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CONTENTS

Indian J. Anim. Nutr. 2021. 38 (4): 330-345
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Review

Belim *et al.*

Recent Advances and Future Directions in Poultry Nutrition - A Review

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ABSTRACT

The poultry industry in India has made a remarkable growth and it is currently emerging as a sunrise sector with a growth rate of 8.51 and 7.52% in egg and broiler production, respectively. Also, the per capita consumption of eggs has gone up from 30 to 68 eggs per annum and demand for processed chicken meat has been growing by 15- 20% per annum. The worldwide consumption of poultry products such as meat plus eggs are currently high and it is growing continuously as compared to other livestock products. So, it is important to know what the recent advances that were made and what will be the future directions in poultry nutrition in a brief. Recent advances that have made includes defining nutrient requirements and nutrient composition, least cost feed formulations based on amino acid and energy, use of biotechnological products in poultry feeding as well as nano minerals supplementation. There are certain future directions like use of alternative raw material, use of phytogenic feed additives as alternative to antibiotic growth promoter, focus on gizzard development and gut health, use of next generation feed enzymes, split feeding and in ovo nutrition are of prime importance.

Key words: Antibiotic alternative, Feed additives, Gut health, Nutrition, Poultry, Supplements

INTRODUCTION

According to the 20th Livestock Census-2019, the total poultry population is 851.81 million and it is increased by 16.81% as compared to previous livestock census (Anonymous, 2019). The poultry industry in India has made a remarkable growth and it is currently emerging as a sunrise sector with a growth rate of 8.51 and 7.52% in egg and broiler production, respectively. India is the third largest egg and fourth largest broiler producing country in the world and it has an estimated production of 103.3 billion eggs and 4.1 million tons of broiler meat per annum. Broiler and layer segment constitutes about 65.3% and 34.7% with the monthly turnover of 400 million chicks and 8,400 million eggs, respectively (Kolluri *et al*., 2020). Production standards of broilers and layers have continuously improved and the male broilers are currently reaching a live weight of 2.5 kg at 33 to 35 days of age, and white egg layers capable of producing 330 eggs in 52 weeks of lay. The body weight of broilers at 42 days has increased by 25 to 50 gram per year and the feed conversion ratio (FCR) to 2 kg body weight has improved 2 – 3 points annually (Ravindran and Abdollahi, 2017).

The global average of FCR in 2013 was around 2.0 for weight gain (live weight) and 2.8 for slaughtered meat (carcass weight). When slaughtered, the world average layer flock as of 2013 yields a carcass FCR of 4.2, still much better than the average backyard chicken flock (FCR 9.2 for eggs, 14.6 for carcass) (Macleod *et al.*, 2013). Since past poultry producers have been able to reduce their feed conversion ratios from producing a bird weighing 2 kilograms in 70 days with an FCR of 2.5 to a bird weighing the same in comparatively less number of days with an FCR of 1.5 today. The industry made this leap possible using genetic improvements most probably and rapid disseminations of improved chickens.

In India, the per capita consumption of eggs has also gone up from 30 to 68 eggs per annum and that of chicken from 400 gms to 2.5 kg per annum in the

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last five years. Demand for processed chicken meat has been growing by 15- 20% per annum in recent years. Poultry meat and eggs are cherished worldwide and are consumed in various forms. They are rich in proteins and a major source of essential micro-nutrients such as vitamin A, vitamin B12, riboflavin, calcium, iron and zinc. The Indian Council of Medical Research (ICMR) recommends the consumption of 180 eggs per capita per year. The primary objective of nutritional research include selection of the good quality ingredients for the healthy growth of birds and as a result of which there will be elimination of certain disease condition that is causing economic losses to farmers and ultimately it will be reducing the cost of production.

The poultry sector has advanced remarkably and poultry meat production had been the most successful than any other in the animal industry. So, the main purpose of this overview is to make researchers, young scientist and students know about the various recent advancements and future directions in the field of poultry industry. Thus, this overview will form a base of generalized knowledgeful information.

RECENT ADVANCES IN POULTRY NUTRITION

Defining nutrient requirements

Defining nutrient needs is bit of difficult because they are influenced by several factors. The factors influencing nutrient requirements are of two main types, the first one is bird related ones, such as genetics, sex, and type and stage of production; and the second are external ones, such as thermal environment, stress and husbandry conditions. One must be very much precise in both this areas for defining nutrient requirement accurately. Defining requirements for the ten essential amino acids has been made easier by acceptance of the ideal protein concept. The ideal protein concept generally uses lysine as the reference amino acid, and the requirements for other essential amino acids are set as percentages (or ratios) of the lysine requirement. This has now become accepted practice for setting the amino acid specifications in the poultry industry for feed formulations (Ravindran, 2013).

Nutritional requirements are not static but dynamic. Thus, with the change in genetic makeup, feeding system and feeding management there is a need for verification, updating and fine tunning of nutritional requirements on a continuous basis (Reddy, 2015). The latest feeding standards adopted are Indian council of agriculture research (2013) which includes nutrient requirements of broiler breeders and layer chicken including cockerels and breeder males, Bureau of Indian standards (2007), Nutrient requirement of poultry (1994) by NRC of National Academy of Sciences (USA). Historically, the industry has utilised the nutrient requirements recommended in the publication by National Research Council (NRC). Currently, the recommendations suggested by commercial breeding companies provide guidelines that more closely match the requirements of modern bird strains than those recommended by NRC (1994) (Ravindran and Abdollahi, 2017). However, NRC standards under Indian conditions may not be appropriate as the requirements differ due to several factors such as genetic makeup, environmental temperature, managemental practices, metabolic characteristics, feedstuff qualities and dietary variables. Substantial differences also exist in the estimates of nutritional requirements for chicken and other poultry species of tropical countries from that of temperate climate (Reddy, 2015).

Defining nutrient composition and ingredient quality

There is the availability of rapid tests, such as the near-infrared reflectance analysis, to predict gross nutrient composition and to access the variability in ingredient supplies (Ravindran, 2013).

(a) Near-infrared reflectance spectroscopy (NIRS)

Recent developments in near infrared spectroscopy (NIRS) are extremely cost-effective, near instant and provide on-site analysis. This is beneficial to animal nutritionist to formulate better diets. Currently the NIRS may be used as a tool to predict the digestible amino acid and likely the energy content of feedstuffs. Also, NIR has a potential to deliver bird's nutrient requirements with unfailing accuracy, without over or under supplying. Latest NIR technology software and hardware developments can help nutritionists better

understand their feed ingredients. Emerging technologies such as centrally maintained on-line calibrations, pay-as-you-use calibrations, portable NIR and affordable in-line NIR installations are making NIR technology more accessible across the entire feed industry. Rapid sample analysis by NIR allows nutritionists to better understand the variation in raw materials. It will adjust ingredient safety margins.

Near infrared spectroscopy is a technology that uses the infrared region of the electromagnetic spectrum (from about 800 nm to 2500 nm) to study the physio-chemical properties of samples in a non-destructive way. The technique is rapid, non-destructive, precise, and cost-effective, compared with other laboratory techniques (Yakubu *et al.*, 2020). Calibration is the key for successfully using the NIRS technique. Accurate, robust calibration models are difficult to obtain as their construction requires the use of a large enough number of samples to include all variations of physical and/or chemical properties (Restaino *et al.,* 2008). So, calibration transfer between instruments is needed because usually calibration equations which are suitable for one kind of spectrophotometer are not working properly on spectra acquired by other instruments. Marchesini *et al.* (2017) concluded that the transfer of calibration curves is good enough to obtain accurate predictions about the composition of the chopped maize plant, using portable NIR instruments. Soto *et al.* (2013) concluded that feed formulation using NIRS as the source of information for amino acid content improved broiler body weight at 21 and 42 days without affecting FCR.

(b) Feed formulations based on amino acid

Dietary protein sources are often a high expense component of poultry diets. As animals require specific amino acids, formulations requiring specific amino acids often lead to crude protein (CP) levels that are overly costly and result in excess nitrogen being excreted as an environmental pollutant (Sigolo *et al.*, 2017). So, using low protein diets can be more economical. Harn *et al.* (2020) showed with their studies result that the crude protein content of grower and finisher diets can be reduced by 2.2-2.3% units without adverse effects on growth performance of broilers, while crude protein reduction seems promising to reduce nitrogen excretion from broiler houses, improve bird welfare, and reduces dependence on vegetable protein sources. Also, Kamely *et al.* (2020) concluded that moderate reductions in dietary crude protein in the starter phase of broilers did not significantly affect growth performance or relative organ weights in either the starter phase or beyond until 35 days of age.

Formulating diets based on digestible amino acids makes it possible to increase the range of ingredients that can be used and the inclusion levels of alternative ingredients in poultry diets. This improves the precision of formulation, may lower feed costs and ensures more predictable bird performance (Ravindran, 2013).

(c) Feed formulations on energy basis

Despite its limitations, apparent metabolisable energy (AME) has been the system of choice for describing available energy. Net energy (NE) system which is a refinement of the AME concept, has received attention from time to time. To be acceptable to the commercial industry, formulations based on NE values should demonstrate an economic advantage over the current system, which is yet to be proven (Ravindran and Abdollahi, 2017).

(d) Better feed formulations and processing

Once the nutritional needs are defined, the next step is to match these needs by using combinations of ingredients and supplements. The objective of feed formulation is to derive a balanced diet that will provide appropriate quantities of available nutrients at least cost. Over the previous years, feed formulation has evolved from a simple balancing of few feedstuffs for a limited number of nutrients to computer-aided linear programming systems. Currently, newer systems of stochastic non-linear programme are becoming popular with the commercial availability of this formulation software. Because variability in ingredient composition is non-linear, stochastic programmes address this issue in the most cost-effective manner possible. Newer approaches, which predict the most profit for a given ingredient combination are being increasingly used by the commercial industry (Ravindran and Abdollahi, 2017).

Kasturi feed formulation is simple and practical

feed formulation software, is developed by K. Chandra Shekhar in the year 2002. It is meant for least cost feed formulation and generally users friendly. It comes with two functions: (1) Optimize: where it uses liner programming to optimize feed formulation at least cost, (2) Analyse: if one does not want least cost formulation but only want to know the nutrient values, this will calculate the nutrients values and the formula cost on entering the ingredients quantity and rate. It is mostly suitable for egg producers, broilers, hatcheries, feed manufacturers etc. Another software is WIN FEED, it is the cheapest least cost feed formulation software developed in the year 2012. It is equally useful for ruminants and non-ruminants such as poultry, cattle, sheep, horses, dogs, cats, fish and aqua culture etc. WinFeed works in two modes, Linear Mode: suitable for conventional feed formulation and Stochastic Mode: specifically for probability based least cost feed formulation. Eco-Mix is user friendly windows-based software which prepares any kind of balanced feed with least cost. This software can be used for poultry, cattle, horse, fish, pets, ruminants and non-ruminants etc (Patil *et al.*, 2017).

Growth models are used to simulate feed intake and production parameters under a given husbandry condition. Such models are effective tools to compare actual versus potential performance, which can indicate the extent of management or health problems in the flock and it will also provide economic analysis of alternative feeding regimens.

Modelling approach is possibly the best way to determine the optimum economic feeding program for broilers and laying hens and for predicting performance in the future (Sakomura *et al.,* 2015). The models are only as good as the datasets we use to develop them (Ravindran, 2012). Offering feed to poultry in pellet or crumbled form has improved the economics of production by bettering feed efficiency and growth performance. These improvements can be decreased feed wastage, higher nutrient density, reduced selective feeding, decreased time and energy which are spent for eating, destruction of pathogenic organisms and more importantly, increased feed consumption (Amerah *et al.,* 2007).

The requirements for energy and amino acids may also be affected by the feed form (pellet or mash). The foremost advantage of pellet feeding is facilitation of easy ingestion. Increasing the diet density through the pelleting process has been reported to markedly increase productive energy. Broilers fed with pellets have lower heat increment and utilise more of the feed energy for productive purposes than those fed with mash (Latshaw and Moritz, 2009). Lemons *et al.* (2021) concluded that broiler birds fed crumbles had the highest body weight and body weight gain/bird, regardless of crumble feed quality and birds fed intact pellets achieved increased weights when feed quality was highest similar to birds fed crumbles.

Use of Biotechnological Products in Poultry Feeding

The ultimate goal of using biotechnology in animal nutrition is to improve the nutritional plan by increasing the availability of nutrients from the feed and reducing the wastage of feed (Asmare, 2014). Some of these applications are already in use (for example, new feed ingredients like single-cell protein and yeast protein, designer ingredients like high-oil maize, high-methionine lupins and low-phytate maize, feed additives like antimicrobials and crystalline amino acids, feed enzymes like microbial phytases, gut ecosystem enhancers like probiotics (direct-fed microbials), prebiotics like mannan oligosaccharides and others whose potentialities are known but are yet to be commercially applied because of technical limitations and public concerns such as bioactive peptides: improved growth and efficiency (e.g. growth hormone-releasing peptides), improved gut function, immune-modulation and antibacterial properties, nucleotides: stimulation of intestinal development, tissue growth and better immune response, antimicrobial replacers: antimicrobial enzymes (e.g. lysozyme) and delivery of specific antibodies via spray-dried plasma and egg products, transgenesis: to modify nutrient metabolism and improve growth efficiency by transfer of genes (Ravindran and Abdollahi, 2017)

Phase Feeding

This is a feeding system in which dietary amino acid levels are reduced steadily over time in an attempt to reduce costs associated with excess dietary protein or amino acids (Ravindran and Abdollahi, 2017). The wider implementation of

phase/precise feeding, however, is limited by several issues like information on metabolizable energy and digestible amino acid requirements for different classes of poultry is lacking, we do not have objective rapid tests, which the industry can use to estimate metabolizable energy/digestible amino acids as the raw materials are received at the feed mill, information is still needed on the comparative digestibility of amino acids for different classes of chickens - layers and broilers of different age groups (Ravindran, 2012).

Meremikwu and Obikaonu (2020) concluded that extending the growing period of the broiler using a high-low-high phase feeding led to the production of roasters (heavy broilers) that achieved identical body weights with the control group at 12 weeks of age and there was zero mortality noted. Also, the nutrient restricted birds had about 25% lower cost of feed per kilogram weight gain than the control group.

Split feeding

Split feeding is generally alternative system for feeding layer birds. It means to provide different morning and afternoon diets to the hens. This responds to their physiological feeding behaviour and nutrient intake according to the different requirements throughout the day. Benefits of split feeding includes: cost savings, greater profits and increased egg quality and number. Better sustainability is the result of improved nutrient efficiency. El-Razek *et al.* (2020) concluded that split feeding of Dandarawi layers at their late phase of egg laying cycle had led to decreasing feed consumption, improving feed conversion ratio, saving in feed cost and increasing egg number and egg mass.

Nano minerals

Nano-minerals are specially synthesized mineral particles with its particle size ranging from 1 to 100 nm. Like nanoparticles, nano-minerals possess higher physical activity and chemical neutrality, which may be the reason for efficient absorption in the animal system (Hassan *et al.,* 2020) and they are reported to be stable under high temperature and pressure as well.

Nano-minerals as feed supplement can increase the feed efficiency, diminish feed cost by reducing the supplemental doses, and simultaneously intensifying the yield and value of animal products by virtue of their superior bioavailability (Patra and Lalhriatpuii, 2019). Different beneficial biological effects of nano-minerals in poultry are, growth: improvement in the body weight gain and feed efficiency, immunity: improve the cell mediated and humoral immunity, meat production: improvement in meat quality and also improvement in meat keeping quality, egg production: improve egg production, egg weight, hatching percent, egg quality parameters, hematology and blood biochemistry: improves antioxidant enzymes hormones related to growth and reproduction, miscellaneous: reduce environment pollution, retained more efficiently, less excretion (Swain *et al.,* 2021). Data given in table 1 represents meta analysis of nano minerals supplementation.

Table 1. Meta analysis of nanominerals supplementation

Nano minerals	Experimental bird	Effects	References
Zinc oxide nanoparticles	Broiler bird	Improves antioxidatives capabilities, Improves growth performance	Zhao *et al.,* (2014)
Nano zinc	Layer bird	Improved average body weight and FCR	Mishra *et al.,* (2014)
Nano calcium carbonates	Laying hens	Stronger egg shell strength, promoted water intake to dissipate heat	Wang *et al.,* (2017)
Zinc oxide nanoparticles	Laying hens	Improve egg quality, bone parameters, antioxidative status	Abedini *et al.,* (2018)

Organic Minerals

Organic mineral is an organic compound in mineral form. Organic trace minerals have higher bioavailability than inorganic trace minerals. Hence, supplementation of organically complexed/chelated trace minerals could help avoid the use of higher dosages of inorganic trace minerals in poultry feed and prevent environmental contamination because they have lower inclusion rates and reduced excretion (Bao *et al.,* 2007; Bao and choct, 2009). In market there is availability of high quality supplement of chelated minerals product like chelated Asocal and mineral mixture like Sermin for better growth of birds.

Rao *et al*. (2016) concluded that chicks supplemented with chelated trace minerals had significant increase in body weight gain, deposition of minerals in the tissue and improved feed conversion ratio (FCR) compared with that among chicks supplemented with inorganic trace minerals at similar dose. Also, broiler birds fed with a diet supplemented with organic chromium (0.5 ppm) showed increased body weight gain compared with birds supplemented with inorganic chromium sources (Mohammed *et al.,* 2014).

FUTURE DIRECTIONS IN POULTRY NUTRITION

Sometime in the future, we should modify feed formulations that will accommodate science-based as well as the needs of the society. The impact of social issues (use of antibiotic growth promoters, environmental issues) will have direct impact on decision-making from farm level to retail distribution of poultry products.

Sustainability

Previously, when feeds were formulated, the main objective was how to supply the nutrients (nutrient input). Today, there is much more public concern about what comes out of the bird (nutrient output) (Ravindran, 2012). A major problem facing intensive poultry production in India is the disposal of litter (Bolan *et al.,* 2010). Poultry manure can also become a serious environmental pollutant and contributor to greenhouse gases. There are environmental and health issues linked to bio-aerosols (e.g. microbes, endotoxins and mycotoxins suspended in air) generated at production, manure storage facilities and during land spreading of poultry litter (Bolan *et al.,* 2010).

Central Pollution Control Board (CPCB) has updated the norms that apply to all types of poultry farms. Smaller poultry farms which is having over 5,000 birds at a single location will need an approval to establish and operate under the Water Act of 1974 and the Air Act of 1981 from the State Pollution Control Board (SPCB) or Pollution Control Committee (PCC). According to the CPCB, new poultry farms should be constructed 500 metres from residential zones to reduce stink and flies, and 100 metres from important watercourses such as rivers, lakes, canals, and drinking water sources to avoid pollution due to leaks and spillages. The updated standards also address environmental concerns raised by poultry farms, such as reducing smell and gaseous pollution through effective ventilation, managing solid waste and hatchery debris, collecting, storing, and composting manure. Poultry houses should be well ventilated to dissipate heat and prevent build up of gases. Excreta should be scratch once in two days and manure collected shall be stored for processing. Manure storage facilities shall be minimum 2 m above water table.

Results from various studies have shown increased phosphorous digestibility and its utilization, and reduced phosphorous excretion into the environment as a result of phytase addition (Applegate *et al.,* 2003; Penn *et al.,* 2004; Angel *et al.,* 2006; Leytem *et al.,* 2007). Supplementation of commercial enzymes can enhance the nutritional value of crops containing high contents of soluble non-starch polysaccharides (NSPs). The NSPs digestibility is very low in poultry and a large amount is voided via the excreta. The NSPs can also bind to large quantities of water and as a result, the fluid viscosity increases. Increasing viscosity may cause problems in the digestion of carbohydrate, protein and fat. Furthermore, high viscosity of intestinal content increases the sticky dropping amounts. These problems can be overcome by addition of enzymes to poultry diets. An increased use of exogenous enzymes is expected not only from the nutritional and economic aspects but also from the health and environmental point of view (Alagawany *et al.,* 2018).

Alternative raw materials

Poultry production costs have continually increased because of the fluctuation prices of high-quality raw materials such as soybean, corn, and others. Many attempts have been made to decrease the cost of feeding to the minimum levels. These attempts include replacing the expensive feedstuffs by cheaper and more abundant by-products to support the sustainability of poultry production (Aftab, 2009).The discovery of alternative protein sources for poultry feeding diet has become a particular focus in the current scenario to decrease dependence on soyabean meal as the main ingredient of protein in poultry feeds (Ashayerizadeh *et al.*, 2018). Dried distillers' grains with solubles (DDGS), sunflower meal (SFM), decorticated cotton seed meal (gossypol free) and rapseed meal are new feed ingredients and may be used as an alternative source of protein in animal and poultry diets. Body weight gain and nutrient digestibility significantly improved by 60% soyabean meal replacement with maggot meal during starter phase in broiler chicks (Khan *et al.*, 2018).

Antibiotic free nutrition and gut health

The use of antibiotics in poultry rations for the purpose of stimulating growth is useful in improving production and preventing infections but the excessive use of these antibiotics led to raise in bacterial resistance to diseases in addition to the accumulation of remnants of these drugs in animal products and therefore they were dispensed (Nisha, 2008).The main characteristic of a good antibiotic growth promoter (AGP) alternative is practicality; it must consistently improve animal performance (Huyghebaert *et al.*, 2011). Antibiotic alternatives may have some positive regulatory and antioxidant effects on intestinal flora in poultry and these compounds can also be considered as growth stimuli in poultry production. Meta analysis of combined or synergistic effect of additives is given in table 2.

Combination of other strategies including modifications in husbandry and nutritional management need to be considered to promote gut health and good gut flora and these may include: Use of highly digestible pre-starter diets, use of lower dietary protein levels and better balance of amino acids, use of coarse particle size or whole grain feeding to enhance gizzard development, maintenance of good litter quality and maintaining proper stocking density etc. When gut health is compromised, digestion and nutrient absorption are affected (Jha and Berrocoso, 2015), which in turn, may have a detrimental effect on feed efficiency and greater susceptibility to diseases leading to economic loss. Some important alternative to antibiotics are discussed below:

Probiotics are single or mixed culture of living microorganisms which when administrated in adequate numbers have health benefits for the host by improving the host intestinal microbial balance, enhancing of colonization resistance against pathogens and improving the immune responses (Das *et al.*, 2012). The species of microorganisms being used in probiotic preparations are varied and LAB, i.e., Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp., are the most common type of bacteria used as probiotics (Kabir, 2009).

Prebiotics are non digestible feed ingredients that beneficially affect the host by selectively altering the composition and metabolism of the gut microbiota. Prebiotics may provide energy for the growth of endogenous favorable bacteria in the gut, such as bifidobacteria and lactobacilli, thus improving the host microbial balance (Das *et al.*, 2012). The most often used prebiotics are fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), pyrodextrins, and lactulose (Alloui *et al.*, 2013). When both probiotic and prebiotic are combined, they form symbiotic (Huyghebaert *et al.*, 2011). The functional benefits of symbiotic, are resistance to gastrointestinal bacterial infection, antimicrobial activity, and improvement of immune system are envisaged in the development of symbiotic products (Saminathan *et al.*, 2011).

Various exogenous enzymes including b-glucanase, xylanase, amylase, a-galactosidase, protease, lipase, phytase, etc. have been supplemented in poultry diets (Bedford and Cowieson, 2012). Preparing poultry diets with a

mixture of glucanase and xylanase improved the feed conversion ratio and nutrient digestion in the ileum, so, adding two types of enzymes produces a synergistic effect than if only one type of enzyme was used (Cowieson *et al.,* 2010). Organic acids are commonly used as acidifiers in poultry nutrition. They act directly against bacteria that are present in the feed. Organic acids can also act indirectly by reducing the pH in the gut. Butyric acid is nowadays commonly used. Lactic acid is more effective against bacteria. Formic acid, propionic acid have broader antimicrobial activities and can be effective against bacteria and fungi, including yeast. Butyric acid is nowadays commonly used.

Phytogenics are a group of natural growth promoters (NGPs) or non-antibiotic growth promoters used as feed additives, derived from herbs, spices or other plants (e.g. garlic, oregano, thyme, rosemary, coriander and cinnamon) as well as to their respective plant extracts in the form of essential oils. They are commonly regarded as favourable alternative feed additives to antibiotic growth promoters (AGPs) in poultry nutrition (Windisch *et al.,* 2008). Phytogenic relieve the host animals from immune defense stress during critical situations and increase the intestinal availability of essential nutrients for absorption, thereby helping animals to grow better (Hashemi and Davoodi, 2010). Karangiya *et al*. (2016) concluded that supplementation of garlic improves the performance of broilers when added at the rate of 1% of broiler ration and can be a viable alternative to antibiotic growth promoter in the feeding of broiler chicken.

Bacteriophage are virus that infect and kill bacteria. An 0.05% bacteriophage cocktail dietary supplementation during the finisher period would be economical and effective as a safe alternative to antibiotics for raising broilers under intensive farming systems (Upadhaya *et al.,* 2021). Immunomodulators are agents which specifically modulate immune system regulating immunity and disease resistance. In poultry it is most important for the growth, disease resistance, FCR, body weight gain as production output mainly depends on the health and immunity of the birds. Different types of immunomodulators are prebiotics, probiotics, phytochemicals, turmeric rhizome powder, cinnamon, thyme, essential oils and carvacrol, vitamins (A, C, E), minerals, adjuvants, polysaccharides, herbs like aloe vera etc. The primary objective of immunomodulation is to improve host resistance to external and internal attacks by the microbes or other infectious agents Immunomodulators can used as alternatives to antibiotics, antimicrobials etc. for the improvement of the immune system. As immune system is directly related to the disease resistance, gut health etc hence they must be used in combination and/or alone according to the needs. They also improve the qualities of feed and immune molecules enhancing all possibilities to fight against diseases as well as maintain health of birds. However, they are not popular and not included in the poultry feed on routine basis. Hence, more studies should be done and efforts should be made to popularise it (Das *et al.,* 2020).

Table 2. Meta analysis of combined effects of additives

Effects	References
Synbiotic product containing Enterococcus faecium bacteria and FOS as a prebiotic, and immunomodulating substances from marine algae (ficophytic substances) significantly increase the average daily body weight gain, improve the gut health and can be used as a growth promoter in broiler diets.	(Awad *et al*., 2009)
Probiotics and phytobiotics can have beneficial synergistic effects on the intestinal microbiota in young chickens	(Ren *et al*., 2019)
Combination of 1% herbal mixture with enzyme feed additives in broiler feed can be effective alternative to antibiotic growth promoter	(Singh *et al*., 2020)
Supplementation of probiotics and/or organic acids enhanced humoral and cell mediated immune response in broilers.	(Ebeid *et al*., 2021)

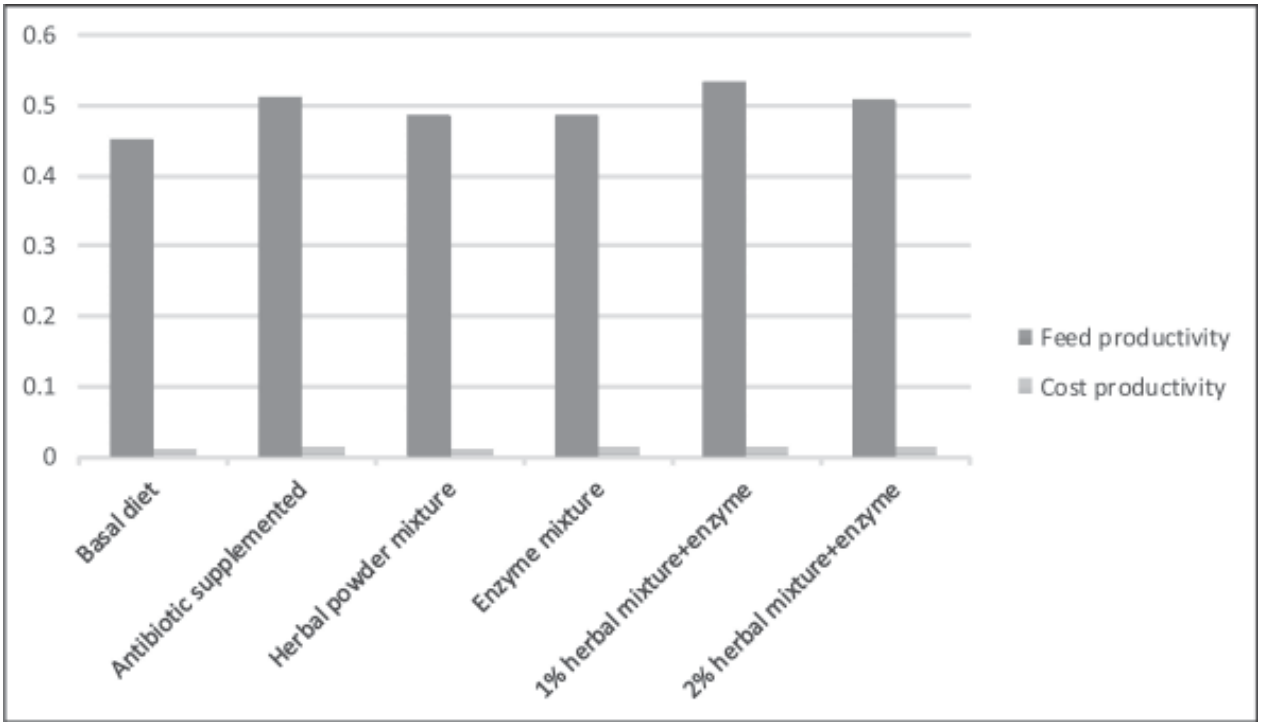

Fig. 1. Representation of feed productivity and cost productivity (Singh *et al.*, 2020)

Focus on gizzard development

Fore gut, particularly gizzard, if fully developed, can be regarded as an important barrier in preventing pathogenic bacteria from entering the distal intestinal tract (Svihus, 2011).The inclusion of dietary structural components, such as coarse particles, insoluble fibre sources and whole grains, in poultry diets should be given consideration. The major motivation for inclusion of structural components in poultry diets is to stimulate gizzard development and its functionality, which will favourably influence gut health and the bird's ability to better utilise nutrients (Ravindran and Abdollahi, 2017).

A large and well-developed gizzard is able to grind feed particles more thoroughly (Amerah *et al.*, 2007), to elevate pancreatic enzyme secretion through increased release of cholecystokinin (Svihus, 2011), to increase proteolysis by pepsin, trypsin and other endogenous proteases in the small intestine, to improve gastrointestinal tract motility and to improve nutrient digestibility (Amerah and Ravindran, 2008).

Synthetic amino acid supplementation

The careful supplementation of synthetic amino acids has the potential to boost the overall amino acid balance and to decrease the level of crude protein in the poultry diet. Dietary synthetic amino acid supplementation to poultry diets improved feed conversion efficiency and reduced nitrogen excretion (Beski *et al.*, 2015). Synthetic DL-Met and Met analogue are now widely used in commercial production diets to reduce crude protein (CP) levels (Vieira *et al.*, 2016).

Natural sources of tryptophan (chick peas, sunflower seeds, pumkin seeds), herbal lysine (spirulina, fenugreek seeds, soyabean), herbal methionine (amla, soya bean, garlic, silybum, bhringraj), herbal choline (soyabean, almonds, red potatoes, cruciferous vegetables like cauliflower, brocolli) are also used. Herbal Methionine is the natural alternate to dl-Methionine as it is obtained from those plants which have same action mechanism in the body as that of synthetic dl-methionine. Herbal methionine (HerboMethione) as a source of active methionine is effective in its optimum activity for proper protein accretion and other functions in poultry birds so that they can reach better growth and performance potential. Thus, Supplementation of Herbomethione has a positive influence on the performance of broiler chickens, when compared to synthetic DL-methionine (Halder and Roy, 2007). Also, Khose *et al.* (2018) concluded that supplementation of herbal choline at 0.500 kg/

ton of feed was more beneficial in terms of improving the bird's performance.

Feed enzyme technology – Next generation

The 'next-generation' enzymes will be close to being 'perfect', with : A high specific activity per unit of protein, good thermostability during feed processing, high activity in the typical pH range of the animal gut, resistance to gastric proteases and good stability under ambient temperatures. Preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilisation in poultry diets. For increasing enzyme thermo stability, put a "coating" around the enzyme. Coated enzyme preparations can easily withstand the pelleting process, however they are more complicated to produce and the preparation quality is very dependant of the coating quality. Coated enzyme preparations are known to show slower release kinetics within the animal gut, by contrast the naturally thermostable enzymes start working immediately after ingesting.

Supplementation of exogenous enzyme in the diets is considered modifications to overcome the adverse effects of Non-Starch Polysaccharide (NSPs). Enzymes break down the NSPs, reduce intestinal viscosity and subsequently get better nutrients digestibility by improving gut performance (Amerah, 2015). Technologies are also being evolved to maintain enzyme activities in their dry enzyme products in order to protect them from the heat, moisture and high pressures generated during feed processing and a number of thermostable enzymes, especially phytases, are now commercially available. Supplementation of phytase had positive effect on production performance and egg weight was comparatively higher in phytase supplemented group in free range lohman brown layers (Akyurek and Orhan, 2016).

Another recently used exogenous enzyme is proteases of microbial origin. The economic benefits of these exogenous proteases are achieved through improved digestibility of dietary protein. Multi protease enzyme application supports protein digestibility throughout the gastrointestinal tract by combining three types of proteases, each having different pH optima. So, multienzyme product containing multiple proteases can be used to reduce protein waste in the poultry ration and deliver considerable economic value. A product Kemzyme® map is a dry, a multisubstrate enzyme an example of multienzyme containing NSP (Non-Starch Polysaccharide) enzymes, multi-proteases and multi-amylases from Kemin. KEMZYME protease is a patented multi-protease solution containing acid, neutral and alkaline proteases. The product has an innovative thermostable and enteric coating for protection during feed milling and targeted release in the gastrointestinal tract.

Importance of early nutrition

Early nutrition means providing the required nutrients to the birds either during the period when the embryo is developing or immediately after hatch until birds attain a fully matured digestive system. The delayed intake of water and nutrients to chicks could lead to a diminishing of their overall growth performance with adverse effects on breast meat. The most extreme consequence of delayed feeding is increased mortality (Willemsen *et al.*, 2010). Early feeding strategies have been suggested and developed to diminish or possibly reverse the negative effects of delayed feeding. These strategies range from in ovo feeding to specially designed post-hatch diets.

The administration of digestible nutrients into the amnion of embryos can bring an improvement in bird quality, increased glycogen reserves, fast development of the total digestive tract superior skeletal health, better muscle growth rate, higher body weight gain, improved feed conversion and enhanced immune function. For example, probiotics supplementation in early life prevent pathogenic infections, amino acids (L-arginine, L-lysine, L-histidine, threonine) are beneficial in growth performance, vitamin C and E boost immunity, carbohydrates increase glycogen stores and creatine supplement promotes muscle growth (Peebles, 2018). Future early nutrition would be feeding complex symbiotic that would replace feed additives and supplements in the post-hatch feed and is more beneficial to the overall poultry industry (Jha *et al.*, 2019). Zhu *et al.* (2019) concluded that in ovo feeding of 3 mg vitamin C at day 15 could improve the antioxidant activity to some extent and immune function in plasma.

Nutrigenomics

The introduction of genomics approach in nutritional sciences lead to the scientific area called 'nutrigenomics' (Asmare and Negewo, 2019).The emergence of nutrigenomics has helped to develop feeds that can be matched to genotypes of animals for a better productivity and health (Ghormade *et al.*, 2011). Nutrigenomics will relate optimal diet to choose from many and different nutritional availability. To evaluate the interaction between diets and genes, DNA microarray techniques and quantitative real-time Polymerase Chain Reaction (PCR) and DNA sequencing can be applied (Morozova and Marra, 2008). There is limitation in nutrigenomic information how to effectively analyze and correlate genes and nutrition conversion.

Gut microbiota

Gut integrity is as important as good microbiota balance. Intestinal integrity for commercial poultry can be defined as the maintenance of intestinal health to enable the expression of the full genetic potential for growth and yield, and to fully utilise the dietary nutrients. Normal biota plays a significantly major role in maintaining gut structure, strengthening the gut mucosal barrier and protein metabolism of the gut. In situations where the profiles are shifted by various pathogenic biota (e.g. clostridium and coliforms), there is significant inflammation and damage to the mucosal layer and the barrier function. Coccidiosis is a major cause of poor gut integrity, and an effective anti-coccidial programme must be in place for maintaining good healthy conditions. Raw material quality is another contributing factor. Thus, raw materials that irritate the gut must be closely monitored (Ravindran and Abdollahi, 2017). However, the development of molecular biotechnology has offered new tools to study the composition, diversity, predicted function and interaction of gut microbiota in different sections of the gastro intestinal tract. 16S rRNA based next generation sequencing is a powerful tool to investigate the biological and ecological roles of the gastro intestinal microbiota in chicken (Shang *et al.*, 2018).

Skewing of sex

In general, gender of animal offspring is quite important to livestock producers. In poultry, egg produced farmers would choose hens because only hens will produce eggs. Poultry sperm cannot be manipulated to preselect the sex of offspring (Saleh and Iriyanti, 2010). The method of providing basic element like sodium, potassium, calcium and phosphorous in diet is quite economical, safe and can be done at home (Vahidi and Sheikhha, 2007). But, Saleh and Iriyanti (2010) concluded that different amount of ions in the diet of hens could not have a significant effect on the percent fertility, hatchability and sex ratio of chicks.

CONCLUSIONS

NIRS is important rapid test to predict nutrient composition of diet. Production prediction models may remain a valuable tool for formulation of practical diet solutions. Supplement of exogenous enzymes is beneficial to poultry industries and environment too. Alternative raw material may be used to increase efficiency of bird with low cost for economic benefits. Natural growth promoter must be used as alternative to antibiotics in diets. Supplementation of bacteriophage is a safe alternative to antibiotics in poultry. For increase in productivity and to obtain highly feed efficient birds, the nutritional demand of embryos must be taken into consideration. Feed and good husbandry practices must be provided to the exploit full genetic potential of birds.

FUTURE PROSPECTS

Future nutritional research should focus on issues related to identify barriers for effective digestion and for utilization of nutrients. Poultry nutritionist must combine their expertise with those specialised in other biological sciences to improve production efficiency with reduced issues of food safety, environment and bird welfare. Efforts need to be focus on more pre and perinatal nutrition.

