter intake was similar in crossbred calves fed different protein levels (100, 75, and 125 % of protein requirement) and it ranged from 2,053.66 to 2,279.22g. Similar results were reported by Chantiratikul et al. (2009), Queiroz et al. (2012), Kumar et al. (2013) and Javaid et al. (2015). They found that the dry matter intake was not affected by the protein content of the diet. However, Yuangklang et al. (2010) reported that feed intake (kg DM/day) was 7.37, 7.26, 7.15 and 6.96 respectively for 8, 10, 12 and 14 % CP in Brahmin bulls which indicated that feed intake was linearly decreased with increasing the protein levels in the diet.

Unlike the observations in the current study, Shahzad et al. (2011) found significant difference (P<0.05) in feed intake in Nili- Ravi buffalo calves fed with different dietary protein levels (CP; 10.5, 12.20, 13.80 and 15.55 %) and energy levels (1.72, 2.11 and 2.5 Mcal/kg of metabolizable energy). They observed higher feed intake for buffalo calves fed 12.20 CP and 2.11 Mcal/kg ME. On the contrary, Tauqir et al. (2011) concluded from their experiment in 36 growing male Nili Ravi buffalo calves that dry matter intake was reduced in calves that were fed a ration containing high CP (16.5 %) than those fed low (11.85 %) or medium (14.2 %) CP. Paengkoum et al. (2019) also detected an increase in dry matter intake linearly with increasing undegradable intake protein (UIP) levels in growing Thai indigenous beef cattle receiving different levels of crude protein (10 and 12 % of dry matter (DM) and undegradable intake protein UIP (15, 25 and 35 % of CP).

Data on cumulative FCR of Vechur cattle maintained on three different treatments is presented

in Table 2. Statistically, no significant difference was observed in the cumulative feed conversion ratio of Vechur cattle maintained on various treatments. Similarly, Sharma et al. (2010) observed that there was no significant difference in feed conversion efficiency in crossbred calves provided with complete feed in block (7.59), mash (7.47), or conventional feed form (7.93). In accordance with the results, Kumar et al. (2015) also observed no significant difference in FCE in Murrah buffaloes under the traditional feeding system and feeding of total mixed ration. Kavya et al. (2025) also observed similar FCR in Punganur calves fed with concentrate mixtures containing varying levels of CP.

Digestibility of nutrients

The digestibility coefficients of nutrients such as dry matter, crude protein, crude fibre, ether extract, nitrogen-free extract, neutral detergent fibre and acid detergent fibre are shown in Table 3. The data revealed non-significant effect ($P>0.05$) on the digestibility of nutrients with TMR comprising varying levels of crude protein. Similar to this study, several researchers reported that dietary crude protein levels did not change the digestibility of nutrients (Malik et al., 1998; Mehra et al., 2001; Chantriatikul et al., 2009; Verma et al., 2009; Tatsapong et al., 2010 and Kavya et al., 2025)

Lohakare et al. (2006) evaluated the effect of different protein levels (normal protein (NP) - 100 %, low protein (LP) - 75 and high protein (HP) - 125 % of protein requirement) in 30, three to five months old male crossbred calves on digestibility and reported that the dry matter digestibility was higher in the HP fed animals. The digestibility of CP, CF, OM and NFE was significantly higher on HP diets compared to LP or NP diets.

Table 3. Digestibility coefficients of nutrients[1] in TMR with varying levels of protein fed to Vechur cattle, %

Parameter	T1	T2	T3	p- value
Dry matter	62.32 ± 0.96	62.48 ± 1.62	61.8 ± 1.78	0.945^{ns}
Organic matter	66.86 ± 0.73	66.83 ± 1.56	65.57 ± 1.68	0.760 ^{ns}
Crude protein	60.49 ± 1.30	60.65 ± 1.50	60.65 ± 1.40	0.995 ^{ns}
Crude fibre	55.35 ± 1.44	55.41 ± 1.77	56.33 ± 2.22	0.915 ^{ns}
Ether extract	82.06 ± 1.14	81.43 ± 1.07	82.36 ± 1.18	0.838 ^{ns}
Nitrogen free extract	72.11 ± 0.55	71.65 ± 1.46	69.16 ± 1.72	0.285 ^{ns}
Neutral detergent fibre	52.53 ± 1.28	52.94 ± 2.11	52.24 ± 2.51	0.971 ^{ns}
Acid detergent fibre	36.73 ± 1.19	36.42 ± 2.45	39.15 ± 2.65	0.639 ^{ns}
CPI (kg)	0.41 ± 0.066	0.35 ± 0.047	0.32 ± 0.031	0.463^{ns}
DCPI (kg)	0.25 ± 0.039	0.21 ± 0.026	0.19 ± 0.018	0.441^{ns}
TDNI (kg)	1.63 ± 0.265	1.61 ± 0.211	1.63 ± 0.148	0.998^{ns}
CPI (kg/kg W0.75)	0.014^{a}±0.001	0.012^{b}± 0.001	0.011^{b} ± 0.001	0.006*
DCPI (kg/kg W0.75)	0.008^{a} ± .000	0.007^{b} ± .000	0.006^{b} ± .000	0.007*
TDNI (kg/kg W0.75)	0.055 ± 0.002	0.054 ± 0.003	0.054 ± 0.002	0.983^{ns}

*Means bearing different superscripts in a row differ significantly ($p < 0.05$)

Tauqir et al. (2011) concluded from their experiment in 36 growing male Nili Ravi buffalo calves that digestibility of NDF and CP were similar among treatment groups (six experimental diets with three levels of crude protein (CP; 11.85, 14.20 and 16.50 %) each with two levels of metabolisable energy (ME; 1.86 and 2.23 Mcal/kg) whereas digestibility of DM was significantly higher in groups supplemented with higher protein levels. Purushothaman (2018) noticed that the digestibility of DM, CP, CF and EE were similar among the two rations (TMR containing soya sauce waste (TMR 1 CP-15.2 %) and TMR containing tapioca starch waste (TMR 2 with CP-14.7 %), whereas the digestibility of OM and NFE were increased significantly in TMR 1 than in TMR 2. The nutrient intake such as CP, DCP and TDN were found to be similar among the groups, whereas the CP intake and DCP intake per kg metabolic body size were found to be significantly ($P<0.05$) lower in T2 and T3 compared to T1.

Urinary purine derivatives and microbial protein production

The urinary allantoin, urinary uric acid and total purine derivative excreted by experimental Vechur cattle retained on T1, T2 and T3 are presented in Table 4. Statistically, there was no significant difference ($P>0.05$) observed in these parameters among the three treatment groups. The values are comparable to those of Purushothaman (2018), who reported total purine derivatives - 11.66 mM/L in Vechur heifers fed on grass based TMR and Jasmine (2021) who reported 12.28 mM/L in cross bred cows fed on straw based TMR. Similarly, Chacko (2015) observed no significant difference in the excretion of urinary total purine derivatives in crossbred cattle fed with complete feed containing varying levels of neutral detergent fibre (25, 30 and 35 % NDF).

Table 4. Urinary purine derivative excretion[1], microbial protein production[1] and PDC index[1] of Vechur cattle fed on TMR with varying levels of protein

Parameter	T1	T2	T3	p-value
Allantoin, mMol/L	11.13 ± 0.38	10.85 ± 0.55	10.81 ± 0.44	0.873[ns]
Uric acid, mMol/L	0.76 ± 0.03	0.81 ± 0.04	0.85 ± 0.02	0.215 [ns]
Purine derivative excretion, mMol/L	11.89 ± 0.35	11.66 ± 0.53	11.66 ± 0.43	0.916 [ns]
Creatinine (mMol/L)	3.52 ± 0.21	3.42 ± 0.29	3.39 ± 0.45	0.960 [ns]
Purine derivative: Creatinine Index	97.75 ± 4.99	100.79 ± 5.95	106.96 ± 7.89	0.597 [ns]
Duodenal flow of Microbial Nitrogen, g N/day	32.60 ± 4.18	31.40 ± 2.58	33.27±2.5	0.915 [ns]
Microbial Protein production, g/day	203.71 ± 26.11	196.22 ± 16.09	207.95 ± 15.64	0.915 [ns]
Efficiency of Microbial nitrogen production, g N/kg DOMI	33.8 ± 1.65	33.23 ± 2.29	34.31 ± 1.85	0.927 [ns]

[1]Mean values are based on five replicates with SE. ns- non significant

Urinary creatinine excretion values were similar (P>0.05). The concentration of creatinine remained unaffected by the different protein levels in the diets. The excretion of creatinine was constant per metabolic body weight and was proportional to muscle mass, but the season affected its excretion (Whittet et al., 2004). Ashwin (2015) and Srinivas and Ramesha (2017) noted the urinary creatinine ranged from 4.2 to 4.7 mMol/L in Malnad Gidda cows and 3 to 5 mMol/L in Deoni cows, respectively. The PDC index was 97.75, 100.79 and 106.96 for the three treatments T1, T2 and T3, respectively, and the values were similar (P>0.05) statistically. The findings indicated that the calculated PDC index for the treatments showed no significant (P>0.05) response to varying protein levels in the diet. The PDC index values obtained in the present study are comparable to those of George (2012) found a PDC index in the range of 115.05 to 162.21 in crossbred calves of nine months of age fed with concentrate mixtures (incorporating with and without urea and slow-release urea) and green grass. Ashwin (2015) observed a PDC index of 223.56 in Deoni cows weighing 370 kg fed ragi straw and concentrate. Cetinkaya et al. (2006) found that the purine derivatives-creatinine index differed significantly across treatment groups (37.6, 51.2, 59.3 and 75.0 respectively) when given a diet containing 125g/kg DM crude protein at four different levels of the voluntary feed intake (40, 60, 80 and 95 %) in Yerli Kara crossbred cattle.

Microbial protein production was 203.71, 196.22 and 207.95 g/day for the three treatments T1, T2 and T3 respectively. The efficiency of microbial protein production for the treatment T1, T2 and T3 were 33.80, 33.23 and 34.31 gN/ kg digestible organic matter intake, respectively. Statistically, there was no significant difference (P>0.05) observed in these parameters among the three treatment groups. Similarly, Chacko (2015) conducted experiments in crossbred cattle fed with complete feed containing varying levels of neutral detergent fibre (25, 30 and 35 % NDF) and noticed microbial protein synthesis was similar among three dietary treatments. Purushothaman (2018) conducted studies in Vechur heifers fed with different total mixed rations (TMR) containing soya sauce waste (TMR 1 CP- 15.2 %) and tapioca starch waste (TMR 2 with CP-14.7 %) and observed higher concentration of microbial protein production in animals fed with TMR 2 (364.59 g/day) than TMR 1 (227.77g/day). The microbial protein production (MPP) values obtained in the present study are comparable to those of George (2012) found MPP values in the range of 61.51 to 196.30 g/day in crossbred calves fed with concentrate mixtures (incorporating with and without urea) and green grass.

In the current study, the observed microbial protein efficiency was slightly above the recommended optimal efficiency of 30 gN/ kg of digestible organic matter intake, as advised by ARC (1980). Srinivas and Ramesha (2017) observed that the efficiency of microbial protein production ranged from 27.96 to 49.83 g/kg DOMI in the dwarf cattle breed, Malnad Gidda, maintained on different feeding systems. Similarly, Ashwin (2015) also observed the efficiency of microbial protein production of 33.57 gN/kg DOMI for Deoni cows fed ragi straw and concentrate.

CONCLUSION

From the results obtained on the present study, it could be observed that cattle in T2 and T3 had similar growth performance as that of T1. Microbial protein production was also similar among the treatment groups. These findings suggest that a total mixed ration containing 12% CP might be adequate to meet the nutritional requirements of growing Vechur cattle without compromising performance and digestibility, thereby offering a potentially economical feeding strategy.

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Effect of Dietary Synbiotic as a Replacement for Antibiotics on the Growth Performance, Gut Health and Immune Response in Broiler Chickens

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ABSTRACT

This study examined the impact of dietary supplementation with a synbiotic on the growth performance, carcass traits, blood biochemical profile, gut microbiome, gut morphology, and immune response of broiler chickens. A total of 162 day-old VenCobb-400 broiler chicks were randomly divided into three dietary groups, each with six replicated pens (n = 6), housing nine broiler chickens: 1) basal diet without any growth promoter (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) at 500g/ton feed (AGP), and 3) basal diet with synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructo oligosaccharide) at 1 ml/bird in drinking water (SYN). Body weight, feed intake, and feed conversion ratios (FCR) were monitored weekly for 42 days. Blood biochemistry was assessed on day 28, and immune response was evaluated on days 28 and 35. Carcass traits, gut microbiome, and gut morphology were examined at the end of the trial. The SYN group exhibited significantly higher final body weight and average daily gain compared to the CON and AGP groups. Feed conversion ratio was improved in the SYN and AGP groups compared to the CON group. Total *Escherichia coli* and *Salmonella* counts were significantly lower in the SYN group, while Lactobacillus counts were significantly higher. In conclusion, synbiotic supplementation could serve as a promising alternative to antimicrobials in broiler production, demonstrating beneficial effects in broilers fed an antibiotic-free diet.

KEYWORDS: Broiler chicken, Growth performance, Gut microbiota, Immune response, *Lactobacillus rhamnosus.*

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INTRODUCTION

The non-therapeutic use of antibiotics in the poultry industry is a common practice to increase yield, promote growth, and protect birds from harmful bacteria. However, this practice has led to the development of antimicrobial-resistant microbes in birds and poultry products, leading to raising environmental concerns (Hayden et al., 2020). Some countries have banned antibiotic use, and consumer demand for antibiotic-free poultry products is increasing. Consequently, there is a growing need for alternatives to non-therapeutic antibiotic use in the poultry industry. Researchers are exploring alternative strategies, such as incorporating feed additives like essential oils, organic acids, enzymes, prebiotics, probiotics, synbiotics, and postbiotics, to replace antibiotics as growth promoters while maintaining poultry growth and well-being (Das et al., 2016; Reuben et al., 2021).

Synbiotics are the combination of probiotics and prebiotics. Probiotics rely on prebiotics as a substrate. The viability of probiotics is enhanced by supplementing them with appropriate non-digestible prebiotics (Sekhon and Jairath, 2010). Prebiotics improve gut health, increase beneficial bacterial count, lower pH and enhance the host animal's immunity (Reuben et al., 2021). Using synbiotics as a feed supplement could improve feed efficiency compared to using prebiotics and probiotics separately (Dakhil and Al-Shammari, 2023; Song et al., 2022). Therefore, safe and cost-effective strategies to reduce the microbial load are crucial for poultry producers (Œli¿ewska et al., 2020). With the increasing focus on safer and healthier chicken production, efforts have been ongoing to find AGP alternatives as feed supplements. While many studies have investigated the effects of probiotics and prebiotics separately on broilers, limited data is

available on the impact of synbiotics on broiler chickens' growth performance, gut health, and immune response. Most studies on synbiotics in broiler chickens have utilized powder form supplements, with limited research on liquid forms. This research intends to evaluate the impact of a synbiotic formulation featuring a distinctive blend (*Lactobacillus rhamnosus* NCDC 298 + fructo oligosaccharide) on the growth efficiency, intestinal health, carcass traits, and immune reaction of broiler chickens as an antimicrobial substitute in poultry farming to address global health security concerns.

MATERIALS AND METHODS

Broiler chickens, experimental design and diets

A total of 162 one-day-old mixed-sex commercial broiler chickens (Vencobb 400, Venkys, Pune, India) were randomly divided into three dietary groups, each with six replicated pens (n=6) containing nine broiler chickens per pen. The groups were: 1) basal diet without growth promoter (CON), 2) basal diet with antibiotic bacitracin methylene disalicylate (AGP) at 500g/ton feed, and 3) basal diet with synbiotics (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) at 1 ml/bird in drinking water (SYN). The basal diet (Table 1) was formulated in mash form using maize and soybean to meet or exceed the nutritional requirements of broiler chickens at different stages (starter, grower, and finisher) based on recommendations for Vencobb 400 broiler chickens (Venkys, 2017). Experimental diets were prepared weekly, packed in high-density polyethylene bags with inner liners, and provided ad libitum along with water. The ingredient and chemical composition of the basal diet are detailed (Table 1).

Table 1. Composition of basal diets

SL. No.	Ingredients	Starter (Day 1-14)	Grower (Day 15-28)	Finisher (Day 29-42)
1	Maize	57.289	59.381	62.519
2	Deoiled Soybean Meal (45%).	37.247	34.035	30.003
3	Vegetable oil	1.841	3.143	4.208
4	Dicalcium Phosphate	1.503	1.375	1.261
5	Limestone Powder	0.756	0.835	0.828
6	Salt	0.322	0.324	0.326
7	DL-methionine	0.314	0.260	0.231
8	L-Lysine HCL	0.226	0.154	0.131
9	L-Threonine	0.084	0.055	0.055
10	Toxin Binder	0.050	0.050	0.050
11	Sodium bi-carbonate	0.100	0.100	0.100
12	Choline Chloride, 60%	0.050	0.070	0.070
13	Trace mineral mixture	0.100	0.100	0.100
14	Vitamin premix	0.100	0.100	0.100
15	Antioxidant	0.010	0.010	0.010
16	Phytase 5000	0.010	0.010	0.010

Nutrient composition				
1	Metabolizable energy (kcal/kg)$^{+}$	3000.00	3100.00	3200.00
2	Crude protein (%)$^{++}$	22.19	20.80	19.20
3	Ether extract (%)$^{++}$	4.49	5.83	6.97
4	Crude fiber (%)$^{++}$	4.00	4.24	5.36
5	Dry matter (%)$^{++}$	90.32	91.29	90.28
6	Total Ash (%)$^{++}$	11.48	13.51	11.54
7	Acid Insoluble Ash (%)$^{++}$	0.82	0.82	0.66
8	Calcium (%)$^{++}$	0.92	0.91	0.87
9	Total phosphorus (%)$^{++}$	0.79	0.76	0.72
10	Available phosphorus (%)$^{++}$	0.45	0.42	0.39
11	Lysine (%)$^{+}$	1.22	1.09	0.98
12	Methionine (%)$^{+}$	0.6	0.53	0.49
13	Methinine + cysteine (%)$^{+}$	0.88	0.80	0.74
14	Threonine (%)$^{+}$	0.77	0.70	0.65
15	Sodium (%)$^{+}$	0.16	0.16	0.16
16	Chloride (%)$^{+}$	0.18	0.18	0.18

$^{+}$ Calculated values ++ Analysed values

Preparation of synbiotics

The active probiotic culture *Lactobacillus rhamnosus* NCDC 298 was sourced from the Department of Dairy Microbiology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences in Mohanpur, Nadia, West Bengal, India. Sub-culturing was carried out in MRS broth at 37°C for 15-18 hours for inoculation. To facilitate feeding to broiler chickens, the synbiotic was prepared in liquid form. The synbiotic liquid base was prepared by dissolving skim milk powder (30 g), dextrose (10 g), fructooligosaccharide (50 g), and a nutrient mix (2.5 g) in 1000 ml of distilled water. The mixture was then distributed into conical flasks and heated at 121°C for 5 minutes. After cooling to room temperature, the active MRS broth *Lactobacillus rhamnosus* NCDC 298 (10^9 cfu/ml) culture was inoculated at a rate of 1% and incubated at 37°C for 15-18 hours to produce the synbiotic preparation. The synbiotic preparation was stored at 5-7°C for a maximum of 7 days.

Broiler Trial

For the first two days, the chicks had continuous lighting from compressed fluorescent lamps, followed by a lighting schedule of 23 hours of light and one hour of darkness each night. The temperature in the poultry house was controlled using heating elements, starting at 32°C on day 1 and gradually decreasing to 24°C by day 22. Proper ventilation was maintained with exhaust fans throughout the trial. All birds were vaccinated against Newcastle Disease virus (NDV) at 5 and 21 days of age and infectious bursal disease virus (IBDV) at 12 days of age.

The initial body weight of each chicken was noted on the trial's first day. Average daily feed intake (ADFI) was determined by dividing the total feed eaten daily by the number of chickens present in each pen. The feed conversion ratio (FCR) was determined by evaluating the total feed consumption and weight increase for every replicate pen.

Blood samples for biochemical analysis were collected from broiler chickens on day 35 after a 12-hour fasting period. The samples were obtained from the wing vein. Twelve birds were randomly selected for each treatment, with two birds taken from each pen. Blood was collected without an anticoagulant, and the serum was stored at -20°C

before analysis. The serum levels of glucose, total protein, albumin, uric acid, triglycerides, and cholesterol were measured using commercially available kits from DiaSys Diagnostic India Pvt. Ltd., based in Mumbai, India.

Humoral Immune Response

The humoral immune response was assessed by measuring antibody levels after the administration of vaccines for Newcastle disease virus (NDV) and infectious bursal disease (IBDV). The B1 strain (0.2 mL) and LaSota strain (0.2 mL), both live lentogenic strains obtained from Venkateswara Hatcheries Private Limited in India, were administered via the ocular route on days 5 and 21. The intermediate plus vaccine for IBD (0.2 mL) was given on day 14. Blood samples (2 mL) were collected from the wing vein of two randomly selected birds from each replicate pen on days 28 and 35. The samples were quickly transferred to centrifuge tubes without anticoagulant, and serum was collected through centrifugation. Antibody levels for NDV and IBDV were measured using an ELISA kit supplied by IDEXX Laboratories Inc. USA. The optical density (OD) for each sample was measured twice with a microplate reader from Meril Life, India, and the mean OD values were used for analysis.

Carcass characteristics

On day 42, two birds (one male and one female with body weights near the mean for that replicate) were randomly selected per replicate and euthanized by cervical dislocation for carcass trait assessment. Different body parts were precisely measured and documented using a digital scale.

Caecal Microbiota

The caecal contents of chickens slaughtered on day 42 were collected aseptically in a sterile sample collection bag (HiMedia, India). The samples were processed on the same day for bacteriological analysis to quantify *E. coli, Salmonella*, and *Lactobacillus* using a standard colony counting procedure (Quinn et al., 1994). One gram of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10ìL of the diluted sample was spread onto specific agar plates for each bacterium: *E. coli* on sorbitol-MacConkey agar, *Salmonella* on Xylose Lysine Deoxycholate agar, and *Lactobacillus* on Lactobacillus agar (all from HiMedia, India). The agar plates were then incubated at 37°C for 24 to 48 hours, and the characteristic colonies for each bacterial group were counted using a digital colony counter (HiMedia, India). The results were expressed as Log10 colony-forming units (cfu) per gram of the sample.

