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Rumen-Protected Amino Acids: Their Importance and Effects on Performance, Heat Stress and Immunity in Cattle

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ABSTRACT

Balanced nutrition is crucial for optimum dairy cow production, with protein quality being particularly important for productivity and reproductive performance. Ruminal microorganisms utilize diet components to synthesize microbial protein (MCP), which is then digested in the small intestine (SI). The protein and amino acids produced by microbial processes are inadequate for optimal daily production, leading to the current practice of supplementing ruminant diets with additional amino acids.Bacteria in the rumen can break down free amino acids, necessitating the supplementation of amino acids in a protected form to ensure their stability in the rumen and absorption post-ruminally for metabolic purposes. The small intestine (SI) is the primary site of amino acid absorption, so it is crucial to protect amino acids from ruminal degradation and direct them to the absorption sites. Beyond their role in milk production, amino acids are important for immune function in animals and have been shown to alleviate oxidative stress. Low-protein diets supplemented with rumen-protected amino acids (RPAA) can replace high-protein diets in summer, as increased protein metabolism can result in higher heat production and subsequent stress.

KEYWORDS: Limiting amino acids, Milk production, Oxidative stress, Rumen-protected amino acids, Ruminants

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INTRODUCTION

Protein is a major macromolecule composed of amino acids (AAs).Amino acids, the building blocks of proteins, are classified based on their structure, the electrical charge of side chains, and their essentiality in nutrition.The essential amino acids (EAAs) which cannot be synthesized in the body and are required by ruminants and non-ruminants in diet include lysine (Lys), methionine (Met), histidine (His), isoleucine (Ile), leucine (Leu), phenylalanine (Phn), threonine (Thr), tryptophan (Trp), Arginine (Arg) and valine (Val). Among these essential amino acids, some of them including lysine, methionine etc are low in practical diets termed as critical amino acids. High-producing animals often require more dietary essential amino acids than those supplied by microbial and escaped protein pools, making the supplementation of rumen-protected protein (RPP) and amino acids crucial to meet their physiological and productive needs (Ali et al., 2009). This has led to the trend of supplementing ruminant diets with amino acids (Schwab and Broderick, 2017). However, supplementing free amino acids in ruminants is ineffective because rumen microorganisms utilize them before they reach the small intestine for absorption (Chen et al., 2020). Since the small intestine (SI) is the main site of amino acid absorption, amino acids need to be supplemented in a protected form to ensure stability in the rumen and post-ruminal absorption for metabolic purposes. Shielding amino acids from degradation in the rumen allows for the supplementation of diets with specific amino acids that can be absorbed in the intestine (NRC, 2001).Rumen-protected protein (RPP) supplementation is vital for productive ruminants relying on high crude protein (CP) diets, as inefficiency arises when highly degradable concentrates like groundnut or mustard cake are used, converting amino acids into non-protein compounds (Ali et al., 2009). Encapsulated nutrients are particularly beneficial for dairy animals during transition and peak lactation, meeting higher nutrient demands.

Thus, supplementing nutrients in protected forms is an effective strategy for improving dairy production under stressful conditions like transition, peak lactation, and heat stress.Understanding the use of rumen-protected amino acids (RPAA) and their benefits is crucial. Additionally, the benefits of using different amino acids and RPAA in animal growth and production are emphasized.

Principles of rumen protection and mechanism of action:

Physical or Chemical Protection

Techniques like heat treatment, tannin inclusion, or chemical additives (e.g., sodium hydroxide, formaldehyde, alcohols etc) are used to modify proteins, making them resistant to microbial breakdown (Mazinani et al., 2022).Non-enzymatic browning (Maillard reaction) increases protein resistance to ruminal enzymes.

Selective Degradation

Encapsulation is defined as the "entrapment of a component (the core) within a secondary material (encapsulant matrix). Encapsulation methods ensure specific amino acids, like methionine and lysine, bypass the rumen while being available for absorption in the intestine(Ji et al*.,* 2016).

Optimized Utilization

Protection mechanisms ensure the availability of essential nutrients like lysine and methionine for absorption in the small intestine, promoting tissue protein synthesis and enhancing overall feed efficiency.

The process of rumen-protected amino acids, such as lysine and methionine, being safeguarded by a protective coating to prevent rumen degradation. The coating dissolves in the intestine, allowing amino acids to be absorbed for tissue protein synthesis.

Importance of rumen-protected amino acids

Rumen-protected amino acids are vital nutrients that improve the biology of cattle and optimize the use of other dietary components. This enables the fulfilment of health, production and reproductive demands of cows across all stages of lactation and production levels. They supply essential nutrients, such as lysine and methionine, more efficiently and reliably than conventional feed sources like blood meal and soybean meal, while avoiding the oversupply of nutrients that the cow may not need. Rumen-protected amino acids offer several advantages for livestock nutrition. By incorporating these amino acids into the feed, farmers can reduce total ration costs, as they provide essential nutrients more efficiently than traditional feedstuffs. This efficiency helps mitigate the impact of rising raw material prices. Additionally, rumen-protected amino acids optimize ration space, allowing for the inclusion of other vital nutrients like energy and fiber (Amrutkar et al., 2014;Mazinani et al.,2022). This results in a more balanced and nutrient-dense diet that supports optimal cattle growth and health. Another benefit is the consistent nutrient flow they provide, reducing variability in nutrient supply often caused by traditional feed ingredients. This consistency helps maintain steady milk production and overall herd health. Compared to heat-treated ingredients like blood meal and soybean meal, rumen-protected amino acids offer a more reliable nutrient supply because they are designed to withstand the rumen environment, leading to better nutrient absorption. Furthermore, their inclusion in diets promotes environmental and health benefits by improving nitrogen use efficiency, reducing nitrogen excretion, and lowering environmental pollution, while also supporting better animal health and preventing metabolic disorders.

Effects of Rumen-Protected Amino Acids on Cattle Performance

A. Milk Production and composition

Milk production in dairy cows is closely tied to their ability to meet nutritional needs, particularly in terms of essential amino acids like lysine (Lys) and methionine (Met), which play a pivotal role in protein synthesis and metabolism. During early lactation, cows experience increased demand for these amino acids, making their supplementation crucial to achieving optimal milk yield and composition. Rumen-protected amino acids (RPAA), especially RP-Lys and RP-Met, have been widely studied for their ability to bypass degradation in the rumen and improve amino acid availability in the small intestine, directly influencing milk production.

Research consistently shows that supplementing diets with RPAA can enhance milk yield and improve the concentrations of milk fat and protein, particularly in high-producing cows. Studies like Liu et al. (2016) demonstrated that the inclusion of RP-Lys in lactating cows' diets increased milk yield and fat-corrected milk (FCM), especially when the cows were fed rapeseed meal. This finding is consistent with Pas et al.(2014), who observed enhanced milk fat and protein concentrations in Holstein Friesian cows producing more than 36 kg of milk per day. These studies highlight a critical insight: the efficacy of RPAA is often influenced by the cow's milk production level. In cows with lower milk yields, the response to RPAA supplementation may be less pronounced, possibly due to a reduced metabolic demand for these amino acids or a balanced amino acid profile in the basal diet.Additionally, studies on individual supplementation of the metabolizable protein deficit (MPD) diet with RP-Met, RP-Lys, and RP-histidine found no significant impact on milk yield, echoing similar observations regarding Lys (Robinson et al., 2000).

Amrutkar et al. (2015) took this a step further by investigating the effects of combined supplementation of RP-Lys and RP-Met in periparturient dairy cows. Their findings illustrated a significant increase in milk yield by 14.21% compared to the control group, along with improvements in milk fat, protein, and lactose concentrations. The increased milk production in response to RPAA is largely attributed to the role of Lys and Met in enhancing protein synthesis, which is particularly critical during the early lactation period when cows experience negative energy balance (NEB).Recent studies have shown that the addition of a combination of three RPAA to the metabolizable protein deficit (MPD) diet numerically increased milk yield, albeit still below that of the metabolizable protein adequate (MPA) diet. This result can be attributed to the trend for increased dry matter intake (DMI) due to RP-histidine, along with the positive independent and additive effects of the three RPAA on milk production (Lemosquet et al., 2009). Positive responses in milk yield have also been reported with similar combinations of essential amino acids (Kim et al., 2000; Lee et al., 2012).

Contrasting findings, such as those from Awawdeh (2016), who found no significant impact of RPAA on milk yield and composition except for milk protein, suggest that the response to RPAA supplementation may depend on multiple factors, including the cow's lactation stage, the overall protein content of the diet, and the adequacy of other nutrients such as energy. In contrast, Mohsen et al. (2011) found that RP-choline (RPC) supplementation had a notable impact on nutrient digestibility, milk yield, and composition, pointing to the diverse roles different rumen-protected compounds can play in enhancing milk performance.

The combination of RP-Lys, RP-Met, and other essential amino acids such as histidine has shown synergistic effects, particularly in diets deficient in metabolizable protein (Lee et al., 2015). These amino acids not only promote milk protein synthesis but also improve nitrogen utilization efficiency, reducing nitrogen excretion into the environment. This finding has been supported by multiple studies (Giallongo et al., 2016 and Wang et al.,2022), emphasizing the environmental benefits of RPAA in minimizing nitrogen pollution while maintaining milk production. Such findings are particularly significant in modern dairy production systems, where sustainability and environmental impacts are of increasing concern.

Beyond amino acids, rumen-protected choline (RPC) has also gained attention for its role in supporting milk yield, particularly by improving liver function and fat metabolism. Huang et al. (2023) consistently demonstrated that the supplementation of rumen-protected amino acids positively influences both milk yield and milk protein synthesis in dairy cows.However, the effectiveness of RPC supplementation appears to vary, as demonstrated by studies like Hartwel (2000), Piepenbrink and Overton (2003) and Leiva et al. (2015), who found mixed results regarding its ability to enhance milk yield and composition. These studies suggest that while RPC may offer metabolic benefits, its effects on milk production are more context-dependent, requiring careful consideration of diet composition and physiological state.

B. Growth performance

In addition to lactation, the supplementation of RPAA has been shown to positively impact growth performance in cattle, particularly during the critical stages of development, including the growing and finishing phases. Growth performance is highly influenced by the availability of essential amino acids in the diet, which are required for muscle accretion and tissue development. Rumen-protected forms of Lys and Met have proven effective in increasing the efficiency of amino acid absorption, leading to improved body weight gain and feed efficiency.

Several studies have demonstrated that RPAA supplementation enhances dry matter intake (DMI) and average daily gain (ADG) in growing cattle. Amrutkar et al. (2015) showed marked increases in nutrient intake and body condition in lactating cows receiving RP-Lys and RP-Met. These improvements are likely linked to enhanced protein metabolism and nitrogen utilization, allowing the animals to convert dietary protein into muscle tissue more efficiently.

Similarly, studies on buffaloes and crossbred bulls (Gami et al., 2015; Xue et al., 2011) further support the role of RPAA in promoting growth. The incorporation of RP-Lys and RP-Met into the total mixed ration (TMR) improved average daily gain and body condition, demonstrating that RPAA is effective across different cattle breeds and production systems. Sai et al. (2014) reported a 16.1% higher average daily gain in female calves fed a basal diet supplemented with 2 g rumen-protected methionine (RPM) and 17 g rumen-protected lysine (RPL) per calf daily for 90 days. Singh et al.(2015) found improved average daily gain in heifers supplemented with RPM and RPL at varying levels, highlighting better growth performance compared to a control group. However, the benefits of RPAA supplementation are not universally observed. Heiderscheit and Hansen (2020) reported a negative effect of RP-Lys supplementation on ADG in Angus crossbred steers, suggesting that over-supplementation of Lys, particularly in the finishing phase, may disrupt the balance of amino acids and hinder growth. This underscores the importance of carefully balancing amino acid levels in cattle diets to avoid overconsumption and ensure optimal growth performance.Cabezas et al. (2023) studied two groups of 40 Montbéliard steers fed either a control diet or a diet with rumen-protected lysine and methionine. No significant differences were observed in body weight, or average daily gain (ADG) during the first 112 days or overall (0–202 days). However, from days 112 to 202, steers on the reduced protein diet had a higher ADG than those on the control diet.

Recent research, like that of Almallah et al. (2021), has further elucidated the mechanisms through which RPAA improves growth performance, particularly by enhancing nitrogen retention and reducing the energy cost of nitrogen excretion. By promoting more efficient nitrogen utilization, RPAA allows animals to allocate more energy towards growth, reducing the waste associated with excess nitrogen excretion. This is particularly beneficial in high-growth phases when nutrient efficiency is critical for achieving target body weights.

The role of rumen-protected amino acids in heat stress amelioration

Heat stress is a major concern in dairy and beef cattle production, especially in tropical and subtropical climates where high temperatures and humidity can severely impact animal performance. Heat stress reduces feed intake, lowers milk yield, impairs reproductive performance, and can lead to increased susceptibility to diseases. One of the emerging strategies to mitigate the adverse effects of heat stress is the use of RPAA, which can improve nutrient utilization and reduce the energetic cost of nitrogen metabolism.

Research has shown that feeding excess protein during heat stress can exacerbate the energy deficit by increasing heat production during nitrogen excretion. Studies like Abdi-Benemar et al. (2015) have demonstrated that low-protein diets supplemented with RP-Lys and RP-Met can alleviate some of these effects by improving nitrogen efficiency and reducing urinary nitrogen excretion, thereby conserving energy for milk production. This is crucial in hot climates, where energy conservation is key to maintaining productive performance.

Cheng et al. (2014) also explored the role of rumen-protected γ-aminobutyric acid (GABA) in alleviating heat stress. Their findings indicated that GABA supplementation reduced rectal temperatures in heat-stressed dairy cows, suggesting that certain amino acids can play a thermoregulatory role. Likewise, Izquierdo et al. (2024) found that RP-Met supplementation lowered internal body temperatures in Nellore cows, highlighting the potential of RPAA to reduce heat stress and improve animal welfare during hot conditions. These findings are significant for improving cattle resilience in regions prone to extreme weather, pointing to the broader benefits of RPAA beyond production and growth.

Effect of rumen-protected amino acids on immune function

The transition period, particularly the weeks surrounding calving, is a critical time for dairy cows, marked by metabolic and physiological challenges that can compromise immune function. During this period, cows experience a drop in dry matter intake (DMI) and enter a negative energy balance (NEB), leading to increased susceptibility to metabolic disorders and infections. Recent research has explored the role of RPAA, particularly RP-Met, in enhancing immune function during this critical time.

Methionine, as a precursor to key metabolites like glutathione, taurine, and polyamines, plays a crucial role in maintaining antioxidant capacity and supporting immune responses. Studies like Sun et al.(2016) demonstrated that RP-Met and RP-choline supplementation improved antioxidant status in transition cows by increasing blood vitamin E concentrations, glutathione peroxidase (GSH-Px) activity, and total antioxidant capacity (T-AOC). These improvements in antioxidant markers contribute to better immune defense mechanisms, reducing the risk of infections during the peripartum period.

Other research, such as Batistel et al.(2018), further confirmed the role of RP-Met in supporting liver function and immune response. Cows supplemented with RP-Met showed higher plasma levels of cholesterol, albumin, and IL-6, along with reduced haptoglobin, indicating a more robust immune status. Additionally, Zhou et al. (2016) highlighted the beneficial effects of RP-Met on phagocytic capacity and oxidative burst, both of which are critical components of the immune response in dairy cows.

However, not all studies have reported positive effects. Abdelmegeid et al. (2017) studied neonatal calves and found that methionine supplementation reduced the expression of interleukin-10 (IL-10) and IL-6 genes in Toll-like receptors (TLR-4) and NFêâ pathways. This suggests that methionine attenuated the inflammatory response in PMN leukocytes.

Cost efficacy of rumen-protected amino acids

Studies on the cost efficacy of rumen-protected amino acids (RPAA) highlight their dual benefits of reducing feed costs and maintaining livestock production. RPAA supplementation allows a reduction in dietary crude protein levels while preserving performance, leading to significant savings in feed costs (Mazinani et al., 2022) . RPAA also improved nitrogen utilization efficiency, minimizing nitrogen excretion and enhancing environmental sustainability alongside economic gain (Kamalak et al., 2005)Additionally, Amrutkar et al.(2014)concluded that supplementing rumen-protected methionine (RPM) and lysine (RPL) in the diet of high-yielding lactating cows during early lactation improved net returns by 12.3% compared to a standard diet, with similar feed costs and cost-benefit ratios, enhancing the profitability of dairy operations by reducing input costs while optimizing production .

CONCLUSION

Rumen-protected amino acids (RPAAs) are vital for optimizing dairy cow nutrition by ensuring essential amino acids like lysine and methionine are absorbed in the small intestine. They enhance milk production and quality, improving yield and composition. RPAAs also boost growth and feed efficiency, positively affecting body weight and condition. They help mitigate heat stress by reducing metabolic heat production and nitrogen excretion. Additionally, RPAAs support immune function and reduce oxidative stress, aiding overall animal health. Their use represents an effective strategy for improving high-yielding dairy cows.

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Effect of Feeding Rubber (*Hevea brasiliensis*) Seed Meal Based Diets on Intake, Nutrient Digestibility and Growth Performance in Goats

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ABSTRACT

The study investigated the processed rubber seed meal (RSM) as an alternative feed ingredient in goat kids. The chemical analysis indicated that RSM contained 30.85 % CP and 14.13 MJ ME / kg DM. The methods employed to detoxify raw RSM were soaking, boiling with and without decanting and fermentation for different time periods and the residual hydrogen cyanide levels were below the detectable levels in all processed RSM. The *in vitro* true digestibility (%) of raw and boiled RSM with respect to DM, OM and NDF was 92.11, 92.47 & 63.48 and 87.88, 88.38 & 59.64, respectively. For feeding trial, 15 kids of comparable body weight and age were distributed into three groups of 5 each in completely randomized design. The compounded feed mixture (CFM) containing groundnut cake (GNC) as a protein supplement was served as control (T1) and test CFMs contained boiled RSM replacing 12.5 % (T2) and 25 % (T3) of GNC nitrogen in control. Each CFM was fed to one of the three groups while the COFS-29 hay was offered *ad libitum* for a period of fourteen weeks. The total dry matter intake (DMI) in terms of g/d (273.4 to 307.9) or % BW (2.68 to 2.94) was similar among the groups. However, the DMI in terms of g/kg$W^{0.75}$ was lower ($P<0.05$) in T3 (47.82) compared to control (52.72) which might be due to lower hay intake in T3 (57.66 g/d) compared to T1 (81.12 g/d). The average daily gain [22.75 (T3) to 32.24 g (T1)] and feed conversion ratio [3.85 (T2) to 4.07 (T3)] were non-significant among the treatments. The digestibility trial conducted during terminal week revealed that the apparent digestibility (%) of DM, OM, CP, EE, NDF and ADF were comparable among treatment diets. The digestible OM in DM was non-significantly ranged between 74.58 (T2) and 71.35 % (T1). It was concluded that boiled RSM can replace conventional protein source upto 25% on nitrogen equivalent basis in CFM of goat kids for sustainable small ruminant production.

KEYWORDS: Detoxification, Digestibility, Goat kids, Growth, Intake, Rubber seed meal

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INTRODUCTION

Goats play a crucial role in the livelihood of small and marginal farmers in India, particularly in ecologically fragile regions like arid, semi-arid, and hilly areas. India ranks second in the global goat population with 148.88 million goats (20th Livestock Census, 2019). Feed accounts for about 75% of livestock production costs, and there is a current net shortage of 44% of concentrate feed ingredients, 10.95% of dry crop leftovers, and 35.6% of green fodder (ICAR-IGFRI, Vision 2050). The rise in both human and livestock population has led to food insecurity, with a high demand for conventional feed ingredients with escalating prices. This necessitates the identification of alternative, nutrient-rich, and cost-effective feedstuffs. Rubber seed meal (RSM) offers a promising alternative and rubber seed trees are predominantly found in Southeast Asia and West Africa (Lukman et al., 2018). RSM contains 30.68% crude protein, 22.27% crude fat, and 33.53% nitrogen-free extract (Aguihe et al., 2017). Rubber seed has high protein content and also has numerous anti-nutritional factors such as hydrogen cyanide (HCN), phytic acid, oxalic acid, tannin, saponin and trypsin inhibitor. Among these, cyanogen compounds are more prominent and HCN content in raw RSM can lead to adverse effects such as growth depression, decreased protein synthesis, and immune system suppression (Ukpebor et al., 2007; Deng et al., 2015). This necessitates detoxification processes such as heat treatment, storage, enzymatic reaction, and fermentation for inclusion of RSM in animal diets.

Udo et al. (2021) reported that the diet incorporated with 10% boiled RSM was better utilized by West African dwarf bucks and could be used to formulate alternative concentrate for goats. However, the available literature is not sufficient to recommend the safe inclusion level of RSM in ruminant diets. Given limited research on using detoxified RSM in small ruminants, this study aims to evaluate its effects on nutrient intake, digestibility and growth performance in goats.

MATERIALS AND METHODS

Processing of RSM

Sufficient quantity of RSM for the study was supplied by M/s Cardolite Speciality Chemicals Pvt Ltd., Mangalore, India. At laboratory level, raw RSM was processed by soaking, boiling with and without decanting and fermentation for different time periods. Two hundred grams of raw RSM was soaked in water for 24 h in two closed containers. Then, the supernatant was discarded and the samples were dried in an oven at 100±2° C for overnight to obtain soaked RSM. Another two samples, each weighing two hundred grams of raw RSM were placed in separate cooking containers where the water had reached a temperature of 100° C and then boiled separately for 30 minutes and 45 minutes. Boiled samples were allowed to cool and samples were wrapped in aluminum foil and kept in an air tight container and fermented for 7 days. These fermented samples were dried in hot air oven at 100±2° C for overnight. Next two samples, each weighing two hundred grams of raw RSM were placed into separate cooking containers where the water had reached a temperature of 100° C and then one boiled for 30 minutes and another for 45 minutes and samples were dried in an oven at 100±2° C for overnight to obtain boiled without decanting RSM. Another two samples, each weighing two hundred grams of raw RSM were placed in separate cooking containers where the water had reached a temperature of 100° C and then boiled separately for 30 minutes and 45 minutes and supernatant was discarded and samples were dried in an oven at 100±2° C for overnight to obtain boiled with decanting RSM. Finally the products obtained were milled as soaked, boiled cum fermented, boiled and boiled with decanted RSM, respectively. The raw and processed RSM samples were analyzed for proximate composition, fibre fractions, *in vitro* digestibility, residual HCN and mycotoxins.

Chemical analysis and *in vitro* study

Representative samples of raw and differently processed RSM including other feed ingredients, compounded feed mixtures and roughages used in the experiment were collected for analysis. The ash content was determined by measuring the residue obtained after the samples were incinerated at 600° C for a duration of 3 hours in a muffle furnace. The crude protein content (N x 6.25) was assessed using Gerhardt digestion and distillation unit adhering to macro Kjeldahl standards (AOAC, 2005). Following the procedure outlined in AOAC (2005), the ether extract (EE) content of the samples was determined through extraction with petroleum ether. Fibertherm was used to assess the fibre fractions, neutral detergent fiber (NDF) and acid detergent fiber (ADF), as outlined by Van Soest et al. (1991). Various mycotoxins, including aflatoxin (B1, B2, G1 and G2) were analyzed using a two-dimensional thin-layer chromatographic method based on the approach suggested by Tapia (1985). Hydrogen cyanide was analyzed based on the approach suggested by Beek and De Jong (2011) using HPLC with fluorescence detection.

Further, all the samples were analyzed for *in vitro* true dry matter digestibility (IVTDMD), *in vitro* organic matter digestibility (IVOMD) and neutral detergent fibre digestibility (NDFD) according to Goering and Van Soest (1970) modification of Tilley and Terry (1963) in *in vitro* batch fermentor. For the purpose, approximately 400 mg of dried sample was weighed into F57 Ankom Filter bags and incubated for 48 h in sealed Erlenmeyer flasks with a mixture of Mold's buffer and rumen fluid (4:1 ratio) in triplicate. After incubation, leftover residues in bags were treated with neutral detergent solution in an Ankom200 fiber analyzer, and the remaining dry residues were weighed.

Experimental animals

Fifteen goat kids of about 3 to 4 months of age with comparable body weight were selected from the flock maintained at Livestock Research and Information Centre (Sheep), Nagamangala, Mandya Dist., Karnataka. The kids were divided into three groups of five each and treatments were allocated randomly to individual group. The procedures employed in the animal trial were duly approved by Institutional Animal Ethics Committee (IAEC) of Veterinary College, Hebbal, Bengaluru vide No.

VCH/IAEC/2023/02 as per Article No. 13 of the CPCSEA rules laid down by Government of India.

Experimental diets and feeding management

In accordance with ICAR (2013) guidelines, diets were formulated to fulfill the energy and crude protein requirements for maintenance and growth of the experimental kids. The diets were comprised of sorghum hay (COFS-29) and compounded feed mixtures (CFM). Animals of control (T1) group were fed GNC based CFM and T2 and T3 groups of experimental animals were fed CFM incorporated with boiled RSM to substitute 12.5 and 25 % of GNC on nitrogen equivalent basis, respectively. The ingredient composition of CFM T1 was 57% maize and 40% GNC, CFM T2 was 55% maize, 35% GNC and 7% boiled RSM and CFM T3 was 53% maize, 30% GNC and 14% boiled RSM. The mineral mixture and common salt was included at 2 and 1%, respectively in all the CFMs. The daily requirement of CFM was provided in two equal portions and offered, at 09.00 and 16.00 hours. The sorghum (COFS-29) hay served as the sole source of roughage and offered *ad libitum* to all the animals. To assure good intake, the COFS-29 hay was weighed and provided to each individual kids three times a day at 09.30, 14.00 and 17.00 hours. Clean drinking water was provided to individual kids in a plastic bucket thrice a day. The feeding trial was carried out for fourteen weeks with an initial adjustment period of ten days. The body weights of the animals were taken at weekly interval in the morning before offering any feed and water.

Digestion trial

A digestion trial employing all the animals was conducted for six days during the terminal week of the experiment to assess the digestibility of nutrients by following standard procedures. The representative feed, faecal and ort samples were subjected for proximate analysis (AOAC, 2005) and detergent system of feed analysis (Van Soest et al., 1991). The digestibility of nutrient was calculated as the difference in nutrient intake and outgo and expressed as percentage intake.

Statistical analysis

The data obtained during feeding and digestion trial was analysed statistically (ANOVA) using SPSS version 16.0. Individual differences between means were tested using Tukey ‘t’ test and results were interpreted accordingly (Snedecor and Cochran, 1995).

RESULTS AND DISCUSSION

Chemical composition and *in vitro* digestibility

The mycotoxin analysis of raw and boiled RSM revealed that the concentration (as µg/kg) of aflatoxin B1, B2, G1 and G2 in raw and boiled RSM were 133.8, 1.0, 137.4 & 0.5 and 17.0, 1.6, 96.3 & 3.7, respectively. Romero-Sanchez et al. (2024) observed that the thermal cooking (boiling with water at 100^{o}C for 12 min) significantly reduces the aflatoxin content. The maximum permissible level of aflatoxin in ruminant diet is 20 ppb/kg DM. Thus the inclusion level of BRSM in T2 and T3 at 7 and 14 per cent on w/w basis in the CFM ensured the aflatoxins below the specified limit in the diets. The HCN in raw and processed RSM were below limit of quantification (<0.05 mg/kg). Udo et al. (2016) reported that boiling rubber seeds for half an hour, followed by sun drying, effectively eliminated HCN. Similarly, Agbai et al. (2021) observed that fresh rubber seeds initially contained a substantial amount of HCN (12.41 mg/ 100 g), but boiling reduced it to 4.71 mg/100 g, and fermentation further lowered HCN to 1.97 mg/100 g and these findings were similar to observations of Farr et al. (2019) and Diptanu and Samanta (2020). RRSM contains oxalates, saponins, phytates, trypsin inhibitor in addition to HCN. Boiling not only reduces HCN but also reduces heat labile toxins such as trypsin inhibitor. Thus boiling makes a better option of processing RRSM in comparison to other detoxification methods (Farr et al. 2019). These studies collectively emphasize the effectiveness of boiling in removing HCN and other heat labile anti-nutritional factors like protease inhibitors from RSM, thereby improving its safety for use as protein supplement in ruminant diets.

Table 1. Chemical composition[1] (% DMB) of different feedstuffs and compounded feed mixtures (CFM)

Attribute	Maize	GNC	RRSM	BRSM	CFM			COFS-29 hay
					T1	T2	T3	
Proximate principles								
Organic matter	96.5	95.4	94.3	93.9	95.6	95.6	95.1	90.2
Crude protein	9.43	39.8	30.9	30.2	22.7	22.0	22.0	8.95
Ether extract	2.92	9.86	17.1	15.1	5.17	6.11	6.89	2.34
Total ash	3.46	4.56	5.72	6.16	4.42	4.40	4.90	9.79
Fibre fractions								
Neutral detergent fibre	15.7	19.3	40.3	41.7	19.6	21.1	23.1	65.9
Acid detergent fibre	6.22	10.4	17.9	18.5	8.72	9.98	10.7	38.6
Hemicellulose	9.49	8.92	22.4	23.2	10.9	11.1	12.5	27.3

The proximate composition and fibre fractions of concentrate feed ingredients, compounded feed mixtures (T1, T2 and T3) and sorghum (COFS-29) hay fed to kids during the growth trial are presented in Table 1 and the *in vitro* digestibility values in Table 2. The proximate composition of boiled RSM was comparable with the values reported by Suprayudi et al. (2015) and Aguihe et al. (2017) except for crude fat. The proximate composition with respect to all nutrients was comparable with the values reported by Mmereole et al. (2008) and Ijaiya et al. (2011) except for ether extract which varied. The ether extract was lower in the present study which might be due to lower levels of oil in RSM and it also depends on the oil extraction method followed. The composition with respect to fibre fractions of RSM was comparable with values reported by Nongyao et al. (1991) and Nguyen and Duong (2003). This indicates that detoxified RSM has considerable amounts of absorbable nutrients than many conventional seed meals and exhibits better alternative for protein supplements in livestock diets. The proximate principles of COFS-29 hay were in agreement with the findings of Iyanar et al. (2015) and Senthilkumar et al. (2009). The fibre fraction composition of COFS-29 hay was comparable with the values reported by Wadhwa et al. (2010). The *in vitro* dry matter digestibility of RSM was in agreement with Jayanegara et al. (2018).

Table 2. *In vitro* digestibility (%) of different feed stuffs and compounded feed mixtures (CFM)

Feedstuff	IVTDMD	IVOMD	NDFD
Maize	93.8	94.2	64.3
GNC	81.8	82.8	79.5
RRSM	92.1	92.5	63.5
BRSM	87.9	88.4	59.6
COFS-29 hay	63.7	44.0	39.7
CFM T1	94.5	94.9	75.0
CFM T2	93.3	93.7	71.3
CFM T3	91.5	91.9	69.9

Dry matter and nutrient intake

The mean intake of DM and other nutrients in different treatment groups are presented in Table 3. The total DMI (as gram per day and per cent body weight) for T1, T2 and T3 groups were found non-significant among the treatment groups. The observed DMI in T1, T2 and T3 groups was comparable with the average daily intakes of DM (T1-2.5, T2-2.8 and T3-2.7 %) as reported by Nguyen and Duong (2003) who used three levels of rubber seed cake replacing 0, 50 and 100 % of groundnut cake in the concentrate supplement and found non-significant difference in DMI in growing goats.

The DMI observed in this study was in agreement with the findings of Chanjula et al. (2011) where rubber seed kernel was included at levels ranging from 20 to 30 % to replace palm seed kernel in goats and found no significant difference in DMI at 20 % inclusion level. However, increasing rubber seed kernel levels beyond 20% resulted in a slight reduction in daily DMI. Simillar observations were noted by Tean et al. (2002) where RSM was incorporated at various levels in the experimental diets of pigs, replacing wheat bran at 0, 10, 20 and 30 % and found non-significant DMI among different treatment groups. Ouppamong et al. (2022) also found that the use of yeast fermented rubber seed kernel in the diet of lactating dairy cows did not affect feed intake when included at 0, 10 and 20 % in concentrate portion of diets. In contrast, the observed values were not in agreement with Udo et al. (2021) who included boiled RSM from 0 – 40 % in CFM (0 %, 20 %, 30 % and 40 % inclusion of boiled RSM in compounded feed mixtures) in West African dwarf bucks.

Intakes of OM, NDF and ADF were not significant, whereas the intakes of CP and EE were found to be significant ($P<0.05$) among the treatment groups (Table 3). In all the three groups the intake of NDF and ADF was adequate, for optimum ruminal fermentation (Van Soest et al., 1991).

Although there was no significant difference in DMI but the significant ($P\leq0.05$) difference in CP intake was due to less consumption of COFS-29 hay in RSM based diets T2 and T3 groups compared to control. CP intake through CFM was similar in all the treatment groups but intake through sorghum hay differed significantly among the treatment groups, this could be the reason for significant difference in CP intake. The T3 group of animals consumed higher EE as RSM incorporated CFM has more oil compared to control CFM with GNC.

Table 3. Nutrient Intake during feeding trial in different treatments

Attribute	T1	T2	T3	SEM	P-value
DM					
g per day	307.9	278.8	273.4	6.42	0.061
% of body weight	2.94	2.82	2.68	0.01	0.073
g/kg $W^{0.75}$	52.7^{b}	49.8^{ab}	47.8^{a}	0.77	0.035
Organic matter					
g per day	290.9	263.6	257.2	6.05	0.054
% of body weight	2.77	2.67	2.53	0.01	0.058
g/kg $W^{0.75}$	49.8^{b}	47.1^{ab}	45.0^{a}	0.72	0.026
Crude protein					
g per day	58.9^{b}	52.0^{a}	52.6^{ab}	1.21	0.035
% of body weight	0.56^{b}	0.52^{ab}	0.51^{a}	0.00	0.036
g/kg $W^{0.75}$	10.1^{b}	9.26^{a}	9.19^{a}	1.36	0.014
Ether extract					
g per day	14.1^{a}	14.4^{ab}	16.2^{b}	0.33	0.021
% of body weight	0.13^{a}	0.14^{a}	0.15^{b}	0.00	<0.001
g/kg $W^{0.75}$	2.41^{a}	2.56^{a}	2.83^{b}	0.03	<0.001
NDF					
g per day	98.1	90.4	87.9	3.34	0.200
% of body weight	0.93	0.91	0.86	0.01	0.226
g/kg $W^{0.75}$	16.8	16.2	15.4	0.42	0.160
ADF					
g per day	48.7	45.9	44.4	1.41	0.081
% of body weight	0.46	0.46	0.42	0.01	0.121
g/kg $W^{0.75}$	8.36	8.23	7.62	0.19	0.064

[ab]Mean values in a row bearing different superscripts differ significantly ($P<0.05$ / $P<0.01$)

Body weight gain and feed conversion ratio

The average daily body weight gain (ADG) and feed conversion ratio (FCR) for three treatment groups during the growth trial is presented in Table 4. In the present study, there was no significant difference in the initial body weight, final body weight and ADG of the kids among the treatment groups. The observations are in corroboration with results of Nguyen and Duong (2003) who found non-significant difference among the treatment groups. In the present study goat kids were fed under intensive system of rearing which might have caused stress as these animals are primarily browsers and need more flexibility in feeding practices. Although, diet (CFM and COFS-29 hay) for the treatment groups (T1, T2 and T3) in the present study was formulated to achieve a target ADG of 75 g in non-descriptive goats (ICAR, 2013), individual variations were observed with respect to intake, digestibility and efficiency of utilization in goat kids which are generally sensitive to any change in feeding regimen.

Table 4. Mean body weights, average daily gain (ADG) and feed conversion ratio (FCR) of experimental kids during the growth trial

Attribute	T1	T2	T3	SEM	P-value
Initial body weight (kg)	9.5	9.28	9.68	0.53	0.960
Final body weight (kg)	12.7	11.7	11.9	0.62	0.837
Body weight gain (kg)	3.16	2.46	2.23	0.26	0.366
Overall ADG (g)	32.2	25.1	22.8	3.36	0.490
Overall FCR	4.00	3.85	4.07	0.73	0.993

There was no significant difference in ADG among the treatment groups which indicates that replacing GNC with boiled RSM on nitrogen equivalent basis did not affect the growth performance of kids. However, negative ADG in both GNC and boiled RSM based diets in a few weeks of the study was primarily due to low DMI and associated stress in stall fed conditions. In the present study, FCR (T1: 4.00, T2: 3.85 and T3: 4.07) was found to be non-significant among the treatment groups which indicates that replacing GNC with boiled RSM did not affect the feed efficiency of growing kids.

Apparent digestibility of DM and its nutrients

There was no significant difference in the apparent digestibility of different nutrients viz. DM, OM, CP, EE, NDF and ADF among the experimental groups (Table 5). In the present study apparent digestibility of DM, OM, CP, EE, NDF, ADF and digestible organic matter in dry matter (DOMDM %) were similar in all the groups indicating no adverse effect on digestibility of nutrients (Table 5). The nutrient digestibility (%) values for T1, T2 and T3 are in agreement with the reported values by Vishwanathan (1977) in growing calves for digestibility for DM, OM, CP and EE. The results are comparable with the findings of Chanjula et al. (2011) who found non-significant difference among the treatment groups in the digestibility of DM, OM and CP. However, the apparent digestibility of NDF, ADF and EE differed significantly ($p \leq 0.05$) among treatment groups and such a trend was not observed in the present study. The apparent digestibility co-efficient of DM and EE in goats were in corroboration with the findings of Udo et al. (2021) who found non-significant difference among the treatment groups.

Table 5. Apparent digestibility (%) of different nutrients during the digestion trial

Attribute	T1	T2	T3	SEM	P-value
DM	73.4	76.9	76.8	0.02	0.180
OM	75.4	78.7	78.3	0.01	0.201
CP	70.0	72.4	71.6	0.01	0.637
EE	70.4	74.8	75.0	0.01	0.130
NDF	46.0	52.3	52.0	0.01	0.120
ADF	31.2	37.6	36.1	0.03	0.219
DOMDM[1]	71.4	74.6	73.7	0.01	0.219

[1] digestible organic matter in dry matter

CONCLUSION

Considering the overall performance of the experimental kids, feeding of boiled rubber seed meal based diets resulted in similar nutrient intake and digestibility, body weight gain and feed conversion ratio as compared to groundnut cake based diet. It is inferred that, boiled rubber seed meal can be utilized as a component of compounded feed mixtures up to 25 % of the conventional protein source on nitrogen equivalent basis for feeding ruminants to ensure efficient utilization of available non-conventional feed resources and reduce the cost of livestock production.

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Assessment of Paddy Straw Availability as a Feed Source for Sustainability of Livestock Farming in Guntur, Bapatla and Palnadu Districts of Andhra Pradesh

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ABSTRACT

This study focuses on the availability of paddy straw, a critical fiber source for dairy animals in Andhra Pradesh (AP), with a particular emphasis on the Guntur, Bapatla, and Palnadu districts. These districts represent three distinct irrigation scenarios in paddy cultivation: highly irrigated (Bapatla), rain-fed (Palnadu), and bore well-based (Guntur). Data on paddy straw usage and availability were collected from 84 dairy farmers directly or indirectly involved in paddy cultivation, spanning nine villages (three from each district). The questionnaire data indicated that the main reasons for mechanical harvesting and straw burning were a shortage of labour and high labour costs. The average yield of paddy straw was estimated at 8.75 tonnes/ha for manual harvesting and 3.74 tonnes/ha for mechanical harvesting. On average, dairy farmers feed 6 kg of paddy straw per animal per day, depending on the availability of green fodder and grazing grass. This data was applied to assess paddy straw requirements and availability in three districts and also in Andhra Pradesh based on the data reported by Agriculture and Animal Husbandry Departments. The study found that machine harvesting of paddy results in deficiencies of 53.62%, 60.82%, and 94.83%, while manual harvesting results in deficiencies of -8.23%, 8.58%, and 87.93% in Guntur, Bapatla, and Palnadu districts, respectively. Compared to Guntur and Bapatla, Palnadu faces a more severe paddy straw shortage due to its higher cattle and buffalo population and lack of canal irrigation. Overall, the study estimated that machine harvesting results in an 87.59% deficiency, while manual harvesting leads to a 71.04% deficiency in Andhra Pradesh for the year 2023-24.Given the livestock population in Andhra Pradesh, the current paddy straw shortage and disposal issues necessitate the urgent adoption of alternative strategies to ensure the sustainability of dairy farming.

