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Indian Journal of Animal Nutrition

Dietary Quercetin for Enhanced Broiler Performance- A Review

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ABSTRACT

Quercetin, a naturally occurring flavonoid, has received consideration for its possible benefits in chicken nutrition because of its anti-inflammatory, gut-health-promoting, and antioxidant properties. This review summarizes current research on quercetin supplementation in broiler chickens, focusing on its implications on growth performance, immunological response, gut health, and carcass quality. Quercetin has been shown in studies to improve body weight gain, feed conversion efficiency, and nutrient absorption while also reducing oxidative stress by upregulating antioxidant enzymes like as superoxide dismutase and glutathione peroxidase. Furthermore, it aids immunological regulation by lowering inflammatory cytokines and increasing disease resistance. Quercetin also improves gut microbiota composition and shape, resulting in greater digestive health. Furthermore, it enhances meat quality by lowering lipid peroxidation, improving texture, and increasing shelf life. Findings indicate quercetin as a possible natural substitute to antibiotics in broiler feed supplement, which aligns with the growing need for sustainable, antibiotic-free poultry production. However, additional research is needed to enhance its bioavailability, administration approaches, and long-term effects in commercial applications. Future research should concentrate on improving its bioavailability and determining its economic viability in large-scale commercial applications.

KEYWORDS: Antioxidant, Broiler chicken, Gut microbiota, Poultry feed additives, Quercetin

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INTRODUCTION

A naturally occurring flavonoid, quercetin can be found in a wide variety of fruits, vegetables, and grains. Chemically, it is a polyphenol, with a hydroxylated aromatic structure that contributes to its strong antioxidant properties. Quercetin is valued for its ability to scavenging free radicals, reducing oxidative damage and lipid peroxidation. Quercetin reduces inflammation by modulating the signalling pathways like NF-κB decreasing pro-inflammatory cytokines. Liu et al. (2021) found that quercetin binds to the γ-subunit of AMPK in a manner structurally similar to ZMP, a known AMPK activator, suggesting that quercetin induces allosteric activation of AMPK and thereby triggers downstream signalling events; in turn, activated AMPK increases cellular NAD+ levels, which enhances the deacetylase activity of SIRT1, leading to deacetylation of the NF-κB p65 subunit and suppression of its nuclear translocation and pro-inflammatory transcriptional activity. As a result, through this AMPK/SIRT1/NF-κB axis, quercetin significantly reduces the production of key pro-inflammatory cytokines, such as TNF-α (by 35–48%), IL-1β (by 28–42%), and IL-6 (by 31–50%) in experimental models, thereby exerting potent anti-inflammatory effects.

Studies have demonstrated that quercetin can protect broiler chickens from inflammatory damage and restore gut microbial diversity, hence promoting intestinal barrier functioning (Sun et al., 2022). It also increases antioxidant enzyme activities including superoxide dismutase and glutathione peroxidase, which improve the oxidative stability of chickens under stress (Dong et al., 2020). Quercetin's biological properties make it a good choice for usage as a natural feed supplement, replacing synthetic growth promoters and antibiotics while meeting the growing demand for sustainable chicken production (Saeed et al., 2017). Quercetin has been found to increase meat quality, growth performance, and gut

microbiota, all of which contribute to the general health of chicken (Benzertiha et al., 2019; Hussien et al., 2021; Walk et al., 2021; Janocha et al., 2022; Selle et al., 2023).

Various bio-enhancers and nano-formulations (Beski et al., 2015, Divani et al., 2018; Marcincak et al., 2018) has been tried as additives in broiler nutrition. In addition, it has been highlighted that mechanistic research and long-term trials are necessary to gain a deeper understanding of quercetin's molecular activities and cumulative effects over time.

Challenges in Broiler Production

Broiler production has several challenges that have a substantial impact on growth, health, and total output. Among these obstacles, oxidative stress and gut health concerns are especially significant. An imbalance between the production of reactive oxygen species (ROS) and antioxidant defence leads to oxidative stress, which damages cells and reduces physiological activity. (Tompkins et al., 2022). This illness is aggravated by environmental stressors such as high temperatures, which are frequent in tropical and subtropical areas. Furthermore, gut health is essential for good food absorption and overall well-being in chickens. Gastrointestinal system is sensitive to many pathogens, which can cause illnesses such as necrotic enteritis and dysbiosis (Obianwuna et al., 2023). These gut health conditions limit feed efficiency and raise mortality rates, creating considerable economic challenges to chicken breeders. In order to overcome these challenges, antibiotics as growth promoters have long been utilized; however, growing customer demand for antibiotic-free products and worries about antibiotic resistance have led to a change in approach. This has resulted in an increased the search for natural feed additives, such as phytochemicals, for improving chicken performance along with their health (Moustafa et al., 2021).Various alternatives to antibiotic growth promoters which is equally effective on growth performance, nutrient utilization and carcass characteristics for rearing broiler poultry like plant extracts and essential oil blend (Devi et al., 2018); dietary neem (*Azadirachta indica*) and kadi (*Murraya koenigii*) leaf powder (Khulbey et al., 2015); garlic (*Allium sativum*) and tulsi (*Ocimum sanctum*) leaf powder supplementation (Kumar et al., 2016) has been tried and found efficient in their role.

Quercetin as a Potential Solution

Quercetin, a flavonoid with five hydroxyl groups, is a potent antioxidant and anti-inflammatory compound that scavenges free radicals, boosts antioxidant enzymes, and reduces oxidative stress, thereby improving broiler growth and health (Table 1) (Vinnarasi et al., 2020; Ying et al., 2020; Tan et al., 2022). It enhances gut health by moderating inflammation, preserving intestinal barrier integrity, and promoting beneficial gut microbiota, making it an effective natural alternative to antibiotics in poultry nutrition (Ying et al., 2020; Moustafa et al., 2021). Quercetin also upregulates antioxidant enzymes (SOD1, GSH-Px), nutrient transporters, improves nutrient digestibility, immune response, calcium and phosphorus metabolism, and meat quality, thus boosting production efficiency in broilers when supplemented at 200–400 ppm (Abdel-Latif et al., 2021; Wang et al., 2022)

Metabolism of quercetin

Quercetin is extensively metabolized mainly in the liver and intestines, commonly existing as glycosides which enhance its solubility and bioavailability. After ingestion, it undergoes phase I metabolism (oxidation, reduction, hydrolysis) and phase II metabolism (glucuronidation, sulfation, methylation) resulting in diverse metabolites that influence its biological activity and pharmacokinetics (Materska, 2008; Ozgen et al., 2016). In poultry, quercetin absorption is affected by diet, phytochemicals, and gut microbiota, with dietary fibers and flavonoids enhancing uptake, but gut environment complexity requires tailored formulations for optimal bioavailability (Materska, 2008; Jurasekova et al., 2014; Ilyich et al., 2018; Brovarets & Hovorun, 2019; Tyagi et al., 2020).

Despite its many advantages, quercetin has low water solubility, limiting its gastrointestinal absorption (Materska, 2008; Ozgen et al., 2016; Shanmugasundaram and Roza, 2022). It undergoes rapid liver metabolism forming quercetin glucuronides and sulfates, resulting in low plasma levels and reducing its active biological availability (Rupasinghe et al., 2010; Seruga & Tomac, 2017; Sun et al., 2022). This extensive first-pass metabolism significantly diminishes the amount of quercetin available for biological effects.

Strategies to improve quercetin's efficacy include encapsulating it in nanoparticles to enhance solubility, stability, and sustained release, as shown by Algan and Karatas (2023). Co-supplementation with compounds like piperine or curcumin blocks metabolic enzymes and boosts bioavailability; Patel et al. (2021) demonstrated improved pharmacokinetics of marbofloxacin in broilers with quercetin-piperine. Additionally, dietary inclusion of quercetin-rich extracts such as apple skin enhances absorption (Rupasinghe et al., 2010). Variations in dosage (100–600 mg/kg) and supplementation duration, as well as environmental factors like temperature and housing, influence its effects on meat quality, immune response, and growth performance (Rupasinghe et al., 2010; Patel et al., 2021; Sun et al., 2022; Ahammad & Kim, 2024). Nanoparticles, such as quercetin-loaded Fef O$_{,,}$, significantly improve immune and antioxidant defenses in broilers, reducing pathogen burden and enhancing performance (Al-Nasser et al., 2024).

Quercetin as antioxidant agent

Quercetin enhances antioxidant status in broiler chickens by increasing enzyme activities such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), while reducing oxidative stress markers like malondialdehyde (MDA), which is essential for maintaining health and production, especially under stress (Dong et al., 2020). Supporting evidence from other models shows quercetin's effectiveness in lowering oxidative damage, such as reduced MDA in rat spleen tissue exposed to titanium dioxide nanoparticles and decreased oxidative stress in elderly rats' cerebral cortex via enhanced antioxidant enzymes combined with calorie restriction (Jaber, 2023).

Oxidative stress is a major constraint in poultry production, commonly triggered by nutritional imbalances, heat stress, and pathological conditions (Mishra & Jha, 2019). Quercetin, a potent antioxidant, effectively scavenges reactive oxygen species (ROS), thereby reducing oxidative damage linked to metabolic and degenerative diseases (Bukowska & Duchnowicz, 2022; Bellavite, 2023). It also enhances the activity of endogenous antioxidant enzymes—glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD)—and inhibits pro-oxidative enzymes such as xanthine oxidase and lipoxygenase (Chiu et al., 2023; Olla et al., 2023).

In broiler chickens, quercetin supplementation is well recognized for mitigating oxidative stress and improving overall health and performance. Abdelrahman et al. (2022b) reported significant reductions in malondialdehyde (MDA), a key marker of lipid peroxidation, alongside improved growth and meat quality. Similarly, Liu et al. (2013) demonstrated that quercetin suppresses lead (Pb)-induced oxidative stress by reducing ROS production, increasing total antioxidant capacity (TAC), and upregulating phosphoinositide-3-kinase (PI3K) and phosphorylated protein kinase B (PKB/Akt) activity. Quercetin-containing seaweed supplements have also shown strong antioxidant effects, with brown seaweed (BS) and green seaweed (GS) enhancing antioxidant activity by 55.19% and 25.74%, respectively, compared to unsupplemented controls (Zhong et al., 2020; Azizi et al., 2023).

Table 1. Effect of dietary quercetin on health, meat quality and growth performance of chicken

Characteristics	Dose rate of quercetin	Observed effect	References
Antioxidant	1g/kg with high energy challenged diet	Reduced metabolic stress, oxidative stress, and lipotoxicity from high-energy diets	Parmar et al., 2022
	0.02%, 0.04% and 0.06% with Streptozotocin	Mitigated STZ-induced oxidative stress by regulating antioxidant enzymes and lowering MDA and NO levels.	Ying et al., 2020
	200-800 ppm	Reduced oxidative stress from oxidized oil and prevented pathogen invasion	Dong et al., 2020
Antioxidant and anti-inflammatory	200 mg/kg along with LPS challenged diet	Alleviated LPS-induced inflammation and apoptosis by modulating gut microbiota and immunity.	Sun et al., 2020
	0.5 g/kg	Antioxidant, anti-inflammatory, and antiapoptotic properties and reduced nephrotoxicity	Abdelrahman et al., 2022a
	250, 500, and 1,000 mg /kg	Increased body weight gain, antioxidant capacity, and SOD; reduced MDA levels	Zhang et al., 2020
Growth promoter and antioxidant	200-600 ppm	Increased weight gain, feed intake, immunity, and antioxidant status	El-Kazaz et al., 2024
	0.5mL /kg	Improve growth performance and feed conversion ratio	Ugwuoke et al., 2024
	200-400 ppm	Improved the intestinal morphometry and growth traits	Abdel-Latif et al., 2021
Meat quality and anti-oxidant	0.2, 0.4, and 0.6 g/kg	Enhanced meat quality and protected against lipid oxidation and fat deposition	Wang et al., 2022
	500 and 1000 mg/kg	Improved antioxidant capacity, muscle protein stability, and edibility.	Deng et al., 2024

Quercetin boosts key antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), helping neutralize reactive oxygen species and reduce oxidative stress. Studies in rats exposed to doxorubicin or hypertension show that quercetin increases SOD and GPx activity, thereby limiting oxidative damage and strengthening endogenous defence mechanisms (Farag et al., 2021; Maksymchuk et al., 2023).

Regulation of Inflammatory Routes

Quercetin plays a key role in controlling inflammation (Table 2) by inhibiting nuclear factor kappa B (NF-êB), a major transcription factor involved in producing pro-inflammatory cytokines such as IL-1â, TNF-á, and IL-6 (Ozbek et al., 2015). It also modulates mitogen-activated protein kinase (MAPK) pathways, reducing inflammation and promoting tissue repair. In broilers, quercetin supplementation decreases serum markers like creatinine, BUN, and AST, lowers inflammatory cytokine expression, and enhances immune response and health (Kim et al., 2010; Al-Nasser et al., 2024). Additionally, quercetin exhibits antimicrobial effects by suppressing pathogenic bacteria, improving gut health, and reducing infection risks. These anti-inflammatory and antimicrobial actions are vital for mitigating stress-induced inflammation in poultry production.

Table 2. Role of dietary quercetin in regulating inflammatory response in broiler chicken

Quercetin dose	Anti-inflammatory action	Reference
LPS-treated, 200 -500 mg/kg	Lowers pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, TLR-4) and boosts protective proteins (ZO-1, Bcl-2)	Sun et al., 2022
Basal diet + quercetin @1 g/kg	Reduces liver expression of IL-1β, IL-6, and TNF-α mRNA.	Parmar et al., 2022
10 μg/mL LPS+1 mM ATP and 5, 10, and 20 μM quercetin	levels of TLR4, NLRP3, caspase-1, gasdermin D, IL-1β, IL-18, IL-6, TNF-α; inhibits NF-κB p65 phosphorylation; increases cell migration and ZO-1/claudin expression; lowers late apoptotic cells	Zhang et al., 2022

Effects on Immunomodulation

Quercetin enhances immune function in broilers by increasing anti-inflammatory markers and reducing pro-inflammatory cytokines, thus strengthening disease resistance (Table 3) (Khampeerathuch et al., 2018). It also raises blood immunoglobulin levels, indicating a stronger humoral immune response (Sun et al., 2020; Abdel-Latif et al., 2021).

Table 3. Immunomodulatory effect of quercetin in chicken

Quercetin Dose	Inflammatory markers involved	References
20-200 ppm	Decreased IgA and IgM levels	Kim et al., 2015
50 μM quercetin hydrate of >95% purity	Increased anti-inflammatory gene expression (IL6, IL8L1, IL8L2, IL18, CCL4, LITAF, MIF)	Khampeerathuch et al., 2018
0.02- 0.06%	Raised TNF-α, TRAF-2, TNFRSF1B, NF-κBp65, IFN-γ mRNA; decreased IκB-α mRNA ($P < 0.05$)	Yang et al., 2020
200 mg/kg	Reduced inflammatory markers and apoptosis genes induced by LPS; increased tight junction proteins and Bcl-2	Sun et al., 2022
0.02-0.06%	Modulated oxidative stress, raised insulin, activated PI3K/PKB pathway genes for glucose metabolism and oxidative damage reduction	Ying et al., 2020; Abdelrahman et al., 2022b
200-600 ppm	Enhanced IgM, IgA secretion; stimulated humoral immunity and upregulated IL-4, IFN-γ, TLR2, and TNF-α in spleen	El-Kazaz et al., 2024

Li and Xu (2008) identified quercetin as the key antimicrobial agent in lotus leaves extract, with minimum inhibitory concentrations of 0.625 mg/mL for *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*, 1.25 mg/mL for *Actinomyces viscosus* and *Porphyromonas gingivalis*, and 2.5 mg/mL for *Actinomyces naeslundii*. Rauha et al. (2000) reported quercetin's inhibition against various pathogens including Aspergillus niger, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*. In broilers, quercetin supplementation (200–800 mg/kg) enhanced immunoglobulin levels, reduced *coliform* and *Clostridium perfringens* counts, and increased beneficial *Lactobacillus* populations, improving gut health and immune status (Abdel-Latif et al., 2021). This immunomodulatory and antimicrobial action is critical for poultry health under intensive production stressors (Table 4).

Quercetin supplementation in broiler diets has been shown to enhance disease resistance, particularly against *Clostridium perfringens*, a primary cause of necrotic enteritis. Quercetin-loaded nanoparticles significantly reduced intestinal colonization of *C. perfringens* while boosting the birds' immune and antioxidant defenses (Al-Nasser et al., 2024). Additionally, micellar quercetin improved immunological responses and growth performance in broilers (Ahammad and Kim, 2024).

Table 4. Effect of dietary quercetin on gut microbiota in broiler chicken

Dose	Bacteria type	Possible mechanism	Reference
400 ppm	Promote *Lactobacillus*	Strengthened intestinal barrier by increasing MUC2 expression and secretion	Dong et al., 2020
250-1000 mg/kg	Favours *Lactobacillus* growth	Unabsorbed dietary phenolics and metabolites inhibit pathogens and promote beneficial bacteria like *Lactobacillus* and *Bifidobacterium*	Zhang and Kim, 2020
200, 400, and 800 ppm	Decrease total *coliforms* and *Clostridium perfringens* and increase *Lactobacillus*	Antibacterial action through inhibition of DNA gyrase, bacterial membranes, motility, FAS II pathway, and Ddl enzyme, acting bacteriostatically	Abdel-Latif et al., 2021
0.025% to 0.100%	Reduces *E. coli* levels without affecting *Lactobacillus* or *Salmonella* count	Fermented flavonoids contain lactic and organic acids with antimicrobial synergy	Ahammad and Kim, 2024
300 mg/kg	*Clostridium perfringens* colonization	Quercetin-loaded Fe_3O_4-NPs reduced C. perfringens colonization and virulence gene expression, increased Lactobacillus and Bifidobacterium, upregulated host antimicrobial peptides, and downregulated intestinal inflammatory genes	Al- Naseer et al., 2024

Growth Performance

Quercetin supplementation has been found to significantly enhance growth performance in broiler chickens. Ahammad and Kim (2024) reported that micellar quercetin supplementation dramatically increased body weight gain and feed conversion ratio. Ugwuoke et al. (2024) observed that dietary quercetin at 0.5 mL/kg in the basal diet resulted in the highest final body weight and average daily weight gain. Importantly, quercetin did not affect feed intake significantly, suggesting improved nutrient utilization efficiency.

Feed Conversion Ratio (FCR)

Quercetin supplementation has been shown to improve the feed conversion ratio (FCR) in broilers, reflecting better efficiency in converting feed into body mass. Guar meal which is good source of flavonoid quercetin (Sharma et al., 2011), when included at 15% fermented toasted guar meal in broiler diets, improved FCR comparable to supplements (Lakshmi et al., 2025). Ahammad and Kim (2024) reported that micellar quercetin significantly enhanced FCR, indicating improved feed-to-weight conversion. Goliomytis et al. (2014) also found FCR increases at 0.5 and 1 g/kg quercetin supplementation, suggesting enhanced metabolic feed utilization. Ugwuoke et al. (2024) observed the highest weight gains at 0.5 mL/kg, indicating optimal dosage ranges for maximizing growth. Quercetin may also interact beneficially with other compounds like vanillin and 7-hydroxycoumarin to improve broiler meat mineral content and overall performance (Zavialov et al., 2023).

Gut Health

Quercetin supplementation improves gastrointestinal health in broilers by enhancing intestinal morphology, including increased villus height and villus height-to-crypt depth ratio, which supports better nutrient absorption (Abdel-Latif et al., 2021; Sun et al., 2022). It positively modulates gut microbiota by increasing beneficial bacteria and suppressing harmful strains, crucial for optimal feed efficiency and overall health. Agarwal et al. (2022) showed quercetin enhances brush border membrane architecture, increasing goblet and Paneth cells vital for gut function. Amevor et al. (2022) found dietary quercetin and vitamin E improved intestinal structure in older hens, promoting nutrient absorption through increased crypt depth and villi height. Studies also highlight quercetin's antioxidant properties improve nutrient utilization and overall health, reducing heat stress impacts in broilers (Parmar et al., 2019; Attia et al., 2023).

Quercetin effectively regulates gut microbiota, promoting beneficial bacteria while inhibiting pathogenic strains, which is vital for gut health and nutrient absorption (Shi et al., 2022). Ghimire et al. (2021) found that combining rice bran and quercetin increased beneficial gut bacteria and reduced harmful *Enterobacteriaceae*. This microbiota balance, along with quercetin's antioxidant properties, supports optimal gut health and nutrient utilization in broilers.

Improvement of Meat Quality Parameters

Quercetin effectively reduces lipid oxidation in broiler chicken meat, enhancing its shelf life and sensory qualities. Delles et al. (2014) showed that quercetin and other dietary antioxidants significantly lowered lipid oxidation markers such as thiobarbituric acid reactive substances (TBARS) in chicken breast meat. Similarly, Gumus and Gelen (2023) reported that supplementation with quercetin-containing thyme and rosemary essential oils dramatically decreased TBARS levels in both drumstick and breast meat during storage, thereby improving oxidative stability and meat quality. This antioxidant action of quercetin is crucial for maintaining meat freshness and extending shelf life in poultry production.

Quercetin supplementation has been shown to improve broiler meat tenderness by reducing shear force in thigh and breast muscles, likely through its effects on protein metabolism (Wang et al., 2022). Janocha et al. (2021) also found that quercetin-rich feed additives enhance meat quality, including tenderness, by protecting muscle proteins from oxidative damage during storage. The antioxidant properties of quercetin help maintain muscle integrity, contributing to improved meat softness and consumer acceptance.

Quercetin's antibacterial properties contribute significantly to improving meat safety in broilers by lowering microbial load. Faluyi et al. (2020) reported that red onion, rich in quercetin, reduced microbial counts in unrefrigerated broiler meat, maintaining quality during storage. Dietary quercetin-rich foods decreased bacterial counts in broiler meat. These reductions help safeguard poultry products and reduce food borne illness risks by controlling pathogenic bacteria in meat.

Future Perspectives: Exploring Quercetin's Potential in Broiler Chickens

Although quercetin shows promise as a dietary supplement in broiler nutrition (Table 6), further research is needed to fully understand its mechanisms, long-term benefits, and practical applications. Molecular-level studies are essential to elucidate how quercetin influences gene expression, cellular pathways, and metabolic processes related to immune response, growth, gut health, inflammation, and oxidative stress in broilers (Ahammad and Kim, 2024). Long-term trials are necessary to evaluate cumulative effects on meat quality, health, growth performance, and possible adverse effects or nutrient metabolism alterations, as emphasized by Sierzant et al. (2023).

Table 5. Summary of quercetin role in broiler chicken nutrition

Property	Effect on Broiler chickens	Mechanism involved	Reference
Antioxidant	Improves meat quality and oxidative stability	Enhances antioxidant enzymes, reduces oxidation	Deng et al., 2024
Anti-lipid peroxidation	Reduces liver MDA content	Reduces oxidized lipid by-products	Sierzant et al., 2023
Growth promoter	Increases body weight gain	Enhances T-SOD, T-AOC, reduces MDA	Zhang and Kim, 2020
Gut microbiota modulator	Increases *Lactobacillus*, reduces *coliforms* and *C. perfringens*	Modifies gut environment, upregulates SOD1, GSH-Px	Abdel-Latif et al., 2021
Feed efficiency improver	Improves feed conversion ratio and weight gain	Enhances nutrient utilization	Onu et al., 2024
Lipid metabolism regulator	Reduces abdominal fat content	Downregulates L-FABP, SREBP1, HMGR; upregulates CPT1	Wang et al., 2022
Intestinal morphometry improver	Increases villus height, decreases crypt depth	Enhances gut structure	Sun et al., 2020
Intestinal protector	Reduces LPS-induced oxidative stress in intestines	Activates MAPK/Nrf2 pathway	Sun et al., 2020
Nutrient transporter up-regulator	Upregulates GLUT2, PEPT1, FAS genes	Enhances nutrient absorption	Abdel-Latif et al., 2021
Meat quality enhancer	Reduces cooking and drip loss, improves breast muscle	Improves muscle structure, increases α-helix content	Ahammad and Kim, 2024
Meat tenderness	Increases tenderness and juiciness	Activates PI3K/PKB/AMPK pathway	Wang et al., 2022
Muscle flavor enhancer	Increases inosinic acid (IMP) in muscle	Modifies muscle biochemistry	
Immunomodulator	Ameliorates immunotoxicity from ochratoxin A	Activates PI3K/AKT pathway, reduces apoptosis	Abdelrahman et al., 2021b
Immune response enhancer	Increases TNF-α, improves antibody response	Modulates cytokine production	Zhang and Kim 2020
Blood profile modulator	Increases thyroxine (T4) and lymphocyte levels	Influences hormone and immune cell levels	Ahammad and Kim, 2024
Nephroprotective	Protects against ochratoxin A-induced nephrotoxicity	Suppresses apoptosis via PI3K/AKT pathway	Elhady et al., 2022
Mitochondrial protector	Upregulates mitochondrial DNA copy number genes	Enhances mitochondrial function	Sun et al., 2020
Anti-apoptotic	Reduces proapoptotic gene expression in kidney	Modulates apoptosis-related genes	Elhady et al., 2022

CONCLUSION

Quercetin exhibits diverse benefits in broiler production, including antioxidant effect, growth-promoter agent, factor for enhancing meat quality, modulation of gut microbiota, immunomodulatory factor, and also with metabolic regulatory effects. Its potential as a sustainable, cost-effective feed additive makes it a promising alternative to synthetic feed additives. However, further research is needed to determine optimal dosages, supplementation forms, long-term safety, and mechanisms of action through controlled field trials to ensure its effective integration into commercial poultry production.

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Nutrient Utilization and Energy Value of Maize Oil Cake in Ration of Goat Kids

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ABSTRACT

A study was conducted to assess the effects of dietary inclusion of maize oil cake on nutrient digestibility, nutrient intake, FCR and energy value of feeding maize oil cake in kids. 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. Ether extract digestibility (%) was significantly (P<0.05) higher in T2 (78.45) and T3 (80.77) groups as compared to the control group (76.38) in both experiments. DM, OM, CP and NFE digestibility did not differ significantly among different treatment groups. Nutrients intake (g/day) in terms of DCP and TDN were found significantly (P<0.05) higher for maize oil cake added groups (T2 and T3) than control group. Nitrogen intake and nitrogen balance reported higher (p<0.05) in T2 and T3 groups than T1 group. Overall mean FCR of experimental kids was found similar among all dietary treatments. GE, ME, DE values were significantly (P<0.05) higher in maize oil cake added treatment groups. Results of the study showed that inclusion of maize oil cake improved ether extract digestibility and intake of total digestible nutrients as well as energy value of feed due to its high energy and protein content.

KEYWORDS: Beetal kids, Digestibility, FCR, TDN, Maize oil cake

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INTRODUCTION

Small ruminants comprise approximately 41.5% of the country's total livestock population, as reported in the 20th Livestock Census (BAHS, 2019). Goats are among the main meat-producing animals in India, whose meat (chevon) is one of the choicest meats and has huge domestic demand. 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). Due to its good economic prospects, goat rearing under intensive and semi-intensive system for commercial production has been gaining momentum for the past couple of years.

The unfortunate reality is that the availability of quality feed resources is increasingly over shadowing the genetic potential of livestock, as their production heavily relies on both the quality and quantity of feed and fodder. Currently, the country faces a net deficit of 61.1% in green fodder, 21.9% in dry crop residues, and 64% in concentrate feeds. There is also a deficiency of 26.5% in crude protein (CP) and 23.7% in total digestible nutrients (TDN) (Datta, 2013). The gap between feed requirements and available resources, along with the need for climate-smart farming, has compelled us to seek non-conventional feed sources that do not compete with human consumption, such as agricultural by-products that are available year-round but are underutilized.

Maize oil cake is a by-product derived from the extraction of maize oil from full-fat maize germ, which is a coproduct of the starch industry. It is nutrient-dense, containing high levels of protein and fat, and possesses excellent functional properties, making it a valuable concentrate source for livestock (Bakke and Vickers, 2007). It is highly digestible by the livestock. Maize oil cake improves production as it contains high amount of ether extract which provide energy to the animals. The fat stored in ruminant adipose tissue primarily consists of triglycerides, mainly saturated fatty acids, with low levels of polyunsaturated fatty acids. This lipid profile has contributed to a decline in the consumption of meat and meat products in some regions (Bas et al., 2007), due to the strong association between the quality of

dietary fats and human health. Corn germ meal has been shown to enhance the nutritional quality of the lipid fraction by enriching it with compounds that are beneficial to human health (Urbano et al., 2014).

Cheaper supplies of feed containing good amount of fat and protein would surely have a substantial impact on designing cost-effective meals, affecting chevon pricing. The product's ease of availability, reasonable market pricing, and high protein and oil necessitates a trial for goat feeding. Present research was thus undertaken to study the effects of dietary inclusion of maize oil cake nutrient digestibility, nutrient intake, FCR and energy value of feed.

MATERIALS AND METHODS

The experimental work of the study was carried out at the Animal farm, Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). Eighteen Beetal kids of comparable body weight were randomly divided into three groups of 6 in each. 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.

They were housed in semi covered sheds. An adjustment period of fifteen days was given before the start of experiment. All the kids were 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 G - II was included with maize oil cake @15% of the concentrate mixture while maize oil cake was included @ 30% of the concentrate mixture in the diet of group - III. Feed ingredients used for ration formulation were evaluated for various proximate nutrients viz. dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and total ash (TA). 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 interval by subtracting residue from the offered amount of feed throughout the experiment. 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. The gross energy (GE) of oven dried feed and excreta samples was determined by standard procedures using Bomb Calorimeter. The gross heat of combustion in calories per gram of the material was computed by substituting values in the following equation.

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 lambs to new system of housing and management, followed by a collection period of 5 days. The proximate composition of feeds and faecal samples was determined (AOAC, 2013). The digestibility coefficient of any given nutrient was calculated as the difference in its intake and its output through faeces and expressed as the proportion of intake. The total digestible nutrients (TDN) and digestible crude protein (DCP) content of any particular diet was calculated by taking into account of all the prevailing digestible nutrients in the diet.

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).

Table 1. Chemical composition of feeds offered to experimental kids offered (%DM basis) of experimental diet

Feed offered	DM	CP	EE	CF	Total Ash	NFE
Green fodder	17.42	7.97	2.65	26.31	9.53	53.54
Gram straw	91.27	5.52	1.59	38.41	7.67	46.81
Conc. Mix. T1	90.55	20.07	4.16	5.84	6.52	65.40
Conc. Mix. T2	90.63	20.20	5.32	6.14	6.11	64.22
Conc. Mix. T3	92.75	20.16	6.56	6.56	5.81	62.89

RESULTS AND DISCUSSION

Nutrient digestibility

Digestibility forms the single most important parameter to determine the nutritive values of ration. The data pertaining to nutrient digestibility under different dietary treatments are summarized in Table 2. Among all dietary treatment groups, there was no significant effect on dry matter and organic matter digestibility. Crude protein digestibility ranged from 70.64 to 71.92 % and had no statistically significant difference on crude protein digestibility amongst different dietary treatment groups having maize oil cake inclusion as compared to control group. It can be inferred from the result that maize oil had no negative impact on digestibility of feed and can be used for formulating ration upto 30% of the concentrate in ruminants.

In agreement to our study, Albuquerque et al., 2014 reported that the control diet, CGM 20% and CGM 30% diets had no effect on coefficients of apparent metabolizability of dry matter (CAMDM) in the layers during 8 days experimental period. Kelzer et al., 2009 reported that OM digestibility and NDF digestibility was similar across experimental groups. i.e. control, dried distillers grains plus soluble, dehydrated corn germ meal and high-protein dried distillers grains in Holstein cows. Kaur (2017) also found that the NFE digestibility (%) in control (90.04), T1 (91.17) and T2 (90.21) group varied non significantly. Detray (2016) also reported that the digestibility of ADF was not affected by CGM and DDG (P e"0.18) diets fed to the calves.

In contrary to our results, Kaur (2017) reported that DM digestibility (%), OM digestibility (%), CP digestibility (%) and NDF digestibility (%) was higher ($P<0.05$) in control and T1 groups than T2 group. Lopez et al. (2003) also reported that apparent DM digestibility decreased ($P<0.05$) from 90.8 to 85.5%, when the corn was replaced by defatted corn germ meal at 0, 10, 20, 30 and 40% level in the diets of growing pigs. They also reported that CP digestibility decreased ($P<0.05$) and NFE digestibility decreased ($P<0.05$) from 93.37 to 86.85% by replacing the corn with increasing levels of defatted corn germ meal in the diets of growing pigs. Zhang et al. (2018) also reported that the ATTD (apparent total tract digestibility) of DM, OM and CP linearly decreased ($P<0.01$) as dietary CGM increased in the diet of growing pigs.

However, Kumar et al. (2018) who reported that DM digestibility in group I (58.34) was lower ($P<0.05$) than group II (59.99) and group III (62.37) and also digestibility of OM , CP, ADF and NDF varied significantly ($P<0.05$) and increased when the calves were fed maize germ oil cake (MGOC) at increasing level of 1.3, 1.7 and 2.1 kg/d.

It was observed that ether extract (EE) digestibility of T3 group was maximum and significantly ($P<0.05$) higher followed by T2 group and the control group T1. Increased ether extract digestibility may be due to high ether extract content of maize oil cake as well as high unsaturated fatty acids which increase formation of micelle formation.

Similar to our findings, Kumar et al. (2018) reported that digestibility of EE increased significantly ($P<0.05$) between the groups when the calves were fed maize germ oil cake at 1.3, 1.7 and 2.1 kg/d.

However, Kaur (2017) found that the EE digestibility (%) in control (79.60), T1 (77.82) and T2 (79.52) groups was similar and there was no significant ($P>0.05$) difference in the EE digestibility among the groups. Similarly, Kelzer et al. (2009) reported that digestibility of ether extract was similar and averaged 85.1±1.9 across experimental treatments i.e. control, dried distillers grains plus soluble, dehydrated corn germ meal and high-protein dried distillers grains fed to lactating Holstein cattle.

Table 2. Nutrient digestibility (%) of growing kids under different dietary treatments

Attributes	Treatments		
	T1	T2	T3
DM%	71.50±0.65	72.50±0.67	72.96±0.87
OM %	72.84±0.44	73.29±0.48	74.30±0.59
CP%	71.04±0.56	71.72±0.28	72.81±0.58
EE%*	76.38^{a}±1.05	78.45^{b}±0.67	80.77^{c}±0.68
CF%	66.20±0.45	66.62±0.41	66.98±0.36
NFE%	75.20±0.30	75.61±0.34	76.68±0.36

*Mean bearing different super scripts in arrow differ significantly (P<0.05)

Nutritive value of different experimental rations

Statistical analysis of the data revealed that TDN% differ significantly (P<0.05) between different dietary treatments as compared to the control group was recorded higher in T2 and T3 groups than control group. Similarly, DCP % in groups fed maize oil cake significantly as compared to the control group (Table 3). Increased nutrient intake in kids fed maize oil cake was may be due to improved ether digestibility as well as high energy content of these rations as compared to control group. Hence, it can be concluded that maize oil cake has improved the nutritive value of feed.

The results of the present study are in agreement with Silva et al. (2023), who reported that cows fed corn germ quadratically increased (p< 0.05) intake of dry matter, crude protein, and total digestible nutrients. Nagpur (2011) reported that the per cent total digestible nutrients (TDN) values of diets were 62.36 ± 1.5 for T1 ,72.99 ± 2.0 for T2 and 86.88 ± 1.8 for T3 diets. Significant (P d" 0.05) difference was recorded among the three experimental diets, which may be due to the varying levels of GNC, CGM and other ingredients in different diets.

However, Kumar et al. (2018), who reported that CP intake did not vary significantly among the different groups of crossbred calves fed maize germ oil cake at 1.3, 1.7 and 2.1 kg/d levels for 28 days. Kaur (2017) also determined that the overall mean CP intake(kg/animal/d) was similar in control, T1 and T2 groups and ranged between 0.570 to 0.588. The CP intake did not vary significantly among the groups.

Table 3. Mean nutritive values of experimental rations under different dietary treatments

Attributes	Treatments		
	T1	T2	T3
DCP%	9.00^{a}±0.20	9.42^{b}±0.11	9.59^{c}±0.09
TDN%	70.73^{a}±0.37	71.97^{b}±0.52	73.50^{c}±0.55

*Mean bearing different super scripts in arrow differ significantly (P<0.05)

Nitrogen balance under different dietary treatments

Statistical analysis showed significant (P<0.05) increase in N intake in groups T2 and T3 than the control group. Both the faecal and urinary outgo were found non-significant among different dietary treatment groups. Nitrogen balance values were maximum for group supplemented with 30% maize oil cake i.e. T3 followed by T2 and T1. Nitrogen balance amongst different treatment groups was observed to be significant (P<0.05). Significant

increase in nitrogen intake and nitrogen balance was may be due to increased percent of digestible crude protein in kids fed maize oil cake.

The results of the present study were in contrary with Li et al. (2018), who reported that nitrogen retention did not differ between corn germ meal, corn gluten feed, peanut meal, dehulled sunflower meal and full-fat rice bran and averaged 25.4 g/d whereas the urinary nitrogen output was the highest (P<0.01) in the peanut meal diet as compared to the corn germ meal, corn gluten feed and full-fat rice bran when fed to the growing pigs. Nascimento et al. (2022) also reported that the inclusion of WCG in the diet did not influence N recycling, and retained N remained similar between the treatment groups. Netto et al. (2023), reported that the nitrogen intake and daily excretion in urine and feces decreased, while nitrogen use efficiency increased linearly by replacing ground corn (GC) with full-fat corn germ (FFCG) in cows. There was no significant effect of diets on nitrogen balance or microbial protein synthesis and efficiency. Silva et al. (2023) reported that cows fed corn germ reduced ($p < 0.05$) the excretion of urea-N in milk and N excretion via urine. Bakshi et al. (2023), determined that animals fed diet containing MPIBs based concentrate mixture as compared to those fed control diet showed comparable urinary excretion of purine derivatives and N-retention in both the groups.

Table 4. Mean values of Nitrogen balance (g/day) under different dietary treatments

Attributes	Treatments		
	T1	T2	T3
N_2 intake(g)*	18.13± 0.30	19.28± 0.39	19.94± 0.35
N_2 faecal outgo(g)*	4.99 ± 0.10	5.15 ± 0.06	5.24 ± 0.11
Urinary outgo (g)*	1.64 ± 0.07	1.71 ± 0.15	1.95 ± 0.09
N_2 balance (g)*	11.49 ± 0.30	12.42 ± 0.25	12.75 ± 0.24

*Mean bearing different super scripts in arrow differ significantly (P<0.05)

Feed conversion ratio (FCR)

FCR reflects the efficiency with which animal convert feed into body weight gain. The analysis of data of this study represents that the overall mean values of FCR did not differed significantly (P<0.05) as compared to the control group. However, the overall mean of FCR of maize oil cake fed groups T2 and T3 were numerically improved as compared with control group with no maize oil cake supplementation. No significant effect observed may be due to numerical increase in feed intake in kids along with increased body weight gain. During different fortnight periods also there was no significant difference between the treatments except during 5th fortnight in which FCR was significantly improved for group T3 as compared to the control group with no supplementation. The high nutrient content of maize oil cake likely, enabled the kids to convert feed more efficiently into body weight gain. Qi et al.(2022), fed Cherry Valley ducks diets containing 0, 3, 6, 9, or 12% CGM and results showed that compared with other groups, ducks fed 12% CGM significantly increased ($P < 0.05$) the feed to gain ratio.

The results of the present study are in consistence with Ezequiel et al. (2006), also reported that no significant differences were observed for feed conversion (7.88 kg of DMI/kg of weight gain) and carcass dressing (54.52%) across treatments in Nellore steers. Similarly, Moreira et al. (2002) reported no influence of defatted corn germ meal at increasing levels (0, 15, 30 and 45%) on the average feed conversion in the crossbred pigs. Leeuw et al. (2009) also reported no significant difference in feed conversion ratio when the steers were fed defatted maize germ meal at 0, 25, 50, 75 and 100% levels, replacing hominy chop for 124 days. Likewise, Lakshmi et al. (2015) investigated that replacing maize and soybean meal with CGM (at 0, 15, 20 and 25%) had no significant effect on FCR in the coloured broilers.

Table 5. Mean values of FCR (Fortnightly) of growing kids under different dietary treatments

Period (Fortnight)	Treatments		
	T1	T2	T3
1st	7.09±0.16	7.25±0.33	6.62±0.21
2nd	7.78±0.22	6.68±0.41	6.59±0.40
3rd	7.75±0.28	6.95±0.24	6.80±0.30
4th	7.77±0.42	8.14±0.25	7.85±0.45
5th	7.71±0.36	6.94±0.41	5.84±0.27
6th	7.70±0.45	7.17±0.47	6.72±0.52
7th	8.22±0.40	7.82±0.38	8.57±0.60
8th	8.52±0.26	8.25±0.26	7.56±0.26
Overall mean FCR	7.82±0.17	7.40±0.18	7.07±0.26

*Mean bearing different super scripts in arrow differ significantly (P<0.05)

Energy values and efficiency ratios

Statistical analysis of GE showed significant (P<0.05) difference between different dietary treatments T2 and T3, as compared to control group. Mean values of digestible energy (DE) of dietary treatment group T1, T2, and T3 having maize oil cake inclusion were significantly (P<0.05) higher than the control group having no maize oil cake inclusion in the diet. Similarly ME of various dietary treatments T2 and T3 also showed significant (P<0.05) higher values as compared to the control group. Gross energy of the corn co-products ranged from 4,397 to 5,811 kcal/kg of DM and GE of corn germ meal was found 4767 kcal/kg (Rochell et al. (2011). GE value of MOC used in our experiment was reported 4900 kcal/kg. Due to high GE of this ingredient it increased (P<0.05) GE values of diets offered to kids in the group T_2, and T_3. Likewise, mean values of DE and ME also found significantly increase in the group in which maize oil cake was added @ 15% and 30% . According to Li et al. (2018), the ME and NE contents also increased (p<0.05) as the BW of pigs increased but without significant changes in energy digestibility and ME/DE and NE/ME ratios. Kim et al. (2008) determined significantly higher true metabolizable energy (TMEn) and amino acid digestibility in corn germ meal compared to high protein DDGS, while 'Phosphorus' bioavailability was significantly less for CGM (25 %) when compared to high protein-DDGS (60 % vs. 58 %, respectively).

Table 6. Energy values of feed and efficiency ratios under different dietary treatments

Attributes	T1	T2	T3
GE(kcal)	3064.04^{a}±65.75	3227.47^{ab}±73.44	3459.56^{bc}±83.88
DE(kcal)	2198.82^{a}±42.50	2332.83^{bc}±48.91	2526.92^{b}±35.98
ME(kcal)	1803.03^{a}±34.85	1912.92^{b}±40.10	2072.07^{b}±29.50

*Mean bearing different super scripts in arrow differ significantly (P<0.05)

CONCLUSION

Inclusion of maize oil cake did not have negative impact on FCR. It improved ether extract digestibility and nutritive value of feed in terms of DCP and TDN % in kids. Energy value of feed was also improved due to addition of maize oil cake.

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Effect of Feeding Berseem Fodder Grown Using Natural Farming Practices on Growth, Serum Metabolites, Interleukins and Semen Qualities in Barbari Goats

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ABSTRACT

Berseem fodder (*Trifoliumalexandrinum*) was cultivated using natural farming practices and evaluated in Barbari goats for their effect on growth, serum metabolites, interleukins and semen quality. Ten growing Barbari goats (Avg. BW 19.15± 0.87) age about 10 months were divided into two groups (CON and NAT) of five each as per completely randomized design. Animals of both the groups were fed with Bengal gram Straw, concentrate pellet and green Berseem fodder. The goats of CON group was fed with green Berseem cultivated using conventional practices while goats of NAT group was fed with green Berseem cultivated using Natural farming practices The experiment was conducted for 120 days out of which 60 days was for growth study. Serum metabolites and semen quality was studied after 120 days of feeding. The average daily gain (ADG) was 90.33 g for Gr CON while 74.33 g for Gr NAT. Dry matter intake (g) was 766.71 and 759.16 for Gr CON and Gr NAT respectively. Among serum metabolites glucose, protein, albumin, triglycerides, urea was statistically similar among groups. Aspartate aminotransferase (AST) and cholesterol was lower in goats fed with naturally grown Berseem fodder. Immunity indicators like IL 1, IL6, TNF and total antioxidant was also similar among groups. No difference was reported on semen quality parameters like semen volume, mass motility, live percent and post thaw motility between groups. Present study concluded that feeding Berseem fodder grown using Natural farming practices had no significant effect on growth, metabolites and semen quality.

KEYWORDS: Berseem fodder, Goat, Growth, Natural practices, Semen, Serum

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INTRODUCTION

Green fodder is essential component of goat ration in intensive system of production. Among green fodder Berseem (*Trifoliumalexandrinum* L.) an annual leguminous fodder forms a significant portion of animals ration particularly during rabi season from November to April months. Cultivation of fodder requires input of chemical fertilizers and pesticides which can negatively affect the soil, animal health and their products. Consumption of animal products having residues of pesticides, insecticides can affect the human health and there may be increased incidence of diseases like cancer in human population. There is also involvement of money for the production of fodders with the use of chemical fertilizers and pesticides in intensive fodder production practices. Therefore farmers are now encouraged to cultivate the crop as well as fodder crops using natural farming practices which uses different decoctions like brijamrit, jeevamrit to improve soil, animal and human health. Natural farming techniques can significantly enhance fodder production by improving soil health, utilizing on-farm resources, and reducing input costs (Niti Ayog). This approach focuses on using natural methods to boost yields and the quality of fodder crops, benefiting both animal health and the environment. Work has been conducted on the fodder quality with the application of natural farming practicesin their cultivation (Arif et al., 2023), but scanty information is available about the effect of feeding these fodder crops on animal growth, blood metabolites and reproductive performance. Keeping in view the present experiment was conducted to evaluate the effect of feeding Berseem fodder grown using Natural farming practices in Barbari goats.

MATERIALS AND METHODS

Study site

This experiment was conducted at Animal Experimental unit, Animal Nutrition Management and PT Division, ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura, India. It is located at 27° 10'N latitude and 75° 28'E longitude and 169 m above sea level. The feeding trial was conducted from 21 Dec 2023 to 18 April 2024 (120 days)

Berseem (*Trifoliumalexandrium*) was cultivatedat Agriculture farm of ICAR-CIRG, Makhdoom during the rabi season of 2023-24.The soil of the experimental field was nearly neutral in reaction (pH 7.2) with EC of 0.27 dS/m. The soil was low in organic carbon (0.24 %) and available nitrogen (238 kg ha-1); and medium in available phosphorus (40 kg ha-1) and potassium (167 kg ha-1). Berseem fodder was cultivated using two practices. First,conventional agronomic practices (CON) and second, natural farming practices (NAT) with application of Beejamrit and Jeevamrit formulations. For natural farming, we first prepared Beejamrit and Jeevamrit formulations. Beejamrit was prepared by mixing 5 liters cow urine, 5 kg cow dung, 50 gram lime powder and one handful of soil in 20 liters of water and keeping it for 12 hours. Similarly Jeevamrit was prepared by mixing 5 liters cow urine, 10 kg fresh cow dung, 2 kg gram flour, 2 kg jaggery and one handful of soil in 200 liters of water and keeping it for 48 hours in shaded area. Berseem seed was first treated with Beejamrit solution and dried before cultivation. Jeevamrit formulation was sprayed on naturally grown Berseem fodder after each cutting. These fodders were cut and carry for feeding to goats of experimental groups.

Ten growing Barbari goats (Avg. BW 19.15± 0.87) age about 10 months were divided into two groups (CON and NAT) of five each as per completely randomized design. Animals of both the group were fed with Bengal gram Straw, concentrate pellet and green Berseem fodder. Goats were fed with concentrate pellet at the rate of 1.5% of body weight, one kg of green berseem and *ad lib* gram straw. Animals of both the group were offered with concentrate pellet in the morning. After complete consumption of pellet fixed quantity of Berseem fodder was fed. Berseem fodder was fed as such without chaffing. Gram straw was fed free of choice and refusal was noted in next day morning (8.00AM) daily to calculated daily dry matter intake.CON group was fed with green Berseem cultivated using conventional practices while NAT group was fed with green Berseem cultivated using natural farming practices. The different feeds were provided separately in conventional form.The duration of experimental feeding was 120 days, out of this period growth was studied up to 12 months of age (60 days of feeding) and serum metabolites and semen attributes were studied after 120 days of feeding. Goats were housed in well ventilated sheds under uniform management. Weighed quantities of concentrate pellet were offered to both the group of goats at 08:00AM daily. After complete consumption of pellet roughage portion of ration was offered to the goats. *Ad libitum* water was provided and was changed twice daily throughout the experimental period.

The serum samples were analyzed for different biochemical constituent's viz. glucose, total protein, albumin, cholesterol,urea, triglycerides and Aspartate aminotransferase (AST) using diagnostic commercial kits (Autospan, Span diagnostic LTD.). There concentrations were quantified using end-point assay using double beam spectrophotometer (UV-Vis spectrophotometer, Optizen, 3220UV, Mecasys.co. Ltd, Korea). The procedure provided by the company on kits leaflet was strictly followed for analysis. IL 1, IL6, TNF and total antioxidant were analyzed using ELISA kits of BT Labs (Bioassay Technology laboratory)

After attainment of the age of around 12 months the bucks were trained for mounting and after 4 weeks of training each animal was subjected to semen collection. Semen ejaculates from each buck were collected twice at weekly intervals with the help of artificial vagina in the morning hours. A dummy non-oestrous doe was used for buck mounting, and semen was collected into the graduated cups. Semen samples were evaluated immediately after collection for colour, consistency, mass motility. Immediately after collection, semen was maintained in hot water bath at 37°C and subjected to evaluation. Volume of each ejaculate was recorded with the graduated collection cup. Mass motility was estimated at low power magnification (10×) using a compound microscope with neat semen on thermo stage maintained at 37°C. Semen samples were diluted and after dilution evaluated for live/dead count and abnormalities. Live and dead sperm count was estimated as per standard staining procedure as

described by Hancock (1951). A drop of diluted semen mixed with 2-3 drops of stain (Eosin (0.67 g/ 100 ml) and Nigrosin (5 g/100 ml)) was incubated at 30 æ%C for 1 min. Then smears made on pre-warmed slides were allowed to dry at 30 æ%C. Then smear was observed under 400× objective lens of the phase contrast microscope. Approximately 200 sperm were counted.After that semen samples were cryopreserved in liquid nitrogen and evaluation of post-thaw qualities likePost-thaw motility, live/dead sperm count were conducted to study the effect of feeding on the semen freezability.

Nutrient composition of concentrate, gram straw and Berseem were analysed using the protocol described by AOAC (2012).Neutral detergent fiber (NDF), Acid detergent fiber (ADF) were determined as per the method of Van Soest et al (1991).

The data collected during study were analyzed by independent sample t-test as per Snedecor and Cochran (1989) according to a complete randomized design using statistical software package (SPSS version 20). Individual animals were considered as experimental units. The difference between means was significant at 95% level of significance ($P<0.05$).