Chemical analysis of feeds samples

The feed samples were analyzed according to AOAC (1995) methods. Dry matter (DM) was determined using method 934.01. Crude protein (CP) was analyzed with Kelplus equipment from Pelican Equipments in Chennai, India, following method 968.06. Crude fiber (CF) was measured using the Foss Fiber Cap 2021 Fiber Analysis System from Foss Analytical in Hilleroed, Denmark. Ether extract (EE) was determined with Socsplus equipment from Pelican Equipments in Chennai, India, following method 920.39. Calcium content was determined as per Talapatra et al.(1940). Additionally, the AIA levels of the diets were measured using the technique outlined by Furuichi and Takahashi (1981).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) with the SPSS software (SPSS Inc, 1997). The study followed a completely randomized design, with treatment as the main factor and pen as the experimental unit for body weight gain, feed intake, and FCR. Individual birds were considered as the experimental units for other parameters. Mortality data met homogeneity criteria and did not require transformation for statistical analysis. A significance level of $P<0.05$ was used to determine significance, while $P<0.01$ was considered a trend. When treatment had a significant effect, the Duncan test was used to detect differences among treatment means.

RESULT AND DISCUSSION

Average Daily Gain, Feed Intake and Feed Efficiency

The average daily gain (ADG) of the birds significantly increased ($p < 0.05$) in the synbiotic group compared to the control and antibiotic groups throughout the entire 42-day experiment period. The final body weight was significantly higher ($p < 0.05$) in the synbiotic group compared to the antibiotic and control groups. The average daily feed intake (ADFI) of the chickens did not vary significantly among the treatment groups during the different experimental phases or over the entire 42-day period. There were no significant differences in feed conversion ratio

(FCR) during the starter (1–14 days) and grower (15–28 days) phases. However, the FCR during the finisher phase (29-42 days) and overall period (1–42 days) was significantly improved ($p < 0.05$) in the synbiotic group compared to the control and antibiotic groups (Table 2). The study revealed that supplementing synbiotics led to a significant increase in final body weight and average daily gain, as well as an improvement in feed conversion ratio over a 42-day period. However, feed intake did not differ significantly among the treatment groups. Similar results were obtained by Dakhil and Al-Shammari (2023), showing significant improvements ($P<0.05$) in body weight, cumulative weight gain, growth rate, and feed efficiency in the synbiotic group compared to the control group. Song et al. (2022) also observed a higher average daily gain in the synbiotic-supplemented group compared to the control group ($P< 0.05$). Aparna et al. (2022) reported that supplementation with *L. salivarius* at 10^{12}cfu/kg showed significant ($P< 0.05$) increase in weight gain and improvement in feed conversion ratio (FCR). These studies suggest that synbiotic supplementation positively impacts weight gain and feed conversion ratio. This improvement in weight gain may be attributed to probiotics' ability to secrete digestive enzymes like lipase, protease, and amylase, which help break down feed nutrients for better digestibility of starch, fat, and protein, resulting in increased availability of nutrients for the broilers and leading to higher live weight gain (Bedford, 2000). Additionally, improved FCR might be attributed to the combined effect of prebiotics and probiotics preserving normal microbial populations while simultaneously enhancing ileal digestibility (Nisar et al., 2021; Aziz Mousavi et al., 2018).

Table 2. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chickens.

Attribute	Treatment[1]			SEM	P-Value
	CON	AGP	SYN		
ADG (g/d)					
d1-14	25.26	25.52	28.19	0.816	0.284
d15-28	73.61	76.43	76.27	1.299	0.636
d29-42	70.88	74.62	78.49	1.400	0.077
d1-42	56.58^{c}	58.86^{b}	60.98^{a}	0.567	0.001
Final BW	2419.01^{c}	2541.55^{b}	2603.81^{a}	23.810	0.001
ADFI (g/d)					
d1-14	29.09	28.99	28.58	0.070	0.796
d15-28	105.57	104.50	102.39	1.218	0.584
d29-42	133.13	129.97	129.85	1.107	0.409
d1-42	89.26	87.78	87.11	0.626	0.382
FCR (g intake/g gain)					
d1-14	1.16	1.14	1.06	0.025	0.222
d15-28	1.44	1.37	1.36	0.031	0.535
d29-42	1.89^{a}	1.74^{b}	1.67^{b}	0.036	0.029
d1-42	1.58^{a}	1.49^{b}	1.43^{b}	0.018	0.000

[abc] Means bearing different superscripts in the same row differ significantly (p d" 0.05).

[1] The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). [2] SEM, standard error of means (n=6)

Blood serum biochemical profile

The levels of glucose, total protein, albumin, uric acid, triglyceride, and cholesterol in serum showed no significant variation (P > 0.05) among the different dietary treatments in this study (Table 3). The study found that supplementing with synbiotics did not lead to significant changes in serum glucose, total protein, albumin, uric acid, triglyceride, and cholesterol levels. This aligns with previous studies by Œli¿ewska et al. (2020), Abdel-Hafeez et al. (2017) and Khalil et al. (2021), which also reported no significant impact on the blood biochemical profile when synbiotics were added to the broiler diet.

Table 3. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on blood biochemical profile of broiler chickens at day 35

Attribute	Treatment[1]			SEM[2]	P-Value
	CON	AGP	SYN		
Glucose (mg/dl)	282.61	240.01	289.67	12.563	0.229
Total protein(mg/dl)	3.34	3.77	3.24	0.105	0.084
Albumin(mg/dl)	2.99	3.24	3.13	0.063	0.285
Uric acid (mg/dl)	3.26	3.11	3.34	0.083	0.543
Triglyceride (mg/dl)	173.50	170.43	159.90	8.607	0.680
Cholesterol (mg/dl)	171.12	177.08	157.89	9.001	0.83

[1] The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). [2] SEM, standard error of means (n=6)

Immune Response

There were no significant differences (p > 0.05) in antibody titers for the infectious bursal disease (IBD) and Newcastle disease (ND) vaccines between the dietary treatment groups on days 28 and 35 (Table 4). In this study, there were no significant differences (p > 0.05) in antibody titers to the infectious bursal disease (IBD) and Newcastle disease (ND) vaccines between the dietary treatment groups on day 28 and day 35. This result is consistent with previous studies by Salehimanesh et al. (2016), Rehman et al. (2020) and Silva et al. (2009) which also reported no significant differences in antibody titers when synbiotic supplements were added to broiler chickens' diets. However, Pappula, (2021) reported that Humoral immune response (NDV titers) was higher (P<0.05) in lauric acid+*Bacillus subtilis* group followed compared to control.

Table 4. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on antibody titre (log_{10}) against infectious bursal disease vaccine (IBDV) and New castle disease vaccine (NDV) of broiler chickens at day 28 and day 35.

Attribute	Treatment[1]			SEM[2]	P-Value
	CON	AGP	SYN		
IBDV					
d28	3.07	3.10	3.18	0.046	0.621
d35	3.13	3.25	3.33	0.053	0.313
NDV					
d28	2.49	2.67	2.70	0.801	0.542
d35	2.79	2.81	2.89	0.478	0.682

[1] The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). [2] SEM, standard error of means (n=6)

Carcass characteristics

There were no significant differences (P>0.05) observed in slaughter body weight, eviscerated carcass weight, dressing percentage, and weights of various body parts (breast, frame, thigh, drumstick, wing, neck, gizzard, liver, heart, spleen, bursa, and abdominal fat) in grams among the treatment groups (Table 5). The current study found no significant differences in carcass characteristics when synbiotics were added to the diet of broiler chickens. These results are consistent with previous studies by Oliveira et al.(2022), which also found no significant differences in carcass yield or breast yields with varying levels of synbiotics in broiler diets. Similarly, studies by Nisar et al. (2021), Sarangi et al. (2016), Ghasemi et al. (2014) and Ashayerizadeh et al. (2011) reported that supplementing synbiotics at different levels did not significantly affect carcass traits, such as thigh, breast, or wing weights. However, Hossain et al. (2024) reported that supplementation with MOS and yeast showed a positive effect (P < 0.05) on the breast, thigh, wing, back, and gizzard weight.

Table 5. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on carcass characteristics of broiler chickens at day 42.

Attribute	Treatment[1]			SEM[2]	P-Value
	CON	AGP	SYN		
Slaughter BW (g)	2443.75	2462.27	2509.26	20.898	0.479
Eviscerated BW (g)	1664.85	1684.91	1690.84	13.704	0.773
Dressing Percentage (%)	68.12	68.45	67.38	0.292	0.358
Breast (g)	598.67	606.67	585.00	11.199	0.779
Frame (g)	307.00	285.67	304.67	7.158	0.470
Thigh (g)	227.67	222.00	214.67	6.331	0.758
Drumstick (g)	217.00	228.33	224.00	3.624	0.498
Wing (g)	126.33	134.67	128.67	3.182	0.610
Neck (g)	58.17	67.33	62.00	2.107	0.218
Gizzard (g)	44.51	47.43	50.63	1.504	0.281
Liver (g)	33.07	39.51	38.03	1.889	0.395
Spleen (g)	1.94	2.26	2.31	0.227	0.820
Bursa (g)	1.43	1.43	0.69	0.234	0.622
Abdominal Fat (g)	38.04	35.93	42.84	1.919	0.367

[1] The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). [2] SEM, standard error of means (n=6)

Gut microbes

The count of *E. coli* and *Salmonella* were significantly lower (p<0.05) in the synbiotic group compared to the control and antibiotic groups. Moreover, the count of *Lactobacillus* was significantly higher (p<0.05) in the synbiotic group than in the control and antibiotic-supplemented groups (Table 6). This study showed that supplementing with synbiotics increased *Lactobacillus* levels and a reduced in *E. coli* and *Salmonella* levels compared to the antibiotic and control groups. These results align with previous studies by Nopparatmaitree et al. (2022)**;** Dibaji et al. (2014); Wein et al. (2020); Ayalew et al.(2022); Abdel-Wareth et al. (2019); Mookiah et al. (2014) and Erdoðan et al. (2010), which also found that synbiotic supplementation boosted beneficial bacteria like *Lactobacilli* while decreasing harmful pathogens like *E. coli* and *Salmonella* in poultry.

Table 6. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on viable bacteria numbers (log_{10}cfu/g) in caecal content of broiler chickens at day 42

Attribute	Treatment[1]			SEM[2]	P-Value
	CON	AGP	SYN		
E. coli	6.92[a]	6.66[b]	6.39[c]	0.068	0.002
Salmonella	6.77[a]	6.57[a]	6.18[b]	0.071	0.000
Lactobacillus	6.97[c]	7.23[b]	7.54[a]	0.064	0.000

[abc] Means bearing different superscripts in the same row differ significantly ($p \leq 0.05$).[1] The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). [2] SEM, standard error of means (n=6)

CONCLUSION

Birds supplemented with synbiotics exhibited higher final body weight and average daily gain, along with an improved feed conversion ratio (FCR) throughout the experiment compared to the control group. Furthermore, synbiotic supplementation led to decreased caecal *E. coli* and *Salmonella* counts in comparison to other groups. *Lactobacillus* counts were notably elevated in birds receiving synbiotics compared to those in the control and antibiotic groups. These findings suggest that synbiotics could serve as effective alternatives to antibiotics in poultry production, particularly for broilers on an antibiotic-free diet, thereby promoting overall broiler health. Further research is necessary to fully comprehend and optimize the utilization of synbiotics in poultry production. This study underscores the potential of synbiotics as a promising option for improving gut health in poultry without the reliance on antibiotics.

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Nano Zinc Supplementation: Its Influence on Growth Performance, Feed Intake and Haematobiochemical Parameters in Male Wistar Rats

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ABSTRACT

A study was conducted to investigate the effect of supplementation of nano zinc (ZnO-NPs) from zinc oxide source on growth performance, feed intake and hemato biochemical parameters in male Wistar rats. A total of 36 male Wistar rat were taken, where 6 animals were sacrificed at zero days for hemato biochemical studies and rest of the rats were divided into five groups of 6 animals in each. The group T1 was fed purified diet without Zn supplementation. In group T2, 12 ppm inorganic Zn was given while the rats in groups T3, T4 and T5 were supplemented with nano Zn @ 3, 6 and 12 ppm, respectively for five weeks. The average daily gain and feed conversation ratio improved in groups supplemented either with 12 ppm inorganic Zn or with 3, 6 and 12 ppm nano Zn. The plasma ALP activity increased ($P < 0.05$) with increase in ZnO-NPs while there was a reverse trend for AST and ALT activities. The concentration of total cholestrol and triglycerides decreased ($P< 0.5$) due to inorganic or ZnO-NPs addition in the diet, however, the effects of 3 ppm ZnO-NPs addition were comparable to those of 12 ppm inorganic Zn or 6 and 12 ppm of nZn. Therefore, supplementation of 3 ppm nano Zn improved growth performance and biochemical parameters in the male Wistar rats and the results were comparable to the groups given either 12 ppm inorganic Zn or 6 and 12 ppm nano Zn.

KEYWORDS: Biochemical parameters, Feed intake, Performance, Wistar rats, ZnO-NPs

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INTRODUCTION

Zinc is one of the most important essential trace elements for animals and its deficiency is a worldwide phenomenon (Chander Datt and Chhabra, 2005). In all species, Zn deprivation is characterized by inappetence, retardation of growth, skeletal and reproductive disorders (Suttle, 2010). Deficiency of Zn from the extracellular space resulted in decreased activity of deoxythymidine kinase and reduced levels of adenosine tri phosphate. Hence, Zn may directly regulate DNA synthesis (Mc Donald, 2000). The acute Zn deprived rats were unable to nibble while little addition of Zn (3-6 ppm) avoided this complication (Apgar et al., 1993). Zinc deficient rats (1 ppm) showed decrease ($P<0.05$) in body weight gain and feed intake and showed reduced growth with bone abnormalities (Roth, 2003 and Cho et al., 2013). The optimum growth and feed intake was observed in rats with 12 ppm Zn supplementation as $ZnCO_3$ (Nagalakshmi et al., 2013) and no further beneficial effects were observed on performance with increase in Zn supplementation (24 to 48 ppm). The organic Zn supplemented in nicotinate form (6, 9 and 12 ppm) in female Sprague Dawley rats had no significant effect on body weight gains and daily feed intake (Nagalakshmi et al., 2015). Zinc-deficient diet groups (19 mg/kg diet, 1/2 of control and 3.8 mg/kg diet, 1/10 of control) gained less ($P<0$ .05) than the control groups (38 mg/kg diet, control) in growing male and female rats for 10 weeks (El Hendy et al., 2010).

In India, common practice is to supplement inorganic mineral sources like sulphates, carbonates and chloride forms (Batal et al., 2001). Interaction of inorganic forms with other nutrients and minerals often leads to higher level of supplementation for

better performance which might result in toxicity and environmental pollution (Cheng et al., 1998). Recently, organically complexed or chelated forms of minerals are being used for livestock because of higher bioavailability (Edwards and Baker, 1999). However, with the advent of nanotechnology, it is possible to use nanoparticle as a source of mineral supplement in animal rations. The nano sized minerals increased absorption (Davda and Labhasetwar, 2002). Numerous interfaces where the atomic configurations differ from those of the crystal lattice define nanoparticles. (Ghosh, 2012). There is scanty information on ZnO-NPs supplementation in animals, Hence, this work was undertaken to examine the effect of ZnO-NPs addition in the diet on growth performance and biochemical parameters in male Wistar rats.

MATERIALS AND METHODS

Animal selection

Thirty-six weaned healthy Wistar male rats (*Rattus norvegicus*) weighing 111±0.5g were selected from Small Animal House, ICAR-National Dairy Research Institute, Karnal, Haryana, India and adapted to the new environs by keeping them for 7 day on a normal diet prior to the start of the actual trial. At zero day, six animals were sacrificed for collection of blood for estimation of hematobiochemical parameters. Rats were divided into five groups of six animals each i.e. T1 (fed with purified diet without Zn supplementation); T2 (fed as per group T1 supplemented with inorganic Zn at 12 ppm level); T3 (fed as per group T1 supplemented with nano Zn at 3 ppm level); T4 (fed as per group T1 supplemented with nano Zn at 6 ppm level) and T5 (fed as per group T1 supplemented with nano Zn at 12 ppm level). The Institutional Animal Ethics Committee (IAEC) of ICAR-NDRI, Karnal, Haryana approved the study and the protocol was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

Housing and feeding management

All the animals were caged in group of two animals and housed in a well-ventilated room (22-25°C, 40-60% relative humidity, 12-hour light/dark cycle) adopting strict management and hygienic practices throughout the experiment. Clean drinking water was provided *ad lib*. throughout the experiment in nipple fitted polypropylene bottles. The animals were offered a common Zn free purified diet according to ICAR (2013) for a period of 35 days (Table 1). The experimental feeding was similar in all five groups except for the sources of Zn. The feed intake and body weights of rats were recorded weekly. Analysis of basal diet was done for proximate principles and minerals (AOAC, 2005).

Table 1. Composition of purified diet

Ingredient	Proportion
Sucrose	45.0
Casein	20.0
Corn starch	15.0
Corn oil	5.0
Cellulose	5.0
Mineral premix*	3.5
Vitamin premix**	1.0
DL-Methionine	0.3
Choline bitartarate	0.2
Wheat flour	5.0

*Mineral premix(g/kg): Calcium phosphate, dibasic ($CaHPO_4$) (500.00); Potassium citrate, monohydrate ($K_3C_6H_5O_7.H_2O$) (220.00); Sodium chloride (NaCl) (74.00); Potassium sulphate (K_2SO_4) (52.00); Magnesium oxide (MgO) (24.00); Ferric citrate (6.00); Manganous carbonate ($MnSO_4$) (3.50); Zinc carbonate ($ZnCO_3$)§ (1.60); Chromium potassium sulphate ($CrK(SO_4)_2.12H_2O$) (0.55); Cupric carbonate ($CuCO_3$) (0.30); Potassium iodate (KIO_3) (0.01); Sodium selenite ($Na_2SeO_3.5H_2O$) (0.01); Sucrose powder(118.03).

§Only added in mineral mixture supplemented to animals of group T_2.

**Vitamin premix (g/kg): Nicotinic acid (3.000); Calcium d-pantothenate (1.600); Pyridoxine hydrochloride (0.700); Thiamin hydrochloride (0.600); Riboflavin (0.600); Folic acid (0.200); d-Biotin (0.020); Cyanocobalamine (0.001); Retinyl acetate (400,000 IU); Alfa-tocopheryl acetate (5000 IU); Cholecalciferol (0.0025); Menaquinone (0.005); Sucrose powder (To make <1000 g).

Haematological and biochemical parameters

At the beginning and end of the experiment, blood samples were collected from the sacrificed animals after being slightly anesthetized with diethyl ether. One mL of blood was anti coagulated with EDTA shortly after collection for analysis of red blood cell (RBC), haemoglobin (Hb), haematocrits (PCV), total leukocyte count (TLC), mean corpuscular hemoglobin (MCH %), mean corpuscular volume (MCV %) and mean corpuscular hemoglobin concentration (MCHC %) by an automatic hematology cell counter (Nihon Kohden, Celltaca, Tokyo, Japan). Four mL of blood was transferred into sterile collection tubes without any addition of anticoagulant. The samples were centrifuged (Sigma, Laborzentrifugen, 3K15) at 5000 rpm for 10 min. at 4ºC. The serum samples were collected, stored at -20^{0}C and used for analysis of different biochemical constituents (glucose, total protein and albumin, globulin, cholesterol, triglyceride, ALP, AST, ALT) using diagnostic kits (Recombigen Laboratories Pvt. Ltd, India).

Statistical analysis

Statistical analysis of data was carried out by one way analysis of variance (ANOVA) model (Snedecor and Cochran, 1994). This statistical ANOVA model was incorporated with General Linear Models (GLM) procedure of SPSS 16.0 computer packages. Comparison of data was done with Tuckey's honest significant difference (HSD) test method (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Body weights, average daily gain and feed conversion ratio in different groups

The DM, OM, CP, CF, EE, total ash and NFE contents of purified diet were found to be 95.08±1.06, 96.93±0.01, 19.50±0.08, 5.22±0.05, 4.89±0.08, 3.06±0.01 and 67.85±0.08 %, respectively. The Ca and P level was found to be 0.70±0.05 and 0.37±0.02 % in purified diet. The concentration of Zn, Cu, Fe and Mn was 1.76±0.29, 6.92±0.15, 40.61±0.73 and 10.10±0.87 ppm, respectively. The DM, OM, CP, CF, EE, total ash and NFE content of purified diet were found in prescribed range of nutrient requirement for rats (ICAR, 2013). The average initial body weights of the experimental rats were recorded to be 111.43±0.36 g. At the end of 5th week of the experiment, average body weights of rats were similar in all the groups (Table 2). There was no difference in average daily feed intake among the groups (Table 2). The average feed intake varied from 16.77 to 18.45 g/d. The feed conversion ratio improved in groups T2, T3 and T5. The significantly highest FCR value was seen in group T1 and lower FCR value in group T5 (Fig. 1). There was no beneficial effect of total replacement of Zn (12 ppm) from $ZnCO_3$ with other organic sources (Zn-Met, Zn proteinate and Zn propionate) on feed intake in rats (Nagalakshmi et al., 2012). Further, it was reported that organic Zn (zinc nicotinate) supplementation (6, 9 and 12 ppm) compared to inorganic Zn (12 ppm) in female Sprague Dawley rats had no significant effect on daily feed intake (Nagalakshmi et al., 2012). Zn deficiency in animals might reduce appetite by impairing the taste because the taste is mediated through salivary Zn dependant polypeptide gustin (Droke et al., 1993). Zinc deficient rats (1 ppm) showed significant ($P<0.05$) decrease in food intake (Cho et al., 2003) compared with Zn adequate rats (35 ppm).

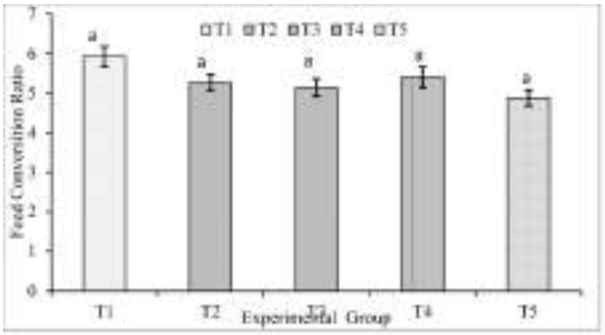

Fig. 1 Feed conversion ratio in rats supplemented with different levels and sources of Zn.