KEYWORDS: Area of cultivation,Dairy farming, Feed resources,Paddy harvesting, Paddy straw

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INTRODUCTION

Livestock sector has been the primary source of energy for agriculture operations and also is a major source of animal protein for the masses. Sustainability of dairy farming in developing countries like India majorly depends on availability of feed and the economics of feeding, as feed alone accounts for 60-70% of production cost (Reddy et al., 2018). Paddy/rice straw is natural crop residue, which is easily available to most farmers worldwide (Dobermann and Fairhurst 2002). Often considered as an agricultural waste, it is the world's third-largest residue from agriculture after sugarcane bagasse and maize straw(McLaughlin et al., 2016). Rice straw holds immense potential as a feed source for cattle and buffaloes of south and south east Asian countries. In India 70% livestock feed is being contributed from paddy and wheat straw only due to their practical, abundant and economic availability. In India, 43.95 million hectares of land are under paddy cultivation, which produced nearly 106.54 million tonnes of rice and 160 million tonnes of straw in the last few years(Trivedi et al., 2017) With contribution of 12.6 % of total India's total rice production Andhra Pradesh ranks 3rd and produces 12.89 million tonnes of rice. Paddy straw is abundantly available after the harvest season. The ratio of rice grain to straw produced ranged from 56.29–100.03t / ha, straw yield 92.19–135.43t / ha 1:1.5 and availability of paddy straw was expected to be 20 million metric tonnes (MMT)(Akay, 2022)

However, the actual availability of paddy straw reduced drastically due to rapid mechanization of harvesting and field burning of crop residues. According recent study (Bhattacharyya et al., 2022)around 60% of paddy straw is being burnt in fields. Shortage of labour for harvesting, short crop window period, uncertainty of weather conditions and many other factors are contributing towards the field burning of crop residues. It has been observed that from the past 5 years the paddy farmers in Andhra Pradesh are resorting to mechanical harvesting with tyre and chain seed pickers of paddy instead of manual cutting resulting in greater biomass losses. These practices along with the annual inflation lead to increase of paddy straw price from Rs. 1400 per ton in the year 2010 to Rs. 8000 per ton in 2024 as per the information by the paddy farmers of AP.

Harnessing this resource effectively not only addresses waste management concerns but also offers a sustainable solution to meet the nutritional needs of dairy animals. Assessment of availability plays crucial role before planning and adaptation of different strategies to improve the straw availability (Chaudhary et al., 2019). Hence the present study was conducted to estimate the availability of paddy straw for livestock feeding in the year 2024-25 in the state of Andhra Pradesh with special reference to Guntur, Bapatla and Palnadu districts of Andhra Pradesh.

MATERIALS AND METHODS

This study was conducted based on data collected from 84 dairy farmers directly or indirectly involved in paddy cultivation, spanning nine villages (three from each district) and Livestock population statistics provided by the Andhra Pradesh Animal Husbandry (APAH) department, alongside paddy cultivation data sourced from the Andhra Pradesh Agriculture Commissionerate for the years 2022-23 and 2023-24.

The location of study represents Guntur, Bapatla and Palnadu districts of Andhra Pradesh with reported with average rain fall (mm) of 812.2, 837.5 and 697.7 respectively from June 2023 to April 2024 (Andhra Pradesh Water Resource Information Management System). To assess the availability of paddy straw of Andhra Pradesh the area of paddy cultivated during rabi season of 2022-23 and kharif season of 2023-24 was considered. This step was followed by collection of information from Guntur, Bapatla and Palnadu districts considering the paddy cultivation area for the year 2023-24. While Guntur district represents bore well and mixed irrigation facilities, in Bapatla district majority of cultivation land is covered under Krishna western main canal with continuous availability of canal water for irrigation. In Palnadu district the cultivation land is primarily rain fed and largely depends on availability of Nagarjuna sagar right bank canal (NSRBC) water. Due to reduced rain fall in the year 2023-24 resulting in absence of canal irrigation most of the Palnadu farmers opted for rain fed and commercial crops over paddy cultivation. The three districts were considered as representative of Andhra Pradesh agriculture scenario with three different crop irrigation methods i.e. rain fed, canal and borewell irrigations.

Regional differences may exist for the variables of interest, surveys were conducted in multiple locations in the three districts to collect data from farmers on yield of straw in manual/mechanical harvest and quantity of straw offered to dairy animals.

The survey questionnaire was developed by distributing questionnaire forms consisting of 10 questions to 84 dairy farmers engaged directly or indirectly in paddy straw cultivation. A pre-tested and semi structured questionnaire was designed to collect data oncultivation area of farmer, Irrigation source, major crops (Area of fodder cultivation), method of harvesting paddy, Yield per hectare, number of dairy animals (Cattle/buffalo), quantity of straw offered/animal, seasonal availability and utilization of green and dry fodders, major constraints in paddy harvesting and in animal feeding.

For calculation of paddy straw availability from these three districts kharif and rabi crop data of 2023-24 was considered. The yield of paddy straw in manual harvesting was considered as 8,750 kg/ha based on the information collected from paddy farmers practicing manual harvesting and paddy straw sellers who sold the straw to dairy farmers. Due to mechanical harvesting where the seed pickers harvesting with tyre/chain harvesters the yield of straw reduced drastically, and the paddy straw yield was calculated based on bales number. Farmers are opting for 30 and 50 kg bales based on the baler capacity. There is an average yield of 120-130 bales/ha weighing around 30 kg each and 70-80 bales/ha of 50 kg each. Based on interview data mechanical harvest yield of paddy straw was considered as 3750 kg/Ha to assess the paddy straw yield.

Paddy straw requirement of cattle and buffaloes

For estimating the paddy straw requirement of cattle and buffaloes, the cattle and buffalo population data of APAH statistics 2022 were considered. Irrespective of animal type the body weight of cattle and buffalo was taken as 400 kg to calculate feed requirement from dry roughage. The paddy straw was assumed to be supplied @6 kg per day per animal representing 1.5% of body weight. For 365 days supply of paddy straw to one cattle/buffalo there is a requirement of 2,190 kg paddy straw. These figures may vary depending on availability of green fodder, which increase during lean seasons due to reduced green fodder availability. The requirements were calculated district wise and also state wise to check the areas of severe feed shortage.

Paddy straw requirement versus availability

The dry fodder deficiency assessment was done by calculating the yield of straw from reported areas of paddy cultivation. The data on area of paddy that were subjected to manual and mechanical harvesting was not recorded anywhere hence separate calculation was done considering total area under manual harvesting and mechanical harvesting to derive the fodder deficiency percentage under manual and mechanical harvesting scenarios. The paddy straw requirements of AP large ruminants and three districts and the availability of paddy straw calculated were considered to estimate the paddy straw deficiency percentage.

RESULTS AND DISCUSSION

Dietary fiber is vital component in the diets of dairy animals that regulates rumen function and major contributor of rumen bulk . Slow digesting fiber from paddy straw helps in synthesis of acetic acid a major volatile fatty acid in the rumen that plays key role in milk fat synthesis (Woodford et al., 1986). The fiber requirements of dairy animals in Andhra Pradesh are majorly met from paddy straw. Assessing the requirement and availability of paddy straw is crucial for devising strategies to mitigate field burning and conserve straw for animal feeding purposes Paddy straw holds significant importance as a feed resource for large ruminants in Andhra Pradesh. However, the rapid mechanization of paddy harvesting, coupled with the traditional practice of field burning paddy harvest residues, has led to a substantial increase in the price of paddy straw. From the survey report collected from 84 dairy farmers it was understood that surge in prices has prompted the farmers with livestock to opt for baling (mechanical harvest) the long fiber residues of paddy straw instead of burning them in the fields. While this shift in practice conserves paddy straw for animal feeding, it exacerbates the dry fodder deficit in Andhra Pradesh. The survey analysis from 74% of paddy farmers depicted that the high wages for labour as the main driving force for burning of straws in the fields and 28 % farmers reported longer inter cropping interval in decomposition of residue in mechanical harvesting led them to field burning of paddy straw (Table 1).

The yield of paddy straw was considered as 8.75 and 3.75 tonnes/ha in manual and mechanical harvesting respectively, on the basis of data provided by farmers and dairy farmers who purchase paddy straw for feeding the dairy animals on hectare basis. The response from 87% of interviewed farmers indicated that the major cause of higher prices of paddy straw was due to shortage of green fodder, faced by dairy farmers of KVK jurisdiction. Similar to current study a survey conducted (Debela et al., 2017) reported that more than 98.9% of the livestock owners response indicated that feed shortage is a seasonal problem and 82.2% of the respondents reported that feed shortage is a serious problem during dry season. From the information provided by farmers it was inferred that the livestock of Palnadu region largely depended on grazing and paddy straw as compared to Guntur and Bapatla districts.

Table 1. Survey analysis of dairy farmers and average yield of paddy straw in Guntur, Bapatla and Palnadu districts

	No. Of dairy farmers surveyed	% Farmers holding paddy fields	% Farmers adopted different disposal methods			Yield of straw in manual harvest ton/ha	Yield of straw in mechanical harvest ton/Ha	Quantity of paddy straw/animal/day
			Manual harvest	Mechanical harvest	Field burning			In kg*
Guntur	18	61.1 (12	27.3 (3)	36 .4 (4)	45.5 (5)	9.0	3.8	6.17
Bapatla	27	100 (27)	25.9 (7)	40.7 (11)	33.3 (9)	8.8	3.5	5.04
Palnadu	39	33.3 (13)	15.4 (2)	76.9 (10)	7.7 (1)	8.5	3.3	8.89

*The values varied depending on seasonal availability of green fodder and the values were taken as average of values provided by farmers

In Bapatla district the number of farmers cultivating fodder crops during kharif and rabi season were higher than Guntur and Palnadu districts and the dairy animals of this district were fed with paddy straw from March to August months. On an average, farmers of Guntur Bapatla and Palnadu regions were feeding 6.17, 5.04 and 8.89 kg of paddy straw/animal/day (Table 1). These values vary with season, rainfall and grazing grass availability.

In Andhra Pradesh, the capacity for paddy cultivation spans 2,343,473 hectares, encompassing both Kharif and Rabi seasons.However, due to reduced rainfall and the unavailability of canal water for irrigation, there has been a significant decline of 66.54% in paddy cultivation area. Particularly, the Rabi season suffered a severe 87.63% reduction in cultivated land compared to the expected area. The paddy straw assessment was calculated considering average yield of 8.75 ton/ha in manual harvesting and 3.75 ton/ha or 125 bales of 30 kg in machine harvesting. The anticipated yield from manual and mechanical harvesting was projected at 6.86 and 2.94 million metric tonnes (MMT) of straw, respectively (Table 2). With a livestock population of 10,819,586 cattle andbuffalo in Andhra Pradesh, the annual requirement for paddy straw is estimated at 23.69 MMT (Table 3) for the year 2024-25. This indicates an expected deficiency of 71.04% and 87.59% in manual and mechanical harvesting, respectively (Table 4). The deficit percentage may further escalate as many farmers resort to burning straw after mechanical harvesting rather than baling it for livestock feed.

According to Chandrasekhar et al. (2019), there are four primary methods for disposing of mechanically harvested paddy straw: removing loose straw and burning stubbles, burning both loose straw and stubbles, removing straw and incorporating stubbles into the soil, and incorporating both loose straw and stubbles into the soil. Depending on feasibility, farmers in Andhra Pradesh are adopting one of these methods for straw disposal, leading to a substantial shortage of fodder for livestock feeding.

Table 2. Paddy straw availability assessment for Guntur, Bapatla and Palnadu districts and Andhra Pradesh state

District	Area sown in Ha	Expected availability in tonnes	
		Manual harvest	Mechanical harvest
Guntur	55,776	4,88,040	2,09,160
Bapatla	92,373	8,08,264	3,46,399
Palnadu	15,949	1,39,554	59,809
Andhra Pradesh[#]	7,84,119	68,61,041	29,40,446

Table 3. Cattle and buffalo population and paddy straw requirement Andhra Pradesh and Guntur, Bapatla and Palnadu districts

District	Cattle	Buffalo	Total	Paddy straw requirement in tonnes*
Guntur	16,899	1,89,009	2,05,908	4,50,938.5
Bapatla	21,930	3,81,764	4,03,694	8,84,089.9
Palnadu	64,240	4,63,921	5,28,161	11,56,673
Andhra Pradesh	46,00,087	62,19,499	1,08,19,586	23,694,893 (23.7 MMT)

Data courtesy: Department of Andhra Pradesh Animal Husbandry statistics on livestock population 2022-23
* Straw requirement calculated considering average data provided by farmers of three districts as 6 kg straw per animal) per day for 365 days

Despite being characterized by a low nutritive value primarily constituted of cellulose and lignin, paddy straw remains a crucial component in livestock feeding. It accounts for over 70% of the fiber requirements for Indian large ruminants (Mahesh and Mohini, 2013). Extensive research has been taken up to improve the nutritive value of these residues (physical chemical and biological treatments) at national agricultural institutes and state agriculture universities (Dhanda et al., 1994; Nagalakshmi and Reddy, 2012). However, the nutritive value of paddy straw bales can be improved by addition of fermentation medium at the time of baling process and was found 11.15% profitable over conventional baling practice (Singh and Rani, 2024).

Table 4. Paddy straw requirement and expected availability to livestock in Andhra Pradesh

District	Paddy straw requirement (tonnes)	Manual harvesting		Machine harvesting	
		Expected yield (tonnes)	Deficiency %	Expected yield (tonnes)	Deficiency %
Guntur	4,50,939	4,88,040	8.23	2,09,160	53.62
Bapatla	8,84,090	8,08,264	8.58	3,46,399	60.82
Palnadu	11,56,673	1,39,554	87.93	59,809	94.83
KVK, Guntur jurisdiction	24,91,701	14,35,858	42.37	6,15,368	75.30
Andhra Pradesh	2,36,94,893	68,61,041	71.04	29,40,446	87.59

The once esteemed straw, now relegated to waste destined for burning, faces a host of challenges including technical, structural, socioeconomic, and institutional barriers (Bhattacharya et al., 2021). Aside from contributing to air pollution, the field burning of paddy straw leads to the depletion of soil organic matter and essential nutrients, reduces microbial activity, and heightens the land's susceptibility to erosion (Kumar et al., 2023). Apart from livestock feeding in order to reduce field burning many alternative and sustainable bio energy models have also been proposed to prevent the field burning of paddy straw (Trivedi et al., 2015). Urgent measures are required to tackle the practice of field burning rice straw, ensuring its availability for feeding large ruminants in India to sustain the dairy sector amidst severe forage shortages

CONCLUSION

Paddy straw, often overlooked as a by product of rice cultivation holds potential as a valuable feed source for livestock. However, factors like a reduction in cultivation area due to decreased rainfall in Andhra Pradesh during 2023-24 and the rapid mechanization of harvesting have caused a decline in the availability of paddy straw for livestock feed. Practices such as field burning and field decomposition of post-harvest paddy waste have worsened the situation, leading to a sharp rise in the price of paddy straw, which challenges the sustainability of dairy farming. This pressing scenario necessitates urgent strategies to augment the availability of paddy straw for viable dairy farming.

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Effect of Feeding Maize Oil Cake on Feed Intake, Body Weight and Economics in Goat Kids

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ABSTRACT

Eighteen Beetal male kids were randomly divided into three groups of 6 animals each. The kids of group I were maintained on basal ration comprising of green fodder, gram straw and concentrate mixture. Maize oil cake was included in the ration of group II and group III @ 15% and 30% of concentrate mixture, respectively. The feeding trial lasted for a period of 120 days. Body weight changes and feed intake of animals were recorded at fortnightly intervals. The dry matter intake (DMI) recorded every fortnight did not vary significantly between the groups. The body weight gain over 120 days and average daily weight gain (ADG g) was found significantly ($P<0.05$) higher among the groups offered maize oil cake @ 15% and 30% of the concentrate. The mean values of nutrient intake (g/day) i.e DCP and TDN of experimental kids under different dietary treatments differed significantly and were highest in group III followed by group II and group I. The feed cost per kg body weight gain was reduced by 2.7% in T2 (15% maize oil cake) and 4.3% in T3 (30% maize oil cake) group respectively. Results of the study showed that inclusion of maize oil cake increased body weight gain as well as reduced the cost of feeding male kids per kg weight gain.

KEYWORDS : Beetal kids, Body weight, Feed intake, Maize oil cake

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INTRODUCTION

Goat meat popularity is increasing worldwide due to a growing demand for lean and nutritious meat. It is a great source of nutrients, including protein, iron, vitamin B_{12}, zinc, potassium as well as low in total fat and saturated fat compared with other forms of red meat (Pophiwa et al., 2020). Goat kids grow rapidly, have similar digestive system to other ruminants, are relatively small and easy to handle. All these characteristics make them a convenient choice for field experiments. Nutritional inadequacy for livestock is currently referred as one of the most burning global problems of agricultural countries (Katoch, 2022) due to reasons like degraded grazing lands as a result of industrialization and urbanization, diversion of feed and fodder for industrial and human use, seasonal scarcity of fodder and rising national demand for animal protein.

Maize oil cake is one such agricultural by-product obtained after extraction of maize oil from full-fat maize germ, a co-product of the starch industry (Toghdory et al., 2024). Maize germ is separated, dried, and sent to a germ plant for extraction of the corn oil. After the oil is extracted, the remaining feed by-product is called maize oil cake, which used as an energy source in animal nutrition (DeTray, 2016) and rich in nutrients (protein & fat), has excellent functional properties and can be used as a concentrate source in the livestock (Bakke and Vickers, 2007).Crude protein content of corn germ meal varied from 10.13 (Ramos et al., 2007) to 24.79 (Almeida et al., 2012). Ether extract content of corn germ meal varied from 0.2 (Beran et al., 2007) to 49.48 (Albuquerque et al., 2014).DDGS and maize oil cake have been recognized as an excellent source of energy due to their high digestibility and high concentration of fat (Jun et al., 2014).The product's ease of availability, reasonable market pricing, and high protein and oil content necessitates a trial for goat feeding. Present research was undertaken to study the effects of dietary inclusion of maize oil cake on feed intake, body weight gain and economics of feeding kids.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with guidelines approved by the

Institutional Animal Ethics Committee, 12/CPCSEA Dated 29.10.2022 via protocol number IAEC/ LUVAS/26/10 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar. Eighteen Beetal kids of comparable body weight were randomly divided into three groups of 6 in each and average body wight of kids among three groups were 15.85 kg for T1, 15.81 kg for T2 and 15.89 kg for T3.. An adjustment period of fifteen days was given before the start of experiment. All the kids of group I i.e control (T1) was maintained on basal ration comprising green fodder, gram straw and concentrate mixture (Maize grain, groundnut cake, barley, mineral mixture and common salt in the ratio of 35: 35: 27: 2: 1). The experimental diet of group T2 was included with maize oil cake @15% of the concentrate mixture (Maize grain, groundnut cake, barley, maize oil cake, mineral mixture and common salt in the ratio of 30: 29: 23: 15: 2: 1)while maize oil cake was included @ 30% of the concentrate mixture in the diet of group T3 (Maize grain, groundnut cake, barley, MOC, mineral mixture and common salt in the ratio of 23: 26: 18: 30: 2: 1).The diets were kept iso-nitrogenous. Table 1 depicts the chemical composition of different feedstuffs used in preparing the experimental diets. Feeding trial lasted for a period of 120 days and kids were fed as per ICAR, 2013. Feed intake was calculated at fortnightly intervals. The kids were weighed individually at fortnightly intervals before feeding and the body weights were recorded to calculate body weight gain up to 120 days of the experimental period. Economics of feeding under different dietary treatments was calculated by taking into account daily feed consumption and by considering the cost of feed, fodder and supplements used.

At the end of the feeding trial, a metabolism trial of 5 days duration was conducted to assess the effects of dietary inclusion of maize oil cake on growth performance in kids. A preliminary period of 3 days was given for adaptation of the kids to new system of housing and management, followed by a collection period of 5 days. The proximate composition of feed and faecal samples was determined (AOAC, 2013).

Table1. Chemical composition of feeds offered to experimental kids offered (%DM basis) of experimental diet

Feed offered	Chemical composition (%DM basis)					
	DM	CP	EE	CF	Total Ash	NFE
Green fodder	17.42	7.97	2.65	26.31	9.53	53.5
Gram straw	91.27	5.52	1.59	38.41	7.67	46.8
Conc. Mix. T1	90.55	20.07	4.16	5.84	6.52	65.4
Conc. Mix. T2	90.63	20.2	5.32	6.14	6.11	64.2
Conc. Mix. T3	92.75	20.1	6.56	6.56	5.81	62.9

Statistical analysis

Data were analyzed statistically using general linear model procedure of operational statistics, Statistical Package for Agricultural Scientists (OPSTAT) and comparison of means tested using Duncan's multiple range test (DMRT) and significance was considered at $P < 0.05$ (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Feed intake

Present study revealed no significant effect of maize oil cake inclusion on feed intake (Table 2) amongst different treatment groups, although, numerically it was higher in kids fed maize oil cake. The DMI recorded every fortnight (kg/animal/day) amongst all treatment groups was also found similar statistically, which shows that maize oil cake had no negative impact on palatability and intake of feed in kids, rather it slightly increased the intake by making it more palatable. Furthermore, during metabolic trial dry matter consumption in terms of grams, percent body weight (% BW) and grams per kg metabolic body weight ($g/kgW^{0.75}$) also did not differ significantly and were determined to be equivalent (Table 3).

The results in the current study were in agreement with Toghdory et al. (2024) who reported no significant difference between the experimental treatments (control and groups in which soybean meal was replaced with 50% and 100% corn germ meal) in terms of dry matter intake in lambs(P>0.05). Similarly, Bakshi et al.(2023) found comparable daily DM intake in both the groups i.e control group and maize processing industry byproducts fed cattle group. Noormohammadi et al.(2022) also reported that treatments fed corn germ to Holstein cows @12% of DM had similar dry and organic matter intake to control group. A study conducted by Kaur et al. (2019)to assess the effect of partial replacement of soybean meal and mustard cake with corn germ meal (CGM) @ 12% and 18 % in the concentrate mixture in male crossbred calves, also reported that the overall mean DMI (kg/animal/ day) in control, T1 and T2 groups was similar and did not vary significantly. Kumar et al. (2018), also reported that dry matter intake did not vary significantly among different groups when the crossbred calves were fed maize germ oil cake at 1.3, 1.7 and 2.1 kg/day levels for 28 days. Furthermore, Miller et al.(2002), reported that dry matter intake did not differ among the experiment diets of control (at 3.5% fat), whole cottonseed (at 5.1% fat), tallow (at 5.1% fat) and full-fat corn germ (at 5.1% fat) in lactating dairy cows during feeding trial of 28 days. Similarly, Detray (2016) concluded that there was no significant effect of corn germ meal at 24.5% inclusion level with increasing levels of nucleotide additive (0, 2 and 4 g/d) on dry matter intake during 85 days experimental period in crossbred heifers.

However, Galaeno et al. (2022) found increased DM intake in goats fed extra-fat whole corn germ @9.5% of DM. Likewise, Zeeshan et al.(2022) found increased feed intake in goat in groups fed maize oil cake than the control group goats and reason behind this is perhaps the palatability of oil seed cakes.Sulpizio et al.(2003), also reported that dry matter intake was greater (P= 0.10) for cattle fed corn germ (16.4 lb/day) than for those fed tallow (16.4 lb/day) for 110 days. Kelzer et al.(2009) also reported that the DMI in Holstein cows fed dehydrated corn germ meal (24.3kg/d) was higher (P<0.05) than control group (22.9kg/d) during the experimental period of 21 days. Inconsistency in the obtained results may be related to the different species in different experiments and fat content of the used CGM in each study.

Table 2. Mean values of feed intake (g/d, DM basis) of growing kids at fortnight interval under different dietary treatments

Period fortnightly	Treatments T1	 T2	 T3
	Feed intake (g/d)		
1st	536.21±16.6	553.71±17.2	568.75±26.9
2nd	568.73±19.5	593.92±15.2	605.50±21.3
3rd	627.00±20.7	649.00±15.03	687.03±21.3
4th	673.20±19.7	699.32±15.3	736.67±21.9
5th	725.72±20.7	755.08±15.2	794.69±22.2
6th	793.80±22.2	808.13±15.5	837.43±21.6
7th	835.50±21.0	870.77±14.1	890.31±20.01
8th	874.90±20.1	907.69±15.4	936.50±20.9
Overall Mean feed intake (g/d)	711.81±20.3	735.09±20.1	769.59±20.1

Nutrient intake

The mean values of nutrient intake (g/day) of experimental rations under different dietary treatments has been presented in Table 3. Statistical analysis of the data revealed that TDN intake (g/d) differ significantly (P<0.05) in dietary treatments T2 and T3 as compared to the control group. Similarly, DCP intake(g/d)of maize oil cake containing treatment groups (T2 and T3) also differ significantly ($P<0.05$) as compared to the control group. The mean values of DCP and TDN intake were highest for 30% maize oil cake added groups followed by 15% MOC fed group and lowest for the control group.

Results of present study were in agreement with Silva et al.(2023) reported that cows fed corn germ quadratically increased ($p<0.05$) intake of dry matter, crude protein, and total digestible nutrients, and linearly reduced ($p<0.05$) the intake of total non-fiber carbohydrates. Somashekhar. (2013) who fed corn germ meal to rabbits, also found increased DCP% in group II than group III and group I and higher TDN % in the groups III followed by group II and group I. Similarly, Nagpure.(2011) evaluated nutritional value of corn germ meal based diets in rabbit by substituting 25% and 50% of GNC protein with expeller pressed CGM on nitrogen equivalent basis in group T2 and T3 respectively and found highest TDN% in group T3 followed by group T2 and T1.

Table 3. Mean nutrient intake (g/day) of experimental kids under different dietary treatments

Attributes	Treatments			CD value
	T1	T2	T3	
		Nutrient intake		
DMI (g/d)	878.42±20.1	910.62±15.4	947.63±20.9	-
DMI (% BW)	3.33± 0.02	3.34±0.02	3.35± 0.03	-
DMI(g/kgW.75)	74.73± 0.63	75.19± 1.01	75.41± 0.75	-
DCP (g)*	79.38^{a}±1.56	88.78^{b}±1.73	90.93^{c}±1.12	4.75
TDN (g)*	619.71^{a}±14.2	655.89^{b}±19.5	696.34^{c}±19.2	40.57

abc*Mean bearing different superscripts in a row differ significantly ($P<0.05$)

Body weight changes

The body weight of kids at the beginning of experiment was similar in all the three treatment groups whereas body weight at the end of 120 days experimental period was found significantly ($P<0.05$) higher of kids fed maize oil cake @ 15% and 30%than the control kids (Table 4). The total body weight gain during experimental period significantly ($P<0.05$) increased in kids supplemented with 30%maize oil cake, followed by treatment group supplemented with 15%maize oil cake as compared to control group. Average daily gain was also observed significantly ($P<0.05$) higher in groups with maize oil cake i.e . 106.11g in T_3 followed by 98.06g in T2than the control group (90.48g). Increase in body weight gain may be due to increased ether extract content of diet containing maize oil cake due to its high EE content (12.1%) and increased intake of DCP and TDN in the groups fed maize oil cake.

The results of the present study are in agreement with Brunelli et al.(2006), who reported significant ($p<0.01$) increase in weight gain of birds with increasing level of defatted corn germ meal inclusion in the diets. Likewise, Somashekhar.(2013) also found significantly higher growth rate in rabbits in which 50% wheat bran was substituted by corn germ meal.

However, in contrary Toghdory et al.(2024), indicated that replacing soybean meal with corn germ meal in the diets of fattening lambs did not significantly affect body weight gain on days 28, 56, and 84. Additionally, total body weight and daily weight gain throughout the experimental period were unaffected by the dietary replacement. Likewise, Leeuw et al.(2009), reported no significant difference in the steer weight when the steers were fed defatted maize germ meal at 0, 25, 50, 75 and 100% levels, replacing hominy chop for 124 days. Jun et al.(2014) also reported that replacing forage fiber sources with non forage sources from dried distiller grains with solubles and corn germ meal at 0, 9, 18 and 27% had no effect on the average body weight in Holstein calves. Furthermore, no effect ($P> 0.05$) of dietary corn germ meal was found on the body weight, average daily gain in meat ducks during the experimental period by Qi et al. (2022).Contrary to these findings, Strighini et al.(2009), observed significant ($p<0.05$) decrease in body weight gain with increasing level of corn gluten meal inclusion in diets.

Table 4. Mean values of Body weight (kg) of growing kids at fortnight interval under different dietary treatment

Fortnight Weight(kg)	Treatments		
	T1	T2	T3
Initial Body Weight	15.85±0.55	15.81±0.50	15.89±0.55
1st	16.99±0.56	16.96±0.55	17.18±0.59
2nd	18.09±0.60	18.31±0.52	18.64±0.63
3rd	19.30±0.89	19.71±0.30	20.40±0.15
4th	20.66±0.66	21.20±.46	22.19±.70
5th	22.09±0.65	22.88±0.46	23.97±0.70
6th	23.64±0.63	24.40±0.45	25.70±0.66
7th*	25.17^{a}±0.61	26.14^{b}±0.45	27.14^{c}±0.62
8th*	26.71^{a}±0.61	27.81^{b}±0.46	29.22^{c}±0.63
ADG(g)*	90.48^{a}±0.72	98.06^{b}±0.44	106.11^{c}±0.93
Total Gain(kg)*	10.85^{a}±0.08	12.00^{b}±0.05	13.33^{c}±0.11

abc*Mean bearing different superscripts in a row differ significantly ($P<0.05$)

Economics of feeding

The cost of concentrate mixtures fed to calves in control, 15% and 30%maize oil cake included groups was worked out to be Rs. 12.43/kg, Rs. 13.39/kg and Rs. 14.18/kg, respectively (Table 5). The total weight gain during the experimental period was higher($P < 0.05$) in maize oil cake containing groups than the control group. Thus, inclusion of maize oil cake at 15% and 30% levels resulted in lower cost of feeding /kg body weight gain by 2.7% and 4.3%, respectively. Feeding maize oil cake at both levels turned out to be economical and profitable as per the results obtained.

The results are in agreement with Moreira et al.(2002), who carried out the economic analysis of defatted corn germ meal (DCGM) in crossbred pigs in the growing and finishing phases, and observed that there was no effect ($P> 0.05$) on the average cost per kg of live weight. Similarly, Kaur et al.(2019) concluded that inclusion of CGM at 12% and 18% levels partially replacing SBM/MC resulted in a net saving of Rs. 0.43 and Rs. 3.86 per kg BW gain in T1 and T2 groups, respectively.

Table5. Economics of feeding (Rs/day) of growing kids under different dietary treatments

Attributes	Treatments		
	T1	T2	T3
Cost of concentrate mixture	12.4	13.4	14.2
Cost of green fodder	2.64	2.74	2.83
Cost of dry fodder	0.92	0.98	1.13
Total cost of feeding	15.9	16.9	17.92
Av. BW gain (g/d)	90.4	98.81	106.25
Cost of feeding (Rs/Kg BW gain)	176.71	171.89	169.10
Relative Profit	0.00	2.7%	4.3%

CONCLUSION

Maize oil cake inclusion at 15 and 30 % levels in concentrate mixture did not have any negative impact on feed intake. Further, it improved body weight gain in kids as well as reduced the cost of feeding/kg body weight gain ration more economical for rearing goat kids.

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Effect of Feeding Exogenous Buffer (Sodium Bicarbonate) as Feed Additive on the Performance of Feedlot Lambs

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ABSTRACT

In the current experiment sodium bicarbonate was used as exogenous buffer to assess its effect on growth performance, nutrient utilization, economics of feeding, blood-biochemistry and rumen fermentation pattern of finishing lambs. A total of fifteen (15) crossbred male lambs (around 11 months age, 20.49±0.74 kg body weight) were selected and randomly allocated in to 3 treatments equally. The lambs in control group (C) were fed basal diet containing roughage and concentrate (R:C) in the ratio of 60:40, while treatment groups fed feedlot rations having R:C ratio of 20:80 without (F) or with Sodium bicarbonate supplementation (FSB) @ 1% dry matter intake (DMI). A feeding trial of 74 days (including 14 days acclimatization period) was conducted, followed by a 10 days metabolism trial at the end of the experiment. The results revealed non-significant differences ($p>0.05$) in terms of dry matter intake (DMI), average daily gain (ADG, g), feed conversion ratio (FCR), and digestibility coefficients of nutrients. The digestible crude protein (DCP%) values of rations were significantly higher ($p\leq0.05$) in the FSB group, but no significant difference ($p>0.05$) was observed in total digestible nutrients (TDN%) values among treatments. Non-significant differences ($p>0.05$) were observed in digestible DMI (kg/d), DCP intake (g/d), and TDN intake (kg/d) between the experimental groups. Nitrogen balance showed non-significant differences ($p>0.05$) among the groups. In FSB group, the reduction in feeding cost and production cost per kg live weight was lesser than F and C group. The haemato-biochemical parameters viz., Haemoglobin (Hb), packed cell volume (PCV), blood glucose, alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), and blood urea nitrogen (BUN) showed improved effect on incorporating Sodium bicarbonate as a feed additive in feedlot ration, however, the results were non-significant ($p>0.05$), but serum creatinine values were significantly higher ($p\leq0.05$) in feedlot groups than control. Rumen pH, total volatile fatty acids (TVFA), ammonia nitrogen, Trichloroacetic acid (TCA) precipitable nitrogen and non-protein nitrogen (NPN) values showed no significant difference ($p>0.05$) among the treatment groups, however, total nitrogen values of rumen liquor were significantly ($p\leq0.05$) higher in feedlot groups than control. So, the present experiment successfully demonstrates the positive effect of incorporating Sodium bicarbonate as a feed additive on the performance of feedlot reared lambs without any adverse effect.

KEYWORDS: Exogenous buffer, Feed additive, Feedlot, Lambs, Sodium bicarbonate

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INTRODUCTION

India ranks 3rd in sheep production in the world having population of 74.26 million sheep contributing 13.87% to the total livestock population (20th Livestock census, 2019). To fulfil the increasing demand and the deficit, either the number of sheep reared should be increased or the carcass characteristics should be selectively improved according to the consumer demand. Mutton production needs to be increased either by vertical expansion or by increasing the carcass weight through feeding technique like "feedlotting" (Sahib et al. 2023). The feedlot period is usually about 60 to 80 days and the animal will normally gain 15- 20 kg during this period. In feedlot system of rearing, ADG normally goes above 250g, however, 400 g/day or above can also be achieved by animals that have a higher weight gain potential which comes from genetic traits (Gallo et al. 2014).

Digestive problems account for between 14% to 42% of cattle mortality on feedlots, making them the second most common cause of death (Nagaraja and Lechtenberg, 2007). Laminitis, rumen ulcers, liver abscess, and thromboembolic respiratory illness, are examples of sequels to rumen acidosis that can each have a more severe effect on the health and welfare of animals than the original cause). Hence, upholding a proper ruminal pH is fundamental for the well-being of these animals (Calsamiglia et al., 2012). Sodium bicarbonate ($NaHCO_3$) is commonly supplemented to buffer rumen pH, although its buffering activity is reduced in condition of low rumen pH (5.5-5.8), as it has a pKa of 6.25 (Russell, 1998). In addition, $NaHCO_3$ rapidly solubilizes into rumen fluid, with a fast but not long-lasting buffering activity. Because of this, $NaHCO_3$ is required @ 0.7 to 1 % of the DM to buffer the rumen pH of cattle fed high-energy diets (Krause, 2008). Thus, the present study was undertaken to assess the impact of supplementing $NaHCO_3$ an exogenous buffer on nutrient digestibility, body weight and health status of lambs finished in feedlots.

MATERIALS AND METHODS

Experimental animals, diets and experimental design

A total of fifteen (15) healthy crossbred male lambs (nearly 11 months age, 20.49±0.74 kg body weight) maintained at Mountain Research Centre for Sheep and Goat (MRCS & G), Shuhama, Srinagar, Jammu and Kashmir were randomly divided into three treatment groups of five animals (n=05) each in a completely randomized block design (CRD). During the study period, the experimental animals were handled as per approved trial protocol regulations of the Institutional Animal Ethics Committee SKUAST-Kashmir (Registration no.: 1809/GO/ReBiS/ReL/15/CPCSEA) vide order number AU/FVS/PS-23/2022/19465; dated: 28-03-2022. The experimental animals were fed individually in pens. The animals in the control group (C) were fed 60% Oat hay as roughage and 40% concentrate. In contrast to C group, the animals in treatment groups were fed 20% roughage and 80% concentrate without (F) or with (FSB) $NaHCO_3$ supplement. The C and F groups were voided of $NaHCO_3$ supplement in their ration, but in the ration of FSB group $NaHCO_3$ was added @1% of dry matter intake. After grinding the ingredients for concentrate mixture, $NaHCO_3$ was mixed well to ensure uniform integration with the concentrate mixture. The ingredient composition (%) of the concentrate mixture was maize grain, 54; wheat bran, 12, mustard oil cake, 23, cowpea, 8, mineral mixture, 2 and salt, 1. The chemical composition of experimental ration is presented in Table 1. The animals were fed the calculated amount of concentrate and roughage in the morning (08:00 hrs) and evening (16:00 hrs) in equal proportion to meet their requirement (ICAR, 2013).

Table 1. Chemical composition (%) of experimental ration

Proximate principles	Concentrate mixture	Oat hay	TMR-1 (60R: 40C)	TMR-2 (20R: 80C)
Dry matter	89.1	84.9	86.6	88.3
Crude protein	18.9	6.56	11.5	16.4
Ether extract	4.91	1.20	2.68	4.17
Crude fibre	5.65	34.1	22.7	11.3
Nitrogen free extract	65.7	52.1	57.6	63.04
Total ash	4.74	5.92	5.44	4.97
Calcium	0.31	0.54	0.45	0.35
Phosphorus	0.59	0.15	0.33	0.49

Growth studies, metabolism trial and economics of feeding

A feeding trial of 74 days was conducted (including 14 days adaptation period). Fortnightly body weights were recorded. The actual influence of experimental feed was assessed by calculating total body weight gain, ADG (g/d), and FCR. At the end of feeding experiment, a metabolism trial of 7 days was conducted to estimate digestibility of nutrients, nutritive value of experimental feed and nitrogen balance. Representative samples of feeds offered and residue left were analysed for proximate principles (AOAC, 2005).

Haemato-biochemical parameters

The estimation of blood glucose was done on-spot by using Accu-Chek instant Glucometer. The haemoglobin (Hb) and packed cell volume (PCV) was estimated by using Sahli's method and Microhematocrit method, respectively. The estimation of ALT, AST, ALP, BUN and creatinine was done by using commercial diagnostic kits procured from Span diagnostics limited, Surat, Gujarat, India.

Rumen fermentation parameters

The samples of rumen liquor were collected from experimental lambs at initial and final day of trial (before feeding and watering in the morning hours) with the help of perforated stomach tube (50 mm). The pH of rumen liquor was determined immediately after collection of rumen liquor by using digital pH meter. For analysis of total volatile fatty acids (TVFA), ammonia nitrogen, Tricarboxylic acid (TCA) precipitable nitrogen and total nitrogen, the collected rumen fluid was strained using muslin cloth. The estimation of TVFA was done by following the protocols given by Barnett and Reid (1957), total nitrogen and ammonia nitrogen was estimated by Kjeldahl methods, and TCA precipitable nitrogen was estimated as per the method of Cline et al. (1958). The NPN content was calculated by subtracting TCA precipitable nitrogen from the total nitrogen.

Statistical analysis

The data obtained during experiment was subjected to one-way ANOVA (Snedecor and Cochran, 1994), using, SPSS Software, Base 23.0 for macOS, Marketing Department, SPSS Inc. Chicago, USA.

RESULTS AND DISCUSSION

Feed intake and gain in body weight

Dry matter intake (DMI, g/d) and body weight gain (kg) did not reveal any significant difference ($p>0.05$) between the treatments. Average daily gain (ADG, g/d) and FCR also showed no statistical difference ($p>0.05$) (Table 2). The smaller but insignificant improvement in the BW gain of animals fed feedlot diets supplemented with sodium bicarbonate as feed additive was also commensurate with the observations of Den hartog et al. (1989), Damir et al. (1990), Jackson and Hemken (1994), Fauchon et al. (1995) and Mandebvu and Galbraith (1999).

Table 2. Feed consumption and body weight gain

Parameter	Groups			SEM	p-value
	C	F	FSB		
DMI (g/d)	1032.36	858.44	1002.03	35.98	0.101
Initial BW (kg)	20.50	20.53	20.45	0.74	0.999
Final BW (kg)	29.61	29.95	31.68	1.05	0.719
Total Gain in BW (kg)	9.11	9.42	11.23	0.67	0.411
ADG (g/d)	123.10	127.29	151.76	8.95	0.411
FCR	7.53	7.14	6.20	0.32	0.252

Digestibility coefficient of nutrients, nutritive value of experimental rations, plane of nutrition, and nitrogen balance

No statistical significance ($p>0.05$) was observed in the apparent digestibility of dry matter, organic matter, crude protein, ether extract, crude fibre, nitrogen free extract, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose, moreover, apparent digestibility of nutrients also showed no significant difference between treatments (Table 3). The results of present study were in agreement with the observations of Phillip (1983), Hadjipanayiotou (1988), Santra et al. (2003), Sarwar et al. (2007), Soto et al. (2008), Farooq et al. (2014), Martins et al. (2016) and Suman et al. (2018).