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Kumari *et al.*

Blood Metabolites of Murrah Buffalo Heifer on Supplementation of Different Sources of Rumen Bypass Proteins

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ABSTRACT

Eighteen Murrah buffalo heifers, at BRC, LUVAS, Hissar distributed into three treatments to evaluate the effect of supplementation of different sources of bypass proteins on blood biochemical profile. Heifers in all three group fed similar basal diets, in treatment T2 10% of crude protein requirement was replaced with commercially available soya bypass protein and in treatment T3 10% of crude protein requirement was replaced with natural bypass protein i.e fish meal. Blood samples were collected with or without anti-coagulant for biochemical and serum mineral analysis respectively, at the start of experiment and thereafter at monthly intervals for a period of 120 days. Feeding of bypass protein result in significant increase ($P<0.05$) in concentration of plasma glucose, total protein, globulin, and calcium but concentration of other biochemical parameters, serum mineral values and activity of enzymes showed no significant difference among the treatments.

Key words: Buffalo Heifer, Bypass Protein, Biochemical Parameters, Enzymes Activity, Feeding Trials

INTRODUCTION

India has the privilege of having best breeds of buffaloes, of which Murrah is most popular because of their superiority on commercial grounds. Fast and early growth of heifers for heard replacement requires feeding of quality protein ration. Among the available feeds soya bean meal is considered a promising protein source but have high degradability in rumen, result in excessive loss of nitrogen and in hepatic overload of ammonia (Alves *et al.,* 2004). To increase the efficiency of using dietary protein, protected or bypass nutrients are suggested (Garg, 1998). Fishmeal is a high protein and naturally less degradable in the rumen hence can be considered as natural bypass proteins or nutrients (Mercer *et al.*, 1980). Wide spectrums of protein and energy concentrate are being used to manipulate ruminant diets in order to enhance their production through efficient nutrient utilization without, compromising the economics. While recommending the feeds and fodder for livestock, it is essential to assess the biochemical profile after feeding.

Most of the studies on bypass nutrients have been done milk production and composition but, little work have been reported on heifers. Present experiment was carried out with the aim to evaluate the biochemical status of Murrah buffalo heifers, on feeding commercially available (bypass soya protein) and naturally available by-pass protein (fish meal).

MATERIALS AND METHODS

Eighteen Murrah heifers distributed into three treatment groups at Buffalo Research Center (BRC), LUVAS, Hisar. Heifers were offered a diet to meet their protein and energy requirement for growth as per ICAR standards (Ranjhan, 1998) in control group T1 for a period of 120 days. Animals in treatment T2 fed similar basal diet as control but 10% of crude protein requirement in concentrate mixture was replaced with commercially available soya bypass protein (CP % DMB-46 and RUP %- 70.0 min) and in treatment T3 fed similar basal diet but 10% of crude protein requirement in concentrate mixture was replaced with fish meal a naturally bypass protein.

The composition of the experimental diet of different treatment groups and proximate chemical composition is presented in table 1 and 2.

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Table 1. Chemical composition of feed ingredient (% on DM basis)

Ingredients	DM	CP	CF	EE	Ash	OM	NFE
Wheat straw	95.0	2.85	35.6	1.02	12.9	87.0	47.5
Green maize	23.0	7.71	28.3	3.11	9.11	90.8	51.7
Maize fodder	88.0	9.13	2.52	3.44	2.83	97.1	70.1
Ground nut cake (GNC)	93.4	40.2	9.43	9.05	8.90	91.1	25.8
Soybean meal	89.4	46.0	4.67	2.98	7.34	92.6	28.4
Fish meal	89.9	45.8	1.81	11.4	27.0	72.9	13.9
Wheat bran	88.6	14.0	7.99	4.30	93.6	6.36	59.7

Table 2. Ingredient and chemical of composition of different concentrate mixtures

Ingredient (kg/100kg)	T_1	T_2	T_3	Particulars	T_1	T_2	T_3
Maize	40	40	40	Dry matter	93.0	94.0	93.0
Ground nut cake	20	20	20	Crude protein	23.2	23.4	23.2
Soya bean meal	20	15	15	Ether extract	4.97	4.50	4.21
Bypass protein	-	5	-	Crude fiber	6.29	6.46	6.99
Fish meal	-	-	5	Organic matter	89.8	92.1	89.1
Wheat bran	17	17	17	Ash	10.1	7.81	10.8
Mineral mixture	2	2	2	Nitrogen free extract	55.2	57.7	54.6
Salt	1	1	1	Neutral detergent fiber	57.3	57.2	47.9
Total	100	100	100	Acid detergent fiber	13.8	13.4	12.7

Blood samples (10ml) were collected at into a set of sterile plastic tubes with and without anti-coagulant for biochemical and mineral analysis respectively at beginning and thereafter at monthly interval upto four month of experiment period. Biochemical parameters like plasma glucose (mg/dl), total protein (g/dl), albumin (g/dl), globulin (g/dl), A/G ratio, blood urea nitrogen (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl),glutamic pyruvic transaminase (GPT) (IU/l), glutamic-oxaloacetic transaminase (GOT) (IU/l), alkaline phosphatase (ALP) (U/L) creatine kinase (CK) (U/L), plasma calcium (g/dl) and phosphorus (g/dl) were carried outby kits, procured from M/S Transasia Biomedical Limited using fully automated Random Access Clinical Chemistry Analyzer (EM 200TMErba Mannheim -Germany). Digested serum used for estimation of minerals (copper, zinc, and manganese) by atomic absorption spectrometer (Model Pinaacle 900T, S/N PTAS13050201 of PerkinElmer Company). Data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) using Completely Randomized Design (CRD). All the data were subjected to ANOVA using IBM SPSS (2007) version 16.

RESULTS AND DISCUSSION

Plasma glucose concentration was significantly ($P<0.05$) higher in treatment groups as compared to control T1 from 60 onwards. Similarly Chen *et al.*, (2002) reported that feeding heat treated soya bean meal diet increased glucose concentration significantly ($P<0.05$) in Holstein cows. This improved plasma glucose concentration on feeding of rumen protected protein could be due to reduced energy loss which is generally caused by excessive microbial protein turnover rate. Plasma cholesterol and triglyceride concentration showed no significant difference among the treatments and control groups (Table 3) throughout experiment, but slightly higher values were obtained in the treated groups T2 and T3. Similarly Dosky *et al.* (2012) and Chen et al. (2002) reported that feeding heat treated soya bean meal showed no any significant difference in the serum cholesterol and triglyceride concentration.

Table 3. Effect of supplementation of rumen protected protein on blood biochemical parameters

Particular	Treatments	Days of experiment				
		0	30	60	90	120
Glucose (mg/dl)	T_1	60.7±2.20	60.8±1.30	61.4^{a}±1.76	66.1^{a}±0.69	69.6^{a}±3.98
	T_2	63.1±0.95	66.8±2.66	68.4^{b}±1.89	75.1^{b}±3.08	81.9^{b}±0.62
	T_3	61.9±3.50	67.2±2.40	68.7^{b}±1.11	74.3^{b}±2.79	83.6^{b}±1.49
Cholesterol (mg/dl)	T_1	38.1±2.65	50.1±3.95	68.1 ±4.54	73.6 ±2.38	80.5 ±4.02
	T_2	37.5±3.72	52.5±4.99	74.6 ±4.06	75.0 ±3.11	84.3 ±4.36
	T_3	39.3±2.65	55.0±4.31	72.3 ±4.62	77.6 ±4.17	89.3 ±4.46
Triglyceride (mg/dl)	T_1	7.16 ± 0.47	8.00 ± 0.44	14.3 ±0.76	14.7± 0.90	15.5±1.09
	T_2	7.33 ± 0.84	9.33 ± 0.21	16.1 ±1.19	17.5 ±0.72	18.1±1.08
	T_3	7.33 ± 0.42	8.33 ± 0.76	16.5 ±0.43	15.6± 1.05	18.3 ±0.56
Total protein (g/dl)	T_1	4.54 ± 0.21	5.14^{a} ±0.18	6.13^{a} ± 0.14	6.95^{a} ± 0.34	7.33^{a} ± 0.19
	T_2	4.52 ± 0.26	6.14^{b} ±0.25	6.67^{b} ± 0.13	8.05^{b} ± 0.22	8.29^{b} ± 0.16
	T_3	4.41 ± 0.08	5.56^{ab}±0.28	6.33^{ab}±0.07	7.37^{ab}±0.19	7.81^{ab}±0.16
Albumin (g/dl)	T_1	1.89 ± 0.16	2.08 ± 0.14	2.26 ± 0.15	3.25 ± 0.10	3.39 ± 0.04
	T_2	1.98 ± 0.24	2.40 ± 0.18	2.07 ± 0.16	3.38 ± 0.11	3.51 ± 0.09
	T_3	1.99 ± 0.17	2.40 ± 0.10	2.19 ± 0.17	3.48 ± 0.10	3.57 ± 0.13
Globulin (g/dl)	T_1	2.65 ± 0.16	3.06 ± 0.15	3.87^{a}±0.08	3.69^{a}±0.34	3.95^{a}±0.17
	T_2	2.54 ± 0.10	3.7 ± 0.20	4.61^{b} ±0.11	4.67^{b} ±0.24	4.78^{b} ±0.16
	T_3	2.42 ± 0.20	3.16 ± 0.33	4.14^{ab}±0.23	3.89^{ab}±0.24	4.23^{ab}±0.27
Albumin: Globulin	T_1	0.73 ± 0.08	0.69 ± 0.06	0.59 ± 0.05	0.92 ± 0.09	0.86 ± 0.03
	T_2	0.79 ± 0.09	0.66 ± 0.07	0.45 ± 0.04	0.74 ± 0.06	0.74±0.039
	T_3	0.89 ± 0.16	0.81 ± 0.12	0.55 ±0.069	0.92 ± 0.08	0.87 ± 0.08
Urea (mg/dl)	T_1	12.1±1.24	29.6±2.16	29.9±1.59	36.2±2.17	44.1±1.60
	T_2	11.6±0.98	22.3±2.78	25.7±1.01	35.9±2.88	40.2±1.74
	T_3	12.0±1.74	26.0±2.65	27.4 ±1.57	34.1 ±1.29	39.1 ±1.63

The values in a row with different superscripts differ significantly (P<0.05)

Total protein and globulin concentration improved significantly in group T2 supplemented with commercially protected protein compared to control T1, although both the parameters were comparable T3 fed with natural protected protein (Table 3). Albumin concentration and albumin, globulin ratio of treatment groups was similar to control group during trial period (Table 3). The concentration of total protein, albumin, globulin, albumin globulin ratio and urea was within the normal range as has been reported by Abd Ellah *et al.* (2014) in buffalo heifer. Present findings are in accordance with Kumar and Walli (1994) who observed higher plasma protein level in crossbred calves fed with formaldehyde treated ground nut cake. In contrast to this, Bhagwat and Srivastava (1993) reported that feeding bypass protein to ruminants did not show any significant effects on total protein, albumin and globulin concentration. It was observed that group supplemented with protected protein had lower plasma urea concentration during experimental periods as compared to control, though it was not

statistically significant. Present results are in accordance White et al. (1992) where they reported that replacing SBM with fish meal (FM) in steer and lamb diets, did not change the plasma urea concentration. Serum urea nitrogen, which is an indicator of protein status in animals, varies with the protein quality and quantity. Lower level of blood urea nitrogen was observed in growing buffalo heifers fed with protected protein as compared to the control indicating better utilization of protein that may be due to the reduced protein degradation in rumen. However, Bhagwat and Srivastava (1993) found significant variation in the urea concentration due to feeding treated soybean cake compared to untreated in crossed bred calves.

Activity of enzymes like GPT, GOT, ALP and CK found to be similar in all the experimental groups of heifers throughout the experimental periods (Table 4). The plasma activity of all enzymes recorded in the present experiment was within the normal range as has been reported by Abd Ellah *et al.* (2014) in buffalo heifers. The level of SGPT and SGOT was found to be statistically non-significant among the various groups of experimental animals and indicated that the animals remained healthy condition without any cellular dysfunction; otherwise it would affect the cellular synthesis of protein and growth performance (Lehninger *et al.*, 1993). Present finding are in agreement with the results of Bhagwat and Srivastava (1993) who observed non significant variation on serum GOT/ GPT enzymes due to feeding formaldehyde and tannic-acid treated soybean cake compared untreated cake in crossed bred calves.

Table 4. Effect of supplementation of rumen protected protein on plasma enzyme activity

Particular	Treatments	Days of experiment				
		0	30	60	90	120
SGPT (IU/l)	T_1	22.6±1.42	29.4±2.74	32.1±2.50	22.1±2.09	25.5±2.16
	T_2	23.7±2.68	26.2±1.90	27.2±0.80	22.6±1.43	28.9±2.22
	T_3	23.6±1.36	23.9±1.36	25.4±2.59	22.6 ±1.39	24.5±0.78
SGOT (IU/l)	T_1	69.0±4.54	62.2±2.96	60.8 ±3.36	66.6 ±4.77	59.9 ±4.38
	T_2	69.0±4.97	64.6±5.13	60.8±4.64	64.6±2.80	65.2±2.91
	T_3	71.4±3.85	67.6±4.66	69.0±5.71	63.8±6.53	68.4±5.90
AP (U/L)	T_1	115.0±5.62	132.0±18.86	102.8±6.82	125.3 ± 9.27	127.5±8.58
	T_2	120.6±17.80	126.1 ± 8.51	105.1±6.80	119.5 ± 7.97	125.0±7.56
	T_3	113.8±9.04	110.8±6.02	104.6±3.24	138.8±20.17	119.1±8.95
CK(U/l)	T_1	226.6±25.65	194.4±21.20	220.7±13.16	219.0±20.58	184.7±23.81
	T_2	198.1±24.35	187.6±12.67	228.6±10.21	224.9±21.61	226.1±21.41
	T_3	222.1±16.36	206.5±15.72	231.8 ± 4.98	211.3±19.51	205.7±16.03

Values are means ±standard errors

Plasma calcium level was significantly higher (P=0.05) (Table 5) in group supplemented with fish meal compared to control T1 from day 90 onwards, though value of T3 and T2 was comparable. Present experiment suggested that animals of groups T2 and T3 supplemented with protected protein fulfills the required dietary calcium for depositing in bone tissue and maintaining normal blood calcium level. Concentration of phosphorous was higher in treated groups compared to control T1, though statistically non-significant (Table 5). Paengkoum *et al.*, (2004) also observed that phosphorous intake, absorption and retention tended to be increased as a consequence of rumen undegradable protein supplementation in Saanen goats fed oil palm fronds. The concentration of other mineral like copper, zinc and manganese were similar among the groups throughout the experiment (Table 5). Mondal and Chopra (2008) also did not find any change in the blood mineral (Mg, P, K, Cu and Zn) concentration due to feeding different levels of rumen degradable and undegradable protein ratio to crossbred cows.

Table 5. Effect of supplementation of rumen protected protein on mineral concentrations

Particular	Treatments	Days of experiment				
		0	30	60	90	120
Calcium (g/dl)	T_1	5.18 ± 0.24	6.84 ± 0.39	8.50 ± 0.64	8.40^{a} ± 0.28	9.35^{a}± 0.20
	T_2	5.22 ± 0.53	7.35 ± 0.35	8.78 ± 0.82	8.63^{ab}±0.09	10.1^{ab}±0.37
	T_3	4.88 ± 0.37	7.40 ± 0.31	9.25 ± 0.91	9.42^{b} ± 0.47	10.5^{b}±0.27
Phosphorus (g/dl)	T_1	3.35 ± 0.17	4.25 ± 0.24	4.18 ± 0.24	5.78 ± 0.24	6.62 ± 0.13
	T_2	3.23 ± 0.30	4.74 ± 0.16	4.69 ± 0.33	6.12 ± 0.41	6.92 ± 0.26
	T_3	3.19 ± 0.21	4.67 ± 0.21	4.57 ± 0.28	6.22 ± 0.40	7.01 ± 0.24
Copper (mg/l)	T_1	0.50 ± 0.01	0.54± 0.02	0.64 ± 0.02	0.70 ± 0.02	0.88 ± 0.03
	T_2	0.52 ± 0.02	0.60 ± 0.01	0.64 ± 0.02	0.66 ± 0.01	0.90 ± 0.02
	T_3	0.54 ± 0.01	0.64 ± 0.02	0.66 ± 0.01	0.80 ± 0.01	0.84 ± 0.02
Zinc (mg/l)	T_1	0.55 ± 0.06	0.65 ± 0.07	0.70 ± 0.06	0.80 ± 0.05	0.95 ± 0.08
	T_2	0.53 ± 0.08	0.70 ± 0.05	0.78 ± 0.05	0.90 ± 0.04	0.98 ± 0.09
	T_3	0.55 ± 0.09	0.62 ± 0.08	0.74 ± 0.08	0.85 ± 0.06	0.90 ± 0.05
Manganese (mg/l)	T_1	0.02 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.03
	T_2	0.03 ± 0.03	0.04 ± 0.03	0.04 ± 0.00	0.05 ± 0.02	0.06 ± 0.01
	T_3	0.02 ± 0.03	0.04 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.07 ± 0.01

The values in a row with different superscripts differ significantly (P=0.05)

CONCLUSION

From the present investigation we can conclude that supplementation of commercial and natural bypass protein in concentrate mixture result in increased concentration of plasma glucose, total protein, globulin, and calcium concentration in serum. Feeding bypass protein through either source did not result any adverse effect on the system

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Ashraf *et al.*

Mineral Status of Dairy Animals in Pulwama District of Jammu and Kashmir

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ABSTRACT

A total of 206 samples of blood were collected from crossbred jersey dairy cows of Pulwama district of Kashmir Division to assess the blood plasma mineral status with respect to the stage of lactation and milk yield parity. Macro-minerals (Ca, P, Mg, Na and K) were estimated by standard methods using commercial kits, whereas microminerals (Cu, Zn, Fe, Co and Mn) were estimated by atomic absorption spectroscopy (AAS). Significantly (P=0.05) lower blood Ca, P, Mg and Na levels was reported in animals yielding milk upto above 10 kg followed 5-10 kg yielders and upto 5 kg and as compared to dry animals. Plasma P was recorded below critical levels in dairy animals of district Pulwama. There was no significant difference in mean plasma K levels of dairy animals among milk yielding and dry animals. Significant (P=0.05) lower plasma Ca, P, Mg and Na levels were found in animals in early and mid-lactation stages of dairy animals compared to the late lactation stage with a non significant difference in plasma calcium levels in early and mid-lactation. Plasma micromineral (Cu, Zn, Fe and Co) concentration was reported significantly (P=0.05) lower in high yielding animals (5-10kg and above 10kg) as compared to 0-5 kg yielders with the highest in dry animals. However, no significant difference was observed in plasma Mn level in dairy animals of district Pulwama in different production levels (above 10 kg, 5-10 kg, 5 kg yielders and dry) and at the stage of lactation (early, mid and late). The present study therefore indicates the need for the formulation of specific mineral mixtures for different stages of lactation.

Key words: Mineral Status, Parity, Pulwama, dairy, production, Stage of lactation

INTRODUCTION

Balanced nutrition with adequate mineral level is required for proper health, production, reproduction and immune defense of the animals. Minerals are forgotten nutrients in animal diets and their physiological role is often underestimated, even though the role of minerals in animal health is well established (Alonso, 2012). Minerals are necessary to maintain proper growth, reproduction, immune status and production performance in dairy animals. The deficiency of minerals has a negative impact on the health, growth production and reproduction of animals as a slight deficiency of trace minerals results in a considerable reduction in performance and production. On the other hand, in recent years, there are higher demands for appropriate nutrition due to genetic selection for higher milk yield. Animals in higher production stages have more requirements and diets. Body stores cannot cope with higher demands, resulting in metabolic disorders like milk fever and diseases like mastitis, lameness, and prolapses with reproductive disorders like infertility, dystocia and retention of the placenta. As such animals need to be offered mineral supplements to meet the requirements for growth, milk production and reproduce optimally.

In the Kashmir region of Jammu and Kashmir, animals are usually stall-fed on the fodder available from apple orchards and cultivated land during summer and rice straw as the primary roughage source during lean months with a small amount of grain by-products (mainly brans and oil cakes) without any mineral/vitamin supplements except for common salt (Ganai *et al.*, 2004). Hence mineral requirements of dairy cows depend on available feed and fodder resources, which are critically low in P, Mg, Mn, Cu, Na, K and Zn (Dhobi, 2018). Thus the quantity of minerals present in forages fed to dairy

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cows is insufficient for their optimum performance. Dhobi (2018) reported that dairy animals of district Budgam of J&K were deficient in almost all macro (Ca, P, Mg, K, Na) and micro minerals (Zn, Cu, Co, Mn, Fe). Reshi *et al.* (2016) reported that cattle and sheep of district Ganderbal of Jammu and Kashmir are deficient in macro minerals (Ca, P). To know the mineral profile of dairy cattle and recommend mineral supplementation for optimum production of animals, the present study of mineral profiling was carried taking district Pulwama as a sample study area. Since plasma is one of the important indicators of mineral status of an animal, therefore the study was planned to find the plasma mineral profile to have first hand information of mineral status of cattle in the study area. The district has a rich cattle population (1,12,664) with the highest milk (4.88 lakhs per day) production in Jammu and Kashmir state (19th livestock census), as one can estimate its richness of production by nicknames given to it like Anand of Kashmir/ Dudha-kul of Kashmir.

MATERIALS AND METHODS

The study was carried in the Pulwama district of the Kashmir Division. The topography of the district is mixed with both mountainous and plain areas. Paddy is the main crop in plains, whereas wheat and maize are the main crops of hilly areas of Pulwama district. Pulses and vegetables are also grown in different pockets of the district. The district has four veterinary blocks and from each of the four blocks, one animal per 300 animals was selected, which amounted to 206 samples as per the formula given by Thursfield (2007) as shown in table1. Sixteen Villages from the blocks were selected at random.

$$N = \frac{Z2\ Pexp\ (1-Pexp)}{d2}$$

where Pexp = expected prevalence = 35%
d = desired precision = 0.05

Animals were grouped according to milk yield into dry, upto 5 kg, 5-10 kg, 10 kg and above yielders and stage of lactation into Early (0-3 Months), Mid (3-6 months) and Late (until drying).

A total of 206 samples of blood were collected with 28 blood samples from dry, 65 from crossbred jersey animals yielding upto 5 kg, 67 from 5-10 kg yielders and 46 samples from animals yielding milk above 10 kg. 86 were in the early stage from sampled animals, 56 in mid and 36 in the late lactation stage. About 10-15 ml of blood was collected from the jugular vein in heparinized test tubes. These samples were maintained at a temperature of 2-8°C. The test tubes containing collected blood samples were then centrifuged at 3000 rpm for 30 minutes and plasma collected was stored in 5 ml vials at -20°C till further analysis.

Table 1. Number of samples collected population-wise and the places of collection

S.no	Block name	Total cattle population	Breedable cattle population	Samples taken
1.	Pulwama	43,023	23,662	78
2.	Pampore	15,062	8,284	28
3.	Kakapora	21,790	11,984	40
4.	Tral	32,789	18,034	60
	TOTAL	**1,12,664**	**61964**	**206**

Population as per 19th livestock census.

The plasma samples meant for mineral estimation were digested by the modified method of Idera *et al.* (2015). 1 ml of previously-stored plasma samples were taken in digestion tubes and 5 ml of triple acid was added. Triple acid was prepared by mixing sulphuric acid, perchloric acid and nitric acid in the ratio of 1:2:7 (Idera *et al.*, 2015). The samples were digested at 100°C on automatic Kjeltech digestion apparatus (Kelplus-KES 20 LVA DLS AL by Pelican) for 2 hours and at 200°C for 1 hour. The samples were checked for clarity and allowed to cool down. The digested samples were diluted with double distilled water to make a final volume of 10 ml. The digested samples were filtered through Whatman filter paper No. 40 before testing in AAS.

Macrominerals (Ca, P, Mg, Na and K) were estimated by standard methods using commercial kits from Accurex Biomedical Pvt. Ltd., India and microminerals (Cu, Zn, Fe, Co and Mn) by atomic absorption spectroscopy (AAS). Standard solutions for microminerals were prepared using the available reagent grade salts. Each standard solution was run one by one for these minerals and their absorbance was recorded. Standard curves for each mineral were constructed by plotting the absorbance of standards against their concentrations. The concentrations of respective minerals in the samples were calculated from their respective standard curves. Micro mineral (Cu, Zn, Fe, Co and Mn) were estimated by atomic absorption spectroscopy (4141 ECIL) (AAS). Standard solutions for micro minerals were prepared using the available reagent grade salts. Each standard solution was run one by one for these minerals and their absorbance was recorded. Standard curves for each mineral were constructed by plotting the absorbance of standards against their concentrations. The concentrations of respective minerals in the samples were calculated from their respective standard curves.

STATISTICAL ANALYSIS

The data was presented as Mean±SE and the significance of mean differences were tested by using one-way ANOVA followed by Duncan's New Multiple Range Test (DNMRT) using the Statistical Package for the Social Sciences, Base 20.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

Macromineral status in dairy cattle

The macro-mineral status in dairy cattleof district Pulwama is presented in table 2 below. The mean plasma calcium levels of dairy animals of Pulwama district were found above critical level with significantly (P=0.05) lower blood calcium level report in the animals yielding milk upto above 10 kg followed by 5-10 kg yielders and upto 5 kg and as compared to dry (10.06 mg/dl) animals of district Pulwama. The lower calcium levels in lactating animals may be due to drainage of calcium in milk and less bioavailability of calcium from the diet. Inactivation of bone calcium resorption may also contribute to low calcium levels (Kamiya *et al.,* 2005). Sarker *et al.* (2015) and Adedibu *et al.* (2013) found significantly higher calcium in dry (pregnant cows) than in the lactating cows. Hypocalcemia is inevitable after calving and lactation commencement due toa tremendous challenge to the cow's ability to maintain calcium homeostasis (Carlos *et al.,* 2013).

Significant (P=0.05) lower plasma Ca levels were found in animals in early (8.09 mg/dl) and mid (8.31 mg/dl) lactation stages of dairy animals as compared to the late lactation stage (8.91 mg/dl) with a non significant difference in plasma calcium levels in early and mid-lactation. The lower plasma Ca levels in lactating cows, especially in the early lactation stage, might be observed due to the high demand of absorbed Ca per liter of milk produced. As per NRC (2001) lactating cattle needs 1.37 gm of Ca/kg of milk produced in addition to maintenance requirement. Lower Ca level in lactating dairy cows could be attributed to negative calcium balance or excessive secretion of Ca through milk (Asif *et al.,* 1996). This might also be due to dietary imbalances of Ca, higher requirements due to the production phase of cows, and dietary interaction with other minerals (Maynard *et al.,* 1979).

Table 2. Plasma macro-mineral level in dairy cattle of district Pulwama with respect to milk yield and stage of lactation

Parameter	Critical levels	Macro-mineral level (mg/dl)				
		Ca (<0.30)	P (<0.25)	Mg (<0.20)	K (<0.8)	Na (<0.06)
Milk Yield	Dry (n=28)	9.85±0.79^{a}	5.84±0.20^{a}	2.23±0.07^{a}	8.34±0.30	148.78±0.21^{a}
	Upto 5kg (n=65)	9.20±0.82^{b}	5.17±0.13^{b}	2.18±0.52^{a}	8.32±0.11	133.32±0.11^{b}
	5 to 10 kg (n=67)	8.24±0.12^{c}	5.09±0.12^{b}	2.10±0.06^{a}	8.20±0.13	132.21±0.13^{c}
	Above 10 kg (n=46)	8.07±0.12^{c}	5.10±0.23^{b}	1.86±0.09^{b}	8.31±0.08	129.12±0.12^{d}
Stage of Lactation	Early (n=86)	8.09±0.12^{b}	4.58±0.15^{b}	1.90±0.11^{b}	7.85±0.18^{b}	129.04±0.13^{b}
	Mid (n=56)	8.31±0.13^{b}	5.10±0.26^{a}	2.14±0.07^{a}	8.27±0.12^{b}	132.27±0.14^{a}
	Late (n=36)	8.91±0.11^{a}	5.09±0.13^{a}	2.13±0.06^{a}	8.84±0.14^{a}	132.67±0.17^{a}

Values are expressed in Mean±S.E, values with different superscripts (a,b,c) vary significantly along the group(p=0.05)

Plasma Phosphorus was recorded below critical levels of 5.6-6.5 mg/dl plasma in lactating animals compared to dry animals. Significantly lower (P=0.05) plasma phosphorus levels were found in lactating animals than dry animals, with phosphorus levels significantly lower (P=0.05) in early than mid and late lactation stages in dairy animals of district Pulwama. Dhobi (2018) also reported the highest level in high producing dairy animals compared to dry animals. Similarly, Hagawane *et al.* (2007) found the serum Phosphorus concentration in the early stage of lactation was significantly (P<0.05) lowered than mid, late lactation and dry pregnant buffaloes.

In the present study, mean plasma Magnesium levels of dairy animals were found significantly (P=0.05) lower in animals yielding milk above 10 kg as compared to 5-10 kg yielders, upto 5 kg yielders and dry animals of district Pulwama, with a non significant difference between 5-10 kg yielders, upto 5 kg yielders and dry animals.The results agree with the findings reported in a number of studies (Kupczynski *et al.*, 2002; Sharma *et al.*, 2006).

The mean plasma Sodium and Potassium levels of dairy animals of Pulwama district were found above critical level with no significant difference in mean plasma potassium values among milk yielding and dry animals. However, significantly (P=0.05) higher plasma sodium values were reported in dry animals, followed by animals yielding milk upto 5 kg, 5-10 kg and above 10 kg. Lower plasma Sodium and Potassium level were reported in animals in the early stage of lactation compared to animals in mid and late lactation. The low value of Sodium and Potassium may be due to lesser availability from the diet and increased electrolyte loss with increased production. These results are in accordance with Dobi (2018) also reported that in dairy animals of Budgam district, Sodium and Potassium were highest in dry animals and were lowest in animals yielding 10 kg and above milk. Contrary to this, Sheikh (2018) in his study of mineral estimation of district Anantnag of Jammu and Kashmir, reported that dairy animals were deficient in Potassium.

Micro mineral status in dairy cattle

The micro-mineral status in dairy cattleof district Pulwama is presented in table 3. No significant difference was observed in plasma manganese level in dairy animals of district Pulwama in different production levels (above 10 kg, 5-10 kg, 5 kg yielders and dry) and at the stage of lactation (early, mid and late). Similarly, significantly (P=0.05) lower manganese was observed in Dhobi (2018) in animals yielding 10 kg and above as compared to animals yielding up to 5 kg milk. Dhobi (2018) also reported manganese lower than the normal range of 3.2-5.4 µmol/L (Constable *et al.*, 2017) in high-yielding animals.

Table 3. Plasma Micro-mineral level in dairy cattle of district Pulwama with respect to milk yield and stage of lactation

Parameter		Micro-mineral level (µmol/l)				
		Mn	Cu	Zn	Fe	Co
Milk Yield	Dry (n=28)	3.77±0.17	13.87±0.25^{a}	14.13±0.24^{a}	27.41±0.55^{a}	0.36±0.02^{a}
	Upto 5kg (n=65)	3.64±0.11	12.39±0.19^{b}	13.69±0.18^{a}	26.22±0.28^{b}	0.33±0.01^{b}
	5 to 10 kg (n=67)	3.53±0.08	10.57±0.12^{c}	12.30±0.17^{b}	24.95±0.27^{c}	0.30±0.03^{c}
	Above 10 kg (n=46)	3.43±0.08	10.27±0.12^{c}	11.49±0.20^{c}	24.38±0.31^{c}	0.28±0.01^{c}
Stage of Lactation	Early (n=86)	3.56±0.09	10.30±0.15^{b}	11.42±0.23^{c}	24.36±0.36^{b}	0.28±0.01^{b}
	Mid (n=56)	3.58±0.08	10.66±0.14^{b}	12.16±0.21^{b}	24.97±0.30^{b}	0.30±0.12^{a}
	Late (n=36)	3.52±0.10	11.86±0.18^{a}	13.26±0.17^{a}	25.88±0.24^{a}	0.33±0.01^{a}

Values are expressed in Mean±S.E, values with different superscripts (a,b,c) vary significantly along the group(p=0.05)

Copper plasma levels were reported significantly (P=0.05) lower in animals yielding milk above 10 kg and 5-10 kg yielders as compared to 0-5 kg yielders with highest in dry animals of district Pulwama. No significant (P=0.05) difference was found in plasma Copper levels in early and mid-lactation stages, although plasma Copper levels was significantly (P<0.05) higher in mid-lactation as compared to the early stage of lactation. Low levels of copper may be due to the drainage of copper in

milk. Also, more production means more stress, which makes more utilization of copper in the antioxidant role. Dhobi (2018) reported the highest copper in animals yielding up to 5 kg milk and lowest in animals yielding 10 kg and above.

The mean plasma Zinc levels of dairy animals of Pulwama district were found significantly (P<0.05) lower in animals yielding above 10 kg and 5-10 kg yielders milk followed by upto 5 kg and dry animals of district Pulwama. The serum level of Zn was critically low in animals in first parity with a higher production level. Literature data also shows that different dietary Zn amounts cause high variability in blood Zn values, with the effect of diet composition on blood zinc values being undefined, except for the fact that high dietary Ca concentrations reduce blood Zn concentrations in cattle (Mullis *et al.,* 2003; Spears, 2003; Kellogg *et al.,*1998).

The mean plasma Iron levels of dairy animals of Pulwama district were found well above the critical level and were significantly (P=0.05) lower in animals yielding milk above 10 kg as compared to animals producing 5-10 kg milk followed by upto 5 kg and dry animals of district Pulwama with no statistical significance between above 10 kg yielders and 5-10 kg yielders. Significantly (P=0.05) lower serum Fe values were reported in animals in the early and mid-stage of lactation compared to animals in late stage of lactation. This might be due to the abundance of Fe in feed and fodder grown on naturally Fe rich soil. It was in agreement with the earlier findings of many workers Dutta *et al.* (2000) and Mandal *et al.* (2004). It has been reported that iron deficiency is not a relevant problem in grazing livestock as Iron is fairly abundant in all grass species consumed by the animal species and it only occurs when there is some loss of blood (Shisia *et al.*, 2013).

Plasma cobalt concentration in most animals in the surveyed zone of district Pulwama was above the critical level. Significantly (P=0.05), lower Cobalt was reported in animals yielding milk above 10 kg, 5-10 kg yielders as compared to upto 5 kg yielders and dry animals with no statistical significance between animals yielding upto 5-10 kg and above 10 kg milk. Mean plasma cobalt levels of dairy cattle of district Pulwama were significantly (P=0.05) lower in the early and mid-stage of lactation thanthe late stage of lactation. Dey *et al.* (1997) has reported cobalt level in buffalo blood grazing around a fertilizer factory ranging from 0.007-0.034 ppm. Baruah and Baruah (2000) have reported serum cobalt levels in healthy Jersey heifer ranging from 0.05-0.07 ppm in different seasons. A similar finding was also reported by Hussain (2006), Das (2007), Sharma *et al.* (2009) and Turkar (2010) in the Vidharba region of Maharastra, Tripura, Uttar Pradesh and Madhya Pradesh, respectively.

CONCLUSION

The plasma macro and micro mineral status of dairy cattle revealed higher degrees of deficiency in early stages of lactation compared to mid and late lactation. Significantly (P=0.05) lower plasma macro and micro mineral levels were found at higher production levels (Above 10 kg and 5-10kg yielders) than in dry animals. Plasma serum level of all macro and micro minerals except phosphorus in macro minerals and zinc in micro minerals were above critical level. The ration offered was found to be deficient in copper (93.77mg/day), iron (233.70mg/day), zinc (613.14mg/day), cobalt (0.39mg/day) and manganese (93.36mg/day). The present study indicates the need for the formulation of specific mineral mixtures for different stages of lactation. based on the deficiency of macro and microminerals in common feed and fodders sources of the district and their bioavailability in roughages and concentrates using commonly available and economical mineral salts as source of these deficit minerals. The dairy cattle of the district should be fed balanced ration taken proper care of the dry matter requirement. Area specific mineral mixtures in powder form or as urea molasses mineral blocks should be introduced in the district for animal feeding to ameliorate the deficiencies in livestock to enhance productivity.

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Effect of Traditional Curd Supplementation and Probiotics on Growth Performance and Frequency of Diarrohea in Cattle Calves Under Farmer's Field

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ABSTRACT

A trial was conducted to evaluate the effect of supplementary feeding of curd as probiotics on growth performance and frequency of diarrohea in pre- ruminant cattle calves under farmer's field in semi- arid condition of Gujarat, India. Twenty four, pre-ruminant female Gir cattle calves with average age of fifteen days were selected randomly and distributed equally in to four groups, six each in a completely randomized design i.e. T1- basal diet (control), T2 - basal diet + Saccharomyces cerevisiae @ 5 g per calf/ day, T3 - basal diet + Lactobacillus acidophilus @ 1 g (2 x 1010 cfu/ g) per calf/ day and T4 - basal diet + Traditional curd @ 50 ml per calf/ day, respectively for a period of 90 days. The results of the study revealed that the average daily gains and final body weight of calves was significantly ($P<0.01$) higher in curd and probiotics groups as compared to the control group. The average daily weight gains were statistically higher in curd group (410±14.92g) followed by Lactobacillus acidophilus (407±15.12g), Saccharomyces cerevisiae (393±14.86g) group as compared to control group (301±10.74g). There were significant difference in the faecal score between the treatment group and the control after two weeks of the experiment; subsequently, faecal score became constant in the treatment groups and never exceeded the normal value. However, curd and Lactobacillus acidophilus was found to be more effective to control/ reduce the incidence of diarrhea after one weeks of application as compared to Saccharomyces cerevisiae. The incremental Benefit: Cost Ratio was found to be 5.27, 10.55 and 21.77 in group T2, T3 and T4, respectively which appears to be very encouraging. Based on these observations, it may be inferred that the traditionally fermented curd, can be effectively used as a probiotic supplement and has a desirable effect in terms of higher growth rate and checking diarrhea in pre ruminant calves.