Table 2. Growth performance and feed intake (g) of male Wistar rats supplemented with different levels and sources of Zn

Week	Group				
	T1	T2	T3	T4	T5
	Body weight (g)				
Zero	111.00±5.70	110.50±7.02	111.67±5.61	112.67±5.07	111.33±5.46
1	130.50±5.32	131.83±6.38	135.00±5.67	134.08±5.30	134.58±5.09
2	151.33±5.98	155.17±5.24	160.75±4.78	156.67±4.18	161.17±5.64
3	171.68±6.28	179.73±4.66	188.52±5.33	183.02±4.88	188.38±4.80
4	192.60±6.41	204.25±4.43	214.50±5.67	208.32±5.44	214.42±5.10
5	211.90 ^{b}±6.63	229.33 ^{ab}±4.59	239.36 ^{a}±5.99	234.01 ^{ab}±5.55	239.51 ^{a}±5.62
Overall average	161.50±6.29	168.47±7.20	174.46±7.75	171.90±7.32	174.90±7.75
	Average daily feed intake (g)				
1	18.73 ± 0.47	19.82 ± 0.58	20.90±0.55	19.58±0.51	19.42±0.63
2	16.60^{c} ± 0.56	17.42^{bc} ± 0.56	19.12^{ab} ± 0.69	20.51 ^{a}±0.57	19.58^{ab}±0.71
3	16.90^{b} ± 0.56	15.65^{b} ± 0.58	18.86^{a}±0.45	17.21^{ab}±0.45	18.07^{a}±0.58
4	11.87±0.73	15.81±0.84	12.95±0.89	12.27±1.35	12.27±1.32
5	19.75±0.55	18.75 ± 0.57	20.40 ± 1.01	21.43±0.69	19.59±0.78
Overall average	16.77±0.56	17.49±0.40	18.45±0.61	18.20±0.69	17.79±0.63
	Average daily gain (g)				
1	2.79±0.09	3.05±0.20	3.33±0.17	3.06 ± 0.26	3.32±0.14
2	2.98^{b} ±0.15	3.33^{ab}±0.25	3.68 ^{ab}±0.18	3.23^{ab} ±0.18	3.80^{a}±0.19
3	2.91^{b}±0.15	3.51^{ab}±0.14	3.97^{a}±0.19	3.76^{a}±0.16	3.89^{a}±0.14
4	2.99^{b}±0.15	3.50^{ab}±0.14	3.71^{a} ±0.11	3.61^{ab}±0.23	3.72^{a}±0.11
5	2.76^{b}±0.15	3.58 ^{a} ± 0.06	3.55^{a} ±0.12	3.67^{a} ±0.17	3.58^{a}±0.14
Overall average	2.88^{b}±0.06	3.40^{a}±0.08	3.65^{a}± 0.08	3.47^{a} ±0.10	3.66^{a} ±0.07

$^{a, b, c}$ Means with different superscripts in a row differ significantly ($P < 0.05$)

Rats fed with a Zn-deficient diet consumed less feed and showed reduced growth rate (Yamaguchi and Yamaguchi, 1986; Blanchard et al., 2001). Similar findings were documented (Lina et al., 2009; Ahmadi et al., 2013) who showed that nano Zn supplementation resulted in better growth performance in broilers. No effect was observed in goat kids (Ahmadi et al., 2013) and piglets (Jianbo et al., 2013) due to nano Zn supplementation which could be due to differences in species, supplementary level of Zn and other dietary and managemental conditions. Similar results (Nagalakshmi et al., 2015) were reported in rats supplemented with 12 ppm Zn as inorganic source ($ZnCO_3$) and no further beneficial effect was observed in weight gain with increased Zn supplementation (24-48 ppm). Replacement of $ZnCO_3$ (12 ppm) with other organic sources (Zn Met, Zn proteinate and Zn propionate) had no significant effect on body weight of rats (Nagalakshmi et al. 2014; Nagalakshmi et al., 2015). However, Zn supplementation showed higher growth which might be due to the fact that Zn is a part of deoxythymidine kinase which is involved in DNA synthesis and nucleic acid metabolism, therefore,

growth may be hampered in case of Zn deficiency (Suttle, 2010). This was consistent with the results where similar growth rate was found in weanling piglets supplemented with 120 mg/kg of nano-Zn, organic-Zn or ZnO in the diet (Li et al., 2016). There was improvement ($P<0.05$) in FCR due to supplementation of Zn. A better FCR value due to inorganic Zn supplementation which corroborated our findings (Nagalakshmi et al., 2012). In this study, supplementary nano Zn showed better daily average feed intake at a lesser dose which might be due to better absorption by intestinal villi (Hett, 2004; Cho et al., 2013).

Effects on haematological parameters

The Hb concentration ranged from 10.05±0.35 to 11.15±0.26 g/dL and RBC values from 9.30±0.27 to 9.97±0.25 million/mm^3. The WBC values varied from 9.41±0.39 to 10.69±0.31 thousand/mm^3 while PCV values ranged from 35.50±0.95 to 38.50±1.25%. The values of MCV and MCH varied from 37.93±1.10 to 39.83±1.72% and from 10.82±0.53 to 11.67±0.48%, respectively. The MCHC values ranged from 28.19±0.87 to 30.34±0.49%. The values of various haematological parameters were similar in all the treatment groups (Table 3). Haematological parameters of Wistar rats were not affected by source and different levels of nZn supplementation (Raje et al., 2018). There was no significant effect of 100% replacement of $ZnCO_3$ with various sources of organic Zn (Zn-Met, Zn proteinate and Zn propionate) on serum haematological constituents in rats (Nagalakshmi et al., 2014; Nagalakshmi et al., 2016). Organic Zn (Zn nicotinate) supplementation (6, 9 and 12 ppm) in female Sprague Dawley rats did not affect haematological constituents (Nagalakshmi et al., 2015). However, Zn deficiency (19 and 3.8 mg Zn/kg diet) in growing male and female rats for 10 weeks decreased the levels of Hb, total erythrocyte count and PCV while increasing TLC count (El Hendy et al., 2001).

Table 3. Haematological constituents in male Wistar rats supplemented with different levels and sources of Zn

Parameter	Group				
	T1	T2	T3	T4	T5
Haemoglobin (g/dL)	10.85 ± 0.43	11.15 ± 0.17	10.90 ± 0.12	10.05 ± 0.35	11.15 ± 0.26
RBC ($10^6 \times mm^3$)	9.68 ± 0.18	9.97 ± 0.25	9.59 ± 0.15	9.30 ± 0.27	9.52 ± 0.23
WBC ($10^3 \times mm^3$)	9.41 ± 0.39	10.59 ± 0.28	10.69 ± 0.31	10.19 ± 0.29	10.61 ± 0.14
PCV (%)	38.50 ± 1.25	37.75 ± 0.62	36.50 ± 0.50	35.50 ± 0.95	36.75 ± 0.75
MCV (%)	39.83 ± 1.72	37.93 ± 1.10	38.04 ± 0.29	38.27 ± 1.78	38.67 ± 1.36
MCH (%)	11.23 ± 0.58	11.20 ± 0.29	11.36 ± 0.13	10.82 ± 0.53	11.72 ± 0.35
MCHC (%)	28.19 ± 0.87	29.54 ± 0.22	29.87 ± 0.46	28.31 ± 0.74	30.34 ± 0.49

Effect of dietary nano Zn on blood biochemical parameters

The lowest value of serum glucose concentration was found in group T1 and the highest in Zn supplemented groups. The highest value of total protein was seen in groups T5 whereas the lowest level was found in group T1. The values in groups T2, T3, T4 and T5 were comparable. The serum albumin level was the highest in group T5 without any difference with group T2 and T3. The lowest albumin level was observed in group T1. The serum globulin level in different groups ranged from 3.56±0.18 to 3.78±0.29 g/dL, respectively. The A/G ratio was similar in all the groups. The group T1 showed the lowest values of serum ALP and there was no difference among T2, T3, T4, and T5 groups. The highest AST activity was seen in group T1 and the lowest in group T4 and T5. The serum ALT level was highest in group T1 and lowest in groups T2, T3, T4 and T5. The lowest serum cholesterol level was found in group T3, T4 and T5 whereas the highest value was seen in group T1. The values of serum triglyceride levels were lower in groups T2, T3, T4 and T5 compared to group T1.

The increased glucose level in Zn supplemented groups in this study was supported (Mishra et al., 2014). There was decrease in glucose, total protein and albumin level in group T1 (El Hendy et al., 2001). Replacement of inorganic Zn with organic Zn (Zn nicotinate) at 6, 9 and 12 ppm level (Nagalakshmi et al., 2015) and dietary Zn ($ZnCO_3$) with Zn-Met at the rates of 50, 75 and 100% in female Sprague Dawley rats (Nagalakshmi et al., 2016) showed no significant change in serum glucose and total protein level. There was significant variation in the activities of ALT in this study among Zn deficient group (T1) and supplemented groups (T2, T3, T4 and T5), therefore, a decreased level of this enzyme indicated no destruction of liver cells. Similar findings of decreased ALP activity were reported in Zn deficient group (Yousef et al., 2002). The Zn deficiency reduced ALP activity in RBC and plasma of rats (Cho et al., 2007; Lina et al., 2009). Zinc was supplemented @ 0, 30, 60, 90 and 180 mg/kg through Zn-Gly in Sprague-Dawley rat diets and ALP activity increased linearly with supplemental Zn levels (Huang et al., 2016). However, there was no effect of 100% replacement of $ZnCO_3$ with various sources of organic Zn (Zn-Met, Zn proteinate or Zn propionate) on serum biochemical constituents in rats (Nagalakshmi et al., 2014). In this experiment, Zn supplementation either in inorganic or nano form reduced cholesterol and triglyceride level in male Wistar rats (Paul et al., 2001). Elevated levels of triglyceride were recorded due to Zn deficiency (Eder and Kirchgessner, 1995). The reduced levels of serum cholesterol due to organic Zn supplementation (6-12 ppm level) have been reported (Nagalakshmi et al., 2016).

Table 4. Effect of supplementation of different levels and sources of nano Zn on biochemical parameters in male Wistar rats

Parameter	Group				
	T1	T2	T3	T4	T5
Glucose (mg/dL)	57.51^{b}±1.23	80.76^{a}±1.15	79.34^{a}±1.03	81.73^{a}±0.99	83.51^{a}±0.92
Total protein (g/dL)	7.37^{b}±0.14	8.08^{ab}±0.22	7.77^{ab}±0.21	7.91^{ab}±0.11	8.12^{a}±0.14
Albumin (g/dL)	3.67^{c}±0.09	4.30^{ab}±0.09	4.21^{ab}±0.06	3.99^{bc}±0.07	4.54^{a}±0.17
Globulin (g/dL)	3.70 ± 0.20	3.78 ± 0.29	3.56 ± 0.18	3.92 ± 0.18	3.59±0.29
A: G ratio	1.01 ± 0.08	1.17 ± 0.10	1.20 ± 0.06	1.03 ± 0.05	1.33±0.16
ALP (U/L)	93.10^{b} ± 0.65	99.65^{a} ± 1.40	98.87^{a} ± 0.44	$98.2\ 5^{a}$ ± 1.52	100.30^{a} ±1.35
AST (U/L)	84.07^{a} ± 0.34	71.14^{c} ± 0.35	73.50^{b} ± 0.51	61.20^{d} ± 0.48	62.06^{d}±0.43
ALT (U/L)	23.82^{a} ± 0.33	17.54^{b} ± 0.17	18.27^{b} ± 0.28	17.42^{b} ± 0.13	17.25^{b} ± 0.16
Total cholesterol (mg/dL)	85.35^{a}±0.78	80.09^{b}±1.09	70.24^{c}±0.90	68.78^{c}±0.70	67.47^{c}±0.58
Triglyceride (mg/dL)	119.60^{a}±5.45	102.43^{b}±0.91	101.10^{b}±1.64	98.24^{b}±2.48	99.62^{b}±1.24

[a,b] Mean values bearing different superscripts in arow differ significantly (P<0.05)

CONCLUSIONS

The feed intake was not affected either by 12 ppm of inorganic Zn or by 3, 6 and 12 ppm of Nano Zn supplementation. However, supplementation of 3 ppm nano Zn was comparable to the groups given either 12 ppm inorganic Zn or 6 and 12 ppm nano Zn in terms of growth performance and blood biochemical attributes in male Wistar rats.

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Effect of Xanthophyll Rich Marigold Petal Meal in the Ration of Commercial Layers on Egg Composition and Quality Characteristics

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ABSTRACT

The study was conducted to determine the effect of xanthophyll rich marigold petal meal (*Tagetes erectus L.*) on egg composition and quality characteristics. One twenty Hyline commercial layers (24wks old) were divided into five groups of four replication each (six birds in each replication) in CRD. The marigold petal meal (MPM) was incorporated (%) in the diet of commercial layers at 0 (T1), 3 (T2), 4 (T3), 5 (T4) and 6 (T5) levels. The CP content of experimental diets ranged from 16.53 to 17.86 per cent and MPM contained 12.49 % CP , 11.43%EE and xanthophylls 2400mg/kg of petal meal. MPM inclusion did not affect egg weight and egg shell thickness between the groups. Even though there was a significant difference in calcium intake between the treatments, Haugh units, yolk index and albumen index were not affected between the treatment groups. Xanthophyll content of egg yolk, yolk colour and crude protein content were higher as the levels of MPM increased in the diet. The inclusion of MPM as a source of carotene and protein in commercial layer diets up to 6 per cent level improved egg quality parameters without any adverse effects on the performance of the layers.

KEYWORDS: Egg composition, Egg yolk, Marigold petal meal, Xanthophyll, Yolk index.

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INTRODUCTION

In poultry industry, maize is used extensively as a source of energy and invariably it is also a source of xanthophyll. However, due to rise in the cost of maize in the recent past, search for alternate feed resources either as source of pigment or any other nutrients is a major thrust area in the field of poultry nutrition. Marigold petal meal is one among several unconventional feed stuffs available locally to the farmers where it contained 11 to 12 per cent CP and 10-11 per cent EE (Sujatha et al., 2015). In addition, it has 100 times more xanthophylls than maize. Several studies were conducted with supplementation of marigold flower extract in the diets of layers but not many on direct feeding of marigold petals in the form of meal as feed additive. Moreover, India produces around 603.18 TMT of marigold flowers and the share of Karnataka alone is around 64025 tons which can be used as a source of xanthophylls and protein in the diets of layers to improve the egg quality (NHB, 2018). However, studies conducted at higher levels of inclusion in poultry is scarce, therefore, this study was taken up to know the effect of marigold petal meal at higher levels as a component of commercial layer's diet by replacing deoiled rice bran on egg composition and egg quality characteristics.

MATERIAL AND METHODS

One hundred twenty (24 weeks old) White Leghorn pullets of Hyline strain were divided in to five groups of four replications each and six birds in each replication in complete randomized design. The birds were maintained under standard management protocol at Poultry Experimental Unit, Dept. of Livestock Farm Complex, Veterinary College, Shivamogga. The approval of institutional animal ethical committee to conduct the experiment was obtained (IAEC Approval number: No/VCS/IAEC/2019-20/SA-55 Date: 17/02/2020).

Marigold flowers (*Tagetes erectus L.*) were grown at farm section of Dept. of Horticulture, College of Agriculture, KSNUAHS, Shivamogga. The marigold petals were separated from calyx, seeds and shade dried. The dried marigold petals

were incorporated at 0 (T1), 3 (T2), 4 (T3), 5 (T4), 6 % (T5) levels in in the standard layer diet (SLD) formulated as per BIS (2007) specification for layers. The SLD contained 17% CP, 2600ME kcal/kg diet, 3.6 % Calcium; 0.74% Phosphorus and 0.36% available Phosphorus. The experiment was started when layers were 24th weeks of age and the laying phase was divided in to three periods of four weeks each from 24th to35th weeks (Period –I: 24th to 27th, Period-2: 28th to 31st, Period-III: 32nd to 35thweek). The experiment was conducted for a period of 120 days. During the experiment, body weight, feed and nutrient intake, intake of marigold petal meal, hen-day egg production, feed conversion ratio (FCR) and economics of inclusion of marigold petal meal were studied. The samples of feed ingredients, maize, marigold petal meal and eggs were collected and subjected for chemical composition (AOAC, 2016) and xanthophyll content of maize, MPM and yolk was analyzed by using spectrophotometer (Visiscan 167) as described by AOAC (2016). The data collected was analyzed by completely randomized design using statistical package for social sciences (SPSS, 15th version, 2017) and the means were compared by Duncan's multiple range test (Duncan and Ducan, 1955) and interpreted accordingly.

RESULS AND DISCUSSION

The inclusion level of maize (Table 1) in all the treatment diets was kept constant (55%) to know the effect of MPM on changes in body weight, feed and nutrient intake, intake of marigold petal, hen-day egg production, feed conversion ratio (FCR), egg production, egg composition and economics. The MPM was incorporated at different levels in the layers' diet by replacing deoiled rice bran and sunflower meal partially but the level of nutrients kept constant in all the diets according to BIS (2007) specifications so that all the diets should contain 18 per cent crude protein (CP) and 2700 Kcal/kg of metabolic energy (ME). However, the CP content of experimental diets ranged from 16.53 to 17.86 per cent, and crude fibre (CF) ranged from 7.69 to 8.78 per cent, calcium (Ca) ranged from 4.87 to 6.1per cent, and phosphorus (P) ranged from 0.89 to 2.28 per cent (Table 2). However, all the nutrient levels in all treatment groups were well within the recommended level of BIS (2007) specifications. These specifications were similar to the specifications maintained in the diets of commercial layers prepared by the various researchers (Hasin et al., 2006; Moeini et al., 2012; Sujatha et al., 2015).

Table 1. Ingredient composition (% as is basis) of experimental diets of commercial layers

Ingredient	T1	T2	T3	T4	T5
Marigold petal meal (MPM)	0	3	4	5	6
Maize	54.96	54.96	54.96	54.96	54.96
Deoiled rice bran	7	5.5	4	3.5	2.
Sunflower meal	6	3	3	2	2
Soybean meal	21	22	22	22.5	23
Limestone powder	2	2	2	2	2
Shell Grit	7	7	7	7	7
Dicalcium phosphate	1.2	1.2	1.2	1.2	1.2
Salt	0.5	0.5	0.5	0.5	0.5
Vegetable oil	0	0.5	1.0	1.0	1.0
AB_2D_3K[1]	0.015	0.015	0.015	0.015	0.015
B-complex[2]	0.025	0.025	0.025	0.025	0.025
Trace minerals[3]	0.1	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1
Total	100.00	100.00	100.00	100.00	100.00

[1]One gram of Vitamin AB_2D_3K supplement contained 82500 IU of Vitamin-A, 50 mg of Vitamin-B_2, 12000 IU of Vitamin-$D_{3\ and}$ 10 mg of Vitamin-K.

[2]One gram of B-Complex supplement contained 8 mg of Vitamin-B_1, 16 mg of Vitamin-B_6, 80 mcg of Vitamin B_{12}, 80 mg of Vitamin-E, 120 mg of Niacin, 8 mg of Folic acid, 80 mg of Calcium pantothenate, 120 mg of Calcium and 300 mg of Phosphate.

[3]One gram of Trace Minerals contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron,2 mg of iodine and 1 mg of cobalt

The CP content of MPM was almost similar to the milling by products like de-oiled rice bran, wheat bran and any gram husk but MPM had higher level of EE (11.43 %).The xanthophyll content of maize grain and MPM used to prepare the experimental diets was 21 and 2400 mg/kg, respectively.

The average feed intake between the treatments was not significant (Table 3). Irrespective of the level of inclusion, the feed intake was similar in both control and treatment groups which indicated that up to 6% inclusion of MPM in the commercial layers diet did not cause adverse effect on the palatability of feed. Similar feed intake was noticed in Hyline laying hens (104-108 grams/ bird/day) in the experiment conducted by Ariana et al. (2011) and Rezaei et al. (2019). On contrary, lower feed intake of around 90 gram/bird/day was observed in desi chickens as reported by Sujatha et al. (2015). Due to non significance in feed intake the FCR observed in all the experimental groups was also similar, however, the FCR achieved in this study was better than the FCR reported for layers other studies (Hasin et al., 2006; Rowghani et al., 2006; Moeini et al., 2012).

There was a significant difference in hen day egg production between the treatment groups in period I, whereas no significant difference in period II and III. The difference in the period *was* due to early laying phase of the bird where birds take some time to achieve maximum egg production. The egg production achieved in this experiment due to inclusion of MPM was corroborated with egg production achieved in Shaver 579 laying pullets (71%) (Hasin et al.,2006), in Hyline laying hens (71-76%) (Altunta and Aydin, 2014).

Table 2. Chemical composition (% on DMB) of commercial layer diets and marigold petal meal (MPM) used in the experiment

Particular	T1 (Control, 0%)	T2 (3%MPM)	T3 (4%MPM)	T4 (5% MPM)	T5 (6% MPM)	MPM	Maize
DM	93.4	93.63	94.29	92.95	92.62	7.76¢	89.73
OM	85.33	85.19	84.08	85.78	83.80	92.77	98.75
CP	16.58	16.82	16.94	16.53	17.86	12.49	9.17
EE	2.65	4.33	4.07	3.55	4.42	11.43	3.39
TA	14.67	14.81	15.92	14.22	16.20	7.23	1.25
CF	8.78	8.69	8.34	7.69	8.21	23.22	2.58
NFE	57.32	52.35	53.73	58.01	53.31	45.63	83.65
Ca	4.87	4.91	5.22	6.1	5.25	0.95	1.29
P	0.89	1.44	1.31	1.72	2.28	0.72	0.45
ME#, kcal/kg	2555	2546	2561	2547	2539	-	-
Xanthophylls, mg/kg	-	-	-	-	-	2400	21

#ME - Metabolized Energy (calculated), MPM- Marigold petal meal .

Table 3. Effect of dietary inclusion of marigold petal meal onfeed intake, FCR and percent hen day egg production.