The nutritive value of experimental diet revealed statistically significant difference ($p\leq0.05$) in terms of DCP (%) with highest value in FSB group, while

TDN (%) showed no statistical difference ($p>0.05$) among treatments (Table 3). Digestible dry matter intake (DDMI, kg/d), digestible crude protein intake (DCPI, g/d) and total digestible nutrients intake (TDNI, kg/d) also revealed no statistical difference ($p>0.05$) between the treatments, but comparatively higher values were witnessed in groups supplemented with sodium bicarbonate than that of control group. The balance of nitrogen revealed significant difference ($p\leq0.05$) between treatments, showing improved quantity of nitrogen absorbed in sodium bicarbonate supplemented feedlot groups than the control group (Table 3).

Table 3. Average digestibility coefficients, balance of nutrients, nutritive value of experimental rations and intake of digestible nutrients

Parameter	Groups			SEM	p-value
	C	F	FSB		
Digestibility coefficient of gross nutrients					
DM	57.1	61.7	63.3	2.07	0.498
OM	68.9	69.9	72.09	1.11	0.542
CP	62.5	68.5	74.09	3.12	0.356
EE	58.09	59.4	74.4	5.59	0.461
CF	58.2	61.9	67.7	3.43	0.568
NFE	71.1	71.4	72.7	1.99	0.950
NDF	54.01	43.6	50.01	4.13	0.631
ADF	50.2	35.5	45.6	5.25	0.553
Cellulose	56.1	59.2	64.1	3.11	0.620
HemiCellulose	60.5	54.6	55.8	2.94	0.729
DCP (%)	7.21^{a}	11.2^{b}	12.2^{b}	0.78	0.005
TDN (%)	64.9	68.9	72.7	2.58	0.512
DDMI (kg/d)	0.85	0.84	0.86	0.04	0.998
DCPI (g/d)	92.8	137.50	144.04	10.5	0.085
TDNI (kg/d)	0.84	0.83	0.87	0.05	0.947
Nitrogen intake (g/d)	23.7^{a}	30.7^{b}	31.2^{b}	1.42	0.033
Fecal nitrogen (g/d)	10.6^{a}	12.2^{b}	10.4^{a}	0.31	0.015
Urinary nitrogen (g/d)	3.92^{a}	5.65^{b}	7.52^{c}	0.48	<0.05
Nitrogen balance (g/d)	9.10	12.8	13.2	1.13	0.283
Nitrogen retention (%)	38.3	41.7	42.3	2.28	0.845

Note: ^{abc} means bearing different superscripts within a row differ significantly ($p\leq0.05$).

Economics of feeding

The feed cost and production cost per kg live weight in feedlot groups with or without sodium bicarbonate as feed additive were reduced when compared with control (C) group (Table 4). In FSB group, the reduction in feeding cost and production cost per kg live weight was lesser than F and C group. The lower production cost was evident from the relatively better nutrient utilization, improved average daily gain and enhanced feed conversation results, which were shown by sodium bicarbonate supplemented feedlot group. The results of the present study show harmony with the findings of Gibb and Baker (1991) and Horn et al. (1992) who reported that supplementation feed additives in the ration of animals was cost effective in winter.

Table 4. Voluntary feed intake (DMI), total body weight gain, FCR, cost of feeding (Rs.) /kg gain and % reduction in cost of feeding/ kg gain of experimental animals

Attributes	Groups			p-value
	C	F	FSB	
Cost of TMR (Rs.)/kg	26.69	28.38	28.78	-
Total DMI (kg)	76.38±0.91	65.59±7.42	76.99±0.78	0.218
Total gain in body weight (kg)	9.11±0.56	9.42±1.68	11.23±0.96	0.411
FCR	7.53±0.32	7.14±0.80	6.20±0.23	0.252
Cost of feed (Rs.)/kg gain	201.23±8.73	202.71±22.87	178.54±6.47	0.268
% Reduction in feeding cost/ kg gain	-	-0.73	11.28	-

Haemato-biochemical parameters

The dietary treatments showed no significant effect (p>0.05) on Hb (g/dl), PCV (%), blood glucose (mg/dl), ALT (IU/L), AST (IU/L), ALP (IU/L), and BUN (mg/dl) (Table 5). However, the values of Hb (g/dl) and PCV (%) were higher in feedlot group supplemented with sodium bicarbonate as feed additive than control. The blood glucose was marginally higher in $NaHCO_3$ supplemented feedlot group than control but were within the physiological limits. Other research workers (de Valdez et al. 1997 and Abo El-Nor and Kholif 1998) reported improved blood glucose concentration with $NaHCO_3$ supplementation and it was attributed to improvement in gluconeogenesis and increased lactose absorption. Results of ALT and AST showed harmony with El-Marakby (2003) who observed no alterations in their concentration in serum. Among the treatment groups, lowest values of BUN were observed in sodium bicarbonate supplemented feedlot group and highest values was observed in feedlot group without feed additives. However, no significant difference (p>0.05) was observed in BUN among the treatment groups. Only the results of serum creatinine showed statistical difference ($p \leq 0.05$) between treatments, revealing higher values in feedlot group supplemented with sodium bicarbonate (Table 5), this might be due to the higher protein intake in feedlot groups. However, creatinine values were within the normal range and no signs of kidney damage were observed in any of the treatment groups.

Table 5. Haemato-biochemistry of experimental animals

Parameter	Groups			SEM	p-value
	C	F	FSB		
Haemoglobin (g/dl)	8.66	8.48	8.89	0.13	0.499
Packed cell volume (%)	25.9	25.4	26.6	0.41	0.499
Blood glucose (mg/dl)	58.0	63.1	68.0	2.03	0.130
ALT (IU/L)	14.9	16.05	15.9	0.73	0.806
AST (IU/L)	93.5	98.8	96.1	2.34	0.686
ALP (IU/L)	115.14	121.6	119.72	1.99	0.424
Blood urea nitrogen (mg/dl)	15.7	16.4	15.04	0.60	0.652
Serum creatinine (mg/dl)	1.25^a	1.49^b	1.51^b	0.04	0.053

Note: ^{abc} means bearing different superscripts within a row differ significantly ($p \leq 0.05$).

Rumen fermentation parameters

The results of rumen pH showed no statistical significance (p>0.05) between treatments (Table 6). The values of rumen pH were higher in sodium bicarbonate supplemented feedlot groups in comparison to feedlot group without feed additive, which may be due to conversion of stronger lactic acid to weaker propionic acid in bicarbonate supplemented feedlot groups. Previous researchers like Hadjipanayiotou (1988), Santra et al. (2003), Tripathi et al. (2004), Gonzalez et al. (2008), Apper bossard et al. (2010) and Ruchel et al. (2014), observed increase in rumen pH on sodium bicarbonate supplementation. The result of TVFA (meq/dl) also showed non-significant difference between treatments and the study conducted by Kawas et al. (2007) revealed that TVFA were not affected by addition of sodium bicarbonate in the diets of finishing lambs. The rumen total nitrogen was significantly higher (pd"0.05) in FSB, which may be due to higher CP content in the TMR compared to CG. Rumen ammonia nitrogen (mg/dl) was comparable between groups but, marginally higher value was observed in CG in comparison to treatment groups which indicated better utilization of ammonia nitrogen for synthesis of microbial protein in feedlot groups. The values of TCA perceptible nitrogen (mg/dl) and NPN (mg/dl) were non-significant (p>0.05) between groups.

Table 6. Rumen fermentation parameters

Parameter	Groups			SEM	p-value
	C	F	FSB		
Rumen pH	6.83	6.57	6.70	0.06	0.171
TVFA (meq/dl)	9.23	9.50	9.33	0.44	0.977
Total nitrogen (mg/dl)	112.00[a]	144.67[b]	158.67[b]	7.45	0.003
Ammonia nitrogen (mg/dl)	19.6	16.2	16.8	0.96	0.362
TCA precipitable nitrogen (mg/dl)	70.0	93.3	105.47	8.13	0.206
NPN (mg/dl)	42.0	51.3	53.2	6.06	0.775

Note: [ab] means bearing different superscripts within a row differ significantly ($p \leq 0.05$).

CONCLUSION

From the present study it could be concluded that sodium bicarbonate can be used as feed additive in the ration of feedlot sheep without any adverse effect on animals. Incorporation of sodium bicarbonate as feed additive (@1% of dry matter intake) in feedlot ration appears to improve the body weight indices and the health status of sheep.

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Evaluation of Growth, Digestibility and Economic Efficiency of Malabari Kids Fed Complete Diets Containing Spent Cumin (*Cuminum cyminum*) Seeds

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ABSTRACT

A three-month feeding trial was conducted to evaluate the growth performance, digestibility, and economic efficiency of Malabari kids fed complete diets incorporating spent cumin (*Cuminum cyminum*) seeds. Eighteen Malabari kids, aged 3 to 4 months, were selected from the University Goat and Sheep Farm, Mannuthy, and randomly divided into three groups. Each group was assigned one of three complete rations: T1 (Control) – containing 16% CP and 60% TDN, T2 – containing 10% spent cumin seeds, and T3 – containing 20% spent cumin seeds. All diets were isocaloric and isonitrogenous. The average daily gain (ADG) and total weight gain were significantly higher in the T3 group (46.23 g/day and 3.88 kg) compared to the control (33.13 g/day and 2.78 kg), while the T2 group showed similar growth to both T1 and T3. Total dry matter intake was comparable across groups (20.01, 21.65, and 24.75 kg for T1, T2, and T3, respectively). Feed conversion efficiency was also similar, with values of 7.23, 6.47, and 6.50 for T1, T2, and T3, respectively. A digestibility trial conducted at the end of the feeding period revealed significantly higher dry matter (DM) and acid detergent fiber (ADF) digestibility in T2 and T3 compared to the control ($p<0.05$). Crude protein (CP) and crude fiber (CF) digestibility were significantly lower in T1 ($p<0.01$). No significant differences were observed among groups for the digestibility of ether extract (EE), nitrogen-free extract (NFE), organic matter (OM), and neutral detergent fiber (NDF). Feed cost per kg body weight gain was Rs. 164.87, Rs. 136.09, and Rs. 133.12 for T1, T2, and T3, respectively, with no significant differences. It is concluded that the inclusion of 20% spent cumin seeds in the diet is economically viable and enhances growth performance without compromising feed efficiency.

KEYWORDS: Digestibility, Growth performance, Malabari kids, Spent cumin

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INTRODUCTION

In India, goats play a vital role in the livelihoods of landless, small, and marginal farmers who rely on pasture-based grazing. According to the 20th Livestock Census (2019), the goat population stands at 148.88 million, contributing 27.8% to the total livestock population. Despite this growth, agricultural production is increasing by only 1.5% to 2% annually, resulting in significant feed deficits, with rising feed costs further straining the livestock sector. To tackle these feed shortages, alternative feed resources, including herbal and agro-industrial by-products, are becoming essential.

Additionally, Kerala, known for its rich Ayurvedic heritage, specializes in producing herbal by-products derived from medicinal plants after extracting active compounds which provide excellent nutritional value at an affordable cost.The integration of these by-products into a total mixed ration (TMR) or complete diets, a system where various feed ingredients are blended into a single, nutritionally complete diet, has gained popularity for improving feed utilization and reducing costs. Complete diets also facilitate the use of non-conventional feeds and addresses feed scarcity while reducing labor (Schingoethe, 2017). By preventing feed selection, complete diets ensure balanced nutrition and improves overall efficiency (Mendoza et al., 2016).

India stands as the largest producer and consumer of cumin seeds globally with Gujarat and Rajasthan being the largest producers in the country. India is estimated to produce about 70% of the world's cumin, with nearly 800,000 hectares under cultivation, yielding approximately 500,000 tonnes annually (Rahman et al., 2020). After cumin oil extraction from Cumin seeds, a medicinal herb and commercial spice known for its bioactive compounds such as cuminaldehyde and terpenoids (El-Sawi and Mohamed, 2002). It also retains bioactive compounds from cumin seeds that enhance palatability (Arun et al.,2014). However, their potential as livestock feed remains largely unexplored. This study aims to evaluate the effects of complete diets containing spent cumin seeds on the growth performance and cost economics of Malabari kids, addressing both nutritional value and economic viability.

MATERIALS AND METHODS

Experimental Design and Feeding trial

Eighteen healthy Malabari kids, aged three to four months, were selected from the University Goat and Sheep Farm, College of Veterinary and Animal Sciences, Mannuthy, for this three-month study. The kids were weaned and housed individually in a well-ventilated, clean, and dry shed with appropriate facilities for feeding and watering. They were divided into three groups, each consisting of six animals, and were as uniform as possible with respect to age, sex, and body weight. The animals were randomly assigned to one of three dietary treatments: T1 (control, complete feed), T2 (complete feed with 10% spent cumin seeds), and T3 (complete feed with 20% spent cumin seeds), following a randomized block design. They were fed diets containing 16% crude protein and 60% total digestible nutrients (TDN), based on ICAR standards (2013), with feed allowances adjusted fortnightly according to body weight. The feed intake and body weight of each kid were recorded fortnightly.

Digestion trial

A digestion trial was conducted during the last five days of the feeding period. The feed and dung samples were analyzed for proximate principles following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were measured using the procedures of Van Soest et al. (1991). Additionally, calcium and phosphorus content in both feed and feces were determined using standard AOAC methods.

Statistical analysis

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

RESULTS AND DISCUSSION

Chemical composition

Spent cumin seeds is a good source of CP and Ether Extract (Table 1). All the three diets were iso-nitrogenous.

Table1.Chemical composition of spent cumin seeds and complete feeds (on DM basis)

Nutrients(%)	Spent Cumin Seeds	Dietary Treatments T1	T2	T3
Dry matter	95.6±0.43	93.92±0.16	92.67±0.44	93.34±0.21
Crude protein	17.56±0.33	16.11±0.26	16.07±0.19	16.01±0.33
Crude fibre	37.45±0.57	15.89±0.04	16.88±0.02	17.89±0.66
Ether extract	9.02 ±1.21	2.53±0.25	2.29±0.05	2.21±0.81
Total ash	4.82±0.09	10.04±0.04	11.26±0.18	10.98±0.31
Nitrogen Free Extract	31.15±0.42	55.43±0.19	53.5±0.23	52.91±0.38
Neutral detergent fibre	52.07±1.88	42.23±0.91	45.61±0.76	40.14±0.33
Acid detergent fibre	24.38±0.24	23.22±0.44	23.14±0.16	23.17±0.36
Acid insoluble ash	1.02±0.99	5.21±0.02	4.89±0.04	4.67±1.01
Calcium (g per cent)	0.59	1.08±0.003	1.04±0.07	1.07±0.23
Phosphorus (g per cent)	1.47	0.51±0.01	0.57±0.037	0.59±0.06

Growth performance

The total weight gains for kids fed T1, T2, and T3 were 2.78 kg, 3.41 kg, and 3.88 kg, respectively, with corresponding average daily gains (ADG) of 33.13 g, 40.67 g, and 46.23 g (Table 2). However, T3 showed significantly higher ADG and weight gain compared to T1 ($p<0.05$).This improvement in T3 may be attributed to phytogenic substanceslike flavonoids, alkaloids, phenols etc.stabilizing the digestive system by reducing undesirable microbial metabolites like ammonia and biogenic amines,which relieved intestinal difficulties, stabilized the digestive system, and eventually improved weight gain as suggested by Franz et al. (2010) and Singh et al.(2019).Supporting the present findings, Smeti et al.(2015) observed increased ADG in kids of lactating goats fed diets containing rosemary essential oils compared to a control diet. Jami et al.(2015) and El-Naggar and Ibrahim (2018) also reported higher ADG in lambs fed cumin residue and cumin or garlic powder, respectively, compared to control diets. However, Mansoori et al. (2006) found no significant difference in ADG between broilers supplemented with varying levels of cumin seed meal.

Dry Matter Intake (DMI) and Feed conversion ratio (FCR)

The fortnightly average daily DMI of kids fed dietary treatments T1, T2, and T3 ranged from 160–380 gduring the six fortnights of the study. Mir and Kumar (2012) found no significant effect of fenugreek seed inclusion on goat feed intake. El-Essawy et al.(2019) also observed no significant differences in DMI in crossbred cattle and lambs, respectively, with or without essential oil supplementation. However, Al-Kassi (2010) reported increased feed intake with 1.5% cumin seed supplementation in broilers, and Aderinboye et al. (2021) found reduced feed intake in goats fed turmeric compared to controls.Feed conversion efficiency (kg feed per kg body weight gain) for kids fed T1, T2, and T3 was 7.23, 6.47, and 6.50, respectively (Table 2), with no significant differences between groups.

Table 2. Body weight, total weight gain, average dailygain and feed conversion efficiency of experimental kids

Parameters	T1	T2	T3	p value
Initial bodyweight(kg)	5.41±0.35	5.35±0.39	5.66±0.61	0.881
Final bodyweight(kg)	8.20±0.39	8.76±0.49	9.55±0.91	0.342
Total weight gain (kg)	2.78^{b}±0.08	3.41^{ab}±0.25	3.88^{a}±0.39	0.04^{*}
Average daily gain (g)	33.13^{b}±1.03	40.67^{ab}±3.01	46.23^{a}±4.75	0.04^{*}
Total dry matter consumed (kg /animal)	20.01±0.66	21.65±1.38	24.75±2.16	0.09
Average daily dry matter consumed(kg/ animal)	233±0.007	254±0.016	292±0.025	0.09
Feed to gain ratio	7.23±0.38	6.47±0.55	6.50±0.39	0.449^{ns}

*Means bearing different superscripts (a,b)within a row differ significantly ($p<0.05$)

Similarly, Bampidis et al. (2005a, 2005b) found no effect on FCR in lambs fed garlic bulb or dried oregano leaves. In contrast, Toghyani et al. (2010) found improved FCR in broilers fed black cumin seeds, and Aderinboye et al. (2021) reported linear improvements in FCR in goats fed turmeric at 2.5 and 5 g/day compared to control.

Digestibility of Nutrients

Dry matter digestibility was significantly higher ($p<0.05$) (Table 3) in cumin-incorporated diets (T2, T3) and can be attributed to increased bile acid synthesis and enzymatic activities (Platel and Srinivasan, 2004). Similar trend was reported by El-Essawy et al. (2019), though contrasting results were observed by Smeti et al. (2015). CP digestibility was showing significantly higher values ($p<0.01$) in T2 and T3. This is supported by Aderinboye et al. (2021) and Modi et al. (2022) where as Sahraei et al. (2014) observed no effect of rosemary essential oil on CP digestibility.

Crude fiber digestibility was significantly higher in T2 and T3 compared to T1, likely due to the mineral content (iron and zinc) in cumin seeds enhancing rumen microbial activity (Khan and Chaudhry, 2010; Milan et al., 2008). Similar improvements were reported by Zanouny et al.(2013) and Mishra et al. (2020), but Oloche et al. (2013) found no significant change with orange peel meal supplementation. Ether extract digestibility was similar across all the treatments. El-Naggar et al. (2016) observed similar findings where as Aderinboye et al. (2021) found significant improvement with turmeric supplementation. NDF digestibility was similar in all treatments for T1, T2, and T3, respectively. Sahraei et al. (2014) reported similar results, while Ikyume et al. (2020) found significantly higher digestibility with ginger and garlic powder supplementation. Finally, ADF digestibility was significantly higher ($p<0.05$) in T2 and T3 compared to T1. Similar findings were reported by Mir and Kumar (2012).

Table 3.Apparent digestibility coefficient (%) of nutrients of experimental kids

Parameters	T1	T2	T3	pvalue
Initial bodyweight(kg)	5.41±0.35	5.35±0.39	5.66±0.61	0.881
Final bodyweight(kg)	8.20±0.39	8.76±0.49	9.55±0.91	0.342
Total weight gain (kg)	2.78^{b}±0.08	3.41^{ab}±0.25	3.88^{a}±0.39	0.04^{*}
Average daily gain (g)	33.13^{b}±1.03	40.67^{ab}±3.01	46.23^{a}±4.75	0.04^{*}
Total dry matter consumed (kg /animal)	20.01±0.66	21.65±1.38	24.75±2.16	0.09
Average daily dry matter consumed(kg/ animal)	233±0.007	254±0.016	292±0.025	0.09
Feed to gain ratio	7.23±0.38	6.47±0.55	6.50±0.39	0.449^{ns}

*Means bearing different superscripts (a,b)within a row differ significantly ($p<0.05$)

Techno economics

The cost of production per kg body weight was higher in the control group (T1) compared to those fed 10% (T2) and 20% (T3) spent cumin seeds, primarily due to the lower cost of cumin seeds (Rs 5/kg) and improved growth in the supplemented groups (Table 4). Statistically, the cost per kg weight gain was comparable across all groups. Similar trends were reported by Zanouny et al. (2013) in sheep fed *Nigella sativa*.

Table 4.Techno economics of production of experimental kids

Parameter	T1	T2	T3	pvalue
Average complete feed intake(kg/kid)	21.31±0.70	23.37±1.49	26.52±2.31	0.112
Cost of complete feed/kg (Rs)	21.39	19.47	19.10	--
Total cost of feed(Rs./kid)	455.89±15.12	455.03±29.02	506.54±44.30	0.443
Total weight gain(kg/kid)	2.78^{b}±0.087	3.41^{ab}±0.25	3.88^{a}±0.39	0.042*
Cost Rs/kg	164.87±8.82	136.09±11.64	133.12±8.10	0.065^{ns}

*Means bearing different superscript (a,b)within a row differ significantly ($p<0.05$)

CONCLUSION

The inclusion of spent cumin seeds at 10% and 20% in the diet of kids significantly improved average daily gain (ADG), feed conversion ratio (FCR), and nutrient digestibility, particularly for dry matter and crude protein. Additionally, the feed cost (Rs) per kilogram of body weight gain was reduced in the supplemented groups indicating that the 20% inclusion level proved to be a practical and economical strategy, enhancing growth performance and nutrient utilization without adverse effects.

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Effect of Organic Zinc Supplementation on Productive and Reproductive Performance of Transition crossbred Cows

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ABSTRACT

Experiment was conducted for 150 d on forty Holstein crossbred cows (530±30 kg b. wt.) and the experimental period had included one-month prior parturition and four months after parturition and were distributed randomly into four treatments and fed super Napier green fodder, paddy straw and concentrate mixture to meet ICAR (2013) nutrient requirements except for mineral mixture. Four experimental rations were formulated viz. BD, Zn_i, Zn_g, and Zn_m in which BD ration had no supplemented zinc, Zn_i (zinc sulphate), Zn_g (zinc glycinate) and Zn_m (zinc methionate). All the forms of zinc were supplemented as per the NRC (2001) nutrient requirements for dairy cattle. Significantly ($P<0.05$) higher concentration of SOD (units/mg protein) was recorded in Zn_m ration fed cows followed by Zn_g, Zn_i and BD rations. The mean GPx (units/ml) concentration was significantly ($P<0.05$) lower in the Zn_m ration fed cows followed by Zn_g, Zn_i and BD rations. Milk yield (kg/day) was increased significantly ($P<0.05$) in the Zn_m ration fed cows. Estrogen (pg/ml) and progesterone (ng/ml) concentrations were significantly ($P<0.05$) higher in Zn_m ration fed cows when compared to other groups. Reproductive performance was improved by supplementing zinc methionate (Zn_m) and it had shown the best performance in comparison to other treatment groups. With the results, it is inferred that zinc methionate supplementation has improved the productive and reproductive performance by ameliorating the transition stress in crossbred cows.

KEYWORDS: Antioxidants, Crossbred cows, Organic zinc, Reproductive performance, Stress, Transition period.

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INTRODUCTION

The "transition period," the period from three weeks before to three weeks after calving, is extremely stressful for dairy cows or any other livestock species. Oxidative stress in a living organism is a result of an imbalance between reactive oxygen metabolites (ROM) production and neutralizing capacity of antioxidant mechanisms. Oxidative stress leads to peroxidative damage of lipids and other macromolecules with consequent alteration of cell membranes and other cellular components, and can also lead to the modification of important physiological and metabolic functions.

Bakhshizadeh et al. (2019) experimented with different sources of zinc as zinc oxide, zinc glycine and zinc nano @ 60 mg zinc per kg diet in Holstein dairy cows and reported that there was significant effect of time on DMI and milk yield, with an increase in milk yield being observed. Nazari et al. (2019) revealed that, supplementation of trace minerals (as per NRC 2001) had greater antioxidant trace mineral concentration of Cr, Cu, Fe and Zn which were associated with early postpartum period with normal luteal activity, earlier resumption of cyclicity, decreased pregnancy loss and increased conception rate in Holstein dairy cows.

There were lack of reports on the effect of organic compounds in roughage-based diets and only a limited number of studies have investigated the importance of organic zinc supplementation in transition cows. Hence, the present work was carried out to evaluate the amelioration of transition stress in crossbred cows with organic zinc supplementation.

MATERIALS AND METHODS

The protocols adopted in this experimental study were approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Hyderabad and is in accordance with the guidelines for the care and use of Laboratory animals with IAEC vide approval No.22/25/ C.V.Sc, Hyd. IAEC. Forty advanced pregnant multiparous crossbred cows (about one-month pre-partum) with an average body weight of 530±20 kg was selected from farmers of nearby villages of Korutla and allotted randomly into 4 groups of 10 animals each. All the cows were housed in well-ventilated hygienic stalls and stall fed throughout the experimental period. The animals were treated for both ecto and endo parasites well before the initiation of the study.

Conventional practice of feeding concentrates and roughages separately was followed throughout the experiment. Chopped green super Napier (*Pennisetum purpureum*) and paddy straw were used as sources of roughage for feeding of experimental animals in all the groups. Concentrate feed ingredients viz., maize, wheat bran, cotton seed cake, moong dal chunni, mineral mixture and salt were used. Concentrate feed ingredients were maize (average 3.5 kg), wheat bran (average 1.75 kg), cotton seed cake (average 1kg), moong dal chunni (1.75kg), mineral mixture and salt. The cows had free access to fresh and wholesome clean drinking water all the time.

Four different rations were fed to four treatment groups as mentioned below. The particulars are BD, basal diet without Zn supplementation; $Zn_{i,}$ Basal diet + inorganic Zn (Zinc sulphate); $Zn_{g,}$ Basal diet + Zinc glycinate; $Zn_{m,}$ Basal diet + Zinc methionate; Zinc supplementation was done through oral route by feeding the animals individually by preparing the mineral mixture. Mineral mixture was prepared with premixer in feed plant. The mineral mixture was prepared separately for four treatments by adding different sources of zinc and as per zinc bio-availability. All the cows were fed basal diet (roughages and concentrates) in accordance with the ICAR (2013) nutrient requirements for the experimental period of 150 days i.e., 1-month before calving to 4-months after calving. Zinc was supplemented to the mineral mixture prepared, as per NRC (2001) nutrient requirements for dairy cattle in different diets of experiment viz. without zinc (BD) Zn_i with zinc sulphate (27 % of zinc - Ven Vet chemicals), Zn_g with zinc glycinate (26 % of zinc- Avitech) and Zn_m with zinc methionate (16% of zinc-Novus company) .

The blood samples were collected at 15 days before parturition and on 0th, 15th, 30th and 60th day of post calving and the separated serum was used for further analysis. The proximate analysis of feeds was performed as per the procedures described by AOAC (2019). Mineral estimation was done by atomic absorption spectrophotometer using wet digestion procedures. Di-acid procedure was used for feed minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer using Air/Acetylene gas. Tri acid digestion procedures were used for serum minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer using Air/Acetylene gas.

Serum antioxidant parameters SOD concentration was estimated as per the procedure detailed by Madesh and Balasubramanian (1998). The GPx activity was determined by the method proposed by Paglia and Valentine (1967). The Milk yield was recorded from individual animals daily up to four months postpartum. The reproductive performance of cows was recorded as part of routine farm management, the postpartum cows were observed twice a day by trained farm personnel. The animals were examined for various gynecological parameters like involution of uterus, % of animals affected with mastitis and endometritis. These parameters were done using intrauterine probe (scanner).

The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 20th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at $P<0.05$. All the statistical procedures were carried out as per the procedures of Snedecor and Cochran (1994) by programming and processing in computer. Two-way ANOVA was used to analyze the various parameters and if there was an interaction between the diet and time period was observed, one way ANOVA was done and mentioned in the results.

RESULTS AND DISCUSSION

Zinc concentration (mg/kg) in different feed ingredients offered to the animals during experimental period was super Napier green fodder (10.52), paddy straw (34.36), maize grain (21.58),wheat bran (69.41), cotton seed cake(undecorticated) (55.30), moong dal chunni (32.1).

Serum Zinc Concentrations

The decreased plasma zinc concentration (Table 1) on the day of calving might be due to redistribution of zinc in various tissues and this is in agreement with other experiments conducted by Maurya et al. (2014) and Chandra et al. (2014) in Karan Fries cows and buffaloes, respectively. Mean serum zinc concentration was 6.59 %, 18.68 %, 30.76 % higher in CB cows fed Zn_i, Zn_g and Zn_m, respectively when compared to BD fed cows. This might be due to increased bioavailability of zinc in organic sources than inorganic and without zinc supplementation.

Table 1. Serum zinc concentration (µmoles/l) of crossbred transition cows fed conventional rations with supplementation of different sources of zinc and at different time intervals

Day	Zn concentration (µmoles/l)				Mean	P-Value		
	BD	Zn_i	Zn_g	Zn_m	±SEM	Treatment	Period	T*P
-15	1.71	1.72	1.73	1.76	1.73^c ±0.13	0.0001	0.001	0.924
0	0.87	0.94	1.16	1.39	1.09^b ±0.09			
15	0.73	0.81	0.98	1.09	0.90^{ab} ±0.11			
30	0.69	0.75	0.83	0.92	0.79^a ±0.12			
60	0.54	0.65	0.72	0.82	0.68^a ±0.15			
Mean ±SEM	0.91^A ±0.13	0.97^{AB} ±0.12	1.08^B ±0.14	1.19^{BC} ±0.16				

a,b,c Values bearing different superscripts in columns differ significantly
A,B,C Values bearing different superscripts in rows differ significantly

Serum antioxidant enzymes

Super Oxide Dismutase (SOD)

The results of the present study are in agreement with the results reported by Bernabucci et al. (2005) who showed an increased activity of SOD during the last 3 weeks of pregnancy and rapidly declined SOD activity after calving (Table 2). Chandra et al. (2013) reported an increase in the SOD activity due to increased level of free radicles during the last three weeks and declined after calving till day 60 postpartum in Karan Fries crossbred cows supplemented with vitamin E and Zn.

Table 2. Super Oxide Dismutase (SOD) (units/mg of protein) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	SOD (units/mg of protein)				Mean± SEM	P-Value		
	BD	Zn_i	Treatment	Treatment		Treatment	Period	D*P
-15	6.48	7.10	7.34	7.27	7.05^a±0.13	0.044	0.0001	0.0001
0	8.03	7.46	7.52	7.85	7.72^c±0.10			
15	7.67	7.36	7.09	7.42	7.38^b±0.13			
30	7.43	7.26	7.56	7.33	7.40^b±0.13			
60	6.60	7.20	7.41	7.31	7.13^a±0.09			
Mean±SEM	7.24^A± 0.14	7.28^{AB}± 0.08	7.38^{AB}± 0.12	7.44^B± 0.11				

a,b,c Values bearing different superscripts in columns differ significantly
A,B,C Values bearing different superscripts in rows differ significantly

Glutathione peroxidase (GPx)

The GPx concentration was increased at the time of calving in all the groups when compared to prepartum values (Table 3). The increase in the concentration of GPx was least in the Zn_m followed by Zn_g, Zn_i and BD. After parturition there was decrease in the concentration of GPx in all the groups but it was less observed in the cows fed Zn_m followed by Zn_g, Zn_i and BD . Irrespective of supplementation of zinc, the GPx concentration was increased at the time of parturition and decreased regressively after calving. The increase was less in zinc methionate supplemented group when compared to basal diet and other supplemented groups. The present results are in agreement with Maurya et al. (2014) who supplemented zinc and vitamin E in Karan Fries cows.

Table 3. Glutathione peroxidase (GPx) enzyme (units/ml) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	Glutathione Peroxidase (units/ml)				Mean± SEM	P-Value		
	BD	Zn_i	Treatment	Treatment		D	P	D*P
-15	602.69	576.22	560.72	550.89	$572.63^{a}\pm9.84$	0.0001	0.0001	0.005
0	691.16	614.41	589.08	581.89	$619.13^{d}\pm9.12$			
15	648.06	605.71	581.89	573.95	$602.40^{c}\pm9.01$			
30	607.22	594.75	577.73	567.14	$586.71^{b}\pm8.37$			
60	602.65	584.92	571.31	558.07	$579.25^{ab}\pm8.85$			
Mean±SEM	$630.36^{C}\pm$ 9.32	$595.20^{B}\pm$ 8.66	$576.14^{A}\pm$ 9.12	$566.39^{A}\pm$ 9.05				

a,b,c Values bearing different superscripts in columns differ significantly
A,B,C Values bearing different superscripts in rows differ significantly

Milk Yield

Increased ($P<0.05$) milk yield in cows fed Zn_m (Table 4) is in agreement with the report of Kellogg et al. (2004) in which Zn methionine increased the lactation performance by increasing milk yield ($P<0.01$). Zinc plays an important role in maintaining skin health and integrity and has been implicated in reducing somatic cell count in some herds (Kellogg et al., 2004) and zinc is involved with keratin lining of the teat canal that physically traps the bacteria and prevents migration into mammary gland (Boland 2003). The improved performance of the cows supplemented with zinc methionine may be attributed to the superior bioavailability of zinc in this form.

Table 4. Milk yield (kg/d) in crossbred cows with supplementation of different sources of zinc in conventional rations

Month	Average milk yield (kg/d)				P Value
	BD	Zn_i	Zn_g	Zn_m	
1st month	19.43	20.04	20.24	20.62	0.619
2nd month	20.64	21.30	21.44	21.70	0.769
3rd month	20.60	20.70	23.11	24.01	0.009
4th month	19.66	20.00	22.63	23.77	0.002
Mean±SEM	$20.08^{A}\pm1.13$	$20.51^{A}\pm1.23$	$21.85^{AB}\pm1.15$	$22.52^{B}\pm0.52$	0.033

A,B Values bearing different superscripts in rows differ significantly

Reproductive parameters

The supplementation of zinc had shown reduced number of days for coming to heat after calving in crossbred cows (Table 5). In agreement with the present study, Patel et al.(2017) stated that, supplementation of zinc at various levels decreased no. of days to show first heat after calving in Karan Fries cows, In corroboration with the present study, Patel et al. (2017) reported decreased number of animals faced difficulty in shedding the fetal membranes, number of inseminations per conception, service period, number of mastitis cases and number of metritis cases with the supplementation of zinc at various levels in Karan Friess cows. Kassab et al. (2020) reported the importance of selenium, Zn and Mn as their deficiency have been linked to abnormal oestrus cycle, impaired ovulation and decreased conception rates. Concomitantly, Ahola et al. (2004) also found that, cows received trace minerals (Zn, Cu and Mn) had higher pregnancy rates than non-supplemented cows. Zinc is involved in the steroidogenesis and thus helps in improving the reproductive efficiency of the animals.

Table 5. Reproductive parameters in crossbred cows with supplementation of different sources of zinc in conventional rations

Parameter	BD	Zn_i	Zn_g	Zn_m	P Value
First heat after calving(days)	61.80^{C}	55.50^{B}	52.30^{A}	51.80^{A}	0.0001
Percentage of animals faced difficulty in expulsion of placenta after calving	0.4^{B}	0.2^{AB}	0.1^{AB}	0.0^{A}	0.012
Average number of inseminations per conception	1.4	1.3	1.2	1.1	0.131
Service period (days)	84.30^{C}	78.30^{B}	74.80^{A}	72.90^{A}	0.0001
Involution of uterus (days)	55.70^{D}	47.70^{C}	44.70^{B}	42.30^{A}	0.0001
% of mastitis cases	0.4^{B}	0.2^{AB}	0.1^{AB}	0.0^{A}	0.012
% of endometritis cases	0.5	0.4	0.1	0.0	0.153

A,B,C Values bearing different superscripts in rows differ significantly

Zinc oxide supplementation showed higher pregnancy rate in Does (Kundu et al., 2014) and concluded that inclusion of Zn @ 50, 100 and 150 ppm increased pregnancy rates compared to controls. In corroboration with present study Maurya et al. (2011) and Chandra et al. (2013) reported reduced mastitis cases with vitamin E and Zn supplementation. In general, free radicals cause cell damage and might influence the functions of immune cells resulting in increased mastitis risk (Politis et al., 2004). Miller et al. (1993) suggested that, cows with retained placenta had lower total antioxidant status. Reduced incidence of mastitis in the present study might be due to zinc trapping the bacteria on the teat surface and prevented their entry into mammary gland.

CONCLUSION

The crossbred cows exhibited better performance with supplementation of organic zinc sources, with a significant improvement observed in the group supplemented with zinc methionine compared to those receiving zinc glycinate and inorganic zinc sources. This enhanced performance could be attributed to the higher bioavailability of zinc in zinc methionine. Supplementing with zinc methionine reduced oxidative stress in the cows during the transition period, which, in turn, improved both their productive and reproductive performance.

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Effect of Feeding Sorghum Hay, Maize Stover and Areca Sheath Based Total Mixed Ration Feed Block (TMRfb) on Nutrient Intake, Digestibility and Cost Economics in Growing Lambs

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ABSTRACT

A comparative study on sorghum hay, maize stover and areca sheath based total mixed ration feed block (fb) on voluntary feed intake, digestibility, growth performance and rumen fermentation parameters was conducted in growing lambs. Eighteen non-descript lambs aged 4-6 months with an average body weight of 17±0.9kg were randomly allotted to three experimental diets with roughage to concentrate ratio of 60:40. The T1- diet comprised of sorghum hay based TMRfb, T2-diet comprised of maize stover based TMRfb and T3- diet comprised of areca sheath based TMRfb. The average DMI (g/d) of TMRfb varied significantly ($P<0.01$) from 480.81 (T2) to 738.19 (T1) with 34.86 percent lower intake in maize stover based group compare to control. The ADG (g/d) was significantly ($P<0.01$) higher in T1 (85.2), compared to T3 (64.3) followed by T2 (35.9). The digestibility of DM, OM, and CF was higher in T3 compared to T1 and T2. Digestibility of CP was ($P<0.01$) higher in T1 and T3 compared to T2. The intake of DCP (g/d), TDN (g/d) and ME (MJ/d) intake were significantly ($P<0.01$) higher in T1 (69.77, 531.87 and 9.03) as compared to T2 (30.82, 367.07 and 5.39) and T3 (32.85, 362.36 and 5.33). It was observed that sorghum hay based TMR had better feed intake and average daily gain compared to maize stover and areca sheath-based groups. However, cost per kg gain was 36.90 percent higher in areca sheath based TMR groups compared to sorghum hay based group.

KEYWORDS: Average daily gain, Cost economics, Digestibility, Feed block, Hay, Lambs, Rumen parameters, Total mixed ration.

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INTRODUCTION

Crop residues are bulky in nature and to facilitate easy transportation and storage with minimum input, densification technologies are useful. Crop residues and agro industrial by-products play an important role as a source of feed for ruminants, but the utilization of these feeds is limited because of poor nutrient content and digestibility. To make crop residues quality roughage for ruminants feeding, various processing methods like grinding, pelleting, treatment with chemicals like ammonia (Reddy and Reddy, 1985) and sodium hydroxide (Ben-Ghedalia et al., 1982) have been tried to improve the nutritive value. The concept of feeding complete rations or total mixed ration with use of locally available crop residues seems to be ideal and promising for improving the utilization of poor-quality fibrous crop residues.

Total Mixed Ration stabilizes the environment for rumen fermentation, minimizes fermentation losses, and reduces fluctuations in ammonia release, thus enhancing the utilization of low-grade roughages (Venkanna et al., 1997). Further, one of the options for effective utilization of the crop residues is by processing technologies for the commercial manufacture of straw-based complete feed for ruminants. The manufacture of densified total mixed ration blocks (DTMRB), also known as densified complete feed blocks (DCFB), is an innovative technology for supplying balanced feeds to dairy and other livestock farmers in the tropics. The study conducted at ICAR-NIANP on use of areca sheath as dry fodder has shown promising results (Gowda et al., 2016). This study was undertaken with objective to prepare sorghum hay, maize stover and areca sheath based total mixed ration and to evaluate feeding of these blocks on voluntary feed intake, average daily gain, nutrient digestibility and rumen liquor profile.

MATERIALS AND METHODS

The animal trial was conducted at Department of Animal Nutrition, Veterinary College, Shivamogga, Karnataka, India.