RESULT AND DISCUSSION

Chemical composition of feed

The proximate principals and the fibre fractions of concentrate pellet, gram straw, and green Berseem fodder are presented in Table 1. Green Berseem cultivated using conventional and natural farming methods are containing similar dry matter (around 11%).The crude protein content (%) was 15.40 for concentrate pellet, 6.6 for Gram straw and around 17 for Berseem fodder. No significant difference was reported in crude protein content among different Berseem. The fibre fractions (neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose) were also statistically similar in both types of berseem. The ether extract (%) was 6.86 for concentrate pellet, 1.81 for gram straw, 3.65 for berseem (CON) and 3.61 for berseem (NAT). The chemical composition of concentrate pellet and gram straw was within range as reported by Kumar et al (2024). The composition of Berseem fodder was also similar as reported by Arif et al (2022). No significant difference was reported in the chemical composition of fodder Berseem (CON) and Berseem (NAT).

Body weight gain and dry matter intake

The initial body weight (kg) in Gr CON and Gr NAT was 19.06 and 19.24 which increased to 24.48 and 23.70 after 60 days of experimental feeding (table 2). The fortnightly body weight changes are depicted in Fig 1.Total body weight gain (kg) was 5.42 and 4.46 in Gr CON and Gr NAT respectively. The average daily gain (ADG) was 90.33 g for Gr CON while 74.33 g for Gr NAT. There was no significant difference in body weight gain among groups. The average daily gain of goats was in agreement with 50–100 g / day reported by Ranjhan (1998) and Kumar et al. (2015).There was no significant effect on body weight gain in response to Berseem fodder cultivation. Since the nutrient composition of both the fodder is similar little variation is expected on body weight gain. Average daily dry matter intake (g) during experimental feeding period was 766.71 and 759.16 for Gr CON and Gr NAT respectively which is approximately 3.52% of mean body weight during experimental feeding period. Generally in tropical environmental condition dry matter intake ranges from 1.1 to 4.1% in growing goats (NRC 1981, Kearl 1982, Devendra and Bums 1983). The intake of Berseem fodder was 103.60g in Gr CON while 106.49 in Gr NAT and statistically similar between groups (Table 2).This clearly indicated that cultivation of Berseem fodder using natural farming practices did not affect its palatability. Kumar et al. (2015) also reported that incorporation of azolla in the complete pellet did not significantly affect its palatability.The concentrate to roughage intake was around 63:37 in both the groups showing no significant difference.

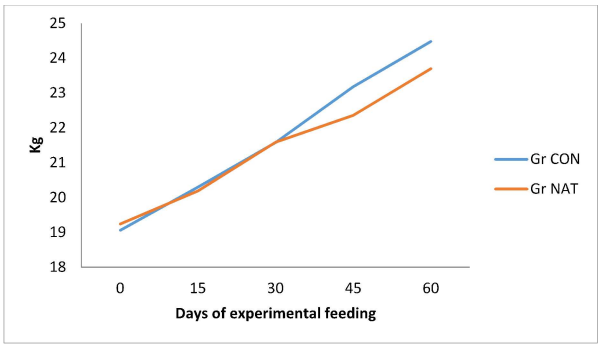

Fig 1: Fortnightly body weight changes in different group of goats

Table 1. Chemical composition of feed (% dry matter basis*)

Attributes	Concentrate pellet	Gram straw	Green Berseem (CON)	Green berseem (NAT)
DM	93.15±0.58	89.41±0.23	11.26±1.07	11.16±0.78
Moisture	6.85±0.58	11.59±0.23	88.74±1.07	88.84±0.78
Organic matter	91.28±0.19	90.10±0.30	81.14±1.79	81.14±1.47
Ether extract	6.86±0.58	1.81±0.19	3.65±0.36	3.61±0.34
Ash	8.73±0.19	9.90±0.30	18.86±1.79	18.86±1.48
Crude protein	15.40±0.36	6.60±0.02	16.58±0.19	16.93±0.27
Neutral detergent fibre (NDF)	24.07±0.14	61.05±0.77	43.58±1.10	46.33±0.88
Acid detergent fibre (ADF)	10.39±0.40	47.62±0.33	29.07±0.97	30.93±0.75
Lignin	2.90±0.25	12.77±0.26	6.90±0.49	6.64±0.30
Cellulose	7.50±0.65	34.86±0.59	22.16±0.91	24.29±0.67
Hemi cellulose	13.69±0.55	13.43±1.11	14.50±0.88	15.40±0.99

*except dry matter and moisture

Table 2. Growth and dry matter intake in different groups of goats

Attributes	Gr CON	Gr NAT
Initial body weight (Kg)	19.06±0.58	19.24±1.73
Final body weight (Kg)	24.48±0.50	23.70±1.53
Average body weight gain (g/d)	90.33±3.78	74.33±3.62
Dry matter intake (g/d)	759.16±7.05	766.71±6.14
Concentrate intake (g/d)	283.31±2.65	281.41±2.68
Berseem intake (g/d)	103.60±2.35	106.49±3.04
Gram straw intake (g/d)	372.24±5.17	378.81±4.19
Roughage intake (g/d)	475.84±6.17	485.29±4.76
Conc: roughage ratio	37.52: 62.48	36.75: 63.24

Serum metabolites and semen quality

Serum metabolites are the indicator of nutritional balance, deficit condition, clinical status and possible indication of major organ efficiency. The data pertaining to the serum metabolites concentration of experimental goats revealed that the mean values of glucose, protein, albumin, triglycerides, urea obtained in the present study was within normal physiological range (Kaneko et al., 1997). There was no significant ($P>0.05$) difference in blood biochemical parameters viz., serum glucose, total protein, albumin, globulin, triglycerides and serum urea concentration among groups. The mean serum Aspartate aminotransferase (AST) values of both the groups were within the reported normal range (66 to 230 (IU/L) suggested for goats (Fraser et al., 1986) but the concentration was lower in goats fed with naturally grown Berseem fodder. Serum cholesterol was also lower in goats fed with naturally grown Berseem fodder. IL-6, IL-1, and TNF-alpha are pro-inflammatory cytokines that play important roles in the body's immune response to injury, infection, and other stimuli. They are involved in the activation of immune cells, the production of other inflammatory mediators, and the development of fever and other acute-phase responses. While these cytokines are essential for fighting infection and tissue repair, excessive or dysregulated production can contribute to chronic inflammation and disease. Elevated serum levels of interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-

alpha (TNF-alpha) are often associated with inflammatory and autoimmune conditions, certain cancers, and infections. These cytokines play crucial roles in the body's immune response, and their dysregulation can contribute to the development and progression of various diseases. IL 1, IL6, TNF and total antioxidant was also similar among groups.

No difference was reported on semen quality parameters like semen volume, mass motility, live, dead percent and post thaw motility between both the groups (Table 3). The values of semen attributes were similar for 12-16 months bucks previously reported (Kumar et al., 2021). The colour was cream to yellow cream and consistency was medium. Other semen qualiy attributes were similar among both the groups. Semen qualities are influenced by the diet. The determinations made on the collected samples indicate non-significant changes ($p < 0.005$) in the fresh as well as post thaw semen qualities. The difference between the two groups has no statistical significance ($p > 0.05$). This can be explained by the fact that both groups received the balanced nutrition during experimental treatment. Have non-significant effect to improve the semen qualities as well as on post thaw seminal parameters. Therefore, in conditions where the experimental treatment was carried out, it is clear that a daily supplementation of feed played an extremely important role, positively influencing not only the quantity but also the qualities of the collected seminal material. Micro nutrients particularly trace elements and bioactive molecules stimulate growth and development of primary and secondary sex organs, spermatogenesis (Underwood and Somers, 1969). Kumar et al (2016) reported that supplementation of fresh azolla improved reaction time and progressive motility of spermatozoa in Barbari bucks without any significant effect on semen volume and live counts. Similar effect was observed in the present study indicating green fodder cultivated using conventional and natural farming practices had similar effect on semen quality attributes.

Table 3. Serum metabolites and semen qualities in different groups of goats

Attributes	Gr CON	Gr NAT
Serum metabolites		
Glucose (mg/dl)	59.53±1.55	61.99±2.69
Protein (g/dl)	6.05±0.22	6.68±0.74
Albumin(g/dl)	4.46±0.17	4.15±0.04
Globulin (g/dl)	1.59±0.07	2.53±0.11
Cholesterol (mg/dl)	90.09±4.60	82.42±6.41
Triglyceride(mg/dl)	97.20±3.13	92.96±2.25
Urea(mg/dl)	73.87±7.30	74.20±2.31
Aspartate aminotransferase (U/L)	89.15±11.46	68.98±8.20
IL1 (ng/L)	2.57±0.36	2.45±0.38
IL6 (ng/L)	1.32±0.25	1.35±0.12
TNF (ng/L)	3.01±0.27	2.65±0.25
Total Antioxidant (u/ml)	2.56±0.38	2.23±0.40
Semen attributes		
Colour	Cream	Yellow-cream
Consistency	Medium	Medium
Volume (ml)	0.75±0.05	0.73±0.09
Mass motility (%)	4.85±0.08	4.45±0.16
Fresh semen Live per cent	93.50±1.78	92.50±1.90
Fresh semen Dead per cent	06.50±0.78	07.50±0.87
Post thaw motility (%)	62.50±0.77	60.00±0.96
Post thaw Live percent	66.10±0.89	65.36±1.16
Post thaw Dead percent	33.90±0.89	34.64±1.16

CONCLUSION

Present study concluded that feeding Berseem fodder grown using Natural farming practices had similar nutrient profiles and exerted similar effect on growth, metabolites and semen quality in goats compared to Berseem grown under conventional agronomic practices.

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Influence of Prepartum Plane of Nutrition on Nutrient Utilization and Performance in Murrah Buffaloes

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ABSTRACT

Maternal nutritional status pre-partum acts as a determining factor not only for the health status of the off-spring but also, for production yield of upcoming lactation phase, thereby feeding during this time period is of utmost importance. The present study investigated the effect of feeding a high plane of nutrition to prepartum Murrah buffaloes on the birth weight of calves and their performance, nutrient utilization, and blood metabolites. Forty pregnant Murrah (4 months before parturition) buffaloes were divided into four experimental groups based on parity, previous lactation yield and body weight as control. Based on the ICAR (2013) requirements for metabolizable protein (MP) and metabolizable energy (ME), four dietary treatments were formulated: (i) a control ration as per ICAR (2013); (ii) a high metabolizable energy (HME) ration containing 30% more ME; (iii) a high metabolizable protein (HMP) ration with 40% more MP; and (iv) a high metabolizable energy and protein (HMEMP) ration with 30% higher ME and 40% higher MP than the ICAR (2013) recommendations. A feeding trial was conducted using these rations until the date of parturition. Concentrate mixture, green fodder (maize) and dry roughage (wheat straw) were offered to individual animal as per experimental protocol. Dry matter intake and metabolizable energy intake were significantly ($P<0.05$) higher for HMEMP, followed by HMP, HME and control group. Average daily body weight gain of pre-partum dams was higher ($P<0.05$) in HMEMP that is 946.08 g/d, followed by HME (761.67 g/d) and HMP (753.17g/d), with lowest for control group (576.08 g/d). It was observed that digestibility coefficients (%) of dry matter, organic matter, ether extract, neutral detergent fibre and acid detergent fibre were higher ($P<0.05$) in groups HMP, HME and HMEMP as compared to the control. It was concluded that group fed HMEMP diet i.e., an additional 40% protein and 30% energy above ICAR, 2013 requirements during last four months of pregnancy resulted in higher body weight gain and better nutrient digestibility compared to groups fed individual diets having high levels of energy (HME) or protein (HMP).

KEYWORDS: Birth weight, Digestibility, P of nutrition, Prepartum buffaloes, Transition nutrition

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INTRODUCTION

Buffaloes are considered the main dairy animal in India, contributing 49% to the country's total milk production (BAHFS, 2019). Murrah leads as the prominent buffalo breed in the country, followed by Mehsana, Surti and Jaffarabadi (Breedwise report of Livestock and Poultry, 2022). Lower birth weight leads to a delay in puberty (generally attained at the age of 34 months in Murrah buffaloes) and retarded performance as the growth of calves depends on birth weight. If the mother is severely underfed during the last three months of pregnancy, it might affect the young, causing death in utero or reducing viability at birth (Mc Donald, 2002, Wallace et al.1999, Godfrey and Barker, 2000). Therefore, balanced feeding taking into account proper nutrition over and above maintenance is of utmost necessity during this phase.

However, experimental studies that substantiate that feeding of higher energy and protein than that recommended by ICAR (2013) during advanced pregnancy might be valuable are sparse. The present study, therefore, aims at monitoring the influence of feeding higher levels of dietary protein and dietary energy than the recommendation of ICAR (2013) during the last trimester of pregnancy on nutrient utilization in buffaloes and the birth weight of their calves.

MATERIALS AND METHODS

Forty Murrah buffaloes in the last trimester (6 months) of pregnancy were randomly distributed into four groups (n=10) based on parity, lactation number and body weight as T1 (Control), T2 (HME), T3 (HMP) and T4 (HMEMP), respectively to minimize variation between the groups. The control group animals were fed as per the ICAR (2013) feeding standards for late gestation. Buffaloes in the HME group received 30% more metabolizable energy (ME) than the ICAR (2013) recommendation, while protein and other nutrients were provided as per the standard. In the HMP group, animals were fed 40% more metabolizable protein (MP), with energy and other nutrients aligned with ICAR (2013). The HMEMP group received both 30% higher ME and 40% higher MP, while other nutrients were maintained as per the standard guidelines. The increase in ME and MP levels in the treatment groups was based on the ICAR (2013) recommendations for late gestation in buffaloes, which served as the baseline. Treatment diets were formulated by increasing the energy and/or protein levels above these reference values to evaluate the physiological response of buffaloes to enhanced nutrient intake during the last four months of pregnancy. Based on ME and MP values four concentrate mixtures were prepared, and four different freshly prepared total mixed ration (TMR) were offered twice a day. The physical compositions of different concentrate mixtures are presented in Table 1. Fresh drinking water was provided ad libitum four times daily.

Table 1. Ingredients and their proportions in the concentrate mixture

Ingredients (kg/100 kg)	Control	HME	HMP	HMEMP
Maize grain	20.0	56.0	16.0	39.0
Oats grain	18.0	7.00	20.00	14.00
SBM	2.00	7.00	9.00	16.00
Wheat bran	40.00	9.00	25.00	5.00
DORB	15.00	0.00	15.00	2.00
MOC	0.00	9.00	0.00	6.00
GNC	2.00	0.00	12.00	8.00
Prilled fat	0.00	9.00	0.00	7.00
Mineral mixture	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
CP%	14.22	14.07	19.80	19.80
ME (Mcal/kg)	2.70	3.55	2.76	3.41
MP%	8.85	9.42	12.14	12.61

A metabolism trial was conducted around one month before expected date of parturition to determine the nutrient digestibility and nitrogen balance. For suitable aliquoting of biological samples representative samples of feed offered, faeces voided, and urine excreted were collected (Schneider and Flatt, 1975). The samples were analyzed for proximate composition (AOAC, 2005), cell wall fractions (Van Soest et al., 1991) and fiber bound protein fractions such as NDF and ADF bound CP (NDICP and ADICP) (Licitra et al., 1996). Total-N content of urine samples were estimated (AOAC, 2005). The TDN, DE and ME value of the fodders was estimated using chemical composition based formulae suggested by NRC (2001) The average of two days was considered as body weight of that fortnight. Difference of body weight for each fortnight was considered as body weight changes for that fortnight. After proper restraining, blood samples were collected from the jugular vein for

immunoglobulin analysis at monthly intervals before feeding. The proximate analysis of the feed, leftover residues, and faecal samples was done to assess their chemical composition (AOAC, 2005) and cell wall constituents (Van Soest et al., 1991). Measured amount of TMR was offered and on the next day collection of residue was done for individual animal on daily basis. DMI was recorded daily by subtracting residual/left-over DM from the quantity of DM offered.

Plasma total immunoglobulin was estimated by zinc sulphate turbidity method (Mc Ewan and Fisher, 1970) Reagents: Zinc sulphate, fetal calf serum, Rabbit gamma globulin Test reagent: 4.1 ml of 5% zinc sulphate solution was taken and final volume was made upto 1 litre with double distilled water. Principle: Albumins, alpha-globulins, beta-globulins and gamma-globulins are the four major classes of protein present in the blood. These proteins have differential precipitability in various concentration of salt such as ammonium sulphate or sodium sulphate. The zinc sulphate turbidity test is based on the principle given by McEwen and Fisher, (1970). Zinc sulphate at a specific concentration precipitates the gamma globulin, this creates a turbidity which is proportional to the quantity of gamma globulin in the sample and can be quantified in the spectrophotometer at 460nm.

Analysis of data assimilated through measurement of various parameters (body weight, DMI) was conducted by one way ANOVA method of Snedecor and Cochran (2004) using the Statistical Analysis System (2012) and presented as average mean ± pooled standard error of means (SEM). 5 % level of probability ($P<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The detailed chemical composition of feed ingredients is presented in Table 2. Dry matter intake (DMI) and digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF) are illustrated in Table 3. The DMI in HMEMP (13.98 kg/d) was higher ($P<0.05$), than HMP (13.36 kg/d), followed by HME (12.39 kg/d) and control (12.21 kg/d). From the third fortnight onwards, the dry matter intake increased in all four groups, however, it gradually decreased around the last fortnight of pregnancy.

Table 2. Chemical composition of feeds and fodder (On DM basis)

Particular	DM	OM	CP	EE	NDF	ME (MJ/kg)	MP (%)
			Roughage				
Maize fodder	18.46	89.25	10.83	2.01	60.67	7.98	7.15
Oat fodder	16.55	90.91	11.83	2.20	42.06	9.26	8.52
Sugargraze	26.13	90.25	10.08	2.07	58.56	7.86	6.88
Berseem	14.62	88.04	17.24	1.82	53.78	8.26	12.58
Wheat straw	90.15	89.0	3.42	1.01	76.73	5.55	0.92
			Energy and protein sources				
Soybean meal	91.10	91.68	46.75	0.99	19.18	13.62	28.07
Maize grain	90.99	98.03	9.76	5.20	17.93	13.11	7.53
Wheat bran	90.14	95.57	14.34	2.07	38.99	11.24	9.28
De-oiled rice bran	92.09	90.7	17.69	1.03	36.93	10.00	8.26
Mustard oil cake	92.39	93.99	36.15	7.79	23.19	11.89	21.26
Groundnut cake	91.53	92.69	46.20	1.06	21.88	13.67	28.77
Oats grain	90.59	97.31	11.22	3.27	24.19	12.68	7.02
Prilledfat	99.99	99.9	-	99.80	-	38.78	

The intake of DM (kg/d), TDN (kg/d), MP (g/d) and MEI (Mcal/d) was significantly higher (P<0.05) in animals fed HMEMP than other treatments. CP intake (g/d) was highest in HMEMP (1336.74) and HMP group (1339.57) followed by HME (876.72) and the control group (878.97). Animals fed with control diets or HME had the least intake of DM, OM whereas, CP intake was minimum in animals offered diets having 30% extra energy (HME).

The digestibility of nutrients is given in Table 3. The DM digestibility was significantly (P<0.05) higher in the HMEMP group followed by HMP, HME and the control group. The OM digestibility was statistically similar in HMEMP and HMP group which was significantly higher than HME and control group. The digestibility of CP, NDF and ADF was significantly higher (P<0.05) in HMEMP and HMP followed by HME as compared to the control.

The mean N intake (g/d) was significantly higher in the HMP group as shown in Table 3. N excretion in faeces was also significantly affected by the supplementation of energy and protein in the diet. Overall N balance (g/d) was significantly high in the group fed with 40% extra protein and 30% extra energy (HMEMP)

Table 3. Intake and digestibility of nutrients and nitrogen balance

Parameters	T1	T2	T3	T4	SEM	p-value
DMI (kg/d)	12.21^{c}	12.39^{c}	13.36^{b}	13.98^{a}	0.15	0.02
DMI (kg/100 kg BW)	1.84^{b}	1.84^{b}	1.97^{a}	2.01^{a}	0.02	0.03
TDN (kg/d)	6.06^{d}	7.50^{c}	6.98^{b}	7.79^{a}	0.06	0.01
CPI (g/d)	878.97^{b}	876.72^{b}	1339.57^{a}	1336.74^{a}	9.21	0.04
MPI (g/d)	469.77	503.70^{b}	765.92	768.99^{a}	4.52	0.01
MEI (Mcal/d)	21.20^{d}	27.45^{b}	25.20^{c}	28.41^{a}	0.23	0.01
Digestibility (%) of nutrients						
DM	62.87^{c}	63.21^{c}	66.81^{b}	68.28^{a}	0.40	0.03
OM	64.96^{b}	65.94^{b}	68.61^{a}	69.72^{a}	0.41	0.01
CP	55.11^{c}	59.69^{b}	64.00^{a}	65.14^{a}	0.54	0.04
EE	67.29^{c}	84.23^{a}	68.32^{c}	80.79^{b}	0.30	0.03
NDF	55.00^{b}	50.07^{c}	57.81^{a}	58.57^{a}	0.55	0.01
ADF	35.67^{a}	31.38^{b}	37.81^{a}	36.76^{a}	0.82	0.01
Nitrogen balance						
Total N intake (g/d)	150.33^{c}	143.57^{d}	233.34	226.69^{b}	1.45	0.02
N outgo through faeces (g/d)	67.43^{a}	57.76^{d}	84.16^{a}	79.06^{b}	1.25	0.01
N outgo through urine (g/d)	58.62^{c}	54.33^{d}	116.82	109.50^{b}	1.60	0.04
Nitrogen balance (g/d)	24.28	31.67^{b}	32.36^{b}	38.13^{a}	0.95	0.03
Nitrogen balance % of total N intake	35.67^{a}	35.67^{a}	35.67^{a}	35.67^{a}	0.77	0.02
Nitrogen balance % of total N absorb	29.01^{b}	36.01^{a}	21.73^{d}	25.82^{c}	0.85	0.01

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

The dietary treatments significantly (P<0.05) influenced the live weight changes over the prepartum period. Body weight increased significantly (P<0.05) in the HMEMP group as compared to HME, HMP group and the control as shown in Figure 1. Average Daily Gain (g/d) was significantly higher in the HMEMP group (946.08) followed by HMP (761.67), HME (753.17) and the control (576.08) as depicted in Figure1.

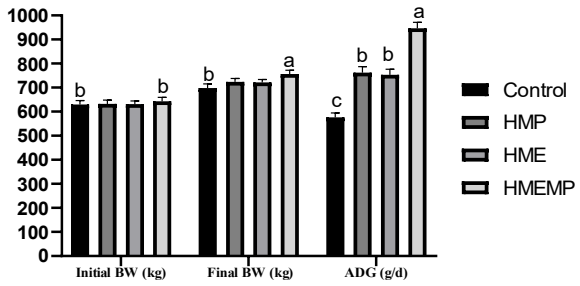

Figure 1. Bodyweight and Average body weight gain in various groups

Figure 2, illustrate the average birth weight (kg) of calves born from dams fed with control, HME, HMP and HMEMP diets and these were 29.36, 31.44, 33.33 and 36.44 kg, respectively.

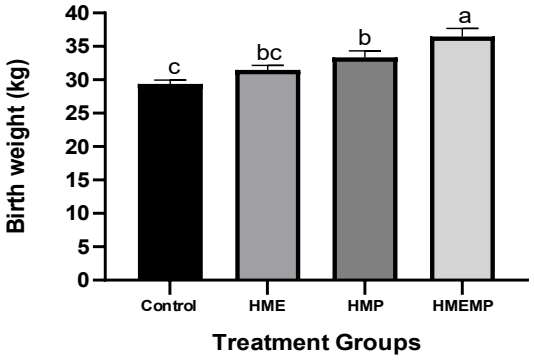

Figure 2. Bodyweight and body weight gain in various groups

Total immunoglobulin concentration (Figure 3) was significantly (P<0.05) higher in HMP and HMEMP groups followed by HME and control group.

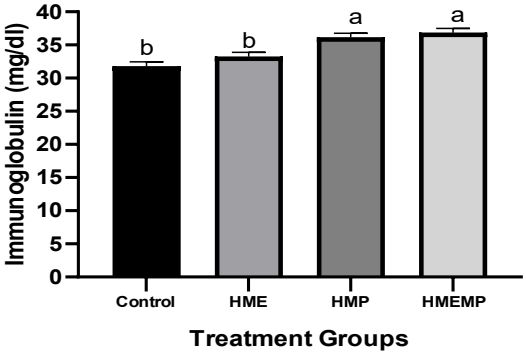

Figure 3. Immunoglobulin concentration in various groups

The present study demonstrated that dry matter intake (DMI) increased progressively with the advancement of pregnancy across all groups, with a significant (P<0.05) improvement observed in the HMEMP group. This aligns with the findings of Singh et al. (2016) and Proto (1993), who reported that DMI in pregnant buffaloes increases during mid-gestation and begins to drop closer to parturition. The higher DMI in HMEMP group might be attributed to the increased nutrient density, better palatability, and a physiological drive to meet the elevated nutrient demands of fetal growth. Contrary to this, Panigrahi et al. (2005) noted no significant effect of different concentrate amounts on DMI in crossbred cows prepartum. Similarly, Shelke (2010) and Silvestre et al. (2011) observed that protected fat and protein feeding in Murrah buffaloes did not adversely affect DMI, although they improved intake of CP and ME. In contrast, Schroeder et al. (2022) and Weiss et al. (2011) reported a reduction in DMI with protected fat supplementation in dairy cows. These discrepancies may be due to differences in dietary composition, physiological status, and breed. Significantly higher intake of CP, MP, ME, and TDN in HMEMP and HMP groups corroborates the enhanced dietary nutrient supply. The observed differences are expected, as animals were intentionally fed 40% more protein and/or 30% more energy in these groups. Similar findings were reported by Mustafa et al. (2017), where transition buffaloes offered high ME and MP diets showed increased nutrient intake. The lowest CP intake in HME animals compared to HMP and HMEMP reflects

that energy supplementation alone without corresponding protein enhancement may not stimulate CP intake.

Digestibility coefficients of DM, OM, CP, NDF, and ADF were significantly higher in HMEMP and HMP groups. These results agree with El-Ashry et al. (2003), who observed improved digestibility in buffaloes fed higher energy diets. Moreover, Jawid (2016) reported improved CP digestibility in buffaloes fed with higher metabolizable protein diets, though digestibility of other nutrients remained unaffected. The improved fiber digestibility (NDF and ADF) in high-protein groups is supported by Lee et al. (2011), who noted low fiber digestibility in animals receiving low-CP diets. Conversely, Christensen et al. (1993) found no improvement in OM, NDF, and ADF digestibility despite increasing dietary CP, suggesting that beyond a certain threshold, digestibility may plateau or depend on factors beyond CP level alone, such as forage quality and rumen microbial efficiency.

Significantly higher nitrogen (N) intake and retention in the HMEMP and HMP groups suggest superior nitrogen utilization efficiency when adequate protein is supplied. These results are consistent with Colmenero and Broderick (2006) and Castillo et al. (2001), who documented a linear relationship between N intake and N excretion, and found that 72% of consumed N is typically excreted via faeces and urine. Additionally, Lee et al. (2011, 2012) and Giallongo et al. (2014) observed that lower MP diets result in higher urinary N loss, implying suboptimal nitrogen retention. The significantly improved nitrogen balance in the HMEMP group indicates that synchronized supply of both energy and protein optimizes ruminal microbial activity and nitrogen retention, leading to better utilization and reduced wastage. Buffaloes fed the HMEMP diet exhibited the highest body weight gain and average daily gain (ADG), followed by HMP and HME groups. These findings are supported by Schoonmaker et al. (2003), Radunz et al. (2010) and Gamit et al. (2024), who observed improved body weight and growth performance in cattle fed higher energy or protein diets during late gestation. However, Vaswani et al. (2025) reported no change in DMI and body weight change after supplementing by pass fat. The enhanced ADG in the HMEMP group suggests that simultaneous supplementation of energy and protein supports both maternal tissue accretion and fetal growth more effectively than individual nutrient supplementation.

The significantly higher birth weights in calves born to buffaloes fed HMEMP and HMP diets are indicative of improved intrauterine growth. These observations are supported by Gunn et al. (2013) and Bolze et al. (1985) who reported higher birth weights in calves from dams fed high-protein diets. Similarly, Radunz et al. (2010) and Pandey et al. (2024) found that feeding high-energy diets and nano minerals, respectively, during late pregnancy enhances calf birth weight. The combined energy-protein supplementation appears to create a more favorable intrauterine environment, thereby promoting better fetal development.

Significant increase in plasma total immunoglobulin levels in the HMP and HMEMP groups suggests improved maternal immunity status and possibly better passive immunity transfer potential to the offspring. This is consistent with the findings of Chatterjee et al. (2003), Aggarwal et al. (2016), and Deka et al. (2014), who noted that improved nutritional status during late pregnancy is associated with enhanced immune response in buffaloes. The elevated Ig levels in protein-supplemented groups reinforce the role of dietary protein in supporting immue function during late gestation.

CONCLUSION

Feeding additional metabolizable energy (30%) and metabolizable protein (40%) above ICAR, 2013 requirements during last four months of pregnancy in buffaloes resulted in higher body weight gain and better nutrient digestibility compared to groups fed individual diets having high levels of energy (HME) or protein (HMP).

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Effect of Combination of Physical Pretreatment on Toxic Factors and Nutritional Value of Canola Meal

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ABSTRACT

The aim of this study was to determine the effect of combination of soaking and heat treatment on nutritional characteristics of canola meal and certain anti nutritional factors. Canola meal was soaked in water for 12 and 24 hours (h) followed by heating at 80°C and 100°C for 30 min and 60 min respectively. Crude protein and fat content of canola meal increased significantly ($P<0.05$) with soaking for 24 h followed by heating at 100°C. Crude fiber content reduced with increasing soaking time and heat treatment. Glucosinolate and erucic acid content reduced significantly ($P<0.05$) with soaking at 24 h followed by heat treatment for 60 min irrespective of the temperature. Results of this study indicate that combination of high temperature heating (100 °C for 60 min) and soaking (24 h) may be necessary to reduce the toxic compounds and improve nutritional characteristics of canola meal.

KEYWORDS: Anti Nutritional Factors, Canola meal, Erucic acid, Glucosinolate, Soaking

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INTRODUCTION

The scarcity generated due to competition for feed ingredients between human and animals and the elevated prices of protein rich ingredients for animal feed emphasize the need for comprehensive studies on all potential protein sources to support efficient livestock production (Ghazalah et al., 2022). Moreover, the declining profitability of the livestock/ poultry industry, driven by rising costs of key raw feedstuffs, has compelled nutritionists to seek more affordable cheaper alternative protein sources. This paves way towards utilization of certain unconventional feeds in animal feeding. However, the efficient use of relatively low-cost unconventional feed ingredients depends on the chemical and physical properties for sustainable animal production (Sinha et al., 2020).

Canola, belonging to the family of Brassica, is a registered name for a type of rapeseed which is highly valued as a component of animal feed (Woyengo et al., 2014). Canola is generally used to prepare the canola oil, and the canola meal is produced as a byproduct of rapeseed during oil extraction process. The presence of high protein content and a well balanced composition of desirable amino acid are favoring canola meal to be a good animal feed. The distribution of amino acid in canola meal is similar to that present in soyabean meal. Canola meal is a rich source of various vitamins and minerals, particularly sulfur, selenium, phosphorus, and B vitamins. It contains less than 2% erucic acid in the total fatty acids of its oil and less than 30 µmoles of glucosinolates per gram (Ravichandran et al., 2008). But, the presence of some anti-nutrients in canola meal limits its potential as a protein supplement. The main antinutritional factors in canola meal are dietary fibre, glucosinolates, phytic acid, and phenolic compounds. But, it is mostly the presence of glucosinolate in canola meal which marks the limitation for its usage in animal feeding. Also, the high fiber content of canola meal restricts its quality and interferes with the digestibility of meal in non ruminant diet.

Glucosinolates are plant secondary metabolites, classified into aliphatic and aromatic glucosinolates, depending on involvement of amino acids for synthesis. Glucosinolates in general play a role in plant defense against insects and diseases; and has anti cancer properties (Sønderby et al., 2010). Glucosinolates themselves are non toxic; however their degradation products are toxic to animal body

(Sharma and Nagra, 2009). High glucosinolate levels and their metabolites, produced through enzymatic breakdown, can have harmful or toxic effects (Cartea et al., 2008).

To minimize the harmful effects on animals and improve incorporation rates in their feed, various processing techniques can be employed. Physical, chemical, and microbiological methods can be used to reduce the glucosinolate (Gls) content. The aim of the present study was to identify combination of various physical techniques which could be beneficial in minimizing the deleterious components present in canola meal and enhancing its nutritional characteristics.

MATERIALS AND METHODS

The GSC07 canola cultivar variety samples were procured from farmer's demonstration unit. The canola meal was obtained after oil extraction by double pressed expeller processing methods. The canola meal samples (10 g) were soaked in water (ratio of meal: water 1:10, w/v) at room temperature (24 °C) for 12 and 24 hours, and then drained by using a vacuum filter. The samples were heated in oven at 80 and 100°C for 30 min and 1 hour based on the treatment (Table1). Each treatment was conducted in 3 replicates.

Table 1. Details of physical treatment of canola meal

Treatment	Soaking time (hrs)	Heating time (min)	Heating temp (^{0}C)
1	12	30	80
2	12	30	100
3	12	60	80
4	12	60	100
5	24	60	80
6	24	60	100
7	24	30	80
8	24	30	100
9	Control	Control	Control

Chemical composition

Proximate analysis: Following different treatments each canola meal sample was analyzed for dry matter (DM), organic matter (OM),ash, crude protein (CP), crude fiber (CF) and ether extract (EE) as per AOAC (1995) procedures.

Estimation of Glucosinolate: Spectrophotometric estimation of glucosinolate was done using methanolic extract prepared by homogenizing 0.1 g defatted seed meal in a 2 ml vial with 80% methanol and centrifuged at 3000 rpm for 4 min. The supernatant collected was incubated at room temperature for 1 hour, absorbance was measured at 425 nm using a spectrophotometer. Total glucosinolates was calculated (Mawlong et al., 2017) by putting OD of each sample taken at 425 nm into the predicted formula:

$$y = 1.40 + 118.86 \times A_{425}$$

Determination of fatty acid methyl ester (erucic acid) was performed with a GC system (Russo et al., 2021) CHROMPACK CP3800, with auto sampler 8200 CX and flame ionization detector. Helium was used as a carrier gas with a flow rate of 1 ml/min. The split ratio was set to 1:20 and injector temperature was 280°C.

STATISTICAL ANALYSIS

The data were analyzed by using one way ANOVA with Tukey's post hoc testing to compare experimental groups test using SPSS (2010) computer package. For all statistical analyses, where the probability values were less than 0.05, were considered as significant.

RESULT AND DISCUSSION

Effect on Chemical composition

Combination of soaking and heat treatment did not result in significant (P>0.05) increase in the moisture content of canola meal, with dry matter content ranging from 91.7% to 95.0% (Table 2). The meal generally have an ability to absorb and retain water and oil helping to improve binding of the structure, enhance flavor retention and reduce moisture and fat losses of food products (Sreerama et al., 2008). However, soaking and heating of canola meal alone also showed no significant change in the dry matter content when heated at 127°C for 15 min in a preheated oven (Aleid et al., 2025). The findings are consistent with those found in heat treated canola meal at 90, 110, 120 and 150° C by Piotr et al., 2020.

Heat treatment is one of the most common methods to reduce ruminal protein degradation and increase post ruminal availability (Sniffen et al., 1992). Water soaking of feedstuffs is beneficial in reducing the antinutritive factors but may also result in loss of soluble protein content (Widharna et al.,2012).Combination of physical treatment significantly improved the protein percentage of canola meal as compared to the untreated group. The author reported an increase in protein percentage by 25% when canola meal was soaked in water for 24 hours over control. This rise in protein percentage when canola meal was soaked in water for an extended period (24 hrs), might be due to the leaching of non-nitrogenous soluble materials into the water (Widharna, 2012). Additionally, heating at higher temperatures (100°C) led to an increase in protein levels, likely due to enhanced synthesis of protease enzymes (Bau et al., 1997). According to Burakowska et al. 2019, degradable fractions of crude protein in CM decreased linearly with increasing temperature of heat treatment which was much higher (110°C) than that used in present experiment. Similarly, protein solubility decreased linearly from 85% to 81%, 61%, 52% and 40% after the toasting (100°C) time increased from 30 min to 60 min (Jensen et al., 1995). The combination of pretreatment in the current study also significantly reduced glucosinolate levels, which may have ensued in enhanced protein levels.

Table 2. Effect of physical pretreatment on proximate composition (%) of canola meal

Treatment	Dry Matter	Protein	Ether Extract	Crude Fiber	Ash
1	94.3±0.33	38.21^{b}± 0.15	11.57^{c}±0.04	11.00^{b}±0.21	5.94±0.18
2	95.0±0.88	39.54^{cde}±0.29	10.63^{b}±0.08	12.60^{c}±0.18	6.12±0.13
3	93.5±0.29	40.2^{de} ±0.15	8.77^{a} ±0.10	11.67^{b}±0.44	6.12±0.07
4	94.0±1.53	39.3^{cd}±0.14	10.20^{b}±0.15	11.77^{b}±0.15	6.29±0.12
5	93.0±0.58	39.3^{cd}±0.15	12.77^{d}±0.15	12.17^{b}±0.60	6.13±0.14
6	94.0±0.58	40.3^{de}±0.15	11.87^{c}±0.09	11.00^{b}±0.06	6.11±0.07
7	93.2±0.60	39.1^{bc}±0.28	13.73^{e}±0.12	12.53^{c}±0.38	5.77±0.13
8	93.3±0.44	40.3^{de}±0.33	13.96^{e}±0.03	11.56^{b}±0.08	6.30±0.36
Control	91.7±0.33	32.0^{a}±0.29	10.40^{b}±0.08	13.10^{d}±0.59	6.52±0.29

Values with different superscript varied significantly (p< 0.05)

Ether extract percentage in untreated and treated canola meal ranged from 8.77 to 13.96%. Significant change (p<0.05) in ether extract was recorded with different soaking time and heat temperature. Increased oil content was recorded in canola meal soaked in water for 24 hours indicating that increased soaking time might promote hydrolysis of fatty acids (Mohamadzadeh et al., 2009). However, maximum

level of fat percentage was recorded when combination of soaking (24 hours) and heat (100°C) pretreatment was applied suggesting increased soaking time and heat, raised the levels of free fatty acids (Mohamadzadeh et al., 2009). The increase in levels may also be associated with heat dissociation of the proteins and denaturation by heat pretreatment, unmasking the non-polar residue from the interior of the protein molecules and enhancing fat absorption (Kinsella, 1976). Contrary, Piotr et al., 2020 reported slightly less fat content for heat treated canola meal owing to higher heating temperatures (150°C).

The effect of different pre treatment on the crude fiber levels compared to control sample is shown in Table 1. The CF content ranged from 13.10 to 11.00 % in untreated and treated canola meal respectively recording significant reduction in CF levels. Both soaking and heat treatment had significant impact on fiber content of canola meal as significantly low fiber levels were recorded in canola meal exposed to 12 h and 24 h of soaking alongwith heating temperature of 100°C.

It has been reported that commercial rapeseed meal contains 12.1% crude fiber, most of which is derived from hulls. Mohamadzadeh et al., 2009 recorded that soaking rapeseed for 100 min followed by hot air drying reduced the crude fiber content significantly correlating the high correlation efficiency of the treatment applied. Also, application of heat has long been deployed in animal feed industry to remove anti-nutritional factors and improve nutrient availability and palatability of animal feed (Allan and Booth 2004).

Physical pretreatment did not alter the ash content in canola meal samples, with ash content ranging from 5.77% to 6.52%. These values are consistent with those found in other varieties of canola meal. Since ash analysis indirectly reflects the mineral content in feed, the lack of significant change suggests that mineral content is not impacted by either moisture or heat treatment in canola meal. This supports the results of Widharna et al. (2012), who reported that pretreatment with heat and moisture did not alter the ash content.

Effect on glucosinolates and erucic acid

The nutritionally undesirable glucosinolates followed by erucic acid in seed meal of rapeseed–mustard cultivars prevalent in India are very high and range between 43 to 57% and 150–240 µmol g-1, respectively (NBPGR, 2003).The data presented in Table 3 indicates that in present study, glucosinolate content of untreated canola variety sample was 77 µmol/g, while glucosinolate content of treated samples varied from 58.04 to 64.95 µmol/g, demonstrating 24% reduction in glucosinolate levels following soaking and heating treatments. Furthermore, significant differences were observed among the treatments, with shorter soaking times resulting in greater glucosinolate reduction (24%) compared to longer soaking times. This finding aligns with Widharna et al. (2012), who recommended shorter soaking times to effectively reduce glucosinolate levels. Tripathi and Mishra (2007) reported heating of canola meal from 10 to 60 min at 100^0 C did not reduce the glucosinolate content significantly whereas heating in combination of moisture reduced glucosinolate content by 18.8%.

Table 3. Glucosinolates and erucic acid of canola meal

Treatment	1	2	3	4	5	6	7	8	Control
Glucosinolate (µmol g−1)	61.11^{b}± 0.20	61.42^{b} ±0.47	58.59^{a}±0 .30	58.04^{a}±0 .09	58.53^{a}±0 .29	60.53^{b}± 0.26	63.30^{c}±0 .44	64.95^{d}± 0.07	77.00^{e}±0 .56
Erucic acid (%)	24.76^{ab}± 0.43	25.91^{b}± 0.39	23.62^{a}±0 .19	23.15^{a}±0 .60	28.20^{c}±0 .44	24.08^{ab}± 0.87	23.41a± 0.36	22.57^{a}±0 .58	33.00^{d}± 0.63

Values with different superscripts varied significantly ($p< 0.05$)

Soaking canola meal in water facilitates the leaching of toxic substances, including glucosinolates, followed by cell lysis and diffusion (Baenas et al., 2020). The glucosinolate levels remained lower and stable when heating was conducted for 1 hour, regardless of temperature. This is consistent with the findings of Nugrahedi (2015), who reported that prolonged processing time increases cell lysis and thermal degradation, leading to greater glucosinolate losses. However, Jensen et al. (1995) noted that heating beyond 60 minutes can reduce both the quantity as well as quality of protein in addition to decreasing glucosinolate content. The decrease in glucosinolates due to thermal and enzymatic degradation has been documented in different studies (Wennberg et al., 2006).

The erucic acid content in both treated and untreated canola meal samples ranged from 22.57% to 33%. A significant decline in erucic acid levels was observed in the treated groups compared to the untreated samples. Additionally, notable differences were recorded among the various treatment groups. Treatments with lower glucosinolate content tended to have lower erucic acid levels, although in some cases, lower erucic acid levels were accompanied by higher glucosinolate content. Zhang et al. (2020) found that erucic acid content is positively correlated with the degradation products of glucosinolates.

Prolonged heating increases the rate of fatty acid auto-oxidation, leading to a decline in erucic acid content (Alireza, 2010). Dawodu et al. (2015) reported a 2–3% increase in free fatty acids when temperatures reached 250°C, which strongly supports the findings of this study. High temperatures also enhance the solubility of targeted phenolic compounds in canola meal (Li and Guo, 2016). Khajali and Slominski. (2012), also reported that higher erucic acid consumption is associated with the myocardial lesions in laboratory animals.

CONCLUSION

The results presented here indicated that glucosinolate content of canola meal was decreased as the soaking time and heating increased. Following the glucosinolate content of canola meal, protein and crude fiber levels were altered by combination of physical treatment. These finding and observed change in anti-nutritive factors propose that, a combination of soaking and heating effect had potential for improving nutritional value of canola meal.

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Effect of Urea and Exogenous Fibrolytic Enzyme Treated Paddy Straw on *In Vitro* Rumen Fermentation Characteristics and Degradability

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ABSTRACT

This study was conducted to evaluate the effect of urea and exogenous fibrolytic enzyme (EFE) treatment on *in-vitro* digestibility and fermentation attributes of paddy straw. In this study, *in vitro* degradability of nutrients in paddy straw as an effect of treatment of urea and EFE at various levels (1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0 and 9.0 % of urea and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 g/kg DM of EFE) were tested. For selecting the best levels of urea and EFE, test samples were incubated with strained rumen liquor and different dosages levels were studied on rumen fermentation parameters like total gas production along with *in vitro* DM and OM digestibility, ammonia nitrogen, pH and some calculated parameters such as partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial protein synthesis (EMPS) by incorporation of urea and EFE treated paddy straw. The result showed significant improvement on *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), *in vitro* total gas production (IVTGP), MBP and PF at 4% and 8 g/kg DM of urea and EFE treatment level in comparison to other levels. The optimum results were obtained at 4% and 8 g/kg DM of urea and EFE treated paddy straw, hence a 4% and 8 g/kg DM dosage, respectively may be selected for *in vivo* study.

KEYWORDS: Exogenous fibrolytic enzyme, *in vitro*, paddy straw, Rumen fermentation, urea

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INTRODUCTION

In many agricultural countries, paddy straw and other agro industrial by products are available in large quantities immediately after every harvest seasons. Thousand tonnes of agricultural residue is set on fire after the harvesting of the crop for preparation of field for the following crop. This problem is much severe in regions where farmers are practicing the mechanized rice-wheat cropping pattern (Mehta et al., 2013). Considerable effort has been made to improve the utilization efficiency and feeding value of cereal straws using pre-treatments to enhance its digestibility, including biological, chemical, and physical pre-treatments (Eun et al., 2006), as well as providing nutritional supplements based on the lack of nutrients in cereal straw (Wei et al., 2019). Ammoniation of rice straw by urea treatment may be suitable way for smallholder-farmers who keep animals in limited quantities because urea treatment can improve intake, crude protein and digestibility with low cost, relatively safe and easy to apply. Therefore, the use of rice straw as an animal feed as well as its treatment is always an economic decision (Sarnklong et al., 2010). Supplementing ruminant diets with fibre degrading enzymes has been shown to improve feed utilization and animal performance (Beauchemin et al., 2003). However, the effectiveness of feed enzymes in ruminant diets is dependent upon substrate-enzyme specificity. Thus, it is important to establish the optimum enzyme activities for the degradation of rice straw.

The aim of the present study was to evaluate the effect of urea and exogenous fibrolytic enzyme (EFE) treatment on *in vitro* fermentation attributes of paddy straw and to select the best levels of urea and EFE for the treating paddy straw for feeding ruminants.

MATERIAL AND METHODS

Urea and EFE were used in the trial i.e. neem coated urea and mafzyme forte. Mafzyme forte was purchased from Meenakshi Agro Farms, Bengaluru - 560043. Karnataka, India. Urea and

EFE were tested at 11 doses (1, 2, 3, 3.5, 4, 4.5, 5, 6, 7, 8 and 9%) and 9 doses (1, 2, 3, 4, 5, 6, 7, 8 and 9 g/kg DM), respectively to determine their effects on *in vitro* rumen fermentation. For *in vitro* trial, feeds were prepared from paddy straw milled by using 1mm screen.

Rumen liquor was collected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura. Animals in LFC were kept on similar feeding like straw and concentrate for about 3-5 days. The rumen liquor strained through four layers of muslin cloth in a glass flask. Flask kept in incubator prior to use and then the required amount of strained rumen liquor used as inoculums. Carbon dioxide gas was passed through the rumen liquor and maintained at 39±1ºC temperature for use of preparation of inoculum. 200 mg substrate was weighed in calibrated glass syringes (Super scientific Co. Bangalore) of 100 ml capacity. Sample was put on the bottom with the help of weighing boat with removable stem. For testing of each treatment group two syringes of substrate blank and two syringes as standard were prepared. The fermentation value for standard was checked with data base available in the laboratory. The incubation medium was prepared as described by Menke and Steingass (1988). 30 ml incubation medium (rumen fluid-medium mixture) was dispensed anaerobically in each preheated (39ºC) syringe. Syringes were closed using clamps and the volume of the mixture was recorded in the syringes. Then syringes were placed vertically in a wooden stand with hole to hold the syringes upright in the incubator ventilated by fan assisted forced air circulation at 39±0.5°C for 24 hr. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe from total gas produced in the syringe containing substrate and inoculum and buffer.

True DM degradability and OM degradability of feed sample of each syringe containing residues after incubation was estimated as per Van Soest et al. (1991). The partition factor (PF) is calculated as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. It provides important information about partitioning of fermentation products. The microbial biomass production (MBP) was calculated by using the degradability of substrate and gas volume and stoichiometrical factor as suggested by Blummel et al. (1997). Daily microbial protein synthesis is the product of the efficiency of microbial protein synthesis (EMPS) (Hoover and Stokes, 1991), which usually is defined as grams of microbial crude protein (MCP) / kilogram or 100 grams of OM digested in the rumen (Hoover and Stokes, 1991). After 24 hr incubation, the pH of the rumen liquor was checked by pH meter. Ammonia nitrogen (NH_3-N) was estimated by using colorimetric method (Weatherburn, 1967).

$$MBP\ (mg) = TDOM\ (mg) - (gas\ volume \times 2.25)$$

$$EMPS\ (g/kg\ DOM) = MCP\ (g) / DOM\ (kg)$$

The generated data were statistically analyzed by ANOVA considering level of urea and EFE as factor using the general linear model procedure (univariate) using SPSS (SPSS for windows, Version 20.0; Inc., Chicago, IL, USA) software.

RESULTS AND DISCUSSION

The effect of urea treated paddy straw on *in vitro* rumen fermentation parameters was shown in Table 1. The *in vitro* dry matter digestibility (IVDMD %) and *in vitro* organic matter digestibility (IVOMD %) was significantly higher ($P<0.05$) in 4% urea treated paddy straw as compared to other dose levels of urea. The present results confirmed the results obtained by Ekani and Wahyono (2020) for urea treatments. Jayanegara et al. (2017) found that *in vitro* study of urea treated rice straw show increased in IVDMD and IVOMD by 18.0% and 17% as compared to control. Vadiveloo (2003) reported that treatment of low degradable variety of rice straw responded better to urea treatments than higher quality straw ultimately resulting increased *in vitro* DM degradability from 45 to 62%. Jayanegara et al. (2017) found increased values of IVOMD which might be due to high quickly soluble and fermentable protein leading to the rapid growth of microorganisms this helped in increasing the breakdown of OM. The *in vitro* total gas production (IVTGP, ml/200 mg DM) (IVTGP, ml/g DM) (IVTGP, ml/g digestible dry matter, DDM) were observed for various levels of urea treatment. The total gas production increases with increasing level of urea treatment. Abo-Donia et al. (2022) carried out *in vitro* study with urea treated rice straw and observed that there was

increase in gas production released. This increased gas release was associated with the improvement in the degradation of OM and NDF which resulted from the increased activity of microorganisms. Sniffen et al. (2006) reported that improved fiber degradation leads to an increase in gas accumulation as well as its release rate. During *in vitro* fermentation, the digestibility of feed OM is positively correlated with gas production. The higher the digestibility of OM, there was increase in the fermentation activity of microorganisms in the rumen, the gas production rate is accelerated, and the gas production is increased (Ma et al., 2020).