Key words: Body weight, Calves, Curd, Diarrhea, Pre-ruminant, Probiotics

INTRODUCTION

Dairy production from centuries has been an integral component of farming system and a primary source of livelihood for farming community. Probiotics are defined as live microbial feed supplements that improve the health of livestock, or in other words, organisms or substances that contribute to intestinal microbial balance referred as probiotics (Parker *et al.*, 1974). Beneficial bacterial concentrates, i.e., probiotics used in feed, have been reported to enhance growth rate and metabolic activities by stimulating digestion and immunity and also to act as prophylactic and therapeutic medium (Rolef, 2000). Microbial probiotics are culture of viable microorganisms and beneficial to host when consumed in appropriate quantities. Although probiotics are present in different form in the market either in freeze dried capsules or spray dried foods but if they provided in live form such as chass (Buttermilk), lassi (sweetened yoghurt), fermented milk dahi (curd) they give more beneficial effects. Dahi (curd), chass (Buttermilk), Lassi (sweetened yoghurt) are some of the traditionally fermented dairy products being used in every household of the Indian subcontinent. Fermented milks offer tremendous potential for improving nutrition, sooth intestinal disorders, improving immune function, optimize gut ecology and promoting overall health (Kore, 2012). In the Ayurveda dahi has been recommended for treatment of diarrhea and other acute/ chronic gastrointestinal disorders from time immemorial. Curds, a traditional

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dish in India, are fermented milk product produced by microbial fermentation by predominantly Lactobacillus species. Apart from well known palatability, nutritious and therapeutic value of curd, it also has a viable bacterial count of 106-107 cfu/ ml which includes lactobacilli, yeast and mould (Sakore, 2007). Traditionally prepared fermented milk dahi contains promising lactic acid bacteria known their probiotic potential with beneficial heath effects on consumers (Harun ur Rashid, 2007). Curd is a natural, cheaper and easily available probiotic supplement could serve as a nutritional intervention for augmenting growth of calves. Resource poor rural farmer cannot procure commercial probiotic from market but if advised them to feed traditionally fermented dahi (curd) as probiotics they would accept it easily due to viability at their home. Therefore, the present experiment was conducted to evaluate the effect of supplementary feeding of traditionally fermented curd as a source of natural probiotic on the growth of cow calves.

MATERIALS AND METHODS

An on-farm trial was conducted under the banner of ICAR- KVK- Panchmahal, Gujarat, India, to assess the effect of supplementary feeding of curd as probiotics on growth performance and frequency of diarrhea in pre- ruminant cattle calves under farmer's field conditions. Twenty four, pre-ruminant female Gir calves with average age of fifteen days were selected randomly and distributed equally in to four groups, six each in a completely randomized design i.e. T1- basal diet (control), T2 - basal diet + Saccharomyces cerevisiae @ 5 g per calf/ day, T3 - basal diet + Lactobacillus acidophilus @ 1 g (2 x 1010 cfu/ g) per calf/ day and T4 - basal diet + Traditional curd @ 50 ml per calf/ day, respectively for a period of 90 days. The commercial probiotics were procured from market. The curd containing lactobacillus species (106-107 cfu/ ml) was prepared fresh daily by incubating the cow's milk with house hold curd culture 1-2 percent for 10-12 h at 35- 37 Co. The experimental group calves were offered curds at the rate of 50 ml/ day throughout the 90 days of the experiment, while the control group did not receive any such supplement. Around 7.00 AM daily, the powder containing probiotics was mixed in milk and curds were offered by drenching the calves with a syringe, taking due care to avoid aspiration. The calves were fed as per the details given in table 1.

Table 1. Feeding schedule of cattle calves during on farm trial

Age of calves (Weeks)	Milk (part of BW)	Concentrate mixture (g)	Mixed green fodder (lucerne & hybrid napier)
2-4	1/10	-	*ad lib*
5-6	1/15	-	*ad lib*
7-8	1/20	400	*ad lib*
9-15	Quantity sufficient to mix the supplement	800	*ad lib*

The basal diet fed to experimental calves consisted of concentrate mixture and mixed green grasses (lucerne and hybrid napier). The proximate composition of feedstuffs used during the on farm trial given in table 2. Fresh and clean drinking water was provided ad libitum throughout the experiment.

Table 2. Proximate composition of feedstuffs used during the on farm trial (% on DM basis)

Particular	Concentrate mixture	Hybrid napier green grass	Lucerne green grass
DM	90.4±1.04	16.2±1.23	22.1±1.31
OM	89.8±2.11	90.6±1.56	85.4±1.82
CP	19.9±1.20	9.12±0.80	18.1±0.64
CF	8.6±1.04	32.1±2.1	19.9±1.67
EE	3.0±1.21	1.34±0.60	2.74±0.49
NFE	58.1±2.10	44.0±2.1	44.6±2.06
Total Ash	10.1±0.91	9.32±0.40	14.5±0.29

All the calves were dewormed with fenbendazole (5 mg/ kg body weight) one week prior to start of the experiment and were maintained under uniform managerial conditions. Deworming was repeated after 60 days of experimental feeding. All the calves of the control and experimental groups were housed in a well-ventilated shed during night time and were left to move outdoors during day time. The body weight and body measurements such as body length, height at withers, heart girth and paunch girth were recorded initially and subsequently at fortnightly intervals before feeding and watering. Fecal scoring for estimation of fecal fluidity was conducted daily in the morning (8.00 AM) according to the procedure of Larson et al. (1977). Fecal scores based on a four-point scale were recorded. Scoring was as follows: for fecal fluidity, 1 = normal (firm but not hard, original form is distorted slightly after dropping to floor and settling), 2 = soft (does not hold form, piles but spreads slightly), 3 = runny (spreads readily) and 4 = watery (liquid consistency). A scour day was recorded if fecal fluidity = 3 or 4. The data were analysed using the statistical software program of SPSS (SPSS, Chicago, Ilinois, USA). Treatments were compared with the Tukey test. Difference between groups were considered significant when P values were <0.05.

RESULTS AND DISCUSSION

The study revealed that, the average initial body weight of calves was 26.40±0.81, 25.90±0.96, 26.10 ±0.84 and 26.30±0.93 kg, respectively in T1, T2, T3 and T4 groups. The results of the study revealed that the maximum body weight of calves was attained in T4 (63.20±1.23kg.) followed by T3 (62.70±1.10 kg.), T2 (61.30±1.27 kg) as compared to control (53.50 ±1.16 kg) after 90 days feeding (Table 3).

Table 3. Growth performance (Mean±SE) of pre-ruminant calves in different feeding groups

Parameter	T1	T2	T3	T4
No. of calves	6	6	6	6
Average initial body weight (kg)	26.4±0.81	25.9±0.96	26.1 ±0.84	26.3±0.93
Average final body weight (kg)	53.5 ±1.16 ^{b}	61.3±1.27^{a}	62.7±1.10^{a}	63.2±1.23^{a}
Average total body weight gain (kg)	27.1±0.96^{b}	35.4±1.21^{a}	36.6±1.09^{a}	36.9±1.26^{a}
Average daily weight gain (g)	301±10.74^{b}	393±14.86^{a}	407±15.12^{a}	410±14.92^{a}

Means with different superscripts in a row differ significantly, P<0.05

The final body weight of calves was statistically (P<0.01) higher in curd and probiotics groups as compared to the control group. The average daily weight gains (ADG) were significantly (P<0.01) higher in curd group (410±14.92g) followed by Lactobacillus acidophilus (407±15.12g), Saccharomyces cerevisiae (393±14.86g) group as compared to control group (301±10.74g). The better growth performance might be due to better intestinal microbial balance in case of curd and probiotic groups which might have lead to increased digestion and absorption of nutrients and minerals from the gastrointestinal tract. During preparation of curd, microbial fermentation produces antibacterial compounds, lowering the pH of the intestines and inhibiting the growth of undesirable organisms and helps improve digestion either due to increased retention of nutrients or partial breakdown of indigestible compounds, ultimately results are increase in body growth. The present study was in agreement with the results of Mudgal and Baghel (2010), Abdel-Raheem *et al.* (2012), Ramachandran *et al.* (2014), Sharma *et al.* (2016), Dar *et al.* (2017) and Khadda *et al.* (2020) those reported better performance in curd and probiotic group with improved final body weight, body weight gain than control group.

Mean diarrhoea score of calves supplemented with or without probiotic is shown in Figure 1. Calve diarrhea was evaluated using the faecal score. A faecal score greater or equivalent to 3 was used as an indicator of diarrhea in the study. Diarrhoea incidence was found to be in both the treated calves and the control calves during first week of the experiment and then reduced speedy in curd and probiotics groups. The fortnightly as well as overall highest faecal score was recorded in control group T1 and the least faecal score was recorded in curd group T4 (Table 4).

Table 4. Fecal score of pre- ruminant cattle calves supplemented with or without probiotics

Period (Fortnight)	T1	T2	T3	T4
First	3.42±0.49	2.67±0.29	2.33±0.29	2.11±0.62
Second	3.50±0.31	2.08±0.66	1.51±0.53	1.25±0.35
Third	3.63±0.47	1.43±0.31	1.26±0.26	1.17±0.29
Forth	2.83±0.43	1.20±0.23	1.20±0.29	1.13±0.21
Fifth	2.97±0.67	1.22±0.24	1.22±0.23	1.14±0.20
Over all	3.27±0.20[a]	1.72±0.37[b]	1.50±0.27[b]	1.36±0.24[b]

Means with different superscripts in a row differ significantly, P<0.05

There were significant difference in the faecal score between the treatment group and the control after two weeks of the experiment; subsequently, faecal score became constant in the treatment groups and never exceeded the normal value. However, curd and Lactobacillus acidophilus was found to be more effective to control/ reduce the incidence of diarrhea after one weeks of application as compared to Saccharomyces cerevisiae. The reduced incidence of diarrhea may be as a result of an improved intestinal bacterial flora in calves supplemented with curd and probiotics. Fermented curd is known to have several desirable effects on regulars, such as prevention of pathogen overgrowth by lactic acid bacteria and improve appetite, vitality and has been recommended for indigestion, dysentery or other intestinal disorder. The similar findings were also reported by Khuntia *et al.* (2002), Frizzo *et al.* (2010) and Khadda *et al.* (2020). Furthermore, Gorgulu *et al.* (2003) also reported that calves supplemented with probiotics were superior with respect to diarrhea than the control groups and concluded that probiotics supplementation before weaning could boost calf health and reduce mortality.

Feed economics was calculated on the basis of partial budget analysis i.e. the cost of production was calculated in terms of 1kg additional body weight gain by supplementations of curd and probiotic above the control group. The cost of probiotics was calculated on basis of market rate prevalent during the study period which was purchased respondent. The cost of traditionally fermented curd was counted on the basis of cost of household milk production, because it was made from cow's milk at home. Selling price of calves was estimated Rs. 100/- per kg live weight. The feed economics and cost of production of 1kg additional body weight over the control group in curd and probiotics has been given in table 5.

Table 5. Feed economics of pre-ruminant calves in control and probiotics and curd groups

Parameter	T1	T2	T3	T4
Additional weight gain above control (kg)	-	8.30	9.50	9.80
Additional feed supplement Consumed (g)	-	450	90	4500
Additional Cost (Rs.)	-	157	90	45
Additional Return (Rs.)	-	830	950	980
Effective Gain (Rs.)	-	672	860	935
Incremental Benefit: Cost Ratio	-	5.27	10.5	21.7
Cost of per kg additional body weight gain (Rs.)	-	18.9	9.47	4.59

The group T2 (Saccharomyces cerevisiae), T3 (Lactobacillus acidophilus) and T4 (Curd) gained 8.30, 9.50 and 9.80 kg more weight than control group. The cost for production of 1kg more weight gain in group T2, T3 and T4 than control was recorded Rs. 18.97, 9.47 and 4.59, respectively. The

incremental B:C Ratio was calculated to be 5.27, 10.55 and 21.77 in group T2, T3 and T4, respectively which appears to be very encouraging.

CONCLUSION

Based on the study, it may be inferred that the traditionally fermented curd can be effectively used as a probiotic supplement and has a desirable effect in terms of higher growth rate and checking diarrhea in pre-ruminant calves. This is of special significance to poor dairy under village conditions wherein, diarrhoea is a common problem for neonatal calves and most of the farmers do not have ready to access commercial probiotics and anti diarrhoeal medicines. Under these situations dahi/ curd can be a good substitute as they are not only readily accessible and economical but also gain higher net income from rearing of calves under field condition.

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Effect of Feeding Rumen Protected Choline and Rumen Protected Fat on Dry Matter Intake and Nutrient Digestibility in Periparturient Gir Cows

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ABSTRACT

The study was carried out on 24 advanced pregnant Gir cows to know the effects of supplementing Rumen Protected Choline (RPC) and Rumen Protected Fat (RPF) alone and in combination on dry matter intake (DMI) and digestibility of nutrients. The cows were divided equally into four treatment groups (T1 to T4; n=6 each). Cows in T1 group were fed with basal diet to meet their nutrient requirement as per ICAR (2013) feeding standard. In T2 group, cows received supplementation of RPC @ 45 g/day, in T3 RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with basal diet of T1, starting from 30 days before expected date of calving to 60 days postpartum. DMI (kg/d) increased significantly ($p<0.01$) in T2 group compared to control. However, no significant differences among the treatment groups were observed in terms of DMI kg/per 100 kg BW or DMI g/kg BW0.75.Digestibility of ether extract was observed significantly ($p<0.001$) higher in T2, T3 and T4 group as compared to control, while that of CP, CF and NFE were not affected in any treatment groups, whereas digestibility of DM and OM significantly increased ($p<0.05$) in T2 group as compared to control. It was concluded that supplementation of RPC significantly improved the DMI and digestibility of DM, OM and EE. Digestibility of EE was also improved by RPF supplementation. However, supplementation of RPF alone or in combination did not have any significant ($p>0.05$) effect on DMI and digestibility of DM, OM, CP, CF and NFE.

Key words: Dry matter, Digestibility, Gir cows, Rumen protected choline, Rumen protected fat

INTRODUCTION

Dairy cows face metabolic challenges during the transition period and also in early lactation. The term "transition period" refers to the period from approximately 3 weeks before calving to 3 weeks after calving during which cows prepare themselves first for parturition and then for lactation along with profound change in their metabolic and endocrine status (Grummer, 1995; Drackley, 1999). The dietary energy intake during this period is not sufficient to meet the demand for maintenance and milk production due hormonal changes and reduction in appetite around calving period (Shahsavari *et al.,* 2016). So during this period, dairy cows show a marked decrease in dry matter intake (DMI) and that is related to physical, behavioural, metabolic and hormonal changes around parturition (Grummer, 2008). Hayirli et al. (2002) also reported that the decline in DMI during the last 3 weeks of pregnancy can reach 32%, with 89% of the decline occurring during the final week. At this time, sudden increase in energy demand, by both foetus and lactogenesis put the animal in negative energy balance (NEB) state (Esposito *et al.*, 2014). Dairy cows try to adapt with NEB by mobilizing adipose tissue reserve through lipolysis resulting in production of non-esterified fatty acids (NEFA) which are drained towards liver (Cooke *et al.,* 2007; Drackley *et al.,* 2014). As a result, TAG accumulates in liver and cow is likely to experience

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fatty liver (FL) syndrome (Bobe *et al.*, 2004). The failure of the liver to use the excessive amounts of NEFA as fuel leads to a ketotic state that is associated with increased production of ketone bodies, primarily ß-hydroxybutyrate (ß-HB) and acetoacetate and to a lesser extent acetone (Goff, 2006). About 50–60% of transition dairy cows experience moderate to severe FL and ketosis, and this remains a major challenge for production, health and welfare of dairy cows (Duffield, 2000).

Choline [(CH3)3N+CH2CH2OH], also called as trimethyl ethanolamine, is a multi-functional B-complex vitamin (Jayaprakash *et al.*, 2016) required for the synthesis of the neurotransmitter, acetyl choline which is involved in the metabolism of FA in the liver and serves as a methyl donor (Shahsavari *et al.*, 2016). Choline plays an important role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver (Acharya *et al.*, 2019b). Choline can indirectly affect DMI in transition cows by restricting the detrimental effects of fatty liver syndrome (FLS) and ketosis on general health (Esposito *et al.*, 2014). As dietary choline gets degraded rapidly in the rumen, it must be supplemented in the protected form (Atkins *et al.*, 1988; Elek *et al.*, 2008). Supplementing RPF to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity (Jenkins and Palmquist, 1984; Thakur and Shelke, 2010) and reduces the deleterious effect of NEB during early lactation (Drackley, 1999; Ganjkhanlou *et al.*, 2009).

Hence, the present study was carried out to record the effects of supplementing rumen protected choline and rumen protected fat alone and in combination on dry matter intake and digestibility of nutrients in periparturient Gir cows.

MATERIALS AND METHODS

The study was carried out during the period from August, 2020 to May, 2021 at the Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh in collaboration with Cattle Breeding Farm, Junagadh Agricultural University, Junagadh (India), following ethical approval by the Institutional Animal Ethics Committee, vide No. JAU/JVC/ IAEC/LA/64/2020. Twenty four advanced pregnant Gir cows in their first to third lactation were randomly selected and divided into four equal treatment groups, T1 (control), T2 (RPC), T3 (RPF) and T4 (RPC and RPF) comprising 6 animals in each on the basis of their parity, body weight and previous lactation yield. In T1 group, cows were fed with basal diet of 250 g maize bhardo, 10 kg green sorghum, mature pasture grass hay ad lib. along with compound cattle feed and cotton seed cake to meet their nutrient requirement as per ICAR (2013) feeding standards. In T2, each cow was supplemented with RPC @ 45 g/d, in T3 with RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with basal diet starting from 30 days before expected date of calving to 60 days postpartum. All the cows were maintained in well ventilated hygienic sheds and ad lib. wholesome drinking water was made available 24 hrs. to them. RPC and RPF were purchased from Kemin Industries South Asia Pvt. Ltd. Daily dry matter intake was calculated by recording the daily feed offered and feed left over during the experimental period. The dry matter of different feed ingredients was recorded fortnightly. A digestibility trial of seven days duration was conducted by total collection method during the experiment to see the effect of RPC and RPF supplementation on nutrient digestibility. All the samples of feed offered. Left over and faeces voided were analyzed for proximate composition as per methods of AOAC (2005).

The data on dry matter intake were analyzed by two way analysis of variance (ANOVA) for treatment and period effects and on nutrient digestibility by one way ANOVA for treatment effect (Snedecor and Cochran, 1994). Pair-wise mean differences between groups were compared by Tukey's post-hoc test for significance at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition of feeds and fodders offered to experimental cows was recorded (Table 1) and found to be within normal range (Ranjhan, 2001; Garg *et al.*, 2012; NDDB, 2012).

Table 1. Proximate composition of feed ingredients (% DM basis)

Nutrients	Compound cattle feed	Cotton seed cake	Maize bhardo	Sorghum green	Dry mature pasture grass
DM	90.3	92.5	91.0	24.3	92.1
OM	87.6	94.2	97.4	92.1	91.3
CP	20.4	20.7	10.8	5.58	3.10
EE	3.65	8.43	3.41	2.90	1.16
CF	10.6	33.2	2.97	32.0	39.8
NFE	52.8	31.7	80.2	51.6	47.3
Total Ash	12.4	5.79	2.57	7.87	8.61

Data on fortnightly average DMI (kg/d), DMI kg/100 kg BW and DMI g/kg BW0.75recorded in different groups of experimental Gir cows are presented in Table 2 and depicted in Figure 1. The overall mean DMI for T1, T2, T3 and T4 groups were recorded as 8.13 ± 0.25, 9.28 ± 0.29, 8.88 ± 0.47 and 8.77 ± 0.72 kg/d; 2.26 ± 0.14, 2.28 ± 0.11, 2.08 ± 0.08 and 2.31 ± 0.16 kg/100 kg BW and 98.13± 5.04, 102.43± 4.44, 94.35± 3.36 and 101.75± 6.78 g/kg BW0.75, respectively. DMI (kg/d) was significantly ($p<0.01$) higher when cows were supplemented with RPC, while supplementation of RPF alone or in combination with RPC showed non-significant ($p>0.05$) numerically higher values for DMI as compared to control group. The significant effect of the treatment on DMI was observed from 45th day onwards. The DMI was observed significantly ($p<0.05$) lower on the day of parturition in all groups, which significantly ($p<0.05$) increased during 1st fortnight in T2, 2nd fortnight in T3 and 4th fortnight in T1 and T4 groups.No significant differences among the treatment groups were observed in terms of DMI kg/per 100 kg BW or DMI g/kg BW0.75. Further, although the values of DMI kg/per 100 kg BW and g/kg BW0.75 were lowest on the day of calving followed by prepartum phase as compared to postpartum periods, the differences between periods were non-significant ($p>0.05$) in all the groups.

Hayirli *et al.* (2002) reported 32% decline in DMI during the last 3 weeks of pregnancy, with 89% of the decline occurring during the final week. Increased DMI in RPC supplemented cows suggests early recovery of RPC supplemented animals from various hormonal and physiological mechanisms that decrease DMI during transition period (Grummer, 1995). Choline had also been reported to affect DMI indirectly in transition cows by restricting the detrimental effects of fatty liver syndrome and ketosis on general health (Esposito *et al.*, 2014). Zom *et al.* (2011) reported significantly increased dry matter intake from 14.4 to 16.0 kg/d in HF cows supplemented with RPC @ 60 g/d (15 g of choline chloride) from week 3 before calving to week 6 after calving. Similarly, Soltan *et al.* (2012) reported 8.4 % increase in the DMI due to RPC supplementation @ 30 g/d in the first 12 weeks of lactation in Holstein cows. Increased in the DMI in RPC supplemented cows was also reported by several other workers earlier (Ardalan *et al.*, 2011; Gupta *et al.*, 2019). A meta-analysis of thirteen studies by Grummer (2012) showed that feeding RPC does not affect feed intake before calving, but increases DMI by 0.8 kg/d in early lactation. However, Acharya *et al.* (2019a) and Anonymous (2020) reported no significant effect of supplementation of RPC on DMI in periparturient dairy cows. Similar non-significant findings were also reported by several other workers (Elek *et al.*, 2008; Rahmani *et al.*, 2014; Pineda and Cardoso, 2015) for dietary supplementation of RPC on DMI in early lactating dairy cows.

Table 2: Effect of feeding RPC and RPF on fortnightly average DMI in periparturient Gir cows (Mean ± SE)

Peripartum Days	Dietary treatment groups			
(kg/d)	T1	T2	T3	T4
-30	7.85AB± 0.35	8.16ABC± 0.16	8.17AB± 0.40	7.94AB± 0.50
-15	7.35AB ± 0.11	7.65AB ± 0.12	7.82AB± 0.55	7.85AB 0.61
0	6.56A ± 0.07	6.72A ± 0.50	6.65A ± 0.30	7.15A ± 0.62
15	8.41AB ± 0.71	9.67BCD ± 0.40	9.19AB ± 0.59	9.54AB± 1.33
30	8.89AB± 0.63	11.0D ± 0.53	10.0B ± 1.01	9.35AB± 0.90
45*	8.57aAB± 0.42	11.2bD± 0.71	10.1abB± 0.70	9.56abAB± 0.82
60	9.29 B ± 0.59	10.3CD ± 0.55	10.1B ± 0.67	9.97B ± 0.94
Overall** (kg/100 kg BW)	8.13a ± 0.25	9.28b ± 0.29	8.88ab ± 0.47	8.77ab ± 0.72
-30	1.93± 0.10	1.87± 0.08	1.79± 0.03	1.90± 0.07
-15	1.79± 0.10	1.71± 0.09	1.66± 0.06	1.85± 0.16
0	1.77± 0.11	1.62± 0.15	1.53± 0.09	1.86± 0.20
15	2.38± 0.27	2.35± 0.12	2.18± 0.14	2.50± 0.25
30	2.64± 0.31	2.79± 0.17	2.41± 0.20	2.61± 0.28
45	2.55± 0.17	2.91± 0.24	2.50± 0.22	2.64± 0.18
60	2.74± 0.11	2.73± 0.14	2.47± 0.04	2.79± 0.17
Overall (g/kg BW0.75)	2.26 ± 0.14	2.28 ± 0.11	2.08 ± 0.08	2.31 ± 0.16
-30	86.5± 3.33	85.3± 2.67	82.7± 1.91	85.8± 3.19
-15	80.5± 3.32	78.5± 3.40	77.1± 3.29	84.0± 6.65
0	77.6± 3.63	73.0± 6.36	69.7± 3.78	82.3± 7.94
15	102.9± 10.80	105.7± 4.94	98.7± 5.73	110.3± 11.89
30	112.8± 11.65	124.4±7.08	108.6± 9.21	113.4± 11.68
45	108.9± 6.37	129.0± 9.94	112.3± 8.87	115.1± 7.85
60	117.4± 4.99	120.6± 5.93	111.1± 2.87	121.1± 7.84
Overall	98.1± 5.04	102.4± 4.44	94.3± 3.36	101.7± 6.78

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF. *p<0.05, **p<0.01
Means bearing different small superscripts (a, b) within the row and capital superscripts (A, B, C, D) within the column differed significantly (p<0.05)

Non-significant changes in DMI in cows supplemented RPF alone or in combination of RPC were well supported by study conducted by Garg *et al.* (2012) where they did not find any significant effect on daily DMI in lactating crossbred cows receiving 100 g of RPF alone and along with 10 g of RPC per day as compared to control. Likewise, Shankhpal *et al.* (2016); Kumari *et al.* (2018) and Anonymous (2020) also reported no significant effect of supplementing dairy cows with bypass fat during peripartum period on their DMI. Similarly, Sirohi *et al.* (2010) and Singh *et al.* (2014) observed non-significantly higher DMI in lactating crossbred cows receiving RPF @ 300 g/d and 75 g/d, respectively as compared to control. Thus findings of present study were in agreement with above reports and also earlier findings of various researchers in cows

during early and mid-lactation (Strusinka *et al.*, 2006; Theurer *et al.*, 2009; Silvestre *et al.*, 2011).

The mean values of digestibility (%) for dry matter, organic matter, crude protein, crude fiber, ether extract and nitrogen free extract in different groups of experimental Gir cows were recorded (Table 3).The digestibility of DM and OM were significantly ($p<0.05$) higher in T2 group as compared to T1, while value of T3 and T4 groups were at par with T1 and T2 groups. The digestibility of EE was significantly ($p<0.001$) higher in all the treatment groups as compared to control, while differences among T2, T3 T4 were non-significant. No significant effect of any treatment was observed on digestibility of CP, CF and NFE.

Table 3. Effect of feeding RPC and RPF on nutrient digestibility (%) in periparturient Gir cows (Mean ± SE)

Particulars	Dietary treatment groups			
	T1	T2	T3	T4
DM*	57.5± 0.48^{a}	59.4± 0.30^{b}	58.3± 0.27^{ab}	58.2± 0.15^{ab}
OM*	59.8± 0.44^{a}	61.8±0.23^{b}	60.8± 0.32^{ab}	61.4± 0.49^{ab}
CP	56.3± 1.41	57.0±0.46	56.5± 0.60	56.3± 0.75
CF	56.8± 1.06	56.7±0.61	56.1± 0.42	56.8± 0.42
EE***	68.7± 1.71^{a}	76.5±0.72^{b}	77.1± 0.46^{b}	76.1± 0.37^{b}
NFE	55.8± 0.20	56.8±0.37	55.9± 0.06	56.1± 0.19

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF. *$p<0.05$, ***$p<0.001$
Means bearing different superscripts within the row differed significantly ($p<0.05$)

Mohsen *et al.* (2011) observed significantly ($p<0.05$) increased digestibility of DM, OM and EE in RPC supplemented group when compared with control. However, digestibility of CP, CF and NFE was also significantly ($p<0.05$) higher in their study, whereas in present study no significant effect of RPC on digestibility of CP, CF and NFE was observed. Significantly increased digestibility of OM, CP and non-significantly increased digestibility of DM and EE were also reported by Gupta *et al.* (2019) due to RPC supplementation. On the contrary, Sai *et al.* (2016) found non-significant effect of RPC on digestibility of DM, OM, CP, EE, NDF and ADF in growing crossbred calves.

In the present study digestibility of EE was significantly ($p<0.001$) higher in the cows receiving RPF as compared to control, while the digestibility of DM, OM, CP, CF and NFE were not affected by RPF supplementation. Sirohi *et al.* (2010) also reported significant ($p<0.05$) increase in digestibility of EE when lactating crossbred cows were supplemented with 300 g RPF/ day, while the digestibility of DM, OM, CP, CF, NDF and ADF was not affected. Similarly, Shankpal *et al.* (2016) did not observe any significant effect of bypass fat supplementation on digestibility of DM, OM, CF and NFE, however, the digestibility of CP ($p<0.05$) and EE ($p<0.01$) were significantly higher in bypass fat supplemented group, as compared to control. Digestibility of EE was also reported to increase significantly without affecting digestibility of other nutrients, when bypass fat was supplemented in the diet of the dairy animals (Naik, 2013; Rajesh *et al.*, 2014).

CONCLUSION

Dry matter intake (kg/d) increased ($p<0.01$) in cows supplemented with RPC alone as compared to control, while supplementation of RPF alone or its combination with RPC did not have any significant effect. However, DMI in terms of kg/ per 100 kg BW or DMI g/kg BW0.75 was not affected by any supplements either alone or in combination. Supplementation of both RPC and RPF alone or in combination improved ($p<0.001$) the digestibility of EE, but it did not have any significant effect on digestibility of CP, CF and NFE. While digestibility of DM and OM increased ($p<0.05$) with the supplementation of RPC alone.

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Evaluation of Feeding Practices of Lactating Jaffrabadi Buffaloes in Their Home Tract

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ABSTRACT

This study was undertaken to investigate the management practices of Jaffrabadi buffaloes in the Junagadh district to find the type of feeding and management practices followed by the owners. The present study was carried out in five talukas viz - Visavadar, Manavadar, Mendarda, Junagadh and Mangrol of Junagadh districts of Gujarat state. Four villages from each selected taluka were selected randomly for the study purpose. The personal interview technique was used to collect first-hand information from three owners from each selected village of selected talukas of Junagadh district. Basic statistical tools were used to draw the inferences. The survey revealed that most respondents reared the animals on stall feeding and supplementation of green maize, marvel grass, jowar hay, groundnut haulms, and cottonseed cake. Generally, 18.23±1.70 kg available green fodders, 11.72±0.43 kg available dry fodders and 7.29±0.23 kg concentrate were fed daily to lactating Jaffrabadi buffaloes. The average DMI of lactating Jaffrabadi buffaloes is 20.04±0.28 kg/day.The concentrate was fed twice a day at the time of milking, and most respondents (95%) used readymade concentrate mixture. Soaking of concentrate was practiced by the majority (65%) of respondents. The majority of respondents were not practicing mineral supplementation (88.33%), salt supplementation (81.67%) and deworming (51.67%) to their buffaloes.

Key words: Feeding practices and Jaffrabadi buffaloes.

INTRODUCTION

The buffaloes are considered the main dairy animals in India, contributing 51% to the country's total milk production (BAHFS, 2015). India is the leader in buffalo population in the world, with 108.7 million buffalo heads, which is 56.75 per cent of the world's buffalo population (20th livestock census, 2019). Jaffrabadi is considered one of the best dairy buffalo breedsinthe world. They are the heaviest and massive type of riverine buffalo. They are good milkers and thrive well on grazing due to their greater feed conversion efficiency. The native breeding tract of Jaffrabadi buffaloes is the Saurashtra region of Gujarat, viz. Junagadh, Bhavnagar, Amreli, Gir Somnath, Rajkot, Morbi and Jamnagar district, and some parts of Surendranagar district. It is also known as "Bhavnagri", "Gir", or "Jaffari" by the local people (Kathiravan *et al.*, 2007). Feeding is crucial in any livestock development programme, and the optimum expression of an animal's genetic potential depends on adequate nutrition. Nutritional inadequacies often limit animal production in many countries of the world.Over the last two decades, although there has been a considerable increase in milk production and productivity, the productivity per animal is much lowerthan in many developed and developing countries (Singh, 2018) because most Indian farmers do not follow scientific feeding practices to fed their animals.

Limited information was available about the extent ofnutrient availability from different feeds andfodder fed to lactatingJaffrabadi buffaloes in their home tract. Therefore, the present study was undertaken to evaluate the existing feeding practices followed by the owner of lactating Jaffrabadi buffaloes in their home tract and recommend necessary modifications.

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MATERIALS AND METHODS

The present study was carried out in five talukas viz - Visavadar, Manavadar, Mendarda, Junagadh and Mangrol of Junagadh districts of Gujarat state of India. Four villages from each selected taluka were selected randomly for the study purpose. Thus, an entire twenty villages were chosen randomly from each selected taluka.Thus the study was confined to a total of sixty respondents from Junagadh districts. The personal interview technique was used as a tool through which first-hand information was collected.While selecting farmers, due care was taken to ensure that selected farmers were evenly distributed in the village and truly representedthe animal management practices of the village. The recorded parameters from each farmer included the number of livestock, land area, irrigated facilities, fodder and other crops being grown etc. In addition, information regarding the amount and types of feeds and fodder offered to their milch animals, rate of actual daily feed intake, milk yield and fat percent, physiological status of animal etc., were collected with a fair degree of precision on a questionnaire, using standard sampling procedure.

The proximate composition of feeds is usually considered a preliminary index for assessingthe quality of feed. Routinely used feed for lactating Jaffrabadi like concentrate, green roughages and dry roughages were collected from the surveyed area. Samples were dried in the oven, ground (1 mm) and stored in airtight bags. Further, it was analyzed according to the standard guideline given by AOAC (2005).

Data were collected from Jaffrabadi buffalo owners on proforma recording sheets, were processed and analyzed as per Snedecor and Cochran (1994). Statistical tools like frequency distribution, percentage, mean and range were used to draw the inferences. Overall differences between treatment means were considered significant when $P<0.05$. The data have been presented as mean±S.E.

RESULTS AND DISCUSSION

The term herd size indicates the numbers of Jaffrabadi buffalo kept by Jaffrabadi buffalo owners. Respondents are classified according to herd size and are distributed into three different groups and are presented in table 1.

Table 1. Distribution of respondents according to their herd size

Sr. No.	Herd size	Frequency	Percentage
I	Small size (up to 5 buffaloes)	45	75.00
II	Medium size (6 to 10 buffaloes)	08	13.33
III	Large size (above 10 buffaloes)	07	11.67
	Total	60	100

This variable is operationalized as the total number of Jaffrabadi buffaloes maintained by the respondents for milk production. It was found that 75 per cent of respondents had small herd size, 13.33 per cent of respondents had medium herd size, and 11.67 per cent of respondents had large herd size. However, 88.33 per cent of dairy farmers had small and medium herd sizes. These findings are well supported by that of Shinde *et al*. (1998), Mande and Thombre (2009) and Thombre *et al*. (2010, 2012).

Data presented in Table 2 indicate that frequency of feeding four times in a day is being practiced by only 5 per cent, five times in a day by 36.67 per cent, six times in a day by 38.33 per cent of buffalo owners. However, 20 per cent of the Jaffrabadi buffalo owners practised more than six times a day. Concentrates were mostly offered twice a day at the time of milking.

Table 2. Feeding practices followed by Jaffrabadi buffalo owners in their home tract

Feeding practices	Particulars	Frequency	Percentage
Frequency of feeding			
Feed offered/day	Four times	03	05.00
	Five times	22	36.67
	Six times	23	38.33
	More than six times	12	20.00
	Total	60	100
Concentrate mixture used			
Concentrate	Homemade	03	05.00
	Readymade	24	40.00
	Mixture of homemade and readymade	36	55.00
	Total	60	100
Soaking of concentrate mixture			
Soaking	Prefer	39	65.00
	Not prefer	21	35.00
	Total	60	100
Chaffing of fodder			
Chaffing	Prefer	04	06.67
	Not prefer	56	93.33
	Total	60	100
Mineral mixture supplements			
Feeding of mineral mixture	Regular	07	11.67
	Never	53	88.33
	Total	60	100
Salt supplements			
Feeding of salt	Regular	11	18.33
	Never	49	81.67
	Total	60	100
Feeding of special supplements after parturition			
Feeding of special supplements	Regular	57	95.00
	Never	03	05.00
	Total	60	100
Frequency of watering			
Watering	Two times	13	21.67
	Three times	40	66.67
	Four times	05	08.33
	Ad libitum	02	03.33
	Total	60	100
Deworming of lactating animals			
Deworming perform	Regular	12	20.00
	Sometimes	17	28.33
	Never	31	51.67
	Total	60	100

The proximate composition of various feed samples was within the normal range, and the proximate composition value is given in table 3.

Table 3. Proximate composition (%DMB) of feeds and fodder of Junagadh district.

Feeds/Fodder	Dry matter %	Crude protein %	Ether Extract %	Crude fibre %	Nitrogen free extract %	Total Ash %
Green maize	20.4	09.68	02.82	24.7	54.2	08.59
Marvel grass	27.3	09.85	03.19	28.9	47.3	10.7
Green jowar	24.9	11.0	02.76	26.7	50.3	09.06
Gajraj grass	17.0	10.0	07.40	29.6	40.0	12.8
Green bajra	33.6	11.2	02.24	24.8	52.7	08.97
Sugarcane	21.7	07.69	03.14	29.5	52.9	06.74
Groundnut haulms	89.2	11.2	02.90	33.0	41.8	11.0
Jowar hay	89.2	10.2	0.92	26.3	53.0	09.42
Cottonseed cake	90.4	24.8	06.84	30.5	32.4	05.32
Cotton seed	92.7	23.8	18.7	31.0	22.7	03.65
Maize cake	92.3	14.1	10.4	13.7	60.1	01.59
Concentrate mixture	91.3	20.8	07.98	23.1	41.8	06.15

Mostly Jaffrabadi buffalo owners of the district used readymade concentrate feed like cottonseed cake and maize cake manufactured by private cattle feed plants which had on an average 22.2 to 29.0 % crude protein, 5.98% to 24.6 % ether extract, 29.4 to 33.0 % crude fibre, 4.47 to 9.98 % total ash and 22.9 to 36.5 % NFE. The basal roughage, i.e. green maize, marvel grass, jowar hay and groundnut straw, contained an average of 9.12% to 12.9% crude protein, 1.66 and 7.40% ether extract, 23.4 to 35.5% crude fibre, 43.0 to 56.0 nitrogen-free extract, 8.27 to 12.8% total ash. The different types of straws showed normal values of proximate constituents. The data on proximate composition of the feedstuffs are in agreement with the reports of Desai et al. (1984, 1985), Garg *et al.* (1999, 2003), Chavda (2003) and ICAR (2013).