Source of roughage for TMR block preparation:

Sorghum hay was grown in fodder plots of Veterinary College, Shivamogga. The hay was harvested at 75 days, chaffed, sun dried for 6 days and were ground by using shredder (8mm sieve size) and stored in gunny bags. Dried Maize stover was ground by using shredder and stored in gunny bags. Areca sheath trimmings were collected from nearby areca sheath plate industry. The sheath was dried for 2 days under sun light. Once it was completely dried, ground by using shredder and stored in gunny bags. The compounded feed mixture comprising maize, DORB, cotton seed cake, ground nut cake, salt, mineral mixture and urea were procured from local market. The molasses premix was done at M/S Sri Devi Feeds, Honnali, Karnataka, India Composition of ingredients in depicted in Table 1.

Table 1.Ingredient composition (% DM basis) of TMRfb

Ingredients	T1 (%)	T2 (%)	T3 (%)
Sorghum hay	60.0	0.00	0.00
Maize stover	0.00	60.00	0.00
Areca sheath	0.00	0.00	60.0
Maize	19.2	19.2	19.2
DORB	4.00	4.00	4.00
Cotton seed cake	5.60	5.60	5.60
Ground nut cake	6.00	6.00	6.00
Mineral mixture	0.80	0.80	0.80
Salt	0.40	0.40	0.40
Urea	0.40	0.40	0.40
Molasses	3.60	3.60	3.60
Total	100.00	100.00	100.00

T1-Sorghum hay based total mixed ration feed block (60:40):(SH-TMRfb)
T2-Maize stover based total mixed ration feed block (60:40):(MS-TMRfb)
T3-Areca Sheath based total mixed ration feed block (60:40):(AS-TMRfb)

Preparation of Total Mixed Ration Feed Block

The TMR was prepared by mixing roughage and concentrate in the ratio of 60: 40 (Table 1). TMR blocks of 500g-1500g was prepared using Commercial Feed Block making machine. This block making machine works on the principle of hydraulic compression. The machine works at a capacity of 3000 psi pressure and equipped with 7.5 HP motor. Blocks were prepared by applying compression pressure at 3000 psi for about 15-20 sec. Blocks of 500g-1500g were stored in plastic bags.

Experimental animals and feeding trial

Eighteen male lambs of non-descript breeds (average body weight of 17±0.9 kg) of four to six months old were divided into three groups in a complete randomized design (CRD). Group I (T1) was fed with Sorghum hay based total mixed ration feed block (60:40): (SH-TMRfb), group II (T2) was fed with Maize stover based total mixed ration feed block (60:40): (MS-TMRfb) and group III (T3) Areca Sheath based total mixed ration feed block (60:40): (AS-TMRfb). Initial adjustment period of two weeks and feeding trial was carried out for a period of twelve weeks followed by a digestion cum metabolism trial for a period of five days was conducted. The ADG and DMI were calculated. Experimental diets were subjected to proximate (AOAC 2016) and Fiber analysis (Vansoest et al., 1991).

Every week body weight of lambs was recorded using digital platform balance before offering feed and water. The average daily gain (ADG) of lambs was calculated on the basis of weekly change in the body weight. Daily intake of TMRfb was recorded. Dry matter intake of lambs was calculated by deducting the left over from the mean daily offered quantities of TM fb.

Statistical analysis

The data regarding DMI and nutrient intake, ADG, digestibility co-efficient, Nitrogen balance and plane of nutrition were subjected to statistical analysis, one way ANOVA by using SPSS statistical software (IBM SPSS Statistics 20).

RESULTS AND DISCUSSION

Chemical composition of experimental diets

The chemical composition of sorghum hay, maize stover and areca sheath are presented in Table 2. The DM, OM, TA and AIA of sorghum hay and areca sheath were similar except CP, CF, EE, NFE, NDF, ADF, HC and C. The CP contents of sorghum hay and areca sheath were similar 4.47 and 4.35, respectively which were similar to the values recorded by Vikas Dharigoud, (2019). The DM, CP, CF, EE and NFE of maize stover were 88.9. The CP (%) content of SH-TMRfb, MS-TMRfb and AS-TMRfb were 10.01, 7.88 and 8.54, respectively. The CP content was different in all the treatment because roughage: concentrate ratio of 60:40 was maintained to prepare a practically and economically feasible feed block. The composition of areca sheath is like the reported values of Gowda et al., (2016).

Table 2. Chemical composition (% on DMB) of CFM, roughages and TMRfb.

Parameter	CFM	SH	MS	AS	T1	T2	T3
DM	89.8	87.8	88.9	87.4	87.5	88.3	86.3
OM	92.2	93.2	93.9	93.0	92.1	93.3	93.0
CP	18.9	4.47	3.41	4.35	10.01	7.88	8.54
CF	10.1	26.7	34.6	38.2	20.7	24.0	21.8
EE	3.10	2.00	1.60	1.40	2.70	2.10	2.40
NFE	60.1	60.03	54.3	49.05	58.7	59.3	60.2
TA	7.80	6.80	6.10	7.00	7.90	6.70	7.00
AIA	1.40	2.60	2.40	2.60	2.60	2.50	1.90
NDF	32.2	44.7	61.3	67.7	42.3	55.2	58.1
ADF	10.5	25.1	35.4	35.7	24.3	28.0	31.4
ADL	3.30	5.30	7.20	5.60	5.30	5.60	5.90
HC	21.7	19.6	25.9	32.0	18.0	27.2	26.7
C	7.20	19.8	28.2	30.1	19.00	22.4	25.5
Ca	2.61	0.30	0.60	0.20	1.20	1.10	1.00
P	1.09	0.14	0.19	0.06	0.50	0.60	0.50
Density (g/cm^3)	-	-	-	-	480.00	540.00	570.00

SH- Sorghum hay; MS-Maize stover; AS-Areca sheath;

Weekly dry matter intake

The average DMI (g/d) of TMRfb varied from 480.81 (T2) to 738.19 (T1). In terms of *per cent* of bodyweight TMR feed block varied from 2.57 (T3) to 3.64 (T1). The sorghum hay based TMR (T1) fed group of animals has significantly ($P \leq 0.01$) higher voluntary feed intake compared to maize stover (T2) and Areca sheath (T3) based groups because of more ME (MJ/kg DM), CP (%) in T1 group and is more acid detergent lignin (ADL) in T2 and T3 group. Similar observations were recorded by Sivala et al. (2024) where there was a substantial increase in the DMI (g/day) of kids fed with feed block containing very low protein and polyethylene glycol compared to kids fed with low protein and high protein feed block. Raghuvansi et al. (2007) reported that DM intakes of Malpura lambs fed on complete feed block were considerably higher than those animals maintained on grazing followed by supplementing of 250g concentrate mixture.On the contrary, Hozhabri and Singhal, (2009) recorded that DMI and average daily gain (ADG) was similar between the groups fed with wheat straw based complete feed block (CFB), sugarcane bagasse containing CFB and formaldehyde treated rapeseed cake containing CFB. Chaudhary et al. (2017) also reported that, the dry matter intake was comparable between the group fed with paddy straw based complete feed block (733.40 g/d) and kinnow mandarin (*Citrus nobilis x Citrus deliciosa*) waste included paddy straw based complete feed block (733.75 g/d).

Average daily gain (ADG)

The overall gain during the 12 weeks feeding trial was 7.7, 3.2 and 5.8 kg concomitantly 85.2, 35.9 and 64.3 ADG (g/d) for the dietary groups T1, T2 and T3, respectively (Table. 3). The average daily gain of T1 was higher compared to T3 than T2. The T2 group observed significantly lower body weight gain than T3.

The change in body weight (gain/loss) of the lambs was rather inconsistent in the treatments (T2 and T3) during the experimental period. Due to lower intake of CP in both the treatment groups, the animals didn’t meet the protein requirement as per the standard specifications (ICAR, 2013). Hence, feeding of crop residue based TMRfb for T2 groups could not support appreciable body weight gain during feeding trial.

Table 3. Body weight changes and Average daily gain of lambs during feeding trial

Parameters	T1	T2	T3	SEM	P value
Initial body wt.(kg)	17.0	17.4	17.0	0.43	0.91
Final body wt. (kg)	24.7^{b}	20.7^{a}	22.8^{ab}	0.69	0.05
Total weight gain(kg)	7.73^{c}	3.22^{a}	5.8^{b}	0.54	0.01
ADG (g)	85.2^{c}	35.9^{a}	64.3^{b}	5.95	0.01

$P \leq 0.05$,Means bearing different superscripts between the columns.

However, such crop residue based TMRfb can be considered to support the requirement of animals with low to medium growth potential and to overcome the feed scarcity situations during Natural calamities like flood and drought conditions. Vikas Dharigoud (2019) reported slightly higher gain compared to present study which might be due to inclusion of CFM was higher (50%) in their total mixed ration diet. Reddy et al. (2018) recorded higher growth rate with feeding of crop residue based total mixed ration.

Nutrient digestibility

The digestibility of DM, OM, CP, CF, EE, NFE, NDF, ADF, HC and C was significantly higher in T3 as compared to T2 and T1, indicating areca sheath was having higher digestibility as compared to sorghum hay and maize stover as the DMI of areca sheath was low and prolonged ruminal stay for effective digestion (Table 4).

The digestibility values obtained in the present study for areca sheath was in concurrence with the reports of Raguvansi et al. (2007a) where OMD and CPD were significantly higher in stall fed goats than goats fed on grazing. Similar observations were also recorded by Reddy et al. (2018). In the present study, higher digestibility of the areca sheath as compared to sorghum hay and maize stover may be attributed to high longer ruminal stay, fiber digestibility consequently higher utilization. Similar observations recorded by Vikas Dharigoud, (2019) where in areca sheath was having higher digestibility as compared to paddy straw. Similarly, the digestibility of cellulose was significantly higher in areca sheath as compared to paddy straw and further confirmed the higher fermentability of areca sheath. On the contrary, Das et al. (2004) fed complete feed in mash form and complete feed in block form to crossbred calves and Babu et al. (2014) fed three complete rations formulated with roughage to concentrate ratio of 60:40 using sorghum stover, maize stover and sweet sorghum stover to Nellore ram lambs reported no significant difference in digestibility of DM, OM, CP, CF, EE and NFE.

Nutrient density and their intake

The DCP, TDN and ME content of T1 rations were significantly higher compared to T2 and T3. Whereas, TDN and ME content of T3 rations were significantly higher compare to T1 and T2. The energy (TDN and ME) and protein (DCP) content of the experimental rations were computed for their intake in which, group T1 animals received significantly higher as compared to T3 and T2 (Table 5).

Table 4. Nutrient digestibility (%) of experimental lambs

Parameter	T1	T2	T3	SEM	P-value
DM	54.48^{a}	55.00^{a}	65.71^{b}	1.57	0.01
OM	55.61^{a}	58.42^{a}	67.52^{b}	1.51	0.01
CP	71.35^{b}	58.77^{a}	69.41^{b}	1.58	0.01
CF	44.01^{a}	45.59^{a}	56.49^{b}	1.77	0.01
EE	73.53^{a}	79.43^{b}	86.28^{c}	1.34	0.01
NFE	57.46^{a}	60.48^{a}	70.49^{b}	1.59	0.01
NDF	50.01^{a}	57.32^{b}	65.58^{c}	1.77	0.01
ADF	50.47^{a}	58.05^{b}	58.61^{b}	1.28	0.01
HC	49.39^{a}	56.40^{b}	62.21^{c}	1.58	0.01
C	53.66^{a}	59.62^{b}	62.22^{b}	1.23	0.01

P<0.01, Means bearing different superscripts between the columns differ significantly.

Table 5. Mean nutrient density and intake of experimental lambs

Parameter	T1	T2	T3	SEM	P
DCP (%)	7.14^{c}	4.63^{a}	5.93^{b}	0.26	0.01
TDN (%)	54.4^{a}	52.2^{a}	65.4^{b}	1.48	0.01
ME (MJ/kg)	9.25^{b}	7.99^{a}	9.62^{c}	0.17	0.01
DCP intake (g/d)	69.7^{b}	31.4^{a}	32.85^{a}	4.44	0.01
TDN intake(g/d)	590.3^{b}	352.5^{a}	348.4^{a}	21.38	0.01
ME intake(MJ/d)	9.03^{b}	5.39^{a}	5.33^{a}	0.45	0.01

P<0.01, Means bearing different superscripts between the columns differ significantly.

In comparison to recommendation of ICAR (2013) for a rams weighing 15 kg with ADG of 100 g/d (DCP intake 65 g/d and TDN intake 373 g/d), the DCP intake was very low in T2 and T3 group which has reflected in lower body weight gain. TDN intake was slightly lower in T2 and T3 than the recommendations. The lower feed formulation of roughage: concentrate in the ratio of 60:40 might affect the plane of nutrition in experimental lambs of T2 and T3 and found to be inadequate.

Cost economics of feeding TMRfb in lambs

The total cost incurred towards feeding of sheep with TMR feed blocks was worked out for unit live weight gain. The average cost of production was found to be Rs.190.2, 200.7 and 120.0 per kg live weight gain in T1, T2 and T3, respectively. The lambs maintained on T3 (RS. 120.0) recorded a reduced feed cost per kg live weight gain compared to lambs fed on T1 (Rs. 190.2) and T3 (Rs. 200.7).

CONCLUSION

Areca sheath-based TMR feed blocks demonstrated better fiber digestibility compared to sorghum hay and maize stover. The cost of feeding per kg gain was 36.9% lower in areca sheath-based TMR feed blocks compared to the sorghum hay-based group. Areca sheath could be conveniently incorporated as a replacement for traditional crop residues in TMR feed blocks, making it suitable for ruminant feeding.

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Impact of Area Specific Micronutrient Supplementation in Peri-Parturient Malvi Cows on the Udder Health and Milk Production

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ABSTRACT

Objective of this experiment was to evaluate the effect of supplementation of area specific micronutrients on udder health, milk yield, and its quality in Malvi cows under field conditions. Eighteen healthy multiparous advanced pregnant Malvi cows, having similar body weights (317.95±5.93kg), parity (3 and 4), and feeding conditions were divided into 2 equal groups in which diet was supplemented with or without a micronutrient. This supplementation study was continued for 2 months during advanced pregnancy and thereafter for 2 months post-calving. Dry matter (DM) intake during the pre- and postpartum remained comparable ($P>0.05$) between two groups. Udder health, milk pH and somatic cell count improved ($P<0.05$) in the micronutrients supplemented group. The average milk yield was increased ($P<0.05$) by 16.42 % in supplemented group. There was no effect of micronutrient supplementation on milk chemical composition (Fat, TS (Total solid), SNF (Solid not fat), lactose and protein) in dairy cows. These findings suggest that supplementation of micronutrient in the ration of peri-parturient Malvi cows not only improved the udder health by reducing the occurrence of mastitis but also increased the milk yield without affecting milk quality.

KEYWORDS: Area specific micronutrients, Malvi cows, Mastitis, Milk quality, Milk yield, Udder health

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INTRODUCTION

Mastitis ranked 1st among the disease in India for dairy industries because it reduces the milk yield, lowers the quality of milk and shorten the productive life of animal thereby leading to heavy economic loss (NAAS, 2013). Subclinical mastitis has been estimated to account for 57.93% (4151.61crores) of total economic loss due to mastitis. Nutrition plays an important role in the control of mastitis. Cows with sub clinical mastitis produce up to 4.6 kg/d less milk compared with healthy herd mates (Green et al., 2006), represent a reservoir of infection for other cows in the herd and are at a greater risk of being culled or developing clinical mastitis (Barlow et al., 2009). Ensuring adequate trace mineral availability is a potential strategy to reduce the effects of mastitis. Trace minerals are critical for proper immune response and play an important role in udder health. Micronutrients remain the most important factor for limiting the transition related disorders and its consequences on general health status due to their cellular roles in immunity, metabolism, growth as well as specific role in free radical control in dairy cows.

Due to lack of awareness in Malwa region of Madhya Pradesh, traditional feeding practices are followed by dairy farmers. The majority of farmers offer straw (wheat straw/masoor straw/gram straw) along with cottonseed cake (un-decorticated) as concentrate without any mineral and vitamin supplementation to lactating animals. The pregnant animals even in an advanced stage of pregnancy fed solely on straw without any concentrate. Few farmers offer a little amount of concentrate/green fodder depending upon the availability. Under such feeding conditions, the availability of antioxidant minerals (Zn, Cu and Se) and vitamins (â carotene and vitamin E) which are having the importance in udder health may greatly be reduced to animals (Weiss et al., 1997; Gangwar et al., 2008). Thus, the incidence of mastitis in cows was high in Madhya Pradesh (Mourya et al., 2020).The present study was therefore, planned to suggest an appropriate area specific micronutrient supplementation for improving udder health and milk production to increase the profit of farmers.

MATERIALS AND METHODS

Formulation of area specific micronutrient supplement

Micronutrient availability to the Malvi cows was calculated and compared with the requirements given by NRC (2001) to find out the deficiency or excess of micronutrients. For the preparation of micronutrient supplement, copper sulfate (Cu), zinc sulphate (Zn), and vitamins A and E (commercial preparations) were used. The measured quantity of trace mineral and vitamin was supplemented in the ration of cows of treatment group daily for 120 days (2 months during advanced pregnancy and thereafter for 2 months post-calving).

Eighteen multiparous healthy advanced pregnant Malvi cows (around last 2 months of gestation), identical in body weights (322±8.57kg) and feeding conditions, were selected randomly from a dairy farm in the village Rau and Indore (Madhya Pradesh, India). Body weight was determined based on body length and girth by using Schaeffer's equation (Sastry et al., 1982). Feed samples collected at the start of the experiment were analyzed for dry matter (DM) and trace minerals (Ca, P, Mn, Zn, Cu, Co and Se) with the calculation of availability of carotene and vitamin E using reported values. Trace minerals were estimated by atomic absorption spectrophotometer (AAS Plus; Motras Scientific).

The selected animals were divided into two groups, control and treatment, including nine Malvi cows in each group based on their body weights. The control group was fed as per the practice of farmer, while the ration of cows of the treatment group was supplemented with a micronutrient supplement. This supplementation study was conducted for 2 months during advanced pregnancy and continued for 2 months post-calving. Feed samples collected during the supplementation study were fortnightly analyzed for DM (AOAC, 2005). The average daily milk yield (kg/day) of individual cows was recorded Milk fat, protein, solid not fat (SNF), lactose and total solid were analyzed by Indiz milk analyzer (Smart 305). Udder health indices; pH (Model pHep-HI 70300), Somatic cell count (Dang and Anand, 2007) and Modified California Mastitis Test (MCMT) (Sastry, 1978) were measured fortnightly. The statistical analysis was carried out using statistical package SPSS (20.0).

Table 1. Average minerals and vitamins content of feedstuffs (On DM basis)

Feedstuffs	Ca (%)	P (%)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Co (ppm)	Se (ppm)	Carotene (ppm)	Vit. E (IU/kg)
Wheat straw	0.37±0.01	0.06±0.01	464.75±0.17	9.37±0.01	8.41±0.10	31.00±1.08	0.11±0.01	0.27±0.02	1.0	0.7
Masoor straw	1.00±0.02	0.06±0.01	621.42±0.75	6.54±0.03	23.53±0.14	39.87±1.27	0.36±0.01	0.21±0.03	1.0	0.9
Cotton seed cake	0.39±0.01	0.38±0.03	216.44±0.86	8.26±0.01	42.52±0.03	25.53±1.40	0.39±0.02	0.45±0.01	0.2	12
Wheat bran	0.17±0.02	0.48±0.02	251±0.95	8.63±0.02	67.51±0.02	131.90±1.56	0.12±0.01	0.12±0.05	0.1	21
Concentrate mixture	0.76±0.01	0.41±0.03	882.21±0.64	20±0.57	34.16±0.54	102.59±0.85	0.88±0.02	0.13±0.01	Trace	Trace
Maize fodder	0.27±0.03	0.21±0.01	294.5±0.85	8.37±0.01	9.84±0.01	190.33±1.29	0.14±0.01	2.0±0.02	285	80-200

RESULTS AND DISCUSSION

Average minerals and vitamins content of feedstuffs

The mineral content of the feedstuffs was given in Table 1 which formed the basis for calculation of availability of minerals in different feedstuffs.

Body weight and dry matter intake of advanced pregnant Malvi cows

The average BW of 18 advanced pregnant cows was 317.95±5.93 kg. The daily DM intakes in those cows from dry roughage, maize fodder, cotton seed cake, wheat bran and concentrate mixture were 5.28±0.05, 0.43±0.15, 0.67±0.23, 0.91±0.16, 0.95±0.16 kg, respectively, with total DM intake of 8.23±0.08 kg/day.

Availability and status of micronutrients

The availability of individual micronutrients was calculated on DM basis. Average availability of Ca, P, Fe, Cu, Zn, Mn, Co and Se is 54.31±4.09 and 14.86±0.29 g, 4322±199.44 mg, 75.40±0.63 mg 224.33±14.34, 511±12.76 mg, 2.73±0.21 mg, 2.60±0.40 mg, respectively. It indicated the shortage of Zn (63.5 %) and Cu (2.83 %) as per NRC (2001) requirements while an excess of Se (37.56 %) and other minerals like Ca, P, Mn, Fe, and Co was observed. Likewise, the average availability of vitamin A and E were 50736±43.19 and 113.13±24.79 IU/day, respectively in prepartum cows, which tunes up with the shortage of 74.6 % and 81.2 %, respectively, for vitamin A and E.

Thakur et al. (2016) observed a shortage of Cu and Zn by 2% and 65%, respectively, in the ration of advanced pregnant buffaloes from the Malwa region of Madhya Pradesh. The average daily supply of carotene and vitamin E in animals also remained deficient by 88.4% and 95.7%. While Jain et al. (2012) reported a 54% deficiency of vitamin A, Thakur et al. (2016) observed shortage of vitamin A and vitamin E by 45% and 80%, respectively, in the ration of advanced pregnant buffaloes of Malwa region of Madhya Pradesh. Variation in feeding practices due to the local availability of different feed ingredients remained a major cause of variation in nutrient intake.

Designing of area specific micronutrient supplement

Micronutrient supplement developed to supply the deficient levels of antioxidant micronutrients includes 1069 mg of zinc sulphate, 8.64 mg of copper sulphate, 2986, IU of vitamin A, and 8626 IU of vitamin E. The level of other minerals estimated in feeds was not deficient, hence avoided to include in the micronutrient supplement.

Effect of area specific micronutrient supplementation on dry matter intake of Mavi cow

No significant difference was observed in DMI during pre- and post-parturient periods between two groups (Table 2) may be due to otherwise nutritionally appropriate ration of the control group, except micronutrients, while other studies indicated increased DMI in animals supplemented with micronutrients (Saini et al., 2010; Righi, 2016). In corroboration to our study, Dhami, (2022); Singh et al. (2021) also reported that the dietary supplementation of micronutrients did not have any significant effect on voluntary feed intake of animals.

Effect of area specific micronutrient supplementation on milk yield and its composition

In the present experiment, overall milk yield was higher ($P<0.05$) in supplemented group as compared with the un-supplemented group (3.41±0.04 kg/head/day) with improved milk production of 0.56 kg/head daily (Table 2). Results of milk yield clearly indicate that micronutrient supplement has potential to increase ($P<0.05$) milk yield by 16.42%. The overall milk (%) fat, protein, lactose, solid not fat, total solids were comparable and statistically non-significant ($P>0.05$) between the groups. Tiwari et al. (2012) also recorded around 15.32 to 18.80% increase in milk production in cows fed strategic dietary mineral mixture supplementation in cattle and buffaloes under field condition. Kantwa et al. (2021) also reported a 15.82 % increase in milk yield in buffaloes fed chelated mineral mixture @ 40 g/buffalo/day till 90 days of early lactation period. Similar results were also reported by Sahoo et al. (2017), Singh et al. (2020) and Jadoun et al. (2024).

As per the composition of milk is concerned, no significant difference in milk SNF and lactose was reported due to supplementation of either mineral or vitamin in dairy animals by Singh et al. (2021), However, they observed significantly higher milk fat and protein % in supplemented group. Similar to the present study, no change in milk composition by supplementation of minerals was also reported by Gowda et al. (2004).

The present results indicating that supplementing of area specific micronutrients could increase milk yield of cows due to having impact on the milk production cells in the udder. Their micro and macro element contribute in the working of memory cell to enhance their production (Pal et al., 2020).

Table 2. Effect of micronutrient supplementation on dry matter intake, milk yield (kg/day) Milk pH, Somatic cell count and milk composition (%) of Malvi cow

Constituent	Control	Treatment	P-value
DM intake, kg/d	8.38±0.05	8.80±0.15	0.080
Milk yield, kg/d	3.41±0.04	3.97±0.03	0.04*
Milk pH	6.85±0.01	6.53±0.03	0.012*
SCC (10^5/ml)	3.25±0.13	2.30±0.07	0.02*
Milk composition (%)			
Fat	3.18±0.03	4.02±0.027	0.717
Lactose	4.46±0.02	4.56±0.02	0.263
SNF	8.42±0.03	8.47±0.03	0.571
Total protein	3.48±0.02	3.56±0.03	0.492
Total solid	12.25±0.07	12.21±0.05	0.36

SCC-Somatic cell counts; SNF- Solid not fat
*Show significance at 5% level as compared to control group (P<0.05)

Effect of area specific micronutrient supplementation on udder health indices

The overall milk pH was significantly (P < 0.05) higher in the supplemented group (6.85 vs. 6.53) in comparison with the un-supplemented group. The MCMT was positive in 6 cows out of 9 cows of the un-supplemented group, while due to supplementation of areas specific micronutrients, it reached up to a level of only 1 case out of total 9 cows in the treatment group. SCC was significantly (P<0.05) lower in cows of the supplemented group than un-supplemented cows, and the numerically same was maintained throughout the study period.

The increased milk pH in the subclinical and clinical mastitis than the normal cases might be a consequence of increased permeability of the blood capillaries during inflammation of the mammary gland, which allows alkaline blood constituents (sodium and bicarbonate ions) to enter the milk and consequently to increase milk pH (Luck and Smith, 1975). The milk pH was significantly (P < 0.05) higher in the unsupplemented group in comparison with supplemented group it indicates reduction in subclinical mastitis in strategic micronutrient supplemented group (Singh et al., 2020), which are in agreement with the present findings. Similarly, Khan et al. (2022) also reported that supplementation of selenium and vitamins E decreased incidence of bovine mastitis by positively impact on immunoregulation and relieve the oxidative and inflammatory status in dairy cattle during the Peri-parturient phase.

Somatic cell count is usually used as an inflammatory indicator to diagnose mastitis. A decrease in SCC count in milk is an indicator of the success of management and hygienic control programme. In the present study, lower SCC in the supplemented group also highlighted the importance of supplementation in the treatment group. A potential factor contributing to the reduction in SCC is the role of Zn keratin formation; the keratin lining of the teat canal entraps bacteria and prevents their upward movement into the mammary gland. High SCC in mastitis-affected milk may adversely affect the quality of fresh and pasteurized liquid milk and reduced its shelf life (Sobhanirad et al. 2010). Zinc may affect mammary gland health status. Micronutrients are part of proteins with specific functions at the immune system level. Zinc is usually bound to metallothionein, whose effects are related to the proliferation, adherence and invasivity of macrophages. Zinc blood concentrations often decrease around calving and when animals go through mastitis. Moreover, animals' requirements vary, and supplementation with zinc around the peripartum period can boost immunity and prevent mastitis.

Considering the immune system and mastitis microorganisms, copper is believed to exhibit antibacterial properties against bacteria isolated from mastitic cows. According to Reyes-Jara et al. (2016) Cu concentration as low as 250 ppm inhibits the growth of common mastitis microorganisms such as Escherichia coli and coagulase negative Staphylococci. Previous researchers also reported a reduction in SCC supplemented with trace minerals, vitamin E, and vitamin A (Nevidita et al., 2017; Maurya et al., 2014).

CONCLUSION

It may be concluded that the ration of advanced pregnant Malvi cows was deficient in copper and zinc; vitamin A and vitamin E. The supplementation of these micronutrients in the ration of cows improved udder health, milk yield without affecting milk quality.

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Evaluation of Poultry Droppings Based Concentrate mixtures For Ruminants by *In Vitro* Method

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ABSTRACT

The study was planned to ascertain the effect of inclusion of dried poultry droppings (PD) at graded levels on chemical composition, *in vitro* utilization and degradability of concentrate mixtures. Dried poultry Droppings were added @ 0,5,10,15,20 and 25% in various concentrate mixtures. *In vitro* utilization of concentrates having dried poultry droppings (PD)@ 10 &20% showed a significant increase (P<0.05) in Net gas production (NGP), Organic matter digestibility (OMD%), Dry matter digestibility (DMD%), Metabolisable energy (ME), Neutral detergent digestibility (NDFD %), Microbial mass Production (MMP) and partition Factor (PF). At 72 h incubation time, cumulative gas production (ml) in 5% poultry droppings-based concentrates was signiûcantly (P < 0.05) lower than those of 15 and 20 % poultry droppings based concentrates. The maximum rate of degradation (k) was observed in control concentrate (9.16%/h) and lowest in 25% poultry droppings based concentrate (7.46%/h). The value of " b" (gas production from insoluble fraction was lowest in 5% poultry droppings based concentrate (30.92 ml) and was highest in 15% poultry droppings concentrate (39.29 ml). and in control (39.5). On the basis of above results it can be concluded that dried poultry droppings can be included upto 20% in concentrate mixtures for cost-efficient livestock production.

KEYWORDS: Concentrate mixture, *In vitro*, Paddy straw, Poultry droppings,

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INTRODUCTION

In India, the poultry farming system generates a substantial amount of waste, with 38.33 million MT of poultry manure, 9486 MT of hatchery waste, and 1.74 million MT of slaughter house wastes produced during the 2018-2019 period alone (Prabakaran and Valavan, 2021). Large-scale accumulation of wastes such as manure in the poultry industry in the absence of suitable environmentally and economically sustainable management technologies, may leads to environmental pollution (Bolan et al.,2010). Animal excreta can be a valuable resources if used judiciously. The waste has the greatest economic value when used as animal feed (Fontenot and Hancock, 2001). Poultry litter waste contains various amounts of nutrients and these nutrients can be affected animals' health. However, dried poultry manure waste contains a substantial amount of digestible energy, crude protein, crude fat, crude fiber, cobalt, iodine, and other nutrients that enable ruminants to utilize the urea nitrogen of poultry and convert it into production (Ghaly and Macdonald, 2012) Consequently, poultry manure waste is readily available at a lower cost than other sources of feed offered to livestock, making it an attractive option for livestock farmers. Paul et al., (1993) reported that poultry manure contains a wide array of minerals such as phosphorous (0.56 - 3.92%), potassium (0.73 - 5.17) and calcium (0.81 - 6.13). Others elements such as magnesium (Mg), sulphur (S), manganese (Mn), copper (Cu), zinc (Zn), chlorine (Cl), boron (B), iron (Fe), and molybdenum (Mo) may also be present in the poultry manure. Poultry droppings can be a cost-effective feed for beef cattle ration. Beef producers are mainly worried about the safety of feeding huge amounts of poultry droppings to beef cattle and are susceptible to pathogenic microorganism infection (i.e., *Salmonella typhimurium*). Literature suggests that when poultry droppings dried, there is a possibility of fewer pathogens in it (Martin et al., 1998). The inclusion of caged poultry droppings in the diet may offer potential cost savings, contributing to the economic viability of buffalo farming and small ruminants (Ramaniah et al., 1998).The utilization of poultry droppings as feed in growing buffalo calves holds promise as a sustainable and environment-friendly feeding practice. Hence, the current study was planned to see its effect on *in vitro* utilization of varying levels of poultry droppings based concentrates.

MATERIALS AND METHODS

The current research was conducted at the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The various concentrate mixtures were prepared by adding different graded levels of dried poultry droppings as given in Table 1.

Table 1. Composition of Concentrate Mixture (kg)

Parameters	0%	5%	10%	15%	20%	25%
Maize	34.2	35.2	34.7	34.7	35	33.7
Rice Kani	5	5	5	5	5	5
Mustard Cake	22	21	23	23	23	25
Soybean meal	5	5	5	5	4	0
Wheat Bran	8	8	2	2	2	2
Rice Polish	10	10	10	10	5.1	3
DORB	10	5	5	0	0	0
By Pass Fat	1	1	1	1	1.6	2
Mineral Mixture	2	2	2	2	2	2
Salt	1	1	1	1	1	1
Urea	1.5	1.5	1	1	1	1
Poultry droppings	0	5	10	15	20	25
Additives*	0.3	0.3	0.3	0.3	0.3	0.3

*yea sac, toxin binder and buffer @0.1 gm as Additives

Chemical Analysis

Proximate principles like crude protein, ether extract were determined as per procedures set by Association of Official Analytical Chemists (AOAC, 2007). Cell wall constituents like NDF and ADF were determined (Robertson and Van Soest. 1991). The difference between NDF, ADF and ADL was used to compute the hemicellulose and cellulose concentrations.

In vitro evaluation

The *in vitro* gas production analysis was done according to the method devised by Menke and Steingass (1988). After 24 hours, volume of gas produced in each treatment was determined. The following solutions were employed and incubated in a waterbath at 39°C: macro mineral solution, buffer solution, micro mineral solution, resazurine, reducing solution, and rumen fluid obtained from a male fistulated animal. The amount of gas produced after 24 hours was measured in each syringe. The calculation of ME is based on the gas output was the partition factor (PF) calculated as the ratio of the substrate's true in vitro degradation weight (mg) to the gas volume (ml) it produced, For digestion kinetics, incubation was carried out at 39°C and gas production was observed at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 hours after incubation. Gas production kinetics was calculated by Neway Program software.

Statistical Analysis

Data was analysed by Snedecor and Cochran (1994) by using SPSS version 2019. The differences in means were tested by tukey B and Duncan. The computer program Graph Pad prism 2007 was used to calculate the rate and extent of gas production in the non linear equation.

RESULTS AND DISCUSSION

Chemical composition of Concentrate mixture with graded levels of poultry droppings

Different concentrate mixtures (CM) were prepared using varying levels of poultry droppings (0, 5, 10, 15, 20 and 25%). The chemical composition of the concentrate mixture is given in Table 2. The CP content of control CM and poultry droppings varied from 25.04% to 25.75%. All the concentrate mixtures prepared were isonitrogenous in nature. The NDF content varied from 29.90 % to 38.43 %. The fat content of CM was between 5.10% to 6.25 %. The ash content in control concentrate was 7.85 %while in poultry droppings incorporated concentrates it varied from 7.88 to 12.60 % and OM varied from 87.40% to 92.12% in poultry dropping incorporated CM and in control concentrate it was 92.15%. The ADL content varied from 3.5 to 4.6 % in poultry dropping incorporated concentrates while in control it was 5.2 %.

Table 2. Chemical composition of Concentrate mixture having graded levels of poultry droppings (%)

Parameters	Concentrate Mixtures Level of Poultry droppings					
	0%	5%	10%	15%	20%	25%
DM %	92.0	91.0	92.0	92.0	92.0	91.5
CP %	25.04	25.3	25.1	25.75	25.51	25.6
EE %	5.10	6.00	6.25	6.05	6.10	6.2
Ash %	7.85	7.88	9.65	10.42	11.37	12.6
OM %	92.15	92.1	90.35	89.6	88.63	87.4
NDF %	29.9	33.5	36.8	38.4	37.4	38.4
ADF %	19.2	18	15.8	15.7	14.1	13.7
ADL %	5.12	4.6	4.5	3.8	3.9	3.5
Hemicellulose %	10.7	15.5	21.00	22.73	16.3	14.7

Effect of different levels of poultry dropping on *in vitro* utilization of nutrients of concentrate mixture

Evaluation of *in vitro* utilization (Table 3) showed that treatments with varied levels of poultry droppings (PD) groups showed non-significant differences among them with NGP values varying from 64.74 to 67.75 ml and NGP varying from 172.66 to 188 ml/g DM.

The net gas production (ml/gDM) during 24 hr was statistically comparable in Concentrate mixtures (CM) CM 0, CM 5,CM 10 and CM 25% with poultry droppings (PD). It varied from 176.66 ml to 180.66 ml. The Net gas production (NGP, /24 h) also followed the same trend. The net gas production during 24 h was significantly lower in Concentrate mixture 15 and was observed highest in CM 20 % with poultry droppings. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher. The partition factor calculated *in vitro* provides useful information for predicting the dry matter intake, microbial mass production in the rumen and the methane emission of the whole ruminant animal. In this study, PF value (mg/ml) was significantly ($P<0.05$) higher in CM10 and lowest in CM 25 % with poultry droppings However ,it was statistically comparable in other CM with poultry droppings.. The OMD % was significantly higher in concentrate mixture 20% (86.88%) followed by CM 10 % and CM 25% (84.20%) with PD. The NDFD % was significantly lower ($P<0.05$) in CM 0 (40.24%) and highest in CM 10 (61.45%) followed by CM 20% with poultry droppings. The dry matter digestibility (DMD, %) was significantly higher in concentrate mixture 20 (86.66%) followed by CM 25 % (84.26%) with poultry droppings (PD). The metabolizable energy (ME, MJ/kgDM) was significantly lower in CM 0 (9.60) and significantly higher in CM 20 (10.31)followed by CM 25 % (9.95) with poultry droppings. A significant lower ($P<0.05$) microbial mass production (MMP, mg) was observed in concentrate mixture 25 (124.76) and was found significantly ($P<0.05$) higher in concentrate mixture 20 % (139.00) with poultry droppings. The efficiency of microbial mass production (EMMP,%) was significantly (P<0.05) lower in CM 25 (44.57%) and was significantly higher in CM 20%(48.81%) with PD. The Short chain fatty acids (SCFA, mmole) was significantly found lower in CON 15% (0.762) and was higher in CON 20% (0.780) with poultry droppings .

Table 3. Effect of different levels of poultry dropping on *in vitro* utilization of nutrients of concentrate mixtures

Parameters	Concentrate mixtures						SEM	p-Value
	Level of poultry droppings							
	0%	5%	10%	15%	20%	25%		
NGP, ml/24hr	67.75^{ab}	66.75^{ab}	66.75^{ab}	64.75^{a}	70.50^{b}	66.25^{ab}	0.658	0.181
NGP,ml/g DM	180.66^{ab}	178.00^{ab}	180.66^{ab}	172.66^{a}	188.0^{b}	176.66^{ab}	1.75	0.181
TDS, mg	345.48	345.56	338.81	335.92	332.36	327.75	1.96	-
OMD, mg	278.48	279.56	277.81	280.42	279.86	284.75	0.801	0.116
PF, mg/ml	4.11^{ab}	4.18^{ab}	4.33^{b}	4.10^{ab}	4.29^{ab}	3.97^{a}	0.043	0.107
OMD, %	80.60^{a}	80.90^{a}	83.47^{b}	81.99^{a}	86.88^{c}	84.20^{b}	0.66	0.00
NDFD, %	40.24^{a}	47.46^{b}	61.45^{e}	55.79^{cd}	59.04^{de}	53.94^{c}	2.19	0.00
MMP, mg	129.43^{ab}	132.71^{ab}	137.97^{b}	128.76^{ab}	139.00^{b}	124.76^{a}	1.76	0.073
EMMP, %	46.47^{ab}	47.47^{ab}	49.19^{b}	46.35^{ab}	48.81^{b}	44.57^{a}	0.56	0.109
ME, J/kgDM	9.60^{a}	9.74^{a}	9.88^{a}	9.61^{a}	10.31^{b}	9.95^{a}	0.07	0.00
DMD, %	80.80^{a}	80.26^{a}	83.46^{bc}	82.40^{b}	86.66^{d}	84.26^{c}	0.65	0.018
SCFA, mmole	0.797^{ab}	0.786^{ab}	0.797^{ab}	0.762^{a}	0.830^{b}	0.780^{ab}	0.0077	0.181

Means bearing different superscripts in a row differ significantly (P<0.05).