In the present study, the gas production of each urea treated groups were higher than that of the control group, indicating that the urea treatment can increase the soluble carbohydrate of rice straw and increase the gas production rate and the gas production. The results of *in vitro* studies further indicate that the higher the urea content, the greater gas production *in vitro*, which is consistent with the findings of Senthilkumar et al. (2010). In present study, pH did not differ significantly which was similar with the findings of Jihene et al. (2022) who also reported that the urea and Urea + EFE supplementation had no significant effects on ruminal pH. Sheikh et al. (2017) also reported non-significant difference in the rumen pH in sheep fed urea molasses treated rice straw. In the present study, ammonia nitrogen (mg/100 ml) and Total-N (mg/100 ml) were found significantly lower ($P<0.05$) at 4.0 % with compare to other levels when the TMR rations treated with urea. Total nitrogen and NH_3-N recorded at different hours post feeding the urea molasses treated rice straw were found significantly ($P<0.05$) higher in treatment groups than that of control (Sheikh et al., 2017). Gomaa et al. (2012) who revealed that lambs fed urea-molasses treated paddy straw has higher total nitrogen concentrations.

Table 1. Effect of urea treated paddy straw on *in vitro* rumen fermentation parameters

Parameters	Urea level (%)												Pooled SEM	P value
	Control	1.0	2.0	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0		
IVDMD (%)	71.46^{a}	71.90^{a}	73.30^{ab}	75.18^{b}	75.88^{b}	77.49^{c}	77.69^{c}	77.89^{c}	77.06^{c}	76.90^{bc}	75.19^{b}	75.25^{b}	2.56	0.038
IVOMD (%)	72.34^{a}	73.88^{a}	75.19^{ab}	76.70^{ab}	77.09^{ab}	79.12^{b}	78.89^{b}	77.15^{b}	78.31^{b}	77.28^{b}	76.61^{b}	77.17^{b}	1.95	<0.001
IVTGP (ml/200 mg DM)	29.52^{a}	30.53^{a}	31.97^{a}	34.29^{ab}	36.2^{b}	39.41^{c}	40.09^{c}	40.14^{c}	38.72^{c}	36.5^{b}	33.78^{ab}	29.42^{a}	1.16	0.011
IVTGP (ml/g DM)	189.25^{a}	195.7^{a}	206.25^{ab}	210.8^{b}	226.05^{c}	227.55^{c}	230.75^{c}	231.41^{c}	226.05^{c}	227.5^{c}	223.55^{bc}	189.25^{a}	14.29	0.045
IVTGP (ml/g DDM)	264.83^{b}	272.18^{bc}	281.38^{bc}	280.39^{bc}	297.90^{c}	293.65^{c}	297.01^{c}	297.10^{c}	293.34^{c}	295.84^{c}	297.31^{c}	251.50^{a}	13.66	0.004
pH	6.97	6.92	6.76	7.01	6.82	6.88	6.91	7.01	7.16	7.28	7.26	7.35	0.53	0.893
NH_3-N (mg/100 mL)	12.75^{ab}	12.50^{ab}	12.21^{a}	11.90^{a}	12.30^{ab}	11.89^{a}	12.31^{ab}	12.45^{ab}	13.05^{b}	14.55^{bc}	16.51^{c}	17.30^{d}	0.94	0.024
Total N (mg/100 ml)	52.25^{a}	54.00^{a}	56.24^{ab}	58.51^{ab}	60.22^{b}	63.18^{bc}	63.25^{bc}	65.51^{c}	67.26^{d}	67.39^{d}	68.25^{de}	70.51^{e}	2.52	0.006
PF	3.88	3.90	3.84	3.72	3.66	3.50	3.47	3.34	3.38	3.42	3.37	3.45	0.07	1.000
MBP (mg/200mg)	78.26^{e}	79.07^{e}	78.45^{e}	76.25^{e}	72.73^{bc}	69.57^{b}	67.58^{b}	63.99^{a}	69.50^{b}	72.44^{bc}	77.22^{e}	88.15^{d}	4.21	0.037
EMPS (g/kg DOM)	540.92^{fg}	535.11^{f}	521.66^{e}	497.05^{d}	471.72^{c}	439.63^{b}	428.30^{ab}	414.68^{a}	443.75^{b}	468.65^{c}	503.95^{d}	571.11^{g}	27.39	0.009

In present study NH_3 concentration was lowest for diets based on 4% urea-treated straw which was similar with findings of Trach et al. (2001) who reported rumen NH_3 concentration was lowest for diets based on 4% urea-treated straw. Akinfemi et al. (2020) estimated that gas volume at 24 hours of incubation progressively increased with increase in urea-molasses treatment which might be due to high crude protein in feed enhances microbial multiplication in the rumen, which in turn determines the extent of fermentation. Wanapat et al. (2013) reported that EMPS was enhanced by urea and urea-calcium hydroxide treated rice straw. In contrast, Sommart et al. (2000) found that MBP and EMPS were significantly lower in urea treated straw than cassava in an *in vitro* study using cassava, rice straw, urea treated rice straw and dried ruzi grass as substrates. MBP was found to be lowest in fermented rice straw (Yulia and Sari, 2021).

The effect of EFE mixture supplemented paddy straw on *in vitro* rumen fermentation parameters was shown in Table 2. Tang et al. (2008) found that fibrolytic enzyme supplementations improved the IVDMD and IVOMD of rice straw. Treatments of rice straw with increasing level of cellulase significantly improved IVDMD and IVOMD compared to untreated rice straw (Selcuk et al., 2016). Another reason might be due to the synergy between EFE and the ruminal flora and increase in ruminal bacteria (Kholif et al., 2022). Kumar et al. (2013) reported that fibrolytic enzymes had increasing effects on total gas production of feeds. Application of EFE both during feed ensiling and directly during feeding increased *in vitro* gas production (Selcuk et al., 2016). In the present study, increase in the amount of gas production might be due to the increase in the activity of rumen microorganisms resulting as an increase in OMD (Selcuk et al., 2016). Nitipot and Sommart (2003) stated that there was a positive correlation between the volume of gas released during fermentation and *in vitro* OMD. Sujani et al. (2017) reported that cellulase and xylanase enzymes were supplemented alone and as a mixture with rice straw stated that all enzymatic treatments enhanced the IVTGP significantly ($P<0.05$) when compared with the control, indicating active microbial fermentation. The IVTGP increased with increasing doses of enzymes (Sujani et al., 2017). Increased IVTGP was noted by Yang et al. (2011) for rice straw treated with fibrolytic enzymes, and Jalilvand et al. (2008) found similar outcomes for wheat straw supplemented with fibrolytic enzymes. Liu and Orskov (2000) found increased IVTGP in steam-treated rice straw supplemented with non-starch polysaccharide enzyme, which is consistent with the current findings.

In the present study NH_3-N concentration was decreases with supplementation of EFE. These results are in agreement with those reported by Silva et al. (2016) showing that the addition of xylanase to the dairy cow diet decreased the NH_3-N concentration. Likely, an *in vitro* trial conducted by Almaraz et al. (2016) stated that EFE supplementation of a diet decreased the ruminal NH_3-N by 11%. Jihene et al. (2022) showing the ruminal NH_3-N concentration decreased ($P<0.05$) by EFE supplementation alone. In the present study average concentration of NH_3 obtained were in the range from 11.49 to 13.17 (mg/100ml), still within the normal range of rumen microbial growth which was similar to earlier findings reported by Lamid et al. (2013) who stated that the average concentration of NH_3 obtained were in the range of 11.70 to 16.79 mg N/100ml.

Rumen pH, NH_3-N and VFA contents are vital parameters in ruminants that illustrate the normal functioning and steady state of the rumen (Jia et al., 2018), and VFA are principal products of rumen fermentation, which directly related to the balance of energy in ruminants (Sun et al., 2013). The addition of lignocellulolytic enzymes and bacterial lignocellulolytic on rice straw resulted in significant improvement of all the fermentation products (acetate, propionate, butyrate, and ammonia) between control and supplemented groups (Lamid et al., 2013).

Table 2. Effect of EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters

Parameters	EFE mixture level (g/kg DM)										Pooled SEM	P value
	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0		
IVDMD (%)	51.03^{a}	51.52^{a}	51.88^{a}	52.62^{a}	54.99^{ab}	55.92^{ab}	58.42^{b}	60.02^{bc}	61.41^{c}	60.96^{bc}	6.39	0.017
IVOMD (%)	54.14^{a}	54.32^{a}	55.98^{a}	58.61^{ab}	59.99^{ab}	61.11^{b}	63.29^{c}	65.32^{d}	66.72^{d}	65.13^{d}	4.06	0.038
IVTGP (ml/200 mg DM)	30.18^{a}	30.51^{a}	31.99^{ab}	33.21^{b}	34.41^{b}	36.15^{c}	38.77^{d}	39.27^{d}	41.34^{e}	41.43^{e}	3.19	0.005
IVTGP (ml/g DM)	189.25^{c}	150.91^{a}	152.55^{a}	159.95^{ab}	171.9^{b}	176.5^{bc}	180.75^{c}	193.85^{cd}	196.35^{d}	206.7^{e}	12.53	<0.001
IVTGP (ml/g DDM)	370.86^{d}	292.90^{a}	294.04^{a}	303.97^{ab}	312.60^{b}	315.63^{b}	309.40^{b}	322.98^{bc}	319.74^{bc}	339.07^{c}	18.29	0.049
pH	6.19^{a}	6.22^{a}	6.34^{ab}	6.52^{ab}	6.69^{b}	6.79^{bc}	6.89^{c}	6.90^{c}	6.92^{c}	6.98^{c}	0.83	0.011
NH_3-N (mg/100 mL)	13.16^{b}	12.64^{ab}	13.23^{b}	12.99^{ab}	12.81^{ab}	12.44^{ab}	13.17^{b}	11.49^{a}	12.54^{ab}	12.39^{ab}	1.07	0.028
Total-N (mg/100 ml)	95.21^{a}	95.06^{a}	99.28^{b}	114.03^{c}	119.10^{c}	119.52^{c}	117.26^{c}	124.37^{cd}	129.39^{d}	130.51^{d}	6.29	0.044
TVFA (mM/100 ml SRL)	78.18^{a}	79.82^{a}	86.28^{ab}	97.23^{b}	99.12^{b}	112.25^{c}	116.89^{cd}	119.30^{cd}	126.16^{d}	125.85^{d}	7.41	0.039
Acetate (mM/100 ml)	50.82^{a}	51.88^{a}	56.08^{b}	63.20^{c}	64.43^{c}	72.96^{d}	76.22^{e}	77.55^{e}	82.00^{f}	81.80^{f}	4.50	0.008
Propionate (mM/100 ml)	19.55^{ab}	17.56^{a}	19.84^{ab}	23.34^{b}	22.30^{b}	27.39^{bc}	29.55^{c}	28.75^{c}	30.53^{c}	31.71^{c}	1.82	<0.001
Acetate: propionate	2.60:1	2.95:1	2.83:1	2.71:1	2.89:1	2.66:1	2.58:1	2.70:1	2.69:1	2.58:1	0.39	0.005
Butyrate (mM/100 ml)	7.91^{a}	7.82^{a}	10.38^{b}	10.35^{b}	10.56^{b}	10.60^{b}	11.90^{c}	11.49^{c}	13.00^{d}	13.63^{d}	1.48	0.033
PF	3.59	3.56	3.50	3.53	3.49	3.38	3.26	3.33	3.23	3.14	0.55	0.889
MBP (mg/200mg)	70.38^{a}	79.99^{b}	79.98^{b}	82.50^{bc}	92.56^{c}	90.88^{c}	79.35^{b}	82.28^{bc}	80.43^{b}	77.04^{ab}	4.18	0.018
EMPS (g/kg DOM)	649.98^{c}	736.28^{f}	714.36^{ef}	703.80^{d}	771.46^{g}	743.58^{g}	626.88^{b}	629.82^{b}	602.74^{ab}	591.43^{a}	36.29	0.047

$^{a-g}$Means within each column under the same subheading bearing different superscript letter are significantly different at P<0.05

Mohamed et al. (2005) evaluated the effect of an enzymatic mixture (cellulase, xylanase and protease) activities on the fermentation of substrate found that all acetate and propionate production were increased by all enzymatic treatments. Similarly, Giraldo et al. (2008) also stated that EFE supplementation increased propionate concentration.

Colombatto et al. (2007) studied the impact of fibrolytic enzymes on the rate and extent of fermentation of alfalfa stems (*in vitro*) and found that addition of these enzymes linearly increased *in vitro* OMD and DMD. Gado et al. (2007) evaluated the effect of biological treatments (cellulase; rumen liquor and Cellumonas cellulasae) of bagasse on lambs indicated that total VFA's values for treated bagasse by cellulase enzyme, rumen liquor and Cellumonas cellulasae were higher than that for untreated bagasse. Yang et al. (2000) revealed that the final concentration of VFAs increased by almost 9% with the enzymes. Arriola et al. (2011) found that supplementation of EFE @ 3.4 mg/g TMR dry matter to HF cows significantly increased TVFA concentration. EFE supplementation related to an increase in *in vitro* DMD and increases in the proportions of propionic acid and VFA (Gado et al., 2011). Kholif et al. (2022) reported an increased concentration of ruminal acetate with fibrolytic enzymes, which could be the result of improved apparent fiber degradation. Giraldo et al. (2007) reported that EFE supplementation significantly increased the microbial protein synthesis (MPS). Similar result obtained by Elwakeel et al. (2007) showing exogenous enzymes increase MPS which was an indicator that the bacterial population of the rumen is increased. Giraldo et al. (2008b) stated that supplementation of EFE increases the MPS which is justified that the EFE can release reducing sugars randomly which act as a readymade source of available energy and promotes rapid multiplication of the microbes (McAllister et al., 2001). Patel et al. (2015) reported that EFE supplementation was significantly increase total N concentration.

Table 3. Effect of urea and EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters

Parameter	Treatment		Pooled SEM	P value
	0.0	Urea (4.0%) + EFE mixture (8.0 g/kg DM)		
IVDMD (%)	52.68[a]	62.93[b]	4.38	0.048
IVOMD (%)	53.93[a]	68.59[b]	3.92	0.002
IVTGP (ml/200 mg DM)	30.11[a]	43.18[b]	2.80	0.016
IVTGP (ml/g DM)	150.55[a]	215.90[b]	11.47	<0.001
IVTGP (ml/g DDM)	285.78[a]	343.08[b]	14.28	0.031
pH	6.47	6.58	0.42	0.938
NH_3-N (mg/100 mL)	11.69[a]	13.32[b]	1.06	0.014
Total-N (mg/100 ml)	93.21[a]	128.53[b]	8.39	0.028
PF	3.50[b]	2.91[a]	0.15	0.044
MBP (mg/200mg)	70.38[a]	79.99[b]	2.81	0.039
EMPS (g/kg DOM)	668.00[b]	635.55[a]	42.06	0.049

[a-b]Means within each column under the same subheading bearing different superscript letter are significantly different at P<0.05

Effect of urea and EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters was shown in Table 3. In the present study, IVDMD, IVOMD and IVTGP were significantly increased. Similar observations found by Eun et al. (2006) and he stated that *in vitro* degradability of DM was greatly increased by addition of various types of enzymes along with ammonia treated straw indicating a synergistic effect between the ammonia treatment and enzyme application. The gas production was higher ($P<0.05$) in urea (4.0%) + EFE mixture (8.0 g/kg DM) treated paddy straw than that of the untreated paddy straw. The present study findings show non-significant difference in pH which was similar with the findings of Jihene et al. (2022) who also reported that the urea supplementation had no significant effects on ruminal pH. Sheikh et al. (2017) also reported non-significant difference in the rumen pH in sheep fed urea molasses treated rice straw along with fibrolytic enzymes. Author also reported that addition of enzymes in ammonia treated straw significantly improve IVTGP starting at 18 h of incubation, resulting in a 15-18% increase after 24 h. Wang et al. (2004) stated that alkali treatment alone significantly increased the amount of phenolic compounds, but not soluble carbohydrates, released from straw particles. In contrary, exogenous enzymes increased the release of soluble carbohydrates but not the release of phenolic compounds from straw particles.

Urea treatment/ ammoniation has been shown to give higher water- holding capacity to straw (Goto and Yokoe, 1996), resulting in softening of rice straw which was easily accessible to exogenous and rumen microbial enzymes. Thus, changes in the structural integrity of the cuticle by ammoniation/ urea treatment could facilitate the action of the exogenous enzymes, thereby resulting in substantial increases in rice straw degradation. Hence, ammonia or urea treatment of rice straw prior to enzyme application exerts the positive effects of enzymes, resulting enhanced microbial degradation of the rice straw (Eun et al., 2006). Jabri et al. (2022) reported improved IVTGP and IVOMD by addition of EFE to urea treated oat straw. This improvement is relevant for both the extent and the rate of gas production for so many studied enzymatic complexes, increasing estimated mostly used digestive parameters like OMD and VFA. Those findings were similar to the findings of kholif et al. (2022), and Abid et al. (2022) in which the EFE supplementation (xylanase and cellulase) effect on by-products commonly used for ruminant feeding (rice straw, barely straw, date palm leaves, and brewer's spent grain) were observed *in vitro* on OMD and microbial protein production.

CONCLUSION

The result showed improvement in *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, total gas production, microbial biomass production and partition factor. The optimum results were obtained at 4% and 8 g/kg DM of urea and EFE treated paddy straw, hence a 4% and 8 g/kg DM dosage, respectively may be selected for *in vivo* study.

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Impact of Moringa Fodder Supplementation on Serum Mineral Profile in Anaemic Goats

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ABSTRACT

A field study was designed to evaluate the efficacy of feeding *Moringa oleifera* tree fodder on the recovery rate of anaemia in desi kids under semi-intensive rearing. Twenty Desi kids 6-8 months of age were selected and anaemic status of animals was assessed using FAMACHA chart, further confirmed by haematology study. Twelve animals were selected based on the levels of Haemoglobin and stratified into control (T0) and treatment groups (T1). The samples of *Moringa oleifera* fodder samples were collected and analysed. Chemical composition of *Moringa oleifera* fodder in terms of DM, CP, EE, CF and total ash were 20.24 ± 0.30, 25.79 ± 0.99, 5.89 ± 0.12, 11.2 ± 0.51 and 9.63 ± 0.13 percent, respectively. The mineral profile of the Moringa fodder was calcium (1.42 ± 0.06), magnesium (90.23 ± 2.19), copper (6.77 ± 0.59), zinc (45.81 ± 2.64) and iron (512.52 ± 4.4) in ppm. The provocative cause of anaemia eliminated by deworming and ectoparasitic drugs. The feeding trial was started after 48 hours of deworming offering 200g of fresh Moringa in the morning to T1 before allowing for grazing. Simultaneously, T0 was allowed for regular grazing without any supplementation. Haematological parameters after 45 days revealed a significant elevation of serum iron level in the T1 (P = 0.00019) with consequential increase of Haemoglobin (P = 0.01) and PCV (P = 0.02) level when compared to the T0. Zinc level (P = 0.003) in serum also significantly increased reflecting the high concentration of zinc in moringa. The study observed enhancement in recovery rate of anaemic kids with substantial increase of zinc level.

KEY WORDS: Anaemia, Haemoglobin, Iron, Moringa fodder, Zinc

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INTRODUCTION

Livestock sector contributes around 37% of global protein supply following agricultural sources (Smith et al. 2024) and it is crucial for the food resilience and economic stability of developing countries, as farming act as a backbone of their endurance and developments. Ruminants are the key factors of livestock sector providing all forms of essential nutrients in the form of milk and meat. Among ruminants, small ruminant rearing is more popular; particularly, goat rearing is abundant globally owing to their adaptability and survivability at different agro-ecological zones. Chevon is one of the most consumed meats in the developing countries of Asia, Africa and Middle East (Lamri et al., 2022). From the recent global livestock census an increase of 10.14% of goat population from 135.17 million to 148.88 million within 6 years was observed (Wakchaure and Sethi, 2024). The same is reflected in Indian statistics with 4.6 percent increase in goat population (Modi et al., 2024). This increase in goat population across the world and India is attributed to their versatility, less complexness, high fertility and disease resistance.

Goats are considered as 'Poorman's cow' as goat rearing is more affordable and profitable than cattle rearing which enables the small-scale farmers to prefer goat rearing (Ibrohimovna, 2024). Being browsers, goats mostly quench their nutritional requirements from natural vegetation making goat farming a more profitable one. In rural areas, few farmers rearing extensive and semi-intensive system

experience economic losses mostly due to high kid mortality and poor growth rate followed by low fertility rates (Roy and Patbandha, 2024). Under these circumstances, the main factor affecting the folk is kids' mortality. One of the major factors contributing to kids' mortality is anaemia. Red blood cells, carriers of oxygen to vital organs are comparatively less in anaemic kids. Therefore, anaemia has been considered as the one of the most important causes for mortality in kids due to lack of oxygen supply to the vital organs (Pawaiya et al., 2017). Primarily, parasitism is the primary cause for these types of anaemia, resulting in the greater economic loss to the farmers. This problem stems from lack of knowledge on controlling ecto and endo parasites in young animals. Though these kids are treated periodically for ecto and endo parasites by farmers, it takes a longer period to revive from anaemic condition (Rizwan et al., 2023). This longer duration is crucial accounting for high requirement of iron for the incorporation onto the haemoglobin structure during erythrocytes synthesis. Iron supplementation during this critical period could augment the recovery stage (Pasini et al., 2021).

Moringa oleifera tree is a common fodder rich in protein, fibre, calcium, magnesium particularly iron (Hossain et al., 2020). *Moringa oleifera* is also a multipurpose tree possessing high significance in both human and animal nutrition for their nutritive value and phytochemicals (Singh et al., 2021). Since Moringa fodder aids in the treating anaemia, in this study we aimed to reduce the lag period of recovery of anaemic animals through oral supplementation of natural source of iron from easily available low-cost nutritious tree fodder.

MATERIALS AND METHODS

Selection of animals

Forty-five days of experimental study was conducted in non-descriptive goats, 6 to 8 months of age with an average body weight of 8 kg (8.0 ± 0.1 kg) reared in semi-intensive system of Pondicherry region. Twenty vaccinated animals under semi-intensive rearing were selected and their anaemic status was assessed by FAMACHA chart and confirmed by haematology. Twelve anaemic animals were identified based on low haemoglobin content and split into control (T0) and treatment groups (T1) each group consisting of six animals. All the animals were tagged for identification and dewormed with a combination of Albendazole and Ivermectin at a dose rate of 25mg/kg body weight.

Chemical composition and Mineral Profile

Samples of *Moringa oleifera* from six different Municipalities of Pondicherry region - Thirukanur, Villianur, Nettapakkam, Kalapet, Bahour, Ozhuvarkarai were analysed for their proximate principles (AOAC, 2023), fibre fractions (Van Soest et al., 1991) and mineral profile using Atomic Absorption Spectrophotometer (AAS Model ICE 3000 series).

Experimental Design

After 48hrs of deworming, animals in treatment group (T1) were offered with 200g of fresh Moringa fodder in the morning hours before allowing them for grazing. The daily voluntary intake of fresh moringa leaves was calculated for the entire experimental period of 45 days. Simultaneously, the control group animals (T0) were allowed for grazing with the treatment group animals without the supplementation of fresh Moringa fodder.

Blood collection and Analysis

Blood collection from the jugular vein was carried out aseptically from all the animals initially before deworming to identify the Hb level and at the end of feeding trial. Whole blood (2ml) was collected using Ethylene Diamine Tetra-acetic acid (EDTA) coated vacutainer for the Haematological analysis. Similarly, 2ml of whole blood was collected in a Clot activator vacutainer for serum separation. Serum separation was accomplished after clotting of blood with the help of centrifugation at 2500 RPM for 20 minutes and stored at -20°c. Whole blood samples was analysed for Haemoglobin (Hb) and Packed cell volume (PCV) immediately after the collection using Sahli's haemoglobinometer and Wintrobe tube, respectively. The stored serum was thawed to a room temperature and subjected to mineral analysis (Fe, Cu, Mg, Zn) in the Atomic Absorption spectrophotometer (AAS Model ICE 3000 series).

Statistical analysis

The data obtained from the blood and serum samples of two groups of goats was subjected to statistical analysis using SPSS (Version 29.0, 2022). Paired t-test and ANCOVA were used and Statistical significance was weighed when P value is less than 0.05.

RESULTS AND DISCUSSION

The Moringa tree is a multi- purpose species with a high nutritive value in their every parts. In addition to their use as human food and medicine they are also used as animal fodder for the ruminants across the world (Lata and Mondal, 2021). Since Moringa fodder is a rich source of iron (El-Massry et al., 2013), in this study we have attempted to ameliorate the anaemic condition of non- descriptive goat kids reared in the semi-intensive system by supplemental feeding of fresh Moringa fodder. Initially, proximate principles and fibre fractions of *Moringa oleifera* from different municipalities of Pondicherry region were analysed and the values are represented in the Table 1. Proximate analysis showed higher crude protein level in Moringa fodder (25.79 ± 0.99) on DMB in accordance with the Sultana (2020) equivalent to other nutritive green fodders and tree leaves. The fibre fraction revealed a moderate level of NDF (44.5 ± 0.45) and ADF (27.31 ± 0.72) in the Moringa fodder which implies its enhanced digestibility as higher NDF and ADF in a fodder can decrease the digestibility and feed intake (Schulze et al., 2014). The low lignin content (13.25 ± 0.50) in the fiber fractions with a higher amount of cellulose (14.06 ± 0.60) and hemicellulose (17.18 ± 0.95) implies a better digestibility. This in turn may be attributed to the fact that low lignin level is not favourable for strong ligno-cellulosic bonds in cell wall components of the fodder (Zeng et al., 2014).

Table 1. Chemical composition of Moringa fodder (DMB%) (n=6)

	Thirukanur	Villianur	Nettapakkam	Kalapet	Bahour	Ozhuvarkarai	Mean ± SE
Moisture	78.73	79.82	79.93	81.55	78.57	79.96	79.76 ± 0.44
CP	23.78	29.69	26.00	22.69	26.75	25.82	25.79 ± 0.99
EE	6.500	5.660	5.73	5.820	5.80	5.870	5.89 ± 0.12
CF	9.810	10.95	11.75	10.85	10.47	13.39	11.20 ± 0.51
TA	9.900	9.43	09.79	9.140	9.940	9.58	9.63 ± 0.13
AIA	0.360	0.36	0.240	0.550	0.150	0.33	0.33 ± 0.06
NDF	45.56	42.76	45.15	44.97	45.04	43.52	44.5 ± 0.45
ADF	26.32	29.48	29.59	25.75	26.85	25.89	27.31 ± 0.72
ADL	12.07	15.00	13.37	11.90	12.86	14.27	13.25 ± 0.50
Hemicellulose	19.24	13.28	15.56	19.22	18.19	17.63	17.18 ± 0.95
Cellulose	14.25	14.48	16.22	13.85	13.99	11.62	14.06 ± 0.60

The mineral composition (Copper, Iron, Magnesium, Zinc) of Moringa fodder from different areas of Pondicherry region was carried out in Atomic Absorption Spectrophotometer using diluted acid soluble extract of dried Moringa fodder (Table. 2) The results from the Table 1 projects only less variations in the chemical composition of Moringa fodder sampled from different municipalities of Pondicherry which implies the more or less identical nature of plant across the region of Pondicherry. However, the mineral composition of Moringa fodder showed mild variations in the present study (Table 2). This mild variation reflects the peculiarity of the soil composition of minerals as different soil conditions like clay soil, red soil and sand areas possess dissimilar concentrations of macro and micro minerals as stated in Osinuga et al. (2024). Moringa fodder is rich in iron content (512.52 ± 4.4 ppm) compared to commonly available fodder grasses Hybrid Napier grass – 308.22 ppm (Shankhpal et al., 2019) and tree fodder Subabul- 397 ppm (Jain and Rane, 2011). Since iron is the major element in erythropoiesis, the rich iron content in Moringa could play a major role in the recovery of anaemia by contributing to the increased need. In addition, the Zinc level in the Moringa fodder is high (45.81 ± 2.64 ppm) with low copper (6.77 ± 0.59 ppm) and Magnesium level (90.23 ± 2.19 ppm).

Table 2. Mineral composition of Moringa fodder (in ppm)

Area	Ca (in g%)	Cu	Fe	Mg	Zn
Thirukanur	1.452	7.04	522.66	93.03	42.89
Villianur	1.48	5.98	522.24	95.16	57.57
Netapakkam	1.6	8.79	498.57	93.36	44.94
Kalapet	1.24	5.65	510	93.00	42.67
Bahour	1.23	5.13	510.06	82.53	38.92
Ozhuvarkarai	1.49	8.06	521.75	84.30	47.88
Mean ± SE	1.42 ± 0.06	6.77 ± 0.59	512.52 ± 4.4	90.23 ± 2.19	45.81 ± 2.64

The kids reared in the semi-intensive condition showed a slow rise in the average daily feed intake (FI) of Moringa fodder during the experimental period of 45 days (Fig.1). Initially the FI of fresh Moringa fodder for six goats was low (114.9 ± 1.31 g/day) for the first 10 days and slowly the FI increased and reached a maximum of 155.16 ± 1.25 g/day by 45 days. The numerical differences among the groups T0 and T1 have been proved insignificant ($P > 0.05$) by independent t-test on comparing the means of Haemoglobin (Hb) and Packed cell volume (PCV) from the Hematological analysis on pre-experimental period (Table 3). However, an increase in the Haemoglobin as well as the PCV in both the groups of goats on 45th day of the trial which was proved statistically different from the 1st day Hematological values. The statistical significance (T0- P= 0004, T1 – P=0.000015) was observed in both the groups in which the Hb and PCV values on 1st and 45th day were analyzed using paired t-test. This health improvement in both the groups of goats may be due to the removal of the etiology of anemia in goats as the animals were treated with the broad spectral dewormer and ectoparasitic drug assuming that the anemia is solely due to parasitic infestation in addition to feeding Moringa fodder.

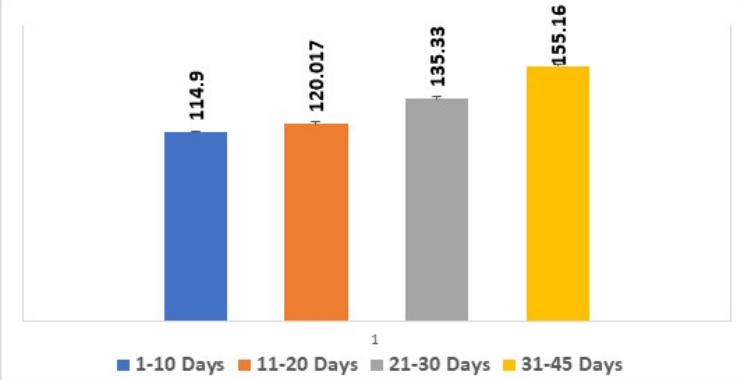

Fig. 1: Voluntary intake (g/d) of Moringa during the course of experiment

Table 3. Comparison of Haemoglobin and PCV in the Control group (T0) and Treatment group (T1) (n=6)

		Haemoglobin (g%)		Packed Cell Volume (%)	
Animal	Body weight (kg)	1st day	45th day	1st day	45th day
Control	8.06 ± 0.14	5.10 ± 0.46	9.67 ± 0.56	15.37 ±1.42	29.07 ± 1.72
Treatment	7.94 ± 0.16	5.33 ± 0.34	6.65 ± 0.41	16.1 ± 1.0	20.03 ± 1.27
Significance	NS	***	***	***	***

*** Significant at P<0.001; NS-Non Significant

Microscopic examination of Blood smear of selected animals revealed a small sized paler erythrocytes pointing to microcytic hypochromic anemia wherein the iron deficiency is a common factor. Supportive studies by Furtado et al. (2024), on the etiology of anemia also stated that the intestinal parasitic infection is one of the major causes of anemia in animals especially in young animals. The intestinal parasites adhere to the intestinal mucosa sucking the blood of host animal for their growth and reproduction resulting in deterioration of animal's health finally leading to anemia. These intestinal worms also cause malabsorption of nutrients including iron by reducing the height and depths of crypts of lieberkuhn (Pearson et al., 2012). Ectoparasitic infestations is another important principal cause of anemia in goat kids which sucks blood as like the helminths in the intestine (Insyariati et al., 2024). So, the process of deworming and removal of ectoparasites invariably removed the cause of anaemia allowing the animal to slowly recover from anaemic condition. This might be the reason for the improvement in haematocrit values of both the groups on 45th day.

To substantiate the hypothesis the effect of Moringa fodder in the T1 group on 45th day was evaluated and the results of two groups were compared to identify the influence of Moringa fodder feeding on the treatment group. By statistical comparison using ANCOVA, the feeding of Moringa fodder to the T1 group of goats had resulted in a very high significant rise in the Haemoglobin (P = 0.01) and PCV (P = 0.02) values in the T1 group from the T0 group of goats. Meel et al. (2018) also observed a consequential rise of hematocrit values on 100% replacement of concentrate by moringa leaves.

Bearing a resemblance to the hematological values on the 1st day in two groups, there was statistical similarity (P = 0.957) in the serum iron level between the two groups. The average serum iron level in the control and treatment group on 1st day were 87.7 ± 0.03 µg/dl and 87.91 ± 0.24 µg/dl. Since the normal serum iron level of goat ranges from 16–35 µmol/l of iron which is equivalent to 89 – 195 µg/dl (Suttle, 2010; LABOKLIN, 2022), both the groups had slightly lower level of iron in their serum which might be one of the major reasons for anaemia as iron forms the crucial component of haemoglobin. The serum iron indices in T0 had increased from 87.7 ± 0.03 µg/dl to 127.0 ± 0.05 µg/dl (P= 0.00019). Similarly, the serum iron concentration has been augmented from 87.91 ± 0.24 µg/dl to the maximum level 193.54 ± 0.08 µg/dl (P = 0.00004). The rise of iron levels in both the groups might be result of deworming as the internal worms interfere in the iron absorption at the intestinal villi (Hall et al., 2008). The kids in the T1 group offered with Moringa fodder had significantly high rise in iron concentration from 87.91 ± 0.24 µg/dl to a maximum level of 193.54 ± 0.08 µg/dl, compared to the control (T0) group wherein only a minimal rise of serum iron level from 0.88 ± 0.03 µg/dl to 1.27 ± 0.05 03 µg/dl was revealed. On comparing the values serum Fe (Table 4) significant difference (P = 0.001) was observed in the mean of serum iron levels between two groups on 45th day implying the high mean serum iron in the T1 group. These comparative results from T0 and T1 groups envisioned a quick and better amendment in the Moringa fodder supplemented group.

Table 4. Comparison of Serum Mineral Profile in the Control group and Treatment group (in ppm) (n=6)

Group	Fe (1st Day)	Cu (1st Day)	Mg (1st Day)	Zn (1st Day)	Fe (45th Day)	Cu (45th Day)	Mg (45th Day)	Zn (45th Day)
Control	0.8770 ± 0.03	1.8699 ± 0.22	18.7067 ± 1.55	0.9626 ± 0.14	1.2639 ± 0.06	2.341 ± 0.12	38.7272 ± 3.31	1.5988 ± 0.15
Treatment	0.8791 ± 0.03	1.213 ± 0.12	26.3786 ± 1.14	2.692 ± 0.55	1.9355 ± 0.09	2.1491 ± 0.26	39.5657 ± 1.09	8.5487 ± 1.41
Significance	***	**	***	*	***	**	***	**
P value	0.00019	0.009	0.0003	0.015	0.00004	0.005	0.00014	0.002

*** Significant at P<0.001; ** Significant at P<0.01; * Significant at P<0.05; NS-Non-Significant

The serum copper levels in two groups differ significantly (P = 0.029) on the 1st day itself outlining the irrelevance in their serum copper concentration. The serum copper concentration in T0 (P = 0.009) and T1 (P = 0.005) groups has been increased in the period of 45 days irrespective of their diets. This progressive escalation observed owing to the removal of cause of malabsorption as stated earlier. Moreover, P value for copper (0.088) describes that there is no significant effect on copper concentration of treatment goats while feeding Moringa fodder. Analogous to copper concentration, the initial serum magnesium level diverges more among the two groups signifying their dissimilarities. As like other minerals and health parameters, magnesium also steadily increased in both group of kids, but there were high remarkable differences in control group (20.02ppm) which is much higher than the treatment group (13.18ppm) signalling the opposing effect of Moringa fodder on the serum magnesium concentration. This dismissive effect may be due to different mineral interactions that reduce the bioavailability of magnesium at the luminal site of intestine. High levels of iron, calcium and zinc in the feed reduces the availability of magnesium in light of high interactions among them. (Pallavi et al., 2022). Since the zinc level is higher in Moringa fodder, the supplemental feeding of the same is reflected in the augmented levels of serum zinc concentration at a faster rate in T1 than the T0 group (P = 0.003). The serum zinc level in the control group has increased from 0.96 ± 0.14 ppm to 1.6 ± 0.15 ppm, while the treatment group has experienced an enormous escalation of serum zinc concentration from 2.7 ± 0.55 ppm to 8.5 ± 1.40 ppm. This significant increase of zinc levels in treatment group can be attributed to the high bioavailability of zinc from moringa fodder (Oladeji et al., 2017). The high dietary zinc levels might act as immunomodulators enhancing the cell mediated immunity in treatment animals. Zinc was reported to have a positive effect on reproductive efficiency in goats by augmenting the regular oestrous cycles, and increasing the fertility rates (Oladeji et al., 2017; Habeeb et al., 2013).

The results from this experimental feeding trial forecasted that the Moringa fodder feeding to the anaemic animals hastened the recovery as they are rich in iron than most of the commonly available feedstuffs. This high rise of serum iron level and Hb level in a short time is crucial for the survival and optimum production of the animal as anaemia affects both the growth rate and immunity of the animals. Since the copper content is less and almost equals the other fodder and tree leaves, the Moringa fodder feeding had no effect on the serum level of T1. Consequently, there were slight rise in serum copper level in both groups as a result of elimination of parasites. Interestingly a slight decline in the progressive rise of magnesium level was observed in the treatment group on comparing with the control group. This effect was observed as a consequence of magnesium interactions with the other major and minor minerals reducing its bioavailability. Additionally, the Moringa fed group of animals had experienced a rapid amplification in serum zinc concentration which could improve the growth rate by strengthening the immune status of the animal (Wang et al., 2013).

CONCLUSION

The experiment proved that the Moringa fodder supplementation is useful improving the anaemic condition of goats as well as augmented the recovery period with much economical and beneficial results. Moringa fodder feeding also improved the zinc level in the animals which could have a positive effect on the immune status of animals.

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Effect of Feeding Earthworm Meal As an Alternative Protein Source Replacing Fish Meal on The Performance of White Pekin Ducks

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ABSTRACT

An experiment was conducted to study the impact of replacing fish meal with earthworm meal (EWM) on performance of White Pekin meat type ducks. 120 no. of day old white pekin ducks (40-52g) were randomly distributed to 3 equal groups (4 deep litter pens per group, 10 ducklings per pen). These were reared following standard management practices for a duration of 8 weeks. The EWM utilized in this investigation had been prepared from *Eisenia foetida*. The earthworms were grown using cow dung as biomass and mixture of duck litter. The matured earthworms were euthanized by dipping in warm water (50-60^{0}C) and dried in hot air oven (50-60^{0}C for 72 h), ground to powder and mixed in diet. Ducklings have been assigned to one of three treatments i.e. 1) Control without EWM; 2) Diet with 1 % EWM replacing fish meal;3) Diet with 2 % EWM replacing fish meal. The EWM contained 55.76 % protein. Body weight gain and FCR improved significantly ($P<0.05$) due to inclusion of EWM at 1 and 2% by replacing fish meal in the diets of white pekin ducks at 6th and 8th week of age. The feed intake increased significantly ($P<0.05$) at both levels of inclusion of EWM during 6th and 8th week of age. The feed cost to produce kg body weight gain was significantly ($P<0.05$) reduced due to inclusion of EWM in the diet at both levels at 6th and 8th week of age. It is concluded that 2 % EWM could be included in diet of white pekin ducks replacing fish meal of the control diet for better FCR, improved body weight gain, and reduction in price of feed for kg body weight gain.

KEYWORDS: Earthworm meal, Feeding cost, Performance, White Pekin ducks

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INTRODUCTION

Protein feed consumption is a crucial component of sustainable production methods, especially for intense production. Because of the associated availability, rising costs, and environmental impact, the real consumption of fish and soybean meal is not sustainable. Since lysine is 2nd limiting amino acid in diets based on soybean and corn meal, animal protein sources offer a more balanced amino acid profile (Chasmidari et al., 2021). Feed cost accounts for 70-80 % of the total cost of production in the intensive system of poultry production (Panda et al., 2024). To minimize the cost of production locally available alternate feed ingredients such as broken rice, azolla, tubercrop, insect meal and earthworm meal are used for feeding of ducks (Naik et al.,2023). Further, fish meal can be replaced with other important protein sources like soybean meal provided the diet is supplemented with 0.10 % methionine (Aziz et al., 2001). Thus far, there has been limited focus on utilizing fresh earthworms or EWM as a protein feed for animals that are monogastric. In past 20 years, research centres and private companies have centered their attention on algae, insects, and other invertebrates. One way to achieve environmental sustainability through cleaner technology is to use earthworms as alternate protein source for poultry feed. Poultry production becomes more cost-effective, environmentally sustainable, and productive when EWM is utilized as a substitute source of protein (Parolini et al., 2020). Studies on utilization of EWM meal are fairly old, particularly those conducted in Europe, but some broad conclusions can be made for both fish and broilers, the parameters typically assessed are feed conversion rate, feed intake, growth rate and body weight gain; level of acceptability of EWM in broiler diet is less than 15 percent. Good productive performances can be achieved without compromising quality of finished food items when EWM is added to diets at an inclusion level below acceptability threshold. EWM

is a novel protein source (Kose and Ozturk, 2017) and could offer a feasible source of protein for poultry feed (Veldkamp et al.,2012;Khan et al.,2016) as it has rich protein content (73 percent) and can be manufactured commercially (Rumpold and Schluter, 2013) and increased concentrations of vital amino acids (Parolini et al., 2020).

Recently, substantial thought is given upon possible function of intensive vermiculture or earthworm culture (Loh et al., 2005), as an animal protein source. As EWM contains high protein 58-71 percent on dry matter basis and also good in amino acids, it is recommended for usage in fish, poultry, and household pet feed (Sabine, 1978). During late 70s and early 80s research work has been carried out on utilization of EWM as a source of protein for poultry and animals (Sabine, 1978; Mekada et al.,1979; Stafford and Tacon,1984). The outcome of these studies suggests that EWM has potential to partially substitute fish and soybean meal as a source of protein. Results of another investigation suggest that EWM could replace soybean meal and fishmeal partially, i.e. up to 15 % in broiler diets (Loh et al., 2009). Therefore, aim of this investigation was to investigate how performance of white Pekin ducks had been affected by partially substituting EWM for fish meal.

MATERIALS AND METHODS

Bird Management, Diet and Housing

120 day old white pekin ducks (40-52g) were randomly distributed to 3 equal groups (4 deep litter pens per group, 10 ducklings per pen). These were reared following standard management practices. EWM employed in this investigation had been prepared from *Eisenia foetida*. The earthworms were cultured using cow dung as biomass and mixture of duck litter. The matured earthworms were immobilized by dipping them in warm water (50-60^{0}C) and dried in hot air oven (50-60^{0}C for 72 h), ground to powder and mixed in diet. One of the 3 treatments has been allocated to ducklings i.e. 1) Control without added EWM;2) diet with 1 % EM replacing fish meal;3) diet with 2 % EM replacing fish meal (Table 1).

Table 1. Ingredient Composition (kg or g/100 kg) of Experimental Diets

Ingredients	Diet 1	Diet 2	Diet 3
Wheat (kg)	60	60	60
Soybean meal (kg)	29	29	29
Fish meal (kg)	4.0	3.0	2.0
Earthworm meal (kg)	-	1.0	2.0
DORB (kg)	5.3	5.3	5.3
DCP (kg)	1.2	1.2	1.2
Shell grit (kg)	0.5	0.5	0.5
Tracemin mix (g)	100g	100g	100g
L-Lysine HCl (g)	50g	50g	50g
DL-Meth (g)	50g	50g	50g
AB_2D_3K (g)	20g	20g	20g
Vitamin B-Complex	20g	20g	20g
VitE & Se (g)	20g	20g	20g
Toxin Binder (g)	100g	100g	100g
Choline Chloride (g)	100g	100g	100g

The duration of experiment was 8 weeks. All the diets were isocaloric and isonitrogenous. Composition of EWM and experimental diets was analysed (AOAC, 2005).

Growth performance

The data on body weight was recorded on white Pekin ducks on weekly basis. Every day until the age of eight weeks, amount of feed consumed by each replicate was recorded. FCR had been computed as ratio of body weight gain at six weeks and eight weeks to feed intake. Feed cost per kg weight gain was computed by taking in to consideration the feed cost (Rs) and FCR in each replicate at 6th and 8th week.

Statistical Analysis

Data were statistically analyzed utilizing one way ANOVA in Complete Randomized x Design (Snedecor and Cochran, 1994) and Duncan's multiple range test was utilized to compare the treatment means (Duncan 1955).

RESULTS AND DISCUSSION

In present study EWM contained 55.76 % protein (Table 2).

Table 2. Chemical Composition of Fish meal, EWM and experimental diets

Nutrients	T1 (Control diet)	T2 (1 % EM)	T3(2 % EM)	Fish Meal	Earthworm Meal
Nutrient Composition (Analysed values, %)					
Crude Protein	22.54	22.86	23.05	50.32	55.76
Ether Extract	3.26	3.37	3.44	4.78	5.68
Crude Fibre	4.23	4.16	4.08	3.52	4.32
Total Ash	10.07	9.34	9.18	20.45	13.73
NFE	59.90	60.27	60.25	20.93	20.51
Nutrient Composition (Calculated values)					
ME(kcal/kg)	2900	2900	2900		
Lysine	1.00	1.00	1.00		
DL-Meth.	0.40	0.40	0.40		

Similar protein % (55.87 %) of EWM was reported by previous workers (Istiquoma et al., 2017). Lower protein % (51.62) and higher protein% (65.6 %) of EWM were stated by Gunya et al., 2019 and Damayanti et al., 2008, respectively. Moreover, Palungkun (1999) reported higher level of protein i.e. 64-76 % in EWM. Similarly, the protein content of *Eisenia foetida* was reported to be ranged from 58-71 percent on dry weight basis (Zhenjun et al., 1997; Tiroesele and Moreki, 2012). Body weight gain and FCR improved significantly ($P<0.05$) due to inclusion of EWM at a level of 1 and 2% by replacing fish meal in control diet of white pekin ducks at 6th and 8th week of age (Table 3).

Table 3. Effect of feeding EWM by replacing fish meal on the performance of White Pekin ducks at 6th and 8th week of age

Treatments	Body weight gain (g)		Feed intake(g)		Feed Conversion Ratio		Feed cost (Rs)/Kg body wt. gain	
	6th Wk	8th Wk	6th Wk	8th Wk	6th Wk	8th Wk	6th Wk	8th Wk
T1	1429c	1966c	3737c	5960b	2.615b	3.032b	102.73a	119.10a
T2	1517b	2046b	3815b	6104a	2.515b	2.984b	97.99b	116.26a
T3	1624a	2144a	3875a	6109a	2.386a	2.850a	92.20c	110.43b
SEM	25.21	23.33	18.96	25.20	0.03	0.03	1.39	1.20
P value	<0.001	0.005	0.001	0.005	<0.001	0.001	<0.001	0.001

Means bearing different superscripts within a column differ significantly (P<0.05)

The feed intake increased significantly (P< 0.05) at both levels of inclusion of EWM at 6th and 8th week of age. Similarly, Loh et al. (2009) stated that inclusion of EWM at levels of 10 % or 15 % in the diet of broilers led to significant increase in cumulative live weight gain at 6th week of age. In another study, better growth and feed efficiency were reported in Japanese quails due to feeding of 10 % EWM (Prayogi, 2011). Inclusion of 5 % EWM in diet of broilers improved body weight gain (Gunya et al., 2019). Current findings are supported by fact that EWM from *E. foetida* may substitute up to 50 percent of fishmeal in broiler finisher diets (Hassan et al., 2020). Present findings in white pekin ducks where improved performance was recorded due to feeding of EWM replacing fish meal confirmed the earlier report in broiler chickens where it was observed that earthworm meal from *Eisenia foetida* is a rich protein source and can be incorporated into diets without affecting their efficacy (Chashmidari et al., 2021). Similar to results of this study, breast percentage, feed intake, broiler final body weight, and HDL level increased when 2% EWM had been added to diet (Gholami et al., 2016).

CONCLUSION

From the findings of present research, it is concluded that 2 % earthworm can be included in diet of white pekin ducks replacing fish meal of control diet for better FCR, improved body weight gain, and reduction in price (Rs) of feed for kg body weight gain.

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Comparative Assessment of Feeding Regimens on the Performance of Composite Fish Culture in Tribal Villages of Tripura

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ABSTRACT

This study evaluated three feeding strategies in composite fish culture (CFC) systems in tribal villages of Tripura to enhance aquaculture productivity. Fifteen farmer ponds (0.08 ha each) were utilized with three treatments in five replicates: conventional feed (CF: rice polish and mustard oil cake 1:1), floating pelleted feed (FPF), and combination feeding (COM: CF and FPF). Indian major carps were stocked at 10,000 fingerlings/ha with species composition of *Catla catla* (40%), *Labeo rohita* (30%), and *Cirrhinus mrigala* (30%). Feeding rates were progressively reduced from 5% of fish biomass in initial two months to 1% in final two months over a 10-month culture period. Fish production was significantly higher ($P<0.05$) in COM treatment (4.2±0.12 MT/ha) followed by FPF (3.8±0.08 MT/ha) and CF (3.2±0.06 MT/ha). Feed conversion ratio was most efficient in COM (2.1±0.05) compared to FPF (2.4±0.06) and CF (3.1±0.08). Economic analysis revealed highest net profit in COM treatment (₹ 4,49,000±18,750 per hectare) with benefit-cost ratio of 2.6±0.07. The combination feeding strategy demonstrated superior performance by optimizing feed utilization efficiency while maintaining cost-effectiveness. These findings suggest that alternating feeding regimen can significantly enhance fish productivity in tribal aquaculture systems, providing a sustainable approach for livelihood improvement in northeastern India.

KEYWORDS: Composite fish culture, Economic analysis, Feeding strategies, Fish production, Tribal aquaculture

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INTRODUCTION

Aquaculture is a key contributor to food security and livelihoods in India's northeastern states, with Tripura showing significant progress (Debnath, 2024). Fish production in the state rose from 19,840 MT in 2004–05 to 85,805 MT in 2023–24—an increase of approximately 332.5% (Fisheries Statistics of Tripura). Despite this growth, productivity remains at 2.8 MT/ha, below the national average of 3.0 MT/ha (Jayasankar, 2018), highlighting the need for technological interventions to boost efficiency.