	Feed intake (g/day/bird)			FCR #			Hen day egg production (%)		
	Period (weeks)			Period			Period		
Treatment	I (24-27)	II (28-31)	III (32-35)	I (24-27)	II (28-31)	III (32-35)	I (24-27)	II (28-31)	III (32-35)
T1	105.17	105.58	104.57	1.54	1.4	1.58	65.48^{b}	71.73	75.45
T2	104.8	105.77	104.40	1.56	1.34	1.54	71.13^{a}	75.30	83.93
T3	104.73	104.34	104.34	1.4	1.35	1.43	66.07^{b}	72.32	83.04
T4	104.04	103.54	103.91	1.44	1.3	1.47	67.86^{b}	69.20	80.80
T5	104.92	103.73	104.15	1.47	1.35	1.43	58.78^{c}	67.86	78.42
SEM	0.21	0.41	0.12	0.03	0.02	0.03	1.82	1.09	1.35
P value	0.423	0.140	0.478	0.121	0.824	0.118	0.02	0.23	0.27
Significance	NS	NS	NS	NS	NS	NS	*	NS	NS

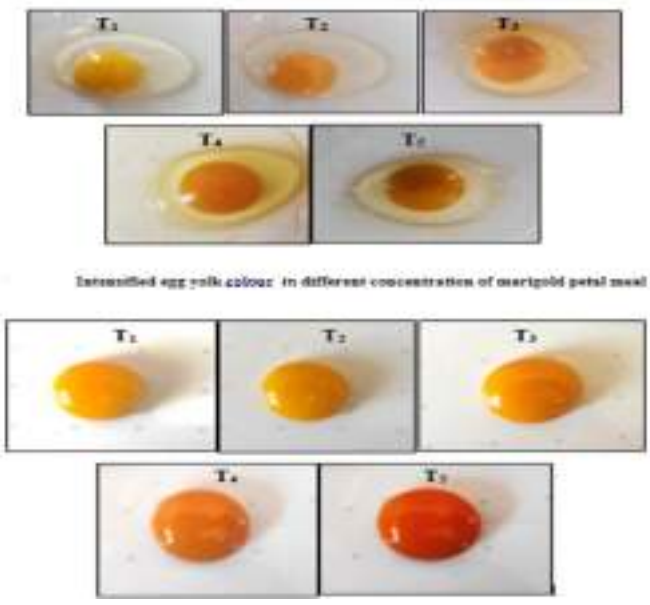
FCR is calculated on dozen egg basis

There was no significant effect on shell thickness of the eggs produced by birds fed with different levels of MPM even though there was a variation in the calcium level among the diets (Table 4) which might be due to efficiency of utilization of calcium in formation of egg shell was similar in all groups. Similar shell thickness values (0.33-0.40mm) were reported in several studies in layers of irrespective breeds fed with various levels of either marigold petal powder or extract from marigold flowers (Hasin et al., 2006; Ariana et al.,2011; Sujatha et al., 2015; Rezaei et al., 2018). The mean haugh units recorded in control and treatment groups in this experiment for commercial layers were not differed significantly. MPM inclusion on haugh units was on par with the maize included diet (T1). Whereas the haugh unit recorded in this experiment were higher (85.93 to 88.48) than the values reported by Hasin et al. (2006) in marigold petal powder at 4 per cent level in Shaver 579 laying pullets (80), in hyline W 36 white hens fed with 1.5 per cent marigold powder (72.26) and in hyline (W-36) birds fed with 1 and 3 per cent marigold flower powder (Moeini et al., 2012).

MPM inclusion did not bring any significant change in the yolk index when compared to the maize included group (T1). The values reported in this study (0.46-0.47) were similar to the values (0.45-0.47) reported in desi chicken eggs (Sujatha et al., 2015) whereas lower than the values (40-41) reported for hyline -5 laying hens (Altunta and Aydin,2014). Not much variation was noticed in albumin index of the eggs between the treatments. In all MPM fed groups, similar values (0.14) were obtained but higher by 1 unit than in T1 group (0.13). Much lower values of albumin index (0.07-0.09) were reported by Altunta and Aydin (2014) and Sujatha et al. (2015).

Table 4. Effect of dietary inclusion of marigold petal meal on egg shell thickness, Haugh unit, yolk index, albumin index, yolk color and xanthophyll content in yolkin commercial layers

		Treatment						
Parameter	Period	T1	T2	T3	T4	T5	SEM	P value
Egg shell thickness, mm	I	0.35	0.35	0.35	0.37	0.33	0.01	0.61
	II	0.34	0.35	0.34	0.34	0.32	0.01	0.53
	III	0.34	0.37	0.35	0.35	0.35	0.01	0.65
Haugh unit	I	81.87	82.62	86.90	86.17	87.37	1.08	0.38
	II	88.13	87.70	85.83	84.88	88.67	0.84	0.60
	III	90.40	87.48	92.73	86.58	87.93	0.91	0.18
Yolk index	I	0.49	0.46	0.48	0.45	0.45	0.01	0.17
	II	0.45	0.47	0.47	0.48	0.48	0.01	0.61
	III	0.43	0.45	0.47	0.45	0.45	0.01	0.38
Albumin index	I	0.12	0.12	0.12	0.14	0.13	0.01	0.67
	II	0.13	0.13	0.15	0.15	0.14	0.01	0.29
	III	0.14	0.16	0.15	0.15	0.14	0.01	0.33
Yolk color	I	8.25^{b}	11.00^{a}	11.50^{a}	11.50^{a}	11.25^{a}	0.3	0.01
	II	9.25^{d}	10.50^{c}	11.50^{bc}	12.25^{b}	14.00^{a}	0.39	0.01
	III	9.25^{b}	12.75^{a}	13.50^{a}	13.75^{a}	14.00^{a}	0.48	0.01
Xanthophyll content (mg/100g of yolk)	I	1.07^{e}	3.02^{d}	5.44^{c}	9.34^{b}	12.69^{a}	0.37	0.01
	II	1.16^{e}	3.45^{d}	5.14^{c}	9.04^{b}	12.94^{a}	0.97	0.01
	III	1.24^{e}	3.35^{d}	5.48^{c}	9.03^{b}	12.87^{a}	0.85	0.01



The yolk color of the egg depends on the carotenoids or any pigments present in the feed ingredients that are used to prepare layers diets. At the beginning of the laying period (Period-I), irrespective of the level of MPM inclusion, birds in all treatment groups imparted same extent of color but significantly higher than control(T1) because birds need some time to stabilize the metabolism of carotenoids in the body. But significantly (Pd" 0.01) higher color in higher level of MPM inclusion was observed (T5) followed by lower levels of inclusion but significantly higher than T1 (control) in period-II. Subsequently, in period-III, the level of color was stabilized among the groups of birds that fed with various level of MPM than control (T1). However, as the level of MPM increased in the diet, the extent of color also increased in yolk. The yolk color obtained in this study was higher than the values reported by Hasin et al. (2006) at 4 per cent level of marigold petal powder (8.2) and orange skin powder (3.3). Rowghwani et al. (2006) also reported lower values in marigold petal powder (8.0 to 9.0), safflower petal meal (5-6) and red pepper meal (9.5-12.5). Moeini et al. (2012) noticed higher yolk color (13.33) in the birds fed with 3% red pepper meal in the diet of layers (Hyline W 36) which matched with 6 per cent level of MPM inclusion in this study.

Xanthophyll content of egg yolk estimated in all the periods was statistically significant (Pd"0.01) between the treatment groups. In all the periods, xanthophyll content was significantly higher in T5 followed by T4, T3, T2 and T1. As the level of MPM increased in the diet of T2 to T5 groups, the xanthophyll content also increased according to the MPM level in the diet. The xanthophyll content of MPM used in the experiment is 2400 mg/kg of dried MP and intake of xanthophyll through the MPM was also increased according to the level of inclusion. However, the transfer of xanthophyll pigment from the feed to the yolk was significantly higher in groups fed with higher level of MPM (T5) followed by T4, T3, T2 but transfer of xanthophyll pigment to the egg yolk was far higher than control group. When xanthophyll content of yolk was compared to the xanthophyll intake through the feed, the extent of transfer of xanthophyll from the feed to the egg yolk in T1 (maize is the only source of xanthophyll) was 95.04 per cent whereas in T2, T3, T4 and T5 was 37.49, 47.60, 66.84, 79.49 per cent where maize and marigold petal meal were the sources of xanthophyll. This clearly indicated that transfer of xanthophyll was more efficient in maize included diet fed group than in groups fed MPM as a supplement to maize where the efficiency was not as good as maize. The relationship between xanthophyll intake and yolk color (Figure 1) in commercial layer was positive (R^2=0.939) where as the relationship between xanthophylls intake and xanthophyll (Figure 2) content in yolk was also positive (R^2=0.843).

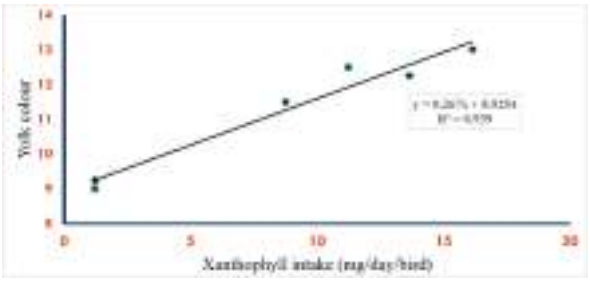


Figure 1: Relationship between xanthophyll intake and yolk color in commercial layers.

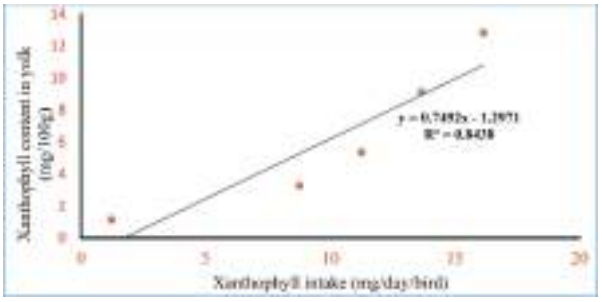


Figure 2: Relationship between xanthophyll intake and xanthophylls content in egg yolk of commercial layers.

The chemical composition of egg without shell and egg yolk of eggs produced during the experiment was analyzed (Table 5). The chemical composition (% as is basis) is given both on 100g, 60g egg weight basis without shell. The CP content of an egg for 100g egg was significantly (P<0.01) higher in T3, T4, T5 groups and lower in T1 and T2 groups. However, the EE, Ca and P contents were also numerically improved in the MPM included groups when compared to the control. The chemical composition expressed on 60g egg also followed the same trend as that of 100g egg because the composition was proportionately converted.

Table 5. Proximate composition (% as is basis) of egg (without shell) of experimental birds.

Particular	Treatment	DM	OM	CP	EE	TA	Ca	P
Egg without shell (100g)	T1	25.34	97.71^{ab}	10.47^{b}	7.89	2.30^{ab}	0.68	0.32
	T2	25.05	97.12^{b}	11.10^{ab}	8.60	2.88^{a}	0.76	0.29
	T3	24.59	98.47^{a}	11.42^{a}	8.67	1.53^{b}	0.83	0.30
	T4	26.54	98.45^{a}	11.84^{a}	9.11	1.55^{b}	0.75	0.33
	T5	26.10	98.23^{a}	11.85^{a}	9.27	1.78^{b}	0.91	0.38
	SEM	0.42	0.16	0.15	0.17	0.16	0.05	0.03
	P value	0.64	0.01	0.01	0.05	0.01	0.61	0.86
Egg yolk (%)	T1	56.38	95.92	14.09^{b}	27.61	4.08	1.43	0.49
	T2	50.62	96.32	15.58^{a}	28.51	3.68	1.52	0.67
	T3	52.50	96.52	15.70^{a}	29.64	3.48	1.38	0.75
	T4	50.76	95.74	16.07^{a}	27.62	4.26	1.43	0.86
	T5	53.84	95.20	15.97^{a}	30.03	4.80	1.64	0.53
	SEM	0.80	0.24	0.23	0.38	0.24	0.14	0.09
	P value	0.12	0.48	0.02	0.12	0.48	0.99	0.69
Egg without shell (60g)	T1	14.70	98.70^{ab}	6.36^{c}	4.69	1.30^{ab}	0.39	0.19
	T2	15.03	98.34^{b}	6.65^{bc}	5.28	1.67^{a}	0.47	0.17
	T3	14.74	99.09^{a}	6.71^{abc}	5.09	0.91^{b}	0.48	0.19
	T4	15.92	99.06^{a}	7.22^{a}	5.41	0.94^{b}	0.52	0.22
	T5	15.66	98.74^{ab}	7.08^{ab}	5.52	1.27^{ab}	0.65	0.23
	SEM	0.27	0.09	0.10	0.11	0.09	0.04	0.02
	P value	0.53	0.023	0.021	0.085	0.023	0.18	0.809

** P≤0.01, * P≤0.05, Means bearing different superscript differ significantly between the rows.

The chemical composition of egg yolk was analyzed separately from the eggs collected during the experiment and there was no significant difference in DM, OM, EE, TA, Ca and P but significantly higher values were observed in MPM added groups when compared to the control group (T1). This indicated that the efficiency of utilization of protein for egg formation was better in MPM fed groups. The CP values of eggs reported in this study were lower than the CP values but higher fat, Ca and P values in the study conducted by Ingrid et al. (2007) in chicken eggs. Rehman et al. (2011) reported almost similar CP values (6.3g) and fat values for chicken eggs of 60g weight. Whereas Ahmad et al. (2017) fed *Moringaolifera* pod meal to Hyline W-36 layers and recorded higher values of CP, EE for the egg yolk than the values reported in this study.

Feed cost per kg of feed for T1, T2, T3, T4 and T5 was Rs. 21.61, 24.12, 25.31, 26.31 and 27.14, respectively (Table 7). Total feed intake per bird (kg) and egg production per bird during the experiment was 8.83, 8.80, 8.78, 8.72 and 8.80 kg; 60, 65, 62, 61 and 57 nos., in T1, T2, T3, T4 and T5 groups respectively. Feed consumed (g/egg) and total cost (Rs.) of egg production during the experiment were 125.00, 123.33, 115.83, 122.50 and 115.83; 190.8, 212.29, 222.11, 229.47 and 238.85 in corresponding groups, respectively. Cost of production per egg (Rs.) was 2.70, 2.97, 2.93, 3.22 and 3.14. Egg sold price (Rs. /egg) was Rs. 4 (control) and Rs. 8 (treatment

groups). Profit by the sale of eggs during the experiment in T1, T2, T3, T4 and T5 groups was Rs. 240.00, 520.00, 496.00, 488.00 and 456.00 Rs, respectively. Benefit Cost Ratio was 1.25, 2.45, 2.23, 2.13 and 1.91 in commercial treatment groups, respectively.

Table 6. Economics of inclusion of marigold petal meal on egg production in commercial layers

Particular	T1	T2	T3	T4	T5
Feed cost (Rs./kg)	21.61	24.12	25.31	26.31	27.14
Total feed intake per bird during the experiment period (kg)	8.83	8.80	8.78	8.72	8.80
Egg production per bird during the experiment (Nos.)	60	65	62	61	57
Feed consumed (g/egg production)	125.00	123.33	115.83	122.50	115.83
Total cost of egg production during the experiment(Rs.)	190.8	212.29	222.11	229.47	238.85
Cost of production per egg (Rs.)	2.70	2.97	2.93	3.22	3.14
Egg sold price (Rs. Per egg)	4.0	8.0	8.0	8.0	8.0
Profit by sale of eggs during the experiment (Rs.)	240	520	496	488	456
Benefit Cost Ratio	1.25	2.45	2.23	2.13	1.91

CONCLUSION

It can be concluded from the above results that higher levels of MPM inclusion in the commercial layer's diets maintained standard egg quality parameters. As the level of MPM increased in the commercial diet, xanthophyll content of the yolk and yolk color increased. Therefore, it can be safely incorporated as unconventional feed resource by replacing some milling by-products without any adverse effect on palatability and egg quality parameters in commercial layers up to 6 per cent.

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Effects of Hot Melt Processed Nano Iron on Growth Performance, Digestibility and Blood Biochemical Profile in Weanling Pig

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ABSTRACT

The objective of this study was to investigate the influence of dietary nano-Fe on growth performance, nutrient utilization and blood biochemical profile in grower pigs. An experiment was conducted considering twenty four (N=24) weaned pigs of HDK-75 having average body weight 21.50±0.38 kg selected from AICRP pig farm, and allotted into four treatment groups. The treatment groups were T0 (Control), T1 (100mg inorganic iron), T2 (75mg organic iron) and T3 (50mg nano iron). Results of 90day feeding trial showed that there was significant difference ($P<0.05$) found in body weight gain and average fortnightly body weight change, which was observed from 75th to 90th day. However, FCR existed significant difference ($P<0.05$) from 60th to 90th day among the different treatment groups. The feed intake and digestibility of nutrient was not showing any significant affect except NFE digestibility which was significantly ($P<0.05$) higher in T3 group. In blood biochemical parameters, serum protein and serum iron was found significantly ($P<0.05$) higher in T3 group and there was no significant difference ($P>0.05$) observed for blood glucose, albumin, globulin, A:G , AST, ALT and BUN. So, it can be suggested that supplementation of nano-Fe@50mg per kg of diet improves overall growth performance, nutrients digestibility and hematological profile to the experimental pig to prevent the occurrence of piglet anaemia.

KEYWORDS: Electron Microscopy, HD-K75 pig, Nano-iron, Nutrients digestibility, Organic iron, Serum profiles,

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Iron (Fe), an essential trace element for pigs, is needed for proper blood hemostasis and count of hemoglobin (Hansen, 2009 and Peri et al., 2016). Iron deficiency anemia in suckling piglets is caused by a low supply of this mineral below the daily requirements; it mainly affects newborn animals due to low iron transfer from the sow to the progeny through the placenta and also due to the low mineral content in milk (Liu et al., 2014). Therefore, weanling piglets eventually suffer from the carry over effects of iron deficiency from the suckling period. In the wild, newborn piglets absorb sufficient iron by rooting in the soil. In conventional pig farming, iron must be supplemented to ensure animal vitality, health, and performance. Sow's milk contains an average of only 1 mg of iron per liter (Brady et al., 1978) which cannot fulfill the iron requirements. As iron is required as a component of hemoglobin in red blood cells which also plays an important role in the body as a constituent of several metabolic enzymes. To avoid piglet anemia, the diet of sows is usually supplementation with inorganic or organic iron which have limitations regarding their bioavailability, and may not meet the mineral requirement. So, nanotechnology is a field of research offering innovative and promising products that, among others, have been recently used to generate nutrients with increased bioavailability. As bioavailability of nano iron is more so could be useful with the objective of this study is to evaluate the effects of dietary nano iron for optimum health and production performance of weaned piglets. In the present study an attempt has been taken to modify the nature of some critical nutrients with an aim to improve its bioavailability and minimize their wastage through excreta. By virtue of their inherent nature of inertness to other interacting nutrients present in GIT, nano-nutrients seems to fit best in precision feeding of livestock. By employing environment benign soft chemistry approach different varieties of dietary nano-shaped iron particles have been synthesized in our lab.

MATERIALS AND METHODS

Laboratory Preparation and Micro-analytical Characterization of synthesized nano-dimensional particles

Nano iron standards are prepared from ferric chloride anhydrous in laboratory under manual control and system and their standardization checked in atomic absorption spectrophotometry (Chatterjee et al., 2007). In order to determine the particle size and potential, Zeta sizer (Malvern Zetasizer Nano, ZS90) was used under room temperature and the peak was observed. Transmission electron microscopy (TEM) analysis was performed to determine the shape and size of synthesized nano-particles.

Micro-analytical Characterization of synthesized nano-dimensional particles

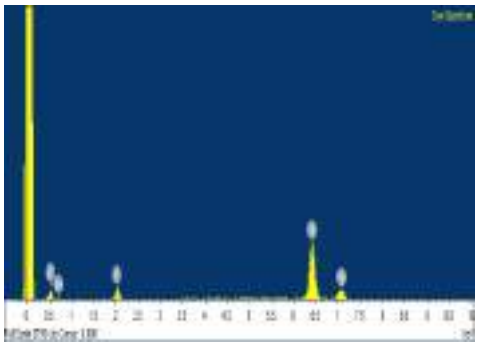

Fig. 1. Depicting the EDx study of FPNP (ferric phosphate nano-particles)

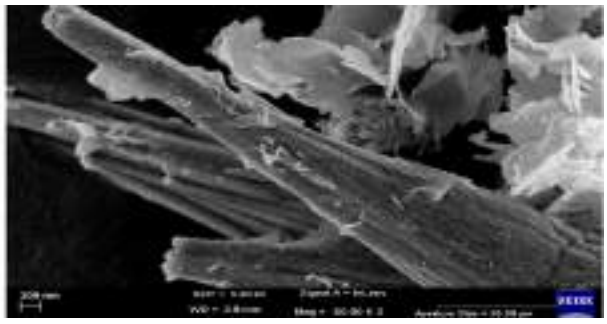

Fig.2. The EDx study of nano-structured $FePO_4$ ensured its elemental composition and purity (bundle shaped appearance)

In the present study an attempt has been taken to modify the nature of some critical nutrients with an aim to improve its bioavailability and minimize their wastage through excreta. By virtue of their inherent nature of inertness to other interacting nutrients present in GIT, nano-nutrients seems to fit best in precision feeding of livestock. By employing environment benign soft chemistry approach different varieties of dietary Nano-shaped iron particles have been synthesized in our lab.

The basal diet was formulated as per the ICAR 2013 recommendation (Table 1) which was fed in the morning (9:00h) by subtracting the equal amount of maize from basal diet. Before housing of the piglets, the floor pens were thoroughly disinfected using fumigants and flame gun. A total of 24 weaned pigs (HDK-75) were randomly allotted with an average initial body weight (BW) of 21.5 ± 0.36 kg on the basis of initial BW according to a randomized block design (RBD) in All India Coordinated Research Project (AICRP) pig farm College of Veterinary Science, Khanapara, Assam. There are four treatments and its treatment having 6 pigs. The treatment included: control (no iron), inorganic iron (100mg/kg of diet), organic iron (75mg/kg of diet) and nano iron (50mg/kg of diet). Prepared experimental diets (table 1) were fed for 90 days among the group and diets proximate composition mentioned.