Estimated parameters of concentrates mixture containing varying levels of poultry droppings

Cumulative gas production profiles from the *in vitro* fermentation of concentrates containing graded levels of poultry droppings (PD) are shown in Figure 1 and the estimated parameters are given in Table 4 . The cumulative volume of gas production increased with increasing time of incubation. The gas produced after 72 h incubation ranged from 28.00 to 34.00 ml per 0.200 g of dry matter. At 72 h incubation , cumulative gas productions (ml) with 5% CM incorporated poultry droppings (PD) has significantly (P < 0.05) lower than those of 15 and 20 % CM incorporated with poultry droppings(PD). However, in all poultry droppings-based concentrates the cumulative gas production at 72 h is statistically comparable (CM 10 to CM 25%) with PD . The gas production in all the concentrates (CM) at 24 h has differed statistically significantly. The gas production was statistically significantly lower in CM 5% with poultry dropping and significantly higher in CM 0 %.(control)

Table 4. *In vitro* gas production from concentrate mixtures when incubated at different time intervals

Treatment	Concentrate mixtures						SEM	p-value
	Level of poultry droppings							
	0%	5%	10%	15%	20%	25%		
2 Hrs	1.75	1.25	0.75	1	1.25	1.25	0.114	0.194
4 Hrs	3.5^{ab}	3.00^{ab}	2.50^{a}	3.25^{ab}	3.50^{ab}	4.25^{b}	0.177	0.031
6 Hrs	9.5	7.0	6.75	8.75	9.25	7.0	0.396	0.064
8 Hrs	15.5^{b}	11.5^{a}	12.0^{ab}	14.0^{ab}	14.2^{ab}	11.0^{a}	0.534	0.018
10 Hrs	19.2^{b}	14.0^{a}	16.0^{ab}	18.0^{ab}	17.7^{ab}	14.5^{a}	0.630	0.025
12 Hrs	22.0^{c}	16.2^{a}	18.0^{ab}	20.7^{bc}	20.5^{bc}	17.0^{a}	0.668	0.004
24 Hrs	29.7^{c}	23.0^{a}	26.2^{abc}	29.25^{c}	28.7^{bc}	24.5^{ab}	0.806	0.007
36 Hrs	31.0^{c}	23.7^{a}	27.5^{abc}	29.5^{bc}	29.5^{bc}	25.5^{ab}	0.796	0.005
48 Hrs	33.75^{b}	26.75^{a}	30.25^{ab}	33.00^{ab}	32.25^{ab}	28.50^{ab}	0.838	0.035
60 Hrs	34.75^{b}	28.00^{a}	31.50^{ab}	34.00^{ab}	33.50^{ab}	30.00^{ab}	0.803	0.042
72 Hrs	34.75^{b}	28.00^{a}	31.50^{ab}	34.00^{ab}	33.50^{ab}	30.00^{ab}	0.803	0.042

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

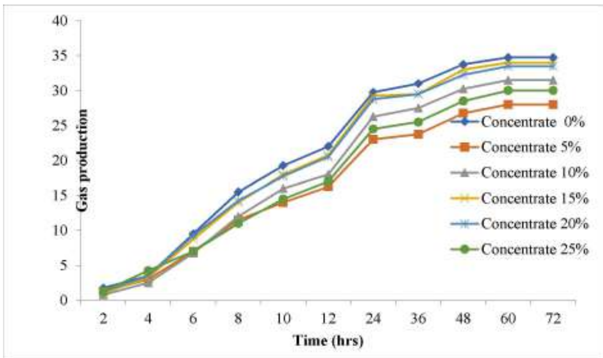

Fig 1. Estimation of gas production in concentrate mixtures when incubated at different time intervals

The maximum rate of degradation (k) was observed in control concentrate (CM) (9.16%/h) and lowest in 25% poultry droppings based concentrate (7.46%/h). The value of 'a' was found lowest in 5% CM (3.40) and highest in 15% CM incorporated with poultry droppings (5.81).(Table 5)

If 'a' is positive, then there is a component which is degraded rapidly and/or a component which is soluble. When a negative value for 'a' is obtained this means that there has to be an initiation period for degradation to start (termed the lag phase)

The value of "b" (gas production from insoluble fraction was lowest in 5% CM incorporated with poultry droppings(PD) (30.92 ml) and was highest in 15% poultry droppings incorporated concentrate mixture (CM) (39.29 ml). and in control (39.5ml). No significant difference was observed in lag time in control and poultry droppings concentrates mixtures.

Table 5. Estimated parameters of Concentrate mixtures of containing varying levels of poultry droppings

Parameters	Treatments						P value
	Con 0%	Con 5%	Con 10%	Con 15%	Con 20%	Con 25%	
a	-5.34^{bc}	-3.40^{a}	-5.25^{bc}	-5.81^{c}	-5.27^{bc}	-3.71^{ab}	0.0057
b	39.58^{a}	30.9^{c}	36.42^{ab}	39.3^{a}	38.23^{ab}	33.38^{bc}	0.0037
c	0.0916^{a}	0.0789^{bc}	0.0804^{bc}	0.0833^{ab}	0.0887^{ab}	0.0746^{c}	0.0046
a + b	34.2^{a}	27.5^{b}	31.18^{ab}	33.4^{ab}	32.9^{ab}	29.67^{ab}	0.0366
Lag time	1.60^{a}	1.50^{a}	1.95^{a}	1.85^{a}	1.65^{a}	1.60^{a}	0.2806
RSD	1.18^{a}	1.04^{a}	1.17^{a}	1.25^{a}	1.04^{a}	1.00^{a}	0.2904
ED	20.4^{a}	15.6^{b}	17.45^{ab}	19.5^{ab}	19.4^{ab}	16.40^{b}	0.0154

c = gas production rate, a = gas production (ml) from quickly soluble fraction, b = gas production (ml) from insoluble fraction, (a + b) = potential gas production

CONCLUSION

On the basis of above results it can be concluded that dried poultry droppings can be included upto 20% in concentrate mixtures for cost-efficient livestock production.

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Effect of Herbal Feed Additives and Sulfate Supplementation on Hematology, Biochemical and Antioxidant Status of Calves

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ABSTRACT

The present study was undertaken to evaluate the effect of herbal feed additives and sulfate supplementation on the hematology, biochemical, antioxidant status and immune response of growing cattle calves. Twenty-four growing cattle calves (12 male Haryana, 8 male Sahiwal, 4 female Sahiwal calves) of 4 to 16 months of age were distributed into four groups with six animals each in a randomized block design. Control (C) group was fed on basal diet without any feed additive, T1 group was fed on basal diet with herbal feed additive (Fennel seed + Clove oleoresin; CLO; @ 1.0% of DMI), T2 group was supplemented with sulfate @ 0.075% of DMI, and group T3 was fed on basal diet with herbal feed additive (Fennel seed + Clove Oleoresin (CLO); @ 1.0% of DMI) and sulfate @ 0.075% of DMI. The basal diet consists of 40% concentrate and 60% wheat straw. Body weight and dry matter intake were recorded fortnightly. Overall body weight, DMI (kg/day), TDN intake (g/kg $W^{0.75}$), and DCP intake (g/kg $W^{0.75}$) were similar in the treatment as well as control group. Hematological parameters like blood hemoglobin concentration and PCV were not impacted by herbal feed additive and sulfate supplementation. Plasmaglucose, triacylglycerol, and cholesterol concentration were found non-significant ($P>0.05$), plasma total protein, Plasma albumin, plasma globulin and PUN concentration were also not affected ($P>0.05$). Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), plasma bilirubin, plasma creatinine were found normal in range and not significantly different ($P>0.05$) between the groups. Total immunoglobulin level and antioxidant activity remain similar in all the groups ($P>0.05$).It may be concluded that supplementation of herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) may be used as rumen modifiers without any adverse effect in the calves.

KEYWORDS: Antioxidant, Calves, Herbal feed additives, Immunity, Sulfate

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INTRODUCTION

In recent years research on plant secondary metabolites (tannin, saponin, essential oils, etc.) has gained interest in animal nutrition due to their beneficial effect on rumen fermentation and methane mitigation. Plant secondary metabolites can be a rational approach to modulating the rumen microbiome and modifying its function. There is immense potential to manipulate the fate of metabolic hydrogen in the rumen, away from methane synthesis toward propionic acid formation, by feeding plants containing secondary metabolites to ruminants (Dey et al., 2016). Methanogenes is also decreases the efficiency of feed utilization in the animals, as 2-12% of the gross energy of feed taken by the animals is wasted in the form of methane (Johnson et al., 1993) leading to great economic losses for the livestock sector. So, the inhibition of methanogenesis in the rumen may result in enhancing the feed conversion efficiency by diversion of hydrogen for propionate synthesis or by reduction of carbon dioxide to acetate by reductive acetogenesis. Reduction of genera such as *Methanobrevibacter* and *Acetobacter* are potential targets for the reduction of CH_4, on the other hand, an increase in the abundance of the genera *Methanosphaera* and *Eubacterium* led to reductions in CH_4 emissions in heifers (Cunha et al., 2019). A mixture of rumen modifiers (RM-7; neem seed cake, mahua seed cake, Fennel seed seed, harad, fruit pulp of bahera, fruit pulp of amla and ajwain seed) in 2:2:2:1:1:1:1 proportion at 5, 10, 15 and 20% with 0.06% sodium sulphate of substrate did not affect total gas production but there was significant reduction in methane production upto 10% level (Lakhani et al. 2019a). Bakshi et al. (2022) also reported that herbal feed additive supplementation reduces methane emission.

In the rumen, sulfate reducing bacteria (SRB) are present which have a higher affinity towards hydrogen moiety than methanogens. Thermodynamically, the reduction of sulfate (“G°= -152 KJ/mole) is more favorable than the reduction of carbon dioxide (“G°= -130 KJ/mole) (Oremland and Taylor, 1978). The threshold values of H_2 (mmol/liter) for SRBs and methanogens are 0.0013 and 0.067, respectively. The SRBs reduce sulfate into hydrogen sulfide by using ruminal hydrogen. The hydrogen sulfide produced by these bacteria can be used by most of the other microbes which cannot use sulfate directly. Sulphide is also involved in the synthesis of S-containing amino acids, therefore, inclusion of sulphate in the diet improves the growth of rumen microbes, hence, the performance of the animals.

Essential oils have shown some antioxidant activity, which may reduce the lipid peroxidation of meat, thus enhancing the tenderness of meat (Aminzare et al., 2019). Kumar (2017) demonstrated the increased antioxidant status of buffalo calves supplemented with a mixture of essential oil rich poplar and eucalyptus leaves.Clove oleoresin, a concentrated extract from clove buds, primarily consists of essential oil eugenol. Fennel (*Foeniculum vulgare*) is a plant rich in volatile components, mainly estragole, eucalyptol and estragole, limonene, trans-anethole, á-pinene, fenchone, and fenchol (Moosavi-Zadeh et al., 2023). Recently, Moosavi-Zadeh et al. (2023) fed Holstein dairy cows on a diet supplemented with Fennel seed@ 25 or 50 g/d for 45 d and observed increased feed intake and milk yield and decreased ruminal acetate proportion.The supplementation of amla fruit powder (*Emblica officinalis*) @ 0.75% enhanced the overall performance of broilers (Gaur et al., 2023).Kumar (2022) reported that the hemoglobin (Hb), packed cell volume (PCV), serum protein (albumin, globulin, and A:G ratio), and serum enzyme (ALT and AST) levels did not differ ($p > 0.05$) in experimental buffalo calves, fed with herbal feed additives. Bombik et al.(2012) studied the effect of herbal extracts on some haematological parameters of calves during rearing and found a significantly higher mean haemoglobin concentration in the treatment groups. Therefore, the present study was conducted to analyze the effect of supplementation of herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) as rumen modifiers on hematology, biochemical, antioxidant status, and immune response of calves.

MATERIALS AND METHODS

Ethics approval

Animal care procedures were approved (approval number IAEC/22/2/18) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per article number 13 of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

Selection of experimental animals, dietary treatment, and experimental design

Twenty-four growing calves of 4 to 16 months (12 male Hariana calves, 8 male Sahiwal calves, and 4 female Sahiwal calves) were selected from the herd maintained at Livestock Farm Complex (LFC), DUVASU Mathura (Uttar Pradesh), India. Calves were divided into four groups (six animals each) in a randomized block design. The four groups of calves were given different treatments. Control (C) group was fed on basal diet without any supplementation, T1group was supplemented with herbal feed additive (Fennel seed + Clove Oleoresin (CLO); @ 1.0% of DMI), T2 groups were supplemented with sulfate @ 0.075% of DMI(sulfate as Sodium sulfate, Na_2SO_4 contains 67% sulphate; Purity-98%), and group T3 were supplemented with diet containing herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) and sulfate @ 0.075% of DMI along with basal diet. The Fennel seed contains tannins and essential oils, and Clove Oleoresin contains essential oils. The basal diet contains 40% concentrate and 60% wheat straw.The chemical composition of the concentrate mixture is given in Tables 1 and 2.The experiment was continued for 120 days where all the calves were managed under similar conditions.

Table1. Composition (% DM basis) of diet fed to calves during the feeding trial

Ingredients			Dietary Group (% DM)			
			C	T1	T2	T3
Wheat straw			60	60	60	60
Concentrate	Ingredients	(in Kg)				
	Maize grain	15	40	40	40	40
	Barley grain	15				
	wheat Bran	33				
	Mustard Cake	35				
	Mineral	2				

Table 2. Chemical composition (% or as mentioned) of diet fed during the experimental period

Attribute (%)	Concentrate	Wheat straw
Dry matter	90.0	92.0
Crude protein	20.2	3.20
Ether extract	6.24	1.31
Total ash	9.25	13.2
Crude fiber	16.1	38.9
Neutral detergent fiber	33.1	82.2
Acid detergent fiber	18.1	49.6
Ca %	1.43	0.38
P %	0.39	0.17

Blood sample collection and analysis

Blood samples were collected before feeding and watering of heifers at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60,and 120 dayspost feeding. A fraction of blood samples was used for analysis of Hb and HITor PCV value as per Sahli's and Wintrobe tube method, respectively. Remaining blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. Plasma samples were stored at -20 °C until further analysis of biomarkers of liver and kidney function (AST, ALT,ALP, bilirubin, and creatinine), biomarkers of protein metabolism (total protein, albumin, globulin, andPUN). The plasma concentration of AST, ALT, ALP, bilirubin, creatinine,total protein, albumin, and PUN was determined byan automated biochemical analyzer (BS-120Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co.Ltd., China) using Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). Plasmaglobulin concentration was determined by subtracting the albumin content from total protein content. Plasma TIg was measured by zinc turbidity method (Mc Ewan et. al., 1970). Total antioxidant status (TAS) was measured as FRAP assay described by Benzie and Strain (1999).

Statistical analyses

All statistical analyses were performed as per the standard method by using the SPSS computer package (SPSS Version 20.0, SPSS Inc, Chicago, USA). The data obtained were statistically analyzed by using one-way ANOVA procedures. Tukey's Honestly Significant Difference test was used to evaluate significant differences between means of treatments.For all statistical analyses, probability values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Total DMI (kg/day), CP intake (g/day), DCP intake (g/day), TDN intake (g/day)werefound similar (P>0.05) in all four, control (C1), T1, T2 andT3 groups. Average BW at starting of the study in (C),T1, T2 and T3 groups were 80.3, 80.3, 81.8, 80.3 kg and at the end of the experiment were 129, 127, 129, 127 kg, respectively. The overall BW (kg) was found to be similar in all the experimental groups.FCR also did not differ significantly (P>0.05) between the groups.

Haematological parameters

The blood haemoglobin concentration and packed cell volume percentage of herbal feed additives and sulfate supplemented calves was not differed significantly (Table 3). Similar to our study, Kumar et al. (2022) studied effects of supplementing phytogenic feed additives composed of a mixture of eucalyptus (*Eucalyptus citriodora)* and poplar (*Populus deltoides*) leaf-meal (EPLM) and found hemoglobin (Hb), packed cell volume (PCV), did not differ ($p > 0.05$) in experimental buffalo calves. Although Bombik et al. (2012) have found significantly higher mean haemoglobin concentration and blood cell count in the experimental calves fed with the herbal extracts. Haematological parameters have no significant effect in the present study, this may be due to the lower doses of herbs and sulfate in the treatment groups.

Table 3. Effect of herbal feed additives and sulfate supplementation on hematocrit value and blood biochemical parameters

Parameters	Control	T1	T2	T3	SEM	P value
Hb (g/dl)	9.13	8.90	9.38	8.71	0.113	0.365
PCV (%)	26.4	26.2	28.8	26.2	0.355	0.799
Glucose(mg/dl)	60.1	59.8	59.5	59.9	0.594	0.988
Triglyceride (mg/dl)	67.3	66.1	68.1	67.2	0.947	0.912
Cholesterol (mg/dl)	198	198	197	198	0.949	0.958
Total protein (g/L)	6.25	6.24	6.27	6.24	0.070	0.999
Albumin (g/L)	3.26	3.28	3.26	3.27	0.049	0.999
Globulin (g/L)	3.10	3.08	3.06	3.09	0.078	0.999

C: Control animals having no feed additives supplementation; T1: Treatment Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate; P>0.05: Non significant

Biomarker of energy, lipid, protein metabolism and liver, kidney function tests

The plasma glucose concentration, plasma triglycerides and plasma cholesterol concentration,plasma total protein, plasma albumin and plasma globulin, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, and bilirubin in control and treatment groups did not differ significantly (Table 3 and Table 4). Similar to present study, Kumar et al. (2022) studied effects of supplementing phytogenic feed additives composed of a mixture of eucalyptus (*Eucalyptus citriodora)* and poplar (*Populusdeltoides*) leaf-meal (EPLM) and found serum protein (albumin, globulin, and A:G ratio), and serum enzyme (ALT and AST) levels did not differ ($p > 0.05$) in experimental buffalo calves. Anantasook et al. (2014) found no change in the level of blood urea nitrogen by feeding rain tree pod meal containing tannins and saponins in growing dairy steers. Kumar et al. (2017) also reported no effect of on blood biochemicals and serum enzymes by feeding tea seed saponins. Lakhani et al. (2019) also reported no effect of on blood biochemicals and serum enzymes by feeding rumen modifier and sodium sulfate.The present study result is no any significant effect on liver and kidney function test and so both herbal and sulfate have no any adverse effect on these vital organs.

Table 4. Effect of herbal feed additives and sulfate supplementation on antioxidant status, liver and kidney function parameters

Parameters	Control	T1	T2	T3	SEM	P value
ALT (U/L)	22.2	22.3	22.1	22.1	0.155	0.953
AST (U/L)	72.9	72.6	72.8	72.8	0.178	0.966
ALP(U/L)	58.3	58.4	58.5	58.6	0.100	0.815
Bilirubin (mg/dl)	0.92	0.935	0.930	0.937	0.009	0.987
Creatinine (mg/dl)	0.910	0.905	0.909	0.905	0.004	0.974
PUN (mg/dl)	6.89	6.86	6.90	6.86	0.032	0.960

Biomarker of antioxidant status and immune response

Total antioxidant activity (TAA) was similar in the group supplemented with herbal feed additives with respect to control groups (Table 5). SOD and FRAP activity remain similar in all groups. Similar to the present study, Passetti et al. (2021) also reported that Adding EO blends (OEO or XEO) and an Emulsifier to barley-based diets of lambs did not change the serum blood oxidative status.Although study of Kumar (2017) demonstrated the increased antioxidant status of buffalo calves supplemented with a mixture of essential oil rich poplar and eucalyptus leaves with increased concentration of major antioxidant enzymes. Total immunoglobulin concentration in plasma was found higher over time period of experiment but no any significant difference among the groups. Similarly to our study, Lakhani et al. (2019b) reported that Supplementing mixture of neem seed cake, mahua seed cake, Fennel seed, harad, fruit pulp of bahera, fruit pulp of amla and ajwain seed in 2:2:2:1:1:1:1 proportion with sodium sulphate at 0.06% of dry matter intake improved immune status of the growing calves. Kumar et al., (2022) also reported that supplementation of phytogenic composite feed additive (EPLM), consisting of an equal proportion of eucalyptus (*Eucalyptus citriodora*) and poplar (*Populus deltoids*) leaves at both the dose levels (50 g and 150 g/h/d) to calves improved the immune response. The present study result is no any significant effect on marker of antioxidant status and immune response, it may be due to lower doses of herbs and sulfate in the treatment groups.

Table 5. Effect of herbal feed additives and sulfate supplementation on antioxidant status, liver and kidney function parameters

Parameters	Control	T1	T2	T3	SEM	P value
SOD (nmol MTT formazan/mg Hb)	381	364	368	344	13.3	0.809
FRAP (µmol/L)	940	983	934	974	21.73	0.822
Totalplasmaimmunoglobulin (mg/ml)	21.7	23.8	23.9	22.6	0.766	0.711

C: Control; T1: Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate.

Effect on plasma mineral levels

The plasma calcium and phosphorus level were numerically higher over time period of experiment but no any significant difference among the groups (Table 6). Similar to present findings, Yadav et al. (2017) reported that Calcium balance was higher in treatment groups but difference was non-significant but phosphorus level here is significantly varied among the groups. A key limitation of this study is the lack of homogeneity among the cattle calves, particularly in terms of breed, age, and sex background. These variations introduced substantial variability in the calves' responses to the nutritional treatments, which likely contributed to the lack of significant differences in the results.

Table 6. Effect of herbal feed additives and sulfate supplementation on plasma mineral profile

Parameters	Control	T1	T2	T3	SEM	P value
Ca (mg/dl)	9.77	9.94	9.97	10.07	0.110	0.816
P (mg/dl)	5.49	5.63	5.53	5.44	0.070	0.790

C: Control animals; T1: Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate.

CONCLUSIONS

The study examined the impact of herbal feed additives and sulfate on cattle calves' hematology, biochemical, and antioxidant status. Results showed no significant differences in blood hemoglobin concentration, packed cell volume percentage, or liver enzymes.Biomarkers of glucose, lipid, and protein indicated no adverse effect of treatment. The study found that all liver enzymes were within the normal physiological range, indicating no detrimental impact on the health of animals. Hence, it may be concluded that the herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) selected as rumen modifiers may be supplemented in the ration of the cattle calves without any adverse effect.

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Effect of Concentrate Mixture Containing Varying Levels of Crude Protein on Growth Performance, Serum Biochemical Profile of Punganur Calves

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ABSTRACT

To arrive the optimum CP content of concentrate mixture for Punganur calves based on their growth performance, nutrient digestibility and serum biochemical profile, a study was conducted in a completely randomized design using fifteen growing Punganur calves of 3-4 months of age with average body weight of 29.72±2.96 kg which were fed on Super napier grass *ad libitum* supplemented with concentrate mixture containing 14 (T1), 16 (T2) and 18% (T3) CP at 1% of live weight. The experiment lasted for 90 days. The total weight gain (kg); 20.80, 20.76 and 21.17 and average daily gain (g) 233.71, 233.26 and 237.95 in T1, T2 and T3, respectively were comparable among different treatments. There was no significant difference in total DMI (g),DMI/kg gain,nutrient digestibility, serum biochemical constituents among the treatment groups. The results of the current study indicated that the concentrate mixture with 14% CP on Super Napier based diet was optimum for Punganur calves.

KEYWORDS: Growth performance, Nutrient digestibility, Punganur calves

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INTRODUCTION

Conserving indigenous cattle breeds requires a clear understanding of their nutrient requirements. Punganur, a dwarf dual-purpose breed native to Chittoor, Andhra Pradesh, is known for its drought resistance, heat tolerance, and ability to thrive on dry fodder. With an average adult weight of 240 kg (males) and 170 kg (females), its milk yield ranges from 200 to 1100 kg per lactation, averaging 550 kg over 153 ± 24 days (Ekambaram et al., 2014).

Indigenous breeds like Punganur are expected to have superior fibre utilization compared to crossbreeds, which can be assessed through digestion trials. Nutritional factors influence key metabolites (glucose, BUN, total protein), impacting growth and metabolism (Todini et al., 2007; Acuti et al., 2009). While dietary protein is essential for growth, excessive intake can lead to increased nitrogen excretion, posing environmental concerns (Klemesrud et al., 2000). However, data on optimal protein levels for Punganur calves remain limited. This study investigates the effects of varying crude protein levels in concentrate mixtures on their growth and nutrient utilization.

MATERIALS AND METHODS

The experimental protocols that were developed in this study were fully complied with the ethical principles of animal experimentation prepared by Institutional animal ethic committee (IAEC) with reference number 281/ go/ S/ 2000/ CPCSEA/ CVSc/ TPTY/ 012/ ANN/ 2020. At Livestock Research Station, Palamaner, Andhra Pradesh, a 90-day growth trial was conducted using 15 Punganur calves (3–4 months old, 29.72 ± 2.96 kg), randomly assigned to three treatment groups (n=5). Calves were housed in a well-ventilated, semi-open shed with individual feeding and watering facilities, ensuring proper hygiene. A seven-day adaptation period was provided before the trial to adjust to the diets. Feed ingredients *viz.,* Maize, Soybean meal and De-oiled rice bran (DORB) were procured from the local market in ground form and concentrate mixture was prepared at the feed mixing plant, Livestock Research Station (LRS), Palamaner. Concentrate mixture was prepared with different levels of crude protein (Table 1) and the claves were fed Super napier green grass *ad libitum* and concentrate mixture with 14% CP (T1), 16% CP (T2) and 18% CP (T3)at 1% of body weight.

Table 1. Chemical composition of concentrate mixture and Super Napier grass fed to Punganur Calves*

Nutrient	Concentrate mixture			Super Napier
	T1	T2	T3	
Dry matter	89.9	89.8	90.1	19.90±0.27
Organic matter	90.9	90.04	90.1	89.2
Crude protein	14.6	15.8	18.5	6.09
Crude fibre	9.0	10.4	10.3	33.5
Ether extract	2.29	1.98	1.39	2.05
Total ash	9.09	9.96	9.88	10.7
Acid insoluble ash	2.71	3.13	2.53	4.2
Neutral detergent fibre	36.02	35.98	35.19	69.65
Acid detergent fibre	12.66	13.39	12.25	40.22
Hemi cellulose	23.36	22.59	22.94	29.43
Cellulose	8.13	10.89	10.51	35.75
Acid detergent lignin	4.81	4.72	4.98	12.57
Gross energy(cal/gram)	4006	4003	3978	-

*DM basis

The calves were fed weighed quantity of concentrate mixture at 9:00 AM daily and *ad libitum* Super Napier green grass twice daily at 10:30 AM and 3:30 PM. The leftover Super Napier grass, if any was recorded next day morning to calculate DMI. Experimental animals were weighed at weekly intervals before feeding and watering to know the effect of experimental diets on average daily gain (ADG) and feed efficiency. A digestion trial was conducted at the end of 90 days growth trial. During the 7-day collection period the daily feed intake, feed left overand faeces voided were recorded.Daily faeces voided of individual animals were collected for 24 h and pooled in air-tight polythene bag. An aliquot of faecal sample for dry matter estimation (1/50) and N estimation (1/500 preserved in 20 % H_2SO_4) was collected daily from individual animal. Dried samples of faeces and leftover grass were ground through a 2 mm sieve using a Wiley mill, labelled and were stored in air-tight polythene bags and used for subsequent analysis.The DM of fresh Super napier was estimated daily and the dried samples were preserved in airtight containers until analysis. About 15 ml of blood was collected at end of the experiment under aseptic conditions from jugular vein. The blood was transferred to clot activator tubes and kept in slant position for 30 minutes and samples were centrifuged at 3000rpm for 5 min. Serum was separated and collected in Eppendorf tubes and stored at -20^{0}C for further analysis.

Dried samples of Super Napier grass, feed ingredients, concentrate feed, faeces and leftover feed were analysed for proximate principles (AOAC, 2005) and cell wall constituents (Van Soest et al., 1991).The serum biochemical constituents (glucose by Glucose oxidase/peroxidise method) , Total protein by Biuret method, Albumin by Bromocresol green method, Triglycerides by glycerol phosphate oxidase/ peroxidase method, Total cholesterol by cholesterol oxidase/peroxidase method and the activity of enzymes (AST and ALT) were estimated using A15 automated biochemical analyser using Biosystems A 15 analyser kits.

The data obtained were subjected to analysis using software (version 23.0; SPSS, 2015) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test with a significance at $P<0.05$ (Duncan,1955).

RESULTS AND DISCUSSION

Growth performance

The total weight gain (kg) of Punganur calves during the 90-day growth trial was 20.80, 20.76 and 21.17 in T1, T2 and T3, and the corresponding average daily gain (g) of 233.71, 233.26 and 237.95 was comparable among treatments (Table 2).Similarly, no significant effect was observed in DMI (Table 3) among treatments. Though superior feed efficiency was observed in T2 group,but the values were statistically comparable. Previous studies, Sharma et al. (2020), Kumar et al. (2016) and Lohakare et al. (2006) also reported no significant difference in growth performance of calves fed the diet containing different levels of crude protein. In contrast to the present study, Brosh et al. (2000) have reported higher weight gain in male HF calves fed high protein diet in comparison to feeding of medium or low protein diets. There was no significant difference in average faecal score and the values were 1.02, 1.02 and 1.04 in T1, T2 and T3 groups, respectively. This indicated that the different levels of crude protein did not show any effect on growth performance of Punganur calves.

Table 2. Effect of experimental diets on growth performance of Punganur calves

Parameter	T1	T2	T3
Average initial body weight (kg)	27.4±4.10	27.6±6.71	28.3±2.76
Average final body weight (kg)	48.2±4.34	48.4±7.71	49.4±2.65
Average total gain (kg)	20.8±1.43	20.7±2.61	21.1±1.14
Average daily gain (g)	233.71±16.10	233.26±29.34	237.95±12.85
FCR(g DMI / g gain)	4.36±0.75	4.09±1.03	4.35±0.58
Faecal score (1-4)	1.02±0.01	1.02±0.02	1.04±0.02

Table 3. Effect of experimental diets on DMI of Punganur calves

Treatment	DMI from Super Napier(g)	DMI from Concentrate mixture(g)	Total DMI (g)	DMI (% Body weight)	CP (%DM)	TDN (%DM)
T1	591.93±153	323.16±47	915.09±196	2.76±0.26	9.26	61.76
T2	534.43±206	336.19±73	860.76±272	2.47±0.32	10.20	64.74
T3	613.03±155	335.04±312	944.92±186	2.79±0.47	10.87	62.82

Nutrient digestibility

The data revealed non-significant effect (P>0.05) on digestibility of nutrients with diet containing varying levels of crude protein (Table 4). Similar to our study, several authors reported that dietary crude protein levels did not alter the digestibility of nutrients (Senger et al., 1985; Mehra et al., 2001; Chantriatikul et al., 2009; Tatsapong et al., 2010 and Andersen et al., 1994).

Table 4. Effect of experimental diets on nutrient digestibility (%) in Punganur calves

Parameter	T1	T2	T3
Dry matter	64.4±0.78	67.1±0.60	65.4±2.32
Organic matter	65.9±0.60	68.5±0.58	66.8±2.31
Crude protein	57.07±2.44	62.7±2.05	62.3±2.27
Ether extract	63.3±2.53	66.2±1.38	64.9±1.63
Crude fibre	57.4±2.80	61.1±0.97	60.3±3.24
Nitrogen free extract	71.7±0.51	73.5±1.09	71.6±2.13
Neutral detergent fibre	54.1±2.37	54.6±1.21	55.1±2.87
Acid detergent fibre	43.1±2.19	46.9±2.32	45.2±2.13
Hemicellulose	69.6±1.37	69.8±1.22	70.9±2.26

Serum biochemical profile

There was no significant difference in serum biochemical profile except for BUN(Table 5). The results agreed with the those of Kumar et al. (2013) and Lohakare et al. (2006) who reported a non-significant difference in serum biochemical profile in calves fed ration containing different levels of crude protein.

In the present study, concentration of BUN was significantly ($P<0.05$) lower in T1 than in T2 and T3 which might be due to higher crude protein content of diets T2 and T3 than T1. This agrees with Dong et al. (1997) and Luo et al. (2000) who reported that the BUN level increased withthe level of dietary crude protein. Concentration of total protein and albumin reflect the availability of protein and their concentration that decline in the face of protein deficiency (Lohakare et al., 2006). The concentration of protein and albumin after three months of feeding experimental diets was comparable which is suggestive of adequacy of the protein even on the low protein diet. However, all the values were reported to be within normal physiological range (Kaneko et al., 2008).

Table 5. Biochemical profile of Punganur calves at the end of growth trial

Parameter	T1	T2	T3
Glucose (mg/dL)	88.8±7.93	96.9±7.58	92.5±3.48
Total protein (g/dL)	6.46±0.24	6.72±0.18	6.92±0.17
Albumin (g/dL)	3.73±0.18	3.93±0.10	3.98±0.06
Globulin (g/dL)	2.74±0.22	2.79±0.11	2.94±0.16
ALT (IU/L)	36.5±6.93	32.12±5.69	33.3±6.49
AST (IU/L)	72.2±6.16	64.8±5.36	67.2±1.84
AST: ALT	2.09±0.21	1.85±0.26	2.44±0.58
BUN (mg/dL)*	7.52±1.09^{a}	9.58±1.08^{ab}	10.5±0.45^{b}
Cholesterol (mg/dL)	141.75±25.47	161.10±37.28	187.10±18.05
Triglycerides (mg/dL)	51.63±6.38	50.30±7.39	58.00±4.41
Cholesterol HDL (mg/dL)	120.46±18.80	124.00±20.51	137.87±8.73
Cholesterol LDL (mg/dL)	15.17±4.72	34.76±14.27	37.63±10.62
VLDL	10.33±1.28	10.06±1.48	11.60±0.88
Ca (mg/dL)	10.65±0.40	10.38±0.34	11.11±0.43
P (mg/dL)	10.14±0.55	8.98±0.53	9.65±0.33
Mg (mg/dL)	2.44±0.20	2.39±0.16	2.28±0.12

CONCLUSION

The present study indicated that, the performance of growing Punganur calves was comparable on low (14% CP), medium (16% CP) and high (18% CP) Protein concentrate mixture supplementation (@1% of body weight) on Super Napier green fodder-based diet.Thus, the concentrate mixture containing 14% CP supplemented at 1% of body weight along with *adlibitum* Super Napier grass is concluded as optimum for rearing Punganur calves from 3-6 months of age to exhibit its maximum growth potential.

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Effect of Varying Levels of Dietary Cation-Anion difference (DCAD) on Nutrient Digestibility and Rumen Fermentation in Deccani Lambs

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ABSTRACT

Experiment was conducted to study different levels of dietary cation-anion differences (DCAD) on nutrient digestibilities and rumen fermentation pattern of Deccani lambs. Eighteen Deccani lambs (27.58±1.99 kg/body weight) were selected and randomly allotted to 3 groups, containing 6 animals in each.A sorghum stover based complete ration (50R:50C) was prepared as a basal diet.Three experimental rations viz., DCAD-160, DCAD-220 and DCAD-280 were prepared by adding sodium bicarbonate at 0.0, 0.477 and 0.955 % to the basal diet to achieve a DCAD level of 160, 220 and 280mEq/kg DM, respectively. Feeding on varying levels of DCAD had similar effects on digestibility of crude protein, ether extract, crude fibre and acid detergent fibre. However, the digestibility of DM, OM, NFE, NDF, hemicellulose and cellulose were lowered (P<0.05) significantly at +280 DCAD level while they were comparable inother two groups. Similarly, the TDN in DCAD-280 ration was significantly (P<0.01) lower than DCAD-160 or DCAD-220 rations. The rumen pH and total volatile fatty acids concentrations were comparable among the three experimental rations while, ruminal ammonia nitrogen concentration decreased significantly (P<0.01) in DCAD-280 ration. Thus, it can be concluded that, the sorghum stover based complete rations containing DCAD level of +220 shall be better for optimum digestibility and rumen fermentation in Deccani lambs.

KEYWORDS: DCAD, Deccani lamb, Digestibility, Fermentation, Nutrients

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INTRODUCTION

Deccani sheep is a dual purpose breed. It is highly climate resilient and is best adapted to the semi-arid tracts of Telangana state. It has a great potential for mutton production under intensive system of management. Now a days, the rearing method of sheep, changing from extensive to intensive and semi intensive systems of rearing in Telangana. It could be due to several reasons such as increased population, urbanization and pressure on land for food crops, industrial and infrastructural projects etc. In the intensive system of rearing feeding management particularly, minerals have great importance as animals have no access to soil unlike in extensive system.

Minerals involve directly or indirectly in all biological processes of an animal. The minerals are either in the form of anions or cations and the balance between cations and anions is necessary for optimum growth and production of animals. Dietary cation-anion difference (DCAD) refers to the numerical difference between major cations (sodium and potassium) and major anions (chloride and sulphate), thus it is defined as milli-equivalents of ($Na^+ + K^+$) – ($Cl^- + S^{2-}$) per 100g of dry matter or per kg of dry matter (Block, 1994). The DCAD values in the diet may be positive or negative. The DCAD is said to be positive if the concentration of dietary cations is more relative to the concentration of dietary anions and vice versa.

Positive DCAD indicates the generation of more blood buffers, accumulation of less hydrogen ions and higher pH, which further affects dry matter intake (DMI), nutrient utilization and growth in animals(Wildman et al., 2007). Positive DCAD diets in ruminants cause an increase in nutrient intake due to favourable rumen dynamics and blood biochemistry (Wildman et al., 2007). Crossbred calves fed with positive DCAD diets of +250 and +350mEq/kg DM during winter and summer seasons improved the nutrient intake, growth of performance, immunity and ameliorated climatic stress (Suman et al., 2018a; Suman et al., 2018b; Suman et al., 2019).Decrease in blood pH (negative DCAD) affects insulin (Robertson, 1987) and growth hormone secretion (Challa et al., 1993) which ultimately decreases feed intake and growth rate of growing lambs. Positive DCAD diet causes slight metabolic alkalosis of cellular environment which reduces the extent of cellular acidity produced by CO_2 and thus allow the cell to work to its maximum capacity and improve the growth of animals (Block, 1994). However, the scientific information pertaining to the effect of the DCAD on performance of sheep is limited in India. Therefore, the present experiment was planned to investigate the effect of different levels of DCAD on digestibility of nutrients and rumen fermentation pattern in Deccani lambs.

MATERIALS AND METHODS

The studywas undertaken to evaluate the effect of varying levels of positive DCAD arrived by adding sodium bicarbonate in a sorghum stover based complete rations. The experiment was conducted at Department of Animal Nutrition, College of Veterinary Science, P.V.Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad.

Experimental Rations

Sorghum stover based three iso-nitrogenous complete rations with 50:50 ratio of roughage and concentrate was prepared as per ICAR (2013) standards (Table 1). The DCAD levels of experimental rations viz., +160, +220 and +280 mEq/kg, respectively were maintained by adjusting sodium bicarbonate level. The roughage and concentrate ingredients were first proportioned and batched to 100 kg as per the formula. Later they were ground separately in a hammer mill and mixed in ribbon type horizontal mixer for 20 minutes. The mineral mixture, dicalcium phosphate and sodium bicarbonate were mixed thoroughly in premixer and then, directly added into the mixer.

The DCAD values of the three experimental feeds were arrived by taking into consideration the Na^+, K^+, Cl^- and S^{2-} ions concentration (%) of all the concentrate ingredients individually. Later the DCAD of the whole ration was calculated using the formula DCAD = $\{[(Na\% / 0.0023) + (K\% / 0.0039)] - [(Cl\% / 0.00355) + ((S\% / 0.0016)\times 0.6)]\}$ mEq/kg DM (Table 2). Here, DCAD was calculated using the basic formula DCAD = $\{[Na^+ + K^+] - [Cl^- + (S^{2-}\times 0.6)]\}$ (Goff et al., 2004), where the absorption coefficient of sulphur was also considered.

Table 1 Ingredient composition (%) of experimental rations

Ingredient %	DCAD-160	DCAD-220	DCAD-280
Sorghum stover	50.0	50.0	50.0
Maize	22.0	22.0	22.0
Cotton seed cake	2.5	2.5	2.5
Soybean meal	15.0	15.0	15.0
Deoiled rice bran	5.60	5.37	5.15
Red gram chunni	2.25	2.00	1.75
Urea	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5
Limestone	1.2	1.2	1.2
Mineral and vitamin mixture	0.2	0.2	0.2
Salt	0.25	0.25	0.25
Sodium bicarbonate	0.000	0.477	0.955

Table 2. Mineral composition (% DMB) of experimental rations

Mineral (%)	DCAD-160	DCAD-220	DCAD-280
Sodium (Na+)	0.28	0.42	0.56
Potassium (K+)	0.56	0.56	0.55
Chloride (Cl-)	0.20	0.20	0.20
Sulphur (S2-)	0.12	0.12	0.12
DCAD (mEq/kg DM)*	+163.99	+224.86	+283.17

Experimental animals

Eighteen growing Deccani lambs of about 6-7 months age and of uniform body weight (23.86±1.70 kg) were selected and divided randomly into 3 groups viz., DCAD-160, DCAD-220 and DCAD-280, containing 6 animals in each by following completely randomized design.All the experimental lambs were housed in a well ventilated pens having individual feeding and watering. Lambs were fed with their respective experimental rations *ad libitum* twice daily.Body weights of animals were recorded at before and after completion of experiment.

Digestion trial

A standard digestion trial was conducted with twenty-one day preliminary period and six days collection period by adopting acid insoluble ash based indicator method (Sharma et al., 1983). The digestibility of nutrients in experimental diets was assessed by analyzing various chemical constituents in the feed and faeces (AOAC, 2012). Cell wall constituents of feeds and faecal samples were determined as per the method described by Van Soest et al. (1991).

Rumen Fermentation

The rumen liquor samples were collected by using stomach tube at 30 min, 2 h and 6 h post feeding for 2 consecutive days. The pH was determined immediately after collection using digital pH meter. Ammonia nitrogen was estimated as perMakkar and Becker, (1996) method. About 50 ml of strained RL was collected in separate containers from each sample and 0.5 ml of saturated mercury chloride was added and preserved at -20 C for estimation of total volatile fatty acids (TVFA) (Barnett and Reid, 1957).