The tribal areas under the Tripura Tribal Areas Autonomous District Council (TTAADC) face particular challenges in aquaculture development, with productivity levels ranging from 1.0 to 1.5 MT/ha/year due to inadequate fisheries resource management and subsistence-level farming practices. These communities often lack access to scientific pond management techniques, appropriate feed management strategies, and modern aquaculture technologies (Debnath et al., 2017). The persistent gap between potential and actual production necessitates targeted interventions to enhance productivity through improved feeding strategies.

Feed represents the most critical input in intensive aquaculture systems, typically accounting for 50-60% of total operational costs (O'Keefe and Campabadal, 2022). The feeding strategy adopted significantly influences fish growth performance, survival rates, and overall production efficiency (De Silva and Gunasekera, 1991; Das et al., 2020; Das et al., 2021). The incorporation of appropriate feed additives has emerged as a crucial factor in optimizing fish performance and aquaculture profitability (Singh et al., 2024). In traditional aquaculture practices prevalent in tribal areas, farmers predominantly rely on locally available feed ingredients such as rice polish and mustard oil cake, which, while cost-effective, often result in suboptimal growth

performance due to nutritional inadequacies and poor feed conversion efficiency.

Commercial floating pelleted feeds have gained recognition for their superior nutritional profile and improved feed utilization efficiency compared to conventional feed ingredients (Yaqoob et al., 2010). These feeds offer advantages including reduced wastage, better feed conversion ratios, and enhanced growth rates. However, their higher cost often limits adoption among small-scale farmers. The development of cost-effective feeding strategies that combine the economic advantages of conventional feeds with the technical benefits of commercial pellets could provide a viable solution for enhancing aquaculture productivity in resource-constrained environments.

Recent research has demonstrated that different fish species respond variably to floating and sinking feeds based on their feeding habits and ecological niches within the pond ecosystem (Hussain et al., 2017). Specifically, surface feeders such as *C. catla* benefit more from floating feeds, while bottom feeders like *C. mrigala* show better performance with sinking feeds. This species-specific response to different feed types suggests that combination feeding strategies might optimize overall system productivity by catering to the diverse feeding requirements of different species in composite culture systems.

The present investigation was designed to evaluate the comparative efficacy of three feeding strategies: conventional feed, floating pelleted feed, and a combination approach involving use of both feed types. The study aimed to determine the optimal feeding strategy for enhancing fish production while maintaining economic viability in tribal aquaculture systems of Tripura.

MATERIALS AND METHODS

The study was conducted in Lembucherra village, located under the jurisdiction of Tripura Tribal Areas Autonomous District Council (TTAADC), Tripura, India. The experimental site was selected based on its representativeness of typical tribal aquaculture practices and the availability of willing participant farmers. The study was implemented under the Schedule Tribe Component (STC) program to ensure direct benefit to the tribal community. A total of fifteen earthen ponds, each measuring 0.08 ha with depths ranging from 1.0 to 1.5 m, were utilized for the experiment. The ponds were randomly allocated to three treatments with five replicates each: Treatment 1 (T1) - Conventional feed (CF), Treatment 2 (T2) - Floating pelleted feed (FPF), and Treatment 3 (T3) - Combination feeding (COM). The experiment was conducted over a 10-month period from June to March. Pond preparation followed standard protocols as described by Ayyappan et al (2019). Ponds were drained completely and lime was applied at 250 kg/ha for pH adjustment and soil conditioning. After 15 days, ponds were filled with water and organic manure (cattle manure) was applied at 2,500 kg/ha to enhance natural productivity. Ponds were allowed to develop natural plankton populations for two weeks before fish stocking.

Indian major carp fingerlings were procured from the ICAR-RC NEH Tripura Centre fish farm. The species composition followed standard composite fish culture practices: *C. catla* (40%), *Labeo rohita* (30%), and *C. mrigala* (30%). Fingerlings with average weights of 8.5±0.5g, 6.5±0.4g, and 4.4±0.3g for catla, rohu, and mrigal respectively were stocked at a density of 10,000 fingerlings/ha. Fingerlings were transported to experimental sites in oxygenated plastic bags and subsequently acclimatized for 30 minutes to equilibrate water temperature before being released into their respective ponds.

Conventional Feed was prepared by mixing rice polish and mustard oil cake in equal proportions (1:1 by weight) with water to form semi-solid dough. The proximate composition showed crude protein content of 24.8±0.5%. Commercial floating pellets (Make: ABIS India, 3mm diameter) procured from local market in Tripura, with crude protein content of 24.6±0.4%. The pellets maintained buoyancy for 45-60 minutes. Combination Feeding was practiced by alternating daily feeding schedule where conventional feed was provided on odd days and floating pelleted feed on even days.

The feeding schedule was designed with progressively decreasing rates based on fish biomass:

- Months 1-2: 5% of fish biomass daily
- Months 3-4: 4% of fish biomass daily
- Months 5-6: 3% of fish biomass daily
- Months 7-8: 2% of fish biomass daily
- Months 9-10: 1% of fish biomass daily

Fish biomass was reassessed bi-monthly by sampling 20-30 individuals of each species using cast

nets from each pond. Based on these sampling assessments, feeding rates were adjusted according to the estimated total biomass. For feeding calculations between sampling periods, survival rates were conservatively estimated at 90% after the first two months and 80% after four months (Ayyappan et al., 2019). Water quality parameters were monitored monthly following standard methods (APHA, 1998). Water temperature was measured using a mercury thermometer, pH was determined using a digital pH meter (Eutech Instruments PCSTestr 35, Singapore), and dissolved oxygen was measured using a portable dissolved oxygen meter (Lutron PDO-519, Taiwan). Total alkalinity was determined through titration with standardized sulfuric acid, and ammonia-nitrogen concentrations were analyzed using the indophenol blue colorimetric method.

Fish growth was monitored bi-monthly by sampling 10 specimens of each species from each pond using cast nets. Fish were measured for total length to the nearest 0.1 cm and weighed to the nearest 0.1 g before being returned to their respective ponds following a prophylactic dip treatment in potassium permanganate solution (10 ppm for 3-5 minutes) to prevent stress-related infections. Final harvest was conducted after 10 months using drag nets, and all fish were counted, measured, and weighed individually.

Growth parameters were calculated using the following formulas:

- Weight gain (g) = Final weight - Initial weight
- Daily weight gain (g) = Weight gain / Culture period (days)
- Specific growth rate (SGR, % per day) = [(ln Final weight - ln Initial weight) / Culture period] × 100
- Feed conversion ratio (FCR) = Total feed consumed (dry weight) / Total weight gain
- Survival rate (%) = (Number of fish harvested / Number of fish stocked) × 100

Economic analysis was conducted considering all input costs, including pond preparation, fingerlings, feed, labour, and miscellaneous expenses. Feed costs were calculated based on market prices: rice polish ₹ 15/kg, mustard oil cake ₹ 20/kg, and floating pelleted feed ₹ 30/kg. Fish were valued at wholesale price of ₹ 175/kg. Net profit, benefit-cost ratio, and return on investment were calculated for each treatment.

Prior to statistical analysis, data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test for mean separation when significant differences were detected. Results are presented as mean ± standard error, and statistical significance was determined at $P<0.05$ level. All statistical analyses were performed using SPSS version 21.0.

RESULTS AND DISCUSSION

Water quality parameters

Water quality parameters remained within acceptable ranges for carp culture throughout the experimental period (Table 1). Temperature ranged from 14.4°C during winter months to 32.8°C during summer months. Dissolved oxygen levels showed variations among treatments, with higher values recorded in FPF treatment (5.8±0.2 mg/L) compared to CF treatment (5.2±0.3 mg/L). pH remained alkaline throughout the study period, ranging from 7.2 to 8.4. Total alkalinity ranged from 68.5±3.2 to 71.2±2.8 mg/L across treatments. Ammonia-nitrogen concentrations were lowest in FPF treatment (0.62±0.03 mg/L) and highest in CF treatment (0.78±0.04 mg/L), with COM treatment showing intermediate values (0.68±0.05 mg/L).

Table 1. Water quality parameters (mean ± SE) in different treatments during the study period

Parameter	T1 (CF)	T2 (FPF)	T3 (COM)
Temperature (°C)	27.6±1.2	27.8±1.1	27.4±1.3
Dissolved oxygen (mg/L)	5.2±0.3^{b}	5.8±0.2^{a}	5.6±0.3^{a}
pH	7.8±0.2	7.9±0.1	7.7±0.2
Total alkalinity (mg/L)	68.5±3.2	71.2±2.8	69.8±3.5
Ammonia-N (mg/L)	0.78±0.04^{a}	0.62±0.03^{b}	0.68±0.05^{ab}

Values bearing different superscripts (a,b) in the same row differ significantly (P<0.05)

Fish growth performance

Significant differences in fish growth performance were observed among treatments across all three species (Table 2). *C. catla* achieved the highest final weights in COM treatment (892±15.2g), followed by FPF treatment (845±12.8g) and CF treatment (678±11.4g). Daily weight gain for catla followed the same pattern, with COM treatment recording 2.92±0.05g, FPF treatment 2.76±0.04g, and CF treatment 2.21±0.04g. *L. rohita* demonstrated similar treatment responses, with final weights of 595±10.2g in COM treatment, 562±9.8g in FPF treatment, and 485±8.6g in CF treatment. Daily weight gain for rohu was highest in COM treatment (1.94±0.03g), followed by FPF treatment (1.83±0.03g) and CF treatment (1.58±0.03g). *C. mrigala* showed distinct performance patterns compared to the other species. The highest final weight was recorded in COM treatment (485±9.4g), while CF and FPF treatments showed similar performance with 425±8.2g and 398±7.8g respectively. Daily weight gain for mrigal was 1.58±0.03g in COM treatment, 1.39±0.03g in CF treatment, and 1.30±0.03g in FPF treatment.

Monthly growth assessments revealed that growth rates were highest during the initial four months across all treatments. The combination feeding strategy maintained consistently higher growth rates throughout the ten-month culture period, with the most pronounced differences observed during months three through six.

Table 2. Fish growth performance parameters

Species	T1 (CF)	T2 (FPF)	T3 (COM)
Catla catla			
Final weight (g)	678±11.4^{c}	845±12.8^{b}	892±15.2^{a}
Weight gain (g)	669±11.2^{c}	836±12.6^{b}	883±15.0^{a}
Daily weight gain (g)	2.21±0.04^{c}	2.76±0.04^{b}	2.92±0.05^{a}
Labeo rohita			
Final weight (g)	485±8.6^{c}	562±9.8^{b}	595±10.2^{a}
Weight gain (g)	478±8.4^{c}	555±9.6^{b}	588±10.0^{a}
Daily weight gain (g)	1.58±0.03^{c}	1.83±0.03^{b}	1.94±0.03^{a}
Cirrhinus mrigala			
Final weight (g)	425±8.2^{a}	398±7.8^{a}	485±9.4^{b}
Weight gain (g)	420±8.1^{a}	393±7.7^{a}	480±9.3^{b}
Daily weight gain (g)	1.39±0.03^{a}	1.30±0.03^{a}	1.58±0.03^{b}

Values bearing different superscripts (a,b, c) in the same row differ significantly (P<0.05)

Fish production and survival

Total fish production varied significantly among treatments (Table 3). The combination feeding strategy achieved the highest production (4.2±0.12 MT/ha), followed by floating pelleted feed (3.8±0.08 MT/ha) and conventional feed (3.2±0.06 MT/ha). Survival rates were consistently higher in FPF and COM treatments compared to CF treatment.

Table 3. Fish production and survival parameters (mean ± SE) by treatment

Parameter	T1 (CF)	T2 (FPF)	T3 (COM)
Total production (MT/ha)	3.2±0.06^{c}	3.8±0.08^{b}	4.2±0.12^{a}
Survival rate (%)	74.2±1.8^{b}	81.6±1.4^{a}	83.8±1.2^{a}
Species-wise production (kg/ha)			
Catla catla	1,286±28^{c}	1,642±32^{b}	1,785±38^{a}
Labeo rohita	875±18^{c}	1,028±21^{b}	1,158±25^{a}
Cirrhinus mrigala	1,065±24^{a}	998±22^{a}	1,257±28^{b}
Feed conversion ratio	3.1±0.08^{a}	2.4±0.06^{b}	2.1±0.05^{c}

Values bearing different superscripts (a,b, c) in the same row differ significantly (P<0.05)

Economic analysis

Economic evaluation revealed significant differences in profitability among treatments (Table 4). The COM treatment achieved the highest net profit of ₹ 4,49,000±18,750 per hectare, followed by FPF treatment at ₹ 3,17,400±18,300 per hectare and CF treatment at ₹ 2,87,400±14,330 per hectare. Total production costs were lowest for CF treatment (₹ 2,72,600±5,630 per hectare), intermediate for COM treatment (₹ 2,86,000±6,250 per hectare), and highest for FPF treatment (₹ 3,47,600±8,000 per hectare). The benefit-cost ratio was highest in COM treatment (2.6±0.07), followed by CF treatment (2.1±0.05) and FPF treatment (1.9±0.05). Return on investment showed similar rankings with COM treatment achieving 157±6.5%, CF treatment 105±5.3%, and FPF treatment 91±5.3%. Feed costs constituted the largest component of variable costs across all treatments, representing 63.7% in CF treatment, 78.7% in FPF treatment, and 73.3% in COM treatment of total variable costs.

The fish production achieved in this study (3.2-4.2 MT/ha) represents a substantial improvement over existing productivity levels in tribal areas of Tripura (1.0-1.5 MT/ha/year). The combination feeding strategy resulted in 31.3% higher production compared to conventional feeding and 10.5% higher than exclusive use of floating pelleted feed. These findings align with Hussain et al. (2017), who reported 19-23% yield increases when tribal farmers adopted floating pelleted feeds over traditional local feeding practices in Arunachal Pradesh. Their three-year study in East Siang District compared floating pelleted feed against local practices involving rice bran, mustard oil cake, and kitchen waste, closely paralleling our conventional feed approach. Hussain et al. achieved an average 21.4% production improvement across study years. The present study extends these findings, demonstrating that our combination feeding strategy achieved superior results with 31.3% higher production than conventional feeding, suggesting that alternating feeding protocols provide additional benefits beyond exclusive use of either feed type.

Table 4. Economic analysis of different feeding strategies

Cost components	T1 (CF)	T2 (FPF)	T3 (COM)
Variable costs (Rs/ha)			
Fingerlings	20,000±0	20,000±0	20,000±0
Feed cost	1,73,600±4,340c	2,73,600±6,840a	2,09,500±5,240b
Labor	20,000±400a	15,000±300c	17,500±350b
Lime and manure	8,500±200	8,500±200	8,500±200
Equipment rental	12,000±240	12,000±240	12,000±240
Harvesting costs	8,500±170	8,500±170	8,500±170
Miscellaneous	10,000±250	10,000±250	10,000±250
Total variable cost	2,72,600±5,630c	3,47,600±8,000a	2,86,000±6,250b
Gross income	5,60,000±11,200c	6,65,000±13,300b	7,35,000±14,700a
Net profit	2,87,400±14,330b	3,17,400±18,300c	4,49,000±18,750a
Return on investment (%)	105±5.3b	91±5.3c	157±6.5a
Benefit-cost ratio	2.1±0.05b	1.9±0.05c	2.6±0.07a

Values bearing different superscripts (a,b, c) in the same row differ significantly (P<0.05)
Fish selling price: ₹ 175/kg based on prevailing wholesale market rates; Rice polish ₹ 15/kg, Mustard oil cake ₹ 20/kg, Floating pellets ₹ 30/kg; Labor costs reflect differential requirements: T1 (₹ 20,000) includes daily feed mixing labor, T2 (₹ 15,000) for standard feeding operations, T3 (₹ 17,500) includes alternate day feed mixing labor; Equipment rental includes cast nets, weighing scales, and feeding implements; Harvesting costs for drag net operations and labor; Miscellaneous costs include fish health management, transportation, water quality monitoring, etc; Fixed costs excluded as experimental ponds were farmer-owned with maintenance performed by participating farmers

The species-specific response was most evident in mrigal, which achieved slightly higher final weights with conventional feed (425±8.2g) compared to floating pelleted feed (398±7.8g), though the difference was not statistically significant (P=0.08). This differential performance among species may relate to the physical characteristics of the feed types and their availability in different water zones. The conventional feed, being a semi-solid dough preparation, settles immediately upon application, making it accessible to bottom-dwelling species for extended periods. In contrast, floating pellets remain at the surface and mid-water column for 45-60 minutes, providing different accessibility patterns for various feeding guilds.

The superior performance of the combination feeding strategy may benefit from established principles in aquaculture nutrition. Silva et al. (2016) noted that dietary variation can influence feeding responses in fish systems, while Webster et al. (1992) demonstrated that fish often exhibit improved feeding responses when offered varied diets compared to monotonous feeding regimens. The alternating feeding schedule in the combination treatment potentially provides both sinking and floating feed options, accommodating the diverse habitat preferences of different carp species in the composite culture system. However, the specific behavioral and physiological mechanisms underlying the observed performance differences in this study would require dedicated investigations to establish definitively.

The FCR demonstrated significant improvement with the combination feeding strategy (2.1±0.05) compared to conventional feeding (3.1±0.08). This 32% improvement in FCR indicates more efficient utilization of feed resources, which contributes to the enhanced economic returns observed in this treatment. The improved FCR in combination feeding may be attributed to better feed utilization and reduced wastage. Floating pelleted feeds remain available in the water column for extended periods (45-60 minutes), providing surface and column feeders with better access to nutrients. Conversely, conventional feeds quickly settle to the bottom, making them readily available to benthic feeders. The alternating schedule appears to provide improved feed accessibility for different species while potentially reducing wastage through sedimentation and leaching of nutrients. These findings are

consistent with Limbu (2015), who reported that floating feeds resulted in lower FCR compared to sinking feeds due to reduced wastage. The present study suggests that strategic combination of both feed types can achieve better FCR performance than exclusive use of either feed type alone.

The economic analysis reveals that the combination feeding strategy generated the highest net profit (¹ 4,49,000 per hectare) and benefit-cost ratio (2.6) despite higher feed costs compared to conventional feeding. This represents a 56.2% increase in net profit compared to conventional feeding and a 41.5% increase over floating pelleted feed treatment. The superior economic performance results from the substantial increase in fish production that more than compensates for the additional feed costs. The feed cost constituted 63.7% of total variable costs in CF treatment, 78.7% in FPF treatment, and 73.3% in COM treatment. While the combination approach had higher absolute feed costs than conventional feeding, the improved production efficiency resulted in better overall economic returns. The floating pelleted feed strategy, despite achieving higher production than conventional feeding, demonstrated reduced economic advantage due to elevated feed costs relative to the production gains achieved (Posadas, 2005; Kumar et al., 2017). This finding is particularly significant for tribal farmers who often face resource constraints but require improved livelihood outcomes. The return on investment was highest in COM treatment (157%), followed by CF treatment (105%) and FPF treatment (91%), indicating that farmers can expect substantial returns from adopting the combination feeding strategy. The moderate additional investment required for incorporating pelleted feed in the combination approach generates returns within a single culture cycle, making it economically attractive for small-scale tribal farmers.

The observed differences in water quality parameters among treatments provide additional context for the production results. Ammonia-nitrogen concentrations were lowest in FPF treatment (0.62±0.03 mg/L) and highest in CF treatment (0.78±0.04 mg/L), with COM treatment showing intermediate values (0.68±0.05 mg/L). Similarly, dissolved oxygen levels were higher in FPF and COM treatments compared to CF treatment. These water quality differences may reflect variations in feed utilization patterns among the different feeding strategies. Boyd and Tucker (1998) noted that water quality management is fundamental in aquaculture systems, as feed inputs and their subsequent breakdown products influence the pond environment. The maintained water quality parameters within acceptable ranges across all treatments throughout the study period contributed to the successful completion of the experimental trials.

This study demonstrates that the combination feeding strategy produced superior results across key performance indicators in composite fish culture systems. The alternating use of conventional feed and floating pelleted feed achieved the highest fish production (4.2 MT/ha), most efficient feed conversion ratio (2.1), and greatest economic returns (¹ 4,49,000/ha net profit) compared to exclusive use of either feed type. The combination approach resulted in 31.3% higher production than conventional feeding and 56.2% higher net profit. The economic analysis revealed that while feed costs were higher in the combination treatment, the increased production efficiency generated better overall returns with a benefit-cost ratio of 2.6 compared to 2.1 for conventional feeding and 1.9 for floating pelleted feed alone.

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Effect of Dietary Protein Substitution of Fish Meal with Insect Larvae Meal on the Growth and Digestive Functions of Koi Carp *Cyprinus carpio* var. *koi* (Linnaeus, 1758) Fry in Nursery Phase

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ABSTRACT

An 8-week feeding trial was conducted to evaluate the effects of two insect larvae meals such as silkworm pupae (*Bombyx mori*) meal and black soldier fly (*Hermetia illucens*) larvae meal as dietary protein substitutes for fish meal on the growth performance and digestive physiology of koi carp (*Cyprinus carpio* var. *koi*) fry. For that, 15 days old induced bred koi fry (2.28/ ±/ 0.24/ cm; 0.26/ ±/ 0.14/ g) were stocked in happas (10/ ×/ 3/ ×/ 1/ m) placed within an experimental earthen pond. Nine iso-nitrogenous (351.07/ g/ kg{ ¹) and iso-lipidic (71.98/ g/ kg{ ¹) experimental diets were formulated with insect meals replacing fish meal at 20%(SWP20,BSF20), 30%(SWP30,BSF30), 40%(SWP40,BSF40), and 50%(SWP50,BSF50) inclusion levels, alongside a control diet containing only fish meal. Results showed significantly higher (P/ </ 0.05) final length and weight gain in fry fed SWP50 (3.26/ ±/ 0.01/ cm; 3.79/ ±/ 0.01/ g) and BSF50 (3.22/ ±/ 0.01/ cm; 3.56/ ±/ 0.02/ g) diets. Growth indices including feed conversion ratio, feed efficiency ratio, protein efficiency ratio and survival rate were also notably improved in these groups. Furthermore, digestive enzyme activities were significantly elevated (P/ </ 0.05) in fry fed SWP50 and BSF50 diets, indicating enhanced nutrient utilization. These findings depicts that both the insect larvae meal can effectively replace 50% of fish meal with a dietary inclusion level of 147 g kg^{-1} in the diet of koi carp fry without compromising growth, survival and digestive function.

KEYWORDS: Digestive enzymes, Fish meal, Insect meal, Koi carp.

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INTRODUCTION

Ornamental fishes have played a significant role in the global pet industry, with their popularity increasing steadily since the 1970s. The industry has seen an annual growth rate of 14%, and over one billion live ornamental fishes are being exported worldwide each year (Maceda-Veiga et al., 2016; Prakash et al., 2017). The ornamental fish trade is valued at approximately US$15-30 billion annually (Novak et al., 2020), with Asian countries contributing around 57% of total global exports (Dey, 2016). India, with its rich biodiversity, harbors over 374 indigenous freshwater and 700 marine ornamental fish species, along with more than 288 exotic fish species in trade (Ghosh et al., 2003; Mahapatra, 2018). Among these, koi carp (*Cyprinus carpio* var. *koi*) stands out for its vibrant colouration and aesthetic appeal, making it one of the most commercially important ornamental species in the global market (Jain et al., 2019). Moreover, the nursery phase remains a major bottleneck in the advancement of ornamental aquaculture, as it is a critical and determining stage influencing the overall success of an ornamental fish production unit. Among the various challenges faced during this stage, nutrition particularly feed quality plays a pivotal role. The performance and survival of fry are closely linked to the nutritional composition of the initial diet. Of the various nutrients, protein is most important during the nursery phase due to the high dependency of fry on protein rich diets to support their rapid growth and development.

Fish meal and fish oil, derived from wild-caught forage fish, have long been the primary protein and lipid sources in aquafeeds due to their high protein content, balanced amino acid profile, absence of anti-nutritional factors and superior digestibility (Daniel, 2018). However, the continued reliance on these

resources poses sustainability concerns, with fish meal demand projected to rise by 75% from 49.7 million tons in 2015 to 87.1 million tons by 2025 (Tacon and Metian, 2015). This has prompted a global search for sustainable protein alternatives. While plant based proteins and fishery by-products show potential, insect meals have gained considerable attention due to their rapid life cycles, high productivity and favorable nutritional profiles (DeFoliart et al., 2009; Hua et al., 2019; Berggren et al., 2019). Among the various species, black soldier fly (*Hermetia illucens*) larvae meal (BSF) and silkworm pupae (*Bombyx mori*) meal (SWP) have emerged as a promising candidates to be used as an effective protein sources to replace the fish meal partially or fully in the aquafeeds (Hodar et al., 2020; Sahib et al., 2024).

Over the past decade, numerous studies have demonstrated the potential of BSF as a sustainable alternative to fish meal in aquafeeds, with no adverse effects on the growth performance. And successful inclusion of BSF has been reported in Jian carp (*Cyprinus carpio* var. *jian*) diets as both partial (Li et al., 2017) and complete replacements (Zhou et al., 2018), as well as in Amur carp with 70% replacement (Amala et al., 2018), and in mirror carp (*Cyprinus carpio* var. *specularis*) with a dietary inclusion level of 131 g/kg (Xu et al., 2020). Similarly, SWP has shown promising results as a fish meal substitute, with studies in rohu (*Labeo rohita*) (Begum et al., 1994), mirror carp (*Cyprinus carpio* var. *specularis*) (Ji et al., 2012), and Jian carp (*Cyprinus carpio* var. *jian*) (Ji et al., 2015) reporting favorable growth outcomes. Since most of the previous studies recommended partial replacement of fish meal with insect meals for optimal performance (Tran et al., 2015; Henry et al., 2015), the present study investigates the effects of dietary inclusion of BSF and SWP upto 50% replacement of fish meal on the growth and digestive performance of koi carp fry during the nursery phase under captive conditions.

MATERIALS AND METHODS

Ethics statement

The experiment was conducted at the Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Erode District, Tamil Nadu, India, in accordance with the ethical guidelines for animal experimentation established by Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamilnadu, India.

Experimental design

The experiment followed a completely randomized design with eight dietary treatments SWP20, SWP30, SWP40, SWP50, BSF20, BSF30, BSF40, and BSF50 along with a control, each in triplicate. Experimental animals were reared in happas (10 m × 3 m × 1 m, 2 mm mesh size) installed in an earthen pond. The happas were thoroughly cleaned and sun-dried prior to installation, and the pond area was enclosed with bird-proof fencing to prevent predation.

Experimental animals

Induced-bred, 15 days old koi carp fry with an average body length of 2.28/ ±/ 0.24/ cm and average body weight of 0.26/ ±/ 0.14/ g were used as experimental animals. Following acclimatization, the fry were stocked into the experimental happas at a density of 100 fry/m² (3,000 fry per happa).

Experimental diets

Nine iso-nitrogenous and iso-lipidic experimental diets containing 35/ ±/ 0.5% crude protein and 7/ ±/ 0.5% crude lipid were formulated by replacing fish meal with varying levels of silkworm pupae meal (20%-SWP20, 30%-SWP30, 40%-SWP40, 50%-SWP50) and black soldier fly larvae meal (20%-BSF20, 30%-BSF30, 40%-BSF40, 50%-BSF50), based on the formulation by Nandeesha et al. (2002). The control diet contained only fish meal as the sole animal protein source. All the feed ingredients were grounded and homogenized using a feed pulverizer and feed mixer. The mash-type diets were then used to fed the fishes at a rate of 5% of their body weight (Paul and Giri, 2015), twice daily, for 60 days. Proximate composition of the diets including crude protein, crude lipid, ash and moisture contents were analyzed in feed analytical laboratory at Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), following the standard protocols (AOAC, 2005) and the gross energy values of the experimental diets were calculated according to Henken et al. (1986). The proximate composition of all the experimental diets and the major protein sources were shown in Table 1 and Table 2.

Table 1. Estimated feed and proximate composition of all the experimental diets

Feed Ingredients	Experimental diets (g/100g)							
	Control	SWP20	SWP30	SWP40	SWP50	BSF20	BSF30	BSF40	BSF50
FM [a]	29.40	23.60	20.60	17.60	14.70	23.60	20.60	17.60	14.70
SWP [b]	-	5.80	8.80	11.80	14.70	-	-	-	-
BSF [c]	-	-	-	-	-	5.80	8.80	11.80	14.70
GNOC [d]	29.40	29.40	29.40	29.40	29.40	29.40	29.40	29.40	29.40
Rice bran [e]	24.70	24.70	24.70	24.70	24.70	24.70	24.70	24.70	24.70
Wheat flour [f]	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40
Vit & Min [g]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Proximate composition (DM %)									
Crude protein	34.95	35.25	34.87	35.07	35.15	35.38	34.83	35.14	35.33
Crude lipid	7.01	6.96	7.11	7.24	7.47	7.08	7.19	7.26	7.47
Moisture	5.60	5.32	4.90	5.54	5.22	5.46	5.28	5.56	5.06
Ash	13.59	12.66	12.87	12.79	13.01	13.21	12.94	12.95	12.76
Dry Matter	94.40	94.68	95.10	94.46	94.78	94.54	94.72	94.44	94.94
Gross Energy (MJ/kg)	1.8844	1.9015	1.8988	1.9043	1.9059	1.8952	1.8991	1.9023	1.9114

Table 2. Estimated proximate composition of the major protein sources

Proximate composition (DM%)	FM	SWP	BSF
Crude protein	55.25	52.50	50.15
Crude lipid	8.30	13.44	14.21
Moisture	8.21	7.11	7.02
Ash	22.50	5.65	12.56
Dry Matter	91.79	92.89	92.98
Gross Energy (MJ/kg)	1.8786	2.2677	2.1505

FM - Fish meal; SWP - Silkworm Pupae Meal; BSF - Black Soldier Fly Larvae Meal; GNOC - Ground Nut Oil Cake; Vit & Min - Vitamin and Mineral Mix

MJ/kg - Mega joules / kilogram

[a] Pearl City Fish Meal Plant, Thoothukudi, Tamilnadu

[b] Silvermine Silk Processors Private Limited, Udumalpet, Tamilnadu

[c] Eco Care Agrovet, Pondicherry

[d, e, f, g] Local market around Bhavanisagar, Tamilnadu

[g] Ingredients included per kg: Vitamin A 700000 IU, Vitamin D_3 70000 IU, Vitamin E 250 mg, Cobalt 150 mg, Copper 1200 mg, Iodine 325 mg, Iron 1500 mg, Magnesium 6000 mg, Potassium 100 mg, Sodium 5.9 mg, Manganese 1500 mg, Sulphur 0.72%, Zinc 9600 mg, DL-Methionine 1000 mg, Calcium 25.5%, Phosphorus 12.75%

Water quality parameters

Water samples were collected fortnightly from the experimental happas to analyze the physico-chemical parameters, following standard methods outlined by APHA (2005). The mean values of the water quality parameters recorded during the experimental period were as follows: Water temperature (27±0.01°C), pH (8.30±0.02), Dissolved oxygen (4.13±0.01ppm), Ammonia (0.01±0.03 ppm), Nitrite (0.01±0.04 ppm), Nitrate (0.1±0.01 ppm), Inorganic phosphate (0.79±0.05 ppm), Free CO_2 (7.33±0.01 ppm), Total hardness (110±0.01 ppm), Total alkalinity (89±0.02 ppm), Total suspended solids (0.08±0.02 ppm) and Total dissolved solids (0.70±0.14 ppm).

Bio-growth indices

Sampling was carried out to assess the growth performance of fishes fed with different experimental diets fortnightly over 60 days of experimental period. Based on the recorded data, the growth parameters such as mean length gain (MLG), mean weight gain (MWG), percentage length gain (PLG), percentage weight gain (PWG), specific growth rate (SGR), feed

conversion ratio (FCR), feed efficiency ratio (FER), the protein efficiency ratio (PER) and survival rate were calculated by using the following formulae (Li et al., 2017).

$$\text{MLG} = \text{Mean final length (cm)} - \text{Mean initial length (cm)}$$

$$\text{MWG} = \text{Mean final weight (g)} - \text{Mean initial weight (g)}$$

$$\text{PLG (\%)} = \frac{\text{Final length (cm)} - \text{Initial length (cm)}}{\text{Initial length (cm)}} \times 100$$

$$\text{PWG (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{SGR} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Experimental duration in days}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gain (g)}}$$

$$\text{FER} = \frac{1}{\text{FCR}}$$

$$\text{PER} = \frac{\text{Total wet weight gain (g)}}{\text{Dry weight of protein fed (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Total number of harvested animal}}{\text{Total number stocked}} \times 100$$

Digestive enzyme assay

At the end of the trial, samples (n=10) from each replicates were randomly collected and their intestines were dissected out for digestive enzyme analysis. A 5% tissue homogenate was then prepared using a pestle and mortar, followed by centrifugation at 5,000 rpm for 10 minutes at 4°C. The resulting supernatant was stored at –20°C for subsequent enzyme assays. Digestive enzyme activities were determined as follows: protease by the casein digestion method (Drapeau, 1976), amylase by the di-nitro-salicylic acid method (Rick and Stegbauer, 1974), and lipase by the titrimetric method (Cherry and Crandall, 1932). Additionally, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured following the method of Wooten (1964).

Statistical analysis

The data collected at the end of experiment were processed and analysed by one-way ANOVA following statistical software SPSS version 20.0 at 5% significance level to test for significant differences between the mean values of various treatments and by using the Duncan Multiple Range test (SPSS Statistics for Windows, IBM. Version 20.0. Armonk, NY: IBM Corp).

RESULTS AND DISCUSSION

Growth performance

The effects of different experimental diets on the growth performance of koi carp fry are presented in Table 3. After the 60-day feeding trial, the highest MLG, MWG, PLG, PWG were significantly higher (P/ </ 0.05) in fish fed with SWP50 (3.26/ ±/ 0.01/ cm, 3.79/ ±/ 0.01/ g,

Table 3. Estimated bio-growth parameters of the fishes recorded at the end of the experiment

MIL – Mean Initial Length, MFL – Mean Final Length, MLG – Mean Length Gain, PLG – Percentage Length Gain, MIW – Mean Initial Weight, MFW – Mean Final Weight, MWG – Mean Weight Gain, PWG – Percentage Weight Gain, SGR – Specific Growth Rate, FCR – Feed Conversion Ratio, FER – Feed Efficiency Ratio, PER – Protein Efficiency Ratio

Growth Parameter	Experimental Diets								
	C	SWP20	SWP30	SWP40	SWP50	BSF20	BSF30	BSF40	BSF50
MIL (cm)	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24	2.28±0.24
MFL (cm)	4.27±0.01 [a]	4.79±0.02 [b]	5.03±0.01 [c]	5.23±0.01 [d]	5.54±0.02 [e]	4.73±0.02 [b]	5.05±0.03 [c]	5.31±0.01 [d]	5.50±0.01 [e]
MLG (cm)	1.99±0.01 [a]	2.51±0.02 [b]	2.75±0.02 [c]	2.95±0.01 [d]	3.26±0.01 [e]	2.45±0.04 [b]	2.77±0.01 [c]	3.03±0.02 [d]	3.22±0.01 [e]
PLG (%)	87±0.01 [a]	110±0.03 [b]	121±0.02 [c]	129±0.02 [d]	142±0.01 [e]	107±0.01 [b]	121±0.02 [c]	133±0.01 [d]	141±0.02 [e]
MIW (g)	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14	0.26±0.14
MFW (g)	1.80±0.01 [a]	2.16±0.01 [b]	2.67±0.02 [c]	2.85±0.01 [d]	4.05±0.03 [e]	2.02±0.01 [b]	2.47±0.01 [c]	2.81±0.01 [d]	3.82±0.02 [e]
MWG (g)	1.54±0.01 [a]	1.90±0.01 [b]	2.41±0.04 [c]	2.59±0.03 [d]	3.79±0.01 [e]	1.76±0.01 [b]	2.21±0.05 [c]	2.55±0.02 [d]	3.56±0.02 [e]
PWG (%)	592±0.02 [a]	731±0.01 [b]	927±0.03 [c]	996±0.01 [d]	1457±0.03 [e]	677±0.02 [f]	850±0.01 [g]	981±0.03 [d]	1369±0.02 [e]
SGR (%/day)	3.22±0.01 [a]	3.52±0.01 [b]	3.88±0.02 [c]	3.99±0.02 [d]	4.57±0.01 [e]	3.41±0.01 [b]	3.75±0.03 [c]	3.96±0.01 [d]	4.47±0.01 [e]
FCR	1.11±0.01 [a]	0.93±0.01 [b]	0.83±0.01 [c]	0.85±0.01 [c]	0.80±0.03 [c]	1.07±0.01 [d]	1.01±0.04 [d]	0.88±0.01 [c]	0.85±0.01 [c]
FER	0.90±0.01 [a]	1.07±0.01 [b]	1.20±0.01 [c]	1.17±0.01 [c]	1.25±0.02 [c]	0.93±0.01 [d]	0.99±0.04 [d]	1.13±0.01 [c]	1.17±0.01 [c]
PER	2.56±0.03 [a]	3.04±0.02 [b]	3.43±0.05 [c]	3.35±0.04 [c]	3.55±0.04 [c]	2.65±0.01 [d]	2.81±0.11 [d]	3.22±0.03 [c]	3.35±0.03 [c]
Survival rate (%)	50±0.01 [a]	60±0.03 [b]	65±0.03 [b]	68±0.01 [b]	70±0.02 [b]	65±0.01 [b]	65±0.02 [b]	68±0.03 [b]	70±0.01 [b]

Values are expressed in terms of Mean±SD.

Values in the same row with different superscripts vary significantly ($p<0.05$)

142/ ±/ 0.01%, 1457/ ±/ 0.03%) and BSF50 (3.22/ ±/ 0.01/ cm, 3.56/ ±/ 0.02/ g, 141/ ±/ 0.02%, 1369/ ±/ 0.02%) diets respectively. Other growth parameters including SGR, FCR, FER, PER and survival rate were also significantly improved (P/ </ 0.05) in fish fed with SWP50 and BSF50 diets, indicating no adverse effects from replacing fish meal with SWP or BSF upto 50%. Furthermore, MLG increased linearly with higher inclusion levels of SWP and BSF, as shown by the regression equations: SWP (y/ =/ 0.0249x/ +/ 1.9954, R^2/ =/ 0.9973) and BSF (y/ =/ 0.0253x/ +/ 1.9841, R^2/ =/ 0.9954), illustrated in Figures 1 and 2, respectively.

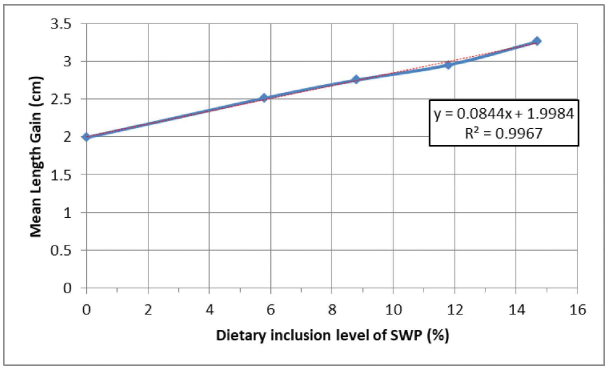

Figure 1. Linear regression of MLG for the SWP included diets

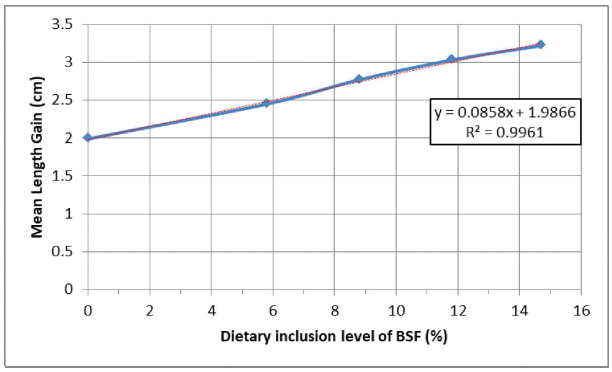

Figure 2. Linear regression of MLG for the BSF included diets

Digestive enzymes

Digestive enzyme activities such as protease, amylase, and lipase were significantly higher (P/ </ 0.05) in fish fed with SWP50 (17.88/ ±/ 0.02, 12.72/ ±/ 0.05, and 22.89/ ±/ 0.01/ µM/ mg{ ¹ protein) and BSF50 (17.01/ ±/ 0.05, 12.99/ ±/ 0.01, and 22.97/ ±/ 0.01/ µM/ mg{ ¹ protein) diets respectively. These results indicate efficient protein utilization in both SWP50 and BSF50 diet groups. Similarly, AST and ALT activities were significantly elevated (P/ </ 0.05) in SWP50 (12.87/ ±/ 0.04 and 13.88/ ±/ 0.02/ nM/ mg{ ¹/ min{ ¹) and BSF50 (12.86/ ±/ 0.01 and 13.69/ ±/ 0.01/ nM/ mg{ ¹/ min{ ¹) diet fed fishes respectively. The digestive enzyme activity data across treatments are summarized in Table 4.

The findings of the present study depicts that the inclusion level of 147 g Kg^{-1} of SWP and BSF in the diet of koi carp fry significantly outperformed other treatments and the control in terms of growth and digestive function. Among the two best performed treatments, SWP50 showed superior performance, with 1.2% and 0.7% higher MLG and PLG respectively, and 6.4% higher MWG and PWG compared to BSF50. This suggests that 147 g Kg^{-1} inclusion level of SWP yields the most favorable growth outcomes. However, BSF50 also proved effective, achieving a 62% greater length gain than the control group, which indicates that it can also be used as an sustainable fish meal substitute. Additionally, growth parameters (MLG and MWG) showed a progressive increase with higher inclusion levels of both SWP and BSF, highlighting their suitability in koi fry diets. A similar study in common carp (*Cyprinus carpio*) also reported improved final body length when fish meal was replaced with 30% of soldier fly (*Ptecticus tenebrifer*) and mealworm (*Tenebrio molitor*) meals (Mamuad et al., 2021). Further, in the present study, the SGR, FCR, FER and PER were significantly better in SWP50 and BSF50 diets. These results are consistent with earlier findings in rainbow trout (*Oncorhynchus mykiss*) where BSF inclusion at 40% (Renna et al., 2017) and SWP at 50% (Dheke, 2013) resulted in improved FCR and SGR without compromising survival.

In addition to improved growth, the present study showed a corresponding increase in digestive enzyme activities with higher dietary inclusion levels of SWP and BSF in koi fry diets. This trend contrasts with findings in Jian carp (*Cyprinus carpio* var. *jian*), where feeding defatted BSF upto 100% resulted in no significant differences (P/ >/ 0.05) in amylase and lipase activities across treatments (Li et al., 2017). Among the enzymes studied in the present study, protease and lipase activities were notably higher than amylase, likely due to the greater reliance of early stage fish on protein rich diets over carbohydrates. The elevated enzyme levels observed in this study suggest enhanced digestive capacity and efficient nutrient utilization in fish fed insect based

Table 4. Estimated digestive enzyme activities of the fishes fed with different experimental diets

Enzymes	Experimental Diets								
	C	SWP20	SWP30	SWP40	SWP50	BSF20	BSF30	BSF40	BSF50
Protease (µmole/mg protein)	14.80± 0.04 ^{a}	15.28± 0.01 ^{a}	16.58± 0.02 ^{a}	17.39± 0.01 ^{a}	17.88± 0.02 ^{a}	16.12± 0.03 ^{a}	16.56± 0.02 ^{a}	16.90± 0.01 ^{a}	17.01± 0.05 ^{a}
Amylase (µmole/mg protein)	8.76± 0.02 ^{a}	10.62± 0.01 ^{b}	11.75± 0.02 ^{c}	12.65± 0.01 ^{c}	12.72± 0.05 ^{c}	11.18± 0.01 ^{b}	12.70± 0.03 ^{c}	12.93± 0.02 ^{c}	12.99± 0.01 ^{c}
Lipase (µmole/mg protein)	18.76± 0.01 ^{a}	20.26± 0.05 ^{b}	21.83± 0.02 ^{c}	22.50± 0.01 ^{c}	22.89± 0.01 ^{c}	20.85± 0.03 ^{b}	22.13± 0.02 ^{c}	22.88± 0.01 ^{c}	22.97± 0.01 ^{c}
AST (nanomoles/mg/min)	8.60± 0.03 ^{a}	10.91± 0.01 ^{b}	11.25± 0.02 ^{c}	12.56± 0.05 ^{c}	12.87± 0.04 ^{c}	10.93± 0.03 ^{b}	12.93± 0.02 ^{c}	12.64± 0.01 ^{c}	12.86± 0.01 ^{c}
ALT (nano moles/mg/min)	9.71± 0.03 ^{a}	11.52± 0.04 ^{b}	12.78± 0.01 ^{c}	13.51± 0.01 ^{c}	13.88± 0.02 ^{c}	12.35± 0.03 ^{b}	12.81± 0.01 ^{c}	13.50± 0.02 ^{c}	13.69± 0.01 ^{c}

Values are expressed in terms of Mean±SD.
Values in the same row with different superscripts vary significantly ($p<0.05$).

diets. Similar findings were reported by Farhoudi et al. (2013), who noted increased protease activity in common carp larvae as an adaptation to protein rich diets. The elevated amylase and lipase levels were attributed to the development of the exocrine pancreas during the growth. Moreover, Rani (2012) emphasized that increased digestive enzyme activities such as protease, amylase, and cellulose support higher nutrient digestibility and retention.

Amino acids serve as essential building blocks of proteins and contribute approximately 14% to 85% of the energy requirements in teleost fish (Ballantyne, 2001). Aspartate aminotransferase (AST) activity is considered a key indicator of amino acid metabolism in fish (Jurss and Bastrop, 1995). In the present study, elevated AST and ALT activity levels observed in SWP50 and BSF50 diet groups may reflect their efficient utilization of amino acids in the experimental diets. Similar findings were reported in Jian carp, where increased AST activity in the hepatopancreas and muscle was linked to active amino acid catabolism (Jiang et al., 2015). Taken together, these findings indicate that the enhanced enzyme activities observed in the present study contributed to improved feed utilization, as reflected in the superior growth performance of fish fed SWP50 and BSF50 diets compared to the control. In addition, an economic evaluation of all experimental diets was conducted using the standard method described by Ardra et al. (2024). The results indicated that SWP50 and BSF50 diets not only enhanced biological performance but also achieved lower production costs and more favorable economic conversion ratios. The economic conversion ratio of all the experimental diets were shown in Table 5.

Table 5. Economical evaluation of all the experimental diets included with graded levels of SWP and BSF as a replacement for fish meal

Experimental diets	Feed cost per kg		Economic Conversion Ratio	
	INR	US$	INR	US$
Control	78	0.93	86.58	1.04
SWP20	72	0.86	66.96	0.80
SWP30	69	0.83	57.27	0.69
SWP40	66	0.79	56.1	0.67
SWP50	63	0.75	50.4	0.60
BSF20	76	0.91	81.32	0.97
BSF30	75	0.90	75.75	0.91
BSF40	74	0.89	65.12	0.78
BSF50	73	0.87	62.05	0.74

ECR, Economic Conversion ratio = FC R × Feed cost

From the above research findings, the results supports the utility of SWP and BSF as effective, eco-friendly alternatives to replace fish meal in aquafeeds. Both insect meals offer a sustainable solution for reducing the aquaculture industry's reliance on wild-sourced fish meal. Additionally, their ability to convert organic waste into high-quality protein makes them environmentally beneficial. Moreover, the enhanced digestive enzyme activities were observed in fishes fed with SWP and BSF included diets, which suggest that these protein sources are efficiently metabolized and utilized by the fishes. Overall, insect larvae meals offer a promising path forward for the development of nutritionally effective and sustainable aquafeeds. In brief both SWP and BSF can be used as a efficient feed ingredient in the nursery rearing of koi carp at a dietary inclusion level of 147 g kg^{-1} with better growth performance and survival rate.

CONCLUSION

The findings of the present study states that insect larvae meals can serve as effective alternative protein sources in aquafeeds, replacing fish meal without compromising growth and survival in koi carp fry. Further, both of the insect meals such as silkworm pupae meal and black soldier fly larvae meal were successfully replaced 50% of fish meal with a dietary inclusion level of 147 g kg^{-1} in the diet of koi carp fry during the nursery phase, resulting in improved growth performance and yield. Moreover, insect meals are more cost-effective than fish meal, which in turn will reduce the overall production costs by lowering feed expenses typically accounting for nearly 60% of the total operating cost in aquaculture.

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Effect of Dietary Supplementation of Lauric Acid on Performance and Carcass Characteristics in Commercial Broiler Chicken

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ABSTRACT

An experiment was conducted to evaluate the effect of dietary lauric acid (LA) supplementation as alternative to antibiotic growth promoter (AGP) at graded levels on performance and carcass traits in commercial broilers. Three-hundred-day-old commercial broiler chicks were randomly allotted to 6 dietary treatments with 10 replicates of 5 chicks in each. Corn soyabean meal based basal diet (BD) was formulated for pre-starter (1-2weeks), starter (3-4 weeks) and finisher (5-6 weeks) phases without antibiotic growth promoter (negative control). Positive control diet was the BD having 0.035% chlortetracycline as AGP. The remaining four experimental diets were formulated by supplementing lauric acid to BD at rate of 0.05% (LA-50), 0.075% (LA-75), 0.1% (LA-100) and 0.2 % (LA-200), respectively. Body weights, feed intake and feed conversion ratio (FCR) were measured at weekly intervals. At the end of 6th week, one bird from each replicate was selected randomly for studying carcass characteristics. Overall body weight gain (BWG) was higher ($P<0.05$) in chicks fed LA supplemented diets compared to BD fed chicks. Highest BWG was obtained in chicks fed LA-50 diet than those fed on either AGP or BD and was comparable to those fed LA-75 diet. Higher ($P<0.05$) feed intake was recorded in birds fed diets supplemented with LA-75 diets followed by LA-50, AGP, LA-200 and LA-100 fed birds. The FCR during all the phases was significantly ($P<0.05$) improved in LA-50 compared to other dietary groups. The dressing percentage was higher ($P<0.05$) with LA-50 compared to AGP fed birds, while at higher levels of LA supplementation, the dressing percentage was either comparable or higher than AGP or BD fed birds. No difference regarding breast, liver and gizzard weight was observed with LA supplementation. The feed cost/kg gain did not vary among the dietary groups. Based on the results it could be concluded that, lauric acid can be a replacer of antibiotic growth promoter and lower dose of lauric acid (0.05%) used in the present study was sufficient in improving performance and dressing percentage in broilers.