Table1. Ingredient and chemical composition of the experimental basal diets (% on air dry basis)

Attributes	% DM basis
Maize	59.0
Wheat bran	13.5
Groundnut cake	11.0
Soyabean meal	15.0
Mineral mixture	1.50
Chemical composition	
OM	92.5
CP	18.6
CF	5.10
EE	3.74
NFE	65.06
Total ash	7.50
Calcium	1.11
Phosphorus	0.23

The body weights were recorded in the first day of this experiment and subsequent fortnightly interval till the end of the experiment. Feed intake was recorded and remaining feed were measured to calculate for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

A digestibility trial was conducted at the end of the feeding trial to investigate nutrient digestibility. Four pigs from each group were selected randomly and place in a digestibility cage. The measured feed is offered and feed residue, feces voided were measured and representative of each sample were dried in hot air oven. The proximate analysis for experimental diets of feed and feces was followed according to (AOAC, 2006).

Blood was collected in start, mid and at end of the trial from each pig from anterior vena cava in the morning (Before watering and feeding) into a vacutainer tube from all the pigs at 0, 30, 60, 90 days of feeding trial. For biochemical analysis blood was collected in a red top clot activator vial, kept slanting with ice pack for 30 minutes and centrifuged it in 1500 rpm for 15 minutes to separate serum for analysis of glucose, total protein, albumin, globulin, A:G, AST, ALT, serum iron and BUN by using commercial kit and by biochemical analyzer. The experimental data were subjected to statistical analysis (SPSS version 20) using a one way analysis of variance described by Snedecor and Cochran (2004). Significance was defined at $P < 0.05$. All the values represent mean ± standard errors of the mean.

RESULTS AND DISCUSSION

Nano iron preparation from ferrous sulphate under laboratory condition examined under FESEM &TEM. The results showed that scanning images of nano iron under field-emission scanning electron microscopy (FESEM) and transmission electron-microscope (TEM) which revealed their structural homology and bundled fashion appearances (Fig.1 and 2). Similarly, Ingole et al. (2010) also reported that by using glucose as a reducing agent, the size of synthesized iron nano particles studied under TEM analysis and results were found in the range of 20-80 nm. In piglet hepatic iron stores are generally low in piglet (Lipinski et al., 2010 and Bessman, 2020).Only limited literature is available on the evidence of oral form of nano iron (Laboratory based) contribution to grower pigs to prevent piglet anaemia. So, in this experiment, the author observed that the final body weight and total gain of the experimental animal significantly ($P<0.05$) affected from 60th day onwards (Table 2) among the different treatment groups.

Table 2. Effect of dietary Fe concentration and source on growth performance in grower pigs

Attributes		T1	T2	T3
Initial body weight	8.50± 0.06	8.65±0.07	8.40±0.05	8.65±0.10
Final body weight	29.04b± 0.35	28.08b±0.32	27.37b±0.325	26.13a ±0.430
Total gain (kg)	29.17b±0.004	29.72a±0.003	30.26a±0.001	32.75c±0.004
ADG (g/ day)*	324b±0.36	330a±0.35	336a±0.11	363c±0.41
Total feed intake (kg)	111.4± 3.20	110.8±3.12	110.66±3.13	108.4±2.84
FCR*	3.72a ± 0.16	3.67b±0.10	3.56c±0.19	3.30d±0.12

*T_0, basal diet; T_1, basal diet with 100ppm inorganic iron (w/w %); T_2, basal diet with 75ppm organic iron (w/w%); T_3 basal diet with 50ppm (w/w%) of nano- iron

*Values bearing superscripts a,b,c,d differ significantly ($P<0.01$)

However, significant (P<0.05) difference existed from 60th day onwards to 90th day among the different treatment groups. No influence (P>0.05) of supplementation was manifested seen from the data presented in the table 3 in terms of the digestibility co-efficient of various nutrients (DM, CP, OM, CF and EE) except nitrogen free extract digestibility which is significantly (P<0.05) higher in T3 group.

Table 3. Effect of dietary Fe concentration and source on nutrient digestibility in grower pigs

Digestibility co- efficient(%)	T0	T1	T2	T3
DM	69.84±0.431	70.28±1.188	70.91±0.147	72.19±1.165
CP	72.38±0.39	73.04±1.08	73.60±0.13	74.90±1.05
OM	77.78±0.98	77.82±0.47	78.23±0.22	79.84±0.42
CF	28.32±1.02	30.08±2.79	30.12±0.35	31.83±2.85
EE	77.014±0.33	77.17±0.91	78.06±0.06	78.43±0.90
NFE	82.27c±0.25	83.14bc±0.67	84.29ab±0.08	84.96a±0.63
N-retention	70.82 ±1.83	72.99 ±0.57	72.76± 0.28	73.18±1.85
Fe-retention	29.76d±0.63	31.61c±0.16	35.41b±0.13	39.68a±0.12

*Values bearing superscripts a,b,c,d differ significantly (P<0.01)
*T0, basal diet; T1, basal diet with 100ppm inorganic iron (w/w %); T2, basal diet with 75ppm organic iron (w/w%); T3 basal diet with 50ppm (w/w%) of nano- iron

In present study, growth performance due to supplementation of iron was affected significantly from 75th and 90th day of experimental period among which nano fed group showed better growth performance compared with the other treatment groups, which may be due to higher bioavailability of iron under nano form, because nano particles having following novel characteristics, such as, greater specific surface area, higher surface activity, high catalytic efficiency and stronger adsorbing ability which has ability to transport directly to target organs by avoiding fast degradability and improved several health benefits. This was in agreement with Ranjan et al., 2012; Deng et al., 2021 and Lee et al., 2019. However, it differs from Bhuyan et al.(2020), Lee et al. (2019). In case of ADG, there is significantly(P<0.05) higher in current experiment (Table 2) which is supported by Bhuyan et al. (2020); Feng et al. (2009); and Lewis et al.(1996). Further, FCR was significantly affected from 4th to 6th fortnight and found better FCR in low doses nano iron with higher iron availability which is supported by Lewis (1999); Kang et al. (2014) and Bhuyan et al.(2020).The effects of dietary Fe concentration and source on serum profiles are presented in Table 4. In phase 1, there were no significant differences in blood glucose, serum protein, BUN level, AST and ALT value.

Table 4. Effect of dietary Fe concentration and source on blood biochemical profiles in grower pigs

Attributes	T0	T1	T2	T3
Serum glucose (mg/dl)	91.4±4.24	92.2±4.24	91.8±4.24	92.3±4.24
Serum protein (g/dl)	6.57±0.17	6.90±0.23	6.93±0.26	7.37±0.30
AST (u/l)	36.49±0.26	36.13±0.31	36.01±0.27	35.98±0.30
ALT (u/l)	43.94±0.32	43.45±0.29	43.45±0.27	43.01±0.30
Serum iron (µg/dl)	130a±4.26	131a±3.92	133b±4.85	135b±4.68
BUN (mg/dl)	14.98±0.23	14.88±0.25	15.19±0.21	15.32±0.21

*T_0, basal diet; T_1, basal diet with 100ppm inorganic iron (w/w %); T_2, basal diet with 75ppm organic iron (w/w%); T_3 basal diet with 50ppm (w/w%) of nano iron

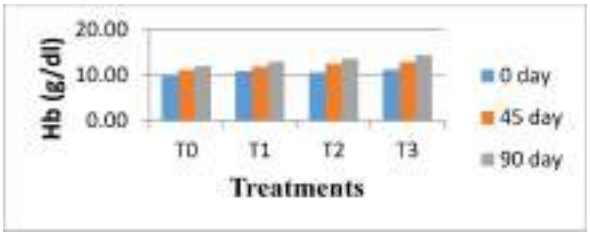

Fig 3. Effect of dietary Fe concentration and source on heamoglobin profiles in grower pigs

However, serum protein and heamoglobin concentration (Fig.3) was significantly higher ($P< 0.01$) in experimental groups of pigs. There was a significant ($p < 0.05$) difference in serum iron (Table 4) between pigs fed the organic iron and nano iron fed diets. From table 4, blood biochemical profile, specially the blood glucose level was not affected significantly among different treatment group which was found similar by Bhuyan et al. (2020) where as total serum protein was influence significantly higher in nano iron fed group may be due to enhance protein digestion which increases protein level in blood as mentioned by Matthews et al.(1998); Bhuyan et al.(2020). Serum albumin, globulin and A:G was not affected by inclusions of iron in pig which was found similar in Bhuyan et al. (2020) findings.

Similarly, AST and ALT level was found within normal physiological range which means there was no adverse effect in the liver of pigs by inclusions of nano iron@50mg/kg of diet which is similar in case of other forms of iron. The present study finding is in good agreement with the findings of Parivar et al. (2018) who reported that there is no significant alteration in AST and ALT level in mice treated with 25, 50 and 75 ìg/mg doses of iron nanoparticles similarly, bhuyan et al. (2020) also found no significant difference ($P<0.05$) in AST and ALT level among the different treatment groups by inclusions of inorganic iron @100mg per kg of diet ($FeSO_4$), organic iron@100mg per kg of diet (Methio-chelated) and nano iron@100 and 50mg per kg of diet ($FePO_4$). Significantly higher serum iron in nano iron@50mg/kg of diet fed group may be due to higher bioavailability of nano iron in blood circulation. Kachuee et al. (2019) also mention that particle size is a key parameter to improve absorption efficiency. The smaller size of Fe nanoparticle (< 100 nm) allows Fe to be absorbed through the intestinal mucus barrier. In addition, this explains the lower serum Fe in inorganic iron@100mg/kg of diet. The present study finding is in good agreement with the following findings (Bhuyan et al., 2020; Leeson et al., 2003; Bruerton, 2005; Lipinski et al., 2010; Li et al.,2018; Yu et al., 2000 and Elshemy, 2018).From the table 4 the BUN level of all the treatment groups was found within normal physiological range which indicates optimal utilization of amino acids (Dukes, 1996) and the present finding is in good agreement with Bhuyan et al. (2020) who also found that BUN level was not significantly affected by inclusion of inorganic iron @100mg per kg of diet ($FeSO_4$), organic iron@100mg per kg of diet (Methio-chelated) and nano iron@100 and 50mg per kg of diet ($FePO_4$).

CONCLUSION

The present investigation can be concluded that supplementation of nano iron@50mg/kg of diet in grower pigs has better advantages in growth performance, nutrient utilization and blood biochemical profiles in grower pigs when compared with other treatment groups fed with inorganic iron ($FeSO_4$) @100mg/kg of diet and organic iron (methio-chelated) @75mg/kg of diet. Inclusions of nano iron @50mg/kg of diet has no adverse effect to the pigs and suggested to the diet of piglet to prevent the occurrence of piglet anemia.

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Effect of Feeding Fermented Rapeseed meal on the Serum Biochemical Constituents and Immune Response of Commercial Broiler Chicken

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ABSTRACT

The study evaluated the impact of raw and fermented rapeseed meal (RSM and FRSM) on serum biochemical parameters and immune response of commercial broiler chicken. Experiment was conducted on day old chicks for 42 days and birds were allotted randomly. Dietary treatments consisted of a control standard corn-soya bean meal diet and 6 test diets where raw or fermented rapeseed meal included at 5, 10 and 15% levels. Blood samples taken at day/ 37 measured total protein, albumin, globulin, creatinine, aspartate amino transferase (AST), alanine transaminase (ALT), and New castle Disease antibody titer, while cell-mediated immunity was assessed via a phyto haemagglutinin phosphate (PHA-P)induced skin response on day/ 40.Results showed no effects on albumin, globulin, creatinine, or AST/ALT ratio across treatments. However, total protein was significantly higher in the 10% FRSM group ($P<0.05$), with other groups comparable to the control. Neither antibody titers nor cellular immunity were affected by the inclusion of RSM or FRSM at any level. The study concludes that incorporating up to 15% of either raw or fermented rapeseed meal in broiler diets has no adverse effect on serum biochemical parameters or immune responses, making these feed components safe for commercial use.

KEYWORDS: Cell mediated immunity, Fermented rape seed meal, Humoral immunity, Serum biochemical parameters.

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INTRODUCTION

Rising soybean meal prices and demand have sparked interest in cost-effective, protein-rich alternative feed resources that support nutritionally sound, least cost rationing (Supriya et al., 2025; Balaji et al.,2025).Rapeseed meal is one such alternative, it is a by product of oil extraction, rich in proteins, but its high content of anti-nutritional factors such as glucosinolates limits its use in animal diets (Elangovan et al., 2001; Tripathi and Mishra, 2006).To overcome the ill effects of RSM various processing techniques such as solvent extraction, heat treatment, use of chemicals like copper sulphate, ferrous sulphate, iodine and enzyme treatment were tried which can remove the anti-nutritional factors. Fermentation process is an effective technique to eliminate the anti-nutritional factors and to improve the nutritional value of vegetable protein supplements (Ashayerizadeh et al., 2018).Fermentation, a biological process involving microorganisms, can degrade these anti-nutritional compounds, increases its nutritional quality, making fermented rapeseed meal (FRSM) a more viable and beneficial ingredient (Supriya et al., 2025; Cheng et al., 2022; Olukomaiya et al., 2019).FRSM has gained attention as a potential feed ingredient in livestock nutrition due to its promising effects on animal health and performance (Shi et al., 2015; Wlaz³o et al., 2022; Fazhi et al., 2011). Fermentation of RSM with Aspergillus niger improved growth and nutrient digestibility in pigs (Shi et al., 2016) and similarly fermentation of RSM with *Saccharomyces cerevisiae* improved antioxidant status and decreased lipid peroxidation in post-weaned piglets (Taranu et al., 2022). Solid-state fermentation (SSF) may be the most promising way to effectively reduce anti-nutritional factors and improve nutritional value of RSM (Van Winsen et al., 2001). Besides, SSF can effectively reduce the glucosinolate level of RSM (Vig and Walia, 2001) so SSF improve the nutritional value of RSM when it is fed to broilers.

The impact of FRSM on serum biochemical parameters has been of particular interest, as it may influence various metabolic and physiological

functions in birds. Additionally, the immune response of birds could be enhanced by fermented products, which are thought to boost immune system efficiency and disease resistance (Zhu et al., 2020; Guo et al., 2021). Several studies have shown that fermented feeds may improve gut health, nutrient absorption, and reduce inflammation, all of which contribute to better overall immune function (Guo et al., 2021; Lian et al., 2024).The studies on inclusion of fermented rape seed meal on different species around the world are available, however work on inclusion of FRSM at higher levels in broilers in India is scarce. Hence, the present study was conducted to explore how Raw and fermented rapeseed meal at level of 5%, 10% and 15% affects both serum biochemical parameters and immune responses in commercial broilers.

MATERIALS AND METHODS

A study was undertaken for 42 days to assess the effect of feeding fermented rapeseed meal on the serum biochemical parameters and immune response of commercial broilers. The experiment was conducted at the Poultry Experimental Station, Rajendranagar, Hyderabad. The laboratory analysis of the biological collected samples was done at Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad and Indian Council of Agricultural Research-Directorate of Poultry Research, Rajendranagar, Hyderabad. The experiment was conducted following the guidelines of Institutional Animal Ethics Committee.

Fermentation of Rapeseed meal

Solid state fermentation of rapeseed meal was done by following the protocol given byRS-L HEALTH.(M/S Loonshot Ventures Private Limited, India). The starter culture contains *Enterococcus faecium* ($1x10^8$CFU/g) and *Lactobacillus plantarum* ($1x10^8$CFU/g). The culture was activated by mixing with lukewarm water (30-35 °C) in bin and incubated at 30-35 °C for 36 hr culture turned from light to bright yellow, which indicates the activation of bacteria. Solid state fermentation of rapeseed meal was done by following the protocol (75ml culture in 75L water for 200kg meal) given by RS-L HEALTH and was standardized for RSM by mixing different levels of water (1, 1.5 and 2X) and culture (1, 1.5 and 2X) in order to achieve the pH of fermented RSM (FRSM) below 4.5 and lactic acid content > 25 g/kg. The RSM was hand mixed with inoculum and water and fermented in air tight vacuum bags and incubated at 33-35! for 84 h and later sampled for pH and lactic acid. Based on pH and LA content in FRSM, the best combination for fermentation of RSM was selected for the broiler experiment. The chemical composition of soyabean meal, raw and fermented rapeseed meal is given in Table 1.

Table 1. Chemical composition (% DM basis) of raw and fermented rapeseed meal

Constituent	Soyabean meal	Rapeseed meal	Fermented[1] rapeseed meal
Dry Matter	88.1	89.3	88.4
Crude Protein	46.2	34.6	35.8
Crude Fibre	7.5	12.4	9.34
Ether Extract	1.2	1.49	2.91
Calcium	0.34	0.63	0.61
Phosphorous	0.23	0.39	0.34
Gross energy (kcal/Kg)	4710	4155	4202
Metabolizable energy (kcal/Kg)	2704	1770	1790

Note**:** ME (Kcal/kg) = 0.57 × GE (Kcal/kg)

[1]Fermented with starter culture containing *Enterococcus faecium* and *Lactobacillus plantarum*.

Serum biochemical constituents

Blood was collected from one bird per replicate on 37th day of age. Blood samples were collected aseptically from brachial vein of birds with the help of sterilized needles and blood was collected in clean sterilized glass tubes and kept in slanted position at

room temperature for serum collection. The collected serum samples were then centrifuged at 3000 rpm for 5 minutes and transferred to 2 ml Eppendorf tubes which were stored at -20°C. The estimation of serum biochemical constituents viz.,total protein, albumin and globulin, creatinine, alanine transaminase (ALT), aspartate amino transferase (AST) was done by auto-analyzer using commercial diagnostic kits (ERBA Diagnostics, INC).

Humoral immunity

Humoral immunity in broilers was estimated by measuring antibody titers to Newcastle disease (ND) vaccine (antibody production against ND virus). Broilers were vaccinated against ND by ocular route at 7thand 28thday of age with Lasota strain (ND Lasota Vac-1000; Ventri Biologicals, Pune, India). On 37th day blood was collected and serum was separated. Subsequently antibiotic specific for ND were detected in sera of chicks by haemagglutination inhibition (HI) test and were expressed as $\log_2$ titers (Allan et al., 1978).

Hummoral immunity was done in 'U' bottom micro well plate. It involves 2 processes i.e., HA estimation and HI estimation. For HA estimation, 50µL of normal saline was pipetted into 12 micro wells, 50µL of ND virus was added into 1st well and serial dilution was done upto 11th well. Now, 50µL of 1 % RBC is added to 12 wells and incubated at 37! for 30-40 mins. After incubation a button was formed at U bottom upto 8th well and this considered as 2^8 (HA). For HI estimation 50 of 4HA virus is added instead of ND virus and remaining procedure is same as HA estimation, the buttons are calculated at end and titre value was expressed in $\log_2$.

Cell mediated immunity

Cell mediated immune (CMI) response was assessed by measuring *in vivo* cutaneous basophilic hypersensitivity (CBH) to phyto haemagglutinin phosphate (PHA-P).On 40thday of experiment, one bird was selected randomly from each replicate to assess the CMI response . The toe web was injected with 100 µg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS). The web swelling of the feet was measured by micrometer at 24 h after injection. The thickness was calculated by using the formula (Edelman et al.,1986)

$$\text{CMI (\%)} = \frac{\text{post injection skin thickness of toe web}}{\text{Pre injection skin thickness of toe web}} \times 100$$

RESULTS AND DISCUSSION

The present study evaluated the effects of dietary inclusion of raw and fermented rapeseed meal (RSM and FRSM) at levels of 5%, 10%, and 15% on serum biochemical constituents and immune responses in broilers. The results indicated in the Table.2 that the serum albumin, globulin, creatinine, and the AST/ALT ratio were not significantly affected by the inclusion of raw or fermented rapeseed meal. However, a significant difference was observed in serum total protein, with birds fed 10% FRSM showing the highest levels, while the lowest levels were recorded in the 15% RSM diet group. This suggests that, while raw and fermented rapeseed meal had little effect on most of the biochemical parameters, FRSM at 10% inclusion may have a positive impact on total protein levels in broilers.

Table 2. Effect of dietary inclusion of raw and fermented rapeseed meal on serum biochemical constituents in broilers

Diet	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	AST/ALT (IU/L)
Control	4.01^{ab}	1.97	2.04	2.073	1.046
5% RSM	3.60^{ab}	1.98	1.62	2.074	1.119
10% RSM	3.61^{ab}	1.96	1.64	2.041	1.189
15% RSM	3.57^{b}	1.96	1.61	2.003	1.302
5% FRSM	4.35^{ab}	2.14	2.22	2.069	1.155
10% FRSM	4.38^{a}	2.13	2.25	2.004	1.129
15% FRSM	4.34^{ab}	2.12	2.21	2.003	1.145
SEM	0.986	0.374	0.981	0.417	0.302
P-Value	0.04	0.62	0.21	0.99	0.46

^{abc} Means with different superscript in a column differ significantly: P<0.05, P-value: Probability value.

The significant effect on serum total protein observed in this study corroborates findings from previous research. Hu et al. (2012) reported that inclusion of 28.41% RSM and 24.27% FRSM in the diet resulted in higher serum total protein levels in birds fed FRSM compared to control groups. Czech et al. (2020) also found that the inclusion of 4% FRSM increased serum total protein and albumin levels in broilers. These studies suggest that fermented rapeseed meal may improve protein metabolism in broilers, potentially due to the breakdown of anti-nutritional factors during fermentation. However, these findings contrast with those of Shi et al. (2016), who observed no significant changes in serum total protein or albumin when 10% RSM or FRSM was included in the diet. This discrepancy may be attributed to differences in experimental conditions, including diet composition and the specific fermentation processes used.

Regarding other serum biochemical markers, including creatinine and AST/ALT ratios, no significant effects were observed in this study, aligning with the results of Elbaz et al. (2021), who found no changes in these parameters when 20% raw or fermented rapeseed meal was included in broiler diets. This suggests that, at the inclusion levels tested in this study, neither raw nor fermented rapeseed meal had a notable impact on kidney or liver function in broilers.