Statistical Analysis

The data was analyzed using the general linear model of the Statistical Package for Social Sciences (version 15), and the means were compared using Duncan's multiple range test (Duncan, 1955). A statistical analysis of the data was carried out by using analysis of variance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The proximate and fiber constituentscomposition (% DMB) of experimental rations was presented in Table 3.

Table 3. Chemical composition (% DM basis) of experimental rations containing different levels of DCAD

Constituents (%)	DCAD-160	DCAD-220	DCAD-280
Proximate constituents			
Dry matter	88.1	88.9	88.1
Organic matter	91.5	90.5	88.2
Crude protein	15.8	15.5	15.7
Ether extract	2.94	2.95	2.98
Crude fibre	22.3	22.7	22.04
Nitrogen free extract	50.4	49.3	47.4
Total ash	8.51	9.45	11.7
Acid insoluble ash	3.76	3.81	4.67
Calcium	0.97	0.97	0.95
Phosphorus	0.49	0.49	0.48
Cell wall constituents			
Neutral detergent fibre	48.4	48.5	48.1
Acid detergent fibre	27.7	29.3	27.5
Hemicellulose	20.6	19.2	20.5
Cellulose	20.8	21.5	21.5
Lignin	4.14	4.17	3.22

Nutrient Digestibility

Digestibility coefficients of dry matter (DM), organic matter (OM) and nitrogen free extract (NFE) were significantly higher (P<0.05) for DCAD-160 and DCAD-220 rations in comparison to DCAD-280 ration(Table 4). However, the digestibility coefficients of crude protein (CP), ether extract (EE) and crude fibre (CF) was comparable among the three experimental rations. In line with our findings,digestibility coefficients of proximate constituents remained unaffected by the feeding varying levels of DCAD in the rations of calves (Suman et al., 2018a), cows (Martins et al., 2016), Goats (Farooq et al., 2014) and lambs (Sarwar et al., 2007).

Among the cell wall constituents neutral detergent fibre (NDF), hemicellulose and cellulose digestibilities were significantly improved (P<0.01) in lambs fed DCAD-160 ration in comparison to DCAD-220 and DCAD-280 rations.In agreement with the present findings, Kawas et al. (2007) observed significant (P<0.05) decrease in NDF digestibility in 0.5 percent sodium bicarbonate supplemented lambs.However, in contrast to present study findings, the NDF digestibility linearly increased as DCAD level increases (+98 to +290 mEq/kg) and reported that positive effect of DCAD on NDF digestibility may be associated with a higher ruminal pH and consequently, a higher cellulolytic activity (Martins et al., 2016).

TDN (%) in DCAD-280 ration was significantly (P<0.01) lower than DCAD-160 and DCAD-220 rations.The per centdigestible CP (DCP) in the three experimental rations and daily intakes of CP, TDN, DM, digestible energy (DE) and metabolizable energy (ME) were not differ significantly among three experimental rations. The intake of CP (g/d), TDN (g/d), DMI (g/d), DE (Mcal/d) and ME (Mcal/d) in three experimental rations ranges 142 to 152, 528 to 552, 900 to 960, 2.33 to 2.43 and 1.91 to 1.99, respectively.According to ICAR (2013) recommendations, the CP, TDN, DM and ME requirements of growing lambs weighing 30 kg with a ADG of 75 g were 122 g/d, 543 g/d, 980 g/d and 1.96 Mcal/d, respectively.

Table 4. Effect of feeding different experimental rations containing varying levels of DCAD on nutrient digestibility (%) in Deccani lambs

Nutrient	DCAD-160	DCAD-220	DCAD-280	SEM	P value
Proximate constituents					
Dry matter	59.1 ^{a}±0.34	58.6 ^{a} ±0.84	55.9 ^{b} ±1.14	0.567	0.040
Organic matter	62.2 ^{a} ±0.37	61.9 ^{ab}±0.90	58.7 ^{b}±1.18	0.602	0.033
Crude protein	75.8±0.56	73.7±0.88	74.2±0.87	0.474	0.189
Ether extract	74.7±0.60	77.4±1.17	77.1±1.42	0.676	0.197
Crude fibre	54.5±0.76	52.7±2.38	52.6±1.88	1.002	0.710
Nitrogen free extract *	60.7 ^{a} ±0.61	59.9 ^{a} ±1.12	55.2 ^{b}±1.24	0.810	0.004
Cell wall constituents					
Neutral detergent fibre	49.5^{a}±0.83	45.05 ^{b}±1.28	43.1 ^{b}±0.81	0.845	0.001
Acid detergent fibre	35.6±0.93	34.9±1.43	31.5±1.64	0.855	0.113
Hemicellulose *	68.2^{a}±1.34	60.4 ^{b}±1.65	58.6±2.84^{b}	1.506	0.011
Cellulose *	51.3 ^{a}±0.84	47.5 ^{b}±1.24	45.05 ^{b}±1.48	0.912	0.008

^{ab} means with different superscripts within a row differ significantly (P<0.05, *P<0.01)

Rumen fermentation

The rumen pH values and TVFA concentrations were comparable among the three experimental rations while, ruminal ammonia nitrogen concentration decreased significantly (P<0.01) in DCAD-280 ration(Table5).The highest (P<0.01) ammonia nitrogen concentration was recorded in DCAD-160 and DCAD-220 and while lowest (P<0.01) was recorded in DCAD-280 rations. The pH values obtained in the present experiment were in consistent with the results reported by Baset et al. (2014) and Kawas et al. (2007) in which the ruminal pH values were also not influenced by DCAD (sodium bicarbonate level) in the ration of lambs.

The concentrations of TVFA not affected significantly neither among DCAD levels nor among time intervals of rumen liquor collection. These results were in accordance with the results reported by Ross et al. (1994), Baset et al. (2014), Hadjipanayiotou et al. (1988), Kawas et al. (2007), Apperbossard et al. (2010) and Gonazalez et al. (2008), who have noticed no effect of DCAD or sodium bicarbonate inclusion on the ruminal TVFA concentration. In contrary to the above findings, Santra et al. (2003) appreciated a significant (P<0.01) increase in TVFA concentration with increasing levels of sodium bicarbonate (0, 0.75, 1.50 and 2.25 per cent). The reason for unaltered TVFA concentration with the change in DCAD level in the present investigation might be due to the increased buffering capacity of sodium bicarbonate in the ration.

Table 5. Effect of feeding different experimental rations containing varying levels of DCAD on rumen fermentation in Deccani lambs

Parameter	DCAD-160	DCAD-220	DCAD-280	SEM	P Value
pH	6.21±0.04	6.19±0.03	6.18±0.04	0.396	0.785
Ammonia nitrogen (mg/100 ml)	25.96^{a}±1.61	25.99^{a}±1.24	6.50^{b}±1.05	1.300	0.001
Total volatile fatty acids (mmol/100ml)	8.45±0.22	8.36±0.20	7.96±0.17	0.201	0.861

^{abc} means with different superscript within a row differ significantly (P≤0.01).

Decrease of ammonia concentration with increase in DCAD level in the present experiment could be due to increased rumen dilution rate (Russel and Chow, 1993) which further decreases the rumen retention time. The results were in consistent with the results of Santra et al. (2003) who reported a decrease ($P<0.01$) in ammonia nitrogen concentration due to the addition of sodium bicarbonate in the rations of lambs.

CONCLUSIONS

Feeding of Deccani lambs with higher DCAD (+280) diets significantly lowered the nutrient digestibility of dry matter, organic matter, nitrogen free extract, neutral detergent fibre, hemicellulose and cellulose and percent TDN, while they were not affected with lower DCAD levels (+160 and +220). Thus, it is concluded that the sorghum stover based complete rations containing DCAD level upto +220 shall be optimum for better digestibility and rumen fermentation in Deccani lambs.

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Effect of Transition Period Nutrition on Lactation Performance, Metabolic Profiles and Reproductive Efficiency in Indigenous Dairy Cattle

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ABSTRACT

A study was conducted to evaluate the effect of Bypass Fat and Vitamin E supplementation on milk production, composition, plasma metabolites, and reproductive performance in postpartum cows during the periparturient period. Twenty-one transition indigenous cows were divided into three groups: T1 (control, n=6), T2 (BF, n=8) and T3 (BF+Vit E, n=7). The Control group received a basal ration as per ICAR (2013) guidelines, while T1 was additionally supplemented with Bypass Fat @ 50 g/animal/day and T2 with Bypass Fat@50 g+Vitamin E @ 3000 IU/animal/day, from 21 days prepartum to 21 days postpartum. Blood samples were collected on days -21, -14, -7, 0, 7, 14, and 21, and milk yield and composition were recorded during the first 21 days of lactation. Uterine involution and ovarian activity were assessed via ultrasonography on days 15, 25, 35, and 45 postpartum, with pregnancy confirmation on days 28-30 and 50 post-AI. Plasma glucose and cholesterol levels were significantly higher ($P<0.05$), and NEFA concentrations were significantly lower ($P<0.05$) in both treatment groups compared to the Control. No significant differences ($P>0.05$) were observed in dry matter intake (DMI), body weight (BW), milk yield, and milk composition, except for a significant reduction ($P<0.05$) in somatic cell count (SCC) in the T2 group. Uterine horn diameter was significantly larger ($P<0.01$) in the Control group on day 45 postpartum. No significant differences ($P>0.05$) were found in the total number and size of antral follicles, calving to first estrus interval, calving to conception interval, or number of services per conception. Pregnancy rates were 50% in the Control group and 66.66% in both T1 and T2 groups. It can be concluded that Bypass Fat supplementation, alone or with Vitamin E, improved energy balance, uterine involution, and conception rates, but did not significantly affect milk yield and composition, except for a reduction in SCC in the T2 group.

KEYWORDS: Bypass fat, Lactation performance, Reproductive efficiency, Vitamin E

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INTRODUCTION

Transition period in dairy cows, encompassing the final weeks of gestation through calving and into early lactation, represents a critical phase characterized by profound physiological adaptations. During this period, dairy cows undergo substantial metabolic, hormonal, and cellular shifts to meet the escalating demands of fetal development, the process of parturition, and the subsequent initiation and maintenance of lactation. These adaptive processes are essential for successful transition. However, the complexity and magnitude of these changes pose significant challenges to the cow's ability to maintain metabolic and physiological homeostasis, thereby predisposing them to various disorders. One of the most critical challenges is the occurrence of negative energy balance (NEB), which results from the increased energy demands of lactation surpassing the intake. NEB leads to extensive mobilization of body fat reserves, contributing to elevated levels of non-esterified fatty acids (NEFAs) and ketone bodies. This metabolic shift increases the risk of various metabolic disorders which can negatively impact milk production, fertility and overall health (Balamurugan et al., 2018). Furthermore, the transition period is frequently associated with delayed uterine involution and other reproductive complications. Studies have shown that Vitamin E, a key antioxidant, declines significantly around calving (Goff et al., 2002). This reduction in antioxidant capacity diminishes the cow's ability to counteract oxidative stress, leading to impaired immune function and an increased susceptibility to intramammary infections. It also exerts positive effects in body metabolism and fertility (Hosnedlova et al.,2017). Various nutrients are utilized in periparturient period to improve NEB. Among these, dietary supplementation of Rumen protected fat serves as a dense energy source, helping to alleviate NEB and support lactation demands, and imparting better fertility by reducing excessive body condition loss and improving hormonal balance while Vitamin E supplementation improves the immune function, reducing the incidence of metabolic and infectious disorders and improve milk quality.Considering these facts, the present study was designed to investigate the effect of Bypass fat and Vitamin E supplementation on milk production, composition, plasma metabolites, and reproductive performance in postpartum cows during the periparturient period.

MATERIAL AND METHODS

Experimental animals, Treatments and management

For this study, Twenty one advanced (8 months) pregnant and healthy cattle in their first to third lactation were selected from the herd maintained at Livestock Farm Complex, DUVASU, Mathura (India). Selected cows were randomly divided into three treatment groups, viz., T1 (control, n=6), T2 (BF, n=8) and T3 (BF+Vit E, n=7) based on their parity, body weight and previous lactation yield. In T1 (control) group, cows were fed with basal diet composed of concentrate mixture, wheat straw and maize green fodder to meet their nutrient requirement as per ICAR (2013) feeding standards followed on the farm. The cows in T2 group received additional dietary supplementation of bypass fat (BF) @ 50 g/d (S.A. Pharmachem Pvt Ltd. Innofeed Rumen Bypass fat supplement (Calcium salt of Long chain fatty acids)) and in T3 group BF along with vitamin E (BF @ 50 gm + Vitamin E @ 3000 IU per animal per day (Microvit E Promix 50, Vitamin E (D-alpha tocopherol acetate)). The treatment began 21 days before expected date of calving and continued for 21 days postpartum. The ingredient composition of concentrate mixture consisted of 20 parts maize grain, 20 parts barley grains, 9 parts oat grain, 10 parts wheat bran, 10-parts gram chunni, 28 parts mustard oil cake and 3 parts mineral mixture. The chemical composition of experimental feed is presented in Table1. Vitamin E powder and bypass fat were premixed with concentrate and was offered prior to providing the ration to ensure its intake.

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

Parameters	Concentrate	Green fodder	Wheat straw
DM	91.3	17.72	90.4
OM	91.2	89.9	88.5
CP	18.2	9.28	3.21
EE	3.12	2.42	1.01
CF	8.12	53.4	34.9
Total Ash	8.75	10.07	11.4

The animals were kept in separate calving pen one week prior to the expected calving date. After parturition; the cows were subsequently shifted to well-ventilated hygienic concrete milking shed. The cows were fed individually and clean, fresh drinking water was available *ad lib*. Daily dry matter intake was recorded for each cow.

Observations recorded

Blood examination

Peripheral blood samples were collected from jugular vein in heparinised vacutainers on days -21, -14, -7, 0, 7, 14 and 21 in relation to the expected date of calving. The samples were brought to the laboratory in ice boxes soon after collection and centrifuged at 1200 g at 4 °C for 20 min to separate the plasma for the analysis. Plasma concentrations of Glucose, total cholesterol, triacylglycerides (TAG) and VLDL were analyzed by using standard kits (Span Diagnostic Ltd, Surat, Gujarat, India) with fully automatic biochemistry analyzer (BS-120 Chemistry analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd.) Plasma Non-esterified fatty acids (NEFA) and beta-hydroxy butyric acid (BHBA) were estimated using commercially available ELISA kits (Bioassay Technologies, China).

Milk yield and composition

Animals were hand milked twice daily and milk production was recorded at each milking during the first 21 days of lactation using electronic weighing balance. Milk samples from each animal, pooled from two consecutive milkings were collected on days 7, 14 and 21 of lactation. Representative sample of each animal was treated with sodium azide, preservative and stored at 5°C until analysis for milk fat, protein, lactose, solids-not fat (SNF), total solids and SCC by using precalibrated milk analyzer (Lactoscan).The 4% fat corrected milk (FCM) was calculated using formula:

4% FCM = milk yield (kg) X 0.4 + fat yield (kg) X 15

Reproductive parameters

During the postpartum period, uterine involution and ovarian activities were monitored using ultrasonographic (USG) examinations at 15, 25, 35, and 45 d postpartum. These evaluations assessed the diameter of the uterine horn cranial to the body and recorded ovarian parameters such as antral follicle counts and the size of the largest follicle, following standard procedures. Routine management practices included monitoring cows for estrus by observing vaginal discharges and behavioural signs, with estrus confirmation conducted through gynaecological examinations of the genitalia. Cows detected in estrus were inseminated using frozen-thawed semen. Pregnancy confirmation was carried out for cows that did not return to estrus, with initial checks performed between days 28 and 30 post-insemination, followed by a second confirmation on day 50 post-artificial insemination (AI). Reproductive metrics, including the date of treatment completion, the first observed estrus or AI dates, subsequent estrus or AI occurrences, pregnancy dates, the total number of services, and the number of services per conception, were recorded.

Statistical analysis

All the experimental data obtained were statistically analyzed by using ANOVA procedures of statistical software SPSS software version 20.0 (Snedecor and Cochran 1994). Significant differences between means of treatments were assessed by the Duncan's test, and the differences among treatments were declared significant at $P<0.05$.

RESULTS AND DISCUSSION

Effect on Plasma metabolites

Glucose and Cholesterol

The mean values of biochemical profile in control and treatment groups of periparturient cattle are presented in Table 3. Supplementation of bypass fat alone or in combination with Vitamin E significantly ($P<0.05$) increased plasma glucose concentration (mg/dL) as compared to the control. Overall values were found to be within the normal range of 45-75 mg/dl in all groups and periods. Blood glucose levels were significantly higher on the day of parturition compared to the peripartum periods across all groups, peaking at calving and declining during prepartum and postpartum periods. This peak is attributed to glucocorticoid release before calving, which stimulates glycogenolysis and gluconeogenesis (Hayirli et al., 2002). Consistent with these findings, prior studies reported no significant effect of bypass fat supplementation on blood glucose levels in periparturient cows (Tyagi et al., 2010; Chavda et al., 2022). Plasma cholesterol levels were significantly higher ($P<0.05$) in the treatment group compared to the control group, attributed to enhanced uptake of dietary fatty acids (Kumar et al., 2007). Cholesterol concentrations were lowest at calving but increased significantly postpartum in the treatment group. Elevated cholesterol levels in periparturient cattle supplemented with bypass fat have been reported (Nirwan et al., 2019) though some studies found no significant changes (Tyagi et al., 2010).

Triglycerides and VLDL

Plasma TAG and VLDL concentrations were significantly higher during the prepartum period compared to postpartum, with the lowest levels observed on days 7 and 14 postpartum, followed by an increase on day 21 (Fig. 1 and 2). No treatment significantly affected overall mean plasma TAG and VLDL concentrations in periparturient cows. Similar findings of non-significant effects of BF supplementation on TAG and VLDL were reported by Chavda et al. (2022).

NEFA and BHBA

NEFA concentration in blood reflects energy balance and body fat mobilization in lactating animals. In this study, mean plasma NEFA levels were significantly (P<0.05) lower in treatment groups compared to controls, with significant variation on the day of calving and days 7, 14, and 21 postpartum. NEFA levels peaked near calving in all groups, indicating increased energy demands and reduced intake. Vitamin E supplementation (T2 vs. T3) had no significant effect on NEFA concentrations. Elevated NEFA levels around parturition reflect negative energy balance (NEB) and mobilization of body reserves (Duffield and Leblanc, 2009). Fat supplementation increases NEFA through hydrolysis by lipoprotein lipase, insulin resistance, reduced lipogenesis, and increased lipolysis (Theodore et al., 2017).Plasma BHBA concentrations did not differ significantly (P>0.05) between treatment and control group, remaining consistent with findings by Singh et al. (2014). However, Dhami et al. (2022) found that Se+Vit E supplementation significantly (P<0.05) reduced BHBA levels from day 15 to 60 postpartum, likely due to improved hepatic oxidative status.

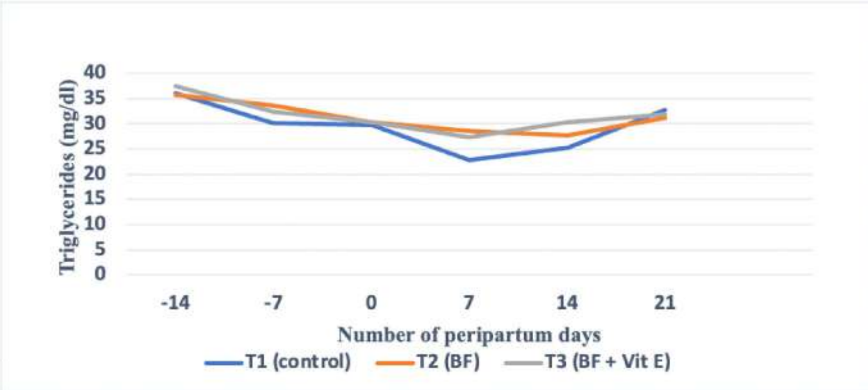

Fig. 1: Effect of supplemental Bypass fat and Vitamin E on plasma Triglycerides concentration of indigenous cows.

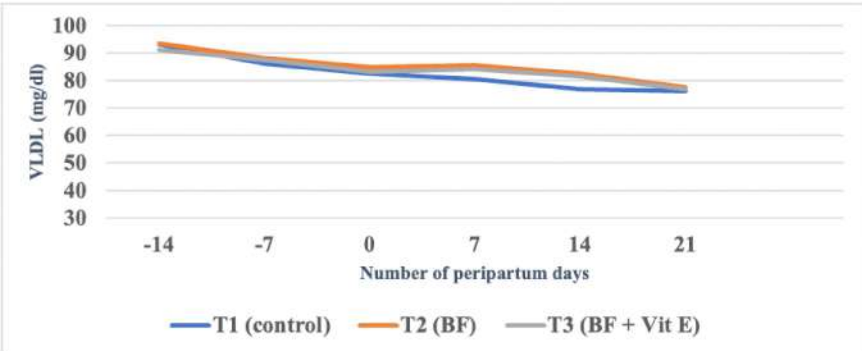

Fig. 2: Effect of supplemental Bypass fat and Vitamin E on plasma VLDL concentration of indigenous cows.

Table 2. Effect of supplemental Bypass fat and Vitamin E on plasma metabolites of transition dairy cows

Days pre-and postpartum	T1 (control)	T2 (BF)	T3 (BF + Vit E)	Overall
Plasma Glucose (mg/dl)				
-21	56.74 ± 1.06^{bB}	55.57 ± 0.88^{abB}	57.87 ± 0.76^{bB}	56.73^{B}
-14	52.89 ± 1.23^{bB}	50.81 ± 1.33^{aA}	52.64 ± 1.05^{bA}	52.11^{A}
-7	53.19 ± 1.66^{aB}	56.42 ± 1.65^{bB}	57.04 ± 1.41^{bB}	55.55^{B}
0	61.76 ± 2.02^{aC}	65.86 ± 1.67^{bD}	64.75 ± 1.53^{bD}	64.12^{C}
7	56.45 ± 1.56^{aB}	60.18 ± 0.89^{bBC}	59.61 ± 1.10^{bC}	58.75^{B}
14	45.08 ± 1.38^{aA}	57.62 ± 1.14^{bB}	56.34 ± 1.42^{bB}	53.01^{A}
21	46.06 ± 2.05^{aA}	58.47 ± 1.65^{bBC}	55.23 ± 1.39^{bAB}	53.25^{A}
Overall	53.17 ± 2.11^{a}	57.85 ± 1.54^{b}	57.64 ± 1.38^{b}	
Plasma Cholesterol(mg/dL)				
-21	133.63 ± 2.34^{B}	136.26 ± 1.98^{B}	140.94 ± 2.24^{B}	136.94^{B}
-14	136.27 ± 1.46^{B}	140.35 ± 1.87^{C}	136.99 ± 1.55^{B}	137.87^{B}
-7	141.27 ± 1.68^{C}	146.02 ± 2.02^{C}	147.42 ± 1.74^{C}	144.90^{B}
0	118.90 ± 1.49^{A}	124.02 ± 2.23^{A}	122.07 ± 1.98^{A}	121.66^{A}
7	135.29 ± 1.56^{aB}	143.65 ± 1.89^{bC}	142.51 ± 1.04^{bC}	140.48^{B}
14	137.17 ± 1.42^{aB}	150.32 ± 2.11^{bD}	154.18 ± 2.42^{bD}	147.22^{C}
21	136.74 ± 2.31^{aB}	143.64 ± 1.79^{bC}	151.21 ± 1.96^{bD}	143.86^{B}
Overall	134.18 ± 1.35^{a}	140.61 ± 2.04^{b}	142.19 ± 1.76^{b}	
NEFA (µmol/L)				
-21	295 ± 2.34^{A}	293 ± 2.53^{A}	292 ± 1.95^{A}	293 ± 2.13^{A}
-14	$271\pm3.12A^{B}$	288 ± 2.14^{AB}	293 ± 2.85^{A}	284 ± 2.25^{A}
-7	313 ± 3.67^{C}	308 ± 1.78^{B}	305 ± 1.34^{AB}	308.66 ± 2.03^{B}
0	381 ± 2.46^{bF}	367 ± 2.22^{aF}	364 ± 3.68^{aE}	370.66 ± 2.87^{E}
7	361 ± 2.23^{bE}	345 ± 2.54^{aE}	351 ± 3.01^{aD}	352 ± 2.77^{D}
14	348 ± 1.98^{bD}	326 ± 1.68^{aD}	332 ± 2.11^{aC}	335.33 ± 1.77^{C}
21	331 ± 2.06^{bB}	318 ± 1.79^{aB}	313 ± 1.76^{aB}	320.66 ± 1.97^{B}
Overall	328 ± 2.02^{b}	320 ± 1.98^{a}	321 ± 2.31^{a}	
BHBA (µmol/L)				
-21	455.25 ± 2.31^{A}	466.54 ± 1.47^{A}	458.66 ± 1.56^{A}	458.82 ± 2.76^{A}
-14	468.47 ± 2.03^{A}	473.87 ± 2.34^{B}	470.32 ± 2.12^{B}	470.55 ± 3.15^{B}
-7	511.45 ± 1.87^{B}	515.43 ± 2.16^{C}	517.86 ± 2.67^{C}	512.91 ± 3.04^{C}
0	517.22 ± 1.68^{B}	522.31 ± 2.04^{C}	526.13 ± 1.98^{C}	521.89 ± 2.87^{CD}
7	543.94 ± 2.05^{C}	542.06 ± 1.87^{D}	546.13 ± 1.77^{D}	542.71 ± 2.77^{E}
14	544.44 ± 1.98^{C}	545.38 ± 1.66^{D}	548.33 ± 2.31^{D}	545.05 ± 3.17^{E}
21	541.12 ± 1.65^{C}	542.78 ± 1.75^{D}	545.56 ± 2.54^{D}	542.82 ± 2.97^{E}
Overall	511.70 ± 2.02	515.14 ± 1.98	516.14 ± 2.31	

Means having different superscripts abc within a row differ significantly ($P< 0.05$). Means having superscripts ABC within a column differ significantly ($P<0.05$).

Table 3- Effect of supplemental Bypass fat and Vitamin E on dry matter intake (DMI), body weight (BW), milk yield and composition of transition dairy cows

Parameters	T1 (control)	T2 (BF)	T3 (BF+Vit E)	SEM	P value
Prepartum					
DMI 21d period before calving	9.24	9.32	9.36	0.431	0.691
Mean BW (kg)	412	423	421	10.23	0.643
Post partum					
DMI from d 1-21 of lactation (kg/d)	10.9	11.2	11.2	0.372	0.788
Mean BW (kg)	376	397	392	11.76	0.322
Yield (kg/d)					
Milk	7.96	8.14	8.21	0.162	0.915
4 % FCM	8.03	8.26	8.38	0.456	0.953
ECM	8.73	9.03	9.13	0.550	0.999
Fat	0.323	0.334	0.340	0.765	0.882
SNF	0.650	0.669	0.680	0.112	0.901
Total solids	0.974	1.001	1.021	0.043	0.999
Protein	0.258	0.271	0.272	0.021	0.671
Lactose	0.390	0.402	0.401	0.102	0.778
Milk composition (%)					
Fat %	4.07	4.11	4.15	0.045	0.878
Protein %	3.25	3.34	3.32	0.064	0.861
Lactose	4.91	4.94	4.89	0.076	0.853
SNF %	8.17	8.23	8.29	0.124	0.775
Total solids	12.24	12.3	12.4	0.342	0.661
SCC (X 10^5 cells/ml)	2.13^b	2.16^b	1.98^a	0.145	0.006

Note: FCM- Fat Corrected Milk, ECM- Energy Corrected Milk, SNF- Solid not Fat SCC- Somatic Cell Count

Effect on DMI, milk yield and composition

The mean values for DMI, body weight (BW), milk yield, and composition are shown in Table 3. No significant differences (P>0.05) were found between the control and treatment groups for DMI, BW, or milk yield during the transition period. These findings are consistent with those of Singh et al. (2014) and Ramteke et al. (2014) who observed increased milk yield on feeding bypass fat to buffaloes during early lactation. Tyagi et al. (2009) also reported similar DMI values in control and treated cows during the transition period. Also, Bypass fat supplementation at 2.5% of DMI did not affect DMI during early lactation (Tyagi et al., 2010). The variation in DMI among animals fed bypass fat is influenced by factors such as the level of fat inclusion, palatability, and the lactation status of the animals (Naik et al., 2009).

During the first 21 days in milk, postpartum diets did not significantly affect (P>0.05) milk or FCM yield. In contrast, Wadhwa et al. (2012) reported a 1.13 kg/d increase in average daily milk yield in the bypass fat supplemented group compared to the control. Bypass fat supplementation has been shown to increase milk production in dairy cattle (Sirohi et al., 2010). The benefits of bypass nutrient supplementation are more pronounced in medium- and high-producing animals. Supplementation with bypass fat (BF) and Vitamin E did not significantly (P>0.05) affect milk fat percentage, SNF, total solids, protein, or lactose yield compared to the control. This lack of effect on milk fat aligns with studies by Sharma et al. (2016) and Ranjan et al. (2012), who also found no significant impact of bypass fat supplementation on milk fat. However, these findings are in contrast with those of Savsani et al. (2015)

who reported changes in milk composition due to bypass fat supplementation. Similarly, no significant differences were observed in milk protein, lactose, or their respective daily yields between treatments, consistent with studies by Shelke et al. (2012). Moreover, significant (P<0.05) difference in SCC values was observed between the control and treatment groups. The group supplemented with bypass fat and vitamin E showed significantly (P<0.05) lower SCC compared to the group supplemented with bypass fat alone or the control group.

Effect on postpartum fertility and reproductive indices

Uterine involution, Follicular size and count

Significant (P<0.05) difference in uterine horn diameter (uterine involution) was observed between the control and treatment groups at days 15, 25, 35, and 45 postpartum. On day 45, the mean uterine horn diameter in the control group was significantly (P<0.05) larger than in the treatment groups (Table 4). However, no significant (P>0.05) difference was noted between the treatment groups. Uterine horn diameter decreases as part of uterine involution, the process by which the uterus returns to its non-pregnant size after parturition.. These findings are consistent with Tyagi et al. (2010), who reported a significant (P<0.05) reduction in the days required for uterine involution in cows supplemented with bypass fat. However, Nirwan et al. (2019) found no significant difference in the number of days required for uterine involution. No significant (P>0.05) differences were observed in the total number of antral follicles and size of the largest follicle in both ovaries between the control and treatment groups.

Postpartum estrus, service period and conception rate

The mean number of days to the first postpartum estrus did not differ (P>0.05) between control and treatment groups. Similarly, no significant (P>0.05) differences were observed for the calving-to-conception interval or the number of services per conception between the control and treatment groups. However, pregnancy rates were higher in the treatment groups, with 50% (3/6) in the control group, and 66.66% (4/6) in both T1 and T2 groups. Previous studies have reported significant positive effects of bypass fat supplementation (Khalil et al., 2012) and Vitamin E and Se supplementation (Hosnedlova et al., 2017) on uterine involution, the onset of postpartum estrus, reduced service period, and improved conception rates. Vaswani et al. (2023) reported increased conception in the groups supplemented with bypass nutrients, with highest conception in group supplemented with bypass fat and proteins than the control. Fat supplementation enhances the energy status of the animals, increasing the precursors for reproductive hormone synthesis, such as steroids and prostaglandins, which positively affects reproduction by modulating ovarian follicle and corpus luteum function (Rahbar et al., 2014).

Table 4. Effect of supplemental Bypass fat and vitamin E on postpartum fertility and reproductive indices of transition dairy cows

Reproductive Attributes	T1 (control)	T2 (BF)	T3 (BF + Vit E)	P value
Uterine horn diameter	25.87±1.28[b]	18.94±1.16[a]	18.85±1.20[a]	<0.01
Antral follicle number on day 45 postpartum				
Right ovary	4.17±0.48	3.88±0.44	3.50±0.40	0.99
Left Ovary	4.83±0.31	4.25±0.67	3.86±0.26	0.87
Antral follicle size (mm) on day 45 postpartum				
Right ovary	10.80±1.50	10.30±0.65	8.58±1.13	0.90
Left Ovary	16.60	9.55±1.48	8.03±1.08	0.76
Service / Conception	1.67±0.58	2.00±0.71	2.00±0.71	0.86
Number of animals inseminated	6	6	6	-
Pregnancy	3 (50%)	4 (66.66%)	4(66.66%)	-

CONCLUSION

Bypass fat supplementation, either alone or in combination with vitamin E, improved energy balance in the treatment groups, as evidenced by significantly lower serum NEFA, higher glucose levels, and increased total cholesterol. Additionally, it enhanced postpartum fertility by reducing uterine involution time. However, no significant effects on milk yield or composition were observed, except for a reduction in somatic cell count (SCC) in cows supplemented with vitamin E.

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Improving Cold Tolerance in *Labeo rohita*: Effects of Job's Tears (*Coix lacryma-jobi* L.) as a Natural Feed Additive

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ABSTRACT

This study investigated the effects of Job's tears (*Coix lacryma-jobi* L.) as a feed additive on cold tolerance in *Labeo rohita* (initial weight: 15.2±0.3 g), marking the first evaluation of its health benefits in fish or aquaculture. Three doses (low: 0.5%, medium: 1%, and high: 2% of diet) were tested over an 8-week period, with fish fed to apparent satiation twice daily. Fish were subjected to a cold stress test (15^{0}C), and physiological, biochemical, and molecular parameters were assessed. Blood samples were collected from the caudal vein of anesthetized fish at 0, 12, and 24 hours during the cold stress test. The medium dose (1%) showed the most significant improvements in cold tolerance, evidenced by enhanced survival rates, reduced plasma cortisol levels, and up regulation of cold-tolerant genes. These novel findings suggest that Job's tears could be an effective feed additive for improving cold tolerance in *L. rohita* aquaculture, opening new avenues for natural stress mitigation in fish farming in subtropical and temperate regions.

KEYWORDS: Aquaculture stress mitigation, *Coix lacryma-jobi*, Cold tolerance, Feed additive, Indian major carp.

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INTRODUCTION

Labeo rohita, commonly known as rohu, is an economically important freshwater fish species widely cultured in South Asian countries including India (FAO 2022). However, its growth and survival are considerably hindered by low temperatures, especially in subtropical and temperate areas (Kausar and Salim 2006; Mridul et al., 2024). Enhancing this species' tolerance to cold is therefore essential to extend its aquaculture viability in colder regions, ultimately improving production efficiency.

Recent studies have shown that certain plant-based feed additives improve stress tolerance in fish (Kuebutornye et al., 2023). Singh et al. (2024) specifically highlighted that feed additives are crucial in aquaculture, serving as functional substances that can improve growth performance, decrease mortality rates, and provide specific medicinal properties. Complementing this perspective, researchers have explored various natural additives' potential in fish cultivation. For instance, Muzaffar et al. (2017) demonstrated garlic powder's efficacy in common carp growth, Khan et al. (2018) investigated Aloe vera's impact on fish performance, and Jain et al. (2020) examined carrot meal's role in enhancing ornamental fish characteristics.

Job's tears (*Coix lacryma-jobi* L.), a grain crop rich in polysaccharides, proteins, and bioactive compounds (phenolic acids, lignans, flavonoids, polyphenols, phytosterols), has demonstrated immunostimulatory and stress-mitigating effects in various animal models (Hsia et al., 2007; Manosroi et al., 2016; Devaraj et al., 2020; Gond et al., 2024). However, its potential to enhance cold tolerance in fish remains unexplored.

This study aimed to investigate the effects of Job's tears as a feed additive on cold tolerance in *L. rohita*. It was hypothesized that dietary supplementation with Job's tears would improve physiological, biochemical, and molecular responses to cold stress, with varying effects depending on the dosage.

MATERIALS AND METHODS

Experimental setup and fish stocking

Three hundred and sixty *L. rohita* fingerlings (initial weight: 15.2±0.3 g) were randomly distributed into 12 FRP tanks (500 liters each tank; 30 fish per tank) in a recirculating aquaculture system. The recirculating aquaculture system was equipped with a centralized aeration system using air blowers and fine-bubble diffusers. Dissolved oxygen was continuously monitored using a digital oxygen meter (Model Lutron PDO-519, Taiwan) and maintained above 5 mg/L through controlled air injection. Oxygen concentration was regulated by adjusting air flow rates and ensuring proper water circulation, with backup oxygen supplementation mechanisms in place to prevent any potential hypoxic conditions. Four treatments were established in triplicate: control (basal diet), low dose (0.5% Job's tears), medium dose (1% Job's tears), and high dose (2% Job's tears). The experiment lasted for 8 weeks.

Feed preparation and composition

Commercial fish feed from ABIS India (32% crude protein, 6% crude lipid) was procured and thoroughly ground using a laboratory grinder. Job's tears grains were separately processed by cleaning, drying, and grinding into a fine powder with particle size <500 µm. The Job's tears powder (moisture content: 10.2±0.3%, crude protein: 15.6±0.4%, crude fat: 5.8±0.2%, crude fiber: 3.5±0.1%, ash: 2.1±0.1%, and carbohydrates: 62.8±0.5%) was precisely weighed and incrementally incorporated into the ground commercial feed at predetermined concentrations (0.5%, 1%, and 2%) using a mixer. The mixing process involved gradual addition and continuous blending for 10 minutes to ensure homogeneous distribution of Job's tears powder throughout the feed matrix. After thorough mixing, the feed was re-pelletized using a laboratory pellet mill to maintain uniform pellet size and consistency, then dried at 40°C for 24 hours and stored at -4°C until use, ensuring feed stability and preventing moisture-induced degradation. The analysis of feed through AOAC methods showed the feeds were isoproteineous and isolipidic (Table 1).

Table 1. The proximate composition (mean ± SE) of the experimental diets was as follows:

Treatment	Crude protein (%)	Crude fat (%)	Ash (%)	Moisture (%)	Gross energy (MJ/kg)
Control	32.1 ± 0.2	6.1 ± 0.1	7.2 ± 0.1	8.5 ± 0.1	17.8 ± 0.2
Low dose (0.5%)	32.3 ± 0.2	6.0 ± 0.1	7.3 ± 0.1	8.4 ± 0.1	17.9 ± 0.2
Medium dose (1%)	32.5 ± 0.3	6.0 ± 0.1	7.4 ± 0.1	8.3 ± 0.1	18.0 ± 0.2
High dose (2%)	32.8 ± 0.3	5.9 ± 0.1	7.5 ± 0.1	8.2 ± 0.1	18.1 ± 0.2

Feeding and management

Fish were fed twice daily (09:00 and 16:00) to apparent satiation, with careful monitoring of feed intake and fish response. Initially, 30 fish were stocked per 500-liter tank, with 20 fish selected for the primary experimental protocol and 10 maintained as a reserve population. Daily feed consumption was calculated by weighing the offered and remaining feed after 30 minutes. Water quality parameters were rigorously monitored using portable meters (Lutron PDO-519, Taiwan; Eutech Instruments PCSTestr 35, Singapore; and API kits, USA), maintaining optimal conditions: temperature 28±1°C, dissolved oxygen >5 mg/L, pH 7.5±0.2, and ammonia-N <0.5 mg/L. Partial water replacement (10-15% daily) was conducted using dechlorinated, temperature-equilibrated water. At the conclusion of the experiment, individual fish were weighed to determine growth performance, with specific growth rate and weight gain calculated using standard aquaculture formulas.

Cold stress test and sample collection

Initially, 30 fish were stocked per tank, and for the cold tolerance test, 20 fish were selected based on uniform size and health status. The remaining 10 fish served as a reserve population for potential replacements and additional measurements. These reserve fish underwent growth monitoring and were used as backup samples if needed. At the conclusion of the 8-week feeding trial, fish were individually weighed to determine final body weight. The initial mean weight was 15.2 ± 0.3 g, and the final mean weights were as follows: control group: 42.5 ± 1.8 g, low dose (0.5%) group: 45.3 ± 2.1 g, medium dose (1%) group: 48.6 ± 2.3 g, and high dose (2%) group: 46.2 ± 2.0 g.

Temperature reduction was conducted using a precision digital temperature control system (Model Lutron PDO-519, Taiwan). The temperature was gradually reduced following a controlled protocol: starting at an initial temperature of 28±1°C, with a reduction rate of 2°C per hour over a total reduction time of 6 hours. The temperature reduction stages were as follows: from 28°C to 24°C during the first hour, from 24°C to 20°C over the second and third hours, and from 20°C to 15°C over the fourth to sixth hours, after which it was maintained at 15°C for 24 hours. Temperature was continuously monitored using calibrated digital thermometers placed at multiple points in each tank to ensure uniform cooling. The survival rate was recorded hourly.