Green fodder intake depends upon the availability of land and irrigation facilities during this season for fodder cultivation. It was found that maize, marvel grass, jowar, gajraj grass, bajro, sugarcane, lucerne, and pasture grassed to the Jaffrabadi buffaloes. Data inTable 4 indicates the average intake of green fodder by lactating Jaffrabadi buffaloes in the respective talukas of the Junagadh district. The quantity of green fodder fed to animals ranged from about 7.8 to 40 kg/day on as such basis to Jaffrabadi buffaloes in the study area. It was found that the Jaffrabadi buffaloes were fed mostly non-leguminous green fodder in the study area. Common dry fodder used for feeding of Jaffrabadi buffaloes during the period were groundnut haulms, stover of maize and jowar, wheat straw and local mature pasture dry grasses. Apart from these, gram / mung / udid bean straw were also used as dry fodder depending on their availability and cost factor in some areas. It was observed that overall, 12.03±0.39 kg/day of dry roughages were fed to the milch animals by farmers of selected talukas of Junagadh district.Data on the average dry fodder intake by the Jaffrabadi buffaloes of respective districts and talukas are presented in table 4.

Table 4. Average green fodder, dry fodder and concentrate intake (kg/day) by lactating Jaffrabadi buffaloes in different talukas of Junagadh district

Sr. No.	Talukas	Average daily feed intake (kg/day)		
		Green fodder	Dry fodder	Concentrate
I	Visavadar	15.6±1.01^{ab} (10 - 22)	7.27±0.56^{a} (8-18)	12.8±0.84^{bc} (5-11.5)
II	Manavadar	17.3±2.59^{ab} (8-35)	11.9±0.81^{ab} (7-18)	7.02±0.56^{a} (4-10)
III	Mendarda	21.6±3.19^{b} (12-38)	7.38±0.58^{a} (6-13.6)	(5-11.3)
IV	Junagadh	19.4±2.97^{b} (7.8 - 40)	11.1±1.02^{ab} (6-18)	7.04±0.41^{a} (6-10)
V	Mangrol	10.5±2.93^{a} (9-12)	14.6±0.48^{c} (12-17)	7.72±0.53^{a} (6.4-11)
	Overall mean Range	18.1±1.27 (7.8-40)	12.0±0.39 (6-18)	7.29±0.23 (4-11.5)

a-c superscript indicates level of significance (P<0.05) within column; Values in parenthesis indicate the range of green fodder, dry fodder and concentrate intake

Special care was taken by the Jaffrabadi buffalo owners for Jaffrabadi buffaloes as far as the feeding of concentrate was concerned. It was observed that almost all the owners fed concentrate to all the Jaffrabadi buffaloes. Most of the owners followed the practice of offering concentrates mixture to the Jaffrabadi buffaloes on the basis of milk production. In addition, Jaffrabadi buffalo owners fed the concentrates like cottonseed cake, cottonseed, maize cake and wheat/maize bran. The practice of feeding compound concentrate mixture was not common in the district. Concentrates were mostly offered twice a day at the time of milking. The death of the young calf also conferred the advantage when buffaloes were accustomed to letting down milk by offering concentrates. Data on the average concentrate mixture intake by the Jaffrabadi buffaloes of respective district and talukas are presented in Table 4. A significant change was not observed in concentrate intake by lactating Jaffrabadi buffaloes.

Common dry fodder, green fodder and concentrate offered by Jaffrabadi buffaloes owners of Junagadh district were consistent with the reports from Akbar *et al.* (1995), Mishra *et al.* (1995), Malik and Nagpaul (1998), Garg *et al.* (2011), Indira and Samuel (2014) and Sherasia *et al.* (2016). The data on dry matter intake of the buffaloes (Table 5) revealed that the overall average DMI of the buffaloes in the Junagadh district was 20.0±0.28 kg/day. Average dry matter intake was at par in Visavadar, Manavadar, Mendarda, Junagadh and Mangrol taluka was 20.2±0.75,20.2±0.52,19.6±0.74, 19.6±0.55 and 20.3±0.55 kg/day, respectively.

Table 5. Average dry matter intake (kg/day) by lactating Jaffrabadi buffaloes in different talukas of Junagadh district

Sr. No.	Taluka	Average dry matter intake (kg/day)
I	Visavadar	20.2±0.75[a] (15.3-23.9)
II	Manavadar	20.2±0.52[a] (16.3-22.7)
III	Mendarda	19.6±0.74[a] (14-22.1)
IV	Junagadh	19.6±0.55[a] (16.8-22.8)
V	Mangrol	20.3±0.55[a] (17.3-22.7)
	Overall mean Range	20.0±0.28 (14-23.9)

a superscript indicates level of significance (P<0.05) within column; Values in parenthesis indicate the range of dry matter intake

In the present study, dry matter intake of lactating Jaffrabadi buffaloes was found higher than the findings of Lal *et al.* (1999), Sing *et al.* (2001), Patange *et al.* (2002) and Sherasiaet al. (2016). It is evident from data table 2 that only 5 per cent of Jaffrabadi buffalo owners used home prepared concentrate, and 40 per cent use readymade concentrate. The majority (55%) of Jaffrabadi buffalo owners used readymade concentrate along with home made concentrate mixture. These findingsare in agreement with the results of Sabapara *et al*. (2010), Kumar and Mishra (2011) and Rangamma *et al.* (2013).

Data presented in table 2 indicate that soaking of concentrate prefers by 65 per cent of the respondent. However, 35 per cent of the Jaffrabadi buffalo owners do not prefer soaking concentrate to feed their buffalo.The findings of the present study were similar to that of Gupta *et al.* (2008) and Rathore *et al.* (2010), while in not agreement with Madke *et al.* (2006) and Kumar and Mishra (2011). Data presented in table 2 indicate that the chaffing of fodder as a daily routine was practiced by only 6.67 per cent of the farmers. However, 93.3 per cent of the Jaffrabadi buffalo owners never chop the fodder before feeding to Jaffrabadi buffaloes. The majority (93.3%) of farmers were unaware of the importance of using chaffed dry and green fodder. It might be due to inadequate knowledge of efficient utilization of feed and fodder. These findings are in agreement with the results of Sabapara *et al.* (2010), Kumar and Mishra (2011) and Rangamma et al. (2013).

Data on feeding of salt, mineral mixture and special supplements are given in table 2. Ration fed to lactating Jaffrabadi buffaloes was supplemented with the mineral mixture by only 11.6 per cent of the respondents. Maximum 88.33 per cent Jaffrabadi buffalo owner never fed mineral mixture to Jaffrabadi buffaloes. The findings of the present study were in agreement with Singh *et al.* (2007), while contrary with Rathore and Kachwaha (2009) and Sabapara *et al.* (2010). Ration fed to Jaffrabadi buffaloes was supplemented with salt by only 18.3 per cent of the respondents.A maximum of 81.6 per cent of Jaffrabadi buffalo owners never fed salt to their lactating Jaffrabadi buffaloes.The present study's findings were agreements with Sabapara *et al.* (2010), while contrast with Singh *et al.* (2007).

Ration fed to Jaffrabadi buffaloes after parturition was supplemented with special supplements like jaggery, ghugari, lapsi, edible oil and vegetable ghee etc. by 95 per cent of the respondents, were only 5 per cent of Jaffrabadi buffalo owner never fed special supplement to Jaffrabadi buffaloes after parturition.These findings

are in agreement with the results of Patel *et al.* (2005), Divekar and Saiyed (2008), Sabapara *et al.* (2010) and Sheikh *et al.* (2011). Regarding the watering frequency, the Jaffrabadi buffalo owners replied that they did not follow a fixed routine of offering water to the Jaffrabadi buffaloes. However, generally, during winters,Jaffrabadi buffaloes offered drinking water two to three times a day, while in summer, the frequency of drinking water was four times a day.These findings agree with the results of Chowdhry *et al.* (2006) Sabapara *et al.* (2010). It is inferred that most Jaffrabadi buffalo owners (51.6%) never dewormed the Jaffrabadi buffaloes. However, 20% of Jaffrabadi buffalo owners regularly deworm, and 28.3 per cent of Jaffrabadi buffalo owners sometimes follow the deworming schedule. Findings of present study was in agreement with the findings of Chowdhry *et al.* (2008), Yadav *et al.* (2009), Kumar *et al.* (2011) and Varaprasad *et al.* (2013) while, did not agree with Pawar *et al.* (2006).

CONCLUSION

Dry matter intake was found to be adequate in the study area. Majority of the respondents reared their animals on stall feeding and fed optimum basal roughages (green maize, marvel grass, jowar hay and groundnut haulms) and concentrate to meet out dry matter requirement of lactating Jaffrabadi buffaloes. Concentrate was fed twice in a day at time of milking. Majority of respondents were not practicing mineral supplementation, salt supplementation and deworming to their buffaloes.

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Praveen *et al.*

Assessment of Milk Yield and Milk Quality on Boron Supplemented Groups in Crossbred Karanfries (Holstein Friesian X Tharparker) Cows During Hot Humid Season

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ABSTRACT

Present study was done on Crossbred Karan Fries (Holstein Friesian X Tharparker)cows (n=18) to evaluate the effect of boron supplementation on milk yield, milk composition and somatic cell count of milk during heat stress, cows were allocated to three treatments, viz, group 1 (n=6), group 2 (n=6) and group 2 (n=6) based on bodyweight, parity and estimated producing ability. All cows received basal feed (roughage and concentrate) based on ICAR feeding standards. Additionally, in group 2 and group 3, boron was supplemented at the rate of 250 ppm and 500 ppm, respectively. The plasma boron was estimated to be significantly higher in boron supplemented groups than control. The milk yield, milk Fat% and milk composition on weight basis (kg) has no significant improvement but other milk composition (viz, milk SNF, milk protein, milk lactose and milk total solids %) did differ significantly between the control and boron supplemented groups. Somatic cell count of boron supplemented groups was significantly low ($p<0.05$) than control, but there was no significant relationship between treatment groups. It is concluded that supplementing boron in diet improved the milk composition (%) and reduced the SCC of milk but no considerable improvement in milk yield was observed.

Key words: Boron, Heat stress, Somatic cell count, Milk yield

INTRODUCTION

The bovine population of India is about 302.79 million according to 20th livestock census (2019) and out of that about 51.36 million animals were crossbred. Exotic cattle with average milk production of about 8.09 kg per day which contribute 28% of total milk produced in India DADH (2021). In dairy cattle when the THI exceeds more than 72, heat stress condition arises, where high producing dairy cattle are more vulnerable to heat stress (Zimbelman *et al.,* 2010). Temperature humidity index (THI) is the combined measure of effect of ambient heat and relative humidity which indicates degree of heat stress experienced by dairy cows (Berman, 2005). Prolonged period of high environmental temperature with high relative humidity reduces milk yield due to reduced dry matter intake (Kadzere *et al.,* 2002) about 0.2 kg per unit increase in THI above 72 (West, 2003). Pragna *et al.* (2017) stated that high temperature and humidity reduces feed intake which leads to reduced reproduction potential and in turn reduces milk yield where high yielding cows are mostly susceptible. Shwartz *et al.* (2009) enacted a significant decrease ($P<0.01$) in Dry matter intake in lactating dairy cows during heat stress condition progressively. Bouraoui *et al.* (2002) observed that with the increase of THI value from 68 to78 % reduces DMI by 9.6 % and also milk yield by 21 %. Aggarwal and Singh (2006) found an increase in THI from 68 to 78 leads to reduced milk production by 21 % and DMI by 9.6 % and also stated that for each unit increase in THI above 69, 0.41 kg milk production per day per cow is decreased. Increased body temperature during heat stress affects the fat synthesis i.e., milk quality is affected. Increase in somatic cell count during heat stress condition of Holstein cows observed (Smith *et al.,* 2013). McDowell *et al.* (1976) showed a decrease of 39.7, 18.9 and 16.9% of milk fat, solids-not-fat, and milk protein percentages respectively with the air temperature from 18 to 30 in lactating Holstein cows.

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Boron is identified as emergency trace mineral and also as an essential mineral in animals (Nielsen, 1994). Boron supplementation increases lambs' cell-mediated and humoral immunity (Bhasker *et al.,* 2017) and also have potential to increase propionate concentration (Bosaglu *et al.,* 2017) and reduce the incidence of ketosis and negative energy balance by reducing NEFA and BHBA Kabu and Civelek (2012). Therefore, the present study was undertaken to determine the effects of supplemental boron on milk yield and milk quality of crossbred Karanfries cows.

MATERIALS AND METHODS

Animal care and conduction of this experiment was approved by the Institutional Animal Ethics Committee (IAEC No. 44-IAEC-19-24) as per the article number 13 of the CPCSEA- rules, laid down by the Government of India.

Present study was conducted at Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal, Haryana, India. NDRI, Karnal is situated at 290 43" N latitude and 760 58"E longitudes at an altitude of 245 meters above the mean Sea level. The minimum ambient temperature falls to near freezing point in winter and maximum goes approximately up to 45^{o}C in summer. The average annual rainfall is close to 700 mm, most of which is received from July to September.

For the present study, 18 pregnant peri-parturient Karan Fries (Holstein Friesian X Tharparker)cows were selected from Livestock Research Centre of NDRI, Karnal and divided into three groups of each six animals on the basis of Expected Production Ability (EPA), parity and body weight (Table 1).It was made sure that the animals used for research were devoid of any anatomical, physiological, or viral diseases.

Table 1. Details of experimental animals (Mean ± S.E)

Groups	Body weight	Parity	EPA
1	447.5 ± 24.39	2.25	3514.50 ± 159.00
2	418.16 ± 22.01	2.00	3524.66 ± 84.21
3	458.66 ± 41.02	2.66	3644.33 ± 384.44

The experimental animals were kept in separate pens during the course of experiment. Experimental animals were maintained as per the standard feeding and management followed at NDRI. All the animals were fed a ration consisting of concentrate mixture and roughage maize. The concentrate mixture consists of maize, oats, wheat bran, GNC, mustard cake, cotton seed cake, gram chunai, rice bran, common salt, mineral mixture and sodium bicarbonate was offered in morning with 20 % crude protein and 70 % total digestible nutrient and the chaffed green fodder (maize) was offered at 10.00 AM. Free access to fresh tap water was made all the times to the animals.

The study was conducted out from month of July to October at the livestock research centre of ICAR-National Dairy Research Institute, Karnal, Haryana (India). Meteorological variables in terms of dry and wet bulb temperatures and relative humidity were recorded throughout the experiment during morning and evening hours and temperature humidity index was calculated by the formula,

THI = 0.72 (Tdb + Twb) + 40.6, Where, Tdb = dry bulb temperature (^{0}C), Twb = wet bulb temperature (^{0}C)

The respective quantities of Boron were weighed accurately based in dry matter intake at the dose of 250 ppm and 500 ppm for two treatment groups and mixed with small quantity of concentrate to be fed to the animals. Boron was supplemented daily as a form of Boric Acid (food grade) 30 days before and 60 days after calving. Digestion of plasma samples was carried out in microwave digester (multiwave 3000, Anton Parr) using polypropylene vessels to avoid possible contamination with boron from borosilicate tubes. One mL of Plasma was added to vessel along with 1 mL of hydrochloric acid (HCl) and 5 mL of nitric acid (HNO3) and digested in closed vessel microwave. After cooling, sample volume was increased to 25 mL using distilled water. Concentration of Boron in the digested plasma

samples was determined via Inductively Coupled Argon Plasma Optical Emission Spectrophotometer (Optima 9000, multitype, Shimadzu, Japan).

Milk samples were collected at weekly intervals in 50 ml sterilized milk sampling bottles from individual animal of each milking for analysis of Somatic Cell Count (SCC) and milk composition. First few strips of milk samples were discarded as it contains higher amount of microbial population and the subsequent stepping from each quarter was collected and pooled to get 50 ml for analysis.

Milk composition viz milk fat (%), milk SNF (%), milk protein (%), milk lactose (%) and total solids was estimated by lactoscan milk analyser. Somatic cell count in milk was estimated by ekomilk scan machine. 10 ml of milk and 5 ml ekoprim solution was added in flask of ekomilk scan machine. After few minutes of adding sample, results were noted. The results were noted down in (x 105 cells/ml).

Statistical analysis was done by one way ANOVA using statistical package for the Social Sciences (SPSS for windows, V21.0; SPSS Inc., Chicago, USA) The data reported as Mean ± SE, and differences were regarded as significant at 5% significant level.

RESULTS AND DISCUSSION

The values of THI at fortnight interval were recorded and given in the table 2.

Table 2. Mean fortnightly temperature humidty index (THI) during the experimental period

Month	Fortnights	THI_{min}	THI_{max}	THI_{avg}
July	I	76.02	83.22	79.62
II	79.14	82.67	80.90	
August	III	80.08	84.15	82.06
IV	79.58	83.37	81.48	
September	V	79.79	85.00	82.40
VI	76.36	82.25	79.30	
October	VII	70.68	80.46	75.57
VIII	66.83	79.09	72.96	

Alhussien and Dang (2017) enacted that THI is the most common indicator to assess heat stress on growth of dairy heifers which is due to combined effect of environmental temperature and relative humidity. THI is common method of analysis for heat stress assessment and value of less than 72 indicate no heat stress, value ranging from 72 to 76 indicate moderate heat stress, 76 to 80 denote severe heat stress and the value more than 82 indicate deadly heat stress (NRC, 1971). The experiment was conducted during July to October and THI calculated during those periods indicated heat stress condition to animals. Kumar *et al.* (2018) stated that the major problem for dairy farmers causing economic loss to animal is heat stress which in most severe form can cause death of animal and also Thatcher *et al*. (2010)observed that when the THI is above 72 and 78, it wasstressful and severe stress in dairy cows. The threshold level of maximum THI for indigenous and crossbred cattle were 75.12 and 73.67 respectively (Choudary, 2017). However, crossbred cattle showed heat stress that affects growth, production and reproduction negatively at THI of 72 and above (Zewdu *et al.*, 2014).

The plasma boron levels of the experimental animals were analysed by digestion followed by plasma optical emission spectrophotometer to evaluate the plasma boron levels in the blood following supplementation. It was found that plasma boron values significantly ($p<0.001$) high in 250 ppm and 500 ppm supplemented groups (Group 2 and 3) as compared (Group 1) to control (only with basal diet) with the Mean ± SE values of control, T1 and T2 groups were 0.57 ± 0.007, 3.21 ± 0.04 and 3.87 ± 0.02 respectively denoted in figure 1.

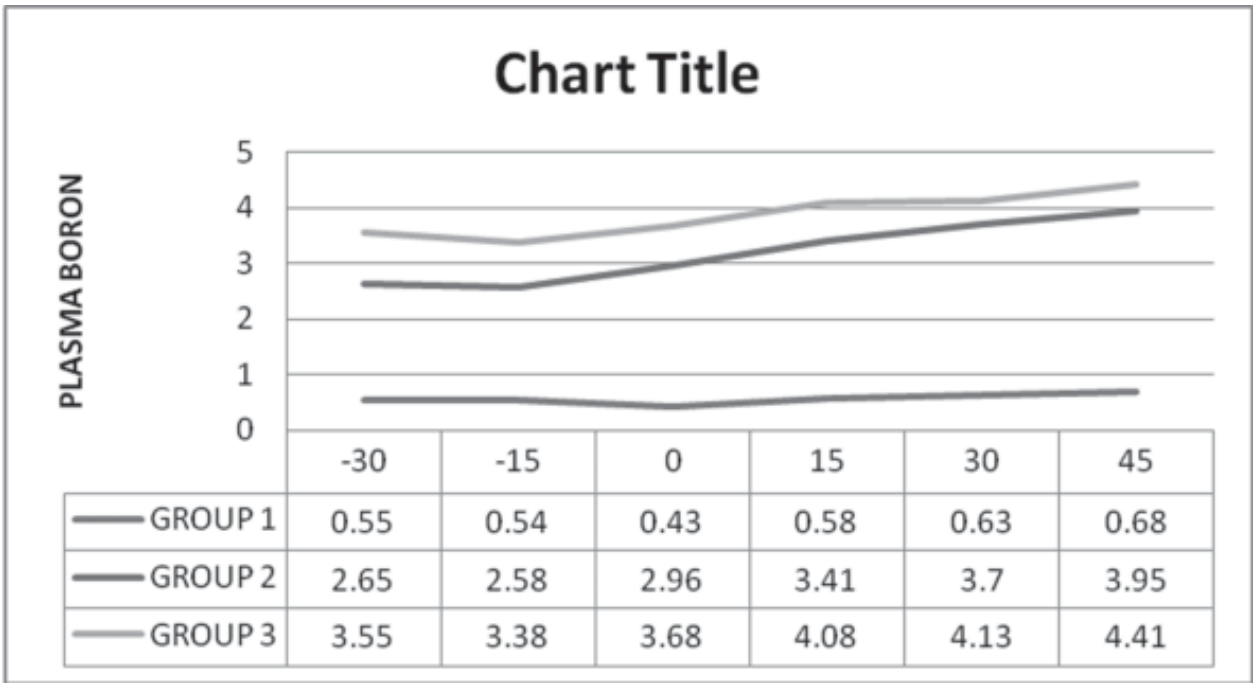

Fig. 1. Average of plasma boron concentration in experimental group of animals

Similar results were reported by Sharma *et al.* (2020), that significantly (P<0.05) higher levels of plasma boron following supplementation of 200 ppm and 400 ppm in the total mixed ration of buffalo diet. Naghii *et al.* (2011) observed that plasma boron concentration increased significantly (P = 0.002) within hours after consumption compared to the placebo. Following oral boron supplementation of 250 and 500 ppm boron in the form of boric acid, there was a greater amount of boron in the blood. seems to be affected the milk composition percentage and milk somatic cell count positively.

Milk yield of animals were recorded daily for each animal among the groups upto 90 days postpartum. The overall Mean (± S.E.) milk yield (Liter/day) were 12.02 ± 0.22, 12.65 ± 0.25 and 12.79 ± 0.25 in Control, T1 (B250) and T2 (B500) groups respectively showing no significant improvement in treatment groups in compared to control (figure 2).

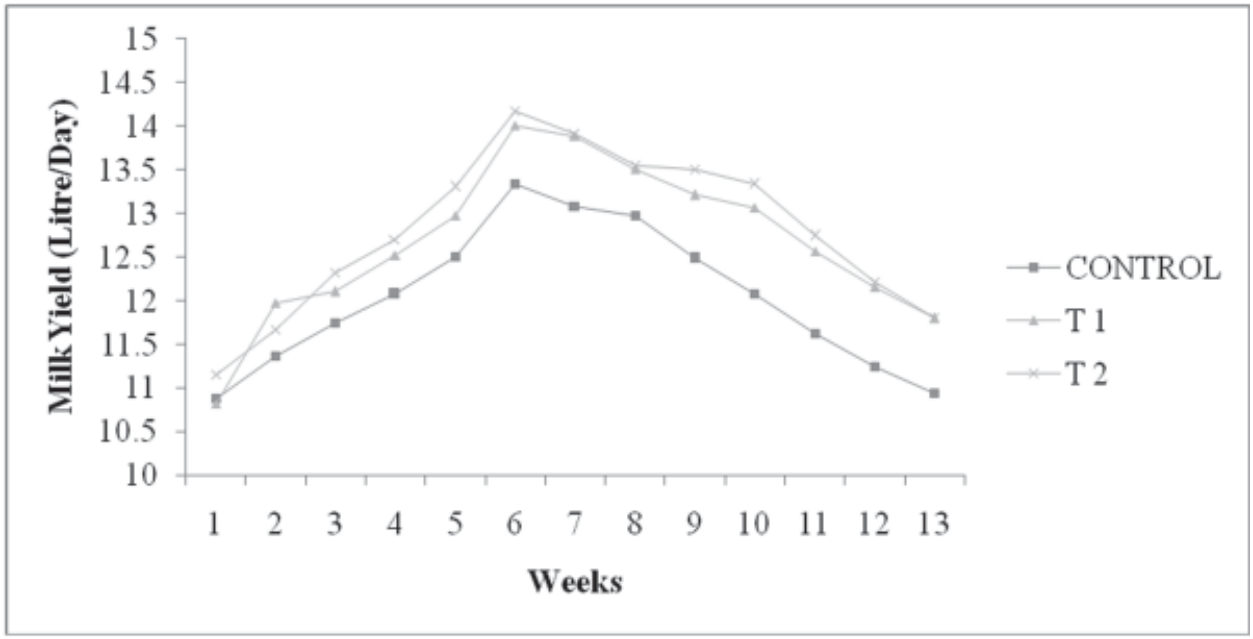

Fig. 2. Average weekly milk yield (litre/day) of experimental groups

The dry period is a critical time for mammary cell turnover in dairy cows. In this period, mammary cells have extensive growth and cell turnover to prepare for the next lactation (Annen *et al.,* 2004). One of the major economic losses resulting from heat stress in dairy industries is a decreased milk yield. Habeeb *et al.* (2014) observed significantly lower DMI at summer compared to winter and the percentage decrease was about 21.96% in purebred and was 24.09% in crossbred calves. Basoglu et al. (2017) also observed no change in DMI in Holstein dairy cows supplemented with boron as Borax in proportion of 567, 1134 and 1071 ppm over control groups. The major reason for reduced milk yield in dairy cows was decreased feed intake during heat stress. OACC, 2014 finding is that a drop of 10 % milk production observed at the temperature and RH of about 27-32 oC and 50-90 % respectively and drop of 25 % observed at 32-38 oC and 50-90 % respectively especially in high producers. Singh et al. (2019) observed that in Karan Fries cows, from THI of 72.2-91.0 there will be reduction in 131.11 ml of milk per unit rise in THI. Smith et al. (2013) observed when THI > 72, Milk yield from 34.8 to 32.9 kg/day in moderate and further to 30.4 kg/day in severe heat stress condition significantly ($P < 0.01$).

In our experiment, peak milk yield for all the groups were found to be at 6th week of lactation and after that reduced reduction in milk yield were noted. Supplementation of Boron has no significant effect on milk yield of different groups of animals which are in line with previous researchers who found no improvement in milk yield by B supplementation (Kabu and Uyarlar 2015; Basoglu *et al.*, 2017). Also, by Owen (1994) that B supplementation at 620 mg boron/100 kg bodyweight per day had no effect on production performance of animals.

Table 3, represents the effect of boron supplementation on milk composition. Results showed that, Milk fat (%) ranges from 3.90 to 4.31 in Control; 3.98 to 4.48 in T-1 and 3.92 to 4.52 in T-2 groups and Milk SNF (%) ranges from 8.13 to 8.50 in Control; 8.48 to 8.86 in T-1 and 8.22 to 8.80 in T-2 groups. Milk protein (%) ranges from 3.10 to 3.23 in Control; 3.28 to 3.38 in T-1 and 3.20 to 3.38 in T-2 groups. Milk Lactose (%) ranges from 4.31 to 4.43 in Control; 4.42 to 4.46 in T-1 and 4.40 to 4.56 in T-2 groups. Total Solids (%) ranges from 12.16 to 12.81 in Control; 12.58 to 13.34 in T-1 and 12.44 to 13.40 in T-2 groups. from the findings shown that there is significant increase in overall average milk SNF %, protein %, lactose % and total solids % but no significance was observed in Milk fat % and milk composition on weight basis of boron supplemented groups.

Table 3. Mean (± S.E.) of milk composition of different treatment groups

Milk parameter	B0 Group 1	B250 Group 2	B500 Group 3	Significance
DMI (kg/100kg bwt)	10.98 ± 0.63	11.23 ± 0.67	11.56 ± 0.62	0.495
4% FCM	12.48 ± 3.65	13.15 ± 2.81	13.36 ± 1.67	0.312
Milk fat %	4.12 ± 0.19	4.18 ± 0.12	4.20 ± 0.15	0.372
Milk SNF %	8.25 ± 0.11^{a}	8.65 ± 0.25^{b}	8.47 ± 0.09^{b}	0.001
Milk protein %	3.18 ± 0.06^{a}	3.32 ± 0.09^{b}	3.30 ± 0.04^{b}	0.000
Milk lactose %	4.37 ± 0.07^{a}	4.49 ± 0.11^{b}	4.50 ± 0.05^{b}	0.011
Total solids %	12.42 ± 0.23^{a}	12.91 ± 0.27^{b}	12.83 ± 0.16^{b}	0.002
Milk fat (kg)	0.50 ± 0.07	0.53 ± 0.12	0.54 ± 0.15	0.309
Milk SNF (kg)	1.00 ± 0.18	1.10 ± 0.10	1.09 ± 0.12	0.054
Milk protein (kg)	0.38 ± 0.03	0.42 ± 0.03	0.42 ± 0.10	0.060
Milk lactose (kg)	0.53 ± 0.03	0.57 ± 0.05	0.57 ± 0.06	0.114
Total solids (kg)	1.52 ± 0.13	1.66 ± 0.15	1.63 ± 0.43	0.154

Our results were similar with Bosaglu *et al.* (2017) observed no change in milk fat % compared to treatmeant groups but contradictory to his findings that non significant in Milk Protein % in periparturient Holstein dairy cows supplemented with boron as Borax in proportion of 567, 1134 and 1701 ppm was over control groups. But there were not traceable research findings to support the results of boron supplementation on SNF and Lactose percentage and weight in kgs. Pragna *et al.* (2017) stated that high temperature and humidity reduces feed intake which leads to reduced reproduction potential and in turn reduces milk yield where high yielding cows are mostly susceptible. Increased body temperature during heat stress affects the fat synthesis i.e., milk quality is affected. Smith *et al.* (2013) observed Milk protein percentage decreased in moderate HS from 3.2 to 3.1% ($P < 0.05$) in Holstein cows. Spiers et al. (2004) stated that the reduced milk fat, milk protein, milk solids and lactose percentage during heat stress is due to elevated core body temperature of animals.

Bosaglu *et al.* (2017) found that boron helps in energy balance and ketogenesis of postpartum milking cows, he also observed that propionate and postpartum lactate concentration was higher than control groups over boron supplemented groups. Intraruminal infusion of propionate increased milk lactose % and reduced milk fat % (Fisher and Eliot, 1966) and milk protein % (Rook and Balch, 1961). High propionate induced improvement in milk composition in aspect of Protein %, lactose % that in turn showed significant improvement of SNF% and total solids %.

Somatic cell count was estimated upto 9th week of lactation. The overall Mean (± S.E.) somatic cell count (x105 cells/mL) were 3.73 ± 0.43, 1.67 ± 0.58 and 1.34 ± 0.34 in control, T1 and T2 groups respectively showing significant ($p<0.01$) decrease in treatment groups in comparison to control (Table 4).

Table 4. weekly Mean (± S.E.) milk somatic cell (SCC) (x 105 cells/mL) of different groups of KF cows

WEEKS Group 1	B0 Group 2	B250 Group 3	B500
1	4.07 ± 0.65	2.32 ± 0.65	2.28 ± 0.78
2	4.01 ± 0.34^{b}	1.81 ± 0.64^{a}	1.55 ± 0.37^{a}
3	4.58 ± 0.73^{b}	2.05 ± 0.86^{a}	1.08 ± 0.19^{a}
4	3.73 ± 0.44^{b}	1.53 ± 0.45^{a}	1.33 ± 0.47^{a}
5	3.44 ± 0.30^{b}	1.78 ± 0.77^{a}	1.30 ± 0.32^{a}
6	4.27 ± 0.65^{b}	2.00 ± 0.87^{a}	1.39 ± 0.43^{a}
7	3.62 ± 0.40^{b}	1.64 ± 0.59^{a}	0.97 ± 0.07^{a}
8	3.12 ± 0.19^{b}	1.14 ± 0.28^{a}	1.28 ± 0.36^{a}
9	2.71 ± 0.20^{b}	0.77 ± 0.07^{a}	0.85 ± 0.08^{a}

^{a,b} Means having different superscripts within a row differ significantly ($p<0.05$)

Milk somatic cell count was reduced significantly by 55.22 % and 64.07 % in 250 ppm and 500 ppm boron supplemented groups, respectively compared to control. Reduced trend of SCC count was observed in all groups of animals from 1st week postpartum upto 9th week postpartum. Somatic cells in the milk are primarily the leucocytes and also the shredded epithelial cells from the lining of the epithelial cells. Paape *et al.* (2002) and Burvenich *et al.* (2007) stated that epithelial cells shedding from the udder and leukocytes as a defence mechanism of infection or injury forms the Milk Somatic Cells. Harmon (1994) stated that milk Somatic cell counts (SCC) is a universal indicator of milk quality and mammary gland health. Smith *et al.* (2013) observed increase in Somatic cell score in milk during heat stress condition of Holstein cows but reduction in SCS from 4.18 to 3.84 during mild to severe heat stress. Alhussien and Dang (2017) found increased significantly ($P<0.05$) SCC during summer months in comparison with Thermo neutral

zone and Winter season and also increased SCC is pronounced in multifarious (above 4thparity) than first lactating animal.

Supplementation of Boron has effectively reduced somatic cell count in 250 ppm and 500 ppm of Boron supplemented groups of animals over control which may be due to immune-modulatory action that is cell mediated and humoral immunity (Bhasker *et al.*, 2017) and antioxidant effect of boron that decreased the somatic cell in milk. We could not compare the results due to unavailability of literature where supplemental Boron has been used in relation to somatic cell count of cattle.

CONCLUSION

During high THI during summer season, the somatic cell count was calculated at weekly interval upto 9 weeks postpartum have found significantly reduced SCC of milk in boron supplemented group than control groups and milk composition percentage but no significant effect on milk yield and milk composition. The reduced somatic cell counts due to immunomodulatory defence mechanism of boron on body which is compromised during heat stress condition due to elevated cortisol level and hormonal imbalances. Boron supplemented groups at the dose of 250 ppm and 500 ppm makes the transition cows adaptable to heat stress by improving the somatic cell count and thus supplementation of boron helps in improving the keeping quality of milk through reducing SCC. These findings will be helpful in developing suitable feeding strategies to combat the harsh environmental climatic condition and its associated stress to improve health and productivity of crossbred animals under the changing climatic scenario.

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Rumen Degradability and I*n Vitro* Fermentation Characteristics of Various Cereal Grains

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ABSTRACT

A study was conducted to evaluate the in vitro gas production (IVGP), degradability and fermentation characteristics of various cereal grains viz., maize, broken rice, jowar, ragi, wheat, barley and bajra by in vitro gas production technique.The in vitro gas production technique conducted indicated significant (P< 0.01) variation for cereal grains with regard to in vitro gas produced (IVGP) volume, in vitro dry matter degradability (IVDMD %), in vitro organic matter degradability (IVOMD %) and in vitro neutral detergent fibre degradability (IVNDFD %). The IVGP and IVOMD were highest for wheat and barley followed by bajra and lowest for maize, broken rice, jowar and ragi. The order of the IVDMD % from highest to lowest was broken rice> wheat>jowar and maize>bajra>barley>ragi. The IVNDFD (%) was highest for wheat and barley followed by bajra then by ragi and lowest in jowar, broken rice and maize. The partitioning factor was higher (P< 0.01) in broken rice, jowar and maize, followed by ragi, then bajra and was lowest in wheat and barley. The microbial biomass production (MBP) (mg/500mg) was lowest (P< 0.01) in maize, broken rice, jowar and ragi, followed by bajra and highest in wheat and barley. The efficiency of microbial biomass production (EMBP) (g/kg DOM) was higher (P< 0.01) and comparable for broken rice, maize and jowar followed by ragi and then bajra and lowest was from barley and wheat. The ME values from in vitro study varied significantly (P< 0.01) and in descending order was wheat >barley >bajra>broken rice >jowar and maize >ragi. The pH was highest (P> 0.01) in maize and jowar and comparable with broken rice and bajra, followed by ragi and lowest was for wheat and barley. The NH3-N concentration from various cereal grains did not vary, but total volatile fatty acid production was significant. The highest (P< 0.01) TVFA production was from wheat, followed by barley then bajra, jowar and lowest in maize, broken rice and ragi.

Key words: Cereal grains, In vitro gas production, Ruminal degradability, Ruminal fermentation

INTRODUCTION

Ruminants obtain most of their energy from cereal grains in order to meet their energy demands and fermentability of these grains largely determines the feeding value for ruminants. The fermentability of cereal grains in rumen has an important effect on the rumen environment due to changes related to ruminal pH, fibre degradability and volatile fatty acid (VFA) production (Sauvant, 1997) and ultimately affecting the intake and milk production (Herrera-Saldana *et al.*, 1990). Cereal grains like maize, rice, wheat, barley, bajra, ragi and jowar are the most important energy sources and among these, maize is the commonly used cereal grain in concentrate mixtures of livestock and poultry due to its high energy content. In our country, there is a huge gap between requirement and availability of maize for livestock and poultry feeding and majority of maize is utilized by the poultry sector (Maize Vision, 2022). Hence there is need to find alternativesfor commonly used maize grain for ruminants. Grains differ in their nutrient composition and their ruminal degradability (Humer and Zebeli, 2017). Optimal rumen environment is essential for improving fermentation endproducts for ruminants use (Wanapat, 2000). Limited information is available on characteristics on nutrient degradation in rumen of commonly available cereal grains in our country. Thus the objective of this study was to determine the degradability of dry matter, organic matter and fibre and fermentation characteristics of 7 available cereal grains using in-vitro gas production technique.

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MATERIALS AND METHODS

Maize, broken rice, ragi, barley, bajra, wheat and jowar grains were procured from local market in Hyderabad. The cereal grains were ground to uniform size by passing through 1mm sieve with Wiley mill, then they were mixed thoroughly to reduce the sampling errors. The cereal grains were analyzed for proximate constituents (AOAC, 2019) and neutral detergent fiber (Van Soest *et. al.,* 1991).

Rumen contents was collected from adult steer immediately after the slaughter and kept in warm water (39°C) until it was carried to laboratory. Then the rumen contents were strained with four layers of muslin cloth and that strained rumen liquor was kept in water bath (39°C)and flushed with carbon dioxide to support the liveability of anaerobic organisms.