KEYWORDS: Antibiotics growth promoter, Broiler chicken, Carcass characteristics, Growth performance, Lauric acid

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INTRODUCTION

Profitability of broiler production depends on the growth rate, improved FCR and good gut health. During the last 50 years, use of antibiotic growth promoters (AGP) at sub-therapeutic dose has been widely practiced in poultry industry to stabilize the balance of gut ecosystem by elimination of intestinal bacteria in the gut and thereby improving the growth performance of chicken (Huyghebaert et al., 2011). But, the prolonged and unregulated use of AGPs has contributed in part to the development of antibiotic-resistant pathogen in poultry as well as in humans which enforced the European Union to ban use of antibiotics as growth promoters in poultry diet since 1st January 2006 (Castanon, 2007). As a consequence, the poultry industry has needed to find alternatives to AGPs in order to stem the spike in infection rates, which are also environmental friendly and safe for both animal and humans consuming animal products (Cabuk et al., 2006). Among all, use of short chain fatty acids (SFCA) such as formic (C_1), acetic (C_2), propionic (C_3), and butyric acid (C_4), and few carboxylic acids such as lactic, malic, tartaric, fumaric, and citric acid are being mostly used in poultry diets (Dibner and Buttin, 2002). Recently medium chain fatty acids (MCFAs) (C_6-C_{12}) were found to be more bactericidal to numerous gram-

negative and gram-positive bacteria than the SCFAs (Hermans et al., 2010). Most of the research conducted in poultry was by using SCFAs and some on caproic acid (C6), caprylic acid (C8) and capric acid (C10) as alternative to AGPs (Skøivanova et al., 2006; Begum et al., 2015). Lauric acid (C12) has broad spectrum activity and causes membrane lysis by increasing cellular permeability (Hemalatha et al., 2023; Ameena et al., 2024). Very less information is available on lauric acid, a medium chain fatty acid as an alternative to AGPs in poultry. Therefore, a study was undertaken to assess the effect of dietary supplementation of lauric acid as growth promoter on performance and carcass characteristics in commercial broilers.

MATERIALS AND METHODS

Three hundred, day-old commercial broiler chicks (Cobb-400) were randomly allotted to 6 dietary treatments with 10 replicates of 5 chicks in each. All replicates were separately housed by positioning at random in identical-sized four-floor electrically heated battery cages having raised wire floors and fitted with feeder, water and a droppings tray underneath. The chicks of all groups were kept under uniform management and standard hygienic conditions throughout the experimental period. Birds were immunized for Newcastle disease on 7th and 28th d of age with Lasota vaccine and for infectious bursal disease on 14thand 21st day of age with Georgia strain vaccine. All replicate groups of chicks were offered the respective diets *ad libitum*. Clean and fresh drinking water was provided *ad libitum* daily. A corn-soybean meal based basal diet (BD) was prepared for pre-starter (1-14d), starter (15-28d) and finisher (29-42d) phases without antibiotic growth promoter (Negative control) (Table 1).

Table 1. Ingredient and nutrient composition of basal diets fed to broilers

Ingredient (g/kg)	Pre-Starter (1 to 14 days)	Starter (15 to 28 days)	Finisher (29 to 42 days)
Maize	539.86	561.51	606.05
Soyabean meal	382.65	345.71	294.66
Rice bran oil	34.86	49.46	59.23
Salt	4.24	4.24	4.23
Dicalcium phosphate	17.47	17.97	15.65
Lime stone powder	12.19	12.18	11.73
DL-Methionine	2.77	2.74	2.34
L-Lysine HCl	1.73	1.61	1.29
L-Threonine	0.12	0.46	0.67
L-Tryptophan	0	0.02	0.06
Nutrient composition (calculated)			
Metabolisable energy (kcal/ kg)	3000	3100	3200
Crude protein (%)	22.5	21.0	19.0
Calcium (%)	0.90	0.90	0.82
Non-phytate phosphorus (%)	0.45	0.45	0.40
Dig. Lysine (%)	1.25	1.15	1.00
Dig. Methionine (%)	0.57	0.55	0.49
Dig. Threonine (%)	0.77	0.75	0.70
Dig. Tryptophan (%)	0.225	0.210	0.19
Sodium (%)	0.18	0.18	0.18
Estimated composition			
Dry matter (%)	90.36	90.43	91.11
Crude protein (%)	22.65	21.02	19.06
Ether extract (%)	4.12	5.31	6.63
Crude fibre (%)	3.85	3.71	3.49

Positive control diet was prepared by supplementing chlortetracycline (0.035% in diet) as AGP to the BD. Remaining four experimental diets were formulated by supplementing lauric acid in powder form (procured from M/S AI Nutritions Pvt Ltd, Malaysia, Rs. 300/kg) to BD at rate of 0.05% (LA-50), 0.075% (LA-75), 0.1 % (LA-100) and 0.2 % (LA-200), respectively. During the experiment, body weights, feed intake and feed conversion ratio (FCR) were measured at weekly intervals. On 43rd day, one bird from each replicate was selected randomly, sacrificed by cervical dislocation after overnight fasting for studying carcass characteristics (weight of total carcass, weight of the breast, gizzard, bursa, spleen and abdominal fat). The results obtained were subjected to analysis through software (version 16.0; SPSS, 2007) by applying one way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test (Duncan, 1955) with a test of significance at 5%. All the statistical procedures were done as per the procedures of Snedecor and Cochran (1980).

RESULT AND DISCUSSION

Performance

Higher (P<0.01) BWG was observed in LA- 50 fed birds during all phases compared to AGP and BD groups (Table 2). The BWG during pre-starter phase and overall period was higher in AGP than BD fed birds. The BWG in birds fed LA-75 diet was comparable to LA-50 during all phases. At higher levels of supplementation of LA (LA-100 and LA-200), the BWG was lower than LA-50, but was comparable to AGP fed birds. Differences with regard to BWG, observed in pre-starter, starter and finisher phases were reflected in overall (0-6 week) BWG in chicks. The broilers fed LA-50 diet grew with highest BWG, followed by LA-75, lowest in BD fed birds and in other groups it was intermediate.

Table 2 Effect of dietary supplementation of lauric acid on body weight gain (g) in broilers during various phases

Diet	Pre-starter (0 to 2 weeks)	Starter (3 to 4 weeks)	Finisher (5 to 6 weeks)	Over all (0-6 weeks)
Basal diet (BD)	346.62^{c}	822.50^{d}	679.85^{b}	1849.0^{d}
AGP	358.68^{b}	848.00^{cd}	777.63^{ab}	1984.3^{bc}
LA-50	374.52^{a}	938.04^{a}	838.32^{a}	2150.9^{a}
LA-75	364.34^{ab}	919.64^{ab}	826.55^{a}	2110.5^{ab}
LA-100	366.28^{ab}	850.64^{cd}	677.89^{b}	1894.8^{cd}
LA-200	369.90^{ab}	875.14^{bc}	761.55^{ab}	2006.6^{bc}
N	10	10	10	10
SEM	2.008	8.304	17.002	22.132
P-value	0.001	0.001	0.012	0.001

^{abcd}Means with different superscript in a column differ significantly : P<0.05, P<0.01,

The overall higher BWG in LA-50 and comparable BWG in other LA (LA-75, LA-100 and LA-200) groups compared to AGP, could be attributed to antimicrobial activity of LA, exhibited by crossing the bacterial cell membranes in their un-dissociated form and reduce the pH inside the bacterial cell causing the cell lysis (Dierick et al. 2002). Antimicrobial activity of LA lowers the load of pathogenic bacteria leading to the reduced metabolic needs of nutrients, thereby increasing the

availability of nutrients for growth. Reduced bacterial load in gut eventually decreases the concentrations of toxic metabolites from the bacteria, reduces bacterial fermentation of essential nutrients like protein and energy. The lower BWG in LA-100 and LA-200 fed groups compared to LA-50 could be due to lower feed intake (Table 3).

Further, these observations were in line with Jadhav et al (2021) in broilers fed diets containing 0.25% LA in comparison to control and AGP fed birds and that of Lipinski et al. (2016) in turkey poults with addition of MCFAs. Del Alamo et al. (2007) observed that inclusion of the MCFA blends (C6-C12) @ 0.20% in the starter (0-7days), 0.15% grower (8-21d) and 0.10% finisher (22-42 d) diets improved the BWG compared to the control group. Another study reported improvement in BWG with supplementation of medium chain fatty acids mixture consisting of 60% caproic acid, caprylic acid, capric acid and lauric acid (Khosravinia, 2015). Issac et al. (2013) reported that supplementation of 0.8 to 1.7 g/kg of MCFA resulted in to higher cumulative gain in weight in starter and grower phase. Similar to the present findings, Mathis et al. (2005) reported significant improvement in weight gain in broilers fed a product containing blend of organic acids (formic, acetic, propionic, sorbic acid) and medium-chain fatty acids (caprylic, capric acid) @ 0.1, 0.2 and 0.5% of diet.

The feed intake was lower in BD fed chicks during all phases (Table 3). The AGP fed chicks had higher ($P<0.01$) feed intake than BD birds, except during starter phase. The supplementation of LA at 0.05 to 0.2% of diet improved the feed intake during pre-starter phase, while during other phases and for the overall period, the feed intake was higher in LA-50 and LA-75 fed birds. While supplementation of LA at higher level (LA-100 and LA-200) resulted in lower ($P<0.01$) feed intake compared to lower supplementary levels of LA but comparable to AGP and BD fed birds.

Higher feed intake in LA supplemented groups could be related to fact that, LA being a MCFA influences the secretion of cholecystokinin and other intestinal hormones, that regulate the feed intake (Mabayo et al., 1992) and reduce the pH of gut that helps to increase pancreatic secretion which increases appetite and palatability of the feed (Cave, 1982). In the current study, the feed intake showed a trend of reduction with increase in dose (LA-100 and LA-200) of LA supplementation that could be attributed to its effect on ghrelin hormone produced by endocrine cells of the gastric mucosa controlling feed intake (Nakazato et al., 2001). Nishi et al. (2012) reported that ghrelin may get acylated by MCFA which changes its activity and reduces feed intake.

Table 3. Effect of dietary supplementation of lauric acid on feed intake (g/bird) in broilers during various phases

Diet	Pre-starter (0 to 2 weeks)	Starter (3 to 4 weeks)	Finisher (5 to 6 weeks)	Over all (0-6 weeks)
Basal diet (BD)	418.70^{b}	1256.10^{b}	1356.05^{c}	3030.9^{d}
AGP	430.88^{a}	1288.08^{b}	1528.9^{ab}	3247.9^{bc}
LA-50	442.80^{a}	1378.84^{a}	1580.43^{a}	3402.1^{ab}
LA-75	437.88^{a}	1384.48^{a}	1617.55^{a}	3439.9^{a}
LA-100	432.70^{a}	1278.10^{b}	1369.05^{bc}	3079.8^{cd}
LA-200	435.74^{a}	1310.74^{b}	1478.52^{abc}	3225.0^{bc}
N	10	10	10	10
SEM	1.856	10.270	25.162	30.719
P-value	0.003	0.001	0.004	0.001

^{abcd}Means with different superscript in a column differ significantly: $P<0.05$, $P<0.01$

Kessler et al. (2009) reported increased (P<0.05) feed intake with replacement of 2.67% corn oil with coconut fat (oil rich in MCFA). Feed intake in broilers improved with dietary inclusion of 0.05% LA during starter, finisher and overall period in study by Pappula et al. (2021) corroborating with the present findings. Reduced feed intake observed in present study with higher doses of MCFA are in line with findings of Cave (1982) who observed depressed feed intake at higher supplementary levels of organic acids or MCFA (0.3% of either acetic, propionic, butyric, caproic, caprylic, capric or lauric acids) to basal diets. Zheng et al. (2006) also reported that supplementation capric acid at 0.08 % of diet reduced (P <0.05) feed intake compared to those fed with 0.05% level.

Feed conversion ratio varied significantly (p<0.05) during all phases and for overall period (1 to 6 week), except during finisher phase (Table 4). Higher BWG with feeding of LA-50 diet resulted in improved (P<0.05) FCR compared to BD, AGP and other LA-fed birds during entire study corroborating with the findings of Hemalatha et al. (2023) when fed diets supplemented with 0.05% LA. In other groups, the FCR was comparable. Nguyen et al. (2018) reported linear improvement in FCR with dietary supplementation of 0.05 and 0.06% blend of OAs and MCFAs levels in broilers. The improved FCR could also be due to reduced pathogenic bacteria load in the gut with MCFA supplementation. Reduction in coliform count might have minimized wastage of nutrients and also diverted them for body weight gain, which eventually resulted in improved feed efficiency in broilers fed MCFA. Addition of LA or other medium chain fatty acids at 0.1% to 0.4% in broiler diets had no effect on FCR (Shokrollahi et al., 2014; Khatibjoo et al., 2017; Jadhav et el., 2021, Demirci et al., 2023) and Japanese quails (Saeidi et al., 2016) corroborating with the present findings. De Los Santos et al. (2008) found no effect on FCR with dietary supplementation of caprylic acid (0.35, 0.525, 0.7, 0.875, 1.05, 1.225, or 1.4%) in broilers. Similarly, dietary supplementation of balance mixture of MCFA (caproic, caprylic and capric acid) (Shokrollahi et al., 2014), blends of MCFAs (C8-10), butyrate or combination of butyrate and MCFAs (Khatibjoo et al., 2017) showed no effect on FCR in broiler chicken.

Table 4. Effect of dietary supplementation of lauric acid on feed conversion ratio (g intake/g weight gain) in broilers during various phases

Diet	Pre-starter (0 to 2 weeks)	Starter (3 to 4 weeks)	Finisher (5 to 6 weeks)	Over all (0-6 weeks)
Basal diet (BD)	1.208^{a}	1.527^{a}	1.995	1.639^{a}
AGP	1.201^{ab}	1.519^{a}	1.966	1.637^{a}
LA-50	1.182^{b}	1.470^{b}	1.885	1.582^{b}
LA-75	1.202^{ab}	1.505^{a}	1.957	1.630^{a}
LA-100	1.181^{b}	1.503^{a}	2.020	1.625^{a}
LA-200	1.178^{b}	1.498^{a}	1.941	1.607^{ab}
N	10	10	10	10
SEM	0.0035	0.0042	0.0176	0.0058
P-value	0.042	0.001	0.369	0.037

^{ab}Means with different superscript in a column differ significantly : P<0.05, P<0.01, P- value: acid acid

Carcass characteristics

The dressing percentage was higher with supplementation of LA @ 0.05% diet compared to AGP supplementation (Table 5).

Table 5. Effect of dietary supplementation of lauric acid on carcass characteristics of broiler chicken

Diet	Dressing %	Breast weight (% LW)	Liver weight (% LW)	Abdominal fat weight (% LW)	Gizzard weight (% LW)
Basal diet (BD)	70.97^{bc}	22.93	1.99	0.95	1.98
AGP	70.46^{c}	22.35	1.95	1.31	1.95
LA-50	72.59^{ab}	21.82	2.02	1.34	1.83
LA-75	71.58^{abc}	22.77	2.21	1.19	1.92
LA-100	72.64^{ab}	22.44	2.15	1.06	1.93
LA-200	73.20^{a}	21.82	2.12	1.06	1.81
SEM	0.261	2.157	0.048	0.049	0.028
N	10	10	10	10	10
P-value	0.010	0.427	0.611	0.164	0.455

^{abc} Means with different superscript in a column differ significantly: P<0.05, P<0.01,

While at higher supplementation of LA, the dressing percentage was either comparable or higher than AGP and BD fed groups. No difference was observed among various dietary groups with regard to liver, breast and gizzard weights and abdominal fat when expressed as percent of live weight. Similar to present findings, Papulla et al. (2021) observed no effect on carcass yield, abdominal fat, liver, gizzard and bursa weight was observed with supplementation of 0.05% LA in diets of broilers. At higher supplementary levels of LA (0.4%) the liver weight increased with no effect on dressing per cent, gizzard and bursa weights in studies of Demirci et al. (2023). Addition of 0.1- 0.3% (Shokrollahi et al., 2014) or 0.1% (Khatibjoo et al., 2017) MCFA in diets did not affect carcass weights and weights of visceral organs in broilers.

Cost Economics

The feed cost per bird though differed among the dietary groups, being lowest on basal diet and highest when fed LA 50 diet, the feed cost per kg gain was not affected with dietary supplementation of lauric acid up to 0.2% (Table 6).

Table 6. Effect of dietary supplementation of lauric acid on cost economics of broiler chicken

Diet	Avg Cost of feed (Rs/kg)	Feed intake (g)	Total feed cost per bird (Rs)	Overall BWG (g)	Feed cost/kg weight gain (Rs)
Basal diet	36.12	3030.9^{d}	109.48^{d}	1849.0^{d}	59.3
AGP	36.23	3247.9^{bc}	117.66^{bc}	1984.3^{bc}	59.3
LA-50	36.26	3402.1^{ab}	123.36^{ab}	2150.9^{a}	57.4
LA-75	36.33	3439.9^{a}	124.98^{a}	2110.5^{ab}	59.3
LA-100	36.42	3079.8^{cd}	112.18^{cd}	1894.8^{cd}	59.3
LA-200	36.71	3225.0^{bc}	118.40^{bc}	2006.6^{bc}	59.1
N	10	10	10	10	10
SEM		30.719	1.11	22.132	0.22
P value		0.001	0.001	0.001	0.063

^{abc} Means with different superscript in a column differ significantly: P<0.05, P<0.01

CONCLUSION

The results of present study indicated that lauric acid (C12) supplementation in broiler diet at 0.05% without any antibiotic growth promoter resulted in higher growth and feed conversion ratio, while higher levels of supplementation (0.075 to 0.2%) of lauric acid had no further beneficial effects on performance of broilers.

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Effect of Dietary Supplementation of *Moringa oleifera* Leaf Meal on Production and Reproduction in Rhode Island Red Chicken

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ABSTRACT

A study was carried out to determine the effect of *Moringa oleifera* leaf meal (MOLM) on production performance, semen quality, fertility, hatchability and chick quality of Rhode Island Red (RIR) chickens. One hundred and eighty, 24-week-old Rhode Island Red layer birds were randomly allotted to four dietary treatments having replicates with 15 birds in each. The treatments were T1 (Control: Basal diet); T2: 1% MOLM in basal diet; T3: 3% MOLM in basal diet; T4: 5% MOLM in basal diet. To study reproductive performance, 24 cocks at the age of 24th weeks were kept separately and equally divided into four groups and were provided same dietary treatments as provided to hens. The results revealed that average daily feed intake per bird (g/day) was lower ($P<0.05$) for hens supplemented with MOLM. No statistical difference for feed conversion ratio (FCR) was observed between groups. The average HDEP % and HHEP % of experiment were significantly ($P<0.05$) higher in T3 and T4 groups as compared to T1. The MOLM diet resulted in significantly ($P<0.05$) increased semen concentration, total sperm count, motility and live sperm than the control, however semen volume and pH were not differed between groups. Fertility % was linearly increased with MOLM but it was not statistically different ($P>0.05$) with control group. Hatchability percentage was higher ($P>0.05$) in T3 and T4 than the others. Hens in T2, T3 and T4 groups had higher mean values for average chick weight and chick length ($P<0.05$) than the control group. Results of the present study indicated positive response of *Moringa oleifera* leaf meal on production and reproduction performance when used in the diet of birds.

KEY WORDS: Fertility, Moringa leaf meal, Rhode Island Red, Semen

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INTRODUCTION

In intensive poultry farming, feed remain to be the biggest challenge, particularly in India where the cost of traditional feed supplies is constantly rising. The scarcity and ensuing high cost of conventional protein sources, which are limiting constraints for the manufacturing of chicken feed, had an impact on poultry productivity. Addition of antibiotic growth promoters to feed, which increased feed productivity, efficiency and financial gain, has been banned from poultry feed (Fallah et al., 2013). Dietary manipulation has been recommended as a method of improving reproduction due to the strong relationship between nutrition and overall fertility (Hudson and Wilson 2013). Peters et al. (2008) reported semen quality as the measure of the capacity of semen to achieve fertilization, which is essential in the production of hatching eggs and can be heavily influenced by nutrition.

One such alternative is phytobiotics, which are defined by Windisch et al. (2008) as plant-derived products added to feed to enhance the performance of farm animals. Tree leaves are a good source of vitamins, vital amino acids, proteins and minerals and have a wide range of nutrients (Fasuyi, 2006). Among the vital elements found in *Moringa oleifera* leaves, protein, vitamin B complexes, vitamin C, beta-carotene, vitamin K and manganese are the most nutrient-dense components (Leone et al., 2015). There is minimal data on the use of *Moringa oleifera* as a protein source in the layer ration for poultry feeding, despite its high nutritional content. Therefore, this study was conducted to determine effects of feeding MOLM on production and reproduction performance in laying hens.

MATERIALS AND METHODS

The experiment was conducted at the Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh Gujarat (latitude 21°29' N, longitude 70°26' E and altitude 60 meters above the mean sea level). Laboratory work was carried out in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. The climate in Junagadh is tropical and arid.

Experimental details

Rhode Island Red layer birds (n=180) of 24 weeks of age were selected at random and divided into four equal groups of 45 birds with three replicates of 15 in each group in a completely randomized design. The birds were raised in cages under uniform management and fed the respective diets from 24th to 40th weeks of age. To study reproductive performance, 24 cocks at the age of 24th weeks were kept separately and equally divided into four groups and were provided same dietary treatments as provided to hens. Research protocol was approved by the Animal Ethics Committee, vide protocol no.: KU-JVC-IAEC-SA-96-2022.

Four experimental diets (isocaloric and isonitrogenous) were prepared with MOLM as T1 (Control: Basal diet); T2: 1% MOLM in basal diet; T3: 3% MOLM in basal diet; T4: 5% MOLM in basal diet (Table 1). Feed was offered ad libitum in weighed quantity twice a day at 9:00 AM and 5:00 PM. Manual turning and mixing of feeds in the feeder were done frequently at least twice daily. Clean, fresh, wholesome drinking water was provided to all experimental birds *ad libitum*. Light bulbs were placed for the lighting system to increase the lighting period to 16 h per day.

Table 1. Ingredient and nutrient composition of the experimental diets

Ingredients (%)	T1	T2	T3	T4
Ingredient composition (% DM basis)				
Maize	53.4	53.4	50	45
Soyabean DOC	27.7	26.7	24.7	22.7
Deoiled Rice Bran	6.41	6.41	9.81	14.8
Moringa oleifera leaf meal (MOLM)	0	1.0	3.0	5.0
Calcite Powder	3.0	3.0	3.0	3.0
Limestone	6.9	6.9	6.9	6.9
DCP	1.63	1.63	1.63	1.63
Salt	0.3	0.3	0.3	0.3
Premix (Vitamins, Enzymes etc.)	0.66	0.66	0.66	0.66
Total %	100	100	100	100
Calculated nutrient composition (%)				
ME (Kcal/kg)	2613	2616	2622	2629
DM	89.5	89.3	89.3	89.8
OM	93.0	93.0	92.9	92.8
CP	18.0	18.4	18.6	18.3
CF	8.17	8.01	7.92	7.94
EE	3.45	3.51	3.67	3.8
NFE	63.4	63.0	62.6	62.7
Total Ash	6.92	6.95	7.1	7.2
Silica	1.11	1.22	1.16	1.19
Calcium	3.05	3.21	3.28	3.36
Phosphorus	0.38	0.39	0.39	0.4

Feed intake, feed conversion ratio and egg production

Feed consumption of each replicate was recorded daily. The amount of feed consumed per bird was determined as the difference between the feed offered and left over to calculate weekly/daily feed intake (g). Feed conversion ratio was determined weekly as a unit feed consumed per unit egg weight (Abou-Elezz et al., 2011).

Eggs were collected two times a day at 9:00 and 17:00 hours. The sum of the two collections along with the number of birds alive on each day was recorded and summarized at the end of the period. Hen-day egg production (HDEP) and hen housed egg production (HHEP) as percentage were determined. Eggs were weighed weekly immediately after collection and average egg weight was computed by dividing the total egg weight to the number of eggs.

Semen quality, fertility and hatchability of eggs and chick quality

At the end of experiment, semen quality was evaluated in 24 cocks (6 cocks in each treatment). Before the commencement of semen sampling, the cocks were trained for a period of four weeks for semen collection. Using an abdominal massage technique, semen was collected from every cock in each replicate and examined for semen quality traits (Lake, 1957). The cloacal region was massaged to induce phallic tumescence; this was followed by a cloacal stroke and a squeeze of the area around the edges of the cloaca to express the semen. Semen was milked down into graduated collecting glass test tubes and its volume was then measured. Each cock's fresh semen samples was measured using a pH strip by dropping the sample on the strip and reading the pH. Semen was diluted to 1:4 with phosphate buffered saline, mixed, and incubated at 37°C to test for individual motility. A cover slip was placed over a drop of diluted semen on a warm slide, and a light binocular microscope set to 400X magnification was used to study it. A thin smear was created by mixing 10 ìl of diluted semen with 10 ìl of eosin-nigrosin stain at 37°C in order to determine the viability. Using a hemocytometer, the sperm cell concentrations were calculated and expressed in billions/ml of semen. Sperm count was done as described by Hafez (1987) with a light microscope (400X).

At the end of experiment, fertility was assessed by performing artificial insemination with standard protocol in experimental birds by semen collected from respective cocks. Fifteen eggs per treatment were randomly selected, sprayed with disinfectant and placed in an incubator with the broad end pointing upwards set at a temperature of 37.5°C and relative humidity (RH) of 65% for 18 days. Candling was done on the 7th and 18th day of incubation, and the fertile eggs only were then transferred into a hatchery at a temperature of 37.4°C and RH 70% until hatching. Average percentage fertility was determined by dividing the total number of eggs found fertile at candling by total number of eggs set times 100. Average percentage hatchability of the fertile eggs were computed by dividing the number of chicks hatched by the number of fertile eggs times 100.

Chick quality assessment was performed by employing chick weight and chick length at hatching. Chick length was determined by stretching the chick along a ruler and measuring the length from beak to the end of the middle toe. Chick weight was measured by weighing the chick at hatching.

RESULTS AND DISCUSSION

Production performance

In the current study, it was found that overall average feed intake per bird (g/day) was higher ($P<0.05$) for hens in T1 than hens in T2, T3 and T4 (Table 2). Hens in T2 had higher ($P<0.05$) average feed intake per bird (g/day) than hens in T4, however, T3 did not significantly differ ($P>0.05$) with T2 and T4. This was similar to the findings of Raphael et al. (2015) noted that addition of 5% and 10% MOLM to the laying hen diet reduced feed consumption. They attributed the significant decline in feed intake with increasing amount of leaf meal due to its high fibre content as well bitter taste. In contrast, Sharmin et al. (2021) observed that average daily feed consumption of laying hens fed diets containing 1.5% MOLM was significantly higher than that of the hens fed diet with 0%, 0.5% and 1% MOLM.

Table 2. Effects of dietary treatment on production parameters of RIR laying hen

Parameter	T1	T2	T3	T4	P value	SEM
Feed Intake, g/day	116± 0.44^{A}	114± 0.36^{B}	112± 0.55^{BC}	111± 0.85^{C}	0.001	3.1
Feed Conversion Ratio (FCR)	2.94±0.03	2.86±0.03	2.93±0.01	2.91±0.05	0.96	0.25
HDEP %	69.08±1.88^{B}	73.76±1.5^{AB}	75.67±1.71^{A}	79.17±1.15^{A}	0.002	4.97
HHEP %	65.96±2.11^{C}	72.13±1.79^{BC}	74.44±1.91^{AB}	78.74±1.28^{A}	0.003	5.83

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability

In the current study, no statistical difference was observed for FCR between groups or within group. Overall average FCR was lowest for hens in T_2. But, there was no statistical differences (P>0.05) among the groups. The results are similar with the finding of Olugbemi et al. (2010) who noted that addition of 5% and 10% MOLM to the laying hen diet had no effect on feed conversion ratio. However, Raphael et al. (2015) gradually replaced soybean with MOLM at 0, 5 and 10% and observed that with 5% MOLM, the FCR was lower; however, with 10% MOLM, the FCR was higher.

The values were significantly (P<0.05) higher in T_3 and T_4 groups as compared to T1 group and numerically higher than T2 group. The values were gradually increased from T1 to T4 and have significantly higher values in T3 and T4 as compared to control. The T3 was non-significant different from T2 and T4. T4 also showed significantly higher value than T2. In the present investigation, an increase in laying percentages with advancement of age was noted in all dietary treatments. Current study was in line with Ebenebe et al. (2013) who reported similar results in response to various levels (0%, 2.5%, 5.0% and 7.5%) of MOLM in diets of laying chickens. Raphael et al. (2015) also noted significant effect on egg production when fed 5% MOLM as compared to 0 and 10% MOLM in Kabir strain chickens. On the other hand, Olugbemi et al. (2010) showed a non-significant effect on laying % for hens fed a diet containing MOLM at 0, 5, and 10% of the diet. Abu and Akangbe (2017) also found that addition of 0, 1 and 5% MOLM Japanese quails' diet had no significant effect on HDEP % when compared to a diet free of MOLM. Improved balanced nutritional supply provided by MOLM in the diet may be the cause of the greater egg production in layers given the diet containing MOLM. Lysine, methionine, and a variety of other amino acids are present in MOLM, which may provide the necessary quantity of nutrients for improved production.

Reproduction performance

Effect of feeding MOLM at different levels on semen characteristics in Rhode Island Red cocks indicated that dietary treatment had significant effect (P<0.05) on sperm motility, live and dead sperm, sperm concentration and total sperm count but did not affect semen volume and pH. Sperm concentration was significantly higher in all level MOLM supplemented groups and higher value was found in 3% MOLM group (Table 3). Total sperm count was also significantly higher in treatment groups, which might be attributed to the increased semen volume and sperm concentration. Sperm motility was recorded to be higher in 5% MOLM supplemented group and it was significantly (P<0.05) higher than control. Live and dead sperm % were also significantly affected with MOLM supplementation.

Table 3. Effects of feeding different levels of MOLM on semen quality parameters of Rhode Island Red cocks

Parameters	T1	T2	T3	T4	SEM	P value
Body Weight (g)	2718 ±85.59	2683 ±134.85	2655.75 ±33.88	2662.75 ±230.98	282	0.9892
Volume (ml)	0.34 ±0.06	0.39 ±0.04	0.46 ±0.04	0.38 ±0.05	0.1	0.3979
pH	7.5 ±0	7.63 ±0.12	7.63 ±0.13	7.63 ±0.13	0.21	0.8015
Concentration ($x10^9$/ml)	1.44 ±0.11^{B}	2.41 ±0.19^{A}	2.49 ±0.19^{A}	2.34 ±0.24^{A}	0.38	0.0001
Total sperm count ($x10^9$/ejaculate)	0.49 ±0.08^{B}	0.93 ±0.15^{A}	1.14 ±0.18^{A}	0.88 ±0.16^{A}	0.3	0.006
Motility (%)	73.75 ±6.25^{B}	81.5 ±0.95^{AB}	86.75 ±2.35^{AB}	88.25 ±1.18^{A}	6.8	0.0443
Live sperm (%)	75.75 ±2.52^{B}	85.25 ±1.65^{A}	85.5 ±2.21^{A}	85.25 ±2.05^{A}	4.27	0.0174
Dead sperm (%)	24.25 ±2.52^{A}	14.75 ±1.65^{B}	14.5 ±2.21^{B}	14.75 ±2.05^{B}	4.27	0.0174

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability.

Sebola et al. (2022) found that addition of MOLM at 70 g/kg diet caused increased sperm motility and elevated (P<0.05) semen pH in Potchefstroom Koekoek (PK) chickens. However, the PK cockerels' semen volume was unaffected by diet (P>0.05). Poku et al. (2023) observed that the dietary MOLM had significant influence (P<0.05) on sperm motility, semen pH and sperm count in Pearl Guinea fowl cock. Increasing levels of MOLM in the diet decreased sperm motility (P<0.05).

Optimal qualities in cock semen are necessary for increased reproductive effectiveness. In this study, dietary MOLM resulted in greater semen pH, sperm count, and decreased dead sperm. Physiologically, higher semen pH, sperm count and low dead sperm obtained with dietary MOLM indicated that increasing MOLM in the diet increased the levels of essential amino acids such as lysine, phenylalanine, valine, histidine and isoleucine. The superior semen quality shown in Rhode Island Red cocks fed MOLM might be due to the high concentrations of healthy antioxidants, phytochemicals, minerals, and vitamins found in MOLM.

Fertility % linearly increased with MOLM but it was not statistically different (P>0.05) with control group. Hatchability percentage was higher (P>0.05) in T3 and T4 than the others (Table 4). Hens in T2, T3 and T4 groups had higher mean values for average chick weight and chick length (P<0.05) than the control group.

Table 4. Fertility, hatchability and chick quality of Rhode Island Red hens fed different levels of MOLM

Parameters	T1	T2	T3	T4	X^2 Value	Df	P Value
Fertility (%)	66.67 ±6.66	86.67 ±6.66	93.33 ±6.66	93.33 ±6.66	5.621	3	0.165
Hatchability (%)	61.11 ±5.55	61.67 ±7.26	65.0 ±5.0	65.0 ±5.0	1.607	3	0.769
Parameters	T_1	T_2	T_3	T_4	SEM		P Value
Chick Weight (g)	37.46 ±0.22^{B}	41.68 ±0.34^{A}	41.61 ±0.1^{A}	41.5 ±0.54^{A}	1.11		0.0001
Chick length (cm)	15.92 ±0.25^{B}	17.34 ±0.13^{A}	17.19 ±0.1^{A}	17.3 ±0.21^{A}	0.49		0.0001

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability

Result obtained in the current study were consistent with the finding of Raphael et al. (2015) using *Moringa oleifera* leaf meal as an alternative feed ingredient in the Kabir strain hens' ration which showed non-significant (P>0.05) effect of MOLM on fertility and hatchability. Similarly, Ashour et al. (2020) found that dietary MOLM had no negative effects on fertility and hatchability in Japanese quails. Contrary to present findings, Alebachew et al. (2016) observed that fertility and hatchability were significantly improved by feeding of MOLM in dual-purpose Koekoek hens. However, average chick weight and chick length were significantly higher (P<0.05) in MOLM supplemented groups. Mousa et al. (2017) also concluded that diets supplemented with MOLM demonstrated significantly (P<0.05) improved fertility and hatchability.

In current study it was found that addition of MOLM in diet of hens improved fertility, hatchability and chick quality in terms of chick weight and chick length. Micro nutrients are key factor in successful poultry reproduction. These nutrients like calcium, phosphorus, zinc, iron, vitamin E, vitamin C etc. are relatively high in MOLM. This result may be due to the fact that herbal plant may provide some compounds that enhance egg quality and fertility.

CONCLUSION

This study concludes that addition of *Moringa oleifera* leaf meal up to 5 % level in the diet of Rhode Island Red laying hens improved production performance and reproduction efficiency.

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Unleashing the Potential of Nucleotide Supplementation on Broiler Health and Performance

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ABSTRACT

The potential benefits of nucleotide supplementation may include an increase in the length of intestinal villi, promotion of nutrient absorption, enhanced weight gain (Yu, 1998), and the rapid turnover of intestinal cells (enterocytes) following damage caused by stress or pathogens. 120-day-old broiler chicks were randomly allocated to 4 experimental groups, each consisting of six replicates of five chicks. The standard broiler diets (Con) were formulated. The ConA: Con+ antibiotic growth promoter. In the N5 and N10 treatments, the antibiotic was replaced with nucleotide @ 5 and 10 mg/L in the drinking water, respectively. The individual body weight and feed intake of the broilers were recorded weekly, and subsequently, the feed-to-gain ratio and production efficiency factor were determined. A metabolic trial was conducted at the end of the experiment to evaluate nutrient utilization. At the end of the trial (42ndday), 02 birds from each replicate were sacrificed to assess carcass traits, nutrient composition of breast muscle, and intestinal morphology. The comprehensive performance evaluation of broilers over the six-week period indicated a significant enhancement ($p<0.05$) in overall performance with the N5 broiler diet. The results further demonstrated a noteworthy improvement ($p<0.05$) in protein digestibility among broilers fed nucleotide supplementation, particularly in the N5 group, signifying a substantial increase in protein utilization. Notably, nucleotide supplementation did not exert any discernible effect on the nutrient composition of the meat. Consequently, based on the findings of this study, it can be inferred that the supplementation of nucleotides at a concentration of 5 mg/L significantly ($p<0.05$) contributed to improved growth performance, enhanced intestinal morphology, and increased intestinal capacity for nutrient absorption in broilers and could be regarded as a viable alternative to antibiotic growth promoters.

KEYWORDS: Antibiotic growth promoter, Broiler, Histo-morphometerical analysis, Nucleotide, Nutrient utilization.

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INTRODUCTION

Nucleotides play essential roles in both physiological and biochemical functions, participating in the encoding and decoding of genetic information, regulating energy metabolism, facilitating cell signaling, and serving as crucial components of coenzymes, allosteric effectors, and cellular agonists in terrestrial animals. These low molecular weight biological molecules are particularly vital as constituents of nucleic acids—Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

Comprising three major components—a sugar, a nitrogen base, and one or more phosphate groups—nucleotides undergo cleavage of the phosphate group by alkaline phosphatase and nucleosidases in the small intestine, resulting in the formation of nucleosides (Carver and Walker, 1995). Remarkably, over 90% of dietary, endogenous, purine, and pyrimidine nucleotides are absorbed into the enterocytes, where they undergo rapid degradation into uric acid and allantoin.

Unlike mammals, poultry can synthesize nucleotides *de novo*; however, it is now believed that the capacity for nucleotide production, particularly in young animals, may not suffice to meet their needs. For this reason, nucleotides are considered "semi" or "conditionally" essential nutrients in animals, especially during periods of stress, rapid growth, health challenges, high stocking

densities, and the replacement or removal of antibiotics (Themburne et al., 2020). In response to the need to mitigate losses in poultry production and the search for natural alternatives to antibiotic growth promoters (AGPs), nucleotides have emerged as promising candidate. Given these considerations, the present experiment was conducted to investigate the effects of nucleotide supplementation on the growth performance and intestinal morphology of broilers.

MATERIALS AND METHODS

Study was approved by Institutional Animal Ethical Committee (IAEC) vide D.No.23/IAEC/Vety/2022 dated 20.05.2022 which was affiliated from CPCSEA, Ministry of Animal Husbandry, India. The experimental design involved the random allocation of one hundred and twenty day-old broiler chicks into four experimental groups, each consisting of 6 replicates with 5 chicks per replicate. Standard isonitrogenous and isocaloric broiler diets (Con) were formulated according to commercial chick feed specifications (2011) for three growth stages: Pre-starter (0-14 days, 22.5% CP and 3000 kcal ME/kg diet), Starter (15-28 days, 21.0% CP and 3125 kcal ME/kg diet), and Finisher (29-42 days, 19.50% CP and 3250 kcal ME/kg diet). The dietary treatment ConA was identical to Con but included an antibiotic growth promoter. In groups N5 and N10, the antibiotic was substituted with nucleotide at concentrations of 5 and 10 mg/L in the drinking water, respectively.

Growth Parameters:

Weekly measurement of bodyweights and feed consumptions was recorded. Accordingly, Feed to gain ratio (F:G) was calculated. Similalry, Production efficiency factor (PEF) as calculated by following formula [Pelicia et al., 2010].

$$PEF = \frac{daily\ weight\ gain\ (kg) \times livability\ (\%)}{Feed\ to\ gain\ ratio\ (FCR)} \times 100$$

Nutrient utilization or nutrient digestibility

To know the utilization of nutrients (DM, CP and EE) from different diets metabolic trial was conducted on all the experimental birds at 6th week of the experiment. During collection period quantity of feed offered and leftover were taken daily. The excreta of each replicate were collected quantitatively at every 24 hours period for 3 days.

Nutrient composition of meat

At the conclusion of the 42-day trial, birds were sacrificed to assess carcass traits. Simultaneously, samples from the breast muscle (two from each replicate) were obtained for analysis of proximate composition, including dry matter, crude protein, and ether extract. These analyses were conducted using AOAC (2012).

Carcass traits

To study the carcass traits, birds were slaughtered and after complete bleeding, weight was recorded. The weight was again recorded after manual defeathering using hot water (50-55°C). The dressed weight was then recorded as follows:

$$Dressed\ wt = Live\ wt - Wt\ loss\ as\ blood, head, feather, shank\ and\ wing\ tips$$

$$Eviscerated\ weight = Dressed\ weight - weight\ of\ viscera$$

$$Drawn\ wt = Eviscerated\ weight + weight\ of\ giblet$$

Various processing losses (% of live weight) such as blood, head, feathers, shank, separable fat and wing tips were also recorded.

The organs (liver, heart, gizzard, spleen and pancreas) and weight of lymphoid organs (bursa of fabricius, spleen and thymus), collected replicate wise at the time of slaughter and their weights (% of dressed weight) were recorded.

Examination of intestinal morphology structure

Duodenum and ileo-jejunum samples were obtained for histo-morphometric analysis. Tissue samples measuring 3-4 mm were collected from the duodenum and ileo-jejunum, fixed in 10% formalin, and processed using the paraffin embedding method. Sections, approximately 4-5 µm thick, were cut and stained with haematoxylin and eosin to reveal the general histoarchitecture. Out of 12 samples of

duodenum and ileo-jejunum from each treatment, total of six intact and well-oriented villi were selected, resulting in 15 measurements for each sample and 60 measurements per replicate. The evaluated gastrointestinal morphological variables included intestinal wall thickness, villus height, crypt depth, the ratio of villus height to crypt depth, and goblet cells. Measurements were taken from each bird and then averaged to derive a mean value for each variable per treatment (n = 6).

Villus height was measured from the top of the villus to the top of the lamina propria, while crypt depth was measured from the base upward to the transition region between the crypt and the villus. Neutral goblet cells were identified through H and E staining. This detailed histo-morphometric analysis aimed to provide a comprehensive understanding of the structural aspects of the duodenum and ileo-jejunum in response to the experimental treatments.

Statistical Analysis

The statistical analysis of the data was performed using analysis of variance (ANOVA) with a Completely Randomized Design (CRD), which was conducted using SPSS version 20.0.

RESULTS AND DISCUSSION

Performance of broilers

The results of the current study clearly reveal a distinct trend of increased live weight and weight gain in broilers supplemented with nucleotides. Notably, the group receiving nucleotide at a concentration of 5 mg/L in drinking water (N5) exhibited the highest and significantly greater live weight and weight gain compared to other groups ($p<0.05$). This observation underscores the positive impact of nucleotide supplementation on the overall growth and weight gain of broilers, suggesting its potential as an effective nutritional intervention in poultry production.

Moreover, minimal feed consumption was observed in broilers fed the N5 diet with nucleotides at 5 mg/L, while diets ConA, N5, and N10 were statistically similar to each other but lower than those fed the Con diet.

Table 1. Performance of broilers in different treatment groups

	Treatments					
Weekly	Con	ConA	N5	N10	SEM	*p-value*
Performance						
Live weight	2672.70^{c}	2738.66^{b}	2839.80^{a}	2727.13^{b}	14.34	0.00
Average weight gain	2616.22^{c}	2682.50^{b}	2783.67^{a}	2670.73^{b}	14.36	0.00
Average FI§	4476.26^{a}	4291.74^{b}	4291.57^{b}	4325.20^{b}	23.85	0.01
Average F:G§§	1.71^{a}	1.60^{b}	1.54^{c}	1.62^{b}	0.01	0.00
PEF§§§	270.80^{b}	316.00^{a}	339.00^{a}	307.50^{ab}	8.03	0.01
Nutrient Utilization (%)						
DM	70.05	70.47	72.90	71.43	2.01	0.96
CP	71.58^{b}	72.58^{ab}	75.10^{a}	73.09^{ab}	0.49	0.06
EE	82.63	82.62	83.37	82.71	1.37	0.99
Nutrient Composition of breast muscle (%)						
DM	24.51	24.55	24.97	24.02	0.47	0.94
CP	19.11	19.13	20.95	19.26	1.19	0.95
EE	5.72	5.48	5.77	5.43	0.23	0.95
Lymphoid organs weight (% of live weight)						
Spleen	0.11^{b}	0.12^{ab}	0.14^{a}	0.12^{ab}	0.00	0.03
Thymus	0.60^{c}	0.63^{bc}	0.84^{a}	0.78^{ab}	0.03	0.02
Bursa of fabricius	0.23	0.21	0.25	0.20	0.00	0.76
Economics of broiler production						
Feed cost Rs/kg	44.02	44.06	44.17	44.32	0.00	-
Feed cost Rs/kg body weight gain	75.30^{a}	70.48^{b}	69.40^{b}	74.54^{a}	0.63	0.00

Means of row with different superscript showing significant difference ($p<0.05$)

At the conclusion of the experiment, a more favorable feed-to-gain ratio was observed in broilers fed the N5 diet, followed by N10 diet which was statistically similar to ConA (Table 01). The production efficiency factor was lower in broilers fed the basal diet (ConA) and highest in those fed the N5 diet containing nucleotides at 5 mg/L. Although the groups ConA, N5, and N10 were statistically similar, they were numerically lower than those fed the N5 diet. Overall, these findings suggest that nucleotide supplementation, particularly at a concentration of 5 mg/L, positively influences weight gain, feed consumption, feed-to-gain ratio, and production efficiency factor in broilers, highlighting its potential as a beneficial nutritional component in poultry diets.

This detailed excerpt offers a comprehensive overview of various studies emphasizing the positive effects of nucleotide supplementation on broiler diets. Thembhurne et al. (2020) observed improved live weight with graded levels of nucleotide rich yeast supplementation. The stimulation of brush border enzyme activity may enhance digestion and absorption, promoting better growth (Villavan et al., 2021). Nucleotide availability may lead to the proliferation of intestinal cells, thereby improving digestion and absorption (Salah et al., 2019). Sheik et al. (2021) noted a numerical reduction in cumulative feed intake with nucleotide supplementation. Jung and Batal (2012) reported no significant difference in feed intake, suggesting variability in results due to management practices, nucleotide sources, and environmental conditions. Sampath et al. (2021) observed an improved feed-to-gain ratio with yeast hydrolysates, highlighting the potential benefits of enhanced intestinal health and increased enzyme activity. Nucleotide supplementation was associated with a numerically increased production efficiency factor (Pelicia et al., 2010; Sheik et al., 2021).

Nutrient Utilization

No notable trend was observed in dry matter and ether extract utilization with dietary nucleotide supplementation in broilers, a significant influence on crude protein utilization was apparent. Specifically, among all the diets, the highest crude protein utilization was recorded in broilers supplemented with nucleotide at a level of 5 mg/L in drinking water (N5). Conversely, the lowest crude protein utilization was noted in the group assigned to the Con diet. This indicates that nucleotide supplementation, particularly at the 5 mg/L level, markedly enhances the utilization of dietary crude protein by broilers, demonstrating its potential to positively influence nutrient utilization in poultry diets.

Improved crude protein digestibility was observed with nucleotide supplementation. Nucleotide supplementation did not significantly influence ether extract utilization (Srinivas et al., 2018; Ahiwe et al., 2020). Balanced microbial populations may play a role in altered metabolism and enhanced digestive enzyme activity.

Weight of lymphoid organs

The study results indicate an enhancement in the weight of lymphoid organs, specifically the spleen and thymus, in broilers supplemented with nucleotides at a concentration of 5 mg/L in drinking water (Table 1). However, the bursa of Fabricius did not exhibit a significant change in weight with nucleotide supplementation. This implies that nucleotide supplementation, particularly at the specified concentration, positively influences the development and weight of certain lymphoid organs in broilers. The spleen and thymus, which play vital roles in the immune system, showed increased weight, potentially indicating a beneficial impact on the immune response of broilers receiving nucleotide supplementation. The absence of a significant change in the bursa of Fabricius may suggest that the effects of nucleotide supplementation are organ-specific within the avian immune system.

Increased spleen weight was noted with yeast RNA supplementation (Deng et al., 2005; Sampath et al., 2021), suggesting potential immunomodulatory effects. Nucleotide deprivation may impact the T-cell cycle and immune responses.

Nutrient composition of meat (breast muscle)

The study data on the proximate composition of broiler meat, specifically dry matter, crude protein, and ether extract, as influenced by the supplementation of different levels of nucleotides is presented in Table 1. The results indicate that there were no significant differences ($p>0.05$) in the nutrient composition of meat among broilers fed basal diets supplemented with various levels of nucleotides compared to those assigned the basal diet alone.

Histo-morphometrical analysis of intestine

The results of the current study, presented in Table 2, indicate that broilers fed basal diets supplemented with varying levels of nucleotide did not show significant ($p>0.05$) effects on duodenum and ileo-jejunum wall thickness, crypt depth, villus height: crypt depth ratio, and goblet cells per field. However, a significant effect was observed on villus height in both the duodenum and ileo-jejunum sections of the intestine for broilers receiving nucleotide at 5mg/L in drinking water (N5).

The intestine serves as the primary site for maximum nutrient absorption in broilers. The villi of the small intestine play a crucial role in this process by projecting into the intestinal cavity, significantly increasing the surface area available for absorption and facilitating the addition of digestive secretions. It is noteworthy that villi are most abundant at the beginning of the small intestine and gradually decrease in number toward the end of the tract.

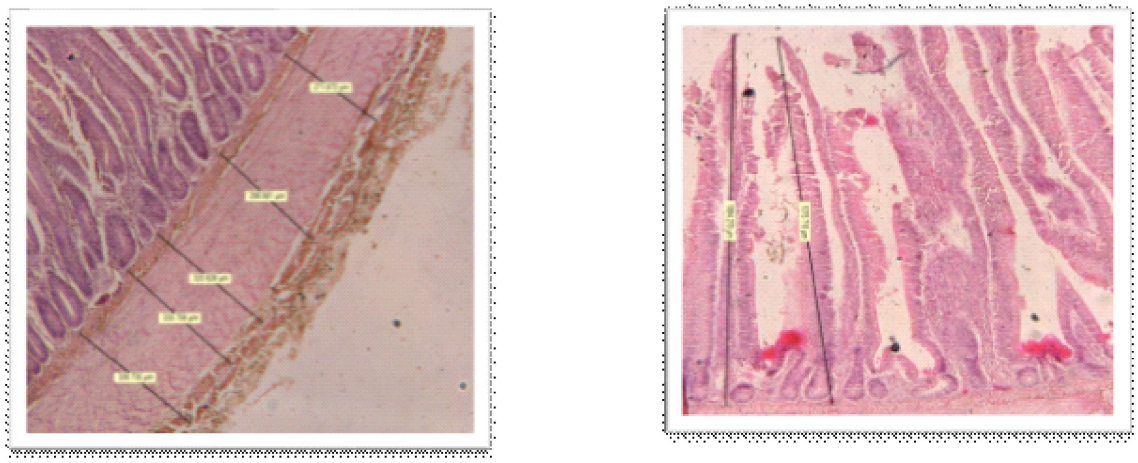
The significant effect on villus height in both the duodenum and ileo-jejunum sections suggests that nucleotide supplementation, particularly at the concentration of 5mg/L, positively influences the structural aspects of the small intestine in broilers. This may enhance nutrient absorption efficiency, highlighting the potential benefits of nucleotide supplementation in poultry diets.

Improved villus height was recorded with nucleotide supplementation, aiding in enhanced nutrient absorption (Daneshmand et al., 2017; Lin et al., 2022). *Saccharomyces cerevisiae* supplementation also improved villus height and the villus height to crypt depth ratio (Lin et al., 2022).