For the collection of blood and liver samples, a subset of six fish per tank group was selected at 0, 12, and 24 hours during the cold stress test. Blood samples, approximately 500μL, were drawn from the caudal vein using heparinized syringes. The blood was immediately centrifuged at 3000× g for 10 minutes at 4°C to separate the plasma. Following blood collection, the same fish were subjected to hepatic sampling. The fish were anesthetized with MS-222 (100 mg/L), and the abdominal region was sterilized with 70% ethanol. A midline laparotomy was performed using sterile surgical instruments, and the liver was carefully excised using pre-cooled surgical scissors and forceps. The liver samples were preserved immediately after excision: portions intended for enzyme activity analysis were snap-frozen in liquid nitrogen, and portions for RNA extraction were stored in RNA later solution. All collected samples were then stored at -80°C for future analyses. All procedures adhered to institutional animal ethics guidelines to minimize animal suffering and ensure ethical standards were met.

Biochemical analysis

Plasma cortisol levels were quantified using a commercial ELISA kit (Thermo Fisher Scientific, USA), which has a sensitivity of 0.1 ng/mL and an intra-assay coefficient of variation of <8%. Antioxidant enzyme activities were assessed using assay kits (Sigma Aldrich, USA). All assays were performed according to the manufacturer's standardized protocols, with spectrophotometric measurements taken using a microplate reader (Thermo Scientific, USA).

Gene expression analysis

Total RNA was extracted from liver samples using TRIzol reagent. cDNA was synthesized and real-time PCR was performed to analyze the expression of cold-tolerant genes: cold-inducible RNA-binding protein (cirbp), fatty acid desaturase 2 (fads2), and glucose-6-phosphate dehydrogenase (g6pd) (Table 2) . â-actin was used as a reference gene (Zhou et al., 2018).

Table 2. Primer sequences for target genes used in gene expression analysis

Gene	Primer Direction	Sequence
cirbp	Forward	5'-TGGATCAGAACGTGGATGAG-3'
	Reverse	5'-CTCCACCACCTTCTCCTTCA-3'
fads2	Forward	5'-ATGGCACTTCAGCTCATCCT-3'
	Reverse	5'-GTCTGCCTGTAGGTCTCCAA-3'
g6pd	Forward	5'-GTCGTCCTCGGTGTCCTCTA-3'
	Reverse	5'-TGCCTTGTCACTCTGGTTGA-3'
β-actin	Forward	5'-CCGTGACCTGACTGACTACCT-3'
	Reverse	5'-CTTGATGTCACGCACGATTTC-3'

PCR reactions were performed in triplicate using a real-time PCR system (e.g., Applied Biosystems 7500). The PCR conditions were as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Melting curve analysis was performed to confirm the specificity of the amplification. The relative expression levels of target genes were calculated using the $2^{-ÄÄCt}$ method, with â-actin as the internal control.

Data normality was first assessed using the Shapiro-Wilk test. Homogeneity of variance was examined using Levene's test to confirm the assumption of equal variances across groups. After confirming that the data met the parametric test assumptions (normality and homogeneity of variance), data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Differences were considered statistically significant at p<0.05. All statistical analyses were performed using SPSS 21.0 (version 21.0, IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

Survival rate

The medium dose (1% Job's tears) group showed the highest survival rate (92.5±2.5%) during the cold stress test, significantly higher than the control (75.0 ± 3.3%), low dose (82.5±2.9%), and high dose (85.0±3.0%) groups (p<0.05) (Table 3). This suggests that the 1% supplementation level of Job's tears provides optimal protection against cold stress in *L. rohita*.

Table 3. The survival percentages of fish under different treatments

Treatment group	Initial fish	Sampling removals	Remaining fish	Survival rate (%)
Control	60	18 (6 fish × 3 time-points)	42	75.0 ± 3.3^{a}
Low dose (0.5%)	60	18 (6 fish × 3 time-points)	42	82.5 ± 2.9^{b}
Medium dose (1%)	60	18 (6 fish × 3 time-points)	42	92.5 ± 2.5^{c}
High dose (2%)	60	18 (6 fish × 3 time-points)	42	85.0 ± 3.0^{b}

Note: Different superscript letters indicate statistically significant differences (p<0.05)

Plasma cortisol

Plasma cortisol levels increased in all groups during cold exposure, but the increase was significantly attenuated in the Job's tears supplemented groups (Table 4). At 24 hours, the medium dose group showed the lowest cortisol levels (89.3±5.2 ng/mL) compared to the control (152.7±8.9 ng/mL), low dose (118.5±6.7 ng/mL), and high dose (105.8±7.1 ng/mL) groups (p<0.05). These results are consistent with the stress-mitigating effects of Job's tears observed in human models (Manosroi et al., 2016; Devaraj et al., 2020).

The elevated cortisol levels observed in the high-dose group suggest a potential hormetic effect. This aligns with findings from Zhang et al. (2022) and Wang et al. (2024), which indicate that excessive plant-based additives can trigger a mild stress response by activating hypothalamic-pituitary-interrenal (HPI) axis mechanisms. Such responses may be attributed to oxidative stress at higher concentrations, challenges in metabolic adaptation, or cellular stress signaling pathways. Similarly, Devaraj et al. (2020) reported dose-dependent stress responses in herbal supplements, emphasizing that optimal benefits are achieved within a narrow supplementation range. In this context, the medium dose (1%) appears to offer a balanced intervention, avoiding the physiological overload observed in the high-dose group.

Table 4. Plasma cortisol levels (ng/mL) during cold exposure

Treatment	0 hours	12 hours	24 hours
Control	45.2 ± 3.1^{a}	98.6 ± 6.8^{a}	152.7 ± 8.9^{a}
Low dose	43.8 ± 2.9^{a}	85.3 ± 5.5^{b}	118.5 ± 6.7^{b}
Medium dose	44.5 ± 3.0^{a}	72.1 ± 4.8^{c}	89.3 ± 5.2^{d}
High dose	44.1 ± 2.8^{a}	79.4 ± 5.2^{bc}	105.8 ± 7.1^{c}

Note: Different superscript letters indicate statistically significant differences (p<0.05)

Antioxidant enzyme activities

Cold stress induced a significant increase in antioxidant enzyme activities across all groups. However, fish fed Job's tears-supplemented diets showed higher SOD, CAT, and GPx activities compared to the control group (Table 5). The medium dose group exhibited the highest enzyme activities, indicating enhanced antioxidant capacity. This aligns with findings by Zhang et al. (2022) and Mohammady et al. (2022), who reported improved antioxidant status in gibel carp (*Carassius auratus*) and Nile tilapia (*Oreochromis niloticus)* respectively fed with herbal extracts.

The reduced enzyme activity in the high-dose group compared to the medium-dose group suggests a potential adaptive mechanism of metabolic regulation. Similar non-linear responses have been reported by Mohammady et al. (2022) in fish antioxidant systems, where excessive supplementation may lead to the down regulation of antioxidant enzyme expression, metabolic recalibration, or the activation of negative feedback mechanisms. This aligns with the hormetic principle, which posits that moderate stressors can enhance physiological performance, whereas excessive stimulation can result in diminished responses. The medium dose appears to strike an optimal balance, effectively stimulating antioxidant defenses without inducing the compensatory downregulation mechanisms observed in the high-dose group.

Table 5. Antioxidant enzyme activities in liver after 24 hours of cold exposure

Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	125.3 ± 7.2^{a}	42.6 ± 3.1^{a}	18.9 ± 1.4^{a}
Low dose	148.7 ± 8.5^{b}	53.8 ± 3.7^{b}	24.5 ± 1.8^{b}
Medium dose	182.4 ± 10.3^{d}	68.2 ± 4.5^{d}	31.7 ± 2.3^{d}
High dose	165.9 ± 9.4^{c}	61.5 ± 4.0^{c}	28.3 ± 2.0^{c}

Note: Different superscript letters indicate statistically significant differences ($p<0.05$)

Gene expression

Expression of cold-tolerant genes (cirbp, fads2, and g6pd) was significantly upregulated in all groups during cold exposure, with the Job's tears-supplemented groups showing higher expression levels than the control (Table 6). The medium dose group demonstrated the highest fold changes in gene expression at 24 hours: cirbp (5.8-fold), fads2 (4.2-fold), and g6pd (3.9-fold) relative to the control group ($p < 0.05$).

Table 6. Gene expression (fold change relative to control) after 24 hours of cold exposure

Treatment	cirbp	fads2	g6pd
Control	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}
Low dose	3.2 ± 0.3^{b}	2.5 ± 0.2^{b}	2.3 ± 0.2^{b}
Medium dose	5.8 ± 0.5^{d}	4.2 ± 0.4^{d}	3.9 ± 0.3^{d}
High dose	4.5 ± 0.4^{c}	3.4 ± 0.3^{c}	3.1 ± 0.3^{c}

Note: Different superscript letters indicate statistically significant differences ($p<0.05$)

The upregulation of cirbp, which plays a crucial role in cold adaptation by stabilizing mRNA (Zhong et al., 2021), suggests enhanced cold tolerance mechanisms in Job's tears-fed fish. Similarly, the increased expression of fads2 and g6pd indicates improved lipid metabolism and cellular redox balance, respectively, which are essential for cold adaptation (Zheng et al., 2014; Xuet al., 2021). Our findings align with broader research in aquaculture feed supplementation. Studies by Muzaffar et al. (2017) on garlic, Khan et al. (2018) on Aloe vera, and Jain et al. (2020) on carrot meal have similarly demonstrated that strategic feed additives can significantly enhance fish performance. These studies collectively emphasize the potential of natural supplements to improve growth, survival, and physiological responses in fish.

The medium dose exhibited significantly higher gene expression compared to the high dose, indicating that moderate dietary supplementation may optimize gene transcription mechanisms. This observation is consistent with findings by Zheng et al. (2014) and Xu et al. (2021), who suggest that moderate supplementation enhances cellular signaling pathways, regulates gene expression more effectively, and promotes a balanced metabolic stress response. Further supported by studies from Kuebutornye et al. (2023) and Wang et al. (2024), the 1% supplementation level appears to create an ideal physiological environment for the expression of genes associated with cold adaptation.

CONCLUSION

This study demonstrates that dietary supplementation with Job's tears, particularly at a

1% inclusion level, significantly enhances cold tolerance in *L. rohita*. The improved survival rate, reduced stress response, enhanced antioxidant capacity, and upregulation of cold-tolerant genes suggest that Job's tears is a promising feed additive for mitigating cold stress in aquaculture. The findings reveal the potential of Job's tears to address the critical issue of cold stress in *L. rohita* cultivation, which could have far-reaching implications for aquaculture in subtropical and temperate regions.

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Effect of Dietary Inclusion of Fermented Toasted Guar Meal on Growth Performance and Carcass Characteristics in Broiler Chicken

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ABSTRACT

This experiment was designed to investigate the effect of dietary inclusion of fermented toasted guar meal (FTGM) on performance and carcass characteristics in broiler chicken. Two hundred and fifty, day-old commercial broiler chicks were randomly allotted to 5 dietary treatments with 10 replicates containing 5 birds in each replicate. Dietary treatments consisted of a control diet and 4 test diets where toasted guar meal (TGM) and FTGM were included at 15 and 20 % levels respectively. Dietary inclusion of TGM or FTGM at 15 and 20 %, shown significant ($P<0.01$) depression on body weight gain (BWG) and feed intake of broilers compared to control group. However, the BWG in FTGM 15 was significantly ($P<0.01$) higher than TGM 15. No difference in BWG of broilers in 20 % TGM and FTGM groups. The feed intake of broilers in TGM or FTGM groups at same inclusion levels was comparable. Significantly poorer FCR was observed in TGM 15 and 20 groups. However, dietary inclusion of FTGM 15, improved the FCR and was comparable to control and significantly ($P<0.01$) better than TGM 15 group. Whereas, the FCR in TGM 20 and FTGM 20 groups was comparable. Dietary treatments did not influence the carcass characteristics of broilers. In summary, the fermented toasted guar meal can be included up to 15 % in the diet of broilers without showing any adverse effect on FCR and carcass characteristics.

KEYWORDS: Broiler, Fermented toasted gaur meal, Growth Performance and Carcass Characteristics

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INTRODUCTION

Inadequate availability and escalating prices of quality feed ingredients have made it increasingly difficult to formulate a balanced and least-cost poultry feed. This scenario encourages the exploration of alternative feed resources instead of traditional sources. Guar meal (GM) emerges as a promising option as protein source owing to its widespread availability and favorable nutritional characteristics (Ahmed et al., 2015). Guar meal, a by-product of gum extraction, is composed of both hulls and germ, with the ground seed typically having an approximate ratio of 25% germ to 75% hull (Lee et al., 2005). Guar meal is regarded as a valuable protein source because of its high crude protein content (35–45%) and its well-balanced amino acid profile, particularly its elevated levels of lysine and arginine which make it a promising alternate ingredient to soybean meal for poultry diets (Saeed et al., 2017). However, guar bean contains some anti-nutritional factors (ANF) like trypsin inhibitor and galactomannan (guar gum) which negatively affect feed conversion ratio and growth performance in broilers (Mohayayee and Karimi, 2012). Effective strategies such as toasting or fermentation can enhance the incorporation of GM into poultry rations. Toasting is a process where guar meal is subjected to a temperature of 100°-140°C for 10 to 40 minutes (Rao et al., 2019a). The process breaks down complex carbohydrates and anti-nutritional factors present in guar meal, making it more digestible and making the meal safer for consumption by birds. Nidhina and Muthukumar (2015) found that heat treatment (toasting) significantly ($P<0.01$) reduced the emulsifying and foaming properties of industrial GM, which may help in better utilization of GM compared to raw guar meal. The inclusion of toasted guar meal in the diet of broilers improved the growth performance and nutrient digestibility (Ahmed and Abou-Elkhair, 2016).

Fermentation is one of the strategy used nowadays to alleviate the detrimental effects of ANFs in guar meal (Xu et al., 2012). The fermentation process improves the nutritional value of protein sources by increasing the crude protein content while decreasing the crude fiber content (Sugiharto et al., 2016). Previous studies by Sun et al. (2013) and Jazi et al. (2017) reported that fermented cotton seed meal can be included upto 8 and 20 % respectively, in the diet of broiler chicken without any adverse effect on performance and carcass characteristics and it improved gut health in chickens. Dinani et al. (2010) reported that dietary inclusion of 15 % FTGM in broiler quails, significantly (P<0.05) improved the performance when compared to unfermented TGM 15 fed group. The studies on inclusion of different fermented protein sources are available in broilers, however works on inclusion of fermented toasted guar meal at higher levels in broilers are not available. Hence the present study was conducted to evaluate the effect of dietary inclusion of fermented toasted guar meal at 15 and 20 % on performance, carcass characteristics and livability of commercial broiler chicken.

MATERIALS AND METHODS

The present research trial was approved by the Institutional Animal Ethics Committee of the College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India vide 02/27/CVSc, Hyd.

Preparation of FTGM

The toasted guar meal used for the study was procured from M/s Mahesh Agro Food Industries (MAFI), Barmer, Rajasthan, India. The cultures of *Lactobacillus fermentum* (MTCC 1745), *Lactobacillus rhamnosus* (MTCC 12920), *Bacillus subtilis* (MTCC 441), *Bacillus clausii* (MTCC 7914) and *Bacillus licheniformis* (MTCC 3244) were used for the preparation of FTGM. For the fermentation process, each kilogram of TGM was mixed with one litre of water containing 10^8 CFU/ml of above organisms. After proper mixing, the material was packed in fermentation bags and incubated for 84 hours. After incubation period, the FTGM was checked for the pH (desirable pH value is below 4.5). If the pH falls below 4.5, it indicates successful fermentation, the FTGM was kept for sun drying for 2-3 days and stored until it mixed in the experimental diets.

Experimental Design and Diets

To conduct the proposed study, two hundred and fifty, (250) day-old commercial broiler chicks were randomly allotted to 5 dietary treatments with 10 replicates containing 5 birds in each replicate. Dietary treatments include T1- Control, T2- Toasted guar meal 15 % (TGM 15), T3- Toasted guar meal 20 % (TGM 20), T4- Fermented toasted guar meal 15 % (FTGM 15) and T5- Fermented toasted guar meal 20 % (FTGM 20). Broiler pre-starter (1-14 days), starter (15-28 days) and finisher (29-42 days) diets were formulated to contain 22 %, 20 % and 19 % crude protein and metabolizable energy was 2950, 3050 and 3150 kcal/kg respectively as per Vencobb recommendations (Table 1). During the experimental period, feed and water were given *ad-libitum* to broilers. The birds were vaccinated for as per standard protocols. At the end of the trial (42d of age), 10 birds per treatment (1 bird per replicate) were slaughtered to study the carcass characteristics. The bird weighing to the mean body weight of the respective treatment was selected, fasted overnight and slaughtered by cervical dislocation to study the relative weights of organs like liver, heart, gizzard and percentage of dressing yield, breast yield and abdominal fat. The data was analyzed using the General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 20th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at P<0.05.

Table 1. Ingredient (kg/ton) and nutrient composition (%) of broiler diets

Ingredient	Pre-starter			Starter			Finisher		
	Control	TGM 15 /FTGM 15	TGM 20/ FTGM 20	Control	TGM 15 /FTGM 15	TGM 20/ FTGM 20	Control	TGM 15 /FTGM 15	TGM 20/ FTGM 20
Maize	562.08	570.59	573.53	621.22	629.73	632.65	633.59	642.19	645.13
Oil- veg	26.68	24.26	23.48	29.68	27.26	26.48	43.00	40.61	39.83
Soybean meal	359.64	201.39	148.40	304.74	146.49	93.54	283.05	124.56	71.56
Salt	3.18	3.25	3.28	3.19	3.27	3.29	2.95	3.02	3.05
Sodium Bicarbonate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Dicalcium Phosphate	26.61	26.94	27.06	16.48	16.82	16.93	14.95	15.29	15.41
Limestone Powder	8.85	7.83	7.49	12.07	11.05	10.71	11.32	10.31	9.97
DL-Methionine	3.44	3.44	3.44	3.28	3.28	3.28	2.88	2.88	2.89
L - Lysine HCL	2.46	4.70	5.46	2.9	5.14	5.89	2.31	4.56	5.31
L - Threonine	1.27	1.77	1.94	0.95	1.46	1.63	0.45	0.96	1.13
L - Tryptophan	0	0	0.11	0	0	0.09	0	0.11	0.22
Choline chloride 75%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Toxin Binder	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace Mineral Mix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Coccidiostat[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Potassium Chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
TGM/ FTGM	0	150	200	0	150	200	0	150	200
Nutrient composition (calculated)									
M.E (kcal/kg)	2950	2950	2950	3050	3050	3050	3150	3150	3150
Protein %	22	22	22	20	20	20	19	19	19
Dig Lysine %	1.26	1.26	1.26	1.16	1.16	1.16	1.06	1.06	1.06
DigMet. %	0.645	0.644	0.644	0.607	0.606	0.605	0.557	0.556	0.556
Dig Met+ Cyst %	0.94	0.94	0.94	0.88	0.88	0.88	0.82	0.82	0.82
Calcium %	0.94	0.94	0.94	0.8	0.8	0.8	0.74	0.74	0.74
Available P %	0.58	0.58	0.58	0.4	0.4	0.4	0.37	0.37	0.37
Sodium %	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.17	0.17
Dig Tryptophan %	0.24	0.21	0.21	0.21	0.18	0.18	0.20	0.18	0.18
Dig Threonine %	0.88	0.88	0.88	0.78	0.78	0.78	0.7	0.7	0.7

[1]Trace mineral mix provided per kg diet: Manganese, 120 mg; Zinc, 80 mg; Iron 25 mg; Copper, 10 mg; Iodine, 1 mg and Selenium, 0.1 mg. [2]Coccidiostat: 1% Maduramicin ammonium at 10g/kg.
[3] Vitamin premix provided per kg diet: Vitamin A, 20000 IU; Vitamin K, 2 mg; Vitamin E, 10 mg; Riboflavin, 25 mg; Vitamin B_1, 1 mg; Vitamin B_6, 2mg; Vitamin B_{12}, 40 mcg and Niacin 15 mg.
*TGM- Toasted guar meal **FTGM- Fermented toasted guar meal

RESULTS AND DISCUSSION

Growth performance

Body weight gain (BWG)

The results showed that body weight gain of broilers was significantly (P<0.01) higher in control group when compared to other treatment groups (Table 2). Dietary inclusion of TGM at 15%, significantly (P<0.01) depressed the BWG of broilers. Whereas at 20% TGM inclusion, BWG was further significantly depressed. The BWG of broilers in FTGM 15 group was significantly (P<0.01) higher than TGM 15 group, but was lower than control group. However, the BWG of broilers in TGM 20 and FTGM 20 groups was comparable with no significant difference among them.

The depression on BWG of broilers with dietary inclusion of TGM at 15 and 20 % in present study was in accordance with the reports of Dinani et al. (2010) who reported dietary inclusion of 15% of TGM in broiler quails, significantly reduced body weight when compared to the control group. Similarly, Reddy et al. (2017) and Rao et al. (2018) found significant (P<0.01) depression on BWG of broilers with dietary inclusion of TGM at higher levels (15, 18 and 20%) when compared to control diet fed birds. The poor growth by birds which are fed on 15 and 20% TGM diet might be due to higher intake of guar gum (galactomannan), which increases the digesta viscosity, suppresses nutrient digestibility and finally causes growth depression in broilers (Rao et al., 2017). Branched-chain amino acids like valine, leucine and isoleucine would likely be limiting with increased inclusion levels of TGM, especially in 15 and 20 % which might be also responsible for growth reduction (Reddy et al., 2017).

Dietary inclusion of 15% FTGM in broilers, significantly (P<0.01) improved the BWG when compared to the unfermented TGM 15 % group. The present findings are in accordance with Dinani et al. (2010) who reported a significant improvement in the BWG of broiler quails that are fed with a diet containing 15% FTGM when compared to the TGM 15% based diet fed group. Similarly, Park and Kim (2019) reported that dietary inclusion of 10% guar meal fermented with *Bacillus subtilis* in broilers, significantly (P<0.01) improved the BWG when compared to birds that are fed on unfermented guar meal. The improved BWG in fermented TGM based diet fed birds might be due to the positive effects of fermentation. The fermentation process enhances the nutritional value of TGM through the degradation of fibers, breakdown of protein (more amino acids available) and non-starch polysaccharides (NSP) leading to a reduction in anti-nutrients and thus increasing the nutrient availability and improved absorption resulting in improved growth performance (Jazi et al., 2019).

Feed intake (FI)

Results of the present study indicated that FI of broilers was significantly (P<0.01) higher in the control group when compared to other treatment groups (Table 2). The inclusion of TGM and FTGM at higher levels (15 and 20 %) significantly (P<0.01) depressed the feed intake. With dietary inclusion of 20 % TGM or FTGM, the feed intake of broilers was further depressed. There was no significant difference between the TGM and FTGM groups at their respective inclusion levels in terms of feed intake.

The present findings are in accordance with the reports of Rao et al. (2018) who found that feed intake of broilers which are fed on diet containing 15 and 20 % TGM was significantly (P<0.01) depressed. Similarly, Rao et al. (2019b) with dietary inclusion of 10 % of TGM and Jazi et al. (2017) with 10 and 20 % of cotton seed meal in broilers observed significant (P<0.05) depression on feed intake. Reduced feed intake of broilers with increased levels of TGM in this study might be due to higher levels of anti-nutritional factors like residual gum, fiber and saponins (Reddy et al., 2017).

Feed conversion ratio (FCR)

The FCR was significantly (P<0.01) poorer with inclusion of TGM at 15 and 20 % in the diet of broilers. The significantly (P<0.01) better FCR was recorded in the FTGM 15 group of birds which is on par with control group and significantly (P<0.01) better than other treatments (TGM 15, TGM 20 and FTGM 20).There was no significant difference between the TGM 15, TGM 20 and FTGM 20 groups in terms of FCR (Table 2).

The present findings of poorer FCR in broilers with dietary inclusion of TGM at higher levels were in accordance with reports of Reddy et al. (2017) who reported TGM inclusion at 15 and 18 % in the diet of broilers, had significant (P<0.05) depression on FCR. Similarly, Rao et al. (2018) found that FCR was significantly (P<0.05) poorer as the level of TGM

(5, 10, 15 and 20 %) increased in the diet of broiler chicken. Similar findings of poorer FCR with higher inclusion level of guar meal in the diet were reported by Gharaei et al. (2012), Nassrallah (2016), Pashamwar et al. (2018) in broilers and Rao et al. (2014) in Vanaraja chicken and Dinani et al. (2010) in broiler quails.

Poorer FCR with higher levels of TGM inclusion in diet might be due to increased intestinal viscosity and the presence of anti-nutritional factors, which hinder nutrient absorption and digestion. This results in reduced growth performance and feed efficiency in the birds.

Similar to the findings of improved FCR in broilers which are fed on FTGM 15 based diet in present study, Dinani et al. (2010) reported that incorporation of 15 % of FTGM in broiler quail diets, significantly (P<0.01) improved the FCR as similar to the control group. Park and Kim (2019) observed an improvement in FCR of broilers with dietary inclusion of FTGM at 10 % when compared to unfermented TGM based diet fed birds. Similar findings of improved FCR with different fermented feeds were reported by Chiang et al. (2010) with fermented rapeseed meal, Nie et al. (2015) and Jazi et al. (2017) with fermented cotton seed meal, Elbaz (2021) with fermented canola meal and Supriya et al. (2023) with fermented soybean inclusion in the diet of broilers.

Improved FCR with dietary inclusion of fermented toasted guar meal might be due to the improved nutritive value of TGM after fermentation by breakdown of anti-nutritional factors, increased nutrient availability and increased activity of digestive enzymes that lead to enhanced feed efficiency (Feng et al., 2007).

Table 2. Effect of dietary inclusion of TGM and FTGM on growth performance of commercial broiler chicken

Diets	BWG	FI	FCR
T1 Control	2297.77^{a}	3500.17^{a}	1.524^{a}
T2 TGM 15	2119.25^{c}	3361.03^{b}	1.586^{b}
T3 TGM 20	2025.73^{d}	3260.16^{bc}	1.610^{b}
T4 FTGM 15	2209.07^{b}	3341.5^{b}	1.515^{a}
T5 FTGM 20	2015.15^{d}	3204.59^{c}	1.590^{b}
P- Value	0.001	0.001	0.001
N	10	10	10
SEM	9.28	13.31	0.006

Values bearing different superscripts within a column are significantly (P<0.01) different
P-value: Probability value, SEM: Standard Error Mean,
Each pen is a replicate of 5 chicks, N: Number of replicates,
TGM- Toasted guar meal, FTGM- Fermented toasted guar meal

Carcass Characteristics

The incorporation of either TGM or FTGM at 15 and 20 % level in broiler diets did not affect the dressing percentage, breast yield, giblet yield and abdominal fat yield (Table 3).

The present findings were supported by Reddy et al. (2017) who found non- significant effect on carcass yield with dietary inclusion of TGM at graded levels (0, 6, 9, 12, 15 and 18 % of diet) in broilers. Rao et al. (2018) with inclusion of TGM at 5, 10, 15 and 20 % of diet in broilers found non-significant effect on carcass yield and abdominal fat yield. Similarly, Rao et al. (2019a) with dietary inclusion of 6, 12 and 18 % of commercial guar meal and Rao et al. (2019b) with TGM 10 % inclusion in diet of broilers found non-significant effect on slaughter variables (ready to cook yield, abdominal fat and giblet yield).

Table 3. Effect of dietary inclusion of TGM and FTGM on carcass characteristics of commercial broiler chicken

Diets	Dressing percentage %	Breast yield %	Abdominal fat %	Giblet %
T1 Control	69.07	22.57	1.10	4.13
T2 TGM 15	69.18	21.97	0.98	4.32
T3 TGM 20	68.25	21.26	1.16	4.61
T4 FTGM 15	69.52	22.77	0.95	4.24
T5 FTGM 20	68.57	21.81	1.19	4.47
N	10	10	10	10
P- Value	0.300	0.324	0.512	0.078
SEM	0.19	0.14	0.05	0.06

P-value: Probability value, SEM: Standard Error Mean, Each pen is a replicate of 5 chicks, N: Number of replicates, TGM-Toasted guar meal and FTGM- Fermented toasted guar meal

Livability and Mortality

The mortality and livability of broilers in different dietary treatments groups was presented in Table 4. Mortality during the experimental period ranged from 4 to 6 % and livability ranged from 94 to 96 % in different dietary treatment groups. It was observed that mortality and livability was not influenced by the dietary inclusion of TGM or FTGM diets. The mortality of birds during pre-starter and finisher phases may be due to unabsorbed yolk conditions and heat stress, respectively. Mortality recorded was within the acceptable range and no specific disease outbreak was observed during the experiment.

Table 4. Effect of dietary inclusion of TGM and FTGM on cumulative mortality (phase wise) in broiler chicken

Treatments	No. of birds housed	No. of birds died	Percent cumulative mortality				Livability
			Pre starter phase (0-2 weeks)	Starter phase (3-4 weeks)	Finisher phase (5-6 weeks)	Overall period (0-6 weeks)	(%)
T1 Control	50	2	-	2.00	2.00	4.00	96
T2 TGM 15	50	2	2.00	-	2.00	4.00	96
T3 TGM 20	50	3	2.00	2.00	2.00	6.00	94
T4 FTGM 15	50	2	-	2.00	2.00	4.00	96
T5 FTGM 20	50	3	-	4.00	2.00	6.00	94

In agreement with these results, Mishra et al. (2013) stated that dietary inclusion of roasted guar korma in broilers did not affect mortality. Sun et al. (2013) reported that dietary inclusion of fermented cottonseed meal up to 12 % in the diet did not show any effect on mortality of broilers. Li et al. (2022) found no significant effect on the mortality of broilers that are fed on fermented feed based diet up to 25 % inclusion level.

CONCLUSION

Based on the overall results, it is concluded that fermented toasted guar meal can be included up to 15 % of the diet without any adverse effect on FCR and carcass characteristics in broiler chicken.

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Performance of Broiler Chicken Supplemented with Zinc oxide Nano Particles

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ABSTRACT

The study aimed to evaluate the effects of zinc oxide nanoparticles (ZnONPs) supplementation on the growth performance, gut microbiota, and immunity of broiler chickens. Two hundred fifty day-old mixed-sex commercial broiler chicks (Vencobb 400) were randomly divided into five groups with five replicates of 10 chicks each. The dietary groups included: 1) basal diet without Zn supplementation (CON), 2) basal diet with 20 ppm Zn from inorganic ZnO (ZnO20), 3) basal diet with 40 ppm Zn from inorganic ZnO (ZnO40), 4) basal diet with 20 ppm Zn from ZnONPs (ZnONPs 20), and 5) basal diet with 40 ppm Zn from ZnONPs (ZnONPs40).There were no significant differences in body weight, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), or carcass characteristics among the groups. However, ZnONPs supplementation significantly reduced the count of *Salmonella spp.* ($P<0.001$) and *Clostridium spp.* ($P<0.001$) compared to the control and ZnO groups. Antibody titers against Newcastle disease vaccine were not affected by zinc supplementation. ZnONPs did not impact zinc concentration in tibia and breast tissues but significantly increased zinc concentration in liver tissues compared to ZnO and control groups. Copper concentration in tibia, liver, and breast was not affected by Zn supplementation. In conclusion, ZnONPs supplementation shows promise in broiler production, with beneficial effects on gut microbiota and immunity in broiler chickens.

KEYWORDS: Broiler chickens, Growth , Gut miocrobiota , Immunity, Zinc oxide nano particles

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INTRODUCTION

Zinc (Zn) is a crucial trace element essential for the overall functioning of all organisms. Zinc is incorporated into chicken diets in inorganic forms, including zinc oxide (ZnO) and zinc sulphate (ZnSO4), as well as organic forms such as zinc acetate, zinc propionate, zinc methionine, and zinc proteinate. However, the bioavailability of zinc from inorganic sources is low, requiring higher levels to meet animal requirements and potentially disrupting mineral balance. The emergence of nanotechnology has introduced zinc oxide nanoparticles (ZnONPs) as a promising alternative to traditional zinc sources in livestock and poultry nutrition. Nanoparticles, with sizes ranging from 1 to 100 nm, possess increased surface area and unique physical, chemical, and biological properties compared to conventional materials. These nano mineral particles offer enhanced bioavailability and absorption due to their larger surface area, allowing for improved interactions with other molecules (Tsai et al., 2016).

Recent studies have highlighted the benefits of ZnONPs in poultry diets, showcasing their superior surface activity, catalytic efficiency, and adsorption qualities compared to conventional sources. Supplementation of ZnONPs at levels of 10 to 100 mg/kg has been shown to enhance broiler growth performance (Zhao et al., 2014; Ahmadi et al., 2017; Yusof et al., 2023). ZnONPs also exhibit antimicrobial properties, with their effectiveness dependent on size and shape, making them versatile for various applications (Siddiqi et al., 2018). Furthermore, ZnONPs offer a potential alternative to antibiotic growth promoters in poultry diets, given their broad-spectrum antimicrobial activity and reduced environmental impact. Research on the effects of zinc nanoparticles on broiler chickens is crucial for optimizing poultry productivity and sustainable agriculture practices. By evaluating the impact of ZnONPs on growth performance, gut microbiota, immunity, and tissue zinc concentrations in broiler chickens, this study aims to provide valuable insights into the role of zinc nanoparticles in poultry nutrition.

MATERIALS AND METHODS

Experimental design and bird husbandry

This study was conducted at the Experimental Poultry Farm in the Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India following CPCSEA Guidelines for Poultry/Bird Facility (2020). Two hundred fifty day-old mixed-sex commercial broiler chicks (Vencobb 400) were randomly divided into five groups with five replicates of 10 chicks each. The dietary groups included: 1) basal diet without Zn supplementation (CON), 2) basal diet with 20 ppm Zn from inorganic ZnO (ZnO20), 3) basal diet with 40 ppm Zn from inorganic ZnO (ZnO40), 4) basal diet with 20 ppm Zn from ZnONPs (ZnONPs 20), and 5) basal diet with 40 ppm Zn from ZnONPs (ZnONPs40).

A basal diet was formulated with maize and soybean meal to meet the nutritional requirements of Vencobb broiler chickens, excluding zinc. The diets were prepared in mash form, with the basal diet lacking supplemental zinc. Zinc oxide (ZnO) from HiMedia Laboratory Pvt. Ltd, Mumbai, India, or zinc oxide nanoparticles (ZnONPs) from the Department of Materials Sciences and Engineering, IIT, Kanpur, India, were added to the basal diet using the top-dressing method and thoroughly mixed. The cryo milling process was used to prepare ZnONPs (Kumar and Biswas, 2015). Chicks were fed a starter diet from 1 to 14 days, a grower diet from 15 to 28 days, and a finisher diet from 29 to 35 days. Feed and water were provided *ad libitum* until 35 days. The ingredient and chemical composition of the basal diet are detailed (Table 1).

Table 1. Ingredient and chemical composition of basal diets

Ingredients (%)	Starter	Grower	Finisher
Maize	62.1	67.9	69.2
Hi Pro Soybean	32.7	27.3	25.5
Soybean oil	1.71	1.54	2.34
Di- Calcium Phosphate	1.36	1.21	0.95
Calcite Powder	0.91	0.88	0.83
Salt	0.27	0.29	0.22
DL-Methionine	0.28	0.26	0.23
Sodium bi carbonate	0.2	0.1	0.2
L-Lysine HCl	0.2	0.24	0.21
L-Threonine	0.11	0.08	0.04
Vitamin premix[1]	0.1	0.1	0.1
Choline Chloride 60%	0.05	0.07	0.07
Coccidiostat	0.03	0.03	0.03
Phytase	0.01	0.01	0.01
Trace mineral premix[2]	0.05	0.05	0.05
Chemical composition (%)			
ME(Kcal/kg)[3]	2925	3025	3100
Crude Protein[4]	21.6	19.3	18.7
Ether Extract[4]	4.57	4.62	5.50
Calcium[4]	0.91	0.85	0.75
Available Phosphorous[3]	0.45	0.42	0.38
Dig. Lysine[3]	1.22	1.12	1.05
Dig. Methionine[3]	0.58	0.54	0.50
Dig. M+C[3]	0.91	0.85	0.80
Dig. Threonine[3]	0.83	0.73	0.66
Zn (mg/kg) [4]	54.4	46.0	44.8
Cu (mg/kg) [4]	6.64	7.58	6.78

[1]Contains (per kilogram): vitamin A, 80,000,000 IU; vitamin D3, 16,000,000 I.U; vitamin E, 64 g; vitamin K, 8 g; vitamin B1, 6.4 g; vitamin B2, 40 g, niacin, 96 g, pantothenic acid, 64 g; vitamin B6, 12.8 g; folic acid 6.4 g; vitamin B12, 0.164 g; and biotin, 0.24g. [2]Contains (per kilogram): 10 g Cu from $CuSO_4$, 80 g Fe from $FeSO_4$, 80 g Mn from $MnSO_4$, 0.70 g I from KI and 0.2 g Se from Sodium Selenite. [3]Calculated value. [4]Analysed value.

The birds were housed in the experimental poultry farm under standard hygienic, management conditions and strict bio-security measures, with each treatment group maintained in their respective pens.

The initial body weight (BW) of all chickens was recorded on the first day of the trial. Weekly weight measurements were taken, with a final measurement on the last day in the morning. Average daily gain (ADG) was calculated for each replicate. Weekly feed intake was determined by subtracting the remaining feed from the total offered per pen. Average daily feed intake (ADFI) was calculated by dividing the total feed consumed per day by the number of chickens in each pen. Feed conversion ratio (FCR) was calculated by considering cumulative feed intake and weight gain for each replicate pen.

One male and one female bird from each replicate, selected based on average body weight, were slaughtered by cervical disarticulation for carcass trait and gut health evaluation. The hot carcass weight was measured without skin and giblets, and various parts were weighed. Dressing percentage and the proportion of different cut parts were measured and expressed as a percentage of slaughter body weight.

Blood samples were collected from one randomly selected bird from each replicate on days 28 and 35 of the trial. A 2 ml blood sample was obtained via vena brachialis puncture under the bird's wing using sterile syringes and needles. The samples were then transferred to centrifuge tubes without anticoagulant and left at room temperature for 1 hour. After centrifugation at 2500 rpm for 5 minutes, clear serum was collected and stored at -20°C for antibody titres analysis. Antibody titres against Newcastle disease virus vaccine at 28 and 35 days were determined using an Antibody ELISA Kit from IDEXX Laboratories, Inc, USA.

The caecal contents of the birds in each treatment group were collected aseptically after slaughter on the 35th day of the experimental trial. After carefully removing the caecal contents, they were stored at 4°C. Bacteriological enumeration was conducted on the same day. One gram of caecal sample was serially diluted tenfold with sterile phosphate-buffered saline (PBS) solution. From the 4- and 5-fold dilutions, 10 µl was plated on EMB agar, *Clostridial* agar, *Lactobacilli* agar, and BGA for E. coli, *Clostridium* spp, *Lactobacilli* spp, and Salmonella spp, respectively (all from HiMedia Laboratory Pvt. Ltd, Mumbai, India). The plates were then incubated aerobically at 37°C for 24 hours, except for the Lactobacillus spp plate, which was incubated for 48 hours. The *Clostridial* agar plates were incubated anaerobically at 37°C. Colonies of each pathogen were counted using a colony counter (hand held digital colony counter LA663, Hi Media Lab Pvt. Ltd., Mumbai, India), and the results were expressed as Log10 colony-forming units (CFU) per gram of sample.

To determine the mineral concentration in tissues, the left drumstick, breast, and liver were collected separately after slaughter and stored at -20°C until analysis. The tibia was extracted and dried in a hot air oven at 100°C until a constant weight was achieved. Subsequently, the tibia, breast, and liver samples were ashed in a muffle furnace for 3 hours at 550°C. The resulting ash samples were dissolved in 0.5 N nitric acid (HNO_3), heated on a hot plate, cooled to room temperature, and filtered using Whatman filter paper No.1. The zinc (Zn) and copper (Cu) content in the digested samples were quantified using an Atomic Absorption Spectrophotometer (PerkinElmer (India) Pvt. Ltd., Kolkata, India) with an air-acetylene flame.

The feed samples were analyzed using the methods outlined by AOAC (1995). Calcium content was determined following the procedure described by Talapatra et al.(1940). Additionally, the AIA levels of the diets were measured using the technique outlined by Furuichi and Takahashi (1981).

The data were analysed by one-way analysis of variance (ANOVA) using SPSS (2017) in a completely randomized design with a model containing treatment taken as the main effect. Single pen was used as an experimental unit. Probability values of $P \leq 0.05$ were declared as significant. When treatment effect was significant, the differences among the treatment means were detected using Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth performance

The BW during the starter, grower, and finisher periods were not significantly affected by zinc supplementation from either zinc oxide (ZnO) or zinc oxide nanoparticles (ZnONPs) ($P>0.05$). Similarly, ADG, ADFI, and FCR were not significantly different between the groups during the starter (1-14 days), grower (15-28 days), finisher (29-35 days), and overall (1-35 days) periods (Table 2). These results suggest that both nano Zn (ZnONPs) and inorganic Zn (ZnO) supplementation did not impact the growth performance of broiler chickens.