Table 3. Effect of dietary inclusion of raw and fermented rapeseed meal on humoral immune response against ND vaccine and cell mediated response against PHA-P in broilers

Diet	Humoral immune response (log_2 titer)	Cell mediated response (thickness index)
Control	6.20	40.16
5% RSM	6.10	45.13
10% RSM	6.10	43.92
15% RSM	6.40	35.87
5% FRSM	6.70	47.84
10% FRSM	6.70	36.01
15% FRSM	6.50	33.16
SEM	0.09	1.947
P-Value	0.427	0.339

In terms of immune responses (Table 3), no significant differences were observed in either humoral immunity, assessed through antibody titres to Newcastle disease virus (NDV), or cell-mediated immunity, evaluated by measuring digital skin thickness after PHA-P injection. These results suggest that raw or fermented rapeseed meal, even at 15% inclusion levels, did not significantly affect the immune function of broilers under the conditions of this study. This is in contrast to the findings of Wlazlo et al. (2021), who observed a reduction in immunoglobulin levels in rabbits fed fermented rapeseed meal at 4%, 8%, and 12% inclusion levels, indicating a potential depression of both humoral and cell-mediated immune responses. However, the current study did not observe such an effect in broilers, which could be due to species-specific differences or variations in study methodologies.

On the other hand, studies by Elbaz et al. (2023) and Zhu et al. (2020) reported that fermented meals, such as fermented canola meal, improved immune responses in broilers, including significant increases in antibody titres against NDV. These contrasting findings highlight that the effects of fermented meals on immune function may vary depending on factors such as the type of fermented meal used, the fermentation process, and the species studied. The differences in results could also be attributed to the bioactive compounds released during fermentation, which may influence immune function in different ways depending on the feed ingredient.

In conclusion, the inclusion of fermented rapeseed meal up to 15% did not significantly affect serum biochemical parameters or immune responses in broilers in this study. However, the variations

observed in other studies involving fermented feeds suggest that the effects of such diets can depend on factors like meal type, fermentation process, and species. Future research exploring a broader range of inclusion levels, types of fermented meals, and immune markers is needed to gain a deeper understanding of how fermented rapeseed meal impacts both nutrient metabolism and immune health in poultry.

CONCLUSION

Results suggest that FRSM can be safely included in broiler diets at inclusion levels up to 15% without any negative impact on the birds' health or immune function.

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Organic Trace Minerals at Lower Concentrations Can Replace Inorganic Trace Mineral Premix in Broiler Chicken Diet

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ABSTRACT

An experiment was conducted to study the performance, immune responses, and bone mineral variables in broiler chickens fed organic trace minerals (oTM) at suboptimal concentrations in the diet. A total of 2080 day-old broiler male chicks were randomly distributed into floor pens (6.25 × 4 feet) at the rate of 26 birds per pen. Maize-soybean meal-based control diet was supplemented with inorganic trace mineral premix (iTM; Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg). The iTM premix was replaced in the test diets with oTM to provide minerals at graded levels in the diet (10, 17.5, and 25% of the control diet). Each diet was fed *ad libitum* to a total of 20 replicates of chicks from 1 to 42 days of age. The body weight gain (BWG) was not affected by supplementing oTM at 20% during 1-14 and 1-28 d of age. Both BWG and feed conversion ratio (FCR) were significantly reduced with an increase in oTM $\geq$35%. Both the production variables in groups fed oTM at 20% were similar to those fed the iTM. The relative weight of breast meat was lower ($p<0.05$) and liver weight was higher ($p<0.05$) in groups fed the higher concentration of oTM (50%) than the iTM group. Similarly, tibia breaking strength and P content in tibia ash in oTM 20% were similar to broilers fed on iTM. Tibia ash and Ca content in tibia ash were not affected by dietary variation in the mineral source (iTM vs oTM) and organic mineral concentration. Based on the results, it is concluded that trace mineral supplementation can be reduced to 20% when fed in organic form in broiler chicken diet to support the optimum growth, mineralization, and HI titer to ND vaccine.

KEYWORDS: Organic trace minerals, Inorganic trace minerals, Weight gain, Feed efficiency, Bone parameters, Immune response, Broiler chicken

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INTRODUCTION

Trace minerals are essential in several metabolic processes in the body, which are required to sustain production, skeletal development, cell oxidation, and immune responses (Branca and Ferrari, 2002) in chickens. Trace minerals are traditionally supplemented in inorganic salt form due to economic reasons and ease of procurement but, are less biologically available (Van der Klis, 1999) to the birds and cause environmental pollution. The higher concentration of minerals in poultry excreta can lead to soil and groundwater contamination (Nollet et al., 2007), a scarce resource in the tropical region. Therefore, the research aiming at more bioavailable minerals that enable higher utilization by the poultry and minimize the lesser excretion to the environment by intensive poultry farming is gaining importance worldwide. Another limitation of using inorganic forms of minerals is potential interaction among the trace minerals, which sometimes leads to mineral deficiency in chicken-fed mineral-balanced diets. On the contrary, the mineral availability from organic/chelated sources of minerals (oTM) is higher than the inorganic trace minerals (iTM) (Aksu et al., 2010, Rama Rao et al., 2013[a], Prakash et al., 2019). Further, the higher availability of oTM leads to reduced excretion of the minerals from birds and minimized contamination of soil and groundwater.

The research data based on inorganic forms of the trace minerals form the basis for arriving at the requirement standards suggested (NRC, 1994) for chicken. Higher inclusion levels of iTM lead to mineral wastage and environmental pollution due to excretion by birds (Leeson, 2003). Considering the

advantage of higher bio-availability of oTM (Yan and Waldroup, 2006, Wang et al., 2007; Rama Rao et al., 2013[b]), it is presumed that the requirement of TM can be reduced considerably to realize the advantage of the higher mineral bio-availability from the oTM compared to the standard mineral requirements suggested. Information regarding the effect of lower levels of oTM on immune responses, bone mineralization, and carcass yield in chicken is limited (Rama Rao et al., 2021), which was based on the limited number of replications (Rama Rao et al., 2013[b]). Therefore, the current experiment was conducted using a higher number of replications to find out the effect of feeding the reduced concentrations of oTM on performance, bone mineralization, immune responses, and carcass yield in commercial broiler chickens.

MATERIALS AND METHODS

Premix analysis for trace minerals

The concentrations of trace minerals i.e. Zn, Mn, Fe, Cu, Se, and Iodine in inorganic trace mineral premix and organic trace mineral premix were estimated using Atomic Absorption Spectrophotometer (AAnalyst 400, PerkinElmer, Shelton, CT, USA) according to methods suggested by the manufacturer. A specific lamp was used for each mineral and the Atomic Absorption Spectrophotometer was calibrated with various concentrations of the mineral standards.

Experimental Birds, Management and Diets

Day-old broiler (*Cobb* 430 Y) male chicks (N=2080) were procured from a commercial hatchery (Venkateswara Hatcheries, Pvt. Ltd, Hyderabad, India) and randomly distributed into four treatment groups. Each treatment was allotted to 20 replicates with 26 chicks in each pen (6.5 × 4'). Birds were maintained in floor pens with a floor space of 1.0 ft^2 per bird. Brooding was provided with incandescent bulbs and additional heat was provided with coal during the initial 3 weeks of age. The room temperature was maintained at about 37 ^oC during the first week and subsequently, the temperature was gradually reduced to the ambient temperature at day 21, after which the birds were reared at ambient temperature (20.7+3.17 to 30.8+3.84 ^oC). The experiment was conducted by following the guidelines of the Institute Animal Ethics Committee (IAEC/DPR/17/1: 21/10/2017).

Feeding regimen and performance

A three-phase feeding regime (starter: 1 to 14 days), (grower: 15-28 days) and (finisher: 29 to 42 days) was followed. Maize-SBM-meat and bone meal-based basal diets were provided *ad libitum* from 1-42 days. Metabolizable energy and crude protein levels in starter, grower, and finisher diets were 3000, 3100, and 3150 kcal/kg and 22.5, 21, and 19%, respectively (Table 1). The diets were formulated as per the (NRC, 1994) recommendations for all the minerals. The oTM premix was procured from a commercial source (P-Min, Varsha Agro Tech, Bengaluru, India), which contained a mixture of trace minerals in chelated form (Zn 50, Fe 50, Cu 5, Se 0.3, Mn 50, Cr 0.5, and I 3g/kg). The control diet was mixed with a commercial premix containing inorganic trace minerals (Trouw Poultry Min Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg) (Trouw Nutrition). Another three diets were prepared by supplementing the basal diet with 3 concentrations of oTM mix to represent 10 (Fe 10, Zn 10, Mn 10, Cu 2, Se 0.05, I 0.25/mg/kg), 17.5 (Fe 17.5, Zn 17.5, Mn 17.5, Cu 3.5, Se 0.88, I 0..44/mg/kg), and 25% (Fe 25, Zn 25, Mn 25, Cu 5, Se 0.125, I 0.63/mg/kg) of the levels in the iTM mix. Concentrations of metabolizable energy, essential amino acids, calcium, and available phosphorus were maintained uniformly in all the diets in each phase. Each of the diets was randomly assigned to 20 replicate pens (201 × 122 cm, 26 birds/pen) and fed *ad libitum* from day 1 to 42. Feed intake (FI) and body weight (BW) were recorded per pen at day 1, 14, 28, and 42 of age and feed conversion ratio (FCR) was calculated as FI/ body weight gain (BWG). The weight of dead birds was recorded as and when the birds died. The weight of dead birds was used while calculating the FCR of the respective pen. Immunization against Newcastle disease (ND) live attenuated Lasota strain (Lasota) and infectious bursal disease (IBD Intermediate, Venkye's India Pvt Ltd, Pune, India) live attenuated strain (intermediate) was carried out on days 7, 28, and 14 of age, respectively.

Table 1. Ingredient and nutrient composition (g/1000g) of broiler diets

Ingredient	Pre starter (1-14d)	Starter (15-28d)	Finisher (29-42d)
Maize	566.5	611.1	662.6
oil-veg	26.1	33	31.360
Soya DOC 45%	336.1	289.4	243.9
Meat cum bone meal	40.0	40.0	40.0
Salt	3.666	3.667	3.665
Sodium bicarbonate	1.000	1.000	1.000
Dicalcium phosphate	10.44	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
Choline Chloride, 75%	1.000	1.000	1.000
Vitamin Premix	0.500	0.500	0.500
Trace Mineral Mixture	1.000	1.000	1.000
Nutrient			
M.E (Kcal/kg)	3000	3100	3150
Protein (%)	22.59	20.77	19.09
Dig. Lysine (%)	1.250	1.120	1.010
Dig.Methionine(%)	0.640	0.560	0.490
Dig TSAA (%)	0.929	0.832	0.750
Calcium (%)	0.880	0.760	0.660
Available Phosphorus (%)	0.420	0.380	0.330
Sodium (%)	0.180	0.180	0.180
Dig. Threonine (%)	0.829	0.742	0.669
dig. Leucine (%)	1.734	1.630	1.536
dig. Iso-leucine (%)	0.842	0.764	0.690
dig. Valine	0.967	0.865	0.787

[1] Supplied per kg of diet: retinol acetate 2.75 mg, cholecalciferol 0.03 mg, á 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.

Carcass traits

Carcass traits including ready-to-cook yield (RTC), and relative weights of breast meat, liver, and abdominal fat were recorded by slaughtering 2 birds per replicate at day 43. The birds weighing nearer the mean (±5%) of each replicate were selected for slaughter traits. The RTC includes the edible portion of the carcass with giblet (gizzard, liver, and heart), which was expressed in grams in proportion to the kg live weight of the respective bird.

Bone mineralization

One bird from each replicate was slaughtered at 43rd day age to study the bone mineral variables of

the tibia and sternum (breaking strength, tibia ash, calcium, and phosphorus in tibia ash). The bones were freed of soft tissue and dried at 70 °C/3h. The bone samples were defatted by soaking in petroleum ether for 48h. The right tibia and sternum of each bird were used to determine the breaking strength. The strength was measured using a 3-point method with a universal testing machine (EZ Test, Shimadzu, Japan). The bone was rested on two points with a gap of 50 mm and pressure was applied with a pressure-sensitive load cell (10 kg) at the center of the two points, which coincided with the center of the bone at a speed of 5 cm per minute. The tibiae and sternum were ashed independently at 600±20 ^{0}C/2h. The bone ash was dissolved in hydrochloric acid (1:10) and the mineral aliquot was estimated for P using molybdovanadate reagent (Fiske and Subbarow, 1925). The bone ash was estimated for Ca using an Atomic Absorption Spectrophotometer (AA Analyst 400, PerkinElmer, Shelton, CT, USA) according to methods suggested by the manufacturer. A specific lamp was used for Ca estimation and the Atomic Absorption Spectrophotometer was calibrated with various concentrations of the mineral standards.

Immune responses

Cell-mediated immunity (CMI) and humoral immunity (HI) (antibody response against Newcastle disease vaccine) were studied. The broilers were vaccinated against ND by ocular route at 7 and 28 d of age with Lasota strain (ND Lasota Vac-500, Indovax Pvt., Ltd., Hyderabad, India). At 20 d of age, 2 mL of blood was collected from one bird per replicate and the antibody titers in sera against Newcastle disease vaccine were measured (Rama Rao et al., 2021) by haemagglutination test.

Statistical analysis

The data was checked for normality and equality of variance using the Shapiro-Wilk test and Lavene's test, respectively. The data that did not meet the normality and equality requirements were square root transformed before statistical analysis. The variations in data of different parameters were analyzed using the general linear model procedure of SAS version 9.2 (2008; SAS Institute Inc., Cary, North Carolina, USA). The statistical model of analysis of variance was the following:

$$Yij = \mu + Ti + eij$$

Where μ is the overall mean, Ti is the fixed effect of ith treatment and eij is the random error. Treatment means were compared using Tukey's test.

RESULTS AND DISCUSSION

Trace mineral concentration in premixes

The concentrations of trace minerals analysed in oTM were close to the concentrations indicated in the premix. The concentrations of Zn, Fe, Cu, Se, Mn, and I were 53, 59, 5.5, 0.32, 56, and 3.1g/kg, respectively. While the concentrations of the respective minerals in iTM premix were 105, 95, 110, 22, 0.46, 2.3/mg/kg.

Groups fed on the lowest oTM (10%) supplemented diet showed similar performance (BWG and FCR) compared to the iTM 100% fed birds and the broiler performance was significantly reduced in groups fed higher concentrations of oTM (17.5 or 25%) compared to the CD (Table 2). The results suggest that oTM at lower concentrations (10% of iTM) achieved the FCR of 100% iTM, whereas the higher concentrations of oTM (17.5 and 25%)) tested reduced the broiler performance. These results agree with the earlier reports with broilers (Saenmahayak et al., 2010, Zhao et al., 2010) and a rural chicken variety (*Vanaraja*) (Rama Rao et al., 2013[a]), where significant improvement in chicken performance was reported when diets were supplemented with oTM. In the present study, the performance was improved significantly in broilers fed the lower levels of oTM. It is reported that the organic form of Se and Zn are known to enhance performance (Prakash et al., 2019), serum antioxidant variables (Rama Rao et al., 2013[a]), and immune response (Georgieva et al., 2011) in chicken. These results are in line with Bao et al. (2007), who reported improved performance in broilers fed a diet containing moderate concentrations of organic minerals, close to the concentrations used in the present study (Cu 4.0, Fe 40.0, Mn 40.0, and Zn 40 mg/kg), which were lower than those recommended by the NRC, (1994). It can be speculated as the reason for the depressed performance observed in broilers fed the higher concentrations of oTM (17.5 and 25%) than those fed the lower concentrations (10%). The first reason is that even though there is no significant variation in FI among the treatment groups, mineral intake varies due to variable quantities of oTM provided in the test diets which resulted in a higher intake of minerals in broilers fed 17.5 or 25% diets than those fed with

10% oTM. Secondly, absorption, transportation, and deposition of minerals are reported to be higher when they are included in organic form. The higher net availability of essential minerals might have elicited a better response in terms of improved BWG and feed efficiency. The bioavailability of oTM is about 1.2 to 1.85 times higher compared to the iTM (Zhao et al., 2010) in broiler chickens.

Table 2. Performance of broiler chicken fed oTM mix in place of iTM premix

Treatment	Pre-Starter (1-14d)		Starter (1-28d)		Finisher (1-42d)	
	BWG	FCR	BWG	FCR	BWG	FCR
iTM	416.8	1.182^{A}	1483	1.443	2372^{A}	1.666^{C}
oTM-10	424.1	1.170^{B}	1486	1.440	2370^{A}	1.670^{BC}
oTM-17.5	423.7	1.173^{AB}	1463	1.450	2326^{B}	1.686^{AB}
oTM-25	421.0	1.178^{AB}	1455	1.458	2321^{B}	1.690^{A}
P	0.660	0.071	0.470	0.199	0.025	0.014
N	20	20	20	20	20	20
SEM	2.287	0.002	8.214	0.003	7.869	0.003

BWG body weight gain; FI feed intake; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals

^{ABC} means having different superscripts in a column differ significantly (P<0.05)

Similar to the current findings, Faria et al. (2020) reported reduced BWG in broilers fed higher concentrations of oTM (920g/ton) compared to those fed the lower concentration (820 g/kg) oTM. The literature and the current data suggest the need to reduce the inclusion levels of TM when included as organic form.

The RTC (ready-to-cook) yield and relative weight of abdominal fat were not affected (P>0.05) by the dietary treatments (Table 3). The breast meat weight decreased, and the relative weight of the liver increased with an increase in the concentration of oTM in the diet. Similar results were reported earlier (Sheikh et al., 2011; Rama Rao et al., 2021).

Table 3. Slaughter variables (g/kg live weight) of broiler chicken fed oTM mix in place of iTM premix

Treatment	RTC	Breast	Abdfat	Liver
iTM	718.4	254.4^{A}	11.05	20.47^{B}
oTM-10	708.1	257.4^{A}	9.474	21.34^{B}
oTM-17.5	697.4	242.4^{AB}	9.059	22.02^{B}
oTM-25	697.6	237.8^{B}	8.932	25.07^{A}
P	0.138	0.026	0.528	0.017
N	20	20	20	20
SEM	3.715	2.751	0.557	0.561

RTC ready to cook yield; Abdfat abdominal fat; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals

^{ABC} means having different superscripts in a column differ significantly (P<0.05)

The CMI response to PHA-P was not affected by the replacement of iTM with lower levels of oTM in the diet (Table 4). The antibody titer against ND vaccine in groups fed the lowest concentrations of oTM (10% oTM/ton) was similar (P-=0.001) to those fed 100% iTM. However, higher concentrations of oTM (17.5 and 25%) in the broiler diet significantly reduced the humoral immune response compared to the group fed on iTM based diet. Similar to these findings, Shawkat et al. (2018) reported that the relative weight of a lymphoid organ (spleen) in broilers fed the lower concentrations of oTM (375g/kg) was similar to those fed higher levels of TM when included as iTM.

Table 4. Immune responses in broiler chicken fed graded concentrations of oTM in place of iTM premix

Treatment	CMI, %	ND titre, log 2
iTM	59.25	8.20[a]
oTM-10	64.30	7.95[ab]
oTM-17.5	60.00	7.55[b]
oTM-25	62.00	7.00[c]
P	0.761	0.001
N	20	20
SEM	1.793	0.103

CMI cell mediated immunity; ND Newcastle disease; P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals
[ABC] means having different superscripts in a column differ significantly (P<0.05)

Tibia ash percent and calcium content in tibia ash were not affected (P>0.05) by the variation in concentration of oTM in the broiler diet compared to those fed the CD (Table 5). The tibia-breaking strength in broilers fed 20% oTM was statistically similar to those fed the CD. An increase in concentrations of oTM progressively reduced the tibia strength and the strength in groups fed 25% oTM was significantly lower than those fed the CD. Similarly, the P content in tibia ash in broilers fed the lower concentrations of oTM (10 and 17.5%) were similar to those fed 100% iTM in their diet. At the highest concentrations of oTM (25%) the P deposition in tibia ash was significantly reduced than those fed the 10 and 17.5% oTM in the diet. Further, the bone parameters (reduced bone strength and phosphorous content in tibia ash) of the current study (Table 5) indicated the negative effects at higher inclusion levels of oTM in broiler diet, which may partly be due to the higher availability of organic form of TM than the inorganic salts.

Table 5. Tibia bone mineral parameters in broiler chicken fed graded concentrations of oTM in place of iTM premix

Treatment	Strength (N)	Ash%	Phosphorus, %	Calcium, %
iTM	69.74[ab]	51.48	18.57[ab]	31.84
oTM-10	84.57[a]	51.72	18.79[a]	32.16
oTM-17.5	48.39[bc]	51.61	19.13[a]	31.60
oTM-25	42.40[c]	52.62	17.70[b]	32.48
P	0.003	0.087	0.045	0.794
N	20	20	20	20
SEM	4.738	0.325	0.190	0.318

P probability; N number of replicates; SEM standard error mean; iTM inorganic trace minerals; oTM organic trace minerals
[ABC] means having different superscripts in a column differ significantly (P<0.05)

Reduced bone mineral variables (tibia strength and P in tibia ash) variables with an increase in concentrations of oTM contradict our previous work (Rama Rao et al., 2021), where the concentrations of Ca, P, and other trace minerals (Cu, Zn, and Fe) in bone ash increased in groups fed diets supplemented with oTM compared to those fed iTM. It is worth noting that in our previous study, the contents of Ca and P in tibia ash were higher in chickens fed diets with lower levels of oTM compared to those fed higher concentrations of oTM. As reported, the retention of oTM is higher compared to iTM (Bao et al., 2007; Ma et al., 2012), which results in higher retention of minerals even at the

lowest levels of inclusion (oTM 100g/ton). The reduced bone mineral variables at higher inclusion levels of oTM in diet (17.5 or 25%) could be due to the higher concentrations of bioavailable elements that might have interacted antagonistically and adversely affected bone mineralization. The higher bio-availability of trace minerals in the organic form (Ma et al., 2012) might have ensured higher/excess availability of the minerals beyond the physiological requirement, which showed some negative responses in bone mineralization, performance, and HI immune response. The similar mineral concentration in birds fed oTM 100g/ton and the CD with 100% iTM also confirms the hypothesis that mineral availability is higher in organic form than in inorganic form. Comprehensively, the data indicated that the TM requirement can be reduced to 10% when supplemented as oTM in commercial broiler diets without compromising the performance, HI titer, bone-breaking strength, and P content in tibia ash.