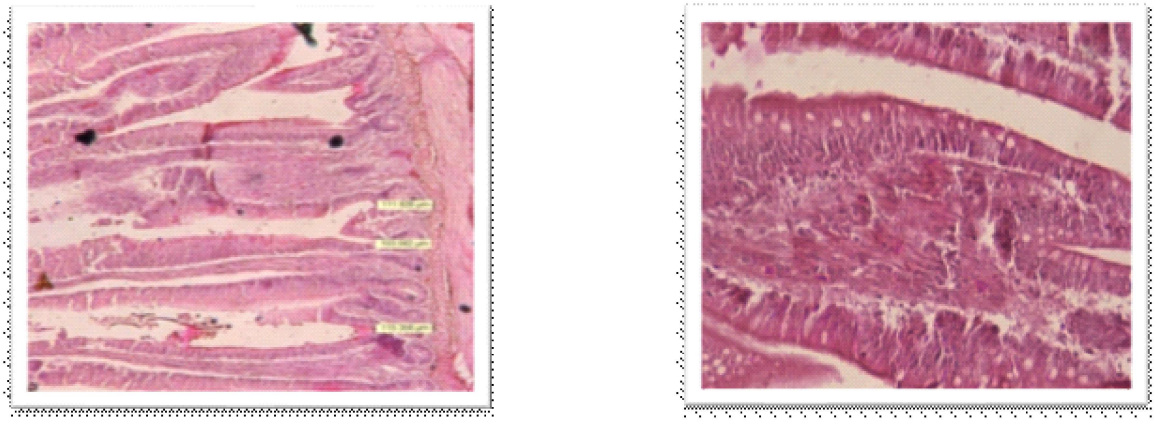
Table 2. Microscopic histological parameters in different segment of small intestine in different treatment groups

Treatment	Con	ConA	N5	N10	SEM	*p-value*
Intestinal wall thickness (µm)						
Duodenum	355.93	354.93	351.48	352.27	11.04	0.99
Ileo-jejunum	330.23	328.92	325.22	325.99	7.20	0.99
Villous height (µm)						
Duodenum	1434.92^{b}	1439.32^{b}	1545.92^{a}	1448.31^{b}	19.10	0.05
Ileo-jejunum	1122.93^{b}	1131.44^{b}	1217.27^{a}	1163.96^{ab}	14.70	0.04
Cyrpt depth (µm)						
Duodenum	115.09	115.38	117.13	116.14	5.18	0.99
Ileo-jejunum	109.92	109.54	110.53	111.36	5.39	1.00
Villous height/Cyrpt depth ratio						
Duodenum	12.82	12.66	13.33	12.65	0.51	0.97
Ileo-jejunum	10.30	10.39	11.39	10.73	0.55	0.92
Goblet cells per field						
Duodenum	9.51	9.62	9.96	9.67	0.21	0.92
Ileo-jejunum	9.50	9.53	9.69	9.62	0.15	0.98

Means of row with different superscript showing significant difference ($p<0.05$)



A B

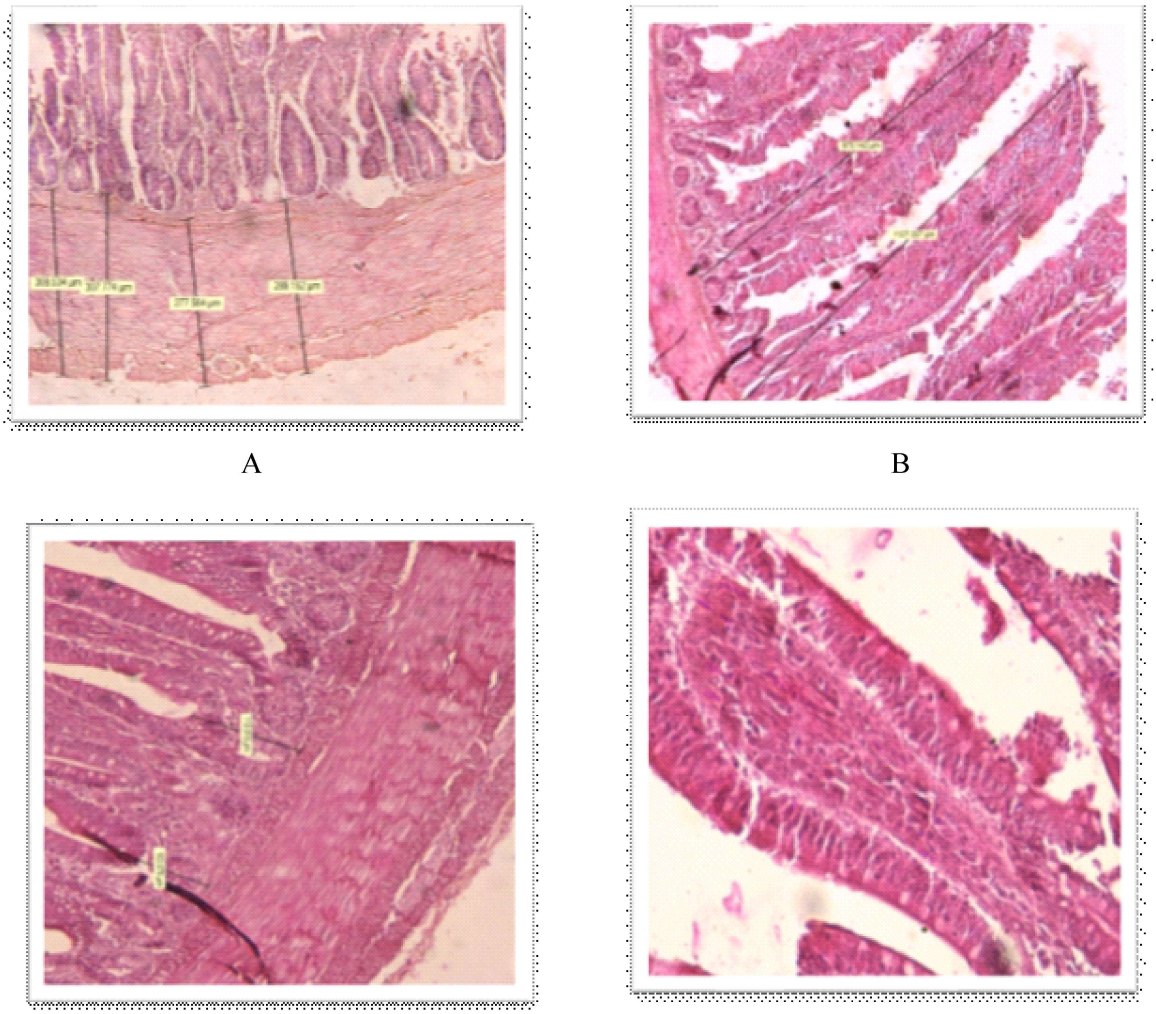


C D

Fig.1. Photomicrograph of duodenum

(A) Intestinal wall thickness, H&E×500 (B) Villus height, H&E×500

(C) Crypt depth, H&E×500 (D) Goblet cells per field, H&E×200



A B

C D

Fig.2. Photomicrograph of ileo-jejunum showing

(A) Intestinal wall thickness, H&E×500 (B) Villus height, H&E×500

(C) Crypt depth, H&E×500, (D) Goblet cells per field, H&E×200

The results presented in Table 3 concerning carcass yield, expressed as a percentage of live body weight, indicate that the eviscerated weight of broilers was significantly affected when fed diets supplemented with nucleotides. However, no significant differences were observed in dressing yield and drawn weight among the various treatment groups. Specifically, the eviscerated weight, expressed as a percentage of live weight, was significantly higher in broilers receiving nucleotide at a concentration of 5mg/L in drinking water. Broilers fed diets ConA and N10 were statistically comparable to those on the N5 diet. These findings suggest that nucleotide supplementation, particularly at the 5mg/L level, significantly impacts the eviscerated weight of broilers, thereby influencing the overall carcass yield in terms of live weight. The absence of significant differences in dressing yield and drawn weight indicates that these specific parameters were not notably affected by the nucleotide supplementation at the studied concentrations.

Table 3.Carcass yields (% of live weight) and serum biochemical parameters of broilers in different treatment groups

	Treatments					
Parameters	Con	ConA	N5	N10	SEM	*p-value*
Carcass weights (% of live weight)						
Dressed weight	77.89	80.21	80.48	79.35	0.48	0.23
Eviscerated weight	67.99^{b}	70.06^{ab}	71.05^{a}	69.26^{ab}	0.49	0.13
Drawn weight	76.55	75.99	77.52	75.60	0.43	0.48
Organ weights (% of dressed weight)						
Heart	0.62	0.57	0.65	0.62	0.02	0.54
Liver	2.00	2.26	2.31	2.28	0.16	0.92
Gizzard	2.31	2.05	2.35	2.34	0.08	0.61
Pancreas	0.22	0.23	0.25	0.24	0.01	0.57
Giblet	7.88	7.39	8.03	7.99	0.23	0.79
Processing losses (% of live weight)						
Blood	2.90	2.77	2.28	2.19	0.14	0.20
Feather	4.18	3.50	4.58	5.26	0.30	0.23
Head	2.14	2.09	2.32	2.10	0.04	0.26
Appendages	4.38	3.94	3.72	3.84	0.21	0.77
Separated fat	1.40	0.90	1.12	1.01	0.09	0.24
Serum biochemical parameters						
Total protein (g/dl)	2.69^{c}	2.72^{bc}	3.06^{a}	2.84^{b}	0.03	0.00
Albumin (g/dl)	1.26^{c}	1.27^{bc}	1.43^{a}	1.33^{b}	0.01	0.00
Globulin(g/dl)	1.43^{c}	1.45^{bc}	1.63^{a}	1.51^{b}	0.01	0.00
Total triglycerides (mg/dl)	49.51	48.95	46.97	48.53	0.43	0.19
Total cholesterol (mg/dl)	158.06^{a}	155.64^{a}	117.64^{b}	126.62^{b}	5.00	0.00
HDL (mg/dl)	88.84^{b}	94.17^{ab}	99.20^{a}	94.53^{ab}	1.26	0.02
LDL (mg/dl)	51.28^{a}	50.62^{a}	45.42^{b}	49.42^{a}	0.61	0.00
ALT/SGPT (U/L)	19.46	19.45	17.96	18.60	0.39	0.49
AST/SGOT (U/L)	167.43	165.02	163.17	162.95	1.18	0.52

Means of row with different superscript showing significant difference ($p<0.05$)

Organs weights

The findings of the current study indicate that birds supplemented with varying levels of nucleotide did not exhibit any significant effects compared to those fed the basal diet. This suggests that the supplementation of nucleotide at different levels did not lead to statistically significant differences in the measured parameters within the study, as detailed in Table 3.

The relative weights of organs, including the liver, gizzard, and abdominal fat, did not exhibit significant changes with nucleotide supplementation (Sampath et al., 2021; Sheik et al., 2021; Ciza et al., 2019; Pelicia et al., 2010).

Processing losses

The findings of the current study, as shown in Table 3, indicate that diets supplemented with nucleotide at 5mg/L and 10mg/L had no significant effect on processing losses. Specifically, factors such as blood, feathers, head, appendages, and separated fat, expressed as a percentage of live weight, did not show statistically significant differences among the various groups. This suggests that the supplementation of nucleotide at these specific concentrations did not lead to notable variations in the measured processing losses compared to the control or other treatment groups in the study.

Blood biochemical indices

The results presented in Table 3 indicate that dietary supplementation of nucleotides, particularly in the N5 group, has a significant effect on certain serum parameters. Specifically, compared to the control group (Con), the N5 group showed a significant decrease in serum concentrations of total cholesterol and LDL cholesterol. Additionally, serum concentrations of total proteins, albumin, and globulin significantly increased in the N5 group ($p<0.05$).

However, no significant effect was observed in total triglyceride levels, nor in the values of SGOT/AST (aspartate aminotransferase) and SGPT/ALT (alanine aminotransferase) across the dietary supplementation of nucleotides among the different groups.

These findings suggest that dietary supplementation with nucleotides, particularly at the specified concentration, may have a favorable impact on lipid profiles by decreasing total cholesterol and LDL cholesterol. Additionally, the increase in total proteins, albumin, and globulin indicates potential positive effects on the overall protein profile in the N5 group. The lack of significant effects on triglyceride levels and liver enzymes (SGOT/AST and SGPT/ALT) suggests that nucleotide supplementation may not have a notable impact on these parameters in the context of this study.

Nucleotide supplementation influenced HDL cholesterol levels (Daneshmand et al., 2017). Monosodium glutamate supplementation significantly impacted total protein, albumin, globulin, total cholesterol, HDL cholesterol, and LDL cholesterol levels (Ciza et al., 2019). Proximate analysis of breast meat did not reveal significant effects from nucleotide supplementation (Majdeddin et al., 2018; ChioFalo et al., 2011). Studies comparing nucleotide supplementation with other additives (e.g., dry yeast) demonstrated significant improvements (Abd El Latif, 2022; Sampath et al., 2021). Proposed mechanisms include altered metabolism, increased digestive enzyme activity, and balanced microbial populations.

CONCLUSION

The conclusion drawn from the current experiment indicates that nucleotide supplementation had a significant positive impact on various aspects of broiler performance and physiology. Nucleotide supplementation led to a notable improvement in growth performance parameters, including body weight, weight gain, feed intake, feed-to-gain ratio, and production efficiency factor. It positively influenced nutrient utilization, Immune function and carcass yield. The cumulative findings indicate that nucleotide supplementation, of 5mg/L can exert significant growth-promoting effects on commercial broilers.

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Effect of Supplementing Feed Emulsifier on the Digestibility of Energy and Performance of Broiler Chicken Fed Sub-Optimal Levels of Metabolizable Energy

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ABSTRACT

Feed emulsifier (FE) improves the digestibility of the feed, thereby reducing dietary nutrient requirements. An experiment was conducted to study the effect of supplementing graded concentrations of FE on the growth performance, carcass traits, and ileal digestibility of energy in broiler chicken (1- 42d of age) fed sub-optimal levels (less 100 kcal/kg) of metabolizable energy (ME). Broiler male chicks (n=1250; Cobb-430) at day old were randomly allotted to five dietary groups, each with 10 replicates having 25 broiler chicks in each pen. Maize-soybean meal-based diet with the recommended concentrations of nutrients (control diet, CD) was prepared. A basal diet (BD) with sub-optimal concentrations of ME was prepared. A commercial FE (combination of equal concentration of 5% lyso-phosphatidyl choline and phosphatidylcholine) was supplemented to the BD at four graded concentrations (0, 100, 200, and 300g/ton). All the diets were fed *ad libitum* from day 1 to 42d of age. The performance data indicated a trend of linear (P =0.056) increase in feed efficiency with the dose of FE. Contrast analysis revealed that the FE at the highest concentration (300g/ton) was comparable to that of the control group. The breast meat weight was reduced in groups fed the BD. The ileal digestibility of energy improved with the emulsifier supplementation (100 or 200g/ton) compared to those fed the CD. Based on the results, it is concluded that supplementation of the feed emulsifier (300g/ton) could reduce the dietary requirement of ME (by 100 kcal/kg) in the broiler chicken diet without affecting feed efficiency and carcass traits. The beneficial effects of feed emulsifier may partly be associated with the increased ileal digestibility of dietary energy.

KEYWORDS: Broiler chicken, Carcass parameters, Emulsifier, Energy digestibility Performance.

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INTRODUCTION

Broiler production is one of the most dynamic and fastest-growing segments in the animal husbandry sector. The broiler industry has taken a quantum leap in growth to meet the dietary protein requirement of the growing population. Fast-growing modern broilers need high-density diets to achieve the expected body weight as per their genetic potential. Modern broilers require high-density diets, and to meet their energy requirements, fats/ oils and concentrated sources of energy are included in the diet (Guerreiro Neto et al., 2011). Digestion and assimilation of dietary fat are limited in young chicks due to limitations in the production of bile and lipase, which are not adequate for assimilating dietary fats (Ghazalah, 2019; Siyal et al., 2017). However, supplementation of oil in poultry diets also adds cost to broiler production. Minimizing feed costs in poultry production requires new research on strategies to reduce the ME requirement without compromising the performance and health of the birds (Brickett et al., 2007).

Supplementation of exogenous feed emulsifiers has been suggested to overcome the physiological limitations of the digestive system during the early age of chicken and reduce the fat globule size into smaller micelles, thereby increasing the surface area for enzymatic action and digestion of fat and other associated nutrients (Gholami et al., 2024; Jala et al., 2024). The beneficial effect of emulsifiers in poultry has been attributed to their ability to improve dietary ME and enhance lipid metabolism (Wang et al., 2020). Emulsifier supplementation in broiler chicken diet reported to increase digestibility of

energy and other nutrients (Ahmadi-Sefat et al., 2022). The improved energy digestibility with the feed emulsifier may allow for the reduction of energy requirement in the diet while maintaining the same level of performance (Khonyoung et al., 2015; Majdolhosseini et al., 2019). It is also reported that the effect of feed emulsifier on performance and nutrient digestibility was reported to be higher in diets containing lower concentrations of energy and other nutrients (Ahmadi-Sefat et al., 2022). Therefore, supplementation of feed emulsifier was reported to sustain the performance of birds fed sub-optimal concentrations of energy (Majdolhosseini et al., 2019; Haetinger et al., 2021; Nemati et al., 2021). Hence, the present study was conducted to evaluate the effect of supplementing graded concentrations of emulsifiers on the energy digestibility and performance of broiler chickens fed low-energy diets.

MATERIALS AND METHODS

Birds, experimental groups, and management

Bird handling, management, and the experimental protocol were approved by the Institute's Animal Ethics Committee (Indian Council of Agricultural Research-Directorate of Poultry Research, India (IAEC/DPR/18/10 dated 29th September 2018/ CPCSEA). Day-old broiler (*Cobb* 430, Venkateswara Hatcheries, Pvt. Ltd, Hyderabad) male chicks (n=1250) were procured and randomly distributed into five treatment groups. Each treatment was allotted to 10 replicates with 25 chicks in each replicate. Birds were maintained in floor pens in an open-sided house with a floor space of 1.1ft^2 per bird. The house's recorded maximum and minimum temperature, humidity, and temperature humidity index were 23.8-28.9^{o}C, 35.2-38.6^{o}C, and 62.8-76.4%, respectively. Brooding was provided with incandescent bulbs and additional heat was provided with coal to provide about 37^{o}C at bird level during the first week. Subsequently, the temperature was gradually reduced to the ambient temperature at day 21, after which the birds were reared at the ambient temperature.

Feeding regimen and performance

A three-phase feeding regime (pre-starter: 1 to 14 days, starter: 15-28 days, and finisher: 29 to 42 days) was followed. Maize-soybean meal-based control diets (CD) were provided *ad libitum* from 1-42 days. Metabolizable energy (ME) and crude protein levels in pre-starter, starter, and finisher diets were 2950, 3050, and 3150 kcal/kg and 22.5, 21.5, and 19%, (Cobb 400 Management Guide) respectively (Table 1). Another set of low ME (-0.42 MJ/kg) basal diets (BD) was prepared with similar concentrations of protein and essential amino acids. The concentrations of essential amino acids, calcium, and available phosphorus were maintained uniformly in all the diets in each phase. The levels of maize and soybean meal were altered to arrive at the desired concentrations of nutrients in the BD. The feed emulsifier was procured from a commercial source (Varsha Group, Bangalore, Karnataka) containing a mixture of lysophosphatidyl choline (5%) and phosphatidyl choline (5%). The BD was supplemented with feed emulsifier at graded concentrations, i.e., 0, 100, 200, and 300 g/ton. Each test diet, along with the CD, was randomly assigned to 10 replicate pens (201×122 cm) and fed *ad libitum* from day 1 to 42. Feed intake (FI) and body weight (BW) were recorded per pen at the end of each phase, and feed efficiency (FE) was calculated as body weight gain (BWG)/FI. Immunization against Newcastle disease (ND) with live attenuated strain (Lasota, 7 and 28 days), and infectious bursal disease (IBD) with live attenuated strain (intermediate) was carried out at 14 days of age.

Table 1. Ingredient and nutrient composition of diets (g/kg) fed to broilers (0-42 days)

Ingredient	Pre-starter (1-14d)		Stater (15-28d)		Finisher (29-42d)	
	CD	BD	CD	BD	CD	BD
Maize	556.11	493.00	603.23	539.83	631.53	568.34
Oil-Veg	23.75	23.70	31.19	31.20	42.13	42.10
Soya DOC 45%	384.50	373.51	331.67	320.66	293.23	282.23
Salt	4.200	4.146	4.215	4.161	4.227	4.173
Dicalcium Phosphate	11.30	11.00	7.17	6.92	6.53	6.28
Phytase 5000	0.100	0.100	0.100	0.100	0.100	0.100
Limestone powder	12.84	12.91	15.73	15.74	15.84	15.84
DL-Methionine	2.591	2.447	2.391	2.246	2.180	2.035
L-Lysine HCl	1.769	1.446	1.471	1.146	1.391	1.067
Premix[#]	2.85	2.85	2.85	2.85	2.85	2.85
Deoiled Rice Bran	0.00	74.89	0.00	75.15	0.00	74.98
Nutrient (g/kg)						
Metabolizable energy, kcal/kg	2950	2850	3050	2950	3150	3050
Crude protein, g/kg	225.0	225.0	205.0	205.0	190.0	190.0
Crude protein, g/kg[##]	221.5	219.8	198.5	199.1	188.8	189.2
Dig. Lysine, g/kg	12.50	12.50	11.00	11.00	10.00	10.00
Dig. Methionine, g/kg	5.70	5.70	5.28	5.28	4.90	4.90
Dig. Arginine, g/kg	13.82	14.02	12.39	12.59	11.33	11.53
Dig. Tryptophan, g/kg	2.56	2.59	2.28	2.31	2.07	2.10
Dig. Threonine, g/kg	7.80	7.94	7.12	7.26	6.61	6.75
Calcium, g/kg	9.40	9.40	9.00	9.00	8.80	8.80
Calcium, g/kg[##]	9.56	9.61	9.05	9.12	8.95	8.86
Available Phosphorus, g/kg	4.50	4.50	4.00	4.00	3.80	3.80
Total phosphorus, g/kg[##]	7.21	7.26	6.52	6.67	6.32	6.18
Sodium, g/kg	1.80	1.80	1.80	1.80	1.80	1.80

CD control diet; BD low energy basal diet

[#] retinol acetate 2.75 mg, cholecalciferol 0.03 mg, á tocopherol 10 mg, thiamin 1 mg, pyridoxine 2 mg, cyanocobalamine 0.01 mg, niacin 15 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, iron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg monensin sodium 50 mg and hydrated sodium calcium aluminosilicate 800 mg

[##] analysed values

Carcass traits

The carcass traits, including ready-to-cook yield (RTC) and relative weights of breast meat, liver, and abdominal fat, were recorded by slaughtering two birds per replicate at day 42. The weights of the carcass variables were expressed as g per kg pre-slaughter live weight of the respective bird.

Ileal digestibility of energy

The apparent ileal digestibility of energy was studied by using Cr_2O_3 as an indigestible marker (0.3% in the diet) (Ravindran et al., 2005). The indicator-supplemented diets were fed *ad libitum* to all the birds in each pen from day 36 to 42. On day 42, five birds in each pen were slaughtered by cervical dislocation after exposure to chloroform for the collection of ileal digesta. The total digesta from the entire ileum, i.e., segment of the small intestine from Meckel's diverticulum to the ileocecal junction, was collected. The digesta was rinsed out of each segment (without squeezing) with demineralized water (4°C) into separate stainless-steel containers. The digesta was pooled per pen and stored at -20°C. Subsequently, the digesta was thawed to room temperature and dried at 70 °C in a forced air oven for 48 hours till the moisture concentration reached about 10% and ground to pass through a 1 mm screen (Cyclotec™ 1093 Sample Mill, FOSS Analytical, Hilleroed, Denmark). Experimental feeds and ileal digesta samples were analysed for gross energy and Cr_2O_3. The gross energy was estimated with an

Adiabatic Bomb Calorimeter (S No 219, CAT. No – CC.01/M3, Toshniwal Brothers (Delhi) Pvt. Ltd, New Delhi, India) using benzoic acid for estimation of energy equivalence of the bomb. The nitrogen (crude protein) content was estimated with Kjeldahl method and Cr_2O_3 content was determined by wet destruction with a mixture of $HNO_3/HClO_4$ (1:1). The absorption of the hexavalent Cr atom, measured at a wavelength of 357.8 nm, is proportional to the Cr_2O_3 concentration in the sample. The following formula was used to calculate the digestibility of energy and nitrogen in different groups.

AIDE (%) = 100-[100 X (Cr_2O_3 diet/ Cr_2O_3digesta) x (Nutrient in digesta/Nutrient in diet)]

Statistical analysis

The data on the effect of emulsifier supplementation on performance and slaughter variables were analysed using a one-way analysis of variance using the GLM procedures available in SPSS (2008). Orthogonal polynomial contrast was performed to study the linear and quadratic effects of emulsifier dose. A simple contrast analysis was performed to compare individual groups with the control and basal diets. For all analyses, the difference between treatment means was considered to be significant when $P<0.05$, whereas a trend was considered to exist if 0.05 £ P £ 0.10. Further, the response in the dependent variables with change in the concentration of feed emulsifier concentration in BD fed groups was fitted by the polynomial equation in the form of $Y=a+bx+cx^2$ to know the trend in the dependent variable in relation to the emulsifier concentration.

RESULTS AND DISCUSSION

Performance

The regression analysis indicated that the performance variables (BWG and FI) were not affected ($P>0.05$) by supplementation of the feed emulsifier to the BD (low ME diets, Table 2) during different phases or overall experiment (1-42d). The FE during 1-42d of age showed a trend of linearity ($P = 0.056$) with the dose of the emulsifier in the BD. The contrast analysis indicated a significant reduction in BWG and FE during the pre-starter phase with a reduction in dietary ME (BD) compared to those fed the CD. However, supplementation of the emulsifier to the BD failed to exhibit any response in the performance parameters during the pre-starter phase compared to those fed the BD (2 vs 3, 4 or 5). The reduction of ME or supplementation of the emulsifier to the low-ME BD did not show any effect on BWG or FE in broilers during the starter or finisher phases compared to those fed the CD. Similarly, during the overall experiment period, the BWG was not affected by dietary treatments compared to the control group. The FE during 1-42d of age reduced significantly in broilers fed the BD compared to those fed the CD (1 vs 2). Supplementation of emulsifier at 100 or 200g showed a trend of improvement ($P = 0.077$ and 0.062, respectively) in FE (1 vs 3 and 4), the FE in groups fed 300g emulsifier was significantly higher than those fed the BD (2 vs 5) and the FE in the later groups was similar to those fed the CD which had the standard energy levels.

Table 2. Effect of supplementing graded concentrations of feed emulsifier (EMR) on the performance of broilers fed standard and low ME diets

Treat	Pre-starter (1-14d)		Starter (15-28d)		Finisher (29-42d)		Overall (1-42d)	
	BWG	FE	BWG	FE	BWG	FE	BWG	FE
	g	FI/BWG	g	FI/BWG	g	FI/BWG	g	FI/BWG
1. CD	480.3	1.108	1201	1.546	1106	2.080	2787	1.680
2. BD	461.2	1.147	1177	1.557	1090	2.124	2728	1.713
3. BD+100 EMR	462.4	1.140	1184	1.550	1104	2.100	2751	1.701
4. BD+200 EMR	458.8	1.153	1187	1.549	1101	2.100	2746	1.702
5. BD+300 EMR	459.5	1.150	1190	1.543	1109	2.068	2759	1.688
SEM	4.316	0.0033	11.25	0.0029	23.25	0.0062	26.45	0.0091
P-value								
Linear	0.673	0.273	0.394	0.105	0.582	0.133	0.458	0.056
Quadratic	0.914	0.529	0.689	0.272	0.854	0.325	0.749	0.163
Simple contrast								
1 vs 2	0.003	0.001	0.142	0.249	0.616	0.264	0.119	0.007
1 vs 3	0.005	0.001	0.308	0.655	0.945	0.608	0.337	0.077
1 vs 4	0.001	0.001	0.384	0.760	0.858	0.622	0.276	0.062
1 vs 5	0.001	0.001	0.517	0.768	0.938	0.760	0.455	0.525
2 vs 3	0.845	0.314	0.647	0.423	0.655	0.506	0.550	0.342
2 vs 4	0.727	0.284	0.545	0.338	0.738	0.492	0.637	0.392
2 vs 5	0.800	0.624	0.409	0.107	0.547	0.125	0.421	0.046

CD control diets having the recommended levels of energy; BD low energy basal diet having -0.42 MJ/kg; BWG body weight gain; FE feed efficiency; SEM standard error mean; N number of replications; P probability

*g emulsifier/ton diet in pre-starter, starter and finisher phases, respectively

The data of the current study suggest that the reduction of dietary ME (100 kcal/kg) reduced the broiler performance (feed efficiency) compared to those fed the energy-adequate CD. Supplementation of feed emulsifier at the rate of 300g/ton sustained the performance of broilers fed the low-ME basal diet similar to those fed the CD. In line with the current findings, a few authors (Bontempo et al., 2018; Kulkarni et al., 2019) and our recent study (Rama Rao et al., 2023) reported significant improvement in BWG, FE, and performance index in broilers fed diets supplemented with feed emulsifier. The improved broiler performance with emulsifier supplementation was attributed to the increased FI and fat assimilation (Kamran et al., 2020; Haetinger et al., 2021). The ileal digestibility data from the current study and our previous findings (Rama Rao et al., 2023) also indicate that feed emulsifiers significantly improve the ileal digestibility of energy and/or N compared to those fed the NC. Increased digestion and absorption of nutrients (fat and energy) with emulsifier supplementation were also reported in the literature (Zhang et al., 2011; Maertens et al. 2015; Ji Seon An et al., 2020; Rama Rao et al., 2023). Significant reduction in the gut viscosity with emulsifier supplementation (Jalal et al., 2024; Gholami et al., 2024), might also be another mode of action of emulsifier to improve bird performance. The data of the current study also further suggested the possibility of maintaining the broiler performance on diets containing reduced dietary ME with feed emulsifier supplementation at par with the performance of broilers fed the energy-adequate CD.

Though the protein digestibility was not estimated in the current study, the published literature indicated a significant improvement in protein and lipid digestibility in broilers fed diets supplemented with feed emulsifier (Saleh et al., 2020; Haetinger et al., 2021). The improvement in nutrient digestibility might be due to an increase in intestinal villi development (Brautigan et al., 2017; Chen et al., 2019; Gazhalah et al., 2021), besides a reduction in fat globular size (San Tan et al., 2016) with emulsifier supplementation.

Contrary to the present findings, Azman and Ciftci (2004) reported a lack of significant improvement in the performance of broilers fed diets supplemented with feed emulsifiers. Soya lecithin was used as a substitute for soybean oil by Azman and Ciftci (2004), who found similar weight gain in broilers compared to that of soybean oils without influencing fat assimilation. The feed emulsifier used in the current study had lysophosphatidylcholine and phosphatidylcholine, which are potent emulsifiers capable of increasing the digestibility of saturated fatty acids. The improved performance observed in the current study could be attributed to the emulsification potential of these two compounds. Results of Gazhalah et al. (2021) demonstrated that the combination of lysolecithin, synthetic emulsifier, and monoglycerides significantly improved the BWG and FE in broiler chicken compared to those fed diets without emulsifier. It is worth noting that the improved ileal digestibility of energy (probably due to fat hydrolysis) was observed in the current study with emulsifier supplementation, demonstrating the probable reason for the improved performance in broilers fed low-ME diets with emulsifier supplementation.

The contrast analysis demonstrated that the performance of broilers fed 300 g/ton emulsifier was significantly higher than that of those fed the low-energy diet, and the FE in 300 g/ton group was similar to that of those fed the CD diet, which implies that dietary supplementation of emulsifiers could reduce the dietary requirement of ME. The FE in groups fed the lower concentrations of emulsifier (100 and 200g/ton) was intermediate. Similarly, Chen et al. (2019) also reported that 250g emulsifier (lysolecithin)/kg diet was optimum for growth performance, and they concluded that the supplementation dose should be recommended according to dietary energy levels. At the reduced dietary ME level (-.42 MJ/kg), a concentration of 300 g/ton diet gave similar performance compared to those fed the CD, which received the recommended levels of energy. Similar to the current findings, few recent studies (Ahmadi-Sefat et al., 2022; Gholami et al., 2024) reported the possibility of reducing dietary energy up to 0.42 MJ/kg diet with the feed emulsifier supplementation without affecting the broiler performance.

Carcass traits

The relative weight of the gizzard was not affected ($P>0.05$) with the emulsifier supplementation to the BD (Table 3). However, supplementation of emulsifier to the BD non-linearly ($P<0.05$) increased the RTC yield, and linearly increased the breast meat weight (BE), and reduced ($P<0.05$) the abdominal fat and liver weight. The contrast analysis indicated that most carcass

variables (except BM) were unaffected by the reduction in dietary ME (0.42 MJ) compared to those fed the CD. The BM weight reduced significantly ($P<0.05$) in broilers fed the BD compared to those fed the CD. Similarly, supplementation of the emulsifier did not affect the carcass variables, except for abdominal fat. The abdominal fat was significantly reduced with the emulsifier at 300g/ton compared to those fed the CD. The contrast analysis also indicated that the emulsifier supplementation to the BD at 200g and 300g/ton, respectively, increased RTC weight and reduced abdominal fat weight.

Table 3. Effect of supplementing graded concentrations of feed emulsifier (EMR) on slaughter variables (g/kg live weight) and energy retention coefficient in broilers fed standard and low ME diets

Treat	RTC	BM	Abd fat	Liver	Gizzard	Energy digestibility coefficient
1. CD	801.0	273.9	10.72	17.27	14.46	0.723
2. BD	783.6	253.7	11.57	17.66	15.92	0.715
3. BD+100 EMR	780.5	268.4	9.71	18.62	15.21	0.773
4. BD+200 EMR	796.2	264.4	10.95	18.26	15.21	0.774
5. BD+300 EMR	790.7	271.9	7.50	16.58	15.41	0.757
SEM	5.179	5.518	0.772	0.722	0.641	1.342
P-value						
Linear	0.591	0.042	0.007	0.249	0.603	0.069
Quadratic	0.011	0.056	0.018	0.018	0.684	0.185
Simple contrast						
1 vs 2	0.337	0.015	0.435	0.704	0.114	0.946
1 vs 3	0.165	0.800	0.364	0.195	0.413	0.034
1 vs 4	0.457	0.229	0.834	0.338	0.413	0.036
1 vs 5	0.168	0.479	0.005	0.499	0.298	0.061
2 vs 3	0.105	0.646	0.120	0.325	0.451	0.027
2 vs 4	0.027	0.615	0.594	0.536	0.451	0.021
2 vs 5	0.681	0.067	0.001	0.262	0.591	0.013

CD control diets having the recommended levels of energy; BD low energy basal diet having -0.42 MJ/kg; RTC ready to cook yield; BM breast meat; AF Abdominal fat; SEM standard error mean; N number of replications; P probability
*g emulsifier/Ton diet in pre-starter, starter, and finisher phases, respectively

Energy digestibility

The regression analysis indicated that there was no significant ($P>0.05$) effect of emulsifier supplementation to the BD on energy digestibility compared to those fed the BD without the emulsifier (Table 3). The contrast analysis between CD and BD indicated no significant effect of dietary ME on the ileal digestibility of energy. However, supplementation of the emulsifier at 100 or 200g/ton significantly improved energy digestibility compared to BD or CD.

In the present study, the relative weight of BM reduced significantly with a reduction in dietary ME. Supplementation of the feed emulsifier improved the breast meat yield similar to those fed the CD. The improvement in meat yields and FE with the emulsifier supplementation to the low-ME basal diet (LF) could be due to the significant improvement in the ileal energy digestibility with emulsifier supplementation. Significant reduction in fat deposition in the abdominal area in broilers fed the highest concentration of feed emulsifier (300 g/ton) also suggests the higher utilization of dietary fat with emulsifier supplementation. Similar to these findings, significant improvements in the carcass traits were reported in broilers fed emulsifier-supplemented diets (Ghazalah et al., 2021). The improvement in energy digestibility may be the probable reason for the improved meat yields in broiler chicken as reported by Ji Seon An et al. (2020).

CONCLUSIONS

Based on the results, it is concluded that emulsifier supplementation (300g/ton) could sustain the performance and meat yield in broilers fed a low-energy diet (-.42 MJ/kg). The improved performance in broilers fed low-energy diets supplemented with

the feed emulsifier was associated with increased ileal digestibility of energy.

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Optimizing Live feed and Weaning Strategies on Growth and Survival of *Mystus cavasius* Larvae in Captivity

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ABSTRACT

The study describes the performance of *Mystus cavasius* larvae by feeding live feeds and weaning time of larval feed during their rearing. The larvae of three days old were fed *Artemia nauplii*, tubifex worm and mixed plankton in triplicate tanks. Tubifex worms were identified as the most effective live feed among the three live feeds evaluated. It significantly ($P<0.05$) enhanced the larval growth (179 mg) and survival (97%) compared to *Artemia* nauplii and mixed plankton. The yolk sac absorbed larvae of 3 days old were also stocked in 12 tanks and were fed *Artemia naupli*. The live feed was withdrawn at the age of 10, 15 and 20 days and fed compound larval feed to know the best age for feeding the larvae with compound feed. A weaning age of 15 days post-hatching (dph) was optimal, facilitating the transition to formulated feed. It significantly enhanced the survival rate compared to the larvae fed with compound feed at the age of 10 dph. These findings may be a framework during large-scale seed production in hatchery. Hence the present study addresses the feeding management in larval rearing of this nutritionally significant species.

KEY WARDS: Catfish, Larval rearing, Live feed, *Mystus cavasius*

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INTRODUCTION

The drastic reduction of many fish species is encountered from the wild waters due to habitat loss, over fishing, pollution and other anthropogenic activities. The small indigenous fish (SIF) species are more affected (Lorenzen et al., 2016), which provides nutritional security to the rural population (Mohanty et al., 2013). *Mystus cavasius*, a near-threatened small indigenous catfish species is one among them (CAMP, 1998), which needs attention for aquaculture. This bagrid catfish is found in the water bodies of Indian sub-continent (Talwar and Jhingran, 1991; Roy and Hossain, 2006). It's delicious taste, fewer intramuscular bones and high nutritional content makes it popular among the consumers of the region (Mohanty et al., 2013; Banu et al., 2020). It is also valued in the ornamental fish trade due to its shiny coloration (Gupta and Banerjee, 2014).

Seed rearing of different stages is considered important in fish production system apart from captive breeding. Key considerations such as larval husbandry practices (Mollah, 1985; Sahoo et al., 2004; Pangni et al., 2008; Nwipie et al., 2015), larval feeding protocols (Verreth and Van Toneren, 1989; Hasim et al., 1992, Hung et al., 1999; Evangelista et al., 2005) and effective weaning strategies (Hung et al., 2002; Liu et al., 2012; Pradhan et al., 2014; Manya et al., 2018) have been identified as essential for the successful rearing of fish larvae. The initiation of larval rearing with live feed and weaning to compound feed thereafter, is a general protocol in larval rearing practice. The importance of live feed during initial feeding of larvae was also reported (Zheng et al., 2018; Radhakrishnan et al., 2020; Melaku et al., 2024). The successful larval rearing with live feed in many catfishes has been documented (Ronyai and Ruttkay, 1990; Hung et al., 1999; Sahoo et al., 2004; Sahoo et al., 2010), which provides energy for the growth and physiological function (Paliñska-¯arska et al., 2014; Radhakrishnan et al., 2020). Different live feeds are considered during larval rearing due to their nutrient profile, ability to remain alive in the rearing environment and, easy digestion and assimilation by the larvae (Damle and Chari, 2011). The use of live feed and weaning them to feed, play a significant role in determining growth and survival rates of larvae in a seed production system. Given these considerations, the present study evaluates the

suitable live feed and weaning time during larval rearing of *M. cavasius* for the large-scale production of high-quality seeds.

MATERIALS AND METHODS

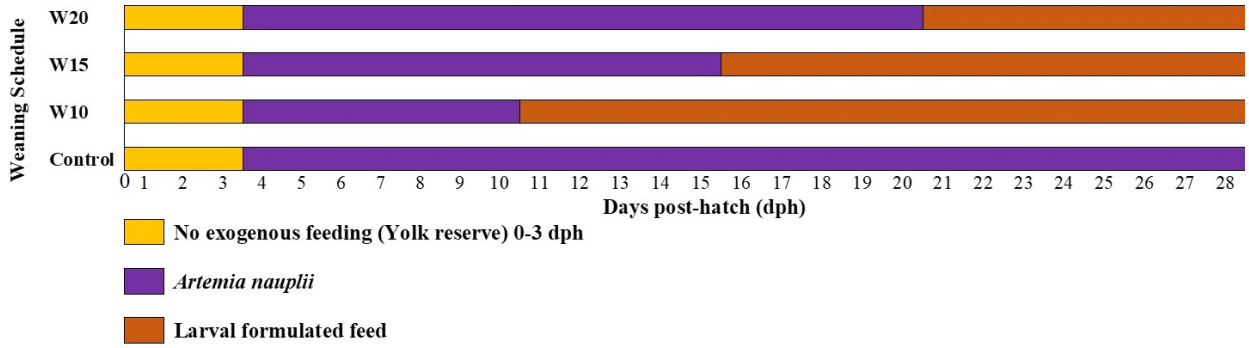
Experiment 1 – Effect of live feed type on growth and survival of *M. cavasius* larvae

The larvae of 3 dph (2.70 mg) were stocked at 5 larvae L^{-1} in triplicate FRP tanks (50 L) to evaluate the growth performance of *M. cavasius* larvae under different live feed based rearing conditions. The study was conducted for 21 days and the *Artemia* nauplii, tubifex and mixed plankton were fed to larvae as per the treatment condition for the entire study period. The sieved mixed zooplankton collected from the nursery pond was fed to larvae, which were dominated by copepods. *Artemia* cysts (OSI red ring, USA) were hatched in salt water (30 ppt). The live tubifex was collected from Ornamental fish unit of the Institute. The collected tubifex were chopped into smaller pieces with the help of scissors before feeding the larvae. All the live feeds were fed *ad libitum* level. The larval tanks were cleaned regularly and 30-40% water exchanged daily and continuous aeration was provided to maintain the water quality to an optimum condition. The larvae in each tank were counted at the end of the experimental period to know the survival rate. Twenty-five fish from each tank were sampled individually to measure the final length and weight through measuring board and electronic balance respectively.

Experiment 2 – Effect of weaning age on growth and survival of M. cavasius larvae

For the weaning experiment, hatchlings of 4 dph (2.85 mg) were used. Healthy larvae were stocked into 12 FRP tanks (50 L; 5 larvae L^{-1}) under a completely randomised design and maintained in four experimental treatment groups in triplicates for a period of four weeks. *M. cavasius* larvae were subjected to four feeding schedules and the weaning was studied at different days of post-hatch such as 0 (C), 10 (W10), 15 (W15) and 20 (W20) dph and feeding of *Artemia* nauplii alone was the control (C) treatment (Fig 1). The experimental tanks were cleaned daily and maintained as described in the previous experiments. After four weeks of experimental study, the larval growth performances and survival rate of different treatment groups were recorded as described in the earlier experiment.

Fig 1. Figure showing the feeding protocol of *M. cavasius* larvae during entire rearing period



Formulation and preparation of larval feed

The experimental feed for *M. cavasius* larvae was formulated (Table 1) and prepared for the weaning study. All the feed ingredients were purchased from the local market at Bhubaneswar, Odisha, India. The feed ingredients were weighed as per the formulation and mixed well with the required volume of water to prepare the feed dough. The dough without the addition of vitamin and mineral mix, fish oil, sunflower oil and carboxy methyl cellulose (CMC) was steam-cooked in a pressure cooker for 25 minutes and cooled at room temperature. The oils, vitamin mineral mixture, CMC were added and mixed uniformly. The feed dough was pelletised through hand pelletiser and the feed pellets were dried at 40 ºC, which were grounded and sieved to obtain particle size of less than 300 microns for feeding *M. cavasius* larvae. Weaning diet were prepared with 30 % inclusion of fish meal and 3% oil sources (fish oil and sunflower oil) and the diet had 37.20 % crude protein and 6.9 % crude lipid. The larvae were fed thrice daily to satiation level in all the experimental groups.

Table 1. Ingredient composition and proximate analyses of the *M. cavasius* larval weaning diet (% of dry matter basis)

Ingredients (%)	Weaning diet
Fish Meal	30.00
Soya flour	30.00
Groundnut oil cake	20.00
Maize	5.00
Wheat flour	6.00
De-oiled rice bran	3.00
Vitamin and Mineral mix	2.00
Fish oil	1.50
Sunflower oil	1.50
CMC binder	1.00
Proximate composition	
Crude protein (% dry matter, DM)	37.20
Crude lipid (% DM)	6.90
Ash (% DM)	5.80
Moisture (%)	8.85

Vitamin and Mineral mix: Each 1kg contains Vitamin A-5000 IU; Vitamin D3-1000 IU; Vitamin B1-10 mg; Vitamin B2-10 mg; Vitamin B6-5 mg; Vitamin B12-15 mcg; Vitamin B3-75 mcg; Vitamin B5-10 mcg; Vitamin C-150 mg; Vitamin E-25 mg; Vitamin H-5mg; Vitamin B9-5mg; Ca-225 mg; Co-20 mg; Mn-60 mg; Fe-30 mg; Cu-2 mg; Zn-2 mg; K-20 mg; Mg-2 mg; Choline Chloride-50 mg.

Analysis of feed proximate composition

The proximate composition of larval diet was analysed (Table 1) according to the standard procedures (AOAC, 1995). Moisture content (%) of the diet was determined by drying the feed in a hot air oven at 105º C overnight. The crude protein was determined by Kjeldahl method (nitrogen×6.25) using the Kjeldahl distillation systems (Vapodest; Gerhardt Analytical System, Germany). The crude lipid content of the diet was estimated by solvent extraction method (SOCS plus, SCS 08 AS, PELICAN Instruments, India). Total ash (TA) content of the diet was calculated by using muffle furnace at 550º C for a period of 6 h.

Statistical analysis

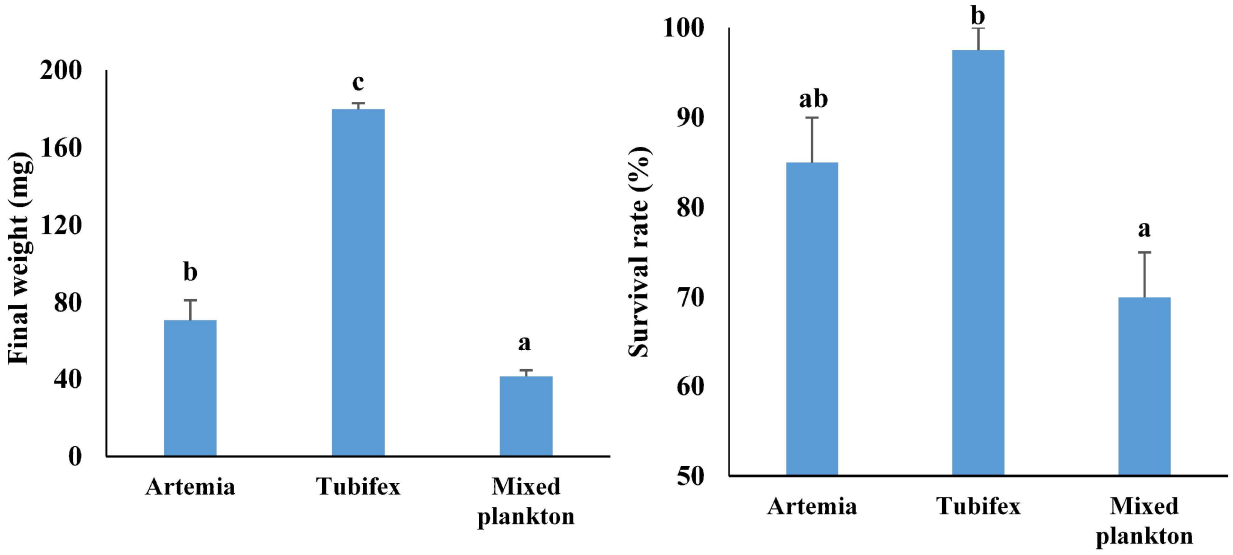
All the data were expressed in mean ± standard error (SE). The data variables were checked for normality using the Kolmogorov-Smirnoff method and tested for homogeneity of variance using Levene's test. The data did not follow a normal distribution and expressed in percentage, were arcsine transformed. Using IBM-SPSS software version 24, the data of different parameters was analysed for any significant difference using one-way analysis of variance (ANOVA) followed by Turkey's HSD test. The values were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Feeding the larvae with a suitable diet is the foremost step for the successful operation of a hatchery. Live feeds are considered most suitable during the initial feeding most of the fish species due to many advantages such as highly digestible, nutritious and will not deteriorate the water quality (Kumar et al., 2022). In the present experiment, significantly ($P<0.05$) higher growth (179 mg) and survival (97%) were observed in *M. cavasius* larvae fed with tubifex compared to *Artemia* and mixed plankton (Figure 2). The growth (70 mg) was reduced significantly in the larvae fed with *Artemia* nauplii, but the survival rate was unaffected. The present result suggests that tubifex is the most suitable live food for *M. cavasius* larvae during initial feeding for enhanced growth, which agrees to the previous works published for other catfish species such as *C. macrocephalus* (Evangelista et al., 2005), *Pangasius bocourti* (Hung et al., 2002) and *Silurus glanis* (Ronyai and Ruttkay, 1990). This is possibly due to the higher fatty acid, amino acid and having more chemoattractant properties in tubifex as also reported earlier (Hashim et al., 1992; Tamaru et al., 1997).

The feeding of larvae with tubifex often reduces the energy cost during feeding, which promotes growth as also reported in *Cyprinus carpio* (James et al., 1993). However, the feeding of *Artemia nauplii* may be the second best option during larval rearing of this catfish if tubifex is not available plenty.