This result align with previous studies by Yusof et al. (2023) and Ramiah et al.(2019). However, in a recent study, Mahmoud et al.(2020) observed higher body weight gain in the 10 mg/kg ZnONPs group compared to the control and other ZnONPs groups. Huang et al.(2007) suggested that optimal live weight gain may be achieved with diet containing 40 mg/kg Zn, as recommended by NRC (1994). In this study, the zinc concentrations in the starter (54.44 mg/kg), grower (46.04 mg/kg) and finisher (44.80 mg/kg) diets were close to the NRC (1994) recommendation.

Table 2. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on growth performance in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
				BW (g)			
Day 14	565.05	552.40	584.10	567.13	573.25	4.201	0.186
Day 28	1529.35	1525.98	1579.18	1534.48	1549.88	11.769	0.646
Day 35	2146.85	2121.25	2126.38	2136.33	2151.93	18.336	0.987
				ADG (g/d)			
Day 1 to 14	37.16	36.19	38.43	37.11	37.83	0.311	0.208
Day 15 to 28	68.88	69.54	71.08	69.10	69.76	0.682	0.893
Day 29 to 35	88.22	85.04	78.17	85.98	86.01	1.701	0.432
Day 1 to 35	60.06	59.30	59.44	59.68	60.24	0.528	0.983
				ADFI (g/d)			
Day 1 to 14	46.53	45.66	47.16	47.13	47.30	0.456	0.812
Day 15 to 28	104.71	106.97	106.71	104.16	106.96	0.905	0.811
Day 29 to 35	153.69	152.12	138.33	146.62	152.53	2.596	0.321
Day 1 to 35	91.23	91.48	89.22	89.84	92.21	0.816	0.809
				FCR			
Day 1 to 14	1.25	1.26	1.23	1.27	1.25	0.009	0.739
Day 15 to 28	1.52	1.54	1.50	1.51	1.54	0.007	0.437
Day 29 to 35	1.75	1.79	1.77	1.71	1.77	0.015	0.541
Day 1 to 35	1.52	1.54	1.50	1.51	1.53	0.006	0.226

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplementalZn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplementalZn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplementalZn at 40 mg/kg feed from ZnONPs.

Carcass characteristics

There were no significant differences (P>0.05) in the percentage of breast, frame, thigh, drumstick, wing, neck, gizzard, heart, liver, abdominal fat, bursa and spleen relative to slaughter body weight among the groups (Table 3). In contrast, Mahmoud et al.(2020)showed that adding ZnONPs (20 mg/kg) to the basal diet increased eviscerated body weight in broiler chickens. Dukare Sagar et al.(2018) also reported increased immune organs weights (bursa, spleen and thymus) in broiler chickens fed nano Zn particles (40 -80 mg/kg). However, our study found no significant effects of heart and liver relative weight, consistent with previous research (Ahmadi et al., 2017; Mohammadi et al,2015).Eskandani et al.(2021) observed that a combination of 50 mg of nano-ZnO and 2g of *Curcuma longa* /kg improved carcass quality, increased protein and decreased fat in broiler breast and thigh. Siddiqi et al.(2018) suggested that dietary Zn supplementation could be reduced to 20 mg/kg when using ZnONPs without affecting growth performance and carcass traits.

Table 3. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on carcass characteristics as percent (%) slaughter body weight in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Slaughter BW (g)	2123.50	2130.00	2138.75	2157.50	2132.75	22.258	0.949
Dressing %	66.72	66.95	65.02	66.91	64.10	0.423	0.116
Breast (%)	25.46	25.82	24.57	24.38	23.13	0.386	0.183
Frame (%)	10.77	11.82	11.24	11.99	11.30	0.154	0.091
Thigh (%)	9.04	9.05	8.43	8.48	8.72	0.144	0.522
Drumstick (%)	9.35	9.38	9.53	9.27	9.17	0.098	0.892
Wing (%)	6.39	5.81	5.46	5.76	5.77	0.104	0.120
Neck (%)	2.99	2.85	2.70	2.34	2.58	0.077	0.095
Gizzard (%)	1.98	2.13	2.09	2.19	1.99	0.048	0.663
Liver (%)	1.85	1.85	1.83	1.87	2.04	0.063	0.889
Heart (%)	0.54	0.63	0.52	0.49	0.59	0.023	0.227
Giblets (%)	4.36	4.61	4.44	4.56	4.63	0.109	0.946
Abdominal fat (%)	1.73	1.55	1.75	1.94	1.97	0.075	0.318
Spleen (%)	0.11	0.10	0.11	0.10	0.12	0.005	0.880
Bursa (%)	0.06	0.05	0.09	0.08	0.09	0.009	0.532

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Caecal bacterial count

There were no significant differences in the numbers of *Escherichia coli* and *Lactobacillus* spp. observed (P>0.05) (Table 4). Supplementation of ZnONPs at 20 and 40 mg/kg significantly decreased the count of *Salmonella* spp. compared to the control group (P<0.001). The ZnONPs 40 group showed a lower count of *Salmonella* spp. compared to the ZnO20, ZnO40, and ZnONPs20 groups. The counts of *Clostridium* spp. were reduced (P<0.01) with the supplementation of either 20 or 40 mg/kg ZnONPs compared to the control, ZnO20, and ZnO40 groups. Overall, ZnONPs supplementation improved intestinal health by reducing pathogenic bacterial counts in the intestine.

Previous researchers have reported the antimicrobial action of metal oxide nanoparticles; Mahmoud et al., 2020; Yusof et al., 2023; Hatab et al., 2024). However, the exact mechanism by which nanoparticles inhibit bacterial growth is not fully understood. Elumalai et al.(2015) proposed that bacterial cell death by ZnONPs is caused by the release of ions that combine with thiol groups of proteins on the cell surfaceleading to protein inactivation, decreased membrane permeability and ultimately cellular death. In the present experiment, ZnONPs reduced the caecal population of *Salmonella* spp and *Clostridium* spp. Similarly, Mahmoud et al.(2020)observed that supplementation with 10, 30 and 40 mg/kg ZnONPs decreased the population of total anaerobic bacteria and coliform in the caecum of broiler chickens. Therefore, the antibacterial effect of ZnONPs observed in this experiment is promising and suggests the potential inclusion of ZnONPs in poultry rations as a replacement for antibiotic growth promoters.

Table 4. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on viable bacteria numbers (log10 CFU/ g) in cecal content in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Escherichia coli	7.55	7.30	7.08	7.33	7.48	0.142	0.883
Salmonella spp	7.65 [a]	7.28 [ab]	7.10 [ab]	6.83[b]	5.85 [c]	0.156	0.000
Lactobacillus spp.	7.53	7.63	7.73	6.90	6.72	0.176	0.261
Clostridium spp.	8.35[a]	8.23[a]	8.55[a]	7.55[b]	7.68[b]	0.107	0.001

[abc]Means bearing different superscripts in the same row differ significantly (P d” 0.05).
[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Immune response

Zinc supplementation, whether from ZnO or ZnONPs, did not have a significant effect (P>0.05) on antibody titres at 28 and 35 days in broiler chickens (Table 5).

This findings aligns with a previous study by Mahmoud et al.(2020), which also found no impact of ZnONPs supplementation (10, 20 and 30 mg/kg) on Newcastle disease haem-agglutination inhibition titre (ND-HI) in broiler chickens. However, the above report mentioned that supplementation with 40 mg/ kg ZnONPs significantly decreased ND-HI titre in compared to the control group. In contrast, Ahmadi et al. (2017) found that replacing ZnO with ZnONPs in the diet improved antibody titre against the Newcastle disease vaccine at 21, 28, 35 and 42 days of age in broiler chickens.

Table 5. Effects of zinc oxide nano particles (ZnONPs) supplementation on antibody titres ($\log_{10}$) against Newcastle disease vaccine in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Day 28	2.74	2.64	2.92	2.65	2.72	0.056	0.527
Day 35	2.99	3.04	3.19	3.07	3.14	0.035	0.501

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Mineral concentration in tissues

Zinc levels in the tibia and breast did not show significant differences (p>0.05) across treatments (Table 6). However, Zn concentrations in the liver were significantly higher (p<0.001) in both inorganic and nano Zn supplemented groups compared to the control group. Cu levels in the tibia, liver, and breast were not affected by Zn supplementation from either source.

The increased zinc concentration in the liver of the ZnONPs groups is consistent with findings from previous studies. Ramiah et al.(2019) observed elevated zinc levels in the liver of broiler chickens supplemented with ZnONPs. The use of ZnONPs in animal diets can affect zinc deposition due to the improved bioavailability of nano zinc particles compared to inorganic sources (Ibrahim et al., 2017). Akbari Moghaddam Kakhki et al.(2017) reported higher zinc levels in the liver and muscle of Zn-supplemented chickens, while Liu et al.(2009) noted increased zinc concentrations in the liver and breast of broiler chickens regardless of the zinc source. This indicates that bone is the most sensitive tissue to dietary zinc, accumulating more zinc than the liver and muscle, acting as a functional reserve that can be redistributed during deficiency (Janet et al., 2000). In this study, zinc supplementation did not affect copper concentrations in the tibia, liver and breast muscle. Similarly, Mishra et al.(2014)found no differences in copper levels in the bone, liver and muscles after varying levels of zinc supplementation in broiler chickens.

Table 6. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on zinc and copper concentrations (mg/kg) in tibia, liver and breast in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
			Zinc				
Tibia	58.5	74.7	74.2	80.2	84.9	4.087	0.329
Liver	12.7^{c}	24.9^{b}	23.6^{b}	34.2^{a}	32.7^{a}	2.041	0.000
Breast	13.1	11.2	13.3	14.6	16.6	0.626	0.063
			Copper				
Tibia	1.10	1.33	1.12	1.39	1.36	0.047	0.125
Liver	2.22	2.46	1.96	2.40	2.05	0.135	0.768
Breast	1.05	0.69	0.89	1.12	0.72	0.069	0.146

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

CONCLUSION

In conclusion, the findings of the study suggest that the supplementation of zinc oxide nanoparticles (ZnONPs) resulted in a notable reduction of pathogenic bacteria residing in the intestinal tract while simultaneously enhancing zinc concentrations within the liver. Despite these positive outcomes, it is important to note that ZnONPs did not yield significant improvements in either the growth performance metrics or the overall immune response of broiler chickens, indicating that while there are benefits related to microbial control and trace mineral status, other performance-related parameters remained unaffected by this supplementation strategy.

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Effect of Organic Acids in Crossbred Weaned Piglets

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ABSTRACT

The present study was aimed to evaluate the effect of dietary organic acids on growth performance, nutrient digestibility and fecal microflora of crossbred weaned piglets. A total of thirty weaned crossbred piglets (75% LWY × 25% Desi) with average body weight of 7.5 ± 0.5 kg (2 months old) were randomly divided (CRD) into five homogenous treatments with six piglets in each. The dietary treatments were T1 (control) - Basal diet as per NRC (2012), T2 - Basal diet + 1.5% Citric acid, T3 - Basal diet + 1.5% Fumaric acid, T4 - Basal diet + 1.5% Benzoic acid, T5 - Basal diet + 1.5% blend of citric, Fumaric acid and Benzoic acids (each at 0.5%). Results revealed that piglets received the diet containing blend of organic acids improved ($P < 0.05$) the ADG, feed efficiency, nutrient digestibility (CP and EE) and reduced fecal *E. coli* counts as compared with piglets received individual organic acids and control. The highest ($P < 0.05$) weight gain was observed in piglets fed T5 or T4 diets, followed by T2 or T3 and lowest in control. There was no effect ($P > 0.05$) of organic acids on average daily feed intake of piglets of different treatments. The digestibility of CF (%) was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared to control. The *Salmonella* count was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared with control. The cost per Kg weight gain (Rs.) was found to be significantly ($P < 0.05$) higher in organic acid supplemented groups as compared to control. In conclusion, dietary supplementation of blend of organic acids at 1.5% indicated beneficial effects on growth performance and nutrient digestibility coexisting with reduced fecal microbiota.

KEYWORDS: Fecal microbiota, Growth performance, Nutrient digestibility, Organic acids and Weaned piglets

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INTRODUCTION

Weaning is one of the most stressful events for pigs, which can cause dysfunction of the gastrointestinal tract (GIT) and immune system, leading to reduced growth performance and feed intake in the first week after weaning (Tang et al., 2017). Use of subtherapeutic doses of antibiotic growth promoters (AGPs) was in practice to alleviate gastrointestinal disturbances and improve microbial balance in the GIT of weaned piglets (Rathnayake et al., 2021). The use of antibiotic growth promoters in swine production contributes to the emergence of antibiotic resistant strains both in livestock and human posing a significant public health threat (Ferronato and Prandini, 2020 and Gois et al., 2016). Thus, exploring the alternatives that can promote growth, improve feed efficiency and reduce enteric diseases in swine has become a trending topic for researchers reduce/ eliminate the use of antibiotic growth promoters.

Among different compounds exploited for the replacement of antibiotics, organic acids (OAs) are potential alternatives as they are known to reduce the load of pathogenic bacteria and modulate the microbial composition in the GIT and enhance the growth performance of pigs (Long et al., 2018; Xu et al., 2018). In recent years, multiple reports have described the beneficial effects of dietary organic acids such as formic, fumaric, citric, butyric, benzoic, lactic and propionic acids by enhancing growth performance (Oh et al., 2018; Xu et al., 2018; Ngoc et al., 2020; Long et al., 2018 and Xiang et al., 2021), nutrient digestibility (Dutta et al., 2015; Yang et al., 2018; Oh et al., 2018 and Bujnak et al., 2021) and modulating the intestinal microflora (Long et al., 2018; Dutta et al., 2015; Bharathidhasan et al., 2022 and Ahmed et al., 2014).

However, the effectiveness of organic acids varies with several factors such as type and dose of their inclusion, dietary composition, age, health status and physiological condition of the animal (Mroz et al., 2006). Hence, the aim of the present study is to evaluate the response of weaned crossbred (LWY×Desi) piglets upon incorporation of single and blend of organic acids.

MATERIALS AND METHODS

Experiment location and ethical statement

The experimental work of the current study was carried out at AICRP on pigs, Sri Venkateswara Veterinary University, Tirupati with the approval of Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Reg No 281/go/ReBi/S/2000/CPCSEA/ CVSc/TPTY/026 /Animal Nutrition /2022 dated 22.06.2022).

Experimental design and animals

In a completely randomized design, thirty crossbred (75% LWY × 25% Desi) weaned piglets with average body weight of 7.5 ± 0.5 kg (2 months old) were divided into five homogenous treatments with six piglets in each.

Housing and feeding of experimental animals

The piglets were housed in individual pens in a well-ventilated semi-opened animal shed having provision for individual feeding and watering. Five iso-nitrogenous and iso-caloric experimental diets were formulated as per NRC (2012) requirements (Table 1). The dietary treatments were T1 (control) - Basal diet as per NRC (2012), T2 - Basal diet + 1.5% Citric acid, T3 - Basal diet + 1.5% Fumaric acid, T4 - Basal diet + 1.5% Benzoic acid, T5 - Basal diet + 1.5% Citric, Fumaric acid and Benzoic acids (each at 0.5%).

Table 1. Ingredient and nutrient composition (%) of experimental diets during weaner phase

Ingredient	T1	T2	T3	T4	T5
Maize	56.9	55.0	55.0	55.0	55.0
Soybean meal	35.6	36.0	36.0	36.0	36.0
DORB	5.0	5.0	5.0	5.0	5.0
Salt	0.5	0.5	0.5	0.5	0.5
Mineral mixture #	1.5	1.5	1.5	1.5	1.5
Lysine	0.5	0.5	0.5	0.5	0.5
Citric acid	-	1.5	-	-	0.5
Fumaric acid	-	-	1.5	-	0.5
Benzoic acid	-	-	-	1.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Proximate composition (%)*					
Dry matter	89.4	88.9	88.8	90.6	90.01
Organic matter	88.2	86.5	87.9	90.1	89.7
Crude protein	18.9	18.9	18.8	18.8	18.9
Ether extract	0.78	1.24	1.01	1.36	1.32
Crude fiber	5.81	7.01	5.02	6.52	6.53
Total ash	8.75	8.43	8.07	7.86	8.28
Acid insoluble ash	2.21	2.95	3.02	2.12	3.06
Nitrogen free extract	65.7	65.4	67.1	65.4	64.9
ME (Kcal/Kg)**	3189.4	3173.5	3179.7	3231.3	3194.8

*On dry matter basis ** Calculated value # Each Kg contained Calcium - 25.5%; Phosphorous - 12.75%; Sulphur - 0.72%; Zinc - 9600 mg; Manganese - 1500 mg; Sodium - 5.9 mg; Magnesium - 6000 mg; Potassium - 100 mg; Iron - 1500 mg; Iodine - 325 mg; Copper - 1200 mg and Cobalt - 150 mg.

Growth trial

Piglets were offered respective experimental rations daily at 9:00 AM and 3:00 PM. Fresh water was offered *ad libitum* daily to all the animals. The growth trial last for 83days (83 days were required for the piglets to reach 25 Kg body weight). The piglets were weighed individually at fortnightly intervals in the morning before feeding and the body weights were recorded to assess the changes in body weight, average daily gain.

Digestion trial

At the end of the growth trial, the piglets were allowed for a period of two days for acclimatization to new system of housing and management followed by a collection period of 7 days. During digestion trial, piglets were offered experimental rations daily at 9:00 AM and 3:00 PM. Fresh water was offered *ad libitum* daily to all the animals. The total quantity of faeces voided during preceding 24 h was weighed, mixed and representative samples (5%) from each piglet was taken separately in a polythene zip lock covers and stored in a deep freezer at -20^{0}C. The faecal sample was dried and ground to pass through 2 mm screen and preserved in air tight bottles for further analysis. The proximate composition of feed and fecal sample was determined as per AOAC (2019).

Fecal microbiota

For enumeration of Faecal microbiota, fresh faecal sample was obtained directly by massaging rectum, collected in an aseptic container and analyzed for faecal pathogens (*E. coli, Salmonella and Staphylococcus spp*). About 1.0 g of composite faecal sample was weighed aseptically and transferred to a sterile test tube and diluted with 9 ml of sterile nutrient broth and was homogenized. Viable count of bacteria in the faecal samples were conducted by plating 10 folds dilution in the nutrient broth on to MacConkey agar, XLD agar and Mannitol salt agar for growth of *E. coli*, *Salmonella and Staphylococcus,* respectively. The plates after incubation at 37^0 C for 24 hours (*E. coli* and *Salmonella*) and 24 – 48 hours (*Staphylococcus*) were counted immediately for the developed colonies. Each colony will account for one viable microbial cell or one colony-forming unit. Therefore, the number of CFU in the sample was determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor.

Cost economics

Economics of feeding was calculated from the records of daily feed consumption and by considering the prevailing market price of feed ingredients and organic acids used for feeding of experimental piglets.

Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) as per the methods of Snedecor and Cochran (1995) and the means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Feed intake of piglets

It revealed that supplementation of different organic acids (either individually or in combination) at 1.5% did not affect ($P > 0.05$) the feed intake of crossbred piglets (Table 2). Similar results by Namkung et al. (2004); Gerritsen et al. (2010) and Zhai et al. (2017) also indicated that the ADFI was not affected by supplementation of organic acids. Contrary to the present study, reduced ADFI upon formic acid supplementation (Ma et al., 2021) and increased ADFI (Diao et al., 2016 and Kludge et al., 2006) upon supplementation of benzoic acid was reported. The variation in these studies might be due to strong pungent odours of some organic acids such as formic acid and propionic acid which may reduce diet palatability, thus decreasing feed intake and thereby negatively impact the growth rate (Kil et al., 2005). In the current study, the organic acids (citric, fumaric and benzoic acids) offered a good acceptability without any considerable difference in the palatability and feed intake of diets in all the experimental groups.

Growth performance and FCR

It was observed that the ADG (g) and feed efficiency during the experimental period of 83 days was highest ($P < 0.05$) in T5 followed T2 or T3 or T4 and lowest in T1 (Table 2). The highest ($P < 0.05$) weight gain was observed in piglets fed T5 or T4 diets, followed by T2 or T3 and lowest in control. Similar results of improved average daily gain and feed efficiency was observed by several researchers (Grilli et al., 2010; Li et al., 2008; Long et al., 2018 and Xiang et al., 2021) in weaner piglets. However, contrary to the present study, Gerritsen et al. (2010)

and Lee et al. (2021) observed no effect of organic acids on growth performance in weaned piglets. These inconsistent results might be attributed to the various factors such as composition of diet, type of acid, purity of the acid, inclusion level of acid, age, breed and health status of the piglets.

Addition of organic acid blend in the current study resulted a positive effect on the growth performance in the weaned piglets. Among several factors which might have involved in improved growth performance in piglets, lowering of gastro-intestinal pH and subsequent modification of intestinal microflora is one of the main reasons for enhanced growth performance in the current study (Kil et al., 2005 and Long et al., 2018). Another possible explanation for improved growth performance was attributed to enhanced digestibility of nutrients. In the current study, increased apparent digestibility of nutrients (CP, EE and CF) was observed in the piglets fed diets containing organic acid/s (Table. 3). As a result of antimicrobial activity of organic acids, there was a reduction in pathogenic microflora such as *E. coli*, *Salmonella* and *Staphylococcus* as evinced by reduction in their count in the fecal matter (Table. 4). This in turn reduces the metabolic needs of microbes, thus enhancing the availability of dietary nutrients to piglets, thereby improved the growth performance. In the current study, blend of organic acids found to be effective as compared to individual organic acid supplementation due to the synergistic activity and dissociation properties (Partanen et al., 2007).

Nutrient digestibility

In the present study, no significant (P > 0.05) difference in digestibility of DM, OM and NFE was observed among different treatments (Table. 3). While there was significant (P< 0.05) difference in digestibility of CP (%) among different treatments and was in the order of T5 > T2 > T3 > T4 > T1. The results agree with several authors who reported improved CP digestibility with supplementation of either single (Diao et al., 2016; Guggenbuhl et al., 2007 and Kiarie et al., 2018) or blend of organic acids (Dutta et al., 2015; Yang et al., 2018 and Bujnak et al., 2021). Contrary to present results, several researchers reported that the digestibility of CP was not affected due to supplementation of either single (Kludge et al., 2006) or blend of organic acids (Kil et al., 2005 and Omogbenigum et al., 2003).

Table 2. Effect of dietary organic acids on growth performance of crossbred weaned piglets.

Particulars	Treatment Groups					P-Value
	T1	T2	T3	T4	T5	
Initial BW (Kg)	7.60±0.53	7.60±0.96	7.50±0.75	7.50±0.54	7.40±0.73	0.863
Final BW (Kg)*	22.6±0.48^{c}	25.4±0.81^{b}	25.1±1.22^{b}	25.7±1.17^{a}	26.2±0.53^{a}	0.031
Weight gain (Kg)*	15.0±0.47^{b}	17.8±0.84^{ab}	17.6±0.67^{ab}	18.2±0.73^{a}	18.8±1.03^{a}	0.032
Feed intake (g)	634.00±2.01	634.30±2.47	636.60±2.29	635.40±2.01	636.60±2.65	0.483
ADG (g/day)*	180.72±7.76^{c}	214.45±10.18^{b}	212.04±8.17^{b}	219.27±8.85^{b}	226.50±12.49^{a}	0.043
FCR*	3.52±0.10^{a}	2.98±0.14^{b}	3.02±0.12^{b}	2.91±0.11^{b}	2.84±0.17^{c}	0.037

^{abc} Values bearing different superscripts in a row differ significantly (P < 0.05) *

Weaned piglets have an unfavorable pH in their stomach because of the limited HCl secretion. Nutrient digestibility is closely related to secretion and functionality of digestive enzymes which is more pronounced at lower pH. Improved CP digestibility associated with organic acid supplementation in the current study might be attributed to the lower pH in the gastrointestinal tract, resulting in increased activity of digestive enzymes (Kim et al., 2005). Low pH is the favorable condition for activation of several digestive enzymes (Mroz, 2005) especially pepsin and trypsin which are the prime enzymes responsible for the digestion of proteins, as evinced in the current study.

Similar trend (T5 > T2 > T3 > T4 > T1) of improved ($P < 0.05$) digestibility of EE was observed in the current research is comparable with the reports of Diao et al. (2016) supplemented with benzoic acid. Contrary to the present study several authors (Bujnak et al., 2021; Dutta et al., 2015; Gerritsen et al., 2010 and Kil et al., 2005) did not indicate any improvement in the digestibility of EE. The improved digestibility of EE observed in the piglets received blend of organic acids could be due to reduced passage rate of digesta into the small intestine and stimulation of the enzyme secretion of the pancreas (lipase, amylase) leading to an increased digestibilities of nutrients (Ma et al., 2021).

The digestibility of CF (%) was higher ($P < 0.05$) in the piglets received organic acids (either individual or blend) as compared with control. Like the present study, Dutta et al. (2015) and Gerritsen et al. (2010) also observed an improvement in CF digestibility. The improved fiber digestion could be attributed to low pH in GIT, inhibiting the activity of enterobacteria (*E. coli* and *Salmonella*), thus creating a better environment for bacteria that ferments crude fibre (Hansen et al., 2007), resulted in better digestibility of CF.

Table 3. Effect of dietary organic acids on nutrient digestibility (%) of crossbred weaned piglets.

Particulars	Treatment Groups					P-Value
	T1	T2	T3	T4	T5	
Dry matter (%)	82.62 ± 1.13	84.37 ± 2.41	84.58 ± 0.91	85.41 ± 0.98	84.67 ± 1.05	0.561
Crude protein (%)*	76.70 ± 4.32^{c}	78.54 ± 5.15^{b}	79.63 ± 4.35^{b}	77.41 ± 3.61^{b}	81.95 ± 2.11^{a}	0.024
Ether extract (%)*	63.76 ± 3.02^{c}	67.93 ± 4.26^{b}	65.45 ± 2.91^{b}	65.23 ± 2.06^{b}	68.78 ± 1.62^{a}	0.043
Crude fiber (%)*	43.14 ± 2.17^{b}	47.54 ± 4.54^{a}	45.78 ± 3.40^{a}	47.58 ± 1.49^{a}	48.19 ± 3.11^{a}	0.048
Organic matter (%)	82.34 ± 1.21	84.34 ± 1.54	85.43 ± 2.43	83.55 ± 2.10	84.94 ± 0.95	0.932
NFE (%)	87.03 ± 1.23	89.35 ± 2.41	87.64 ± 2.42	88.67 ± 3.12	89.47 ± 0.43	0.562

[abc] Values bearing different superscripts in a row differ significantly ($P < 0.01$)**

Fecal microbiota

In accordance to the results of present study (Table. 4), several authors observed a reduction in fecal microbial count by supplementing single (Bharathidhasan et al., 2022) or blend of organic acids (Yang et al., 2019; Long et al., 2018; Dutta et al., 2015; Ahmed et al., 2014). Contrary to the present study, non-significant difference in fecal microbial count was observed due to supplementation of single (Canibe et al., 2001) or blend of organic acids (Lee et al., 2021; Oh et al., 2018; Li et al., 2008; Namkung et al., 2004).

The reduced fecal microbiota in the current study might be attributed to reduced pH in the GIT due to supplementation of organic acid/s. This agrees with Richards et al. (2005) who reported that lower pH in the GIT of weaned piglets resulted in reduced proliferation of pathogenic bacteria (*E. coli*) in the GIT. Supplementation of organic acids influences the intestinal microbiota by changing intestinal environment to a uncongenial conditions for pathogenic bacterial growth (Long et al., 2018) resulting in reduced pathogens as evinced in the current study. Another possible explanation for reduced pathogenic microflora can be attributed to direct anti-microbial activity of organic acids. Organic acids in their undissociated from, passively diffuse through the bacterial cell wall and interfere with bacterial biochemical processes leading to cell death (Kluge et al., 2006 and Graber et al., 2012). Therefore, results of current study demonstrated the favorable impact of supplementation of organic acid/s by reducing pathogenic micro-organisms, thereby contributed to improved growth performance in weaned piglets.

Table 4. Effect of dietary organic acids on fecal microbiota (CFU/ml) of crossbred weaned piglets

Species	Treatment Groups T1	T2	T3	T4	T5	P-Value
E. coli ($\times10^5$)**	5.09±0.24^a	4.93±0.23^b	4.44±0.21^b	4.71±0.22^b	4.15±0.31^c	0.006
Salmonella ($\times10^5$)**	2.18±0.07^a	1.34±0.05^b	1.29±0.12^b	1.38±0.16^b	1.08±0.17^b	0.001
Staphylococcus ($\times10^5$)**	1.54±0.08^a	1.27±0.04^b	0.96±0.04^c	1.26±0.05^b	1.12±0.05^{bc}	0.001

^{abc} Values bearing different superscripts in a row differ significantly ($P < 0.01$)**

Cost economics

There was increased feed cost (Rs./Kg) due to the incorporation of organic acids in the diets as compared to control (Table. 5). The cost (Rs./Kg) was increased by 3.59, 4.04, 2.84 and 3.49 in T2, T3, T4 and T5, respectively as compared to control (T1). The cost variation among the treatments was due to the inclusion of organic acids. The cost of organic acids were in the order of fumaric acid > citric acid > benzoic acid. Hence the diets containing fumaric acid (T3) resulted in the maximum cost and diets containing benzoic acid (T4) resulted in the minimum cost. The cost per Kg weight gain was lowest ($P < 0.05$) in T4 or T5 groups followed by T2 or T3 and highest in T1 group. Though there was an increase in feed cost due to organic acid supplementation, the cost per Kg weight gain (Rs.) was found to be significantly ($P < 0.05$) lower in organic acid supplemented groups as compared to control.

CONCUSION

The present study suggested that dietary inclusion of blend of organic acids at 1.5% improved overall growth performance, nutrient digestibility and reduced fecal pathogenic microbial load. Therefore, the blend of organic acids (citric, fumaric and benzoic acid) can serve as an alternative to antibiotic growth promoters in weaned piglets to promote growth and improve the health status. Further research is warranted to determine if the gut microbiota was altered by organic acids during early stage would lead to long-lasting beneficial effects on swine performance in their later stages.

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Table 5. Effect of dietary organic acids on cost economics of pork production in crossbred weaned piglets

Particulars	Treatment Groups					
	T1	T2	T3	T4	T5	P-Value
Cost of weaners feed (Rs. /Kg)	37.25	40.84	41.29	40.09	40.74	
Total feed intake (Kg /animal)	52.62±1.12	52.64±0.80	52.83±0.63	52.73±1.40	52.83±2.20	0.378
Total feed cost (/Rs./animal)	1960±40^{d}	2149±54^{b}	2189±79^{a}	2114±33^{c}	2152±82^{b}	0.041
Weight gain (Kg)*	15.00±0.47^{b}	17.80±0.84^{ab}	17.60±0.67^{ab}	18.20±0.73^{a}	18.80±1.03^{a}	0.032
Cost / Kg weight gain (Rs.)*	130.69±2.65^{c}	120.78±1.13^{b}	123.94±1.89^{b}	116.15±2.07^{a}	114.49±1.27^{a}	0.032

^{abc} Values bearing different superscripts in a row differ significantly ($P < 0.05$) *

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Occasional Feeding with Mussel Meat Boosts Productive and Reproductive Performance in *Ompokbimaculatus*

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ABSTRACT

This study investigated the effects of occasional mussel meat (*Lamellidensmarginalis*) supplementation on the growth and reproductive performance of *Ompokbimaculatus*, a fish species with high market demand and price in India. Fish with an initial weight of 20.1g were reared in 500-liter FRP tanks for three months. The experimental group received mussel meat supplementation 2-3 times per week in addition to standard feed, while the control group received only standard feed. Results showed significant improvements in growth parameters and reproductive performance in the supplemented group. The experimental group exhibited a 38.3% higher weight gain (38.3 ± 2.5g vs. 27.7 ± 2.1g) and a 23.5% higher specific growth rate (1.42 ± 0.07%/day vs. 1.15 ± 0.08%/day) compared to the control. Feed conversion ratio improved from 1.85 ± 0.12 to 1.53 ± 0.09. Reproductive parameters also showed enhancement, with the gonadosomatic index increasing by 27.6% (15.7 ± 1.3% vs. 12.3 ± 1.1%) and fecundity improving from 4182 ± 312 to 4876 ± 287 eggs per female. Egg diameter increased from 1.12 ± 0.08mm to 1.28 ± 0.07mm. This study suggests that occasional mussel meat supplementation could be an effective strategy to enhance the aquaculture production of *O. bimaculatus*.

KEYWORDS: Aquaculture nutrition, Growth performance, *Lamellidensmarginalis*, Mussel meat supplementation, *Ompokbimaculatus*, Reproductive enhancement.

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Ompokbimaculatus, commonly known as Indian butter catfish, is an economically important freshwater fish species native to South and Southeast Asia (Talwar and Jhingran, 1991). Its popularity in local markets, coupled with its declining wild populations due to overfishing and habitat degradation, has led to increased interest in its aquaculture potential (Mukherjee et al., 2002; NBFGR, 2011; Debnath et al., 2016). However, the successful cultivation of *O. bimaculatus* faces challenges, particularly in terms of optimizing growth and reproductive performance (Raizada et al., 2013).

Nutrition plays a crucial role in fish growth, development, and reproduction (Debnath et al., 2018a,b; Nahakpam et al., 2019). In recent years, there has been growing interest in the use of alternative protein sources in aquaculture to enhance fish performance and reduce reliance on conventional feed ingredients (Kumar et al., 2013; Das et al., 2021). Freshwater mussels, such as *Lamellidensmarginalis*, are rich in proteins, essential amino acids, and micronutrients, making them a potential dietary supplement for cultivated fish species (Sicuro et al., 2024).

The objective of this study was to evaluate the effects of occasional feeding with mussel meat (*L. marginalis*) on the productive and reproductive performances of *O. bimaculatus* under controlled conditions. We hypothesized that supplementing the diet with mussel meat would positively influence growth parameters and reproductive indices compared to a standard feed-only diet.

Experimental design

The experiment was conducted over a period of three months using twelve 500-liter Fiberglass Reinforced Plastic (FRP) tanks. A total of 240 *O. bimaculatus* with an initial average weight of 20 ± 1.5g were randomly distributed among the tanks at a stocking density of 20 fish per tank. The tanks were divided into two treatment groups with three replicates each:

1. Control group (C): Fed with standard commercial feed (Table 1) only

2. Experimental group (E): Fed with standard commercial feed + mussel meat supplementation 2-3 times per week

Table 1. The proximate composition of standard commercial feed

Nutrient	Composition (% dry matter)
Crude Protein	35.2 ± 1.3
Crude Lipid	8.5 ± 0.7
Ash	9.8 ± 0.5
Fiber	4.2 ± 0.3
Nitrogen-free extract	42.3 ± 1.8
Gross energy (MJ/kg)	18.7 ± 0.6

Feed and feeding

The control group received a standard commercial feed (35% crude protein) two times daily at 5% of their body weight. The experimental group received the same standard feed but with the addition of minced mussel meat (*L. marginalis*) at 10% of their body weight, given 2-3 times per week, replacing one of the standard feed meals on those days. Mussels were collected locally and their meat was extracted, minced, and stored at -20°C until use. Feeding rates were adjusted biweekly based on sampling weights.

Water quality management

Water quality parameters including temperature, dissolved oxygen, pH, and ammonia were monitored daily and maintained within the optimal range for *O. bimaculatus*. Partial water exchange (30%) was performed every week to maintain water quality.

Data collection

Growth parameters

Fish were weighed individually at the start of the experiment and every two weeks thereafter. The following growth parameters were calculated:

- Weight gain (WG) = Final weight - Initial weight

- Specific growth rate (SGR) = [(ln Final weight - ln Initial weight) / Days] × 100

- Feed conversion ratio (FCR) = Total feed given (dry weight) / Total weight gain

Reproductive parameters

At the end of the three-month period, three females from each tank were randomly selected for reproductive analysis. The following parameters were assessed:

- Gonadosomatic index (GSI) = (Gonad Weight / Body Weight) × 100

- Fecundity: Total number of mature eggs in the ovary

- Egg diameter: Measured using an ocular micrometer

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. After confirming these assumptions were satisfied, data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post-hoc test to identify specific differences between treatment means.

After three months of rearing, fish in both groups showed substantial growth, with the experimental group exhibiting significantly better performance compared to the control group (Table 2). The experimental group demonstrated a 38.3% higher weight gain (38.3 ± 2.5g vs. 27.7 ± 2.1g) and a 23.5% higher specific growth rate (1.42 ± 0.07%/day vs. 1.15 ± 0.08%/day) compared to the control. The feed conversion ratio improved significantly from 1.85 ± 0.12 in the control group to 1.53 ± 0.09 in the experimental group.

Table 2. Growth performance of *O. bimaculatus* fed with and without mussel meat supplementation

Parameter	Control	Experimental
Initial weight (g)	20.1 ± 1.3a	20.2 ± 1.4a
Final weight (g)	47.8 ± 3.2a	58.5 ± 2.9b
Weight gain (g)	27.7 ± 2.1a	38.3 ± 2.5b
SGR (%/day)	1.15 ± 0.08a	1.42 ± 0.07b
FCR	1.85 ± 0.12a	1.53 ± 0.09b

Note: Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

This enhanced growth performance can be attributed to mussel meat's high nutritional value (moisture 78.5 ± 2.1%, crude protein 12.7 ± 0.9%, crude lipid 2.3 ± 0.3%, ash 1.8 ± 0.2%, and carbohydrate 4.7 ± 0.5%, w/w). Freshwater mussels are rich in proteins, essential amino acids, and various micronutrients (Sicuro et al., 2024). This high-quality protein source likely contributed to better nutrient utilization, as evidenced by the lower FCR in the experimental group (Claessens et al., 2023). These findings align with Ahirwal et al. (2019), who also reported improved growth and feed utilization in catfish fed mussel flesh-supplemented diets.

The reproductive parameters of *O. bimaculatus* were also positively influenced by mussel meat supplementation (Table 3). The experimental group showed a significantly higher gonadosomatic index (27.6% increase) compared to the control group (15.7 ± 1.3% vs. 12.3 ± 1.1%). Fecundity was notably higher in the experimental group, with an average of 4876 ± 287 eggs per female, compared to 4182 ± 312 eggs per female in the control group. Additionally, the eggs from the experimental group were larger in diameter (1.28 ± 0.07mm vs. 1.12 ± 0.08mm), suggesting potentially higher quality.

Table 3. Reproductive parameters of *O. bimaculatus* fed with and without mussel meat supplementation

Parameter	Control	Experimental
GSI (%)	12.3 ± 1.1a	15.7 ± 1.3b
Fecundity	4182 ±	4876 ± 287b
Egg diameter (mm)	1.12 ±	1.28 ± 0.07b

Note: Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

The enhanced reproductive performance in the experimental group is particularly noteworthy. Higher GSI values indicate greater gonadal development, crucial for successful reproduction. The improvement in fecundity falls within the range reported for the fish in previous studies (Banik et al., 2012; Malla and Banik, 2015). Larger egg size in the experimental group is a positive indicator of egg quality, as larger eggs often contain more yolk reserves, potentially leading to larger and more viable larvae (Marimuthu and Haniffa, 2007).

The positive effects on reproductive parameters may be attributed to mussel meat's rich nutrient profile, particularly essential fatty acids and micronutrients. These nutrients play crucial roles in gonadal development, vitellogenesis, and overall reproductive success in fish (Nandi et al., 1999). Nayak et al. (2021) observed improved fecundity in fish (*Amblypharyngodon mola*) by enriching the tank environment with the aquatic plant *Hydrilla verticillata*, creating a modified habitat. A similar approach is recommended for *O. bimaculatus* to further enhance their reproductive outcomes.

Throughout the experiment, water quality parameters remained within acceptable ranges for both groups (Table 4), ensuring that the observed differences in growth and reproductive performance were attributable to the dietary treatments rather than environmental variables.

Table 4. Water quality parameters during the experimental period

Parameter	Control	Experimental
Temperature (°C)	27.5 ± 1.2a	27.3 ± 1.1a
Dissolved Oxygen	6.8 ± 0.4a	6.7 ± 0.5a
pH	7.4 ± 0.2a	7.3 ± 0.3a
Ammonia (mg/L)	0.05 ±	0.06 ± 0.03a

Note: Values are mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$).

CONCLUSION

The combined results demonstrate that occasional mussel meat supplementation produced substantial improvements across multiple performance metrics in *O. bimaculatus*. The experimental group achieved a 38.3% higher weight gain and improved feed conversion ratio (1.53 vs 1.85), indicating more efficient nutrient utilization. Reproductive parameters showed marked enhancement, with a 27.6% increase in gonadosomatic index (15.7% vs 12.3%) and 16.6% higher fecundity (4876 vs 4182 eggs per female). The 14.3% increase in egg diameter (1.28mm vs 1.12mm) suggests potential improvements in offspring quality.

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