The data of the current study also indicated that the levels of trace minerals in 10% oTM are optimum for performance as well as bone development. Similarly, Zaho et al. (2010) also found an optimum response with lower concentrations of TM when included as an organic form. As the performance variables in broilers fed 10% oTM were similar to those fed 100% iTM, the concentrations of TM present in 10% oTM (Zn 10.0, Mn 10.0, Cu 1.0, Fe 5, I 0.6, Se 0.06, and Cr 0.01 mg/kg) appears optimum for broiler chicken. The absence of negative effects in broilers fed the lower concentrations of oTM could be due to the higher bioavailability of minerals in organic form (Wang et al., 2007) and therefore, the absolute availability of minerals even at the lower levels of oTM probably met the broiler requirement.

CONCLUSIONS

The concentrations of oTM can be reduced to 10% of the recommended concentration (Fe 10, Zn 10, Mn 10, Cu 2, Se 0.05, I 0.25/mg/kg) in broiler diet without affecting the performance, bone mineralization and HI response compared to those fed the 100% inorganic TM in the diet (Fe 100, Zn 100, Mn 100, Cu 20, Se 0.50, I 2.5/mg/kg). Higher concentrations of oTM (17.5 and 25%) significantly reduced the broiler performance and bone mineral variables.

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Rapid Estimation of Chlorpyrifos in Feed Samples using Gas Chromatograph-Micro Electron Capture Detector (GC-µECD)

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ABSTRACT

In this research paper, we have standardized a rapid method for estimation of chlorpyrifos residues in feed samples using Gas Chromatograph- Micro Electron Capture Detector(GC-µECD). The extraction method used for this study was QuEChERS viz. quick, easy, cheap, effective, rugged and safe. Validation was done by performing the following parameters; Linearity, Limit of detection (LOD), Limit of quantification (LOQ), Matrix effect and Recovery percentage. A regression equation with regression coefficient (r2) of 0.9913 was obtained indicating excellent linearity. LOD and LOQ of the method were obtained as 0.1 mg/L and 0.4 mg/L respectively. Recovery percentage of the spiked concentration 0.1 ppm was obtained as 85.7% and for 0.5 ppm it was 77.7%. The method qualified the required parameters for analysis of chloropyriphos in the different classes of feeds. Different classes of feedstuffs were analyzed using this method.

KEYWORDS: Chloropyrifos, Gas Chromatograph-Electron Capture Detector, Feeds, QuEChERS.

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INTRODUCTION

Chlorpyrifos (CPF) is a widely used pesticide which comes under the group organophosphorus (OP) (Callahan et al., 2014). They are commonly available in the form of white crystalline-like solids, slightly hydrophobic in nature and having affinity towards oily liquids. In the year 1965, chlorpyrifos was brought into market to be used against insects, termites, mosquitos and beetles, particularly their larval stage (George et al. 2014 and Dua and Joshi, 2014).Chlorpyrifos kill the pest by malfunctioning their nervous system. When the pest is infested with chlorpyrifos, they block the enzyme acetylcholinesterase which results in the accumulation of acetylcholine in the synapse. This leads to overstimulation of neuronal cells and muscle spasms which eventually causes the death of pests (Saunders et al., 2012).

In the present scenario, agriculture practices to increase the crop yield pesticides are still used even though they have some drawbacks if not used properly. The pesticide CPF is mainly used to control soil pests, foliar treatments and directly applied in cattle, sheep etc to control fever ticks, ear ticks, lice and so on (Supreeth et al., 2016; Katuri et al., 2017; Arevalo and Stansly., 2019). The prolonged usage of these pesticides can lead to deposition of its residue in the environment. However, if the residue content is below the MRL (maximum residue limit) it is safe to use(Mackay et al., 2014). The higher dose of lipophilic CPF on agriculture crops may increase the chances of bioaccumulation of pesticide; the leftover residue will remain on vegetables, crops and fruits hence entering into the food/feed chain through the consumption of crop-by products by livestock. Higher doses of CPF exposure can cause several health hazards in humans as well as in animals. Intake of crops and vegetables containing higher amounts of CPF residue can cause metabolic abnormalities, chronic neurotoxicity, gastrointestinal effects, musculoskeletal effects etc. Eventually, over usage of these pesticides can also lead to the damage of aquatic and terrestrial ecosystems. Some of the American and European countries have already restricted the domestic usage of CPF due to the human health risk(Lim et al., 2011; Osterloh et al., 1983; Eaton et al., 2008; Nandi et al., 2022). Thus, proper evaluation and monitoring of these pesticide residues in the natural vegetation and crops is inevitable.

Various methods are available for isolation and detection of CFP such as immunization assays (Hongsibsong et al., 2020), flow cytometry(Zhang et al., 2018) and chromatography. Commonly used chromatographic methods include, Thin Layer Chromatography (TLC), Liquid Chromatography (LC), High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography (GC). Coupled with specific chromatography, various detectors such as mass spectrometry, Flame ionization detector, Electron capture detector, Near-Infrared spectroscopy etc. are used (Chauhan et al., 2017).

Extraction and clean up methods prior to the analysis is an important step. There are many conventional methods available such as Soxhlet extraction, sonication extraction, homogenization with solvents etc. However, these traditional methods have many drawbacks like wastage of solvents, time consuming procedures and so on. Many advanced techniques are available today in order to overcome such drawbacks. Among the advanced methods, solid phase extraction (SPE), solid phase microextraction (SPME) and the quick, easy, cheap, effective, rugged and safe (QuEChERS) dispersive solid phase extraction are the widely used effective methods (Obuseng et al., 2013).Even though SPE offers higher selectivity, it is a time consuming method and have challenges such as batch to batch variability and clogging issues with dirt samples (Rawa-Adkonis et al., 2006). Similarly, SPME which is noted for its simplicity, speed and minimal use of solvents, lacks reproducibility and may cause matrix affects whiles using complex samples(Fernández-Amado et al., 2016). In contrast, QuEChERS method has revolutionized pesticide residue analysis especially in terms of food sample analysis. This method is developed by Anastassides and his team which have many advantages like its simplicity to use, minimal solvent use, good extract recovery and can be used for wide ranges of pesticides (Anastassiades et al., 2013).

In this study we have used QuEChERS extraction methods and Gas chromatography coupled with a micro electron capture detector to estimate the presence of chlorpyrifos pesticides in the feed samples. Since chlorinated organic pesticides contain high electron affinity, detection and measurement of chloropyriphos with GC-ECD was more accurate. Electron capture detector with gas chromatography (GC-ECD) is a suitable method for determining low concentrations in feeds below maximum residue limits.

MATERIALS AND METHODS

Standard preparation

Stock solution (1000 ìg /ml) of Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) (Sigma-Aldrich)standard was prepared by accurately weighing 0.01 g of chlorpyrifos (Shimatzu Analytical Balance with minimum 0.00001 g accuracy) in a 10 ml volumetric flask and volume made up to the mark by adding HPLC grade acetonitrile. A working solution of chlorpyrifos (10 ìg /ml) was prepared from the stock solution. The working solution was then diluted to six concentrations (0.01, 0.1, 0.5, 0.25, 0.75, 1ìg /ml) to prepare a calibration curve for quantitative analysis. For each level of concentration in the calibration curve, four replicates of the standards were injected and chromatograms were obtained to find out the peak area and retention time. . Storage of standards wasdone at -20^{0}C in deep fridge (Velfrost Co.)

Extraction and sample preparation

The sample extraction was performed following the QuEChERS extraction protocol combined with solid phase extraction. From the properly grounded and homogenized feed sample, 2 g was transferred to a 50 ml centrifuge tube and 10 ml of milli Q water was added. 10 ml of acidified acetonitrile was added, the water was then shaken manually for 1 minute. Followed by that, QuEChERS salt mixture (6 g MgSO4 and 1.5g Na Acetate) was added to the tube and vortexed thoroughly. Subsequently the tubes were centrifuged at 5000 rpm for 5 minutes at room temperature. After 5 minutes pH was adjusted 4.5 to 4.8 and again centrifuged at 5000 rpm for 3 minutes at room temperature. From the supernatant 1 ml was transferred into a 2ml dispersive SPE tube (150 mg MgSO4, 50 mg C18EC, 50 mg PSA, 7.5 mg GCB) and further centrifuged at 13000 rpm for 2 minutes at room temperature and dried under a stream nitrogen (99.9995%) in nitrogen evaporator (Speedovap-LV Takahe analytical instruments). Finally, the dried extract in the SPE tube has been dissolved in 1 ml of ethyl acetate and transferred into an autosampler vial and ready for injection.

GC-µECD system

Concentration of Chlorpyrifos pesticides in the samples were determined using an Agilent 7890A

gas chromatographic system coupled with an electron capture detector. DB-5MS (Agilent Technologies, #123-5531) column (30 m x 0.320 mm x 0.10 µm, max. Temp: 325°C) was used for separation of pesticide. Nitrogen was used as the carrier gas (1 mL/min). Inlet temperature was held at 250°C in splitless mode and the purge flow rate was 3mL/min (Table 1).

Table 1. The set values for Inlet, Detector, column and oven parameters.

Inlet		Detector		Column
Temp.	250°C	Temp.	340°C	Mode: Constant Pressure
Mode	Splitless	Makeup flow	60 ml/min	Pressure: 6 psi(1.1846 ml/min)
Purge Flow	3ml/min	Signal	50Hz/0.004	
		Oven programming		
Stage	Rate °C/min	Temperature (°C)	Hold time (min)	Run time
Initial		60	1	1
Ramp1	30	180	0	5
Ramp 2	3	220	0	18.333

Validation of method

Linearity

To determine the linearity of the method 6 different concentrations (0.01;0.1; 0.25;0.50;0.75;1.0 ppm) of chlorpyrifos standards were taken and performed analysis. Linearity equations used for analysis is as given below,

$y=mx+b$

Where, m=Slope

b= y intercept (value of y where x=0)

y= area of peaks plotted on y axis

x= concentration of analyte plotted on x axis

Regression coefficient (r^2) value also calculated to see the goodness of fit of a model

LOD and LOQ. LOD and LOQ were calculated using the following equation.

LOD = 3.3*SD intercept / slope

LOQ =10*SD of intercept / slope

Recovery studies

Determination of the recovery percentage of the method was done using green para grass fodder which is not contaminated with chlorpyrifos. The desired quantity of the samples spiked with 0.1 ppm and 0.5 ppm of chlorpyrifos and further extractions were followed as same as the extraction method along with a blank sample.

Matrix effects

Percentage of matrix effects were calculated using following equation:

$\% ME = 100 - (100 * A_m / A_s)$

Where, A_m = Peak area of analyte with matrix (extract)

A_s = Peak area of analyte without matrix (Standard)

RESULTS AND DISCUSSION

Linearity of the method

Concentration of standards in X axis were plotted in scatter plot against the area of standards (Hz*sec) in Y axis (Figure 1.) and a calibration curve was prepared.

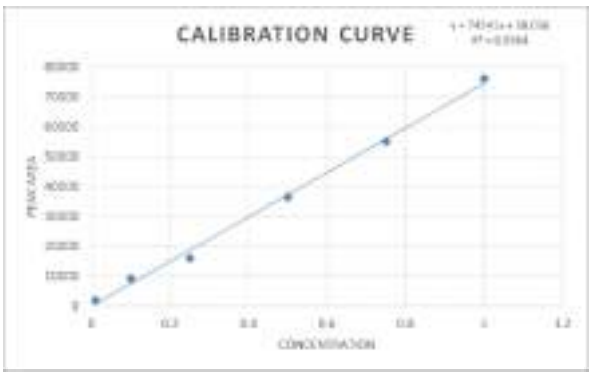

Figure 1: Linearity graph of chlorpyrifos with different concentration of pesticides

A regression equation with regression coefficient (r^2) of 0.99 was obtained indicating excellent linearity. Similar to our findings, several researchers obtained good linearity (0.99) using GC-ECD coupled with QuEChERSmethod in different samples like soil, vegetables, etc. (£ozowicka et al., 2017; Cho et al., 2013).

Limit of detection and Limit of quantification (LOD and LOQ)

LOD and LOQ are the two important parameters that need to be considered to define the performance of the method at lower concentrations. LOD is the lowest quantity or concentration of the analyte that can precisely analyze with the given method whereas LOQ is the smallest concentration of the analyte that can be determined with accuracy and repeatability. In this study, LOD of the method was obtained as 0.1 mg/L and LOQ as 0.4 mg/L (Table 2). Similar values were reported in other studies (Zhang et al., 2018; Schwantes et al., 2020). Some have reported lower and higher values than our studies and those variations due to differences in extraction procedure or instrumentation Tay and Wai 2021)

Table 2. LOD and LOQ values for standardized method of chlorpyrifos estimation in animal feeds

	Coefficients	Standard Error	t Stat	P-value
Intercept	38.0360588	1256.706746	0.030266455	0.97730449
X Variable	74540.85772	2242.034932	33.24696535	4.88E-06
		Calculations		
SD Intercept	SE of intercept * SQRT N			3078.3
LOD	3.3*SD intercept/slope			0.1
LOQ	10*SD of intercept/slope			0.4

Recovery studies

Recovery percentage of the spiked concentration 0.1 ppm was obtained as 85.7% and for 0.5 ppm it was 77.7%. Details of spiking and recovery calculations are given in Table 3. Similarly, 73% recovery at 0.1 ppm in rice straw was obtained (Lee et al., 1993). Chromatograms of 0.5 ppm standard of chlorpyrifos and spiked in the sample have been given in Figure 2 and 3.

Table 3. Spiking and recovery calculations

Spike Concentration (ppm)	Sample RT	Sample Area	Std RT	Std Area	Recovery %
0.1 ppm	12.882	8332.2	12.881	9767.5	
	12.88	8202.5	12.878	10583	
	12.895	10527.3	12.876	10823	
	12.893	9666.6	12.901	11680	
		9182.15		10713.375	85.7
0.5 ppm	12.867	39934.8	12.888	44381.9	
	12.9	33624.4	12.884	48781.4	
	12.897	34859	12.883	47526.6	
	12.899	37299.6	12.911	46801	
		36429.45		46872.725	77.7

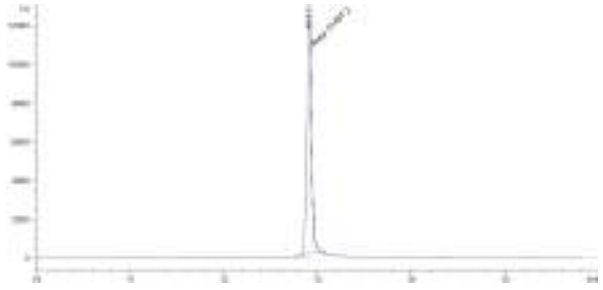

Fig 2. Chromatogram of 0.5 ppm Standard chlorpyrifos

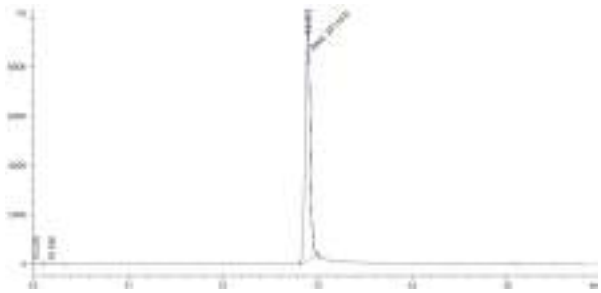

Fig 3: Chromatogram of Chlorpyrifos recovered after extraction

Matrix effect

In Order to determine the influence of undetected matrix components from the sample on the analyte measurement, percentage of matrix effects were calculated. A negative value indicates the suppression of the matrix and the positive value indicates the enhancement. As per the criteria set by SANTE/ 11321/2021 matrix effect less than 20% signal enhancement or suppression need not be addressed in the calibration. In our study we obtained -16% indicates matrix suppression which is a negligible matrix effect.

$\% ME = 100 - (100 * A_m / A_s)$

Where, A_m = Peak area of analyte with matrix (extract)

A_s = Peak area of analyte without matrix (Standard)

Validation of method using field samples

A total of 565 samples collected from dairy farmers from small, medium and large enterprises have been analyzed using Gas Chromatograph- Micro Electron Capture Detector (GC-μECD). Only 37 samples out of 565 samples collected were positive for chlorpyrifos indicating a positivity rate of 6.55%.

CONCLUSIONS

We have described a rapid extraction and analysis of chlorpyrifos residues using Gas chromatography-μElectron Capture Detector. The method qualified all the standardized parameters prescribed for the analytical method and tested using variety of feedstuffs.

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SHORT COMMUNICATION
In vitro Evaluation of Minor Millet By-products
Girisha et al.

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Evaluation of Byproducts of Some Minor Millets for Chemical Composition, Mineral Profile and *In Vitro* Gas Production Kinetics

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ABSTRACT

An *in vitro* evaluation of byproducts of selected minor millets by gas production technique followed by an *in vivo* evaluation of browntop millet (*Brachiaria ramosa*) byproduct-based CFM by twelve-week feeding and metabolism trial in sheep was conducted. *In vitro* study revealed that ME(MJ/kg) and IVOMD (%) of browntop (6.81 and 53.95) and little millet (6.81 and 53.09) byproduct were similar; whereas, lower values were found in foxtail (5.04 and 41.70), kodo millet (5.00 and 40.26), proso millet (5.23 and 43.47) and poor in barnyard (2.90 and 30.79). *In vitro* gas production kinetics of Millet-byproducts study revealed that readily soluble fraction 'a' value was not detectable while, insoluble but fermentable fraction "b" was significantly ($P<0.01$) highest (34.87) in Browntop millet and lowest (7.98) in Barnyard millet. Among various minor millet milling byproducts, Browntop and Little millet by products found to be better whereas barnyard is poor in ME and IVDOM.

KEYWORDS: Digestibility, *in vitro*, Minor millet byproducts,

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In Indian subcontinent, since last many decades, millets are being grown and consumed traditionally due to their rich source of nutrients to human and their residues as fodder to ruminant livestock. Millets are excellent drought resistant crops where highly stainable under rain fed conditions and therefore millets crops are well suited for contingency crop planning in addition to address the issues of climate change (ICAR-AICRP, 2018). Minor millets have high nutritional value in terms of proteins, vitamins, minerals and fiber. Six species viz., brown top millet (*Brachiaria ramosa*), kodo millet (*Paspalum setaceum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), little millet *(Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*) are grown in Indian subcontinent mainly in mountain and hilly areas, semi-arid areas where soil fertility is poor and rain fall is limited. Several tons of millet byproduct is produced every year in India after thrashing and processing of millets traditionally by the farmers and about 20-30 per cent millet byproduct by processing in the milling industries (Nagaraju, 2017). The millet husks are among the millions of tons of agricultural wastes seeking ways of disposal (Rosa et al., 2009). Important characteristics of millet husk include biodegradability, lighter weight and cost effectiveness. Hence, in addition to waste disposal, millet byproducts are found to be potential unconventional sources of feed ingredients in providing nutrients as well as producing cost effective livestock feeds for ruminant livestock. However, very limited information pertaining to nutrient composition, intake and utilization of minor millet byproducts as a potential feed resource are available. Therefore, the present study was conducted to evaluate various millet byproducts by *in vitro* g*as* production technique.

The minor millet milling byproducts comprised millet husk, small quantity of millet bran and broken grains produced during processing of millets grains from six varities viz., Brown top millet (*Brachiaria ramosa*) (BTMBP) (*Korale, Kan)*, Kodo millet (*Paspalum setaceum*) (KDMBP) (*Haraka, kan.*), Proso millet (*Panicum miliaceum*) (PSMBP) (*Baragau, kan.*), Foxtail millet (*Setaria italica*) (FXMBP) (*Navane, kan.*), Little millet *(Panicum sumatrense*) (LTMBP) (*Same, kan.*), and Barnyard millet (*Echinochloa utilis*) (BYMBP) (*Oodalu,*

kan.) were procured from millet processing plant of Shree Kshetra Dharmastala Rural Development Project (SKDRDP), Rayapura of Dharwad district., Karnataka. The byproduct mixture of these millets were ground through a 1-mm sieve by Cyclone sample mill (UDY, Corp., Fort Collins, Colorado, US) stored in airtight polythene bags for further chemical analysis. All the minor millet husks were subjected for chemical composition according to the procedures of AOAC (2016) and fiber fractions (Van Soest et al., 1991). The same were analysed for their mineral composition (Perkin Elmer, Analyst-400). The minor millets husks were subjected for rumen *in vitro* gas production technique (RIVGP) according to Menke and Steingass (1988) to estimate metabolisable energy, *in vitro* digestibility of organic matter and fermentation characteristics. The rumen fluid for in *vitro* gas production test was collected from the cannulated bull weighing 260 kg fed on maintenance ration consisting of concentrate feed mixture (0.75kg with 18% CP and 2600 kcal/ kg ME), green super Napier fodder (5 kg twice a day) and *ad lib*. paddy straw. Data of gas kinetics (exponential decay equation model) and the rumen *in vitro* gas production kinetics were subjected to nonlinear regression by GraphPad Prism (version 7.0, La Jolla California USA, 2016).

The proximate composition and fiber fractions of selected minor millet milling byproducts (husk) are presented in Table 1. The DM and OM content of different minor millet milling byproducts were similar to milling products of pearl millet (Tran, 2015), and sorghum ear husk (Gaur and Taparia,1991). Whereas, crude protein contents were lower than traditional milling byproducts such as wheat bran and rice bran. The CP content of LTMBP and BTMBP were 8.03 and 7.47 per cent which were similar to CP content of finger millet husk (7.10%) (Tran, 2015) and CP of PSMBP and BYMBP were similar to foxtail millet bran (Kadirvel et al., 1994) but lower than for sorghum ear husk (5.38 %) (Gaur and Taparia, 1991) and oat hulls (5.20 %) Heuze et al. (2016). However, the CP values reported for the byproducts of all the millets recorded higher CP values than CP reported for rice husk (3.25%) by Okoruwa et al., (2012). This indicates that the CP values are at higher levels when compared to any byproducts of conventional cereals.

The higher ether extract (EE) level was noticed in BTMBP (3.34 %) and LTMBP (2.24%) when compared to other millet byproducts (0.23 to 1.35%). The EE content of BTMBP was similar to wheat bran (3.14%) as reported by Dineshkumar et al. (2015) and Heuze et al. (2015). The total ash was reported to be little bit higher in all the six verities (8.76 to 13.13) of millet milling by products, however, the values were lower than the values reported for finger millet husk (17.60%) (Tran, 2015) and for foxtail millet bran (14.50%) (Kadirvel et al. 1994). The minor millet milling byproduct had significant crude fiber composition which is almost similar to any of the cereal hulls and the same is also true with NFE content.