The incubations were carried out in 100 ml glass syringes (HaberleLabortechnik, Lonsee-Ettenchieß, Germany) kept in water bath maintained at 39±0.5°C as described by Menkeet al. (1979) and Menke and Steingass (1988). The amount of cereal grains and the volume of incubation medium were 500 mg and 40 ml, respectively.The medium mixture was prepared using double strength rumen buffer (Blummel *et al.,* 1997) and the ratio of medium mixture to rumen liquor was kept at 2:1. The cereal grains were incubated in triplicate and blank was set comprised of rumen fluid-medium mixture alone and was run simultaneously. The gas production was recorded at 0 h, ½ h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 24 h. After removing the syringes at 24 h, the pH was recorded immediately and the contents were transferred to 600 ml capacity spoutless beaker, shaken and allowed to settle. After 15 minutes, 10 ml supernatant was carefully pipetted for ammonia-N (NH3-N) estimation and 5 ml supernatant was stored with saturated mercuric chloride for total volatile fatty acids (TVFA) estimation. Then the syringes were rinsed twice with 25 ml of neutral detergent solution, double strength (Van Soestet al., 1991) and contents were transferred through narrow outlet into the respective beakers and NDF was determined (Blummel *et al.,* 1997).

The in vitro dry matter degradability (IVDMD) (%), organic matter degradability (IVOMD) (%) and neutral detergent fibre degradability (IVNDFD) (%) were determined by

IVDMD% = DM% of the substrate – DM % of the residue

IVOMD % = OM% of substrate incubated on DM basis – OM % of the residue

IVNDFD % = NDF% of substrate incubated on DM basis – NDF % of the residue

The partitioning factor (PF), Microbial biomass protein (MBP) and metabolizable energy (ME) were estimated as follows:

$$\text{Partitioning factor} = \frac{\text{In vitro true DM Digested (mg)}}{\text{Total gas produced (ml)}}$$

MBP (mg) = IVOMD (mg) – (Net gas volume × 2.20)

Where 2.20 is the stoichiometric factor (Blummel and Lebzien, 2001).

In vitro ME (MJ/kg DM) = 2.20 + 0.1356 GP + 0.057 × CPDM/kg (Menke and Steingass, 1988)

Where, GP = Net gas production at 24 h fermentation (ml/0.5 g DM);

CPDM/kg = Crude protein on DM basis × 10

The data was subjected to statistical analysis using software (SPSS, Version 17). One way analysis of variance through generalized linear model was used to analyse all the results. The treatment means were ranked using Duncan's multiple range test with a significance at $P<0.05$ (Duncan, 1955). All the statistical procedures were done as per Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The chemical composition (% DMB) of seven cereal grains is presented in table 1. The chemical composition of cereal grains varied significantly ($P<0.01$) except for the DM. The crude protein (% DMB) was highest ($P<0.01$) in wheat, followed by bajra, then by broken rice, barley and jowar and next by maize, and lowest in ragi. The CP content of maize, broken rice, barley and bajra were lower than the values given by NDDB (8-12,8-10, 11-16 and 12-15 %, respectively), while jowar and wheat are similar to the NDDB values (8-12 and 8-14%, respectively). The cereal grains accordingto ether extract (% DMB) in descending order ($P<0.01$) was bajra> maize> wheat and jowar followed by ragi>

barely andbroken rice. The EE content of maize, jowar, wheat and bajra were higher and barley is lower than the values given by NDDB,while rice is similar. The cereal grains according to crude fibre (% DMB) in descending order (P<0.01) was ragi>jowar = maize = bajra = wheat=barley and broken rice. The total ash(%DMB)was highest (P<0.01) in ragi followed by jowar> then bajra later in wheatand maize followed by barley and lowest in broken rice.The NFE content (% DMB) was highest (P<0.01) in barley and broken rice followed by jowar, maize, ragi later in wheatand lowest was observed in bajra. The NDF (% DMB) was highest(P<0.01)in ragi, followed by barely and decreased linearly in bajra, wheat and maize, then in jowar and lowest in broken rice.

Table 1. Chemical composition (% DMB) of cereal grains

Cereal grain	Dry matter	Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract	Neutral detergent fibre
Maize	89.58	7.44^{c}	5.45^{b}	1.26^{bc}	1.30^{d}	84.54^{b}	12.76^{d}
Broken Rice	89.32	8.75b^{c}	1.53^{e}	0.20^{d}	0.69^{f}	88.83^{a}	6.85^{f}
Jowar	90.67	7.57^{bc}	3.38^{cd}	1.81^{b}	2.02^{b}	85.22^{b}	11.31^{e}
Wheat	90.58	11.03^{a}	3.89^{c}	0.79^{cd}	1.50^{d}	82.78^{bc}	13.68^{d}
Ragi	90.57	5.71^{d}	2.94^{d}	3.78^{a}	3.99^{a}	83.59^{b}	24.61^{a}
Barley	91.94	7.90b^{c}	1.69^{e}	0.36^{d}	1.00^{e}	89.05^{a}	22.43^{b}
Bajra	90.17	9.07^{b}	6.98^{a}	1.07^{c}	1.78^{c}	81.10^{c}	18.02^{c}
SEM	0.635	0.372	0.417	0.258	0.225	0.664	1.318
P Value	0.09	0.001	0.001	0.001	0.001	0.001	0.001

Each value is an average of triplicate analysis.
^{abcdef}Means with different superscripts in a column differ significantly: P<0.01
SEM: Standard Error Mean; P: Probability value

The IVGP volume (ml/500mg) significantly (P<0.01) differed among the cereal grains (Table 2).The IVGP volume was highest for wheat and barley, followed by bajra and lowest from maize, broken rice, jowar and ragi. The IVGP volume among latter 4 cereals was comparable.The gas volume produced is indicating that wheat and barley fermented faster than the other cereal grains investigated (bajra, maize, broken rice, jowar and ragi).The faster fermentation in wheat and barley grains could be due to presence of higher proportion of floury endosperm(Rooney and Pflugfelder, 1986).The present study reports are in accordance with Opatpatanatik *et al.* (1994), who reported higher gas volume with wheat than with maize grain. Amanzougarene *et al.* (2020) reported that barley produced more gas volume than the sorghum grain. But contrary to the present study results, Lanzas *et al.* (2006) reported more gas production for sorghum > corn > wheat and barley. Yoo *et al.* (2020) reported that higher gas production was with rice TMR than with corn TMR which also could be the associative effect of other ingredients present in TMR.

The in vitro degradability of DM, OM, and NDF differed significantly (P<0.01) for various cereals analysed (Table 2).

Table 2. In vitro gas production and in vitronutrient degradability of various cereal grains assessed by in vitro gas production technique

Cereal grain	IVGP (ml/500 mg feed)	IVDMD%	IVOMD%	IVNDFD%	PF
Maize	28.24±0.17^{c}	93.38±0.22^{c}	44.18±0.15^{c}	44.38±2.11^{d}	15.20±0.15^{a}
Broken Rice	28.00±1.21^{c}	96.36±0.25^{a}	44.16±1.08^{c}	45.52±0.36^{d}	15.92±0.69^{a}
Jowar	28.14±2.13^{c}	93.99±0.09^{c}	44.61±1.90^{c}	46.82±0.88^{d}	15.37±1.11^{a}
Wheat	70.40±2.74^{a}	95.05±0.19^{b}	83.41±2.44^{a}	63.72±0.90^{a}	6.34±0.30^{d}
Ragi	31.04±1.23^{c}	87.86±0.36^{f}	47.63±1.10^{c}	50.66^{c}±1.4^{b}	12.94±0.49^{b}
Barley	70.07±0.29^{a}	91.55±0.37^{e}	81.38±0.26^{a}	62.33±0.65^{a}	5.99±0.02^{d}
Bajra	50.07±0.17^{b}	92.53±0.19^{d}	64.63±0.15^{b}	58.56±0.69^{b}	8.46±0.01^{c}
SEM	4.112	0.575	3.710	1.742	0.928
P Value	0.001	0.001	0.001	0.001	0.001

Each value is an average of three observations

^{abcd}Means with different superscripts in a column differ significantly: P<0.01

IVGP-in vitro gas production, IVDMD-in vitro dry matter degradability, IVOMD-in vitro organic matter degradability, IVNDFD-in vitro neutral detergent fibre degradability, PF- Partitioning Factor

SEM: Standard Error Mean; P: Probability value

Broken rice showed highest (P<0.01) IVDMD% i.e., 96.36 followed by wheat (95.05). The IVDMD % for jowar and maize was and, and was comparable, but lower than broken rice and wheat. The IVDMD in other cereal grains, decreased linearly in order (P<0.01) for bajra, barley and ragi. The highest and lowest IVDMD of broken rice and ragi grains was probably due to presence of lowest (%) and highest (%) crude fibre content, respectively observed (Table 1). The higher degradability of wheat was due to presence of floury endosperm. The lower degradability in maize and jowar was probably due to presence of hard seed coat (Rooney and Pflugfelder, 1986). The results obtained in this study are in accordance with values obtained by earlier researchers like Yang *et al.* (2018), who evaluated three total mixed rations containing rice, corn and wheat in each one and reported that rice TMR with higher degradability than the corn and wheat TMRs.Yoo *et al.* (2020) reported that the IVDMD of rice TMR was higher than that of corn TMR (P<0.05). Allister *et al.* (2020) evaluated the ISDMD (In sacco DM disappearance) of barley, maize, sorghum and wheat and reported wheat exhibited the highest ISDMD followed by barley, sorghum and maize, respectively corroborating with the present findings. Rosendo *et al.* (2013) reported that IVTDMD (%) was higher for maize than sorghum grain after 48 hours of the incubation, while in our study, no difference between maize and sorghum grain was observed, which could be due to less incubation time of 24 h in present study.

While the IVOMD % was highest (P<0.01) in wheat and barley followed by bajra in comparison to other cereals (Table 2). The IVOMD % in rest of the cereals viz., maize, broken rice, jowar and ragi was comparable among each other.The main component in cereal grains is (60-80%) starch which is highly degradable compared to fibre, but the proteinaceous matrix surrounding starch granules affects the starch digestibility. Digestibility is negatively associated with the presence of prolamins which are the storage proteins. The prolamins in maize, sorghum, wheat, barley and rice are zein, kafirins, gliadin, hordeins and secalins, respectively and their concentration varies among the cereals. Usually, wheat, rice and barley have fewer prolamins than corn, bajra and sorghum (Momany *et al.*, 2006 and Giuberti *et al.*, 2014).The starch source is also an important factor that affects the degradability of cereal grains. Corn and especially sorghum have a

high proportion of peripheral and horny endosperm resulting in increased resistance to microbial activity (Rooney and Pflugfelder, 1986), unlike wheat, which have higher proportion of floury endosperm. In addition, corn and sorghum have a denser protein matrix (Kotarski *et al.*, 1992). The in vitro experiment by Lanzas *et al.* (2007) measured fractional gas rates, as a measure of starch digestion (Huhtanen and Sveinbjornsson, 2006), reporting 0.26, 0.24, 0.15, and 0.06 h-1 rates for wheat, barley, corn and sorghum, respectively.

The higher (P<0.01) IVOMD observed in wheat and barley in the present study was could be due to the presence of fewer prolamins than the other cereal grains (Momany *et al.*, 2006; Giuberti *et al.*, 2014). The lowest IVOMD of corn and sorghum grain was due to higher prolamins and higher proportion of peripheral and horny endosperm which resulted in increased resistance to microbial activity. The lowest IVDMD and IVOMD of ragi grain might be due to presence of higher crude fibre (Table 1) content.

The IVNDFD % ranged from 44.38 to 63.72 with significant (P<0.01) difference among the cereal grains (Table 2). The IVNDFD (%) was highest in wheatand barley followed by bajra and then inragi and the lowest IVNDFD was observed in jowar, broken rice and maize. The IVNDFD % in latter 3 cereals was comparable. The IVNDFD of cereal grains in this study followed the trend of IVOMD except for ragi grain. The lower IVNDFD of maize, jowar and broken rice was could be attributed to their increased resistance to microbial activity because of their denser protein matrix and higher peripheral and horny endosperm in the grain (Rooney and Pflugfelder, 1986). In disagreement with present study reports, Yoo *et al.* (2020) reported that IVNDFD of rice TMR was higher than corn TMR which could be the result of associative effect of other feed ingredients in TMR. Yang *et al.*(2018) reported that lower IVNDFD was observed with wheat TMR than with corn TMR and rice TMR.

Significant (P<0.01) variation was also observed for partitioning factor (PF) among the cereals. The higher PF was noticed for broken rice, jowar and maize followed by ragi and then by bajra and lowest in wheatand barleyand was in accordance with efficiency of microbial biomass production observed for various cereal grains (Table 3).

Table 3. Microbial biomass production, efficiency of microbial biomass production and metaboliz able energy of cereal grains assessed by in vitro gas production

Cereal grain	MBP (mg/500mg)	EMBP (g/kg DOM)	ME (MJ/kg)
Maize	138.33±1.04^{c}	690.08±2.70^{ab}	10.27±0.02^{e}
Broken Rice	140.47±2.17^{c}	695.42±6.03^{a}	10.98±0.16^{d}
Jowar	137.12±4.65^{c}	689.82±9.04^{ab}	10.33±0.29^{e}
Wheat	230.23±14.37^{a}	597.11±13.15^{d}	18.04±0.37^{a}
Ragi	140.06±1.75^{c}	672.53±5.99^{b}	9.66±0.17^{f}
Barley	215.00±0.39^{a}	582.41±0.83^{d}	16.21±0.04^{b}
Bajra	180.52±0.60^{b}	621.04±0.66^{c}	14.16±0.02^{c}
SEM	8.486	10.225	0.692
P Value	0.001	0.001	0.001

Each value is an average of three observations

^{abc}Means with different superscripts in a column differ significantly: P< 0.01

MBP (mg/500 mg) - Microbial biomass production, EMBP (g/kg DOM) - Efficiency of microbial biomass production, ME (MJ/Kg) - Metabolizable energy

SEM: Standard Error Mean; P: Probability value

The MBP (mg/500mg) ranged from 137.12 (jowar) to 230.38 (wheat) with significant (P<0.01) difference among the cereals (Table 3). The highest MBP (mg/500mg) was noticed for wheat and barley followed by bajra and lowest in maize, broken rice, ragi and jowar. While, the mean EMBP (g/kg DOM) values ranged from 582.41 (barley) to 695.42 (broken rice) showing significant (P<0.01) difference among the cereals. The highest EMBP (g/kg DOM) wasfor broken rice and comparable values were observed among broken rice, maize and jowarfollowed by ragi and then by bajra and lowest EMBP was in barley and wheat.The EMBP calculated from the ratio of MBP (mg) to IVOMD (mg) indicating its negative correlation with the IVOMD (mg). The lower EMPB of wheat and barley grains than the other cereal grains noticed was due to their higher IVOMD values. There will be inverse relationship between in vitro gas production and microbial biomass yield (Blummel *et al.*, 1996). This is in corroboration with the present study findings (Table 2 and 3).

The ME (MJ/kg) contentin these cereal grains significantly (P<0.01) differed ranging from 9.66 (ragi) to 18.04 (wheat). The descending order of the cereal grains according to their ME (MJ/kg) values was wheat > barley >bajra> broken rice >jowar and maize >ragi.The ME content of cereal grains positively correlated with the CP content in grains and gas volume produced (Table 1 and 3).

The mean pH values ranged from 7.00 to 7.23 and were statistically significant (P<0.01) and presented in Table 4.

Table 4. Ruminal pH, total volatile fatty acid and ammonia nitrogen concentration of various cereal grains assessed by in vitro gas production technique

Cereal grain	pH	TVFA(mmol/dl)	NH3-N(mg/40ml)
Maize	7.23±0.03^{a}	3.72±0.16^{d}	17.92±0.01
Broken Rice	7.20±0.01^{ab}	3.45±0.05^{d}	17.17±0.75
Jowar	7.23±0.03^{a}	3.85±0.21^{cd}	19.41±0.75
Wheat	7.00±0.01^{c}	6.50±0.09^{a}	15.68±0.01
Ragi	7.13±0.03^{b}	3.70±0.03^{d}	16.43±1.49
Barley	7.00±0.01^{c}	5.78±0.16^{b}	15.68±0.01
Bajra	7.17±0.03^{ab}	4.17±0.11^{c}	17.17±1.49
SEM	0.022	0.249	0.393
P Value	0.001	0.001	0.11

Each value is an average of three observations

^{abc}Means with different superscripts in a column differ significantly: P=0.001

TVFA- Total volatile fatty acid, NH3-N- Ammonia nitrogen

SEM: Standard Error Mean; P: Probability value

The pH was highest from maizeand jowar followed by broken rice and bajra and were comparable to maize, jowar and ragi. The pH in ragi was lower than maize and jowar but higher than wheat and barley. The pH in wheat and barley was lowest. The pH value is negatively correlated with TVFA production and positively correlated with NH3-N. The lowest pH in wheat is due to the highest TVFA and highest pH in maize and broken rice is due to the lowest production of TVFA. According to the findings of present study results, Amanzougarene *et al.* (2020) reported highest (P<0.05) pH in sorghum and maizethan barley.Opatpatanatik *et al.* (1994) reported that pH was lower (p<0.05) with time for wheat than maize grain. Yoo *et al.* (2020) reported that ruminal pH was higher in corn TMR (p<0.05) than in rice TMR. Yang *et al.* (2018) reported that rice TMR maintained lowest pH value than the wheat and corn TMRs. While, Yang *et al.* (2020) reported that pH was not

affected (P>0.10) with substitution of rice in the TMR instead of corn TMR which is in agreement with the present study findings.

The TVFA (mmol/dl) concentration differed significantly (P<0.01) among the cereal grains, being highest in wheat, followed by barley and then in bajra and jowar. The TVFA in jowar and bajra was comparable and lowest TVFA was observed in maize, ragi and broken rice. This result was in consistent with the results of Opatpatanatik et al. (1994) who reported that total VFA production from wheat was higher than the maize grain due to the higher (P<0.05)starch degradability of wheat.Yang *et al.* (2018) reported that rice TMR with lower VFA concentration than the wheat TMR (P<0.05).In disagreement with the present study reports, Yang *et al.* (2020) and Yoo *et al.* (2020) reported TVFA production from rice TMR was higher (P<0.05) than corn TMR.

In this study, maize, broken rice and sorghum fermented more slowly. The protein matrix was probably the major factor responsible for limiting the access of rumen microbes to starch granules of maize and sorghum (Rooney and Pflugfelder 1986 and McAllister *et al.,* 1993). The composition and kernel structure of sorghum and maize are similar and they have much higher proportion of peripheral endosperm which is extremely dense, hard and resistant to water penetration and digestion (Rooney and Pflugfelder., 1986) and in addition sorghum also contain tannins that could reduce starch digestion. These possible reasons could have resulted in lower starch degradability and lead to lower TVFA production from these grains.

The mean NH3-N (mg/40 ml) ranged from 15.68 to 19.41 and was comparable among various cereal grains.This is in accordance with the results of Yang *et al.* (2020) and Yoo *et al.* (2020) reported that NH3-N was not affected (P>0.10) with substitution of rice in the TMR instead of corn TMR. The present study results are in disagreement with the results of Yang *et al*. (2018), who noticed highest NH3-N concentration for wheat based TMR than the corn and rice based ones.

CONCLUSION

The in vitro degradability and fermentation of maize, broken rice, ragi and sorghum grains are much slower than that of wheat, barley and bajra. The rumen degradability and rumen fermentation pattern of broken rice was comparable to maize grain.

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Murali *et al.*

Effect of Time of Sowing, Seed Rate, and Cultivar on Oat Green Fodder, Dry Matter and Crude Protein Yield

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ABSTRACT

A field experiment was conducted to find out suitable sowing time, optimum seed rate, and right cultivar of fodder oat for Telangana. The treatments consisted of 16 combinations with four times of sowing (first fortnight of October, second fortnight of October, first fortnight of November, and second fortnight of November), two seed rates (80 and 100 kg/ha), and two fodder oat cultivars (JHO 822 and Kent). The experiment was conducted in a split-plot design and replicated thrice in sandy loam soil. Two-year (2016-17 & 2017-18) pooled data analysis results revealed that green fodder yield, dry matter yield, and crude protein yields recorded were significantly ($p<0.05$) higher with the crop sown during the first fortnight of November followed by the second fortnight of October. Plant height and tiller number per meter row length also responded similarly. No significant difference was observed among the two seed rates for plant height, the number of tillers/meter row, green, and dry fodder yields except crude protein where 100 kg/ha seed rate recorded higher crude protein yield than 80 kg/ha seed rate. Cultivars significantly differed in plant height and crude protein yield only. All the interaction effects were found to be non-significant. The best sowing window for obtaining higher fodder yield and quality of oat in Telangana was found to be from the second fortnight of October to the first fortnight of November.

Key words: Cultivar, Fodder oat, Sowing time, Seed rate

INTRODUCTION

Oat (Avena sativa L.) is one of the best cereal fodder crops in North, Central, and Western parts of India in rabi season due to its ideal climate leading to good growth, quick regrowth, and high nutrition for both milch as well as draught livestock. Fodder shortage is common in Telangana State during winter because only a few fodder crops are available to grow in the winter season. One of the best suitable crops is oat which can grow quickly in cool weather and provide quality fodder in the winter months. Oat thrives well in areas where winter temperatures range between 15-25 ^{0}C and it requires a long winter season for their growth and development. Its fodder yield and quality are reduced due to hot and dry weather conditions though it can tolerate frost to some extent. Oat contains 10.0 to 11.5% crude protein in its dry fodder; and also consists, 55 to 63 % neutral detergent fibre, 30 to 32 % acid detergent fibre, 22.0 to 23.5 % cellulose, and 17 to 20 % hemicellulose when harvested at 50 % flowering stage of the crop (Kumar *et al.,* 2012). Oat is known for its multiple uses for livestock as green fodder, straw, hay, or silage. Oat grains provide good feed particularly, for horses, sheep, and poultry.

Shekara and Lohithaswa (2012)reported that October second fortnight sown fodder oats produced higher green forageyield and dry matter yield as well as crude protein yield followed by November first fortnight sown fodder oat. October 25th sown fodder oat has produced higher green fodder and dry matter yields compared to November 25th sown fodder oat, reported by Kadam *et al.* (2019). In many parts of Telangana, low temperatures prevail during the rabi season makes it fit for the production of fodder or grain oats. As oat crop is generally sown in the month of November in Telangana, the effect of early and delayed sowing on green fodder yield and other quality parameters needs to be studied to identify the ideal sowing time/sowing window for exploiting the full production potential. Optimum seed rate is also needed to be identified for getting optimum plant

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population for the production of higher fodder or seed yield. The seed rate of 120 kg/ha has produced higher green forage, dry matter, and crude protein yields. However, no significant difference found when compared this with the seed rate of 100 kg/ha (Shekara and Lohithaswa, 2012). To increase the fodder yield under restricted growth conditions of Telangana, there is a need to identify higher yielding genotypes which may compensate for the much-felt green fodder demand during winter months. With this background a field experiment was initiated in rabi 2016-17 inthe Experimental field of All India Coordinated Research Project on Forage Crops & Utilization Centerat Agricultural Research Institute, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad with the following objectives:

1. To identify a suitable time of sowing (sowing window) and establish a relationship with climatic factors on productivity in Telangana.
2. To identify an optimum seed rate for getting optimum plant population for production of higher fodder yield
3. To identify suitable variety for Telangana which produce higher fodder yield

MATERIALS AND METHODS

A field trial was conducted during rabi 2016-17 and repeated in rabi 2017-18.The experimental location is situated at 17°19' 18" N latitude, 78°24' 18" E longitude and at an altitude of 527m above mean sea level in the Southern Telangana Agroclimatic Zone in Telangana State India.The average annual rainfall of the area was 750 mm and maximum and minimum temperatures ranged between 24.6 to 34.10C and7.6 to 18.6 ^{0}C respectively during the crop growth period. Experimental site was well drained moderately deep sandy loam soil with pH of 7.8 and EC of 0.22 dS m-1. The experimental field was low in available N (152 kg ha-1), medium in phosphorus (26.0 kg ha-1) and high in potash (293.0 kgha-1). The experiment was conducted in a split-plot design with three replications. In the field layout four times of sowing were applied to four main plots (4 main blocks) and seed rates incombination with varieties were randomly allotted to sub plots within each main plot. Recommended dose of nitrogen, phosphorus, and potassium @ 100, 40, and 40 kg/ha was applied in the form of urea, single super phosphate (SSP), and muriate of potash (MOP) respectively. Full dose of SSP and MOP were applied to the experimental plots as basal dose and half of the total nitrogen applied as basal and remaining half was top dresses at 30 days after sowing. The crop was evaluated for plant height (cm), number of tillers/metercrop row, and green forage yield at 50% flowering stage. The crop was harvested plot-wise and fresh green forage yield was recorded in kg/plot, later it was converted into quintals per hectare. A fresh sample of 500 g taken from each treatmentwas sundried initiallyfollowed by oven driedat 60-65^{0}C to a constant weight and estimated dry matter yield, later dried plant samples were finely ground and subsequentlyused for quality analysis in the biochemistry laboratory. The collected data was statistically analysed by analysis of variance (ANOVA) for split plot design. Critical differences were worked out at five percent probability level in LSD, if treatments were significantly differedand if not; NS was denoted (Gomez and Gomez, 1984). Two years data was pooled and statisticallyanalysed using OPSTAT software for theinterpretations of results (Sheron *et al.,* 1998).

Details of the treatments:

Main plot Treatment (Times of sowing: 4)

1) T1: First fortnight of October; T2: Secondfortnight of October

2) T3: First fortnight of November; T4: Second fortnight of November

Sub plot Treatment (Seed rates:2)

1) S1:80 Kg/ha; 2) S2: 100 kg/ha

Sub plot Treatment (Cultivars: 2)

1) V1: JHO-822 ;2) V2: Kent

The total number of treatments was 4x2x2 = 16

RESULTS AND DISCUSSION

Times of sowing have a significant influence on plant height in fodder oat. Two-year pooled data (Table 1) revealed that November 1st fortnight sown fodder oats produced significantly taller plants when compared to October 1st fortnight and November 2nd fortnight sown fodder oats. But it was on par with October 2nd fortnight sown oats. Results of both rabi 2016 and 2017 also shown a similar trend. Similar findings were reported by Shekara and Lohithaswa (2012) and Kadam *et al.* (2019).

Table1. Effect of times of sowing, seed rate, and cultivar on plant height and tiller

Treatments	Plant Height (cm) at 50% flowering			No. of Tillers/m row at 50% flowering		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Main plot: Times of sowing (4)						
T1: First fort night of October	72.0	60.2	71.9	141.3	140.5	140.0
T2: Second fortnight of October	76.9	71.8	76.0	159.0	158.0	158.6
T3: First fortnight of November	77.9	68.8	77.8	169.0	164.5	167.0
T4: Second fortnight of November	62.9	62.2	62.9	135.6	138.3	135.6
SEm+	1.9	1.0	1.9	5.0	4.5	4.9
CD (P<0.05)	6.8	3.5	6.8	15.8	13.1	14.8
Sub plot: Seed rates (2)						
S1: 80 Kg/ha	71.1	63.5	71.2	157.6	152.3	157.6
S2: 100 Kg/ha	73.3	66.5	73.2	165.8	154.4	165.8
SEm+	1.4	0.8	1.4	4.8	1.3	4.8
CD (P<0.05)	NS	NS	NS	NS	NS	NS
Sub plot: Varieties (2)						
V1: JHO-822	74.5	65.4	74.6	157.5	152.7	157.6
V2: Kent	70.0	62.1	70.2	166.0	154.0	161.2
SEm+	1.4	1.0	1.2	4.8	1.3	4.8
CD (P<0.05)	4.2	3.1	3.9	NS	NS	NS

Note: All the Interactions (T x S, T x V, S x V, and T x S x V) were found to be non-significant (NS)

Plant height was 71.2 cm at the seed rate of 80 kg/ha and 73.2 cm at the seed rate of 100 kg/ha, but these two seed rates didn't differ significantly in the pooled data (Table 1). This is in accordance with Droushiotis (1990). Pooled analysis showed that the cultivar JHO-822 produced significantly taller plants (74.6 cm) than Kent (70.2 cm).All the interaction effects (interaction between dates of sowing and seed rate, seed rates and cultivars, and dates, seed rates, and cultivars) were found non-significant in influencing the plant height.

The number of tillers per meter row length significantly differed with timesof sowing. Fodder oats sown in the first fortnight of November produced a higher number of tillers per meter (167.0), followed by the second fortnight of October sown oats (158.6). These two sowing dates were significantly higher than the first fortnight of October and the second fortnight of November sown, treatments in tiller number 140.0 and 135.6 respectively (Table 1). This higher tiller number may be due to conducive vegetative growth for oat.

November 1st fortnight and October 2nd fortnight sown fodder oats didn't differ significantly in tiller number per meter row length. Rabi 2016 and rabi 2017 results individually also followed a similar trend.Two seed rates and two cultivars didn't influence the number of tillers per meter row length significantly. Erega *et al.* (2020) had also reported the similar results. No interaction effect was found significant in affecting the tiller number.

The November 1st fortnight sown fodder oat has produced more green forage yield 525.5 q/ha than other 3 dates of sowing treatments. It was significantly higher than October 1st sown (430.2 q/ha) and November 2nd fortnight sown crop (373.8 q/ha). However, it was at par with the treatment October 2nd fortnight sown oats (478.4 q/ha). Shekara and Lohithaswa (2012) and Kadam *et al.* (2019) reported similarly, but Jehangir *et al.* (2013) reported that September 30th sown fodder oat produced significantly higher green fodder yield compared to October 10th sown crop at in Kashmir conditions; Sood *et al.* (1992) reported higher plant height, tillers per meter row, and green fodder yieldin early sown oat crop (5th October) under temperate conditions. Sharma *et al.* (2017) also reported October 15th sown fodder oats produced than that of October 30th sown and November 14th sown crop at Palampur, Himachal Pradesh (Jehangir *et al.,* 2013).Early sown (September 10th) berseem crop has produced significantly higher green forage yield than late sown crop (October 20th) at Amritsar reported by Singh *et al.* (2021). Kumar *et al.* (2021) also found that different dates of sowing differed significantly in producing green fodder yield in fodder oat.Fodder oat need cool winter season for good crop growth and to produce green fodder and dry matter yields. In temperate regions of India such as Kashmir & HP the fodder oat produced significantly higher yields in mid-September to mid-October sown crop where as in southern States such as Telangana and Karnataka fodder oats produced higher green forage and dry matter yields when sown during mid-October to mid-November. Green forage yield was not significantly influenced by the two seed rates in contrast to it (Shekara and Lohithaswa, 2012) reported and the similar trend was noticed also with the two cultivars used in the experiment. In contrast to it May *et al.* (2004) reported the significant difference between two cultivars they tested in combination with three sowing dates.

The times of sowing have a significant effect on dry matter yield also as November 1st fortnight sown fodder oat yielded more dry matter yield (123.7 q/ha) than other October 1st& 2nd fortnight sown and November 2nd fortnight sown fodder oat. Shekara and Lohithaswa (2012) have reported October 2nd fortnight sown fodder oat produced higher dry matter yield followed by November 2nd fortnight sown fodder oats. October 2nd fortnight sown annual ryegrass produced significantly higher dry matter yield than other treatments (Sidhu *et al.,* 2020). This may be due to congenial climate for better fresh growth that reflected in dry matter accumulation. Two seed rates did not influence dry matter yields. Similar results reported by Erega *et al.* (2020). However, Singh *et al.* (2018) has reported that seed rate 90 kg/ha of fodder oat produced higher dry matter yield than other seed rates viz., 60 and 120 kg/ha.Cultivars (JHO 822 and Kent) didn't significantly differ in producing dry matter yields. In contrast, Dar *et al.* (2014) and Sharma *et al.* (2017) reported that fodder oat cultivars differed significantly in producing dry matter yields.

Crude protein yields significantly differed with dates of sowing. The November 1st fortnight sown fodder oat produced highest crude protein yield which was followed by October 2nd fortnight sown crop. Similar results were also reported by Shekara and Lohithaswa (2012). In contrast to it Dar *et al.* (2014) reported that the crude protein and crude fiber content were significantly higher with delayed sowing (5th November).

Table 2. Effect of times of sowing, seed rate, and cultivar on green forage yield, dry matter yield, and crude protein yield

Treatments	Green Fodder Yield (q/ha) at 50% flowering			Dry Matter Yield (q/ha) at 50% flowering			Crude protein Yield (q/ha) at 50% flowering		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Main plot: Times of sowing (4)									
T1: First fort night of October	453.2	383.5	430.2	120.4	90.5	100.6	9.1	7.8	8.8
T2: Second fortnight of October	552.1	423.4	478.4	123.6	102.5	112.5	9.8	9.2	9.5
T3: First fortnight of November	564.6	448.3	525.5	138.4	113.7	123.7	11.5	10.4	10.8
T4: Second fortnight of November	379.2	369.6	373.8	114.3	92.7	94.4	8.5	8.1	8.2
SEm+	15.2	13.7	13.9	4.8	4.3	4.6	0.2	0.3	0.05
CD (P<0.05)	55.8	47.2	47.8	15.5	13.2	14.4	0.7	1.0	0.20
Sub plot: Seed rates (2)									
S1: 80 Kg/ha	495.3	405.8	445.5	127.6	90.8	102.8	9.3	7.3	8.8
S2: 100 Kg/ha	504.2	415.8	464.7	133.6	93.9	108.9	10.3	7.9	9.4
SEm+	8.8	8.2	8.2	2.1	1.7	1.7	0.2	0.1	0.1
CD (P<0.05)	NS	NS	NS	NS	NS	NS	0.7	0.4	0.3
Sub plot: Varieties (2)									
V1: JHO-822	490.1	405.8	405.8	124.6	91.6	103.6	9.0	8.7	8.8
V2: Kent	509.4	415.8	455.2	131.6	93.2	110.1	10.1	9.5	9.9
SEm+	8.8	7.6	8.2	2.9	1.7	1.7	0.2	0.1	0.2
CD (P<0.05)	NS	NS	NS	NS	NS	NS	0.7	0.4	0.6

Note: All the Interactions (T x S, T x V, S x V, and T x S x V) were found to be non-significant (NS)

Seed rates and varieties have significantly influenced the crude protein yield in fodder oats (Table 2). But Shekara and Lohithaswa (2012) reported that seed rates did not influence significantly in crude protein yields in fodder oats. Dar *et al*. (2014) reported that cultivars significantly influenced crude protein yields in fodder oats.

CONCLUSION

November 1st fortnight sown fodder oat crop produced significantly higher green fodder yield, dry matter yield, and crude protein yields which was followed by second fortnight of October sown fodder oats. These two sowings were superior over the other two times of sowing (October 1st fortnight and November 2nd fortnight sown crop). Plant height and tiller number per meter row length also responded similarly. Hence the best sowing window for fodder oat in Telangana is from October 15th to November 15th. No significant difference was observed among two seed rates for plant height,

number of tillers/meter row, green and dry fodder yields except crude protein where 100 kg/ha seed rate recorded higher crude protein yield than 80 kg/ha seed rate. Cultivars significantly differed in plant height and crude protein yield only. All the interaction effects were found to be nonsignificant.

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Kumar *et al.*

Effect of Dietary Supplementation of Fenugreek Seed (*Trigonella Foenum-Graecum* L.) Powder on Body weight, Blood-biochemical Parameters and Immunity in Broilers

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ABSTRACT

A six-week study was carried out to investigate the effect of dietary supplementation of Fenugreek seeds (Trigonella foenum-graecum L.) powder (FSP) on serological and immunological parameters in broiler chickens. One hundred and forty four day-old chicks were randomly divided into six dietary treatment groups having 24 birds in each group and three replications of 8 birds each. Supplementation with FSP was done at the rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% for T1 to T6. The result of the study revealed that there was a significant ($p<0.05$) decrease in serum total cholesterol and LDL-cholesterol concentrations in the broilers fed diets supplemented with FSP at 1.5% or above. HDL-concentrations were found to be improved significantly ($p<0.05$) upon FSP supplementation at 1.0% and above. Blood glucose concentration was reduced significantly ($p<0.05$) as the supplementation of FSP was increased from 1.0% to 3.0%. Expression of genes controlling antibacterial and anti-viral activities revealed that relative mRNA expression of TLR-2 and TLR-7 of broilers was found to be enhanced upon feeding FSP. But a significant down regulation was seen in case of relative mRNA expression of TLR-4 receptors in FSP supplemented groups. Serum triglycerides, SGPT and SGOT concentrations remained unaffected. It was inferred from the study that FSP fenugreek supplementation in the diets of broiler birds have substantial improvement in the serum biochemical parameters along with showing immunological activities.

Key words: Fenugreek Seed Powder, Broiler, Cholesterol, Blood biochemical and Immunity

INTRODUCTION

The consumption pattern in our society is shifted from red meat (beef, mutton, lamb, pork) to white meat (broiler meat) due to high saturated fat and cholesterol content of the red meat (Daniel *et al.*, 2011). A regular consumption of red meat has been shown to be directly associated with cardiac disease, high blood pressure, arteriosclerosis, stroke, diabetes, obesity and earlier death. Many medicinal and aromatic plants have been used for broilers to increase dietary energy utilization and improve performance efficiency. A large number of plant-derived products are becoming important, due to their antioxidant effects, and in particular, their potential prophylactic benefit in poultry production, as the use of most antibiotic growth promoters as feed additives has been banned by many countries around the world. Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume and widely cultivated in India. Fenugreek has been widely used not only as a constituent in traditional foods, but also as a natural herbal remedy in its leaf and seed forms. Human and animal studies show that fenugreek seeds have various physiological effects, including anti-diabetic, anti-inflammatory, anti-cancer, and immunomodulatory effects (Basch *et al.*, 2003; Srinivasan, 2005; Kawabata *et al.*, 2011; Thomas *et al.*, 2011). Fenugreek seeds are also rich in phenolic compounds, especially flavonoids, which have extensively antioxidant capacity (Kaviarasan *et al.*, 2004). It has also been reported that fenugreek seed have hypolipidemic effects due to high amount of galactomannan, and lipotropic effect due to mainly high choline amount. The aim of the present study was to analyze the effect of fenugreek seed powder supplementation at different level on serum parameters and investigating the relationship between inclusion level of fenugreek seed and expression of genes associated with antibacterial or antiviral activities in broiler birds.

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MATERIALS AND METHODS

This study was conducted with a total of 144 day-old as broiler chickens at poultry shed of Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for a period of 42 days to examine the effect dietary supplementation of fenugreek seed powder on serological and immunological parameter. A total of 144 day-old experimental broilers were weighed, identified by wing bands and randomly distributed to 6 treatment groups. Each treatment was divided into 3 replicates having 8 birds in each. The ration was formulated as per the BIS (2007) Specification. The control group (T1) was fed a basal diet having no fenugreek seed powder. While the rations of broilers under treatment group T2, T3, T4, T5 and T6 was supplemented with fenugreek seed powder (FSP) at the rate of 1.0, 1.5, 2.0, 2.5 and 3.0%, respectively. The ingredient and chemical composition of diets fed during different stages of growth and fenugreek seed powder have been presented in table 1. Fenugreek seed powder contained 28.4% CP, 9.30% CF, 7.14% EE and 3.28% total ash.