Fig 2. Final body weight and survival rate of *M. cavasius* larvae weaned on different age for 21days rearing period



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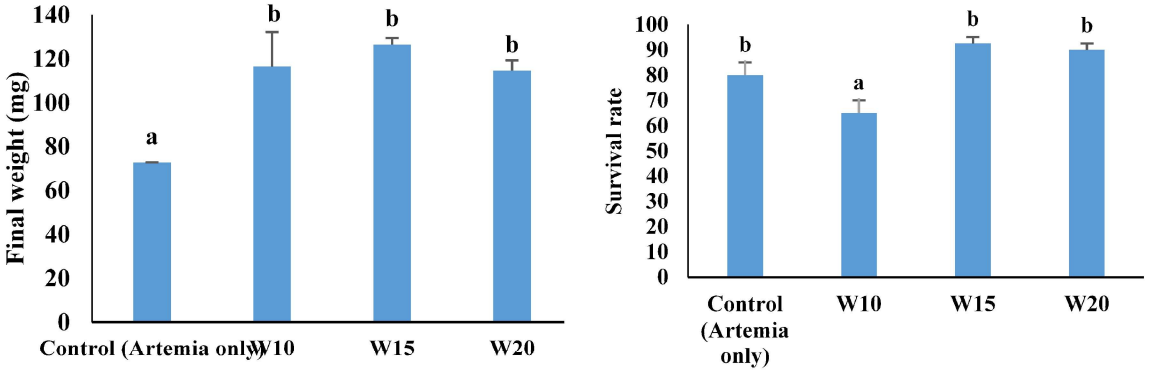
The requirement of live feed in fish larvae culture is unavoidable during initial feeding. Their production and maintenance are often laborious and cost-intensive, accounting for more than 50% of the total operation cost of a hatchery (Drossou et al., 2006). Hence, the larvae must be weaned at the earliest possible age with compound feed for the best growth and survival. In the present study, the final weight and survival rate of *M. cavasius* larvae weaned at different age have shown significant differences among the treatments. The final body weight of larvae was increased significantly in all the treatment groups (10, 15 and 20 dph) than the control (Figure 3). However, the percent survival (65%) was drastically reduced among the larvae weaned at 10 dph, which indicates that the *M. cavasius* larvae require live food until 15 dph. Thereafter the weaning of larvae with formulated feed can be feasible, which resulted higher growth (126 mg) and survival rate (92%). The higher larval mortality was observed during weaning at 10 dph, which might be due to lack of ontogenical development of the digestive system in the larvae. The catfish larvae do not pose a functional stomach and rely solely on intestinal digestion at the initial feeding. Previous studies also reported the early weaning leads to higher mortality of fish larvae due to small mouth size, inadequate development of digestive tract and insufficient production of digestive enzymes (Engrola et al., 2010; Pradhan et al., 2014). The formulated feeds cannot be digested compared to live food organisms due to low moisture concentration (<10%) than in live feed (>90%). It is assumed to be supplying their endogenous digestive enzymes as exo-enzyme to the larvae and facilitate easy digestion in the alkaline pH of the intestine as reported in *Channa striata* (Kumar et al., 2022). The optimal weaning age of different catfishes (Verreth and Van Tongeren, 1989; Fermin and Bolivar, 1996; Hung et al., 2002; Pradhan et al., 2014) and other fishes (Liu et al., 2012; Hien et al., 2017; Minya et al., 2018) varies from few days to weeks. This variation of time in different fishes could be due to the completion of digestive ontogenic development in different times. The present finding indicates that completion of digestive ontogeny and the onset of the functional stomach is relatively leisurely developed in *M. cavasius* larvae compared to other catfish species as mentioned above.

Fig 3. Final body weight and survival rate of *M. cavasius* larvae weaned on different age for 28 days rearing period.



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CONCLUSION

The study reflects a comprehensive protocol for feeding the *M. cavasius*, larvae with live feed and the feasibility of using compound feed during its larval rearing. The feeding of chironomid larvae remains best among the live feeds tried. *Artemia nauplii* may be the other option during larval rearing of this catfish in case of non-availability of chironomid larvae. These larvae may be reared with live feed till 15 days after which they may be shifted to compound feed for higher growth and survival during the large-scale larval rearing of *M. cavasius*.

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Effect of Humic Substance Supplementation on Performance and Gut Health in Broilers

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ABSTRACT

The study was conducted to investigate the effect of dietary supplementation of humic substance (HS) on performance of broilers. 120 day old broiler chicks were randomly distributed into 6 dietary treatments with five replicates of four chicks each. Broiler diets were formulated as per commercial chick feed specification (2011). Antibiotic growth promoters (AGP) group diet was same as CON with addition of AGP whereas, in HS0.1, HS0.2, HS0.3 and HS0.4 replacing antibiotic with HS @ 1, 2, 3 and 4 g/kg feed, respectively. Individual body weight and feed intake were recorded and thus feed to gain ratio and European production efficiency index (EPEI) % was calculated. Birds were sacrificed to estimate carcass traits, intestinal histomorphometry and caecal microbial count. Immunological response and liver function tests (SGOT and SGPT) were estimated. Broiler performance (0-6 weeks) indicate that supplementation of HS0.4 significantly ($p<0.05$) improved body weight. Better feed to gain ratio and EPEI % was observed with HS0.2 compared to CON. Crude protein and crude fat utilization improved significantly ($p<0.05$) in broilers fed with HS0.2 and HS0.4. Supplementation of HS significantly ($p<0.05$) improved intestine villus height (VH), crypt depth (CP) and VH:CD ratio. Caecal *E. coli* count was lowered, humoral immune response, weight of bursa of fabricius, spleen, serum IgG and IL-10 levels were increased in HS supplemented groups compared to CON. HS inclusion had no effect on the carcass traits, nutrient composition of meat and liver function tests. Lower feed cost per kg weight gain and improved economic efficiency was observed in broilers fed on diets supplemented with HS0.2. It can be concluded that supplementation of HS0.2 in feed can be used as an economically efficient alternative to antibiotic growth promoters in broilers diet.

KEYWORDS: Broiler, Histomorphometry, Humic Substance, Nutrient Utilization Performance

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INTRODUCTION

The poultry industry is rapidly expanding, especially in developing countries with both the production and consumption of poultry meat rising steadily. As per the 20th livestock census, the poultry population stands at 851.81 million, reflecting a growth rate of 16.81%. However, despite this growth, the current annual per capita availability of meat in India is only 3.5 kg/year, significantly lower than the recommended 10.5 kg/year suggested by the Indian Council of Medical Research.

Antibiotic growth promoters (AGPs) are widely used in poultry diets to promote growth and control of diseases. However, European Union has prohibited the use of feed grade antibiotic growth promoters due to concerns about potential drug resistance in human pathogens (Anadon, 2006). Consequently, this has led to a need for safe alternative additives to enhance poultry production without relying on antibiotics. The adoption of humic substance (HS) as substitutes for antibiotics in poultry farming has garnered significant attention. Humic substance are natural organic compounds formed from the decomposed plants matter in soil, coal and other sources. These substances have a complex structure and contain humic, humin and fulvic acids (Bezuglova and Klimenko, 2022), and are known for natural growth enhancers, antioxidant, antifungal, detoxifying and antiseptic properties. Their colloidal

nature allows the formation of a protective layer on the intestinal epithelium, reducing pathogen colonization and toxins penetration in intestine (Gonzelez et al., 2018). Additionally, they enhance nutrient utilization and growth performance by promoting villus length and maintaining gut microflora. Furthermore, there buffering capacity also regulate gut pH and inhibit the growth of pathogenic bacteria (Marcincak et al., 2023). Despite their potential benefits, limited reports are available on the supplementation in broiler performance. Therefore, this study aimed to evaluate the effect of dietary supplementation of humic substance on performance, gut health and immune response of broilers.

MATERIALS AND METHODS

The experiment was conducted at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur, India. Study was approved by Institutional Animal Ethical Committee (IAEC) vide D.No.39/IAEC/Vety/2023 dated 01.10.2023. Affiliated with CPCSEA, Ministry of Animal Husbandry and Dairying, India.

Experimental design

A total of 120 day-old broiler chicks were procured from a commercial hatchery in Jabalpur and vaccination were done as per stander protocol/ schedule. (Chicks were vaccinated against Ranikhet disease (F1 strain), infectious bursal disease, and given a booster dose of Ranikhet disease on days 7, 14, and 28, respectively). Experimental diets were formulated as per commercial chick feed specifications (2011). Water were offered *ad-libitum* throughout the experimental period. The experiment was conducted under a completely randomized design with six isonitrogenous and isocaloric dietary treatments, each with five replicates of four chicks: CON (control, VENCOBB 400 specification), AGP (CON + AGP, 0.03%), HS0.1 (CON + 1 g/kg HS), HS0.2 (CON + 2 g/kg HS), HS0.3 (CON + 3 g/kg HS), and HS0.4 (CON + 4 g/kg HS).

Growth performance

Individual live body weight (g) and feed intake of broilers were recorded on weekly basis throughout the experiment. Feed to gain ratio (F:G) was calculated by considering cumulative feed intake and weight gain for each groups. European production efficiency index (EPEI) was calculated as formula given by Hubbard (1999).

Economics of production

Economics of broiler production over feed cost was calculated as per cost of feed consumed and per kg weight gain for each dietary treatment. The price of the diet and HS was calculated according to the local market price.

Nutrient utilization

A 3- day metabolic trial was conducted during 6^{th} week of the experiment and excreta of each replicate were collected in acidic medium quantitatively at every 24 hours. Dry matter (DM), crude protein (CP) and crude fat (CF) of leftover feed and faeces were determined accoding to AOAC methods (2012).

Carcass traits and nutrient composition of meat

Two broilers per replicate were humanely sacrificed on day 35 after a 12-h fasting period to study carcass traits. Dressing yield, eviscerated weight, and drawn weight were recorded. Processing losses (% of live weight; blood, head, feathers, shank, separable fat, and wing tips) were also recorded. Organs (liver, heart, giblet, gizzard, pancreas) were collected replicate-wise, and their weight expressed as % of dressed weight. Breast muscle samples (two per replicate) were analyzed for proximate composition (DM, CP, CF) following AOAC (2012).

Histomorphometrical analysis

Tissue samples (approx.2 cm) from the duodenum, jejunum, and ileum of sacrificed birds were collected and fixed in 10% neutral buffered formalin for 72 h. Samples were processed, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin (Drury and Wallington, 1980). Measurements included intestinal wall thickness, villus height, crypt depth, villus height-to-crypt depth (VH:CD) ratio, and goblet cells per field was measured from middle part of the villi.. Sections were examined using a Leica DM 1000 LED microscope with digital imaging software (LAS v4.10).

Caecal microbial analysis

Caeca from sacrificed birds were aseptically collected, homogenized, and stored at –20°C until analysis. Total *E. coli* and *C. perfringens* counts were determined using standard plate count methods (Markey et al., 2013). Serial dilutions of caecal contents were plated on MacConkey agar for *E.*

coli (aerobic, 37°C, 24 h) and on tryptose-sulfite-cycloserine (TSC) agar with egg yolk emulsion for *C. perfringens* (anaerobic, 37°C, 48 h) in gas-pack anaerobic jars. Colony counts were expressed as log10 cfu/g of caecal digesta.

Immunological response

Lymphoid organs (bursa, spleen, and thymus) were collected and weighed as a percentage of live weight. Blood samples from vaccinated birds were taken on days 30 and 42, and serum was separated and stored at –20°C. Humoral immunity against NDV was assessed by haemagglutination inhibition test (OIE, 2004), with titres expressed as log10 of the reciprocal endpoint dilution. Serum IgG and IL-10 concentrations were measured using chicken-specific ELISA kits (Chongqing Biospes Co., Ltd, China).

Estimation of liver function test

Serum glutamic oxalo-acetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels estimated by (Reitman and Frankel, 1957) spectrophotometrically using commercial diagnostic kits (Erba Diagnostics Ltd., India).

Statistical analysis

Data were analyzed by one-way ANOVA for a completely randomized design (CRD) as per Snedecor and Cochran (1994) using SPSS software (version 22.0). Significant differences among treatment means were compared using Duncan's Multiple Range Test (1955). Results are expressed as pooled standard error of mean (SEM), with significance set at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance, nutrient utilization and economics of production

The effects of supplementing different levels of HS on growth performance, nutrient utilization and economics of production of broilers are shown in Table 1. The current study showed definite trend for increase in live weight of broiler supplemented with humic substance. Maximum and significantly ($p<0.05$) higher live weight was observed in group HS0.4 followed by HS0.2 and HS0.3 compared to the CON. The improved body weight in HS supplemented groups may be due to their effect on improving the intestinal morphometry, which increases intestinal absorption surface available for the nutrients for necessary growth. There are number of studies reporting improvement in growth performance of broilers with HS supplementation in feed (Ghazalah et al., 2022 and Elnaggar and El-Kelawy, 2018) or in drinking water with different levels (Kati, 2018).

There were no significant differences ($p>0.05$) in feed intake among broilers supplemented with different levels of HS. Results of present study are in line with the Arif et al. (2016) and Saleh et al. (2022) who reported no effect of HS supplementation on feed consumption in broilers.

However, significantly ($p<0.05$) lower or better feed to gain ratio was observed in broilers fed with HS0.4 and HS0.2 was best. While the groups supplemented with HS0.1 and HS0.3 were having at par feed to gain ratio with the AGP supplemented group. The European production efficiency index was significantly better ($p<0.05$) in the HS @ 2 g/kg feed group (HS0.2) compared to the control group but with no significant difference ($p>0.05$) between different levels of HS. The improved feed to gain ratio with HS supplementation may be attributed to improved nutrient utilization and body weight gain with similar feed intake. Humic substance stabilizes the intestinal microbiota which enhances nutrient utilization and body weight gain (Lala et al., 2017). Our results were in accordance with Ghazalah et al. (2022) and Arif et al. (2016), who observed improved FCR in humic acid supplemented groups.

Overall, these findings suggest that HS supplementation, particularly at a concentration of 2 g/kg feed, positively influences live weight, feed-to-gain ratio and EPEI in broilers, demonstrating its potential as a beneficial natural growth promoter in poultry diets.

In the present study, significantly ($p<0.05$) higher crude protein and crude fat utilization was observed in broilers fed a basal diet supplemented with HS compared to the control, with maximum values in HS0.2 and HS0.4 groups. However, HS supplementation had no significant effect ($p>0.05$) on dry matter utilization. Improved villus length in HS-supplemented birds likely contributed to better digestibility of crude protein and crude fat/EE. These findings are in agreement with Elnaggar and El-Kelawy (2018) and Ghazalah et al. (2022), who also reported improved apparent digestibility of crude protein and ether extract with humic acid supplementation.

Broilers supplemented with HS0.2 had significantly ($p<0.05$) lower feed cost per kg weight gain and higher economic efficiency compare to the CON and AGP fed group. In present study the reduced cost per kg live weight gain and improved economic efficiency might be due to better feed to gain ratio, higher nutrient utilization and better improved weight gain in broilers fed basal diet with humic substance. These findings are accordance with Hammod et al. (2021) and Omidiwura et al. (2021) who reported that supplementation of humic acid in diet improved economics of broiler production.

Table 1. Growth performance and economics of production in broilers of different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
Growth performance								
Final body weight (g)	2219.37^{d}	2318.85^{cd}	2358.10^{bc}	2469.73^{ab}	2303.57^{cd}	2495.75^{a}	22.99	0.01
Overall feed intake (g)	3779.55	3788.48	3753.73	3730.01	3775.43	3834.37	15.30	0.52
Average F:G	1.74a	1.67^{ab}	1.63^{bc}	1.54^{d}	1.56^{cd}	1.57^{cd}	0.02	0.01
EPEI(%)	297.99^{d}	324.64^{cd}	339.70^{bc}	374.99^{a}	368.05^{ab}	373.44^{a}	6.59	0.01
Nutrient utilization (%)								
Dry matter	70.98	72.69	71.81	72.12	71.79	71.91	0.23	0.43
Crude protein	71.80^{b}	73.93^{a}	72.87^{ab}	74.07^{a}	73.69^{a}	73.81^{a}	0.25	0.05
Crude fat	80.77^{b}	83.07^{a}	82.62^{a}	83.19^{a}	83.18^{a}	83.20^{a}	0.27	0.04
Economics of production								
Feed cost (Rs)/kg body weight gain	72.73^{a}	70.19^{ab}	68.51^{bc}	65.62^{c}	67.36^{bc}	68.31^{bc}	0.59	0.01
Economic efficiency (%)	51.92^{c}	57.00^{bc}	61.09^{ab}	67.74^{a}	52.95^{c}	61.43^{ab}	1.39	0.02

EPEI- Europian production efficiency index
Means within the same row with different superscripts are significantly different ($p<0.05$).

Carcass traits

Effect of different levels of humic substance supplementation on carcass traits of broilers in terms of carcass yield, organs weight, processing losses and nutrient composition of broiler breast meat at 35 days is described in Table 2. Diets with different levels of HS did not significantly ($p>0.05$) affect the carcass yield (dressing yield, eviscerated weight and drawn weight), organs weight (% of dressed weight) and processing losses (% of live weight). Similarly, the proximate composition of breast meat (DM, CP, and EE) was not influenced ($p>0.05$) by dietary treatments.

These findings agreed with the results reported by Nagaraju et al. (2014) and Khalaquzzman (2022) for dressing percentage, Samudovska et al. (2022) and Pistova et al. (2016) for relative organ weight of liver and heart in broilers and Hascik et al. (2018) and Korsakov et al. (2019) for nutrient composition (DM, CP and EE) of broiler meat with humic acid supplementation.

Table 2. Carcass traits of broilers in different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
Carcass yields (% of live weight)								
Dressing yield	82.87	82.97	83.45	83.46	83.23	83.67	0.26	0.97
Eviscerated weight	79.65	79.50	79.79	79.70	79.48	79.67	0.26	1.00
Drawn weight	82.70	82.79	83.29	83.28	83.04	83.49	0.26	0.97
Organ weight (%)								
Heart	0.42	0.55	0.53	0.56	0.57	0.63	0.04	0.85
Gizzard	1.76	1.76	1.84	1.97	1.90	2.07	0.04	0.27
Liver	1.47	1.61	1.86	1.87	1.83	1.86	0.08	0.67
Giblet	3.69	3.92	4.22	4.39	4.31	4.56	0.11	0.22
Pancreas	0.21	0.22	0.21	0.22	0.22	0.23	0.01	0.55
Processing losses (% of live weight)								
Blood	3.75	3.57	3.54	3.68	3.27	3.46	0.09	0.79
Feather	6.26	6.18	5.77	5.85	5.91	5.22	0.29	0.97
Head	2.10	2.21	2.41	2.33	2.55	2.50	0.06	0.25
Separable fat	1.19	1.26	0.95	0.80	1.17	0.66	0.10	0.47
Appendages	5.01	5.07	4.83	4.68	5.06	5.15	0.07	0.36
Meat nutrient composition (on DM basis)								
Dry matter	23.51	24.33	24.00	24.39	25.12	25.03	0.34	0.79
Crude protein	20.93	21.26	20.95	21.13	21.26	21.39	0.11	0.80
Crude fat	5.51	5.55	5.50	5.54	5.69	5.73	0.07	0.88

Means within the same row with different superscripts are significantly different ($p<0.05$).

Histomorphometry

The results of histomorphometrical analysis of small intestine in broilers are given in Table 3 and Figure 1. Broilers fed on basal diets supplemented with different levels of HS did not significantly ($p>0.05$) influenced duodenum, jejunum and ileum wall thickness and goblet cells per field. Maximum and significantly higher ($p<0.05$) villus height was recorded in the broilers fed with HS0.4 in both duodenum and ileum compared to CON. Whereas, in jejunum significantly ($p<0.05$) higher villus height was observed with HS0.3 feed compared to the CON. Diet supplemented with humic substance @ 4 g/kg feed (HS0.4) showed significantly ($p<0.05$) lower crypt depth in duodenum, jejunum and ileum. Similarly, significantly ($p<0.05$) higher villus height to crypt depth ratio was observed in HS0.4 group compared to CON group. Birds fed with HS0.4 showed significantly ($p<0.05$) better intestinal histomorphometry compared to CON but statistically non significant ($p>0.05$) to different HS supplemented groups.

Humic acid lowers intestinal pH and bacterial colonization, reducing intestinal mucosa inflammation and enhancing villus height, thereby improving secretion, digestion and nutrient absorption (Taklimi et al., 2012). It may also promote crypt cell proliferation and tissue turnover (Panda et al., 2009). Several studies agreed with present study who reported improved villus height and crypt depth with HS supplementation (Taklimi et al., 2012, Omidiwura et al., 2021 and Lala et al., 2017).

Table 3. Histomorphometry of small intestine in different treatment groups

Treatment	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p-value
				Villus height (µm)				
Duodenum	1420.43^{c}	1484.25^{b}	1496.40^{ab}	1513.96^{ab}	1508.14^{ab}	1516.18^{a}	8.58	0.01
Jejunum	1125.71^{c}	1218.42^{b}	1225.38^{ab}	1268.30^{ab}	1304.95^{a}	1277.69^{ab}	16.39	0.01
Ileum	732.27^{c}	808.30^{ab}	790.99^{b}	849.09^{a}	823.01^{b}	869.45^{a}	10.94	0.01
				Crypt depth (µm)				
Duodenum	189.20^{a}	171.20^{b}	167.65^{bc}	159.32^{bc}	157.73^{bc}	153.42^{c}	3.29	0.02
Jejunum	170.92^{a}	155.72^{b}	160.81^{ab}	152.06^{b}	156.45^{ab}	147.41^{b}	2.37	0.05
Ileum	169.62^{a}	161.58^{ab}	155.31^{bc}	152.70^{bcd}	149.50^{cd}	144.03^{d}	2.37	0.01
				Intestinal wall thickness (µm)				
Duodenum	345.01	339.75	346.13	341.07	338.01	342.88	1.71	0.79
Jejunum	377.43	376.06	374.25	377.06	363.73	371.75	2.22	0.53
Ileum	426.37	423.18	428.38	420.25	415.09	417.30	4.51	0.97
				Goblet cells per field				
Duodenum	23.66	25.66	24.00	25.00	23.00	23.66	0.72	0.94
Jejunum	35.67	33.67	34.00	34.33	33.33	34.66	0.67	0.96
Ileum	84.67	83.33	84.00	85.00	84.33	83.67	0.64	0.99
				Villus height/Crypt depth ratio				
Duodenum	7.52^{c}	8.68^{b}	8.94^{ab}	9.51^{ab}	9.58^{ab}	9.91^{a}	0.22	0.01
Jejunum	6.61^{c}	7.85^{ab}	7.62^{b}	8.34^{ab}	8.37^{ab}	8.67^{a}	0.19	0.02
Ileum	4.32^{d}	5.00^{c}	5.10^{c}	5.56^{b}	5.51^{b}	6.04^{a}	0.14	0.01

Means within the same row with different superscripts are significantly different ($p<0.05$).

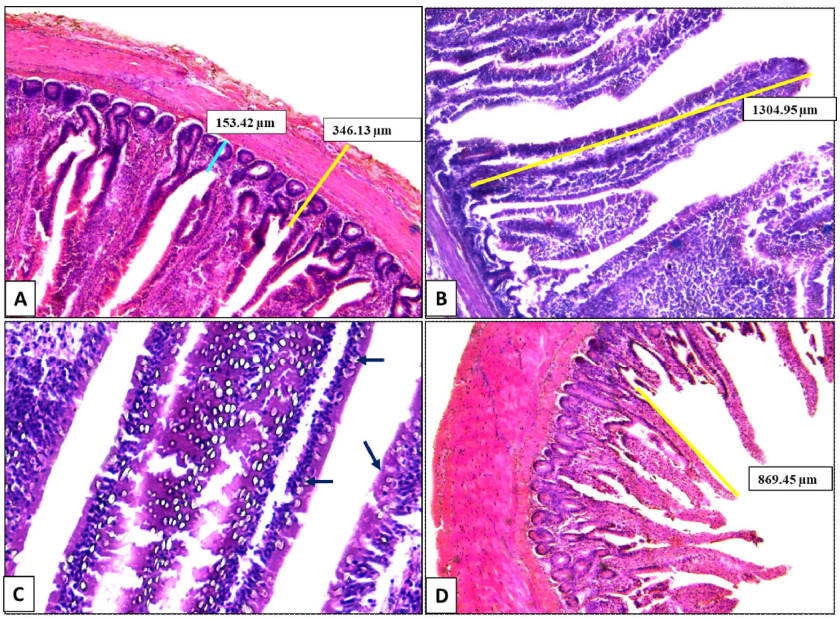

Fig 1. Photomicrographs (H&E) of small intestine showing: A. Total wall thickness of duodenum (yellow bar) and crypt depth (blue bar) X50. B. Villus height measurement of jejunum X50. C. Goblet cells in epithelium of jejunum (arrow) X200. D. Measurement of villus height in ileum X50

The effect of different levels of HS supplementation on caecal microbial count (cfu/g) of broilers are presented below in Table 4. Supplementation of HS0.4 had significantly lower ($p<0.05$) *E. coli* count compared to CON group but statistically ($p>0.05$) comparable to other supplemented groups. There was no significant ($p>0.05$) difference observed in *C. perfringens* count in caecal contents in different dietary treatments. However, numerically lower *C. perfringens* count was observed in HS supplemented groups compared to control group.

Humic substance reduces the gut pH ((Marcincak et al., 2023) which decreases the pathogenic bacteria colonization and toxins production. They may also disturb the microbial proteins and carbohydrates metabolism leads to the destruction of bacterial cells or viral particles (Huck et al., 1991). Studies have reported lower *E. coli* count in the intestinal contents of broilers in HS supplemented groups (Omidiwura et al., 2021, Mudronova et al., 2020 and Khalaquzzman, 2022), although Dominguez-Negrete et al. (2019) found that supplementation of HS had no significant ($p>0.05$) effect on *C. perfringens* count in caecal contents of broilers.

Table 4. Caecal microbial count (log_{10}cfu/g) of broilers in different treatment groups

Caecal microbiota	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
E. coli	8.37^{a}	7.78^{ab}	7.44^{b}	7.18^{b}	6.97^{b}	6.98^{b}	0.14	0.01
C. perfringens	6.11	5.41	5.51	5.43	5.39	5.38	0.09	0.15

Means within the same row with different superscripts are significantly different ($p<0.05$).

Immunity

Humoral immune response, lymphoid organ weight, IgG and IL-10 and liver function test were estimated and presented in Table 5 and Table 6. Antibody titre against NDV on 30^{th} and 42^{nd} day in broilers indicated that humoral immune response was significantly improved ($p<0.05$) by increase of age with dietary supplementation of graded levels of HS compared to CON with significantly ($p<0.05$) higher antibody titre observed in broilers fed HS @ 4 g/kg diet (HS0.4).

Lymphoid organ weights (% of live weight), particularly the spleen and bursa, were significantly ($p<0.05$) higher in broilers supplemented with graded levels of HS in the diet.

Serum IgG and IL-10 levels were significantly improved in birds fed HS at 2, 3, and 4 g/kg feed, whereas SGOT/AST (aspartate aminotransferase) and SGPT/ALT (alanine aminotransferase) showed no significant effect ($p>0.05$).

The improved antibody titre against NDV might be due to their antiviral properties, phagocytic activity of leukocytes, ability to reduce colonization of pathogens in the gastrointestinal tract and improving immune functions. Results of the study agreed with the findings of Ahfeethah et al. (2023) and Mehdi and Hasan (2012) who demonstrated that supplementation of humic acid increases antibody titre against NDV. Increased spleen and bursa weight were observed with supplementation of HS (Korsakov et al., 2019; Elnaggar and El-Kelawy, 2018). Increase in serum IgG levels suggested that HS has capacity of elevating natural antibody production. Findings are agreement with Zhang et al. (2020) who noticed that supplementation with 0.1, 0.3 and 0.5% sodium humate significantly ($p<0.05$) increased serum IgG levels compared to the birds in the control group. Ma et al. (2021) observed that broiler diet supplemented with mixed organic acids has significant ($p<0.05$) effect on IL- 10 levels in the broiler serum. However, there is no increase in serum AST and ALT levels which indicates no negative effect on liver function. Results of our present study agreed with Saleh et al. (2022).

Table 5. Humoral immune response against Newcastle disease virus (log_{10}) of broilers in different treatment groups (30^{th} and 42^{nd} day)

Days	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	Period mean	SEM
30^{th}	1.51	1.66	1.70	1.70	1.80	1.86	1.70^{b}	0.11
42^{nd}	1.61	1.76	1.80	1.86	1.96	2.02	1.83^{a}	0.05
Treatment mean	1.55^{c}	1.71^{bc}	1.75^{abc}	1.78^{ab}	1.88^{ab}	1.95^{a}		
SEM	0.14	0.06	0.05	0.08	0.13	0.05		
p- value	Treatment		Period		Treatment*period			
	0.011		0.032		0.998			

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 6. Immunological parameters of broilers in different treatment groups

Parameter	CON	AGP	HS0.1	HS0.2	HS0.3	HS0.4	SEM	p- value
Lymphoid organs (% of Live weight)								
Spleen	0.07^{b}	0.08^{b}	0.11^{a}	0.11^{a}	0.12^{a}	0.13^{a}	0.12	0.01
Thymus	0.37	0.44	0.50	0.52	0.55	0.58	0.55	0.25
Bursa of fabricius	0.11^{b}	0.15^{ab}	0.16^{a}	0.16^{a}	0.18^{a}	0.19^{a}	0.18	0.04
IgG and IL-10								
IgG (µg/ml)	8.92^{c}	10.30^{bc}	10.08^{bc}	11.72^{ab}	12.15^{ab}	13.25^{ab}	0.43	0.03
IL-10 (ng/L)	15.46^{c}	18.01^{c}	19.05^{bc}	22.29^{ab}	23.39^{a}	25.10^{a}	0.83	0.01
Liver function tests								
SGOT(IU/L)	244.14	215.78	229.42	236.88	256.64	206.72	6.83	0.31
SGPT(IU/L)	8.58	12.78	10.18	11.24	10.44	12.90	0.79	0.63

Means within the same row with different superscripts are significantly different ($p<0.05$).

CONCLUSION

The present study concluded that supplementation of humic substance @ 2 g/kg feed to the broilers diet improved feed to gain ratio and nutrient utilization (crude protein and crude fat) which resulted in better growth. Humic substance improved intestinal histomorphometry, immunological response and lowered caecal *E. coli* count which improves gut health. Substituting AGP with HS0.2 had lower feed cost Rs. per kg body weight gain and higher economic efficiency. Therefore, humic substance @ 2 g/kg feed can be used as an economically efficient alternative to antibiotic growth promoters in broilers diet to reduce the risk of antimicrobial resistance.

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Welfare Evaluation of Pet Dogs Through Feeding Protocols Followed, Nutritional Composition of Available Feeds and Blood Metabolic Profile

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ABSTRACT

A survey was conducted in Central Kashmir covering 91 adult pet dogs with collection of 26 feed and 73 blood samples. Feeding practices followed by the dog owners were recorded and samples of commercial dry adult dog foods collected during survey were subjected to nutritional analysis to assess their effects on blood metabolic profile. Mostly (~63%) the pet dogs were reared on homemade diets with about 90% receiving non-vegetarian type, and only ~5% owners were exclusively feeding commercial dog foods of different brands. Majority (63%) of the pet dogs were fed thrice a day. Among the analyzed commercial dog food samples, the proportion falling below the recommended nutrient standards was 19% for protein, 15% for fat, 31% for fibre and calcium, 12% for total ash, and 15% for phosphorus. The homemade diets had wide variation in composition and were generally imbalanced. Blood metabolic profiling revealed prevalent calcium deficiency in dogs fed homemade diets, while plasma cholesterol (51%), triglycerides (44%), and urea nitrogen (30%) were found elevated beyond normal physiological ranges. It is concluded that homemade diets constitute the mainstay of feeding with little or no provision of supplements to pet dogs, but these diets are nutritionally imbalanced.

KEYWORDS: Commercial feeds, Feeding practices, Homemade diets, Nutritional status, Pet dogs, Survey

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In modern society, the role of companion animals and the relationship between humans and their pets have markedly changed during the last century. Among companion animals, dogs are distinctive for their ubiquitous distribution, being closely associated with human settlements across diverse habitats. In India, the pet dog population has increased by 26% every year and at present, about 17% of the households own a pet dog (Singh et al., 2020). The total pet dog population is 24.74 millions out of which 9.43 millions are pet and 15.31 millions are stray dogs (Anonymous, 2019). While, in Jammu and Kashmir Union Territory the pet dog population is 1.06 lakh contributing a share of 1.22% towards the total pet dog population of the country, being reared predominantly for guarding owners' stocks and as military working dogs.

For better performance of the prescribed activities, health and welfare of the pet dogs require regular care and attention. Proper nutrition and feeding management are the main objectives to ensure the well-being and performance of animals including pet dogs. Normally, homemade foods are the primary means of nourishing pet dogs; however, these diets appear to be nutritionally deficient or imbalanced (Pattanaik and Sharma, 2006), and feeding such diets may lead to some metabolic disorders in pet dogs (Gonzalez et al., 2003). As such, pet owners are now seeking alternative food options to the standard commercial dog foods available in different forms as dry foods, wet foods, semi-moist foods for various physiological conditions that meet their needs. Currently, no data is available on welfare of pet dogs with regards to feeding practices followed by the owners, nutritional composition of the available feeds offered and their nutritional status in Kashmir. Therefore, the present study was planned to ascertain the nutritional practices adopted and their impact on relevant blood metabolic profile of pet dogs reared in Central Kashmir.

The present study based on survey work was conducted in three central districts (Budgam, Ganderbal and Srinagar) of Kashmir valley. A total of 91 owners rearing adult dogs were interviewed with 48 from district Srinagar, 23 from district Ganderbal and 20 from district Budgam.

A single-visit formal survey technique was used through household interviews using a pre-tested survey schedule based on queries regarding the feeding practices like source (homemade or commercial), type (vegetarian or non-vegetarian) and composition of food, use of supplements, and frequency of feeding followed by pet dog owners. Besides, a total of 26 number of dry food samples for adult dogs representing seven different commercial brands (Pedigree, Drools, Royal Canin, Moochie, Himalaya, Canine Greek and PurePet) commonly offered were collected from the pet dog owners in the study areas. The collected samples were packed in sealed bags, carried to the laboratory for further processing and evaluation.

Out of the surveyed pet dogs, a total of 73 blood samples were collected as per the method of Waxman and Lind (2023). The area over the cephalic veins was clipped and scrubbed with a 2% chlorhexidine solution to collect the blood samples under all aseptic conditions into ethylene diamine tetra acetic acid (EDTA) vials for harvesting plasma placed in eppendorf microtubes and stored at "40 <"C for batch analysis.

The collected adult dog dry foods samples procured and offered by the owners in the study areas were ground, and then analyzed for proximate principles as per AOAC (2019) methods and few major minerals (calcium and phosphorus) as per Talapatra et al. (1948).

Blood glucose was estimated in whole blood immediately after collection using SD-Codefree blood glucose meter. After thawing, the plasma samples were analysed for contents of various biochemicals in semi-auto biochem analyzer (Photometer-5010V5+, Robert Riele INC, Berlin, Germany) using commercial kits (DiaSys Diagnostics Pvt. Ltd., Navi Mumbai, India).

The data collected during the survey were compiled and analyzed using frequency distribution analysis through the statistical software program SPSS (2011) for Windows. The frequency data analysis was employed to determine the proportion of diferent samples falling within or beyond the established standard reference ranges. Descriptive statistics including means, standard deviations, and ranges were also computed providing both prevalence estimates and the degree of variation within the population under study.

In the survey conducted across Central Kashmir, female dogs (n=70) comprised 76.92% of the population, whereas males (n=21) accounted for 23.08%. A similar preference for bitches by majority (56%) of the respondents was also reported by Sawaimul et al. (2009). Four major pet dog breeds were identified in the region, with German Shepherds (45.05%) representing the largest group, followed by Labradors (32.97%), Golden Retrievers (17.58%), and Dalmatians (4.39%). Medium-sized exotic breeds were generally preferred by both civilian and military personnel, while none of the surveyed households kept the local Bakharwal dog.

A total of 62.64% of the respondents were found to feed their pet dogs solely on homemade diets, 5.49% exclusively on available commercial dry dog feeds, and rest 31.87% on homemade diets supplemented with commercial feeds (Table 1). Owners in surveyed area used mostly homemade diets for different reasons among which important ones included that homemade food are close to dogs natural diet, the owners preference to offer them the same food which they prepare for themselves, avoid monotonous feeding, with intention to avoid undesired ingredients in their diets, lack of awareness about feeding management of their pets, less availability of commercial dog feeds and even some reported financial constraints due to high price of proprietary pet feeds.

Homemade diets are the common items and constitute core feeding practice of dogs in India (Pattanaik and Kore, 2022). In Central Kashmir, the composition of homemade diets offered to pet dogs varied region wise depending upon the staple foods and preference of the owners. 90.11% of the surveyed dog population were being offered non-vegetarian diets by the owners due to their own dietary pattern as Jammu and Kashmir Union Territory is a main meat consuming region in India with about 80% human population being non-vegetarian.

Only 7.69% of the surveyed pet dogs were being offered dietary supplements in the form of micronutrients (mineral mixtures and vitamins), herbal immunomodulator, anti-oxidants/stress and

some treats as dog biscuits etc., while rest were fed the basal diets alone. Feed supplementation was practiced to bitches during pregnancy and lactation; however, in most of the cases the supplementation did not constitute part of the routine feeding schedule. Majority of the respondents reported that they feed their pet dogs thrice (62.64%) a day, while only 12.09% owners preferred to feed their pets two times a day. Frequency of feeding is an important aspect of metabolism and is known to influence both food intake as well as the metabolic efficiency. An increase in number of meals/day usually results in an increased energy loss as a result of meal-induced thermogenesis (Li and Zheng, 2023).

Table 1. Feeding practices followed by pet dog owners in Central Kashmir

Attributes	Variables	District Srinagar (n=48)	District Ganderbal (n=23)	District Budgam (n=20)	Pooled Avg ± SE (n=91)
Source of feed offered	Home-made	60.42 (29)	60.87 (14)	70.00 (14)	62.64 ± 3.12
	Commercial	6.25 (3)	4.35 (1)	5.00 (1)	5.49 ± 0.56
	Mixed	33.33 (16)	34.78 (8)	25.00 (5)	31.87 ± 3.05
Type of feed offered	Vegetarian	4.17 (2)	13.04 (3)	20.00 (4)	9.89 ± 4.58
	Non-vegetarian	95.83 (46)	86.96 (20)	80.00 (16)	90.11 ± 4.58
Feed supplements offered	Yes	8.33 (4)	4.35 (1)	10.00 (2)	7.69 ± 1.68
	No	91.67 (44)	95.65 (22)	90.00 (18)	92.31 ± 1.68
Daily feeding frequency	Once	0.00 (0)	0.00 (0)	0.00 (0)	0.00 ± 0.00
	Twice	12.50 (6)	13.04 (3)	10.00 (2)	12.09 ± 0.94
	Thrice	64.58 (31)	60.87 (14)	60.00 (12)	62.64 ± 1.41
	Quadraple	22.92 (11)	26.09 (6)	30.00 (6)	25.27 ± 2.05
	ad lib	0.00 (0)	0.00 (0)	0.00 (0)	0.00 ± 0.00

Nutritive value of available adult pet dog foods

The pooled mean values for nutritional parameters of the 26 adult pet dog food samples collected in the surveyed areas were within the normal ranges (AAFCO, 2018) and are presented in Table 2. Dry matter (DM) content of 50.00% of the samples was in the range of 88-90%, while 38.46 and 11.54% of the samples were having more than 90% and less than 88% DM, respectively. 69.23% of feed samples were in the range of 18-22% for crude protein (CP) content; however, 11.54% feed samples were having more than 22% CP and 19.23% feed sample were having less than 18% CP. 80.77% of the feed samples were having fat (EE) content within the range of 5-8%. 57.69% of feed samples were having fibre (CF) content within the range of 3.5-6%, while 11.54% of the samples were having CF more than 6%. 15.38% sample were having more than 0.5% calcium and 30.77% having less than 0.5% calcium. 19.23% of feed samples were seen to have more than 0.3% phosphorus and 15.38% less than 0.3% phosphorus. Average concentration of different nutrients in majority of the commercial dry adult pet dog foods available in central Kashmir were within the ranges of recommended values representing that these were more balanced, uniform, nutritious and within the frames as declared by most of the manufacturers. In contrast, homemade diets offered to pet dog in India were nutritionally inadequate and/or imbalanced with respect to protein, energy and minerals especially Ca and P (Pattanaik and Sharma, 2006).

Blood metabolic profile of adult pet dogs

All the attributes of blood metabolic profile (Table 2) for majority of the adult pet dogs in the surveyed areas were within the normal physiological ranges (Kaneko et al., 1997) except for plasma cholesterol wherein 50.68% of the pet dogs surveyed had above normal values. 43.84% of the plasma samples analyzed were having calcium (Ca) values below the normal physiological range, while none of the samples had above normal values representing wider prevalence of Ca deficiency among the surveyed dogs. This was also reflected by higher percentage (31.51%) of animals with plasma phosphorous (P) levels above the reference range coupled with 24.66% of the dogs having plasma ALP levels below the suggested physiological range referring to metabolic bone disease conditions in the surveyed

pet dogs. Serum ALP levels may act as a useful, non-invasive indicator of skeletal health in dogs, and as a diagnostic and prognostic marker in the management of dogs with musculoskeletal or metabolic disorders (Allen et al., 2000). Hyper-phosphatemia is perceived as a latent display of imbalanced dietary Ca: P ratio. Factors like consumption of meat or grains based foods with high P contents for longer periods due to non-vegetarian feeding practices would be attributed to hyper-phosphatemia and hyper-cholesterolemia of surveyed dogs in the present study.

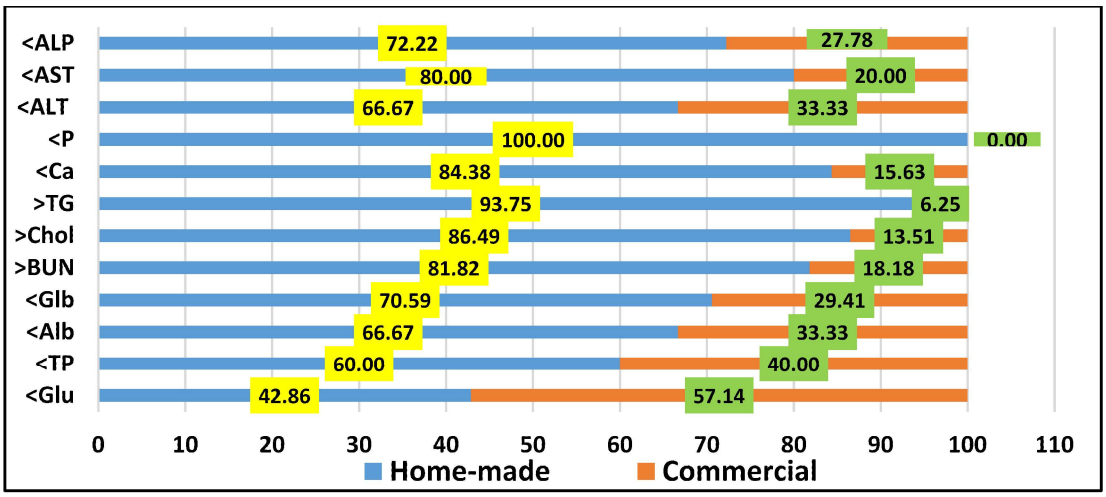
The prevalence of abnormal blood metabolic profiles reveal higher values for all estimated parameters (except blood glucose and serum ALT, AST and ALP) in those pet dogs reared by their owners on homemade compared to commercial diets (Figure 1). The percentage of population with above reference values for plasma cholesterol, triglyceride and urea nitrogen levels were higher in dogs fed homemade than those fed commercially available dog foods. While, percentage of population with below reference values for plasma proteins and minerals were lower in pet dogs on commercial feeds. Higher plasma fats, urea N and blood glucose levels in pet dogs fed on homemade diets could be attributed to greater proportion of grains and meat sources in the said diets. In aggrement to the present findings, Gonzalez et al. (2003) reported that dogs fed a homemade feed exhibited higher values of glucose and cholesterol than dogs feeding on a commercial ration.

Table 2. Nutritional profile of adult pet dog in Central Kashmir

Attribute	Reference value * & **	Pooled Mean of samples
Composition (%) of available commercial foods		
DM	88-90.0	90.87
CP	18.0	19.90
EE	5.0	6.26
TA	2.5-3.0	2.65
CF	3.50	4.71
Ca	0.5-0.6	0.51
P	0.3-0.5	0.44
Blood metabolic profile		
Glu (mg/dL)	65-118	98.45
TP (g/dL)	5.4-7.1	6.97
Alb (g/dL)	2.6-3.3	3.12
Glb (g/dL)	2.7-4.4	2.98
PUN (mg/dL)	10.0-28	18.46
Chol(mg/dL)	135-270	245.75
TG (mg/dL)	23-102	100.48
Ca (mg/dL)	9-11.3	9.25
P (mg/dL)	2.6-6.2	5.84
ALT (U/L)	17-95	25.45
AST (U/L)	18-56	22.71
ALP (U/L)	20-156	42.87

* AFFCO (2018) reference values for adult dog food
** Kaneko et al. (1997) reference values for blood biochemicals in adult dogs.

Figure 1. Prevalence of abnormal blood metabolic profiles in adult pet dogs fed diets of different sources in Central Kashmir



CONCLUSION

Feeding of non-vegetarian type homemade diets were being preferred over proprietary feeds with little or no provision of supplements to the pet dogs in Central Kashmir. These diets were qualitatively inadequate to meet the nutrient requirements which was reflected in their metabolic profiles. Comparatively most of the evaluated commercial adult dry dog foods met the minimum AAFCO requirements for different nutrient contents and their feeding resulted in blood biochemical indices within the normal physiological ranges.

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Effects of Bacterial Inoculants and Xylanase on Silage Fermentation Characteristics of Wheat straw and Pasture hay based Green Maize Silage

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ABSTRACT

Different silages were prepared using green maize fodder and wheat straw and pasture hay separately in the proportion of 10:0 & 7:3 ratio in plastic jar of 3 kg capacity by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silage with nine different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants), PH (green maize and pasture hay in 7:3 ratio), XPH (PH added with Xylanase), LFPH (PH added with *Lactobacillus fermentum*), LPLFPH (PH added with both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6 cfu/g and 2 x 10^6 cfu/g, respectively. All silages were evaluated in terms of their proximate composition, cell wall constituents and quality parameters of silage on 45 days of ensiling. Bacterial inoculants significantly reduce DM content of silage. LPLF silage and XPH and LFPH significantly increases crude protein content. X and LPLFPH shows significant improvement in EE content. LF shows significantly improve values of CF and NFE. X and XPH silage significantly lowers NDF and ADF content. LPLF and XPH silage significantly improves cellulose while additives do not have any significant effect on hemicellulose content. Bacterial inoculants and Xylanase significantly improves TVFA, WSC and LA to lower silage pH for very good silage. Xylanase, *Lactobacillus fermentum* and combination of bacterial inoculants with wheat straw and pasture hay silage, respectively significantly reduces ammonia nitrogen for excellent silage quality. Thus, it is concluded that Xylanase and/or *Latobacillus fermentum* significantly improves overall silage fermentation characteristics and nutrient content in green maize based silage along with wheat straw or pasture hay in 7:3 ratio.

KEYWORDS: *Lactobacillus fermentum*, *Lactobacillus plantarum*, Pasture hay, Silage,Wheat straw

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Wheat straw and pasture hay is inexpensive and available locally, its use in silage along with additives may improve its quality and there by its utilization in animal feeding. Silage additives have been used as a management tool to improve the nutritional value of silage. They are natural or industrial products added in smaller quantities during ensiling to control the preservation process so as to retain as many of nutrients as present in the original fresh forage.

Wheat straw and pasture hay are available locally and in expensive. They are generally burnt in the field itself by the farmers and not utilized in the feeding of animals and thus go as a waste. Incorporating wheat straw and pasture hay in silage production along with different feed additives, will judiciously utilize this poor quality roughage in silage production to enhance the productive performance of animals by reducing the shortage of quality fodder in the present scenario.

Inoculation with homofermentive or facultatively heterofermentive lactic acid bacteria during ensiling rapidly decreases pH of silage and increases content of lactic acid than the other fermentation products in silage (Carvalho et al., 2020). Also, treating forages with enzymes may improve their digestibility via number of mechanisms that include direct hydrolysis of sugar, improvement in palatability, change in gut viscosity and change in the site of digestion (Kung Jr, 2010). Keeping the above facts in view, the proposed experiment was planned to study the effect of Lactobacillus bacterial inoculants and xylanase in maize based green silage along with

different dry fodders like wheat straw and seasonal pasture hay.

Enzyme xylanase was procured from the standard manufacturer company @ 100 [1] /kg. The result showed that percent cost saving was found to be higher in xylanase added wheat straw silage as compared to control (maize alone) silage.

The present study was conducted at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat.

Different silages were prepared using green maize fodder and wheat straw and pasture hay separately in the proportion of 10:0 & 7:3 ratio in plastic jar of 3 kg capacity (3 replication in each) by adding common salt @ 0.5%, urea @ 1% and molasses @ 1.5% in each silage with nine different treatments, *viz.,* Control (only green maize), WS (green maize and wheat straw in 7:3 ratio), X (WS added with Xylanase), LF (WS added with *Lactobacillus fermentum*), LPLF (WS added with both bacterial inoculants), PH (green maize and pasture hay in 7:3 ratio), XPH (PH added with Xylanase), LFPH (PH added with *Lactobacillus fermentum*), LPLFPH (PH added with both bacterial inoculants). Xylanase, *L. plantarum* and *L. fermentum* were used @ 1500 IU/g, 1 x 10^6 cfu/g and 2 x 10^6 cfu/g, respectively. Different additives were spread as per their application rate in different treatments and mixed thoroughly. Fodder mass along with different additives were packed in plastic jar having the capacity of 3 kg and designed with valve at the lid of the jar. Air from the jars was removed with the help of vacuum pump. Jars of different treatments were stored at room temperature for 45 days. The store house was disinfected and appropriate measures were taken to avoid the entry of rats, mice and birds.

All silages were evaluated after 45 days of ensiling. Before ensiling samples of green maize fodder, wheat straw, pasture hay, mixture of green maize & wheat straw (7:3) and mixture of green maize & pasture hay (7:3) were analysed for proximate composition and cell wall constituents. Samples from different experimental silage were evaluated in terms of their proximate composition and cell wall constituents according to the methods of AOAC (2023) and Van Soest et al. (1991), respectively.

Organoleptic criteria are the most important way to judge the silage quality. Silage was observed visually for its colour and categorized in four different grades as: Grade 1 - Golden yellow, Grade 2 - Light brown, Grade 3 - Dark brown, Grade 4 – Blackish.

For estimation of silage pH, total volatile fatty acid (TVFA) and ammonia nitrogen (NH3-N), water extracts of silages were prepared by adding 90 mL of distilled water to 10 g fresh silage sample in a beaker and homogenized by mechanical homogenizer. A few drops of 0.1% mercuric chloride were added into the extract and kept in refrigerator at 4 °C. After 48 hours, material was filtered through four layer muslin cloth and stored at 4 °C. The pH of silage was measured by pen type pH meter. TVFA, lactic acid and NH3-N were analyzed as per the method given by (Prasad, 2015). Total nitrogen was estimated by Kjeldahl method (AOAC, 2023). Silage samples were oven dried at 100 ± 5 °C for overnight. The dried samples were ground to pass through a 1 mm screen for analysis of Water soluble carbohydrate (WSC) as per the standard method (Yemm and Willis 1954).

The data were analysed for descriptive statistics (mean and standard error). Treatment effects on different parameters were analyzed by one way analysis of variance according to Snedecor and Cochran (1994). Pair wise mean differences between groups were compared by Duncan's new multiple range test for the significance at $p<0.05$.