Table 1. Chemical composition and fibre fractions (% DMB) of milling by-product of minor millets

Constituent	BTMBP	LTMBP	FXMBP	KDMBP	PSMBP	BYMBP
DM	89.45	88.37	89.81	92.65	93.61	92.23
OM	87.55	90.92	89.93	91.24	89.66	86.87
CP	7.47	8.03	4.67	4.39	6.05	6.85
EE	3.34	2.24	0.77	1.33	1.35	0.23
CF	24.32	25.01	31.32	24.53	34.00	40.78
TA	12.45	9.08	10.07	8.76	10.34	13.13
AIA	7.23	4.57	5.64	4.26	6.56	8.66
NFE	52.43	55.66	53.18	60.99	48.25	39.02
NDF	75.96	62.39	76.42	70.80	68.54	89.64
ADF	45.04	47.43	48.75	40.76	43.17	59.57
Hemicellulose	30.92	15.36	27.67	30.04	25.37	30.07
Cellulose	31.29	31.16	30.15	29.27	23.34	39.27
ADL	6.35	10.06	11.81	8.64	14.02	13.06

The NDF contents were ranging from 62.39 % (LTMBP) to 89.64 per cent (BYMBP) and The ADF content (%) ranged from 40.76 (KDMBP) to 59.57 (BYMBP). These values were comparable to various cereal husks (Gaur and Taparia, 1991; Okoruwa et al., 2012; Akinfemi and Ayoade, 2017). The cellulose (%) and hemicellulose (%) contents of BTMBP, LTMBP, FXMBP, KDMBP, PSMBP and BYMBP were 31.29 and 30.92**;** 31.16 and 15.36**;** 30.15 and 27.67**;** 29.27 and 30.04**;** 23.34 and 25.37**;** 39.27 and 30.07 respectively. The ADL (%) of different minor millet milling byproducts ranged from 6.35 per cent (BTMBP) to 14.02 per cent (PSMBP).

The calcium and phosphorus contents (g/kg DM) of BTMBP, LTMBP, FXMBP, KDMBP, PSMBP and BYMBP were 12.37 and 9.28, 13.12 and 9.57, 12.91and10.04, 14.07 and 8.66, 13.22 and 9.09, 16.47 and 6.73 respectively. The calcium and phosphorous levels were far higher than the milling byproducts of other major millets (Mopate et al., 2011; Heuze and Tran, 2015)

Table 2. Mineral composition of milling by-products of minor millets

Particular	Ca	P	Na	K	Mg	Co	Cu	Fe	Mn
			%				mg/kg		
BTMBP	1.24	0.93	0.010	0.59	0.24	2.47	9.01	243.10	40.11
LTMBP	1.31	0.96	0.014	0.64	0.23	1.97	8.89	238.10	36.20
FXMBP	1.29	1.00	0.019	0.50	0.29	2.09	12.50	216.08	38.79
KDMBP	1.41	0.87	0.013	0.56	0.30	1.86	8.70	208.79	35.52
PSMBP	1.32	0.91	0.017	0.65	0.29	2.45	9.95	217.86	42.45
BYMBP	1.65	0.67	0.013	0.86	0.31	3.03	7.78	239.77	41.06

The potassium, magnesium, cobalt, copper, iron and manganese for minor millet milling byproducts noticed in this study were similar to the values reported for brans of various cereals (Buerkert et al., 2001; Mopate et al.,2011).

The chemical composition of minor millet byproducts indicated that the fiber content and acid detergent lignin content were significantly of very high level. This was suggestive of these byproducts do not fall strictly under concentrate ingredient category. Due to this fact, the ME value calculated from *in vitro* gas production values using formulae prescribed for concentrates, roughages and also formula for combined category. Among the three formulae, the one used for roughages found to be more appropriate.

The ME values of byproducts were significantly different where BTMBP (6.81 MJ/kg) and LTMBP (6.81MJ/kg) were similar to ME content of MTH (7.91MJ/kg), whereas PSMBP (5.23), FXMBP (5.04 MJ/kg) and KDMBP (5.00 MJ/kg) had low ME content. Among all the byproducts, BYMBP (2.90 MJ/kg) was found to be very poor in ME content. BYMBP showed lesser gas production and ME values which might be due to higher levels of acid detergent lignin, crude fiber and lower level of EE and NFE. Akinfemi and Ayoade (2017) reported that RIVGP and ME values for maize husk were 15 ml and 5.39 (MJ/kg DM) respectively.

Table 3. Rumen *in vitro* gas production (ml/200mg DM) at 24 hours, ME (MJ/kg DM) and IVODM of CFM and by product of minor millets

Particular	24 hTotal Gas (ml/200mgDM)	ME, MJ/kg DM			IVDOM, %
		A	b	C	
BTMBP	31.06^{d}±0.25	6.29^{d}±0.04	6.81^{d}±0.04	7.05^{d}±0.04	53.95^{e}±0.22
LTMBP	32.28^{d}±0.36	6.56^{e}±0.06	6.81^{d}±0.05	7.02^{d}±0.05	53.09^{e}±0.32
FXMBP	20.43^{bc}±0.34	4.01^{b}±0.05	5.04^{b}±0.05	4.72^{b}±0.05	41.70^{c}±0.31
KDMBP	19.91^{b}±0.34	4.14^{b}±0.05	5.00^{b}±0.04	4.75^{b}±0.05	40.26^{b}±0.30
PSMBP	21.52^{c}± 0.35	4.41^{c}±0.05	5.23^{c}±0.04	5.11^{c}±0.04	43.47^{d}±0.31
BYMBP	4.82^{a}±0.16	1.38^{a}±0.03	2.90^{a}±0.02	2.48^{a}±0.02	30.79^{a}±0.14

* Means bearing different superscripts (a,b,c,d) within the columns differ significantly (P<0.01)

IVDMD (%) was found to be higher in BTMBP (53.95) and LTMBP (53.09) as compared to PSMBP (43.47), FXMBP (41.70), KDMBP (40.26) and least in BYMBP (30.79). As the IVDOM values increased the gas production also increased and the ME values calculated based on all three equations followed the same trend where the millet byproducts which had higher IVDOM had higher values of ME. The IVDOM also directly correlated to the content of lignin in the by-products, the byproducts which had lower level of lignin had higher IVDOM values. Akinfemi and Ayoade. (2017) reported lower IVDOM value for maize husk (33.22 %) than the values reported for all byproducts except BYMBP. The 'a' values were negative in the present study (Table 3) for these minor millet byproducts indicating the fraction which could not be measured with non-linear equation used for various parameters of *in vitro* gas kinetics (Blümmel,1994) and do not have any biological meaning -described by Krishnamoorthy et al. (1995). Similarly, 'b' values varied significantly (P<0.01) among various minor millet byproducts with higher values ranged from 34.87 (BTMBP) to 7.98 (BYMBP). Low level of 'b' values was due to higher levels of AIA, cellulose and ADL. The results also indicated that among various minor millet milling byproducts, BTMBP and LTMBP had higher fermentable fraction which was potential to provide higher ME values whereas FXMBP, KDMBP& PSMBP were medium and lowest in BYMBP in providing fermentable fraction. Akinfemi and Ayoade (2017) reported wide variations in the fermentation of the insoluble but degradable fractions (b, ml) with values ranging from 13 (UNMH) to 27.33 (PPMH).

Table 4. Kinetics of gas production of byproducts of minor millets as determined by *In vitro* Gas Production

Feed sample	96h Total Gas (ml/200mgDM)	a	b	D	k	$t_{1/2}$
BTMBP	36.77^{e} ±0.7	-3.22^{b} ±0.2	34.87^{d} ±1.1	31.65^{e} ±1.3	0.082^{bc} ±0.0	8.66^{bc} ±0.6
LTMBP	33.46^{d} ±0.3	-6.36^{a} ±0.4	34.64^{d} ±0.9	28.29^{d} ±0.9	0.113^{d} ±0.0	6.37^{a} ±0.5
FXMBP	26.44^{c} ±0.3	-0.98^{c} ±0.1	24.83^{c} ±0.4	23.85^{c} ±0.4	0.072^{b} ±0.0	9.72^{c} ±0.4
KDMBP	24.25^{b} ±0.2	-2.04^{c} ±0.2	22.89^{b} ±0.5	20.85^{b} ±0.6	0.097^{cd} ±0.0	7.35^{ab} ±0.5
PSMBP	23.42^{b} ±0.4	-1.89^{c} ±0.2	22.35^{b} ±0.4	20.46^{b} ±0.6	0.119^{d} ±0.0	6.21^{a} ±0.6
BYMBP	8.13^{a} ±0.4	-0.95^{c} ±0.1	7.98^{a} ±0.4	7.03^{a} ±0.3	0.043^{a} ±0.0	15.12^{d} ±0.8

a- Rapidly produced gas (ml/200mg DM); b- gas volume from the insoluble but fermentable fraction (ml/200mg DM); D- potential gas production (ml/200mg DM); k- rate of gas production/h; t½ - time at half asymptotic gas production. Means bearing different superscripts within the columns differ significantly (P<0.01)

Among minor millet byproducts, D value ranged from 7.03 (BYMBP) to 31.65 (BTMBP). Fidriyanto et al. (2020) reported that there were reductions in gas production potential (D value/200 mg) from 111.37 ml to 28.16 ml in determining fermentation characteristics of rice bran with various levels of rice husk as the level of rice husk was increased.

Among the minor millet byproducts, the rate gas production (k, h^{-1}) values were comparable with BTMBP (0.0822), FXMBP (0.0722). The values were significantly lower for BYMBP (0.0433). However 'k' values were significantly higher for KDMBP (0.0976), LTMBP (0.1135) and PSMBP (0.1191). Although the 'k' values were high for KDMBP, LTMBP and PSMBP, the overall gas production, ME and IVDOM values significantly low in minor millet byproducts which did not reflect in terms of ME and IVDOM indicating that these minor millet milling byproducts are energetically poor and less digestible. The time at half asymptotic gas production 't½' value is an indicative of time to reach peak gas production or time taken to reach 50 per cent of the total fermentation of organic matter. In the present study, BTMBP (8.658) and FXMBP (9.718), had taken similar time as compared to other minor millets. Further BYMBP (15.12) took maximum time to attain half asymptotic gas production level while LTMBP (6.368), PSMBP (6.211) registered shortest time to attain half asymptotic gas production level. However, gas production kinetic parameters did not reflect on energetic worth of the feed ingredients and also potential digestibility value in the present study.

CONCLUSION

Chemical composition of minor millet milling by products appears to be comparable with traditional crop residues. *In vitro* study revealed that among various minor millet milling byproducts, brown top and little millet by products found to be better whereas barnyard is poor in ME and IVDOM.

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Important Points on Experimental Power to be Considered during Poultry Nutrition Experiments

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In planning poultry feeding experiments, determining the sample size in terms of the number of replicates required per treatment and the number of birds per pen to achieve adequate power for a statistical test is an important question that is often ignored, increasing the probability of a treatment effect not being detectable even if it exists. On the other hand, when a difference is statistically significant, it does not necessarily mean that it is big, important, or helpful in decision-making. To know if an observed difference is not only statistically significant but also important or meaningful, we need to calculate its effect size. We will cover the calculation of effect size in the later part of this document. The results of experiments are affected not only by the effect of the treatments, but also by experimental error introduced by variability either due to biological variation in responses among individual subjects or inaccuracy in estimation of any parameter. Increasing the number of replicates and the number of birds per replicate pen will increase the experimental power, but it comes with a cost. Moreover, the number of animals must be kept at a minimum, considering animal ethics regulations. The number of replicates required depends on several factors like the significance level (α; 1%, 5%, etc.), the power desired (1-β; β is the type II error), the number of treatments to be investigated, the minimum size of the difference between a pair of treatment means that is considered important and the magnitude of the uncontrolled variation expected (standard deviations).

In broiler poultry body weight (BW) and feed conversion ratio (FCR) are important parameters from economic point of view where we wish to detect minimum of 2% difference between treatments for each of FCR and BW which is equivalent to 50 to 60g BW difference or 0.03-point difference in FCR at 6 weeks of age as this makes economic justification in favor of use of any additive or treatment in commercial farms. The subject of the minimum requirement of replicates in poultry has been extensively reviewed (Pesti and Shim, 2012; Demetrio et al., 2013; Nunes et al., 2018). Based on variability, size of smallest difference that need to be determined and extent of precision of estimation of FCR or BW it has been shown that at least 15 replicates (better if 20 replicates used) are required to have 80% chance (statistical power of 0.8) with a significance level of 0.05 in detecting a minimum treatment difference of about 50 g BW in an experiment involving 6 treatments; if there is high variation among individual birds within replicates the required number of replicates can be much higher(Demetrio et al., 2013). Thus, in poultry, replicate requirement is different from that of human or large animal experiments. Statistical power can be estimated while analyzing data in SPSS using GLM Univariate ANOVA by selecting observed power in the option section. Similarly, G*Power - a free software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower) can be used to calculate replicate requirements for experiments. The software uses effect size as an input parameter, besides á error probability (level of significance; 0.05 or 0.01, etc.), power (1- β; β is type II error; power should be 0.8 or higher), and number of groups. Generally, effect size is calculated by taking the difference between the two groups (e.g., the mean of the treatment group minus the mean of the control group) and dividing it by the standard deviation of one of the groups. An effect size of 0.5 or above is considered a moderate to large difference. Similarly, an Excel tool named 'Power and sample size calculator' available at https://www.moresteam.com/university/downloads/Power_SampleSize_Calculator.xls can be used to calculate the replicate requirement as well as the power of an experiment using input data such as mean difference to be detected, standard deviation, number of treatments, etc. Generally, the greater the

number of birds per pen, the lower the variations. In one of our feeding trial datasets involving 6 treatments, we observed that 8 replicate pens, each having 18 birds (floor rearing), had statistical power of 0.98 and detected a difference of 3.5% in FCR as significant at 1% level when weather conditions were favorable, supporting optimal uniform growth with limited mortality. Similarly, when 5 birds/pen were reared in battery brooder cages under the favorable weather conditions and limited mortality or disease incidence, 12 replicates per treatment (5 treatments) had a statistical power of > 0.80 and detected a difference of 4.5 % in BW or FCR as significant at 5% level. However, when there was high within pen variations due to heat stress and disease occurrence, or incorporation of chicks from both sexes, etc. combined with lower mean difference between groups, experimental power tended to fall below 0.8 in experiments involving similar replicates or treatments.

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Prof. Jyoti Palod honoured with Prestigious CLFMA award 2025

Professor Jyoti Palod from the College of Veterinary and Animal Sciences, Pantnagar, has been honored with the prestigious CLFMA Award for her outstanding contributions in the field of Livestock Sector.This honor was conferred during the inaugural session of the 66th National Symposium of CLFMA of India, held at Hotel Taj Deccan, Hyderabad on August 22, 2025.The award was presented by Prof. S. P. Singh Baghel, Union Minister of State for Fisheries, Animal Husbandry & Dairying and Minister of State in the Ministry of Panchayati Raj along with Mr. Vakti Srihari, Minister of Animal Husbandry & Fisheries, Government of Telangana.On this occasion, Mr. Divya Kumar Gulati, President of CLFMA, along with Secretaries of Animal Husbandry from the Government of India and the State of Telangana, and other distinguished dignitaries were present. With over 30 years of professional experience, she is serving as Professor for more than 15 and a half years. She is a Fellow many National Academies/Professional Societies.

Animal Nutrition Society of India congratulates Dr. Jyoti Palod for her outstanding achievement.

Appointment of Dr. D. V. R. Prakash Rao as Honorary Consul of Kyrgyz Republic

Dr. D.V.R Prakash Rao is a PhD in Animal Nutrition and was conferred D.Sc. by GADVASU, Ludhiana, NDRI, Karnal and O.U.A.T Bhubaneswar for his outstanding contribution in Animal Nutrition and Feed Technology. He is a distinguished Scientist, Successful First Generation Entrepreneur and a Visionary Leader in Veterinary and Animal Sciences. He has pioneered advancements in livestock nutrition and introduced innovative products making significant contribution to animal health, rural livelihoods and National food security as a Chairman & Managing Director of Prakash Foods & Feed Mills Pvt Ltd. He has brought visibility and direction to the critical domain of Veterinary Sciences by unique integration of science, enterprise and public policy as the President of National Academy of Veterinary Sciences (India). He constantly promoted science based policy, sustainable animal husbandry and farmer's welfare.

He has been appointed as the Honorary Consul of Kyrgyz Republic in Chennai with jurisdiction over Tamilnadu for fostering bilateral cooperation in trade, education, culture and scientific collaboration between India and Kyrgyzstan which was duly concurred by The Ministry of External Affairs, New Delhi.

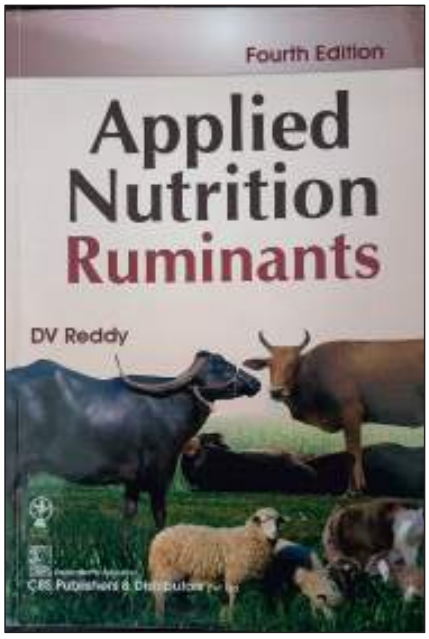
Animal Nutrition Society of India congratulates Dr. Prakash Rao for his appointment as Honorary Consul of Kyrgyz Republic.

Superannuation of Professor Vishnu Sharma

Dr Vishnu Sharma, born on 17 July 1965 at Jaipur and completed his B.V.Sc & A.H. from College of Veterinary and Animal Sciences, Bikaner in 1991. Later on, he completed Post Graduate and Ph.D. He served as Professor and Head , Animal Nutrition and further rose to the level of Dean, Post Graduate Institute of Veterinary Education & Research, Jaipur, Rajasthan, India. He was selected for Vice Chancellor, RAJUVAS and also additional charge of Vice Chancellor, SKRAU, Bikaner and JNVU Jodhpur. He was awarded Honorable Colonel rank by GoI and received CLFMA award and e-Governance by Government of Rajasthan. He was Member of scientific panel of Food Safety Standards Authority of India and member of Veterinary Council of India. He extensively travelled throughout the globe for various academic excursions and author of many peer reviewed scientific publications. He is Fellow of various National Societies and Academies including Animal Nutrition Society of India (ANSI).

ANSI wishes him happy, healthy and peaceful post-retirement life and members would be continuously benefitted by his experience and wisdom for handling various issues of ANIMAL NUTRITION.

BOOK REVIEW



Dr. DV Reddy's **Applied Nutrition: Ruminants (4th edition)** published by **CBS Publishers & Distributors Pvt Ltd.** serves as a comprehensive guide for Veterinary students and Animal Nutrition Professionals. Basically, this book can help future students coping with Minimum Standards for Veterinary Education (MSVE) requirements prescribed by Veterinary Council of India (VCI).

This book has 13 chapters including 5 new chapters on emerging topics of animal nutrition like Prediction of DMI in Dairy Animals; Rumen Modifiers for High Producing Cattle; Nutrition, Reproduction Interaction with special reference to Ruminants; Efficient Feeding; Systems for Ruminants; Nutrition for transition Dairy Animals for efficient Reproduction; All these chapters are a result of extensive compilation of latest happenings in these field. All these chapters provide useful insights into latest concepts in a random manner. At the end, Dr. Reddy has provided very useful appendices for students with regard to readymade calculators for calculation of body weights and model ration formulation for different classes of livestock. This book serves as guide for the students and provides recent references for researchers to refer further. Thus, the book can be recommended for Animal Nutrition graduate Students of Veterinary Science and Animal Husbandry and Agriculture. Since, Research and Development activities are continuous process, improvements can be made in presentation of latest concepts in an easy and understandable manner.

Dr. S. B. N. Rao, Chief Editor

OBITUARY

Dr Asit Das was born on 01-01-1966 in Agartala (Tripura). He obtained BVSc. &A.H. from OUAT (1990), and MSc (1992) and PhD (Animal Nutrition) (1997) from NDRI, Karnal. He joined ICAR service on 13.11.1995 at ICAR-ICAR Research Complex for NEH Region. He joined ICAR-Indian Veterinary Research Institute Izatnagar on 08-09-2003 and ICAR-NDRI Karnal on 16-07-2018 and again ICAR-Indian Veterinary Research Institute on 27-10-2021 and working as Incharge Clinical Laboratory of Animal Nutrition Division.

He was instrumental in streamlining the wildlife nutrition research in India and has standardized diets of 50 endangered species. He has 22 years of teaching experience and had guided 17 post-graduate students (5 Ph.D. and 12 M.V.Sc), published 128 research papers including 31 high impact international journals), 4 books, 8 book chapters, 7 technical manuals and several technical papers. He was the Chief Editor of Indian Journal of Animal Nutrition. He was worked as the Member of Health Evaluation Committee of several zoos. Besides, he is in the Reviewer panel of several high impact international journals like European Journal of Wildlife Research, Zoo Biology, Ecology, Ethology and Evolution, Journal of Animal Physiology and Animal Nutrition, Mammal Study, Tropical Animal Health and Production, and Journal of Veterinary Medical Sciences. He is recipient of several awards and recognitions from national and international organizations, some of the notable ones are Dr. K. Pradhan Young Scientist Award of ANSI, Best Poster award of ANA, Fellowship of ANA and Certificate of Appreciation from Royal Society for Protection of Birds (RSPB), UK. His contributions to Animal Nutrition, Veterinary Science, and Wildlife Conservation were immense and will leave a lasting legacy in the scientific community. His dedication, integrity, and passion for research inspired many and set a high standard for future generations. This is a tremendous loss not only to his colleagues and students, but to the entire scientific fraternity, and above all he was a wonderful human being.

Animal Nutrition Society of India is deeply saddened by sudden untimely demise of Dr.Asit Das, Principal Scientist on 29-07-2025 at ICAR–Indian Veterinary Research Institute, Izatnagar, Bareilly. We express heartfelt condolences to the bereaved family. We all Animal Nutritionists miss him and pray almighty God for his soul to rest in peace.

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