Table 1. Ingredient and analyzed chemical composition of basal diet fed during different phases of broiler production

Attributes	Pre starter	Starter	Finisher
Ingredient composition* ((kg /100 of feed)			
Maize	55	56	57.20
Soybean meal	20	14	14.4
Ground nut cake	12	16	14.4
Fish meal	7	7	6
Vegetable oil	4	5	6
Mineral mixture	2	2	2
Chemical composition**			
Moisture (%)	9.83	10.05	9.97
% DM basis			
Crude Protein (%)	23.12	22.03	20.42
Crude Fiber (%)	2.50	2.59	2.56
Ether Extract (%)	6.32	6.64	8.24
Total Ash (%)	6.08	6.18	6.65
ME (kcal/kg)	3013	3102	3210

*Feed additive mixture was added @ 410 g/100kg of control ration and 310 g/100 kg feed in the rest of treatment rations during different growth phases of broilers. Feed additive mixture was composed of Vitamin Mixture-I-10 g, Vitamin mixture-II-20 g, Coccidiostat-50 g, Choline chloride-100 g, Antibiotic, oxytetracycline (only in T1) 100g, Lysine-50 g and DL-methionine-80 g.

** Each value is mean of three observations.

For proper brooding of chicks, sufficient heat and light was provided by using electric bulbs in each treatment for first three weeks of age. Temperature of brooding was 950F for first week. A weekly reduction of 50F was done till brooder temperature reached to 850F by third week of age. Afterword sufficient artificial light was provided during night hours throughout the experimental period. Birds were vaccinated against F1 strain of Newcastle disease on 3rd day and Infectious Bursal Disease on 14th day through intranasal route. Fresh and clean drinking water was provided ad-libitum. Body weight of the birds was recorded at the end of pre-starter (day 7), starter (day 21) and finisher phase (day 42). All the precautionary measures against diseases were taken throughout the

experimental period of six weeks. At the end of the feeding trial (6th week), blood samples were collected from five broiler per treatment and thus a total of 30 samples were analyzed. About 2 ml of blood was collected from each bird via brachial wing vein puncture using sterilized syringes. Serum parameters were determined by auto analyzer using commercial kits. Serum samples were analyzed for different serum variables like total cholesterol, glucose, triglyceride, SGOT, SGPT high density lipoproteins (HDL) and low-density lipoproteins (LDL). To study the effect of dietary FSP on expression of genes related to antibacterial and anti-viral activities, extracted RNA from fresh blood sample was used for cDNA preparation and for the analysis of temporal expression profile of different genes, real-time PCR was carried out using Step one plus real-time PCR system and the average CT (Threshold cycle) value obtained for the TLRs 2, 4 and 7 (target) gene was normalized (endogenous control). The data obtained were subjected to comparative CT method for the analysis of the expression levels of targeted TLR gene and an endogenous control.

STATISTICAL ANALYSIS

Data was analyzed statistically as described by Snedecor and Cochran (1994). Analysis of variance was used to study the differences among treatment means and they were compared by using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1956).

RESULTS AND DISCUSSION

Body weight of the broiler birds at the start of the feeding trial was similar ($p>0.05$). At the end of pre-starter phase, group T3 had significantly ($p<0.05$) higher body weight as compared to the control and other groups followed by group T4 while at the end of starter phase, group T4 and T5 had statistically ($p<0.05$) higher body weight followed by group T3. At the completion of the trial the overall body weight of birds under group T3 and T4 was significantly ($p<0.05$) higher than control group and numerically higher than other treatment groups (Table 2) Similar results were also confirmed by Yatoo *el al.* (2012).

Table 2. Body weight of the broiler birds at the end of different growth periods

Treatments	Average body weight (g)			
	Initial BW (g)	Pre-starter phase (g)	Starter phase (g)	Finisher phase (g)
T_1	44.37±1.57	96.16±0.66^{b}	501.3±9.72^{a}	2130±1.09^{a}
T_2	44.83±0.75	96.39±0.55^{b}	577.8±5.66^{ab}	2441±4.19^{b}
T_3	44.64±0.58	106.2±0.53^{d}	669.3±3.69^{c}	2457±8.53^{bc}
T_4	44.73±1.12	98.15±0.35^{c}	713.6±8.48^{cd}	2552±13.47^{b}
T_5	44.89±1.77	98.17±1.17^{c}	762.9±8.84^{d}	2478±1.54^{bc}
T_6	44.17±0.78	90.51±2.67^{a}	636.1±3.09^{bc}	2379±8.14^{bc}

*Mean values bearing different superscript within a column differ significantly ($p<0.05$)

There was no statistical variation in serum triglycerides concentrations among various groups. Serum total cholesterol decreased significantly ($p<0.05$) in fenugreek seed powder supplemented groups at the level of 2% and above (Table 3). Anti-cholesteremic effect of fenugreek seed may be due to presence of saponins which either compete with cholesterol at binding sites or interfere with cholesterol biosynthesis in the liver. The results are in agreement with the study of Abbas and Ahmed 2010; Abdel-Rasoul and Yousif, 2003 and El-Ghamry *et al.*, 2002 in broilers. Abdul-Rahman, (2012), Safaei *et al.* (2013) and Mamoun *et al.* (2014) have also observed cholesterol and glucose lowering effects of fenugreek seeds in broilers.

Table 3. Mean values of biochemical parameters of broilers fed different diets

Treatment	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	Glucose (mg/dl)
T_1	96.33±4.61	143.0±2.64^{d}	74.44±2.70^{a}	63.50±1.30^{d}	225.0±10.10	9.40±0.60	230.0±3.70^{d}
T_2	88.33±10.20	140.0±1.70^{d}	74.44±1.70^{a}	52.60±0.80^{d}	220.3±23.10	8.60±0.20	226.3±2.80^{d}
T_3	90.67±8.22	139.0±2.90^{d}	80.06±2.70^{b}	51.30±1.40^{d}	222.0±30.70	8.30±2.10	215.4±2.20^{c}
T_4	83.33±4.12	137.0±2.90^{c}	82.03±1.40^{bc}	49.10±1.40^{c}	210.0±23.40	7.70±0.90	203.3±3.30^{b}
T_5	84.08±6.00	136.0±2.30^{a}	85.48±1.90^{c}	47.00±1.10^{a}	198.9±29.50	7.30±1.20	182.5±1.50 ^{a}
T_6	78.33±1.40	132.0±2.40^{b}	89.03±1.60^{bc}	40.30±1.20^{b}	197.7±19.70	6.90±1.30	177.5±3.20^{a}

*Mean values bearing different superscripts in a column differ significantly ($p<0.05$).

Serum HDL was increased significantly ($p<0.05$) in the broilers fed fenugreek seed powder at or above 1.5% in comparison to control group. LDL-cholesterol decreased ($p<0.05$) on fenugreek seed powder supplementation. In harmony of these results, Mullaicharam *et al.* (2013) also reported that there was a significant suppression in the human serum total cholesterol, triglycerides and LDL cholesterol by fenugreek supplementation.

The mean values of blood biochemical parameters were within normal range. Supplementation of fenugreek seed powder at the level of 1.5% or above in the diets of broilers resulted in significant drop ($p<0.05$) in the blood glucose concentration. The significant fall in blood glucose might be due to increased plasma insulin by a direct stimulatory effect on B-cells. Earlier studies by Sauvaire *et al.* (1998) and Schryver (2002) have suggested that abundant 4-hydroxyisoleucine amino acid in fenugreek has a stimulatory effect on the pancreas to release insulin. Safaei *et al.* (2013) and Mamoun *et al.* (2014) have also reported similar effects.

Dietary supplementation of fenugreek seed powder had no effect ($p<0.05$) on the liver function indicators i.e., Serum glutamic oxaloacetic transaminase (SGOT) and Serum Glutamic pyruvic Transferase (SGPT). In contrast to the current finding, Nakhla *et al.* (1991) reported that SGOT activity was elevated by fenugreek seed saponins diets in boiler chicks while Ali and Ismail (2012) reported that inclusion of fenugreek seeds in the diet of broiler chicken had no significant ($P>0.05$) effect on the liver enzymes. Hepatoprotective role of fenugreek seeds has also been stated by Meghwal and Goswami (2012).

A comparison was made in between the treatment groups T2, T3, T4, T5 and T6 and control group for relative mRNA expression study of TLR2, TLR4 and TLR7 (Table 4).

Table 4. Relative quantification expression analysis of the toll like receptors (TLR 2, TLR 4 and TLR 7) with the reference to the endogenous reference gene ß actin

Sample Name	Target Name	C_T Mean	C_T SD	ΔC_T Mean	$\Delta\Delta C_T$	RQ
T_1	TLR 2	19.54	0.74	2.24	0	1
T_2		18.50	0.34	1.84	-0.4	1.31
T_3		18.77	0.12	1.48	-0.76	1.69
T_4		17.80	0.13	1.28	-0.96	1.94
T_5		18.23	0.77	1.08	-1.16	2.23
T_6		17.54	1.26	1.02	-1.22	2.32
T_1	TLR 4	20.43	0.74	3.12	0	1
T_2		19.45	0.29	2.79	-0.33	1.25
T_3		20.78	0.24	3.48	0.35	0.78
T_4		20.05	0.15	3.52	0.39	0.76
T_5		21.50	0.62	4.34	1.21	0.432
T_6		14.31	0.81	2.21	1.34	0.4
T_1	TLR 7	26.20	0.26	8.90	0	1
T_2		26.59	0.07	9.93	1.03	0.49
T_3		27.53	0.29	10.29	1.33	0.39
T_4		28.04	0.22	11.51	2.61	0.16
T_5		25.13	1.80	7.97	-0.92	1.89
T_6		22.31	0.65	5.78	-3.11	1.07
T_1	ß actin	17.30	0.13	-	-	-
T_2		16.66	0.25	-	-	-
T_3		17.29	5.29	-	-	-
T_4		16.52	0.16	-	-	-
T_5		17.15	6.09	-	-	-
T_6		16.52	0.32	-	-	-

The treatment group supplemented with 3% FSP (T6) had highest RQ value showing maximum expression of TLR2. On the other hand, treatment group supplemented with 2.5% FSP (T5) had highest RQ value showing maximum expression of TLR7. While, in case of TLR4 mRNA expression study, a significant down regulation of RQ value was obtained with the increasing level of supplementation of FSP in broilers' diet. TLR-2 denotes presence of Gram-positive bacteria that are beneficial for the health of the gut e.g., Lactobacillus. Therefore, up regulation of the TLR-2 RQ values in FSP supplemented groups enhanced the population of Lactobacillus bacteria in the gut microflora which is better for competitive exclusion of harmful gut microorganisms. Augmented TLR signaling denotes up-regulation of cell-mediated immunity, improvement of T-cell homing to

mesenteric lymph nodes (Corthésy *et al.*, 2007). TLR-4 depicts the presence of Gram-negative bacteria in the gut microflora e.g., E. coli. The down regulation of TLR-4 RQ values in FSP supplemented groups indicates an elevated anti-bacterial activity against these harmful bacteria. TLR-7 represents antiviral activity of the immune system, thus, up regulation of TLR-7 RQ values for FSP fed groups signifies a boosted immune system response against different viruses. In vitro study by Qureshi *et al*. (2015) also reported antibacterial activity of Fenugreek seed in which they observed a 2.1 mm of zone of inhibition for the concentration of 0.05 mg/ml of extract against E. coli on the Mueller Hinton agar. Dash et al. (2011) also concluded also demonstrated the antibacterial activity of methanolic extract of fenugreek against E. coli due to flavonoids, saponins and phenols present in it. In a similar study, Gao et al. (2008) also reported that probiotics bacteria augment toll-like receptor (TLR) signaling and regulate local mucosal cell-mediated immune responses in broilers.

CONCLUSION

Based on the findings of the study, it was concluded that dietary supplementation of fenugreek seeds powder modulates the cholesterol profile by improving HDL cholesterol and lowering LDL cholesterol and lowering blood glucose. Therefore, it was inferred that fenugreek seed powder can be supplemented at the level of 1.5% or above for its beneficial effects on broiler production.

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Effect of Black Cumin and Ginger Supplementation on Production Performance, Nutrient Utilization, Haemato-biochemcial and immune Parameters in White Leghorn Layers

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ABSTRACT

The effect of ginger and black cumin was evaluated in terms of production performance and nutrient utilization as well as blood-biochemical and immune profile of White Leghorn laying hens. Two hundred and seventy hens were randomly allotted to nine dietary treatments with three replications while adopting 32 factorial design. A feeding trial of 84 days was conducted to evaluate production performance and at the end of experiment six birds from each treatment group were subjected to metabolism trial to study nutrient utilization. Metabolizability of proximate principals except NFE was improved significantly on black cumin supplementation and on ginger supplementation only NFE metabolizability wasimproved. Nitrogen retention due to ginger and nitrogen and calcium retention due to black cumin were improved significantly (P<0.01). Both the herbs have non-significant effect on phosphorus retention. Dietary inclusion of ginger significantly reduced feed intake (P<0.05) and egg production (P<0.01), whereas black cumin has no effect on feed intake but increased the egg production (P<0.01) at 1% level. At the end of experiment, Two birds from each replication were randomly selected for blood-biochemical and one bird for immune characteristics analysis. Supplementation of both the herbs in diet significantly reduced serum cholesterol and triglycerides. Hematological indices, serum enzymes and spleen, bursa and thymus weight index were within normal physiological range and have not been influenced by dietary treatments. On observing egg production among different treatmet grouops it can be sated that highest egg production was in treatment group with 1% level of black cumin. Due to main effect of ginger, there was increase in metabolizability of crude protein and nitrogen retention but decline in egg production were observed. Further due to main effect of black cumin, highest metabolizability of dry matter, crude protein, ether extract and crude fiber, highest nitrogen intake, nitrogen retention, calcium inatke and retention with decline in calcium excretion and increase in the egg production were observed at 1 per cent level. So supplementation of black cumin at 1% level might be promising for profitablepoultry production.

Key words: Black cumin, Ginger, Nutrient utilization, Leghorn laying hen, Production performance

INTRODUCTION

Extreme hot environment and erratic rain fall increases heat stress and causes serious physiological dysfunction that may result in a decline in animal performance. Heat stress is amongst the most significant stressors influencing poultry productivity in hot climate regions, causing substantial economic losses in poultry industry (Abdel-Moneim *et al.*, 2021). Broiler and layers are more sensitive to heat induced oxidative stress because they are devoid of sweat glands, fully covered with feathers, limited capacity to dissipate heat, high metabolic activity and narrow zone of thermal tolerance (Emami *et al.*, 2021). In arid zone during summer, high environmental temperatures along with high moisture increases temperature humidity index (THI) which can be detrimental to laying hens, not only because of mortality, but also because of the reduction in egg production. Laying hen has been genetically improved to give maximum eggs in their egg laying phase, predisposing them to negative energy and nutrient retention as well as various metabolic disorders. These problems in a way reduce farmer's profit not only by affecting birds' performance but also by increasing the egg condemnation. The effects of oxidative stress on lipid metabolism in the avian liver due to stress are a particular concern in modern commercial poultry industry. The economic and effective alleviation of

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stress may achieve by supplementation of antioxidants in feed. The use of phytogenic feed additives has recently received much greater importance in animal and poultry nutrition to enhance performance. Comprehensive investigations of phytogenic plants have indicated their antioxidant, growth promoting, antimicrobial, metabolism-regulatory and anti-inflammatory properties (Swelum *et al.*, 2021). Additionally, they possess a stimulatory effect on the digestive system through increasing the production of digestive enzymes and by enhancing liver functions (Abou-Elkhair *et al.*, 2014).

Ginger root contains several compounds that have biological activities such as antioxidation, antimicrobial and pharmacological effects. The major components of ginger are zingiberen and zingerol that can stimulate the digestive system, digestive pH, digestive enzyme and intestinal microbial activity (Zhao *et al.*, 2011). The chemical constituents found in black cumin seeds are nigellone, nigellicine, nigellimine, nigellimine- N-oxide, melanthingenin, glucosides-melanthin, volatile oil, fatty oil, oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol (Desai *et al.*, 2015). It was demonstrated that black cumin seeds have considerable antioxidant, antibacterial, digestive and appetite stimulant and hepatoprotective and immunomodulative properties (Herve *et al.*, 2019). Thus in arid zones, herbal inclusion in hen diet during the crucial period of peak egg production should improve nutrient utilization and retention of nutrients as they have a positive effect on digestibility as well as nutrient metabolizability. However, our knowledge about their applications in poultry nutrition is still rather limited. Therefore, all these observations encourage the hypothesis that ginger and black cumin may positively affect production performance, nutrient utilization and blood parameters of hens in arid zone.

MATERIAL AND METHODS

The feeding trial was carried out at the Poultry Farm of College of Veterinary and Animal Science, Bikaner, Rajasthan which comes under Western arid Zone of India. A total of 270 White Leghorn layers of 28 weeks old were randomly alloted to nine dietary treatments with three replication of each while adopting 32 factorial design.The T1 i.e. control group was fed on commerically available feed as a basal diet, basal diet of T2 and T3 treatment groups were supplemented with 0.5 and 1.0% level of ginger, respectively. Similarly, basal diet of T4 and T5 treatment groups were supplemented with 0.5 and 1.0% level of black cumin, respectively. The basal diet of T6group have 0.5% level of both the herbs and T7 groups have 1.0% level of both the herbs. In T8 group basal diet was supplemented with 0.5% level of ginger and 1.0% level of black cumin. In T9 group basal diet was supplemented with 1.0% level of ginger and 0.5% level of black cumin.The experimental layers were leg banded for identification. The birds were maintained under standard managemental conditions, free access to water, lighting programme of 16L: 8D and other routine bio-security aspects.The birds were housed in deep litter system and reared under different feeding trials upto 84 days. Fresh and dry wheat straws were used as bedding material. Digital thermohygrometer was used to record temperature and relative humidity. THI values were calculated from observed measurement as described by Kibler (1964). The chemical composition of basal diet, ginger and black cumin was presented in table 1.

Table 1. Chemical composition of basal diet, ginger root powder and black cumin seed powder (DM basis)

Chemical composition	Basal ration	Ginger	Black cumin
Dry matter	91.4	96.2	97.2
Crude protein	18.0	10.8	20.3
Ether extract	5.20	2.37	29.3
Crude fiber	4.80	5.50	8.10
Total ash	15.5	4.48	5.32
Nitrogen free extract (NFE)	56.3	76.8	36.9
Acid insoluble ash	1.59	0.46	0.27
Calcium	5.61	1.42	1.75
Phosphorus	0.90	4.80	0.03
ME calculated (Kcal/kg)	2800	2987	3993

During the present study, the feed offered and residual feed from the feeding trough of each replicate was quantitatively weighed at fortnight interval and actual feed intake of each replicate was calculated. Eggs were collected twice a day and percent egg production was calculated. Egg mass was calculated as following: Egg mass (g) = (Number of egg laid per replicate)/(Number of hen per replicate) X Average weight of egg. Feed conversion ratio was calculated by dividing feed consumption with egg mass. At the end of experiment, five days metabolic trial was conducted to estimate the metabolizability and retention of nutrients. Six birds (two birds per replication) from each treatment group were subjected to metabolism trial. Daily feed intake and fecal droppings were measured. The group wise aliquots from droppings after thorough mixing with the help of spatula were drawn for dry matter estimation. Dry matter determination of excreta was done in triplicate for each group by keeping the weighed excretal material in an oven at 85 ^{o}C till constant weight was obtained. For nitrogen estimation, samples in duplicate were preserved in 5 percent sulphuric acid in wide-mouth glass stoppered bottles and kept in the refrigerator. Metabolizability coefficient of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract(NFE) of the experimental diet were measured. The retention of nitrogen, calcium and phosphorus were determined using the following formula: Retention of nutrient = (Unit nutrient intake)-(Unit nutrient outgo in excreta + Unit nutrient outgo in egg).

In this study, two birds from each replication (six bird from a treatment) were selected for collection of blood samplefrom wing vein.Whole blood was collected in sterile test tubes containing EDTA for hematological study. Haemoglobin, erythrogram and leucogram parameters were estimated as per standard methods viz. Sahli-Hellige haemoglobinometer, microhaematocrit as per Jain (1986). For biochemical studies, blood was collected in sterile tubes having no anticoagulant and kept in slant position in an incubator at 37 ^{o}C for one hour. Blood clots were broken and tubes were centrifuged at 3000 rpm for 30 min. The serum was pipetted out in small tubes which were stored under deep freeze condition (–20°C) until analysis. Serum cholesterol, serum triglycerides, serum glucose, serum alkaline phosphatase (ALP), serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) by Thermoscientific Evolution 220 UV-Visible spectrophotometer by using kit supplied by Coral clinical system and serum triglyceride was determined by using kit supplied by Diasys diagnostic sIndia pvt. Ltd. as per the manufacturer's subscribed procedure.

At the end of feeding trial, one bird per replication (three birds from a treatment) were sacrificed. The birds were kept off fed overnight and water was withdrawn 3-4 hours prior to slaughter. The birds were slaughtered by the Halal method and a bleeding time of 2 minutes was allowed. From the sacrificed birds, spleen, bursa and thymus were removed, weighed with the help of electronic balance and expressed as the percent of pre-slaughter bird weight to observe the effect of different dietary treatments on immune organs.

STATISTICAL ANALYSIS

The data obtained was statistically assessed by two-way ANOVA as per the standard methods ofSnedecor and Cochran (2004). Wherever the variance ratio (F-values) were found significant at 5 percent and 1 percent levels of probability, the significance of mean differences were tested by Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1956).

RESULTS AND DISCUSSION

As shown in table 2, after second fortnight the shed temperature and THI during afternoon hours were higher than the recommended threshold values for poultry in the tropical regions (temperature 18-24 °C and THI 70; Holik, 2009 and Karaman, 2007). The location of present experiment was at Bikaner of Rajasthan which comes under Western arid Zone of India, which is known for its extreme climatic conditions. Being part of the arid zone, the climate is very severe and dry showing erratic patterns. Dry

violent winds with sand storms, wide diurnal and seasonal variations in temperature are the common features of the area. The average recorded rainfall is 25 to 37 cm which occurs mostly in months of July and August in short and stormy showers with a relatively high intensity. The annual minimum and maximum temperatures at Bikaner are recorded close to freezing point and 48°C respectively (Kumar and Khatri, 2021). Such type of extreme enviorment is primerly responsible factor responsible for lesser development of poultry industry in Rajasthan.

Table 2. Temperature(°C), relative humidity(%) and THI in shed at fortnight interval

Periods	Temperature (°C)		Relative humidity (%)		THI	
	8 AM	2 PM	8 AM	2 PM	8 AM	2 PM
I	14.37^{d}	22.70^{c}	72.97^{a}	31.73^{a}	57.59^{c}	66.83^{e}
II	15.32^{d}	24.46^{c}	67.91^{a}	27.97^{a}	59.30^{c}	68.71^{d}
III	19.76^{c}	29.15^{b}	70.70^{a}	30.69^{a}	65.88^{b}	74.03^{c}
IV	20.57^{bc}	30.30^{b}	55.61^{b}	18.90^{b}	65.97^{b}	73.49^{bc}
V	21.91^{ab}	32.62^{a}	45.33^{b}	18.47^{b}	66.94^{ab}	75.53^{ab}
VI	22.99^{a}	33.54^{a}	46.03^{b}	20.77^{b}	68.53^{a}	77.06^{a}
SD	4.04	4.81	19.49	9.33	5.08	4.34
P	0.000	0.000	0.000	0.000	0.000	0.000

Means with different superscripts in a column differ significantly

Apparent metabolizability coffiecients of dietary treatments are illustered in Table (3).Metabolizability of CP (P<0.05), EE (P<0.01) and CF(P<0.05) were significantly differe among experimental treatments and the data indicated that all these nutrient metabolizability values increased for hen fed ginger and black cumin alone and in combination as compared to the control diet. Dietary supplementation of different levels of these herb have no significant effect on DM metabolizability. Significantly (P<0.01)highest NFE metabolizability was observed in T3treatment group, whereas it was lowest in T9 treatment group.

The main effect black cumin indcated that the metabolizability of DM and proximate principles influenced significantlyand these were highest at 1 per cent level. Similarly,due to main effect of ginger, only crude protein and NFE metabolizability were differ significantly among different levels and these metabolizability were highest at 1 per cent level of ginger.

Table 3. Effect of supplementation of ginger root powder and black cumin seed powder on metabolizability of nutrients in White Leghorn layers(%)

Treatment groups	Dry matter	Crude protein	Ether extract	Crude fiber	NFE
T_1	56.70	34.95^{a}	78.30^{a}	16.24^{a}	77.20^{bc}
T_2	60.62	43.30^{b}	80.20^{bc}	22.15^{ab}	76.57^{ab}
T_3	60.60	41.57^{b}	81.12^{bcd}	25.93^{bc}	80.06^{d}
T_4	60.56	44.09^{bc}	80.69^{bc}	22.26^{ab}	76.04^{ab}
T_5	63.31	45.19^{bcd}	82.43^{de}	27.83^{bcd}	77.60^{bc}
T_6	62.77	48.59^{cd}	81.72^{cd}	26.60^{bc}	76.93^{ab}
T_7	65.94	49.89^{d}	83.61^{e}	33.00^{d}	79.70^{d}
T_8	63.69	49.14^{d}	82.57^{de}	28.57^{cd}	79.03^{cd}
T_9	58.60	41.40^{b}	79.62^{ab}	18.37^{a}	75.21^{a}
SEM	1.562	1.698	0.566	1.745	0.648
Significance	NS	S*	S**	S*	S**
Effect of black cumin					
BC 0	59.31^{a}	39.94^{a}	79.88^{a}	21.44^{a}	77.95^{b}
BC 0.5	60.64^{a}	44.69^{b}	80.67^{a}	22.41^{a}	76.06^{a}
BC 1	64.31^{b}	48.08^{c}	82.87^{b}	29.80^{b}	78.76^{b}
SEM	0.902	0.980	0.327	1.234	0.374
Significance	S*	S**	S**	S**	S**
Effect of ginger					
G 0	60.19	41.41^{a}	80.47	22.11	76.94^{a}
G 0.5	62.36	47.01^{b}	81.50	25.77	77.51^{ab}
G 1	61.71	44.29^{b}	81.45	25.77	78.32^{b}
SEM	0.902	0.980	0.327	1.234	0.374
Significance	NS	S**	NS	NS	S*

Means with different superscripts in a column differ significantly, S** = P < 0.01, S* = P < 0.05, NS= Non significant

The retention of nitrogen, calcium and phosphorus are represented in table 4. The intake of nitrogen, excretion of nitrogen and nitrogen in egg were influenced significantly (P<0.01) due to suppelmentation of ginger and black cumin among different treatments groups, however no effect was observed on nitrogen retention on herbal supplementation. On observation of mean values it was observed that highest nitrogen in egg was observed in T5 treatment group which was stastically comparable with T2 group.

Table 4. Effect of ginger and black cumin on retention of Nitrogen, Calcium and Phosphorus (g/head/day) in White Leghorn layers

Treatment groups	Nitrogen				Calcium				Phosphorus			
	Intake	Faecal	Egg	Retention	Intake	Faecal	Egg	Retention	Intake	Faecal	Egg	Retention
T_1	3.34^{cde}	2.18^{d}	0.74^{a}	0.42	6.74^{e}	4.49e	1.75^{a}	0.50^{a}	1.05	0.79	0.078	0.176
T_2	3.14^{ab}	$1.78a^{bc}$	0.87^{bc}	0.49	6.31^{abc}	2.82b	1.97^{bcd}	1.52^{cd}	1.09	0.81	0.073	0.196
T^3	3.10^{ab}	1.80^{bc}	0.86^{b}	0.43	6.22^{ab}	3.10c	2.06^{cd}	1.06^{b}	1.02	0.76	0.079	0.175
T_4	3.03^{a}	1.69^{ab}	0.84^{b}	0.49	6.08^{a}	2.21a	1.86^{ab}	2.01^{e}	1.11	0.81	0.072	0.222
T_5	3.39^{e}	1.86^{c}	0.95^{c}	0.58	6.78^{e}	2.72b	2.15^{d}	1.92^{e}	1.08	0.79	0.075	0.206
T_6	3.21^{bd}	1.65^{a}	0.84^{b}	0.72	6.44^{bcd}	3.25cd	1.94^{bc}	1.26^{bc}	1.05	0.87	0.071	0.110
T_7	3.35^{e}	1.68^{ab}	0.81^{ab}	0.85	6.68^{de}	2.26a	1.89^{abc}	2.53^{f}	1.07	0.77	0.079	0.211
T_8	3.34^{de}	1.70^{ab}	0.82^{ab}	0.81	6.67^{de}	3.03c	1.90^{abc}	1.74^{de}	1.04	0.83	0.071	0.134
T_9	3.27^{cde}	1.92^{c}	0.79^{ab}	0.560	6.55^{cde}	3.39d	1.85^{ab}	1.30^{bc}	1.10	0.78	0.080	0.230
SEM	0.046	0.052	0.028	0.06	0.092	0.091	0.064	0.199	0.022	1.69	0.003	1.745
Significance	S**	S**	S**	NS	S**	S**	S**	S**	NS	NS	NS	NS
Effect of black cumin												
BC 0	3.19^{a}	1.92^{b}	0.825	0.450^{a}	6.43^{a}	3.47c	1.93	1.03^{a}	1.05	0.793	0.0772	0.182
BC 0.5	3.17^{a}	1.75^{a}	0.826	0.590^{b}	6.36^{a}	2.95b	1.88	1.52^{b}	1.09	0.823	0.0748	0.187
BC 1	3.36^{b}	1.75^{a}	0.866	0.751^{c}	6.71^{b}	2.67a	1.98	2.06^{c}	1.06	0.804	0.0751	0.184
SEM	0.026	0.03	0.016	0.036	0.053	0.053	0.037	0.69	0.013	0.980	0.001	1.23
Significance	S**	S**	NS	S**	S**	S**	NS	S**	NS	NS	NS	NS
Effect of ginger												
G 0	3.26	1.91^{c}	0.846	0.499^{a}	6.53	3.14b	1.92	1.48	1.08	0.801	0.075^{a}	0.226
G 0.5	3.23	1.71^{a}	0.847	0.675^{b}	6.47	3.03ab	1.94	1.51	1.06	0.842	0.071^{a}	0.174
G 1	3.24	1.80^{b}	0.825	0.617^{b}	6.48	2.92a	1.94	1.63	1.06	0.777	0.079^{b}	0.196
SEM	0.026	0.03	0.016	0.036	0.053	0.053	0.037	0.69	0.013	0.980	0.001	1.234
Significance	NS	S**	NS	S**	NS	S*	NS	NS	NS	NS	S**	NS

Means with different superscripts in a column differ significantly, S** = P < 0.01, S* = P < 0.05, NS= Non significant

Supplementation of ginger and black cumin have significant (P<0.01) effect on calcium intake, excretion of calcium, calcium content of egg and calcium retention among different treatmen groups. The calcium content in egg was highest in T5 treatment group which was compable with T2 and T3 group. the retention of calcium was highest in T4 tretment group which was comparable with T5 and T8 groups.Non-significant effect was recorded on phosphorus inatake, excterion of phosphorus, phosphorus in egg and retention among different treatment groups on supplemnation of herbs alone and in combination.

On observing main effect it can be stated that, black cumin have significant effect (P<0.01) on nitrogen and calcium intake, their excretion in faeces and retention. Supplementation of black cumin in laying hen diet significantly increased nitrogen and calcium intake along with decrease their excretion in faeces which indicates better availability and retention of these nutrients in body. Highest nitrogen and calcium retention were observed at 1 percent level of black cumin. Phosphorus intake, its excretion and retention and phosphorus of egg were non-significant (P>0.01) among different levels of black cumin. Ginger and black cumin supplementation in laying hen diet have no effect on nitrogen and calcium content of egg.

Due to main effectof ginger supplementation, the nitrogen and calcium excretion in faeces reduced significantly (P<0.01). Lowest nitrogen and calcium excretion was recorded at 0.5 and 1 per cent level of ginger. Nitrogen retention was significantly increased due to supplementation of ginger in laying hen diet. Phosphorus content of egg was significantly (P<0.01) highest at 1 per cent level of ginger.The effecton nutrient utilization on herbal feed supplementation could be due to presence of isoprene derivatives, flavonoids, glucosinolates and other plant metabolites which stabilize the intestinal microflora, control the pathogenic bacteria, modulate the intestinal morpho-physiology and increase the enzyamatic activity, balance of gut microbial ecosystem and stimulation of endogenous digestive enzymes. The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism (Jamroz *et al.*, 2003). Issa and Omar (2012) compared the effect of supplementation of garlic at 0.2% and 0.4% level in the feed of broiler and reported that digestibility of total tract DM, CP and EE digestibility were improved (P<0.05) as compared to that in the control diet.

No difference in feed intake was observed among different treatment groups on ginger and black cumin supplementation in hen diet. On observinng the main effect, non-significant effect was recorded on feed intake among different levels of black cumin. However, ginger supplementation in laying hen diet resulted in significantly (P<0.05) lower feed intake at 0.5 and 1 per cent level as compared non-supplimentation (Table 5).Significantly (P<0.01) highest egg production was observed in T5 treatment group i.e. group having 1 per cent level of black cumin. On observing the individual effect, significantly highest egg production was recorded at 1 per cent level of black cumin, whereas due to ginger, there was significant (P<0.01) decline in egg production at 0.5 and 1 percent level as compared to non-supplementation.

Supplementation of ginger and black cumin among different treatment groups and due to main effect of both the herbs no effect was observed on feed conversion ratio in terms of kilogram of feed consumed to produce one kilogram of egg mass.

Table 5. Effect of supplementation of ginger root powder and black cumin seed powder on performance of White Leghorn layers

Treatment groups	Feed intake (g)	Egg production (g)	FCR (Kg feed/ Kg egg mass)
T_1	112.0	82.40^{a}	2.38
T_2	111.1	86.16^{cd}	2.28
T_3	108.4	86.24^{cd}	2.19
T_4	111.8	87.35^{d}	2.28
T_5	117.0	94.85^{e}	2.15
T_6	107.4	87.28^{d}	2.23
T_7	107.5	84.54^{bc}	2.26
T_8	107.5	84.84^{bc}	2.28
T_9	105.9	83.20^{ab}	2.24
SEM	2.643	0.603	0.062
Significance	NS	S**	NS
Effect of black cumin			
BC 0	110.5	84.93^{a}	2.28
BC 0.5	108.4	85.93^{a}	2.25
BC 1	110.6	88.07^{b}	2.23
SEM	1.526	0.348**	0.036
Significance	NS	S**	NS
Effect of ginger			
G 0	113.6^{b}	88.19^{c}	2.27
G 0.5	108.6^{a}	86.08^{b}	2.26
G 1	107.3^{a}	84.65^{a}	2.23
SEM	1.526*	0.348**	0.036
Significance	S*	S**	NS

Means with different superscripts in a column differ significantly, S** = P < 0.01, S* = P < 0.05, NS= Non significant

In present study the egg production on ginger supplementation was in correspondence with the result of feed intake tended to decreases with supplementation of higher level of supplementation. The effect of herbal supplementation on feed intake may be dependent on its dietary level, taste and birds age. Bitter taste of phenolic terpens present in ginger may also attribute to reduction in feed intake (Rahman *et al.*, 2013). The observed increase in egg production on black cumin supplementation was probably due to the presence of essential nutrients such as crude protein, essential fatty acids, minerals and carbohydrates in black cumin that have resulted in better laying performance. Alagawany *et al.* (2020) stated that feeding quails on rations enriched with

herbal oil of black and red pepper at 0.4, 0.8, 1.2 and 1.6 g/kg diet has linearly improve liver functions. It is also possible that antioxidant property of black cumin might have increase the secretion of egg yolk precursor's from liver, by preserving hepatocytes from oxidative damage. In laying hens under stress of high ambient temperature, the feed conversion ratio was improved by feeding of Cuminum cyminum L., however, feed intake and egg mass were not affected (Saleh *et al.,* 2019). No difference was observed on feed intake whereas the increase in egg production was due to positive effects of black cumin on gastric secretion and digestive enzyme activities along with antioxidant property results in better feed conversion ratio. Boka *et al.* (2014) aslo reported that dietary inclusion of black cumin decreased ($P<0.05$) the concentration of serum cholesterol and triglycerides and increased ($P<0.05$) serum HDL concentration. These effects might be attributed due to the reduction in small intestinal cholesterol reabsorption (Brunton, 1996), hypocholesterolaemic activity of herbs by inhibiting hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (El-Dakhakhny *et al.,* 2000), suppress of lipid peroxidation and enhancement of clearance of endogenous cholesterol via bile acid excretion due to presence of thymoquinone (Badadry *et al.,* 2000). El-Ratel *et al.* (2020) also reported enhanced lipid profile, liver function, immunity and antioxidant activity, with reducing liver lipid peroxidation of heat-stressed growing rabbits on supplementation of herbal feed additive curcumin in diet as comared to control.

The values of hemoglobin and pack cell volume, total erythrocyte count, total leucocyte count, differential leucocyte count, H/L ratio, weight of immune organs viz. spleen, bursa and thymus and serum enzyme parameters viz. ALP, ALT and AST were in the normal physiological range (Banerjee, 1998) and supplementation of both the herbs have no effect on these parameters of laying hens (table 6 and 7).

On observing the main effect of ginger, there was significant reduction in serum glucose at 0.5 per cent level, however serum glucose was comparable between 0.5 per cent level and non-supplementation. However, black cumin at different level in diet showsno difference in serum glucose among different treatment groups. Supplementation of both the herbs resulted in significant decline in serum cholesterol and triglycerides.

Zomrawi *et al.* (2013) supplemented 1, 1.5 and 2 percent level of ginger root powder in broiler diet and reported no effect ($P > 0.05$) on Hb, PCV, RBC percentage, serum triglyceride and inorganic phosphorus, however, serum glucose, cholesterol and calcium decreased significant in group received 2% ginger root powder in diet.