The result regarding proximate composition are presented in Table 1. The dry matter was significantly ($p<0.01$) lower in different silages as compared to WS and PH. Similarly, Jalc et al. (2009), Khota et al. (2017), Dakore (2018) and Yadav (2018). In contrast, Su et al. (2019) reported DM was significantly ($p<0.05$) increase. OM was significantly ($p<0.01$) higher in LF treated silage. It is corroborated with Dakore (2018) while in distinction, Yadav (2018) reported significantly reduced OM. Crude protein content was found to be significantly ($p<0.05$) higher in Xylanase treated pasture hay silage. Similarly, Jalc et al. (2009) noted higher CP in *L. fermentum* added silage. EE was significantly ($p<0.05$) higher in xylanase treated wheat straw silage. Similarly, Khota et al. (2017), Zielinskal and Fabiszewska (2017), Dakore (2018) and Yadav (2018) reported higher EE content. Unlikely, Nkosi et al. (2012) observed significantly lower EE content. CF was significantly ($p<0.05$) lower in LF treated wheat straw silage.

Unlikely, Jalc et al. (2009) obtained significant decrease in the CF. The TA was significantly ($p<0.01$) lower in LF treated wheat straw silage. In contrast, Jalc et al. (2009) reported non significant decrease. NFE was significantly ($p<0.05$) higher in LF treated wheat straw silage.

Table 1. Proximate composition of different experimental silages (% DMB)

Treatments	Parameters						
	DM**	OM**	CP*	EE*	CF*	TA**	NFE*©
C	31.39^{a} ±0.32	84.59^{a} ±0.23	8.06^{ab} ±0.17	1.08^{ab} ±0.05	36.06^{a} ±2.39	15.40^{e} ±0.23	39.40^{abc} ±2.61
WS	41.02^{e} ±0.22	87.57^{cde} ±0.64	7.15^{a} ±0.40	0.90^{a} ±0.03	45.11^{c} ±3.69	12.42^{abc} ±0.64	33.37^{a} ±4.65
X	41.63^{f} ±0.30	86.74^{bc} ±0.52	7.54^{ab} ±0.32	1.48^{b} ±0.27	41.15^{ab} ±0.74	13.25^{cd} ±0.52	37.89^{abc} ±1.08
LF	37.51^{d} ±0.19	88.54^{e} ±0.28	7.72^{ab} ±0.32	1.18^{ab} ±0.05	36.09^{a} ±0.96	11.47^{a} ±0.28	43.54^{c} ±1.00
LPLF	35.17^{b} ±0.48	88.21^{de} ±0.10	9.00^{b} ±0.63	1.15^{ab} ±0.06	42.06^{bc} ±1.53	11.79^{ab} ±0.10	36.00^{ab} ±1.90
PH	40.52^{e} ±0.37	84.76^{a} ±0.32	8.25^{ab} ±0.54	0.94^{a} ±0.08	39.08^{ab} ±0.60	15.23^{e} ±0.32	36.47^{ab} ±0.84
XPH	38.73^{d} ±0.27	86.39^{b} ±0.25	9.01^{b} ±0.89	1.07^{ab} ±0.04	37.85^{ab} ±0.33	13.60^{d} ±0.25	38.45^{abc} ±0.91
LFPH	38.55^{d} ±0.25	87.43^{cd} ±0.11	8.97^{b} ±0.73	1.31^{ab} ±0.18	38.13^{ab} ±0.60	12.56^{bc} ±0.11	39.02^{abc} ±1.42
LPLFPH	36.12^{c} ±0.22	87.04^{bc} ±0.04	7.60^{ab} ±0.26	1.39^{b} ±0.12	37.60^{ab} ±0.46	12.95^{cd} ±0.43	40.43^{bc} ±0.68
p value	<0.001	<0.001	0.017	0.036	0.004	<0.001	0.075

^{abcde}Means with different superscript within a column differ significantly (**$p<0.01$, *$p<0.05$)

Cell wall constituents

Significantly ($p<0.05$) lower NDF was found in Xylanase treated wheat straw and pasture hay silage as compared to WS and PH silage. Similarly, Govea et al. (2013) Khota et al. (2017), Yadav (2018) and Agarrusi et al. (2019) recorded non-significant decrease in NDF content. Also, Jalc et al. (2010) observed significantly lower NDF in LF inoculated silage. The ADF was significantly ($p<0.01$) lower in Xylanase inoculated wheat straw silage as compared to other all inoculated silage. It might be due to the effect of xylanase to release maximum amount of nutrients from indigestible and potential digestible fraction of cell wall. In agreement, Xing et al. (2009) and Dakore (2018) reported significantly lower ADF content. The cellulose was significantly ($p<0.05$) higher in combination of both bacterial inoculants added wheat straw silage. In accordance, Zhao et al. (2021) reported significant increase in the cellulose content of lactic acid bacteria inoculated silage. The hemi cellulose was non-significant ($p>0.05$) among all experimental silage. In the same line, Yadav (2018) noticed numerically higher value of hemicellulose in enzyme xylanase inoculated silage.

Table 2. Cell wall constituents of different experimental silages (% DMB)

Treatments	Parameters			
	NDF*	ADF**	Cellulose*	Hemicellulose
C	67.41^{abc}	47.41^{bc}	33.49^{ab}	20.00
	±1.74	±1.70	±0.63	±0.70
WS	69.89^{c}	49.95^{de}	33.58^{ab}	19.94
	±0.84	±0.47	±0.42	±0.90
X	65.96^{ab}	44.55^{a}	33.37^{ab}	21.41
	±0.72	±0.99	±0.43	±0.74
LF	66.13^{ab}	45.79^{ab}	32.23^{a}	20.34
	±1.45	±0.77	±0.57	±2.10
LPLF	68.85^{abc}	48.21^{bcd}	36.50^{b}	20.64
	±1.37	±0.82	±0.68	±1.64
PH	70.37^{c}	50.49^{de}	33.99^{ab}	19.87
	±0.38	±0.50	±0.94	±0.71
XPH	65.77^{a}	45.99^{ab}	36.85^{b}	19.77
	±0.37	±0.41	±0.56	±0.67
LFPH	68.98^{bc}	49.76^{cde}	34.55^{ab}	19.21
	±0.25	±0.37	±3.15	±0.53
LPLFPH	69.41^{c}	50.83^{e}	34.74^{ab}	18.73
	±0.35	±0.34	±0.64	±0.20
p value	0.005	<0.001	0.013	0.830

^{abcd}Means with different superscript within a column differ significantly (**p<0.01, *p<0.05).

Quality parameters of silage

The results of quality parameter of silage are presented in Table 3. The colour of silage was observed golden yellow in all experimental silage. The pH values of different experimental silage were found to be significantly (p<0.01) lower in all the additives inoculated silage as compared to Control, WS and PH silage. It might be due to the addition of enzyme and bacterial inoculants as they decreased the silage pH rapidly as compared to control. These results are in agreement with the observations of most of the workers, Jalc et al. (2009), Nkosi et al. (2012), Govea et al. (2013), Guo et al. (2014), Khota et al. (2017), Zielinska and Fabiszewska (2018), Dakore, (2018), Yadav (2018) and Zhao et al. (2021) as they observed significantly lower pH in all inoculated silages as compared to control silage. Whereas, Xing et al. (2009) and Marbun et al. (2020) noticed contrary to present finding that pH of silage was not affected significantly by inoculants.

The results of TVFA contents were found be significantly (p<0.01) higher in all additives inoculated silage as compared to Control, WS and PH silage. However, significantly (p<0.01) higher TVFA content was found in *Lactobacillus fermentum* treated wheat straw and pasture hay silage which might be due to better quality fermentation. Corresponding to present findings, Dakore (2018) and Yadav (2018) reported significant higher TVFA content in all additives inoculated silages as compared to control silage. Increase in TVFA content of inoculated silages might be due to better microbial fermentation of carbohydrates.

Ammonia nitrogen content was found to be significantly (p<0.01) lower in all the additives inoculated silage as compared to Control and WS silage which is due to better quality silage. However, numerically lower ammonia nitrogen content were found in Xylanase and *lactobacillus fermentum* treated wheat straw and pasture hay silage, respectively. The results of present finding is well corroborated with findings of Filya (2003), Jalc et al. (2009),Nkosi et al. (2012), Govea et al.(2013), Chen et al. (2019) and Zhao et al. (2021), they reported that ammonia nitrogen content was significantly (p<0.05) reduce in inoculants and

enzyme added silages. In distinction to current findings, Agarussi et al. (2019) noted that ammonia nitrogen content was numerically increased in inoculated silages.

Total nitrogen content was found to be significantly ($p<0.05$) higher in Xylanase, *Lactobacillus fermentum* treated PH silage and combination of both bacterial inoculants LPLF treated wheat straw silage which is due to higher crude protein content in the same silages. The present trend regarding the results of Total N content of this study was in corroborated with the findings of Dakore (2018) and Yadav (2018) as they recorded significantly higher Total N content in additives inoculated silage.

WSC content was found significantly ($p<0.01$) lower in *Lactobacillus fermentun* and combination of both bacterial inoculant inoculated pasture hay silage as compared to WS and PH silage. However, numerically lower values were obtained in all the additives inoculated silage as compared to control, WS and PH silage. WSC contents of all inoculated silage were comparable with control (maize silage). In support to current results, Filya (2003), Xing et al. (2009) and Yadav (2018) recorded significantly lower WSC content in all additives inoculated silage as compared to control whereas, findings of Zhao et al. (2021), noticed non significantly lower WSC content in all additives treated silage as compared to control group. In disparity to present results , Nkosi et al. (2012) and Dakore, (2018) observed significantly higher WSC content in Lactic acid bacteria and enzyme inoculated silage.

Lactic acid was found significantly($p<0.01$) higher in all the additives inoculated silage as compared to control, WS and PH silage. However, significantly ($p<0.01$) higher lactic acid content was found in *lactobacillus fermentum* followed by xylanase added WS silage, LPLF and LF added pasture hay silage. Lower water soluble carbohydrates content and higher lactic acid content in all the additives inoculated silage indicates that more water soluble carbohydrates are utilized for producing higher amount of lactic acid which results in very good silage quality. In accordance with current investigation, Jalc et al. (2009), Xing et al. (2009), Nkosi et al. (2012), Govea et al. (2013), Guo et al. (2014), Zielinskal and Fabiszewska (2017), Dakore (2018), Yadav (2018), Su et al. (2019), Oskoueian et al. (2021) and Zhao et al. (2021) reported that lactic acid content was significantly higher in bacterial inoculants and enzymes added silages as compared to control silage.

Table 3. Quality parameters of different experimental silages

Treatments	Parameters						
	Colour of silage	pH**	TVFA** (mMol/100g DM)	NH3-N** (g/kg silage)	Total N* (%	WSC** (g/100 g DM)	LA** (g/100g fresh
C	Golden yellow	4.41^{c} ±0.06	20.25^{a} ±1.84	4.40^{b} ±0.20	1.28^{ab} ±0.02	1.91^{ab} ±0.26	4.70^{b} ±0.31
WS	Golden yellow	4.15^{b} ±0.07	21.19^{a} ±1.95	4.66^{b} ±0.29	1.14^{a} ±0.06	2.48^{c} ±0.26	3.36^{a} ±0.22
X	Golden yellow	4.11^{ab} ±0.04	42.41^{b} ±3.33	3.03^{a} ±0.43	1.20ab ±0.05	1.82^{ab} ±0.04	5.82^{cd} ±0.28
LF	Golden yellow	4.00^{ab} ±0.07	50.49^{d} ±1.48	3.03^{a} ±0.23	1.23^{ab} ±0.05	1.77^{ab} ±0.31	6.19^{d} ±0.31
LPLF	Golden yellow	4.13^{ab} ±0.04	42.36^{b} ±3.49	3.50^{a} ±0.31	1.44^{b} ±0.10	1.84^{ab} ±0.05	5.67^{cd} ±0.15
PH	Golden yellow	4.31^{c} ±0.04	21.61^{a} ±0.85	3.13^{a} ±0.04	1.32^{ab} ±0.08	2.14^{bc} ±0.05	4.33^{b} ±0.07
XPH	Golden yellow	4.08^{ab} ± 0.07	43.24^{bc} ±1.79	3.08^{a} ±0.03	1.44^{b} ±0.13	1.59^{ab} ±0.07	5.41^{c} ±0.13
LFPH	Golden yellow	3.95^{a} ±0.04	49.43^{cd} ±2.40	3.16^{a} ±0.08	1.43^{b} ±0.11	1.49^{a} ±0.10	5.71^{cd} ±0.07
LPLFPH	Golden yellow	3.98^{ab} ±0.03	41.56^{b} ±1.95	3.21^{a} ±0.10	1.21^{ab} ±0.04	1.46^{a} ±0.19	5.80^{cd} ±0.12
p value	-	<0.001	<0.001	<0.001	0.04	0.007	<0.001

abcdMeans with different superscript in a column differ significantly from each other (**p<0.01, *p<0.05)

CONCLUSIONS

Thus, based on present findings of proximate composition, cell wall constituents and quality parameter of silage, it is concluded that Xylanase and *Latobacillus fermentum,* either alone or in combination significantly improves overall silage fermentation characteristics and nutrient content in wheat straw or pasture hay (3%) based green maize (7%) silage.

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Utilization of *Acacia nilotica* Pod Meal as a Protein Supplement in Growing Goats

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ABSTRACT

A 75-day growth trial was conducted to evaluate the effect of feeding babul pods (*Acacia nilotica*) meal on the growth performance of Black Bengal male goat kids. Eighteen goats (average body weight 10.3 kg, aged 7–8 months) were randomly assigned to three dietary treatments (T1, T2, T3) with six animals per group. All goats received roughage and a homemade concentrate mixture (20% crushed maize, 15% mustard cake, 10% groundnut cake, 15% chana besan, 10% rice polish, 25% wheat bran, 2% mineral mixture, 1% salt, 2% calcite) formulated to meet nutrient requirements as per ICAR, 2013. In T1, the standard concentrate was fed; in T2 and T3, 10% and 20% babul pods replaced mustard cake and wheat bran to maintain isonitrogenous diets. Babul pods contained CP 17.34%, EE 4.15%, CF 15.32%, NDF 34.56%, ADF 28.25% and ADL 2.32%. Average daily gains were 40.72 g (T1), 46.82 g (T2), and 52.04 g (T3), with no significant differences ($P > 0.05$). Feed conversion ratios (DM and CP basis) were also similar. However, parasitic load was significantly reduced ($P < 0.05$) in T2 and T3. Feed cost was highest in T1, while net profit was highest in T3, indicating economic and health benefits of babul pod inclusion.

KEYWORDS: Anti-haemonchus, Babul pods meal, Goats, Performance, Tannin

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Goats are vital to the Indian economy, providing meat, milk, fibre, and skin. India has 148.88 million goats (20th Livestock Census, 2019), mostly reared on poor-quality roughages such as crop residues, tree foliage, and agro-industrial by-products. Seasonal shortages and low nutritive value of feeds limit livestock productivity in arid and semi-arid regions (Gerbu et al., 2018). To address this, alternative feed resources, including tree-based and agro-industrial by-products, are gaining importance (Srihitha et al., 2025). Among these, babul (*Acacia nilotica*), introduced in 1876 for its drought, submergence, and salinity tolerance, now covers ~2 million hectares in India, with mature trees yielding 20–40 kg pods/year (Shukla et al., 1984). While tannins (>5%) can reduce digestibility and nitrogen balance (Kumar et al., 2014), babul pods are protein-rich and abundant in tropical areas, though underutilized for small ruminants. They can serve as an energy source in concentrate mixtures, improving energy utilization, and contain essential amino acids comparable to egg protein (Barman et al., 2006). Studies show *Prosopis juliflora* pods can replace up to 40% of sheep concentrates without adverse effects (Chaturvedi & Sahoo, 2013). Against this backdrop, the present study was designed to evaluate babul pods as a protein source in growing goat rations for growth performance and anti-haemonchus effects.

Animals, feeding, management and dietary treatments

The study evaluated babul pods as a protein replacement in the concentrate mixture of growing Black Bengal male goats. Eighteen goats (average 10.3 kg) were assigned to three treatments (T1, T2, T3; six animals each). All received roughage and a homemade concentrate meeting ICAR (2013) nutrient requirements. T1 contained maize, mustard cake, groundnut cake, chana besan, rice polish, wheat bran, mineral mixture, salt, and calcite (Table 1). In T2 and T3, 10% and 20% babul pods replaced mustard cake and wheat bran to maintain iso-nitrogenous diets. Goats were stall-fed under hygienic conditions, exercised in a confined area, and provided free access to fresh drinking water.

Table 1. Ingredient composition (%) of concentrate

Attributes	T1	T2	T3
Maize crushed	20.0	20.0	20.0
Mustard Cake	15.0	12.0	11.0
Babul pods	0.0	10.0	20.0
Ground nut cake	10.0	10.0	10.0
Chickpea flour	15.0	15.0	15.0
Rice polish	10.0	8.0	6.0
Wheat bran	25.0	20.0	13.0
Mineral mixture*	2.0	2.0	2.0
Common salt	1.0	1.0	1.0
Calcite	2.0	2.0	2.0

*Composition of mineral mixture (% on DM basis): Calcium (20%), Phosphorus (12%), Cobalt (0.012%), Copper (0.10%), Iodine (0.026%), Iron (0.4%), Magnesium (5%), Manganese (0.12%), Sulfur (2.0%), Zinc (0.80%) and Fluorine (0.07%).

Data collection and statistics

During the 75-day experiment, daily feed intake and body weights (recorded every 15 days) were monitored, with weights taken on two consecutive days before feeding and watering. At the end of the trial, three goats per group were selected for faecal examination. Samples were collected directly from the rectum, stored in labeled polythene bags, and examined using the McMaster technique (Coles et al., 1992) to determine eggs per gram (EPG) and oocysts per gram (OPG), indicating infection severity. Dry matter content was determined by oven drying at 100 °C overnight. Pooled feed and refusal samples were dried, ground (2 mm sieve), and analyzed for nitrogen via the Micro Kjeldahl method. Proximate composition followed AOAC (1999) procedures, fibre fractions were determined as per Van Soest et al. (1991), and calcium and phosphorus were estimated using Talpatra et al. (1940) method. All analyses were performed in the Animal Nutrition Laboratory, Bihar Veterinary College, Patna, India.

Data were analyzed using SPSS (Version 20.0, 2011) with one-way ANOVA and Duncan's multiple range test, and means were separated using LSD, following Snedecor and Cochran (1994) to assess significance between control and experimental groups.

The DM content of *Acacia nilotica* pod meal was 94.18 % and it contained CP 17.40, EE 4.15, CF 15.2, respectively (Table 2). The total phenolics (%, DM) of *Acacia nilotica* pod meal were 22.60 comprising of TTPh 19.3, CT 1.94, HT 17.15 and NTPh 3.36, respectively. The concentration of tannin in the diet was 0, 1.93 and 3.86 percent for T1, T2 and T3, respectively. The concentrate mixture prepared was iso- nitrogenous and iso-caloric among the groups. Similarly, Paswan et al. (2017) Reported CP 17.3% and total tannin phenolics 19.1% in *Acacia nilotica* pod meal.

Table 2. Chemical composition (%, DM) of feed stuffs offered to goats

Attributes	Concentrate mixture	*Acacia nilotica* pod meal	Sorghum (green)
Dry matter	93.80	95.20	24.80
Organic matter	90.50	94.38	86.80
Crude protein	19.60	17.40	7.58
Ether extract	3.40	4.15	3.64
Crude fibre	5.60	15.20	28.90
Total ash	9.50	5.62	13.20
Nitrogen free extract	61.90	57.65	46.68
Phenolic constituents (%, DM)			
Total phenolics	-	22.64	-
Total tannin phenolics	-	19.30	-
Non- tannin phenolics	-	3.34	-
Condensed tannin	-	1.92	-
Hydrolysable tannins	-	17.38	-

Dry matter intake (DMI, kg/100 kg BW) ranged from 2.93 to 3.27 across treatments, with no significant differences, indicating that inclusion of *Acacia nilotica* pod meal did not affect palatability (Table 3). Similar findings were reported by Paswan et al. (2017), where 30% pod meal in goat concentrates had no effect on DMI, and Kushwah et al. (2012), who observed no reduction in intake with up to 33% inclusion in lactating goats. Hidosa et al. (2020) also noted no palatability issues with up to 38% *Acacia tortilis* pods. Lalhariatpuii et al. (2022) and Balaji et al. (2025) reported DMI in Black Bengal goats at 2.7–3.0% of body weight. Min et al. (2003) found that condensed tannin (CT) levels >55 g/kg DM reduced intake, while 20–45 g/kg had no effect. In this study, 20% *Acacia nilotica* pod meal (3.86% tannin of TDMI) did not reduce voluntary intake, supporting earlier reports. For a 9.98 kg goat, NRC (2007) recommends 23 g protein/day for maintenance plus 14 g for 50 g/day growth, while ICAR (2013) suggests 55 g/day for a 10 kg goat at the same growth rate. In this study, average protein intake met ICAR standards and exceeded NRC values, ensuring optimal growth. Protein intake, as a percentage of requirement, was statistically similar across groups (92.5–102.5%). However, nutrient utilization efficiency for DM and CP was 25.12% and 24.42% lower, respectively, in the 20% *Acacia nilotica* pod meal group compared to control (Table 3). Paswan et al. (2017) also observed 22.60% lower FCR at 20% inclusion, while Hidosa et al. (2020) reported improved efficiency at higher (38%) *Acacia tortilis* inclusion.

Table 3. Effect of feeding *Acacia nilotica* pod meal on growth performance, feed intake and feed conversion efficiency in goats

Attributes	T1	T2	T3	SEM	P-value
Initial body weight (kg)	10.49	10.39	9.99	3.17	0.987
Final body weight (kg)	13.54	13.90	13.90	2.98	0.990
Body weight gain (kg)	3.06	3.51	3.90	0.51	0.296
Average daily gain (g)	40.72	46.82	52.04	6.75	0.294
DMI (g/day)	308.75	303.13	307.63	23.39	0.968
DMI (% body weight)	3.27	2.93	3.20	0.79	0.901
Concentrate: Roughage	2.13	2.13	2.07	0.65	0.452
Total protein intake (g/day)	51.2	50.3	50.7	12.44	0.683
Protein intake (% requirement)	102.5	92.5	101.8	0.48	0.991
DMI (kg/kg gain)	8.00	6.75	5.99	1.35	0.368
CPI (kg/kg gain)	1.31	1.12	0.99	0.18	0.255
Feed cost (Rs./day)	3.92	3.91	3.92	0.05	0.977
Gross profit (Rs.)	1222	1405	1561	202.54	0.294
Net profit (Rs.)	928	1111	1267	203.16	0.295

DMI- Dry matter intake; CPI- Crude protein intake

The growth rate (g/day) of goats was non-significantly higher in T3 (52.04 ±3.8) compared to T2 (46.8±6.0) and T1 (40.7±4.2), respectively (Table 3). The ADG was 14.98 and 25.48% higher in T2 and T3 as compared to control group. Hidosa et al. (2020) reported higher weight gain in 38% inclusion of *Acacia tortilis* in concentrate mixture of goat. Change in weight of animals at 15 days' interval (fortnightly) up to 75 days has been presented in Figure 1. Hence, supplementation of Babool pods to black Bengal growing goats had no adverse effect on their body growth rate.

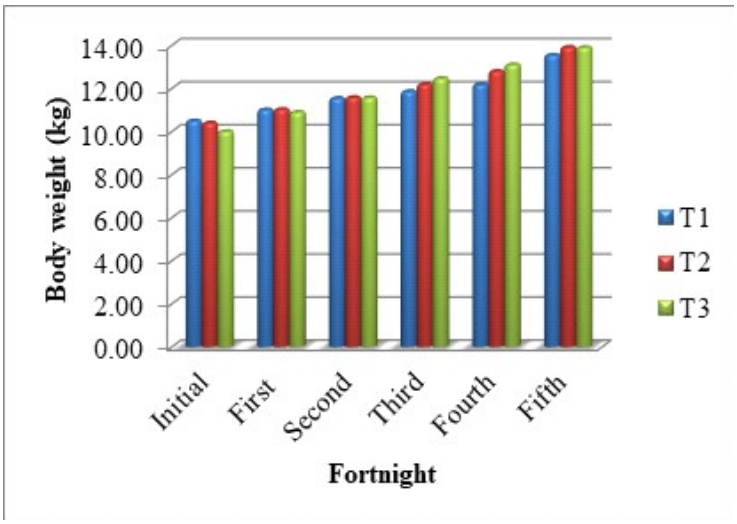

Fig.1. Effect of feeding *Acacia nilotica* pod meal on fortnight body weight of goats

The EPG count was significantly higher in T1 (762.50) than T2 (400.00) and T3 (337.50) group represent anti haemonchus activity of babool pods (Table 4). The maximum EPG was noted in control group and minimum in 20% babul pod meal supplemented group followed with 10% supplemented group. Similarly, Paswan et al. (2016) reported significantly reduced (P<0.05) EPG after 20% supplementation of babul pod meal in goat ration. This study showed that babul pod of this region had antiparasitic effect. This might be possible due to the presence of tannin in babul pod.

Table 4. Effect of feeding *Acacia nilotica* pod meal on eggs per gram (EPG) in faeces of goats

Attributes	T1	T2	T3	SEM	P-value
Initial EPG	562.50	500.00	775.00	246.57	0.529
Final EPG	762.50^{c}	400.00^{ab}	337.50^{a}	173.00	0.074

Feed cost was highest in the T1 group, whereas net profit was maximized in the T3 group (Table 3). Inclusion of babul pods at 10% and 20% of the ration for growing Black Bengal goats resulted in an additional weight gain of 458–849 g and an increase in average daily gain by 6–11 g/day. In the present study, the cost of production ranged from Rs.98 to Rs.75 per kg body weight gain in Black Bengal goats, which is considerably lower than the Rs.169–176 per kg body weight gain reported by Meetu et al. (2025) for Beetal goats.

CONCLUSION

The study demonstrated that babul (*Acacia nilotica)* pods can be included in the concentrate mixture of growing Black Bengal goats at levels up to 20% without affecting feed intake or growth performance. Supplementation significantly reduced parasitic load, indicating strong antiparasitic potential, and improved economic returns by lowering feed cost per unit weight gain.

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Effect of Bypass Fat Supplementation on Productive Performance of Buffaloes

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ABSTRACT

One farm trial was conducted to assess the effectiveness of bypass fat on overall performance of milch buffalo during 2022-23 in the Khijrabad, Sohali and Khubahedi village of SAS Nagar district of Punjab. Twenty lactating Murrah buffaloes were selected and distributed equally in two groups of ten buffaloes in each group, i.e. T1 (control) and T2 (supplementation bypass fat @ 100g/day/ buffalo) up to 150 days. The results of study revealed supplementation of bypass fat had significantly ($P<0.05$) increased the milk production in milch buffalo. The mean milk yield during the recoding was found to be 10.19 ±0.36 and 11.29±0.34 l/day in group T1 and T2, respectively; which was 10.78 percent higher in group T2 than the control. The average length of the postpartum estrus cycle (67.70±6.20 days) and service period (91.43±8.33days) was significantly ($P<0.05$) reduced in T2 than T1 (86.40±11.57days and 131.00±12.33days, respectively). AI per conception was significantly ($P<0.05$) lower in T2 group (1.57±0.40) than the T2 (2.71 ±0.44). The study indicate that supplementing bypass fat in the diet had a significant ($P<0.05$) positive impact on both milk yield and postpartum reproductive performance in milch buffalo.

KEYWORDS: Buffalo, Bypass fat, Milk, Production, Reproduction.

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In many developing countries, including India, animals are primarily fed with agricultural by-products and low-quality crop residues, characterized by their inherently poor nutritive value and digestibility. These feed and fodder don't meet the needs of dairy livestock in terms of nutrients, optimal reproductive function, and long-term milk production (Khadda et al., 2014). The energy demand, especially during early lactation stages, is significantly higher than the available supply, limiting the production potential of animals (Sirohi et al., 2010). Bypass fat technology increases rumen energy density, enabling animals to fulfill key fatty acid requirements, enhance productive and reproductive performance (De Veth et al., 2009). Adding bypass fat to the diet of lactating animals is known to increase energy intake in early lactation, thereby reducing the negative effects of acute energy deficiency on lactation. (Tyagi et al., 2010). Considering the aforementioned details, on farm trail was undertaken to assess the effect of bypass fat supplementation on the overall performance of milch buffalo in Punjab, India.

One farm trial was conducted to assess the effectiveness of bypass fat on overall performance of milch buffalo during 2022-23 in the Raipur village of SAS Nagar district of Punjab. Twenty Murrah buffaloes in early phase of lactation were selected and alienated equally in two groups of 10 buffaloes in each group, i.e. T1 (control) and T2 (supplementation bypass fat @ 100g/ day/ buffalo beside the farmer practices). All animals were managed under farmers' own traditional feeding consisted of 5-6 kg wheat straw as dry fodder and 25 kg green maize fodder with concentrate mixture @ 4.5 kg per day/ animal. The buffaloes were selected at nearly the same body weight, milk yield, second or third parity and early lactation stage. The bypass fat was taken from the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. In the treatment group (T2), consistent supplies of bypass fat as supplementations were offered without interruption during the entire period of study. To manage internal parasites, fenbendazole was used as dewormer and

given to every animal prior to the experiment's commencement. The buffaloes were fed the feed separately, and the percentage intake per head per day was also recorded. The drinking water was offered *ad-lib*. The data recording of trial was taken up to 150 days. The milk recording was conducted in the morning and evening time fortnightly. Milk samples were taken fortnightly at milking time and examined for milk composition i.e. SNF, milk fat, total solid, milk protein and lactose by milk scanner and 6% fat correct milk was computed using the formula (Rice et al., 1970): 6% FCM yield (kg)= 0.308x total milk (kg) + 11.54 x total fat (kg). Reproductive performance concerning the onset of post-partum estrus, service period and AI per conception was also recorded. The significance of the difference between treatment means was evaluated using the Student's t-test after statistically analyzing the data in completely randomized design (CRD) (Snedecor and Cochran 1989).

The proximate composition of wheat straw, green maize fodder and concentrate mixture is presented in Table 1. The dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE), and total ash content in the concentrate mixture were 90.05, 91.94, 21.03 and 11.56, 5.02, 54.33 and 8.06 per cent, respectively.

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

Particular	Concentrate mixture	Wheat straw	Green maize fodder
OM	90.50	91.40	95.00
DM	90.05	91.94	21.03
CP	21.40	3.60	8.10
EE	5.10	0.83	2.28
Cellulose	12.30	41.00	35.10
NDF	34.50	79.20	62.30
ADF	16.30	59.80	38.60
Hemicellulose	18.20	19.40	23.70
ADL	5.40	7.50	4.00
Total Ash	10.10	8.10	4.94

The addition of bypass fat in diet did not affect the body weight of the buffaloes under experimentation (Table 2). The results of this trial are under the findings of Ranaweera et al. (2020) and Sadrasaniya et al. (2022). The data related to dry matter intake (DMI) during the study period was found to be 13.49±0.04 and 13.53±0.06 kg/d in T1 and T2 groups, respectively (Table 2). Supplementary feeding of bypass fat did not influenced the DM intake (in terms of kg/d and DMI %) in lactating buffaloes which could be attributed to inertness of the added fat in rumen because of its low solubility. Corresponding results were also published by Sadrasaniya et al. (2022) for Mehsana buffaloes and Mane et al. (2017) and Sihag et al. (2020) for crossbred cattle.

Table 2. Effect of supplementary bypass fat feeding on production performance of lactating buffalo

Particulars	T-1 (Control)	T-2 (*Bypass fat*)
Initial body weight (kg)	498.2 ±6.39	496.7±8.54
Final body weight (kg)	505.8 ±9.54	504.2 ±5.77
DMI (kg/d)	13.49±0.04	13.53±0.06
DMI (% of BW)	2.67±0.02	2.68±0.01
Initial Milk yield (l/d)	10.10±0.42	10.00±0.26
Av. Milk yield (l/d)	10.19^{b} ±0.36	11.29^{a}±0.34
6FCM yield (l /d)	11.83^{b} ±0.43	13.64^{a} ±0.36
% Increase in milk yield	-	10.78
% increase in FCM yield	-	15.30
Fat %	6.57^{b} ±0.53	7.21^{a} ±0.46
Total fat (kg/d)	0.69	0.81
Lactometer reading	29.92 ±2.23	30.48 ±2.31
Milk protein (%)	3.89	3.86
Lactose (%)	5.31	5.28
SNF %	9.81±0.21	9.73±0.23
Total solid %	16.38	16.94

Group mean with different superscripts differed significantly ($p<0.05$).

The findings showed that the buffaloes supplemented with bypass fat (T2) produced significantly ($P<0.05$) higher average daily milk yield and 6 % FCM yield in contrast to the T1, which was 10.78 percent higher in group T2 than control (Table 3). Buffaloes in group T2 produced 15.30 per cent higher 6% FCM than control (T1). More FCM production in buffalo indicated better utilization of nutrients due to bypass fat. Increased milk yield by feeding of bypass fat were also reported earlier by various researchers (Kumar et al. 2019, Saxena et al. 2019, Hifzulrahman et al. 2020, Butt et al., 2020, Rajneesh et al., 2021). This increase in milk yield is probably attributed to the greater energy density of the ration provided by the bypass fat, which helps mitigate the negative effects of energy imbalance in by dairy animals. Fat % in milk was significantly ($P<0.05$) higher in group T2 (7.21±0.46) as compared to control (6.57±0.53). Kirovski et al. (2015) also reported increased milk fat content as a consequence of feeding protected fat. There was no discernible variation in the content of solids-not-fat (SNF), milk protein, and lactose among the treatment groups, indicating that the supplementation of bypass fat in lactating buffaloes did not affect these parameters. Similar results were also reported by Naik et al., 2009, Tyagi et al., 2009 and Sirohi et al., 2010. However, the total solid content in milk was significantly higher in group T2 compared to the T1. Rohila et al. 2018 also reported increase TS in milk.

Table 3. Effect of bypass fat supplementation on reproductive performance of lactating buffalo

Particulars	T-1 (Control)	T-2 (*Bypass fat*)
Post-partum oestrus (days)	86.40^{b}±11.57	67.70^{a}±6.20
Service period (days)	131^{b}.00±12.33	91.43^{a} ±8.33
No. of services (AI) per conception	2.71^{b} ±0.44	1.57^{a} ±0.40

Group mean with different superscripts differed significantly ($p<0.05$).

The mean duration of postpartum estrus period and service period (67.70±6.20 days and 91.43±8.33 days) was reduced significantly ($P<0.05$) in T2 group than T1 (86.40±11.57days and 131.00±12.33 days), respectively (Table 4). The services per conception was also observed to be significantly ($P<0.05$) higher in T1 group than the T2. The no. of AI per conceptions in T2 and T1 group was recorded 1.57±0.40 and 2.71 ±0.44, respectively. The findings indicated that supplementing bypass fat in the diet significantly ($P<0.05$) affected the postpartum reproductive performance in milch buffalo. The results of this study corroborate with the findings of (Savsani et al. 2013 and Ramteke et al. 2014, Prajapati 2018). The onset of cyclicity appears to be associated with involution process of uterus. Research suggests that the duration of uterine involution may be shortened with supplementation of bypass fat, potentially leading to an earlier commencement of cyclicity. Savsani et al. (2013) and Ramteke et al. (2014) have reported a reduced timeframe for the occurrence of the first postpartum heat in buffaloes supplemented with bypass fat compared to those in the control group. Shelke et al. (2012) also reported that reproductive performance is linked to the energy status of the animal. Dietary fats play a significant role in this aspect by providing fatty acid precursors essential for production of cholesterol and prostaglandins. These compounds exert influence on ovarian and uterine function as well as conception rates conception rates. Thus, the nutritional composition of diet, particularly its fat content, can have a substantial impact on the reproductive health and success of animals.

Table 4. Feed economics of bypass fat supplementation for lactating buffalo

Particulars	T-1 (Control)	T-2 (*Bypass fat*)
Av. Milk yield (l/d)	10.19	11.29
Additional increase in milk yield (l /d)	-	1.10
Av. Feeding cost (Rs./ day)	190	207
Additional cost of supplementary feeding (Rs. / day)	-	17
Av. Feeding cost/ lit. milk production (Rs.)	18.65	18.33
Reduction in cost of milk production/ lit. (%)	-	1.75
Gross return from sale of milk (Rs./ day)	611.40	677.40
Additional income from supplementary feeding (Rs./ day)	-	66.00
Net return (Rs./ day)	421.40	470.40
B:C Ratio	3.18	3.27
Additional B:C Ratio from supplementary feeding	-	3.88

A partial budget analysis approach was applied to those incomes and expense elements (Khadda et al., 2023). As a result, the market price of concentrate, bypass fat and fodder have been taken into account. The family members were used to manage the buffaloes prevalent during the study

period, so, the cost of labor was not taken into account in the computed. The price of used inputs was computation on basis of market price prevalent during the study period. The bypass fat procured from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana @ Rs. 170/- per kg. Selling price of milk obtained by farmers during the experimental period was taken to be Rs. 60/ lit. The average feeding cost/liter of milk produced in T2 and T1 groups was found to be Rs. 18.33 and Rs. 18.65, respectively, which indicates that, in field conditions, dietary supplementation with bypass fat considerably decreased the cost of producing milk (Table 5). Net return over feed cost of milk yield / day/ animal in T1 and T2 group was found to be Rs. 421.40 and 470.40, respectively. Additional cost and income of supplementary feeding to milch buffalo was found Rs.17.00 and 66.00 day/ buffalo, respectively. Benefit-cost ratio of 1: 3.27 was obtained during the trial period with bypass fat supplementation, which seems to be quite profitable compared to traditional feeding methods.

CONCLUSION

The results indicated that the feeding of bypass fat, improved the milk yield and its composition as well as postpartum reproductive performance in milch buffalo at farmer's field.

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National Workshop on Animal Nutrition and the AMR Challenge: A One Health Perspective from Feed to Field

Department of Animal Nutrition, Veterinary College, West Bengal University of Animal and Fishery Sciences, Kolkata in collaboration with Animal Nutrition Society of India (ANSI) organized a National Workshop on 'Animal Nutrition and the AMR Challenge: A One Health Perspective from Feed to Field' on 12th September 2025 at Vivek Bhawan, Belgachia Campus. The workshop was inaugurated by Dr. Tirtha Kumar Dutta, Honourable Vice-Chancellor of the University, Prof. Purnendu Biswas, Former-Vice-Chancellor, WBUAFS, Sri Madan Maity, General Secretary, West Bengal Poultry Federation and Organizing Secretary Prof Guru Prasad Mandal, Department of Animal Nutrition, WBUAFS in presence of Registrar of the university Dr. Partha Das, Joint Registrars of the University Dr. Pankaj Kumar Biswas and Dr. Sourav Chanda, Dr. Santanu Banik, Head, ERS, ICAR-NDRI, Dr. BN Paul, PS, ICAR-CIFA and Dr. Debasish De, PS, ICAR-CIBA. Dr Dutta stated in brief about the importance of the topic in global health perspective and hoped for the best that a solution would come from vivid discussions of this workshop. Sri Madan Maity stated that the present poultry industry growth specially in West Bengal is solely dependent on the research driven balanced nutrition, not the antibiotics. He advised to all the stakeholders to take the poultry meat and eggs without fear. Technical session was chaired by Dr. BN Paul, Co-Chaired by Dr S. Banik and Dr. Stephen Soren, Head, Department of Animal Nutrition acted as the rapporteur for the session. The main speakers for the session were Prof. Indranil Samanta, Head, Department of Veterinary Microbiology, WBUAFS and Dr. Sudipto Halder, Director, Agrivet Research and Advisory Private Limited, Kolkata. Prof. Samanta explained in details about mechanism of antimicrobial resistance, importance of AMR in livestock health sector, data driven findings on role of antibiotic misuse as well as the contaminated environment specially in low-biosecurity farms in generation of superbugs and the social impact of the menace. His findings in West Bengal showed why antibiotics are prescribed in healthy livestock, how paraveterinarians, drug shop owners are involved in making informal prescription, value chain analysis of the antibiotic distribution starting from the wholesale stockist to the paraveterinarians. He stressed on mass awareness program, maintenance of good herd health and high biosecurity of the farms, and incorporation of the issue into school/college curriculum as mitigation strategy. Dr Halder briefed about the role of animal nutritionist as a problem solver with the range of products like probiotics, prebiotics, synbiotics and lantibiotics. His research on direct fed microbials (DFM) showed promising results with the growth of the birds and curbing the usage of antibiotics as growth promoters. He believed on phase-wise antibiotic replacement in animal usage and creation of phage library will make an effective solution for the menace. The workshop ended with comments from the scientists and teachers present and Kemin Industries Private Limited.

ANSICON-2025 at ANDUAT, Kumarganj, Ayodhya
(19th to 21st November 2025)

International Conference of Animal Nutrition (ANSICON-2025) on "Global Resilience in Animal Nutrition: Innovations for a Sustainable Future" was successfully conducted under the joint aegis of the Department of Animal Nutrition, C.V.Sc. & A.H., Kumarganj and the Animal Nutrition Society of India. The conference witnessed the participation of 431 delegates, including leading scientists, academicians, industry representatives, students, farmers and extension personnel from India and abroad, providing a dynamic platform for deliberations on sustainable and climate-resilient animal nutrition. The inaugural session commenced with the traditional Jal Bharo ceremony and homage to Maa Saraswati, followed by welcome addresses by Dr. V.K. Singh, Dr. P.S. Pramanik, Dr. Shivani Katoch and Dr. A.P.S. Sethi, highlighting the significance of innovation, scientific integration and sustainability. The session also featured the presentation of awards, release of the conference Souvenir and Abstract Book and felicitation of dignitaries. Eminent experts such as Dr. A. Sahoo, Dr. D.V.R. Prakash Rao and Dr. N.V. Patil shared valuable insights on emerging trends, translational research and institutional leadership in animal nutrition. The keynote address by Dr. R. Bhatta, DDG (Animal Science), ICAR, outlined strategic directions for climate-smart feeding, precision nutrition and sustainable livestock production systems. The session concluded with remarks by Col. (Dr.) Bijendra Singh, Vice Chancellor, ANDUAT, Ayodhya and a formal vote of thanks proposed by Dr. Dharmesh Tewari.

In the plenary session Dr. Vishnu Sharma highlighted nutrition as a key driver of future livestock development, stressing the importance of safe feed, precision and immuno-nutrition, methane-reducing strategies and welfare-focused feeding in response to environmental pressures and changing consumer expectations. Dr. Vibha Ahuja emphasized the contribution of GM crops and gene-edited plants in strengthening feed security, nutritional quality and climate resilience and Dr. Devender Hooda discussed the transition of Indian poultry nutrition towards precision, antibiotic-free and sustainability-oriented feeding systems, highlighting gut health management, alternative feed resources and collaborative efforts among industry, researchers and policymakers to ensure safe, efficient and welfare-friendly poultry production.

The Advances in Ruminant Nutrition session featured with seven lead, 42 oral and 38 poster presentations. The lead lectures were presented by Dr. Yutaka Uyeno (Japan), Dr. P.K. Malik and Dr. D. Rajendran (NIANP, Bengaluru), Dr. Sohan Vir Singh (NDRI, Karnal) and Dr. Sunil Ekanath Jadhav (IVRI, Izatnagar). The session emphasized climate-resilient and sustainable ruminant production through improved feeding strategies, greenhouse gas mitigation and emerging nutritional technologies. Dr. Uyeno highlighted integrated feeding approaches to simultaneously reduce methane, nitrous oxide and carbon dioxide emissions. Dr. Malik stressed the need for practical, region-specific enteric methane mitigation strategies in Indian livestock systems. Dr. Rajendran presented nano-minerals and nano-additives as novel tools to enhance nutrient utilization, productivity and environmental sustainability, while Dr. Sohan Vir Singh and Dr. Jadhav underscored the role of targeted nutrition, micronutrients and advanced delivery systems in mitigating heat stress and sustaining bovine productivity under changing climatic conditions.

The session on Innovations in Monogastric, Canine and Wildlife Animal Nutrition (IMN) highlighted advanced, welfare-oriented and sustainable nutritional strategies for monogastric livestock, companion animals and wildlife, emphasizing productivity, gut health and animal welfare. Lead lectures by Dr. A.K. Singh, Dr. Udeybir Singh, Dr. Anju Kala, Dr. Sachin Kumar and Dr. Dharmesh Tewari, supported by twelve oral and eleven poster presentations, addressed key challenges such as antimicrobial resistance, metabolic disorders and food hypersensitivity. The session highlighted repro-nutritional management in swine, precision and therapeutic nutrition in dogs, microbiome-based feeding through probiotics and biotics as alternatives to antibiotics, and personalized dietary approaches for managing pet food hypersensitivity.

The Initiatives and Innovations in Poultry Nutrition session was featuring seven lead papers, twenty-nine oral and thirty-one poster presentations, and highlighting the transition toward sustainable and antibiotic free poultry production. Dr. S.S. Paul addressed antimicrobial resistance from a One Health perspective, emphasizing surveillance and nutritional alternatives to antibiotics, while Dr. Shivani Katoch highlighted the role of polyherbal feed additives in improving gut health, immunity and performance. Dr. S. Nayak presented spray-dried animal plasma as a safe and functional protein source, Dr. P. Vasan emphasized scientific sampling strategies for accurate mycotoxin estimation and Dr. Rajesh Nehra reviewed advances in precision nutrition, feed formulation and biotechnology-based additives. The session also featured innovative concepts such as histidine enriched functional broiler meat by Dr. V. K. Singh and bacteriophages as antibiotic alternatives by Dr. Anju Nayak.

The Feed and Fodder Production for Climate Resilience session focused on climate-smart, cost-effective and resilient feed strategies suited to Indian livestock systems. With five lead papers presented, thirty-one oral and eighteen poster presentations, the session highlighted innovations in perennial fodders, crop residue enrichment, agro-industrial by-product utilization and complete feed technologies. Dr. Ravindra Kumar emphasized moringa as a high-yielding, protein-rich perennial fodder for mitigating green fodder scarcity, while Dr. A.P. Dhok demonstrated residue valorization technologies that improve digestibility, performance and environmental outcomes. Dr. Nitin Tyagi presented integrated waste-to-wealth approaches for paddy straw and by-products and Dr. M. K. Gendley highlighted complete feed systems as a practical solution to feed deficits and climate stress. Dr. M. S. Mahesh discussed the potential of DDGS as a sustainable and economical ruminant feed with appropriate quality control. The session highlighted that feed and fodder innovations as key drivers of climate resilience, circular bioeconomy and sustainable livestock production.

The Sustainable Fish Nutrition session focused on how better feeding practices can support sustainable aquaculture, climate resilience and future food security. Dr. K. Ambasankar explained India's fast-growing aquaculture sector and highlighted indigenous feed innovations, cooperative feed models and functional feeds that reduce feed cost, improve feed efficiency and increase farmer income. Dr. S.B.N. Rao discussed the importance of modern feed testing tools such as NIRS, feed microscopy and advanced nutrient evaluation systems to ensure feed quality, safety and precise feeding under changing climate conditions. Dr. Laxmi Prasad presented a global view of aquaculture growth and emphasized the need for balanced,

eco-friendly feeds, alternative protein sources and precision feeding in systems like cage culture, RAS and biofloc. So, the science-based, sustainable fish nutrition is essential for productive, environmentally safe and climate-resilient aquaculture.

The Training and Extension Approaches for Improving Animal Productivity session highlighted extension as the critical link between science and farmers in an era of climate stress and technological change. Through five lead papers supported by sixteen oral and fifteen poster presentations, the session highlighted extension reforms, digital platforms, artificial intelligence and participatory learning as key tools for enhancing livestock productivity and sustainability. Dr. Ram Batuk Singh emphasized inclusive, ICT-enabled and institutionally coordinated extension reforms, Dr. M. J. Chandre Gowda presented AI-driven advisory and precision nutrition tools for real-time decision making, Dr. Subodh Kumar highlighted extension-led pathways for food security and livelihood generation and Dr. Suma N. She focused on climate-resilient livestock systems in hilly regions using indigenous breeds and integrated farming. Collectively, the session reaffirmed that farmer-centric, technology-supported and locally adapted extension systems are vital for resilient livestock development.

The Emerging Trends in Nutritional Research session featuring six lead papers along with oral and poster presentations, the session showcased how modern nutrition research is responding to challenges of climate change, animal welfare, metabolic disorders and evolving consumer expectations. Dr. Debashis Roy addressed ethical and regulatory dimensions of nutrition research, while Dr. S.V. Singh and Dr. Chander Datt emphasized nutrition-based prevention of production diseases and effective transition-phase feeding in dairy animals. Metabolic regulation through improved insulin sensitivity was discussed by Dr. Muneendra Kumar and a circular economy approach using rumen liquor derived prebiotics as antibiotic alternatives was presented by Dr. Ankur Rastogi. Consumer-focused innovation was highlighted by Dr. Avishek Biswas through the concept of designer and functional milk.

The Farmers–Academia–Industry Interface for Animal Welfare session chaired by Dr. Manvir Singh, Chief Executive, Paayas Milk Producer Company, Jaipur, the session set the tone by emphasizing that true animal welfare begins with practical, affordable and locally adaptable nutrition. Dr. Prashant Shinde of Cargill Animal Nutrition & Health highlighted how sound calf nutrition and management form the foundation of lifetime health, productivity and welfare. Active participation from industry leaders including Gyandhara, Fine Organics, Zamira, Kemin, Innovatlef, Sapan Feeds, Dayal Feeds and R.N. Feeds, alongside scientists, students and a large group of progressive farmers, made the discussions dynamic and solution oriented. Farmers openly shared challenges related to feed costs, fodder scarcity and calf survival, while experts responded with welfare-focused, low-cost and farmer-friendly nutritional and management strategies.

Glimpses of ANSICON-2025 Conference

ANSICON-2025 concluded with a perfect blend of celebration and reflection, following two days of rich scientific discussions. The gala evenings provided a relaxed and friendly setting for delegates, scientists, industry members and students to interact and celebrate the spirit of the conference. The valedictory session graced by Dr. V.R.B. Sastry as Chief Guest, along with Dr. A.P.S. Sethi, Dr. Udeybir Singh, Dr. P.S. Pramanik and Dr. V.K. Singh, who highlighted key outcomes, future directions and the importance of linking research with practical field applications. Best presentation awards were presented to recognize outstanding contributions by researchers and young scientists. The conference concluded with a vote of thanks by Dr. Sachin Gautam, leaving participants motivated, connected and committed to advancing sustainable and resilient animal nutrition.

Recommendations

- The clean and safe feeds, precision and immune-supportive nutrition and climate-smart feeding practices are essential for sustainable livestock development.
- Ruminant nutrition must advance toward integrated, climate-adaptive feeding systems that enhance productivity, improve nutrient-use efficiency, reduce emissions and strengthen heat-stress resilience.
- Feeding strategies for monogastric animals, poultry, companion animals and captive wildlife should be precision and microbiome based, prioritizing gut health, antibiotic-free approaches, functional additives and strong feed safety and mycotoxin control.
- Feed and fodder systems should focus on climate resilience and resource efficiency through perennial fodders, crop residue valorization, agro-industrial by-products, DDGS, complete feeds and local resources, while aquaculture nutrition should adopt sustainable alternative proteins and functional feeds.
- For long-term sustainable productivity, welfare and food security interdisciplinary research, transparent regulation, effective extension and coordinated stakeholder collaboration should be strengthened.

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