Table 6. Effect of supplementation of ginger root powder and black cumin seed powder on haematological profile of White Leghorn layers

Treatment groups	Hb (g/dl)	PCV (%)	TEC (106/cumm)	TLC (103/cumm)	H/L ratio	Lymphocyte (%)	Heterophils (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
T_1	9.04	26.09	2.69	29.43	0.39	65.47	25.55	3.19	3.12	2.05
T_2	9.33	26.85	2.68	29.53	0.38	66.89	25.42	2.86	3.17	2.05
T_3	9.50	27.14	2.70	29.57	0.38	66.32	25.41	3.19	3.14	2.04
T_4	9.03	26.09	2.70	29.40	0.38	66.20	25.09	3.35	3.18	2.03
T_5	9.57	27.36	2.70	29.60	0.37	67.23	24.93	3.09	3.11	2.04
T_6	9.18	26.56	2.70	29.42	0.37	66.90	24.84	3.16	3.19	2.04
T_7	9.63	27.53	2.71	29.62	0.37	65.95	24.58	3.22	3.24	2.04
T_8	8.97	25.97	2.68	29.45	0.36	66.73	24.55	3.23	3.04	2.04
T_9	9.63	27.59	2.72	29.48	0.37	66.84	25.30	3.24	3.26	2.04
SEM	0.307	0.793	0.023	0.098	0.011	0.767	0.637	0.211	0.154	0.012
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Effect of black cumin										
BC 0	9.29	26.69	2.69	29.51	0.38	66.23	25.46	3.08	3.14	2.049
BC 0.5	9.28	26.75	2.71	29.43	0.37	66.65	25.08	3.25	3.21	2.041
BC 1	9.39	26.95	2.70	29.56	0.37	66.64	24.69	3.18	3.13	2.047
SEM	0.177	0.458	0.013	0.057	0.006	0.443	0.368	0.122	0.089	0.007
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Effect of ginger										
G 0	9.21	26.51	2.701	29.48	0.38	66.30	25.19	3.21	3.13	2.04
G 0.5	9.16	26.46	2.694	29.47	0.37	66.84	24.94	3.08	3.13	2.04
G 1	9.58	27.42	2.716	29.56	0.37	66.37	25.09	3.22	3.21	2.04
SEM	0.177	0.458	0.013	0.057	0.006	0.443	0.368	0.122	0.089	0.007
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS= Non significant

Table 7. Effect of supplementation of ginger root powder and black cumin seed powder on immune organs and serum biochemical parameters of White Leghorn layers

Treatment groups	Immune organs			Serum biochemical parameters					
	Spleen (%)	Bursa (%)	Thymus (%)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
T_1	0.081	0.075	0.156	139.7	245.2	193.9	262.4	6.00	247.0
T_2	0.054	0.080	0.173	118.8	238.1	185.5	252.2	5.85	238.9
T_3	0.061	0.079	0.190	114.2	239.6	197.4	248.2	5.88	243.1
T_4	0.076	0.077	0.189	114.1	235.0	192.7	247.7	5.75	245.6
T_5	0.062	0.082	0.190	108.9	232.6	196.8	245.4	5.51	241.2
T_6	0.057	0.078	0.165	100.9	221.5	190.1	250.9	5.37	242.5
T_7	0.069	0.076	0.184	101.4	225.9	197.9	249.3	5.45	241.8
T_8	0.065	0.076	0.185	105.1	230.8	187.1	258.1	5.52	248.5
T_9	0.066	0.080	0.188	101.1	221.5	190.4	248.7	5.48	241.8
SEM	0.008	0.002	0.008	5.91	3.78	3.81	5.38	2.32	3.60
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Effect of black cumin									
BC 0	0.065	0.078	0.173	124.2^b	241.0^b	192.3	254.3	5.91	243.0
BC 0.5	0.066	0.078	0.181	105.4^a	226.0^a	191.1	249.1	5.54	243.3
BC 1	0.065	0.078	0.187	105.1^a	229.8^a	193.9	250.9	5.49	243.8
SEM	0.005	0.001	0.004	3.41**	2.188**	2.20	3.10	1.34	2.08
Significance	NS	NS	NS	S**	S**	NS	NS	NS	NS
Effect of ginger									
G 0	0.073	0.078	0.179	120.9^b	237.6^b	194.5^b	251.8	5.75	244.6
G 0.5	0.059	0.078	0.174	108.3^a	230.1^a	187.6^a	253.7	5.58	243.3
G 1	0.065	0.078	0.188	105.6^a	229.0^a	195.2^b	248.7	5.60	242.3
SEM	0.005	0.001	0.004	3.417**	2.188*	2.201*	3.109	1.34	2.08
Significance	NS	NS	NS	S**	S*	S*	NS	NS	NS

Means with different superscripts in a column differ significantly, S** = P < 0.01, S* = P < 0.05, NS= Non significant

CONCLUSION

In conclusion, the results indicate that the dietary supplementation of ginger and black cumin improved the crude protein, ether extract and crude fiber metabolizability, retention of calcium and egg production among different treatment groups as compared to control and highest egg production was recorded in T5 tratment group i.e. group supplemented with 1 per cent level of black cumin.

Further, on observingthe main effect of ginger, there was increase in metabolizability of crude protein and nitrogen retention, decline in faecal excretion of nitrogen and decline in egg production were observed in ginger supplemented group as compared to non-supplementation.Further, on study the main effect of black cuminit was obsercved that highestmetabolizability of dry matter,crude protein, ether extract and crude fiber, highest nitrogen intake, nitrogen retention, calcium inatke and retention with decline incalcium excretion and increase in the egg production were observed at 1 per cent level of black cumin.

On observing the effect of ginger and black cumin among different treatment groups and main effect it can be concluded that supplementation of black cumin at 1% level in laying hen diet might be promising for profitable poultry production.

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Dietary Inclusion of Feed Additives in Broilers: Effect on Carcass Characteristics, Visceral and Lymphoid Organs and Gut Health

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ABSTRACT

The present experiment was conducted on 288-day-old unsexed broiler chicks to evaluate the effect of dietary feed additives viz. neem leaf meal (NLM) and citric acid (CA) on carcass characteristics, visceral and lymphoid organs, and gut health in broiler chickens. The chicks were randomly distributed into 6 dietary groups of 48 birds per group, each having 4 replicates (12 birds per replicate) in a randomized block design. The birds were fed on a standard diet without any supplementation control (T0); and with supplementation of NLM 2.5 g/kg of feed (T1); CA 15 g/kg of feed (T2); CA 25 g/kg of feed (T3); NLM 2.5 g + CA 15 g/kg of feed (T4); NLM 2.5 g + CA 25 g/kg of feed (T5). Eight birds from each treatment (2 per replicate) were randomly selected for slaughter and collection of samples and microbial study at day 42. The yield of the carcass, cut-up parts, and visceral organs, and length of the small intestine revealed comparable values without any adverse effect in the groups. The caecal E. coli population reduced significantly ($P<0.05$) in treatments T2 and T3 as compared to T0. The lower ($P<0.05$) caecal pH was observed in groups T1, T2, T3, T4, and T5 than T0. It can be concluded that feeding CA at 15 g and 25 g/kg of feed improved gut health without any adverse effect on the carcass and internal organs. Hence, supplementation of CA at two dietary levels i.e. 15 g and 25 g per kg of feed could be an alternative to produce healthy chickens.

Key words: Broiler, Carcass, Caecal pH, Citric acid, Gut health, Neem leaf meal

INTRODUCTION

Chickens are the major source of high-quality cheaper animal proteins in a shorter time span. At present, the demand for such cost-effective and high-valued food has been raised enormously due to the ever-growing human population, shifting food habits, and improved purchasing ability of consumers across the world. Hence, to bridge up the gap between demand and supply, cost-effective broiler production using feed additives (Huyghebaert *et al.*, 2011) is of paramount importance. The balanced feed followed by efficient nutrient utilization has significantly exploited the production potential of chickens. Among several factors, 'gut health' is the most vital which significantly affects the productivity and feed efficiency of broilers (Gracia *et al.*, 2007). In poultry, gut microbiota regulates intestinal health by modulating host physiology, mainly through the competitive exclusion of harmful microbes by preventing colonization (Diaz Carrasco *et al.*, 2019). The poultry gut is a vital organ system and plays a key role in digestion and assimilation of feed nutrients besides, it also regulates the immune system of the birds. Apart from nutrient absorption, intestinal mucosa acts as an effective barrier between lumen contents and host splanchnic tissues, and hence; it is a key determinant of gut health and performance of birds (Rinttila and Apajalahti, 2013). For proper functioning of the mucosal barrier, a dynamic balance between mucus layer, epithelial cells, microbiota, and immune cells is essential which might be influenced by diet and microbes affecting the health and productivity of chickens (Sugiharto, 2016).

Currently, a higher growth rate and efficient feed

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conversion are two major goals of the broiler industry. To balance the gut ecosystem for higher growth (Huyghebaert *et al.*, 2011) a subclinical level of antimicrobials has been widely practiced in the poultry sector (Murugesan *et al.*, 2015). But currently, indiscriminate use of antibiotic growth promoters in the poultry industry has been widely criticized due to the development of anti-microbial resistance and transfer of antibiotic resistance genes from animal to human microbes, posing potential threats to human health. Further, withdrawal of such antimicrobial feed additives from poultry feeding reduced the production and increased morbidity (Huyghebaert *et al.*, 2011) and mortality in commercial flocks (Dibner and Richards, 2005). Hence, after the ban on the use of antimicrobials in food species by the European Union, researchers are compelled to explore alternative growth promoters (Pearlin *et al.*, 2020). At present, the broiler industry is looking for some non-therapeutic feed additives to replace the antibiotics for achieving higher gains through efficient feed conversion and less input cost. Therefore, keeping in view the above-cited problems and significance of novel feed additives in poultry, the present study was designed to evaluate the impact of dietary NLM and CA on the carcass, visceral and lymphoid organs, and gut health in broilers.

MATERIALS AND METHODS

The present experiment was conducted at Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari Agricultural University, Campus, Navsari, Gujarat. In this study, two different feed additives like NLM and CA were utilized to formulate experimental diets as per standards of BIS (2007) to meet their energy and protein requirements during the pre-starter, starter, and finisher phases (Table 1). To prepare NLM, medium-sized fresh green neem leaves were harvested within the university campus, dried in a hot air oven (600C for 12 h), and milled to a fine powder. The CA, a crystalline powder was supplied by Aashi Chem, Surat, India. The experimental diets were prepared by incorporating and proper mixing of NLM and CA as per the experimental protocol. The proximate analysis of experiential diets was carried out to determine the dry matter, crude protein, crude fibre, ether extract, total ash, and nitrogen-free extract (Table 1) as per methods described in AOAC (2011).

Table 1. Ingredients and chemical composition of experimental broiler diets

	Experimental diet		
Ingredient (%)	**Pre-starter**	**Starter**	**Finisher**
Maize	51.300	53.900	54.080
De-oiled rice bran	1.000	1.150	9.170
De-oiled soybean cake	42.300	39.550	30.600
Calcite powder	2.235	2.235	1.650
Di-calcium phosphate	2.000	2.000	1.350
Trace mineral*	0.100	0.100	0.000
Lysine	0.100	0.100	0.100
D.L.-methionine	0.135	0.135	0.100
Metabolic activator	0.100	0.100	0.100
Enzyme	0.050	0.050	0.050
Salt	0.300	0.300	0.400
Toxic binder	0.100	0.100	0.100
Maduramycine-1%	0.050	0.050	0.050
Furazolidone	0.030	0.030	0.050
Vegetable oil	0.000	0.000	2.000
Vitamin and mineral supplement**	0.200	0.200	0.200
Chemical composition (%)			
Crude protein	26.89	22.89	21.61
Crude fiber	4.21	4.18	4.32
Total ash	6.53	5.83	6.69
Nitrogen free extract	58.1	61.9	61.0
Ether extract	4.28	5.21	6.35

*Trace minerals: Each kg contains: Copper-15 g, Iodine-1 g, Iron-60 g, Manganese- 80 g, Selenium- 0.3 g, Zinc- 80 g; Metabolic activator: Lecithin extract treated with co enzyme; Enzymes: Each gram contains: Xylanase-2000 IU, Amylase-400 IU, Protease- 4000 IU, Cellulase-500 IU; Toxin Binder: Selected silicates, surfactants, organic acids and salts of organic acids; **Vitamin and mineral supplement: Each 2 kg contains: Vit. A-50 lakh IU, Vit. B2- 2 g, Vit. B6- 0.4 g, Vit. B12- 5600 mcg, Vit. E- 800 IU, Iron- 7.5 g, Vit. D3- 6.25 lakh IU, Choline chloride- 10 g, Copper- 2 g, Iodine- 1 g, Zinc- 15 g, Manganese- 27.5 g, Calcium- 27.25 %, phosphorus- 7.45 %, Calcium pantothenate- 4 g.

A total of 288 day-old vaccinated straight run broiler chicks (Vencobb- 400) were procured from Venky's Hatchery Pvt. Ltd., Anand. These were weighed individually and randomly allocated into six dietary groups based on their body weight. Each group comprised of 48 chicks was further replicated 4 times with 12 chicks per replicate in randomized block design. The grouped birds were fed on a standard diet without any supplementation control (T0); and with supplementation of NLM 2.5 g/kg of feed (T1); CA 15 g/kg of feed (T2); CA 25 g/kg of feed (T3); NLM 2.5g + CA 15 g/kg of feed (T4) and NLM 2.5 g + CA 25 g/kg of feed (T5).

The experimental birds were raised on deep litter (rice husk) with standard managemental practices for floor space, temperature, relative humidity, ventilation, lighting, feeding, watering, and other routine bio-security aspects. Brooding temperature maintained at 34±1°C up to 7 days of age and later on decreased by 3°C per week till it reaches 21°C. A photoperiod of 24 h was provided to the entire flock throughout the experiment. The experimental birds were fed ad-lib using experimental diets. A measured quantity of feed and water was offered twice daily at 08:00 and 18:00 h throughout the experimental period. At the end of the experiment (42 days of age), 2 birds per replicate were randomly selected, fasted overnight, and sacrificed through cutting carotid arteries and partial slicing of the neck. The following measurements were taken to examine the carcass and visceral organs such as pre-slaughter live weight, eviscerated weight, shank, head, neck, liver, heart, empty gizzard, pancreas, spleen, thymus and bursa abdominal fat pad and empty intestine weights, etc. All these were calculated as such (in grams) and as a percent of the live weight of the bird. The different segments of the small intestine like duodenum, jejunum, and ileum were dissected and washed in normal saline to record their length and mass. To measure the pH and microbial counts, samples of caecal contents (1-2 g) were collected into a 2 ml safe lock Eppendorf tube and kept on ice until they were analyzed. The population of E. coli was estimated as the log10 of colony-forming units (cfu) per gram of caecal contents. The E. coli was cultured with Eosin Methylene Blue (EMB) agar (Himedia Laboratories Pvt. Ltd., Mumbai, India) at 37°C for 48 h under aerobic conditions. The colonization of E. coli on EMB agar was indicated by a distinctive metallic green sheen due to the metachromatic properties of the dyes. The colonies of E. coli were counted under the digital colony counter and expressed appropriately (log10×106 cfu/g). After sampling for enumeration of microbial population, caecal contents were drained into a small beaker for estimation of caecal pH with help of a digital pH meter (PHTestr 30, Eutech Instruments Oakton, Thermo Fisher Scientific, USA).

The data were subjected to analysis of variance using Software Package for Social Sciences (SPSS, trial version 20.0, Chicago, USA) and the corresponding means were compared by Tukey-Krammer test at $P < 0.05$.

RESULTS AND DISCUSSION

The perusal of Table 2 revealed that broilers supplemented with NLM and CA did not influence the carcass traits, which might be attributed to the fact that carcass traits are mostly governed by the genotype of individual and not much affected by the environment. Hence, these are considered as the most consistent attributes and least affected by dietary interventions. In agreement with these results, previous studies reported that supplementation of NLM (Egbeyale *et al.,* 2020; Ubua *et al.,* 2019), aqueous fresh neem leaf extract (NLE) (Shaahu *et al.,* 2020) and organic acid (OA) (Kopecký *et al.,* 2012) did not influence live and dressed body weight in broilers. However, contradictory findings were reported by Obun *et al.* (2013) and Hassan *et al.* (2016) in broilers

supplemented with NLM and CA, respectively. The nonresponsive impact of NLM and CA on carcass yield (Table 2) is supported by several authors in broilers supplemented with NLM (Shihab *et al.*, 2017; Egbeyale *et al.*, 2020), aqueous fresh NLE (Shaahu *et al.*, 2020), neem seed oil (Sonhafouo *et al.*, 2019), CA (Hassan *et al.*, 2016) and OA (Fathi *et al.*, 2016) in their diets. However, in contrast to our findings, several researchers stated that feeding of NLM (Singh *et al.*, 2015), NLE (Paul *et al.*, 2020), and OA (ELnaggar *et al.*, 2017), significantly improved the carcass yield of broilers. The yield of cut-up parts did not differ significantly among the groups (Table 2), which is in line with the previous studies which indicated that supplementation of NLM (Ubua *et al.*, 2019), aqueous fresh NLE (Shaahu *et al.*, 2020), and CA (Kopecky *et al.*, 2012) had no impact on the weight of cut-up parts in chickens. Inconsistently, Singh *et al.* (2015) observed higher cut-up parts yield in guinea fowl supplemented with neem leaf powder and increased breast weight was reported by Fik *et al.* (2020) in broilers supplemented with CA.

Table 2. Effect on carcass characteristics of broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Live wt. (g/bird)	2550±64	2625±60	2650±45	2587±46	2643±40	2612±83	0.83
Dressed wt. (g/bird)	1731±43	1768±57	1842±39	1762±33	1785±43	1825±69	0.66
Carcass yield (%)	67.8±0.15	67.3±0.75	69.5±0.94	68.1±0.38	67.5±0.96	69.8±1.93	0.44
Head wt. (g)	52.8±1.78	53.7±1.23	55.5±2.37	52.0±0.84	53.3±1.38	54.5±3.09	0.80
Relative head wt. (LW %)	2.08±0.11	2.04±0.02	2.09±0.07	2.01±0.04	2.01±0.04	2.08±0.08	0.91
Leg wt. (g)	89.8±5.58	82.2±2.13	94.8±5.88	86.3±2.90	89.0±3.85	98.0±5.71	0.29
Relative legs wt. (LW %)	3.54±0.29	3.13±0.09	3.57±0.17	3.34±0.15	3.36±0.09	3.74±0.14	0.23
Cut up parts							
Neck wt. (g)	70.1±3.33	73.6±2.44	79.1±2.20	71.6±4.46	74.0±2.74	76.1±3.16	0.46
Relative neck wt. (LW %)	2.74±0.10	2.80±0.08	2.99±0.11	2.76±0.13	2.79±0.07	2.91±0.12	0.61
Back wt. (g)	179.8±9.2	177.7±7.8	189.8±6.8	180.5±11.	184.2±15.3	179.0±8.5	0.96
Relative back wt. (LW %)	7.04±0.25	6.76±0.23	7.16±0.23	7.00±0.56	6.94±0.48	6.85±0.26	0.97
Thigh wt. (g)	255.7±4.7	27.50±8.9	266.3±14.2	251.6±13.21	268.7±11.86	257.3±9.15	0.68
Relative thigh wt. (LW %)	10.03±0.15	10.49±0.16	10.04±0.44	9.74±0.60	10.15±0.32	9.85±0.19	0.78
Breast wt. (g)	511.7±17.3	539.7±16.6	564.0±17.8	506.8±6.9	486.1±31.6	492.3±11.8	0.09
Relative breast wt.	20.0±0.56	20.5±0.35	21.2±0.52	19.6±0.59	18.3±1.05	18.9±0.98	0.09
Drumstick wt. (g) (LW %)	219.2±9.14	217.1±9.49	234.8±2.82	227.3±3.13	237.0±6.94	229.5±8.52	0.33
Relative drumstick wt. (LW %)	8.59±0.27	8.26±0.25	8.87±0.26	8.78±0.06	8.95±0.15	8.80±0.39	0.53
Wing wt. (g)	59.2±2.03	65.1±2.55	69.6±4.72	65.2±2.24	62.7±1.27	64.2±1.85	0.29
Relative wing wt. (LW %)	2.32±0.08	2.48±0.07	2.62±0.17	2.52±0.12	2.37±0.04	2.46±0.10	0.48

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

Table 3. Effect of NLM and CA supplementation on visceral organs yield in broilers

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Liver wt. (g)	45.0±2.09	45.6±1.43	46.8±1.88	43.2±2.24	46.7±2.42	40.8±4.27	0.496
Relative liver wt. (LW %)	1.76±0.05	1.74±0.08	1.76±0.06	1.66±0.06	1.77±0.10	1.55±0.14	0.409
Heart weight (g)	13.5±0.79	14.1±0.47	14.1±0.72	13.7±0.52	13.0±0.54	13.1±0.69	0.658
Relative heart wt. (LW %)	0.52±0.03	0.53±0.02	0.53±0.03	0.53±0.02	0.49±0.01	0.50±0.02	0.500
Pancreas wt. (g)	3.75±0.32	3.62±0.31	3.50±0.20	3.87±0.24	3.12±0.24	3.87±0.13	0.384
Relative pancreas wt. (LW %)	0.14±0.01	0.13±0.01	0.13±0.01	0.15±0.01	0.11±0.01	0.14±0.01	0.313
Giblets wt. (g)	92.0±3.52	92.2±4.37	97.1±5.22	96.1±3.47	94.2±1.94	90.6±5.65	0.892
Relative giblets wt. (LW %)	3.60±0.06	3.51±0.17	3.66±0.15	3.71±0.08	3.57±0.12	3.46±0.18	0.841
Abdominal fat wt. (g)	29.5±2.72	19.6±2.65	27.0±0.68	23.7±3.20	22.6±4.60	26.5±3.43	0.401
Relative abdominal fat wt. (LW %)	1.15±0.10	0.75±0.12	1.02±0.04	0.91±0.12	0.86±0.18	1.01±0.12	0.393
Gizzard wt. (g)	33.5±1.40	32.5±2.94	36.1±3.43	39.1±1.14	34.5±3.46	36.6±1.64	0.567
Relative gizzard wt. (LW %)	1.31±0.02	1.23±0.11	1.35±0.11	1.51±0.04	1.30±0.14	1.40±0.07	0.450
Duodenum wt. (g)	11.6±1.33	11.8±0.43	12.1±0.55	10.2±0.78	10.7±0.52	10.3±0.66	0.274
Relative duodenum wt. (LW %)	0.45±0.04	0.45±0.01	0.45±0.03	0.39±0.04	0.40±0.01	0.39±0.02	0.241
Jejunum wt. (g)	39.7±0.63	40.2±1.89	37.8±1.66	40.1±2.55	39.7±2.29	43.1±4.90	0.778
Relative jejunum wt. (LW %)	1.56±0.06	1.53±0.06	1.43±0.07	1.54±0.09	1.50±0.08	1.64±0.17	0.594
Ileum wt. (g)	4.87±0.31	4.62±0.47	4.75±0.72	4.12±0.31	4.62±0.63	4.62±0.55	0.847
Relative ileum wt. (LW %)	0.19±0.01	0.17±0.02	0.18±0.03	0.15±0.01	0.17±0.02	0.17±0.02	0.806
Small intestine wt. (g)	54.1±3.20	55.0±2.12	54.7±2.73	51.8±3.84	55.1±3.24	52.8±3.80	0.936
Relative small intestine wt. (LW %)	2.11±0.09	2.09±0.07	2.07±0.12	2.00±0.15	2.08±0.11	2.01±0.11	0.928

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

Likewise, past researches reported that feeding of NLM (Ubua *et al.,* 2019; Egbeyale *et al.,* 2020), aqueous fresh NLE (Shaahu *et al.,* 2020), and OA (ELnaggar *et al.,* 2017) did not influence the yield of visceral organs in broilers. In disagreement to these results, Paul *et al.* (2020) and Abou–Ashour, *et al.* (2021) observed higher weights of different visceral organs of chickens fed on NLE and CA, respectively. In this study, the weight of lymphoid organs was comparable among the groups (Table 4) which is in accordance with the findings of Egbeyale *et al.* (2020) and Hassan *et al.* (2016) in broilers supplemented with NLM and CA, respectively. However, a higher weight of lymphoid organs was observed by Ansari *et al.* (2012) and Abou-Ashour *et al.* (2021) in broilers supplemented with NLM and CA, respectively.

Table 4. Effect on lymphoid organs weight in broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Thymus wt. (g)	4.75±0.48	5.50±0.50	5.25±0.75	5.75±0.48	5.75±0.85	5.50±0.50	0.806
Relative thymus wt. (LW %)	0.19±0.01	0.21±0.01	0.20±0.02	0.22±0.02	0.22±0.03	0.22±0.02	0.902
Bursa wt. (g)	6.00±0.00	6.75±0.25	6.00±0.00	6.25±0.25	6.00±0.00	6.50±0.29	0.072
Relative bursa wt. (LW %)	0.22±0.01	0.25±0.01	0.23±0.01	0.24±0.01	0.23±0.01	0.24±0.01	0.281
Spleen wt. (g)	3.75±0.85	4.50±0.29	4.25±0.25	4.50±0.50	3.50±0.29	3.75±0.48	0.597
Relative spleen wt. (LW %)	0.14±0.03	0.17±0.01	0.15±0.01	0.16±0.01	0.13±0.01	0.13±0.02	0.504

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

The measurement of the small intestine were found comparable in the groups (Table 5) which is supported by several researchers who reported that dietary inclusion of NLM (Ubua *et al.*, 2019; Egbeyale *et al.*, 2020), neem seed oil (Sonhafouo *et al.*, 2019) and acetic acid (AA) (Sorathia, 2016) in broilers did not influence the length of the small intestine. However, Nourmohammadi *et al.* (2010) reported increased (P<0.01) jejunal length in broilers supplemented with 6% CA.

Table 5. Effect of supplementation of NLM and CA on length (cm) of small intestine in broilers

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Duodenum	26.5±0.95	26.8±0.72	28.6±0.92	28.5±0.84	27.3±1.42	27.8±1.01	0.452
Jejunum	109.8±3.39	112.8±1.14	106.1±3.94	110.5±5.83	111.7±3.25	111.8±1.88	0.820
Ileum	12.4±0.19	13.0±0.99	12.6±0.37	13.3±0.66	13.3±0.52	12.3±0.52	0.758
Small intestine	148.8±3.45	152.8±0.81	147.5±4.10	152.3±5.87	152.5±4.02	152.0±2.47	0.883

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

The data presented in Table 6 revealed a significant (P<0.05) fall in caecal E. coli counts in groups T2 and T3 than T0. Similar results were found in broilers supplemented with CA (ELnaggar *et al.*, 2017), OA (Fathi *et al.*, 2016; Eftekhari *et al.*, 2015), and OA blend (Sultan *et al.* 2015). Likewise, the non-responsive impact of NLM on the E. coli population of the present study is in agreement with the findings of da Silva Assunção *et al.* (2019) and Vanessa *et al.* (2019) in broilers supplemented with NLM and neem oil, respectively. In contrast to these results, Esmaeilipour *et al.* (2012) observed a non-significant effect of OA on the E. coli population in broilers. The inhibitory effect of CA on gut E. coli might be due to the lowering of the intestinal pH through dietary acidification. Acidifiers in the feed inhibit the growth of pathogenic bacteria by altering the gut pH and hence, reducing the microbial competition for host nutrients. The proliferation of most pH-sensitive bacteria like E. coli, Salmonella, and Clostridium is minimized under acidic pH. The organic acids (undissociated form) penetrate the bacterial cell and after dissociation cause lowering of cytoplasmic pH. At lower pH, the enzymatic reactions of glycolysis signal transductions and nutrients transport to the microbes are impeded causing energy deprivation on its effort to recoup the normal pH (Mroz *et al.*, 2006). Further, the trapped anions of the acids cause toxicity and disrupt bacterial membranes (Russel, 1992).

Table 6. Caecal microbial count (log10 × 10 6 cfu/g) and pH of broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
E. coli	11.7±1.11[b]	9.75±1.92[b]	7.02±0.02[a]	6.75±0.14[a]	9.87±0.59[b]	9.75±0.63[b]	0.006
Caecal pH	7.15±0.08[c]	6.99±0.05[b]	6.79±0.02[a]	6.83±0.01[a]	6.78±0.02[a]	6.81±0.04[a]	0.000

[abc] values in a row bearing different superscript differ significantly (P<0.05)

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

In the present study, the caecal pH reduced significantly (P<0.05) in treatments T1, T2, T3, T4, and T5 than T0. Likewise, previous workers reported that dietary supplementation of CA (Nourmohammadi and Khosravinia, 2015), OA (Jadhao *et al.*, 2020), a mixture of propionic- acetic acid (Martínez *et al.*, 2021) and AA (Sorathia, 2016) had significantly reduced the caecal pH in broilers. However, contradictorily, Hassan *et al.* (2016) and Esmaeilipour *et al*. (2011) observed a non-responsive effect of CA on caecal pH in ducks and broilers, respectively. The low caecal pH in the present study could possibly be due to more synthesis of volatile fatty acids (VFA) such as acetic, butyric, and propionic acid as a consequence of anaerobic degradation (Jozefiak *et al.*, 2011). Moreover, large numbers of lactobacilli in the caeca add more VFA which further decrease the caecal pH in the poultry gut (García *et al.*, 2008).

CONCLUSION

It may be concluded that feeding of CA at 15 g and 25 g per kg of feed in broiler chickens may improve the gut health without any ill effect on carcass and visceral organs. Whereas, neither carcass characteristics nor intestinal health influenced by supplementation of either NLM or a combination of NLM+CA in broilers. Therefore, the inclusion of CA at two dietary levels i.e. 15 g and 25 g per kg of feed in broiler ration could be a better alternative to produce healthy broiler chickens.

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Calcium intake on Gramasree male bird's semen quality

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ABSTRACT

A study was assigned in 18 Gramasree male birds of 32nd weeks of age, randomly distributed to 3 experimental groups (G1, G2 and G3) with 6 birds in each group and housed separately in male cages for 91 days. During this experimental period, layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to all experimental male birds. Egg shell powder (ES) and shell grit (SG) having more than 2 mm particle size was given as free choice after feeding to G2 and G3 birds, respectively to meet their extra calcium requirement. The feed intake (FI) and body weight (BWT) were significantly different (P<0.01) between the treatment groups. ES and SG intake was 0.67 and 0.72 g/ rooster/day, respectively. Total calcium (Ca) intake was 3.20, 3.44 and 3.55 g/rooster/day in G1, G2 and G3, respectively. Semen was collected by abdominal massage at weekly intervals from 33rd to 44th weeks of age and analysed for semen volume, colour, sperm concentration and sperm motility and significantly no difference was found between the groups. The serum Ca and P content, tibial characteristic and tibial-ash were similar in all treatment groups but the tibial Ca content was significantly (P<0.01) higher in SG group. It can be concluded that, Ca intake (3.20 g/rooster/day) through feed is sufficient for male birds to meet their Ca requirements and extra ES, SG given as free choice feeding had not influenced the semen qualities without any detrimental effects.

Key words: Calcium, ES, Chemical composition, Gramasree Male birds, Semen quality

INTRODUCTION

In India, poultry rearing was primarily a backyard system until the early 1960s, and indigenous desi birds were mainly utilised for egg and meat production, despite their low productivity. The scenario of poultry production has transformed over the last six decades, with indigenous desi birds gradually being replaced by highly specialized improved varieties. Gramasree was one of the important germplasms developed by KAU to exploitation their productivity under field conditions. Their progenies were synthetic crosses of different breeds such as Barred Plymoth rock, New Hampshire, Rhode Island Red and desi breeds.
According to Kanyinji *et al.* (2010) feeding dietary Ca at 3.1 per cent level had shown enhanced seminal Ca level, sperm motility, thermo-tolerance and cryo-survivability due to increase in Ca seminal plasma in Barred Plymouth Rock roosters. Tyler *et al.* (2021) had mentioned high Ca level (3.05%) showed significantly poorer sperm concentration at 42 and 57 weeks of age of broiler breeder male birds.

In backyard poultry farms, usually layer breeder ration was fed to male birds, which contains more Ca content and effect of excess Ca feeding and data on Ca requirements for improved varieties of native chicken male birds are scanty. In this scenario, the aim of the present study was ascertained to find out the maximum level of Ca intake and effect on semen quality of male Gramasree birds by providing free-choice feeding of ES and SG.

MATERIALS AND METHODS

Experimental procedures were approved (approval number, IAEC/COVAS/PKD/8/2021) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the 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.

The chicken ES used in this study was collected

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from bakeries, washed in running tap water and soaked in water for 5 h. Then, shell membrane was manually removed and sundried for 48 h and dried again in hot air oven at 120°C for 24 h, powdered in laboratory mill then packed in bags for further processing. The SG was purchased from the local market.

The particle size of ES and SG was studied by taking 100 g of representative samples of egg shell and SG were manually sieved in BSS 8 (0.75 mm) and BSS 22 (2 mm) sieves for five minutes at one minute interval and quantities were weighed in electronic balance. The solubility of Ca sources was determined by in-vitro method proposed by Zhang and Coon (1997).

The experiment was conducted at Poultry Farm, ILFC, College of Veterinary and animal sciences, Pookode, during May to July'21. Eighteen Gramasree male birds of 32nd weeks of age was randomly distributed to 3 experimental groups (G1, G2 and G3) with 6 birds in each group and housed separately in male cages for 91 days. During this experimental period, layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to all experimental male birds. The ingredient composition was shown in the table-1.

Table 1. Ingredient composition of Layer breeder ration (per cent)

Raw material	Composition
Maize	54.0
De-oiled Rice Bran	5.0
Wheat Bran	4.0
Soya bean meal	25.4
Limestone powder	4.0
Shell grit	5.0
Di Calcium Phosphate	1.4
Dl-Methionine (98.5 per cent)	0.2
Salt	0.5
Trace Mineral Mix	0.125
Vitamin Premix	0.05
Choline Cholride	0.2
Toxin Binder	0.1
Liver extract powder	0.025
Analytical values	
Crude Protein (%)	17.65 ± 0.24
Crude Fibre (%)	9.26 ± 0.15
Total Ash (%)	14.99 ± 0.35
Calcium (%)	4.85 ± 0.27
Total Phosphorus (%)	1.90 ± 0.19

All the birds were maintained under standard management conditions prevailing in the farm during the entire period of experiment. The birds were provided with 2 times feed and ad-libitum water. They were exposed to a 16 h photoperiod (16 Light: 8 Dark) daily and lights were turned off manually. The feed, ES and SG intake were calculated from offered quantity and left over on daily basis and Ca intake from feed and different Ca sources were calculated at weekly interval. The body weight was taken before and after the end of the experiment.

The semen was collected in a sterile glass funnel, between 10.00 - 10.30 am by abdominal massage method (Burrows and Quinn, 1937) from four roosters housed in cages from each group at weekly interval from 33nd to 44th weeks of age. Semen

volume was measured use of a collection tube with graduation to measure 0.01 ml. The semen colour was scored as 1–5 by visual examination (Score 1-Watery or clear semen; Score 2 -Watery semen with white streaks; Score 3-Medium white semen; Score 4-Thick white semen; Score 5-Very viscous chalky white semen) by McDaniel and Craig (1959) procedure.

Immediately after semen collection, the semen was four-fold diluted with semen diluent as per Chaudhuri and Lake (1988) and stored for further semen quality analysis. Sperm motility (per cent) was examined by under the low power of light microscope (40 x) field and scored as per Wheeler and Andrews (1943). Sperm concentration was estimated as per the procedure suggested by Taneja and Gowe (1960).

The serum Ca and P (Farrell E C, 1984) were measured using biochemical kits in a semi-automated clinical chemistry analyser (Merck, Microlab 300). At end of experimental period, four roosters from each group were randomly selected to estimate tibial ash and Ca content (Gongruttananun, 2011). The chemical composition of the layer breeder feed, ES and SG were analysed as per AOAC (2016).

Data of the study were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The chemical composition of egg shell and SG is presented in table 2.

Table 2. Chemical composition of Egg shell powder and Shell grit fed to experimental birds during 33rd to 44th weeks of age (%), on dry matter basis

Attributes	Eggshell	Shell grit
Moisture	0.56 ± 0.24	0.70 ± 0.24
Crude Protein	8.30 ± 0.41	7.47 ± 0.41
Crude Fibre	2.90 ± 0.26	1.58 ± 0.26
Ether Extract	0.70 ± 0.06	0.48 ± 0.06
Total Ash	93.45 ± 0.60	94.86 ± 0.60
Acid insoluble ash	0.34 ± 0.11	0.97 ± 0.11
Calcium	42.16 ± 0.48	39.11 ± 0.48
Phosphorous	1.70 ± 0.32	3.46 ± 0.32

In this present study, egg shell without shell membrane contains 8.30 % CP, 42.16 % Ca and 1.70 % P which is higher than reported values reported by Islam (2021) in kitchen-extruded egg shell and hatchery-extruded egg shell. Islam (2021) reported kitchen-extruded egg shell contains, 4.24 % CP, 29.75 % Ca and 14.82 % available P and hatchery–extruded egg shell contains 13.80 % CP, 25.53 % Ca and 13.87 % available P. The estimated CP in the present study was higher than kitchen-extruded egg shell and lesser than hatchery-extruded egg shell reported by Islam (2021), however lesser value of 2.14 % CP was reported by Lertchunhakiat *et al.* (2016).

In this study, Ca content estimated as 42.16 % on DMB which was higher than the previously mentioned values of 33.5– 34.8 % Ca in eggshell by Muir *et al*. (1976) and Olgun *et al*. (2015) reported value of 32.3 %. Further to that, as explained by Islam (2021), egg shells from hatchery had lower Ca and P content because of Ca and P utilization by embryo during incubation. The variation in nutritive value of egg shell might be due to source of collection, processing involved and study materials used in this study was collected from bakeries which are infertile eggs.

The dietary Ca requirement for birds was highly influenced by particle size, solubility and source of Ca. The relative proportion particle size, in-vitro solubility of egg shell and SG was studied and presented in table 3.

Table 3. Particle size and in-vitro solubility of shell grit and egg shell

Particle size (mm)	Egg shell	Shell grit	SEM	P value
Quantity (per cent, by weight)				
Coarse (> 2.00)	84.13[a]	69.11[b]	0.46	0.001*
Medium (< 2.00)	15.36 [b]	22.56 [a]	0.43	0.001*
Fine (< 0.75)	0.39 [b]	7.01 [a]	0.06	0.001*
In-vitro solubility (per cent)				
Coarse (> 2.00)	40.61 [b]	56.21 [a]	0.54	0.001*
Medium (< 2.00)	57.33 [b]	76.51 [a]	1.35	0.001*
Fine (< 0.75)	55.39 [b]	92.44 [a]	0.74	0.001*

[ab] Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 [ns] non-significant

It was found that coarse particle (>2.00 mm) in ES (84.13 %) was comparatively more than SG (69.11 %) and fed to the male birds in this study. The in-vitro solubility of coarse egg shell (more than 2 mm) used in this study was comparatively lesser than SG. Like that, Pizzolante *et al.* (2011) mentioned that, limestone powder with 0.44 mm particle size had 31 % solubility, 2.40 mm coarse limestone powder with 28 % and 46 % in-vitro solubility was recorded for oyster shell which similar to SG used in this study.

The mean feed intake (FI) of the roosters in this experiment was 87.58, 86.86 and 88.28 g/rooster/day, in control, egg shell and shell group, respectively and illustrated in the table 4.

Table 4. Mean values of Calcium Intake, Semen quality and BWT of Gramasree roosters from 33 to 44 weeks of age

Parameter	Group			SEM	p-value
	Control	Egg shell	Shell grit		
Feed intake (g)	87.58[b]	86.86[c]	88.28[a]	0.22	0.001*
Egg shell powder and shell grit intake (g)	-	0.67[b]	0.72[a]	0.00	0.002*
Calcium intake from feed (%)	3.20[b]	3.18$_c$	3.25[a]	0.01	0.001*
Calcium intake from egg shell and shell grit (%)	-	0.26	0.30	0.00	0.06[ns]
Total calcium intake (%)	3.20	3.44	3.55	0.01	0.04[ns]
Semen characteristics					
Semen volume (mL)	0.13	0.16	0.14	0.04	0.54[ns]
Semen colour (score)	2.35	2.81	2.83	0.25	0.32[ns]
Sperm motility (%)	47.92	61.04	61.46	8.19	0.28[ns]
Sperm concentration (x109 cells /mL)	4.25	5.49	5.31	0.88	0.45[ns]
Serum parameters					
Calcium (mg/dL)	13.66	17.38	17.43	2.34	0.40[ns]
Phosphorous (mg/dL)	6.19	6.32	6.18	0.85	0.27[ns]
BWT (kg/male bird)					
32 weeks	2.307	2.177	2.400	0.09	0.25
44 weeks	2.603[a]	2.311[b]	2.029[c]	0.108	0.01*

[a, b, c] Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 [ns] non-significant

As age increased, control group birds FI was significantly (P<0.01) increased and not evidenced in other treatment group birds. Gongruttananun (2011) reported FI was more in egg shell group (118.56 g/ rooster/ day) and Lertchunhakiat *et al.* (2016) reported that the ES inclusion at 10.8 % level did not influence the FI and BWT. Meanwhile, the BWT was reduced in SG group compared to control and egg shell group birds in this experiment. The present study reports revealed that, male birds consumed 3.46, 3.49 and 3.25 g of Ca/rooster/day in control, egg shell and shell group, respectively during the experimental period and observed male birds consumed only 0.67 g/bird/day of eggshell and 0.72 g/bird/day of SG, which contributed 0.26 and 0.3 g of Ca/bird/day, which fed as free choice source of Ca. However, ICAR (2013) recommends minimum Ca requirements of 0.8 % for Gramapriya male breeder birds.

The mean semen volume of G1, G2 and G3 group roosters are 0.13, 0.16 and 0.14 ml/rooster, respectively during the experimental period and Chauhan *et al.* (2009) reported in Gramapriya cocks average ejaculate volume of 0.39 ± 0.02 ml/rooster with sperm concentration and total sperm count of 2.49 ± 0.03 x 109/ ml and 0.98 ± 0.04 x 109/ ejaculate. Whereas, weekly mean semen concentration recorded in present study was 4.25, 5.49 and 5.31 x 109/ ml in control, egg shell and SG groups, respectively and higher than Chauhan *et al.* (2009) reported value. However, no significant differences were found in semen concentration between the groups and the results were similar to Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

Like that, Gongruttananun (2011) and Lertchunhakiat *et al.* (2016) also reported 0.37 ml/ ejaculate and 0.39 ml/ejaculate, respectively and reported that source of Ca not affected the semen volume, corroborates with our findings. Meanwhile, semen colour average score evaluated during this study are 2.35, 2.80 and 2.83 and results were similar to findings of Shanmugam *et al*. (2012) and Lertchunhakiat *et al.* (2016). The average semen motility reported in this study was 47.92, 61.04 and 61.46 % in control, egg shell and SG groups, respectively and no significant difference in semen motility between the groups was observed and same findings were reported by Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

In agreement to the present findings, Namntu, (2011) reported that the effect of feeding female ration to male broiler breeders and found that it had no influence on male fertility but it increased the male BWT. Like that, Khalil *et al.* (2012) reported that cocks fed with 2 and 3 % Ca levels had superior forward sperm motility, live sperm and fertility percentage compared with those fed with 1 % Ca.

Table 5. Tibia Characteristics of experimental Gramasree male birds

Treatment	Control	Egg shell	Shell grit	SEM	p-value
Tibial weight (g)	6.11^{b}	6.80^{a}	5.58^{c}	0.001	0.001*
Tibial length (mm)	126.27^{a}	126.4^{a}	120.90^{c}	0.269	0.001*
Tibial thickness (mm)	6.10^{b}	6.53^{a}	5.97^{c}	0.072	0.001*
Tibial width (mm)	13.33	13.33	13.07	0.39	0.86^{ns}
Tibial Ash (%)	53.42	56.52	55.82	2.16	0.59^{ns}
Tibial calcium (%)	19.66^{c}	20.96^{b}	26.58^{a}	0.61	0.001*

^{abc} Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 ^{ns} non-significant

It was understood that, extra Ca intake through feed had not influenced the serum Ca, P content and similar in all the treatment groups. The tibial bone weight, length, thickness and tibial Ca content were significantly differed between the groups. Higher tibial Ca content (P<0.01) and lesser tibial bone weight was observed in SG group compared to other groups and similar findings were reported by Gongruttananun (2011). The increased tibial bone Ca content in SG fed group might be due to increase

in bio-availability of Ca as evidenced by in-vitro solubility studies of SG. The fecal Ca excretion studies may clarify further, which not studied in this experiment.

CONCLUSION

It can be concluded that, breeder layer ration with 3% Ca was sufficient to maintain the semen quality without any detrimental effects and Ca intake up to 3.49 g/male bird/day had not influenced the semen qualities and ES can be used as extra Ca source for Gramasree male birds.

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Growth Performance and Brood Stock Management of Small Indigenous Fish Mola Carplet, *Amblypharyngodon mola* (Hamilton, 1822)

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ABSTRACT

Spontaneous spawning of wild caught and domesticated mola carplet, Amblypharyngodon mola was attempted in controlled condition using an aquatic plant hydrilla, Hydrilla verticillata as a modified habitat. Growth performance of hatched larvae revealed that the larval growth (weight gain %) was significantly higher ($p < 0.05$) with a mixed diet (63.76 ± 0.52%) than natural diet (40.42 ± 0.26%) or artificial diet (13.74 ± 0.39%) whereas juvenile mola grew better on the artificial diet. Significantly higher maturity rate (> 80 %) of mola was observed in tanks/ ponds having hydrilla as an additional habitat than the tanks /ponds having no hydrilla. Similarly, higher fecundity of mola (>900) was recorded in tanks having hydrilla. Better spawning performance was also noted using hydrilla based habitat. The findings can be useful in artificial propagation and diversification of small indigenous fish species in captivity.

Key words: Habitat manipulation, Hydrilla, Larval recovery, Spawning, Small indigenous fish

INTRODUCTION

Fish are not only a good source of animal protein but also provide other beneficial micronutrients such as calcium, iron, zinc, phosphorous, selenium, fluorine, iodine, and polyunsaturated fatty acids (Pal *et al.,* 2018; Thilsted *et al.,* 2014). The protein requirement of the global population is partially met from aquaculture. The global annual aquaculture production in the year 2016 was 80 million tonnes that comprised of nearly 598 species out of which 369 are finfish (FAO, 2018). This production is mostly contributed by medium to large size fish. It is pertinent to mention that spawning and culture of small indigenous fish species have received limited attention over the years though they are richer in nutrients and vitamins than other cultured table fish. One of the small fish that requires attention is Amblypharyngodon mola (Hamilton, 1822), commonly referred to mola carplet of Cyprinidae family (Menon, 1999; Saha *et al.,* 2009). It is a small freshwater fish found in the rivers, lakes, reservoirs, slow-moving streams, floodplains, inundated fields, ditches and ponds in the Indian sub-continent. Mola is preferred for its taste (Roos *et al.,* 1999; Saha *et al.,* 2009) and is rich in nutrients: every 100 g of the mola contains 2680 retinol activity equivalent units of vitamin A, 5.7 mg of iron, 776 mg of calcium and 3.2 mg of zinc. Mola contains 20 times more calcium and 80 times more vitamin A than carps such as the silver carp (Hypophthalmichthys molitrix) and rohu (*Labeo rohita*) (Thilsted *et al.,* 2014).

Despite being highly nutritious and particularly suitable for farming and diversification in pond ecosystems, its artificial propagation and culture is hampered by insufficient information and research. The present study aimed at production of mola seed by habitat manipulated spontaneous spawning, evaluation of growth performance and brood stock management to promote farming of mola.

MATERIALS AND METHODS

Wild mola stock was collected using a dragnet in the morning hours from river Mahanadi at Banki in Cuttack district, Odisha, India with the help from local fisherfolk. A total of 30,000 mola conditioned and packed in 10 L capacity plastic bags (50 numbers in each bag) with one part of oxygen and two parts of water (vol/vol) and transported to the Carp Breeding Unit, ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar which is 70 km away from the collection site. After arrival, all the live mola were treated with 5% potassium permanganate

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for 30 to 60 seconds and stocked in cement tanks of 7500 L capacity with 24-h aeration. However, due to its delicate nature only 40% could survive despite the best care. Spontaneous spawning was simulated in two types of habitats viz. earthen pond and cement tank in duplicate with or without addition of submerged weed. One set of ponds/tanks were added with hydrilla (Hydrilla verticillata) to the tune of 20% of their water spread area and other set of tanks/ ponds were devoid of hydrilla that served as control. Hydrilla was used in this study due to the fact that large amount of hydrilla could be seen in natural habitat from where mola was collected.

Healthy brood males (5.2 ± 1.1 cm length, weight 1.3 ± 0.56 g) and females (5.8 ± 1.2 cm length, weight 1.9 ± 0.59 g) mola were identified by their sexual dimorphism and kept together in equal proportions in cement tanks (7500 L; 3.0 m × 2.5 m × 1.0 m deep) and also in 0.01 ha earthen ponds at a stocking rate of 130/m³. To maintain the concentration of dissolved oxygen and to simulate natural environment, 30% of water was removed from the tank and replaced with freshwater every day. Two aerators were used in the tank but not in the pond. Spawning tanks were kept under natural photoperiod (12L:14D). Brood fish were given a commercially formulated powdered feed (protein 37.3%, crude fat 6.7%, crude fibre 3.8%, dry matter 94.7%, total ash 7.0%) at 3% of the body weight twice a day and live plankton (crude protein 43.8%, crude fibre 2.7%, dry matter 8.9%) collected from the wild twice a week. The proximate composition of the plankton and the artificial diet was determined using standard methods (AOAC, 2005).Various parameters such as relative fecundity, hatchlings/ larval recovery (%) were calculated as follows:

To measure the growth of mola larvae during the initial days, 20 individuals of average length 16.2 ± 0.5 mm and average weight 38 ± 0.1 mg were reared in 150 L glass tanks for almost 10 weeks and fed three times a day with plankton and artificial diet at 5% of their body weight/day. Water quality was maintained by exchanging water to the tune of nearly 75% during one-week period and continuous aeration throughout the experiment. Feeding was discontinued 24 hours before sampling to avoid any larval excrement. In every 15 days, samples of the larvae were collected using a hand net and their length was measured with a scale and weight with an electronic weighing balance (Afcoset ER-200A, India). To find out a suitable diet for better growth and survival of mola fry, each time hatchery produced 20 numbers of active mola fry (15.5 ± 0 mm long, weighing 37.5 ± 0 mg) were introduced into each 75L tank. Three treatments were tested in duplicate, namely (1) natural diet (plankton feed) (crude protein 43.8%, crude fibre 2.7%, dry matter 8.9%), (2) artificial diet (Formulated powdered feed) (protein 37.3%, crude fat 6.7%, crude fibre 3.8%, dry matter 94.7%, total ash 7.0%) and (3) mixed diet (plankton feed + formulated powdered feed). The feed was given three times a day at 5% of the body weight of fish. All the tanks were aerated round the clock throughout the experiment for 45 days. Water exchange was done twice a week and uneaten feed and excreta were removed before feeding once in day. At the end of the experiment, 15 larvae were collected from each unit and their length was measured to the nearest millimetre and weight to the nearest milligram. Similarly, 6-month-old juveniles (42.4 ± 0.6 mm long, weighing 701.7 ± 2.9 mg) were also studied for 30 days.

Instantaneous growth rate (mg/day)

$$= \frac{[\text{Ln } \{ \text{ final weight (mg) } \} - \text{Ln } \{ \text{ initial weight (mg) } \}]}{\text{duration of rearing period (days)}}$$

Instantaneous growth rate (mg/day)

$$= \frac{[\text{Ln } \{ \text{ final weight (mg) } \} - \text{Ln } \{ \text{ initial weight (mg) } \}]}{\text{duration of rearing period (days)}} \times 100$$

$$\text{Condition factor} = \frac{\text{weight}}{\text{Length}} \times 100$$

RESULTS AND DISCUSSION

Significantly higher ($p < 0.05$) relative fecundity of mola was recorded in tanks and ponds with addition of hydrilla (937 and 906) than the tanks and ponds without hydrilla. Maturity rate of females were higher (85%) in cement tanks with hydrilla than controls (48%) without hydrilla. In earthen ponds too, the same was higher (80%) with the addition of hydrilla than its absence (60%). Similar trend was also noticed in larval recovery (yolk sac absorbed larvae) rate in cement tanks. (Table 1).

Table 1. Manipulation of habitat for spontaneous spawning of mola with or without addition of an aquatic weed, hydrilla (Hydrilla verticillata)

Parameter	Cement tank		Pond	
	With hydrilla	Without hydrilla	With hydrilla	Without hydrilla
Total number of broodfish stocked (male and female)	700 ± 0[a]	700 ± 0[a]	1000 ± 0[a]	1000 ± 0[a]
Average wt. of female	1.9 ± 0.12	1.9 ± 0.22	1.9 ± 0.21	1.9 ± 0.25
Relative fecundity	937 ± 1.22[b]	145 ± 0.12[a]	906 ± 1.12[b]	123 ± 0.15[a]
Maturity rate (%) (female)	85 ± 2.22[b]	48 ± 0.12[a]	80 ± 2.10[b]	60 ± 1.22[a]
Larval recovery rate (%)	85 ± 1.52	80 ± 1.22	-	-
	700 ± 0[a]	700 ± 0[a]	1000 ± 0[a]	1000 ± 0[a]

Different superscript in same row indicates the significant differences ($P<0.05$).

The fry showed good growth: between 5 dph and 80 dph, the average length increased from 16.2 ± 0.5 mm to 29 ± 0.4 mm and average weight, from 38 ± 0.1 mg to 248 ± 1.6 mg. Significant differences were seen in four parameters related to weight: final weight, weight gain, daily weight gain, per cent weight gain and specific growth rate (Table 2).

Table 2. Growth and survival of mola fry under different diets

Parameters	Natural diet (Plankton feed)	Artificial diet (Formulated powdered feed)	Mixed diet (Plankton feed + Formulated powdered feed)
Initial length (mm)	15.49 ± 0	15.49 ± 0	15.49 ± 0
Initial weight (mg)	37.5 ± 0	37.5 ± 0	37.5 ± 0
Final length (mm)	18.5 ± 0.43	17.47 ± 1.2	19.3 ± 0.23
Final weight (mg)	52.66 ± 0.1[b]	42.65 ± 0.15[a]	61.41 ± 0.2[c]
Weight gain (mg)	15.16 ± 0.1[b]	5.15 ± 0.15[a]	23.91 ± 0.2[c]
Daily weight gain (mg)	0.34 ± 0[b]	0.11 ± 0[a]	0.53 ± 0[c]
Weight gain percent (%)	40.42 ± 0.26[b]	13.74 ± 0.39[a]	63.76 ± 0.52[c]
Instantaneous growth rate (mg/day)	0.008 ± 0.00001[b]	0.003 ± 0.00001[a]	0.011 ± 0.00001[c]
Specific growth rate (%/day)	0.75 ± 0.004[b]	0.29 ± 0.01[a]	1.1 ± 0.01[c]
Condition factor	0.83 ± 0.06	0.82 ± 0.17	0.855 ± 0.03
CV for length (%)	17.09 ± 1.37	15.35 ± 1.37	14.68 ± 0.74
CV for weight (%)	33.15 ± 4.31	27.03 ± 7	22.87 ± 4.57
Yield (mg L-1)	14.48 ± 0.47[b]	9.95 ± 0.68[a]	16.89 ± 0.46[b]
Survival (%)	82.5 ± 2.5	70 ± 5	82.5 ± 2.5
Mortality rate (%)	17.5 ± 2.5	30 ± 5	17.5 ± 2.5
No of larval output	17 ± 0.5	14 ± 1	17 ± 0.5

Different superscript in the same row indicates the significant differences ($P<0.05$).

Mixed diet proved significantly ($p < 0.05$) superior in terms of per cent weight gain, followed by the natural diet and the wholly artificial diet. Similarly, specific growth rate was also significantly higher in fish fed with the mixed diet than that with the other two diets. No significant differences ($p > 0.05$) in terms of survival, mortality and the number of larval outputs were noticed among all the three treated diets. The juvenile mola gained weight and grew longer as days passed in all the treatments. The growth parameters were significantly influenced by the diet (Table 3).

Table 3. Growth performance and survival of mola juvenile (6-month-old) under different diets

Parameters	Natural diet (Plankton feed)	Artificial diet (Formulated powdered feed)	Mixed diet (Plankton feed + Formulated powdered feed)
Initial length (mm)	42.36 ± 0.57	42.36 ± 0.57	42.36 ± 0.57
Initial weight (mg)	701.67 ± 2.89	701.67 ± 2.89	701.67 ± 2.89
Final length (mm)	44 ± 0.1^{a}	43.87 ± 0.07^{a}	45.93 ± 0.07^{b}
Final weight (mg)	817.32 ± 1.31^{a}	922.32 ± 2^{c}	869.25 ± 0.99^{b}
Weight gain (mg)	115.66 ± 1.31^{a}	220.65 ± 2^{c}	167.59 ± 0.99^{b}
Daily weight gain (mg)	3.86 ± 0.04^{a}	7.36 ± 0.07^{c}	5.59 ± 0.03^{b}
Weight gain percent (%)	16.48 ± 0.19^{a}	31.45 ± 0.29^{c}	23.88 ± 0.14^{b}
Instantaneous growth rate (mg/day)	0.005 ± 0.0001^{a}	0.009 ± 0.0001^{c}	0.007 ± 0.0001^{b}
Specific growth rate (%/day)	0.51 ± 0.01^{a}	0.91 ± 0.01^{c}	0.71 ± 0.001^{b}
Condition factor	0.96 ± 0.005^{b}	1.09 ± 0.007^{c}	0.897 ± 0.003^{a}
CV for length (%)	9.7 ± 1.06	11.45 ± 0.6	6.77 ± 2.65
CV for weight (%)	45.11 ± 0.13^{b}	33.81 ± 5.57^{ab}	22.57 ± 6.49^{a}
Yield (mg L-1)	272.44 ± 0.44^{a}	307.44 ± 0.67^{c}	289.75 ± 0.33^{b}
Survival rate (%)	100	100	100

Different superscript in the same row indicate the significant differences ($P<0.05$).

After 30 days of culture, the weight of mola was significantly higher with the artificial diet followed by mixed diet and natural diet. Other growth parameters such as weight gain, per cent weight gain, daily weight gain and specific growth rate were noticed to be higher in artificial diet than other two (artificial and mixed) diets. Juvenile mola reared on the artificial diet showed higher values of the condition factor than those reared on the other two diets. However, the survival rate of fry was not affected by the diets used.

Aquatic macrophytes provides a conducive spawning environment besides being a source of food and recycle of nutrients and maintenance of water quality (Cowx and Welcomme, 1998). In the present study, hydrilla (an aquatic macrophyte) was introduced into the experimental tanks/ponds to create a modified habitat for mola. This resulted in substantial higher relative fecundity (> 900) and larval recovery (85%) in mola brood reared in habitat modified tanks. This may be attributed to the habitat modified environment that provided a favourable environment for spawning in mola. Moreover, the biological nature of these fish is to find a safe place to spawn and also to avoid predators and sometimes cannibalism. Habitats with moderate amounts of aquatic vegetation provide an optimal environment for reproduction, breeding and raising fish nurseries and also protect the fish from predators (Gettys *et al.*, 2014; Petr, 2000). In some fish, spawning occurs only on ideal spawning grounds (Ganapati *et al.*, 1951). Fish like Cyprinus carpio, Colisa sota also require aquatic vegetation such as hydrilla sp., najas, eichornia, commelina sp. etc. for their spawning (Adamek and Pardo, 2015; Mitra *et al.*, 2006). Artificial spawning substrates are also used to enhance spawning and growth of fish. A comparative account of habitat manipulations used for spawning and larval survival of different fish species is presented in table 5.

We obtained the best growth (23.9 ± 0.2 mg) in mola fry using mixed diet than natural diet (15.2 ± 0.1 mg) or artificial diet (5.2 ± 0.2 mg) given alone. Nearly a four-fold higher weight gain was recorded in mola fry using mixed diet than the artificial diet which is attributed to the fact that it provided the required micronutrients and the protein during the developmental stage. When natural diet was provided alone, the growth rate was slower than the mixed one. This may be due to the protein insufficiency. The pattern of other growth parameters viz. daily weight gain, per cent weight gain and specific growth rate were similar. The superiority of the mixed diet may be due to the fact that it provides all the essential amino acids. It is reported that higher dietary protein level could meet the requirements of body protein synthesis in early stages and supports fast growth of larvae (Bengtson *et al.,* Leger and Sorgeloos, 1991). Trout larvae (Oncorhynchus mykiss), for example, grew better when higher amount of protein was provided (Akbary *et al.,* 2010). Similar findings in the larvae of Rhamdia quelen showed a higher survival rate when given a diet that contained brine shrimp (Artemia nauplii) (Salhi and Bessonart, 2012). In common carp, C. carpio, growth was better when fed with a mixed diet than artificial dry food and live zooplankton (Szlaminska and Przybyl, 1986). It is also reported that Labeo parvus showed better survival, weight gain and specific growth rate when fed with a mixed diet (Montchowui *et al.,* 2012).

Natural live food is considered ideal for freshwater larviculture, especially in the initial days (Awaiss *et al.,*1992; Wang *et al.,* 2005). Here, our observations also suggest that just-hatched larvae (yolk sac absorbed larvae) need live plankton because it is easily digested. We provided plankton and artificial diet as a supplement because enzymes in live food help digestion in first-feeding larvae (Lavens and Sorgeloos, 1996; Verreth *et al.*, 1993). Moreover, the formulated diet given to mola larvae helped them to adapt faster to the artificial diet. Feeding only the artificial diet resulted in significantly greater weight gain (220.6 ± 2 mg) after 30 days than with the other two diets, probably because the juveniles adapted more easily to the artificial diet, as evident from the condition factor: the condition factor is an indicator of fish health including growth and feeding intensity (Froese, 2006) was greater than 1 in the artificial but less than 1 in the other two diets. The factor is also used in evaluating the efficiency of feeding regimes, that is to assess how efficiently a given food is used (Weatherlley, 1972). The higher growth and conversation efficiency obtained with the artificial diet could also be due to higher intake of food (given that the consistency of the artificial diet was better than that of the other two diets leading to greater cohesion of particles), greater palatability and higher protein content (Quintana *et al.,* 2008). The mola juveniles grew to nearly 1 g after the experimental period of 30 days, which is a marketable size and can be used for domestic consumption.

CONCLUSION

Taking into account consumer preference and nutritional value, mola deserves to be grown in large scale. The present study provides essential benchmark information on artificial propagation of mola in captivity. By manipulating their habitat, mola can be made to spawn twice in a year. The potentiality of habitat manipulated spontaneous spawning of mola in captive condition and higher survivability of the hatchery produced individuals made it easier for mola breeders to take up this in a commercial scale. Formulated artificial diet may be recommended for the growth of mola juveniles whereas mixed diet was most suitable for fry rearing. This study opens new vistas for sustainable aquaculture production and species diversification.

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Sahoo *et al.*

Chronological Changes in Postprandial Blood Glucose Level of *Labeo rohita* (Hamilton, 1822) Fed with Formulated Diet

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ABSTRACT

In general, fish is considered as a natural diabetic animal. In order to understand how the feed is metabolized to glucose and the time duration for its complete uptake from blood, a study was conducted to estimate the pre and postprandial blood glucose level in Labeo rohita, at two-hour intervals of both fasting and fed state. The blood glucose levels of four replicates at every time point were estimated using glucose oxidase method. The pre-prandial/ fasting blood glucose level was found to be in the range of 10-18 mg/dl. Whereas, two hours post-prandially the blood glucose level had got increased and estimated to be varying between 46-88 mg/dl, which was maximum. Also, it was observed that the sugar levels got reduced back to normal/fasting state after about 10hrs. Time period shows significant inverse relationship with blood glucose level ($p<0.05$). These results will help in establishing and managing a proper feeding schedule for rohu.

Key words: Glucose, Pre-prandial, Post-prandial, Fasting blood glucose, Practical diet, Glucose oxidase

INTRODUCTION

Glucose is a simple sugar or monosaccharide which is preferred as a substrate for energy production for all the cells of the body, and the only energy source for the nervous system. It serves as the key to keeping the body mechanisms in working order and is often referred to as blood sugar. Glucose tolerance is a term that refers to the ability of an organism to rapidly deal with a glucose load. The consequences of glucose intolerance are persistent hyperglycemia and in many cases, reduced growth. Glucose intolerance is a clinical term used in the diagnosis of human insulin-dependent diabetes mellitus. The IDDM and is assessed by glucose tolerance test GTT. A GTT involves administering glucose either orally or intravenously, and if plasma glucose values do not return to baseline within 1-2 h, the person is considered to have impaired glucose tolerance. The GTT has been used by many researchers to study fish glucose tolerance and in all most all cases; persistent hyperglycemia was observed (Moon, 2001). However, the time period of hyperglycemia is species and condition-dependent. Furuichi and Yone (1981) demonstrated the most severe hyperglycemia for longer duration in the omnivorous carp in GTT. According to Wilson (1994) marine species are more tolerant to glucose load than freshwater species, although Garcia-Riera and Hemre (1996 a, b) demonstrated opposite observation. There are differences in tolerance observed between Chinook salmon strains (Mazur *et al.,* 1992). The conditions of the test species are also a factor for its glucose tolerance. Again, Furuichi and Yone (1981) fed different doses of dextrin diet to carp, red sea bream and yellow tail and observed that more the dextrin content there was more hyperglycemia and lower insulin level. Prolonged food-deprivation compared with feeding will generally extend the period of hyperglycemia (Blasco *et al.,* 1996; Legate *et al.,* 2001). And the source of starch can also affect the hyperglycaemic response to a glucose load (Hemre and Hansen, 1998). Although there are many studies on GTT in fish with glucose administration or dextrin in diet, but study of glucose levels with a formulated diet is scarce. In this study we monitored the blood glucose level in Labeo rohita fingerlings after fed with a formulated diet.

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The experiment was conducted at the wet laboratory of ICAR-Central Institute of Fisheries Education (CIFE), Salt Lake, Kolkata Center. 60 numbers of Labeo rohita (Hamilton, 1822) (rohu) fingerlings having an average weight of 12.0 ± 0.25 g procured from the fish farm at Ramsagar, Kolkata. Handling of fish at all stages was done utmost care to give less stress. During the experiment and sampling, all the ethical guidelines of animal care for ICAR-Central Institute of Fisheries Education, Mumbai India were strictly followed. Fishes were treated with 2ppm potassium permanganate to disinfect them following which they were transferred into a 500 litre FRP tank and acclimatized for a few days. After acclimatization, 15 fishes were transferred in each of the four rectangular fibre-reinforced plastic (FRP) tanks (180L capacity). Before blood collection, fish were fasted for 48hrs. After the collection of fasting blood, feeding was carried out with the prepared diet. One hour after feeding, the tanks were siphoned for removal of excess feed and faecal matter before post pandrial blood collection

All fishes were fed with the same diet containing 29% of crude protein, which is optimum protein requirement for carps. The diet was prepared using different common ingredients used for fish feed (Table 1).

Table 1. Composition of formulated diet (g/100 gm)

Ingredients (g 100g^{-1})	In Diet
DSBM[a]	30.00
GNOC[a]	14.25
Wheat flour[a]	6.97
DORB[b]	40.00
Vegetable oil[a]	3
Fish oil[a]	2
Vit-min Mix[d]	2
CMC[c]	1.50
BHT[c]	0.03
Choline chloride[c]	0.25

Notes. Quantities of ingredients are expressed in g/100 g. DSBM: de-fatted soybean meal; GNOC: groundnut oil cake; DORB: de-oiled rice bran; CMC: carboxymethyl cellulose; BHT: Butylated Hydroxy toluene.

[a]Procured from local retail shop. [b]Purchased from Vaighaiagro products, India. [c]Purified ingredients procured from HImedia ltd., India. dComposition of vit.-min. premix (quantity/kg):Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; VitaminB6, 1,000 mg; Vitamin B12, 6 mg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 l-lysine, 10 g; dl-methionine, 10 g; Selenium 50 ppm.

Proximate composition of diets was analysed according to the standard methods of AOAC (1995). The experimental diet was dried to constant weight at 100 ± 2°C in hot air oven to determine the moisture content. Crude protein content was determined using the micro-Kjeldahl method (Kelplus, PELICAN, India), whereas ether extract was calculated by the Soxhlet extraction method (SOCS plus, SAS-AS 08; PELICAN). The ash content was estimated using muffle furnace and fibre approximation was done in Fiber tech (Tulin Equipments) apparatus and further ashing was done using a muffle furnace at 550°C for 5 hr. The proximate composition of experimental diets is given in table 2.

Table 2. Proximate composition of experimental diet (% dry matter)

Proximate composition	Diet (% dry matter)
Dry matter	91.05 ± 1.45
CP[1]	29.02 ± 0.41
EE[2]	7.29 ± 0.07
CF[3]	6.56 ± 0.24
NFE[4]	48.18 ± 0.15
TA[5]	8.95 ± 0.03

Data are expressed as mean ± SE; (n = 3)

[1]CP: crude protein. [2]EE: ether extract; [3]CF: crude fibre; [4]NFE: nitrogen free extract; [5]TA: total ash; NFE (nitrogen-free extract) = 100 - (crude protein + crude lipid + crude fiber +ash).

Each fish was anesthetized with clove oil (50µl of clove oil per litre of water) before taking blood from them. Blood was taken from caudal vein using a medical syringe (N-22) of 2 ml and then immediately centrifuged in a micro-centrifuge (5 minutes at 6000 rpm) for plasma collection.

In order to monitor the changes in blood glucose level, the fishes were examined in quadruplicates after two hours of fasting and then at the interval of two hours postprandial. Each time after blood collection, the fish were transferred to another common tank and a set of four new fishes from the four experimental replicate tanks were used in the next time for blood glucose estimation, i.e. at every intervals four new fish are choosen for blood collection to minimise the stress.

The glucose level in plasma was determined using Erba diagnostic kit (Transasia Biomedicals Pvt. Ltd., India). In the assay, glucose is oxidized to gluconolactone with concomitant reduction of the flavin adenine dinucleotide (FAD)-dependent enzyme glucose oxidase. The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide reacts with 3, 5-dichloro-2- hydroxybenzene sulfonic acid and 4-amino antipyrine to generate a pink dye with an optimal absorption at 514 nm.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{Concentration of standards (mg/dl)}$$

Data of the current experiment were analysed by regression analysis using SPSS program, 22.0 version. Second degree Polynomial equation showed the best fit. R square and P values were calculated.

The pre-prandial/ fasting blood glucose level in the absence of food for 48 hours was found to be in the range of 10-18 mg/dl. Two hours post-prandial blood glucose level was observed to be varying between 46-88 mg/dl (Table 3), which was the maximum estimated blood glucose level, thus indicating the rise in circulating blood glucose by absorption immediately after the digestion of feed and it is reduced back to normal range or fasting blood glucose levels after 10hr (Fig. 1).

Table 3. Blood glucose concentration of replicate

Time	R1(mg/dl)	R2(mg/dl)	R3 (mg/dl)	R4(mg/dl)	Average ±S.E.
Fasting -2 hr	18	16	10	12	14.00±1.83
After feeding +2hr	65	88	46	74	68.25±8.80
+4hr	50	42	42	52	46.50±2.63
+6hr	31	27	23	34	28.75±2.39
+8hr	29	19	17	18	20.75±2.78
+10 hr	<10	15	11	<10	10.75±1.55

Data presented examine blood glucose level changes in rohu (Labeo rohita) at two hrs interval before and after feeding, a commercially cultured fish species. R1-R4 represents four replicates

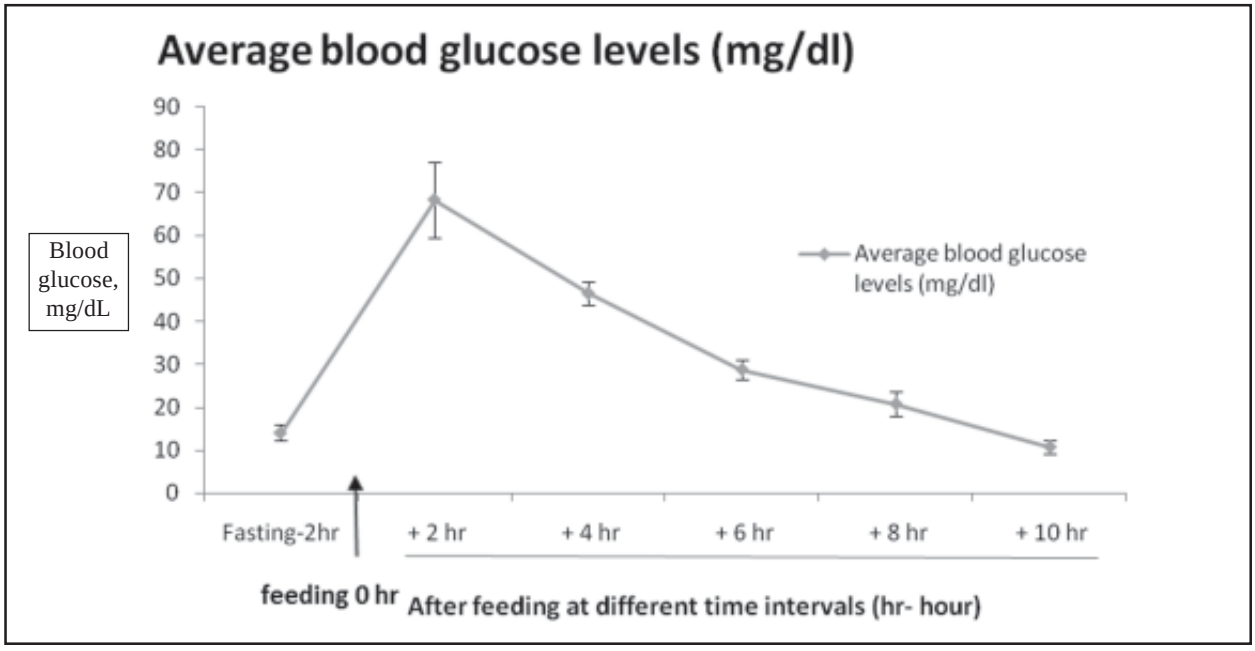

Fig. 1. Pre and post prandial blood glucose level

This result re-establishes the claim that fish is a diabetic animal (Malik, 2020). As per Legate *et al.* (2001) and Falkmer (1961), the pre-prandial glucose level reported in fish blood is 25 mg/ dl to 90 mg/dl while the post-prandial glucose level is 300 mg/100 ml. After every meal, the blood sugar levels increase and in response, insulin, a pancreatic hormone is secreted to reduce the sugar levels until it becomes normal. But, clearance of the blood glucose is very slow in fishes; it takes up to 6 hrs to 7 hrs, while in human beings it takes only 30 min. However, exceptionally in some fishes it takes up to 24 hr for its clearance. The glycaemic changes that occurred clearly indicate the sensitivity of blood glucose levels. Consistent with this, modifications in glucose metabolism after feed intake are often the reasons for these changes, suggesting that glucose is an important substrate that is mobilized as a result of a variety of physiological, metabolic and environmental challenges. Later on, glycaemic decreases were observed with food deprivation. These alterations in blood glucose suggests that glucose metabolism in fish is important. Normal blood glucose levels of fish are within 40-90 mg/dl (Polakof *et al.*, 2012) and the

data recorded also lies approximately within the optimum range within four hrs of feeding. But after that there is gradual decrease in blood glucose level that is as low as 10mg/dl. Regression analysis of the level of glucose vs time gives the best fit curve (glucose level = 93.85 - 14.16 time + 0.594 time*time) has a R-square vale of 99.79%, showing very good fit of the curve with the data. The p-value of 0.004 for the regression model signifies that the predictor variable, i.e. time, significantly varies the response variable, i.e. glucose level.

All the previous studies were based on either pure glucose or dextrin diet base studies. As per our knowledge, this is the first report of such study in Labeo rohita, in which after feeding formulated diet blood glucose has been estimated chronologically. After 8 hrs the level of blood glucose was very low, so feeding of rohu should be done at 8hrs interval.

The study establishes the fact that the fasting blood glucose level of fish can go below 20mg/dl and in this particular study, 10-18 mg/dl is the observed fasting blood sugar level in fish. It takes about 8-10 hrs to fully absorb the blood sugar post feeding. However, further study with other fish species is necessary to validate these findings.

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CONTENTS

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