Review Article

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Sustainable Poultry Production: Enhancing Heat Stress Resilience through Nutritional Management

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Poultry production is a vital aspect of agricultural economy worldwide providing affordable and high quality protein source. However, heat stress acts as a significant threat to poultry production. Heat stress can be defined as a physiological condition in which an animal is unable to adequately dissipate excess body heat due to the combined effect of environmental factors such as high ambient temperature, humidity, solar radiation and low air movement resulting in impaired welfare, health and productivity. Unlike mammals, birds lack sweat glands, so they rely on evaporative cooling through the respiratory system to dissipate excess heat. Relative humidity along with ambient temperature plays an important role to assess thermal stress. Their combined effect expressed as Temperature- Humidity Index (THI), serve as a reliable indicator of thermal load and physiological stress. Thus, heat stress is classified based on THI and duration of exposure. Heat stress induces significant behavioural, morphological and physiological changes in poultry, leading to substantial economic losses. Hence, development and implementation of effective mitigation strategies are important. Among various mitigation strategies of heat stress, nutritional management seems to be easy, affordable by farmers and most effective. Supplementation of electrolyte helps maintain acid-base balance and hydration, while vitamins A, C and E improve egg production, hatchability and overall health. Probiotics such as *Bacillus spp*. improve gut health and stress resilience. Dietary plant extracts, such as rosemary and boldo leaf, provide antioxidant support. *In ovo* feeding can improve thermo tolerance by promoting optimal embryonic growth before hatch. Additional approaches such as controlled feed restriction, wet mash feeding, dietary fat supplementation and specific amino acid fortification further help reduce negative impact of heat stress. Strengthening nutritional and management strategies is essential to improve poultry adoptability and sustain productivity with rising global temperature.

Keywords: Poultry, Heat stress, THI, Nutritional management

Introduction

Poultry production is an essential component of global agriculture, contributing significantly to food security by providing an affordable and dependable source of high quality protein worldwide. However, the sustainability of the sector is increasingly threatened by heat stress (HS) due to rising global temperature in the tropical and subtropical regions of the world including India (Wasti et al., 2021). Poultry are very prone to HS due to their feather covered bodies, high metabolic heat production and the absence of sweat gland which limit their ability to dissipate heat efficiently (Akbarian et al., 2016). HS occur when bird struggle to maintain a balance between heat generation and heat loss and it can impair growth, reproductive and immune function of poultry while also raising production and management cost (Nawaz et al., 2021). High humidity intensifies the effect of heat stress in poultry which further reduces feeding, growth, production and quality of eggs (Awad et al., 2019).

The thermoregulatory capacity of poultry depends on multiple factors such as relative humidity, feeding schedule, age, sex, breed characteristics, body weight and growth rate (Farag and Alagawany, 2018). Generally, the normal body temperature of chickens ranges from 41–42°C while their optimal thermo neutral zone is 18-21 °C ensuring maximum growth and performance (Kumari and Nath, 2018). Exposure to temperature above this range, whether acute or chronic, can disrupt normal physiological function reducing gut immune defense and impairing nutrient digestion. This can trigger gastro intestinal inflammation negatively affecting overall health and increasing mortality risk (Kadykalo et al., 2018). Therefore, understanding and mitigating HS is critical for maintaining poultry health, welfare and sustainable production under changing climatic condition.

Effect of Heat Stress on Growth, Reproduction and Immune Status of Chicken

The hypothalamus plays a central role in coordinating energy balance through complex neuroendocrine signaling pathways. When animals are exposed to HS, the neurogenic system gets activated. During the initial phase of HS, the Sympathetic Adrenal Medulary Axis (SAM) plays an important role in maintaining homeostasis. In contrast, in case of chronic HS, the Hypothalamo Pituitary Adrenal Axis (HPA) becomes activated (Fernandes et al., 2023).

The physiological mechanisms and effect of HS in poultry are shown in Figure 1.

High environmental temperature disturb normal functioning of the hypothalamus which in turn lowers Luteinizing Hormone (LH) levels in the blood stream and reflects disruption of the reproductive hormonal axis (Kala et al., 2017). Ambient temperature adversely influences key reproductive traits in both laying hens and breeder

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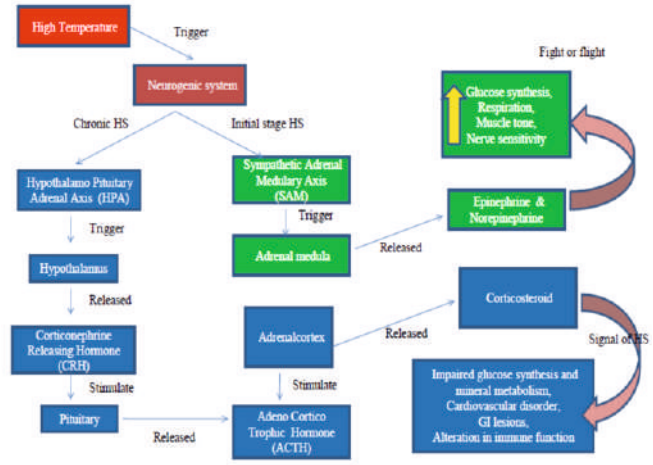

Figure 1. Physiological responses initiated during the initial phase of HS (green section) and prolonged HS (blue section) along with their respective consequence in poultry exposed to HS

flocks including egg weight, egg output, shell quality, hatchability and fertility (Yousaf et al., 2017). Male breeders are generally more sensitive to heat induced infertility than females (Bonato et al., 2014). Exposure to temperature beyond thermo neutral zone, triggers excessive generation of reactive oxygen species which leads to oxidative stress and lipid peroxidation causing damage to testicular tissues. Consequently, semen characteristics decline with reduction in semen volume, testicular size, sperm concentration, motility and viability along with impaired development of spermatogenic cells (Türk et al., 2016).

Beyond reproductive impairment, HS also compromises immune system which is crucial for maintaining poultry bird health and productivity. Figure 2 depicts impact of HS on poultry growth and productivity. During HS, major lymphoid organs such as spleen, thymus and bursa of fabricus shrink in size indicating suppression of the immune system in chickens (Awad et al., 2019).

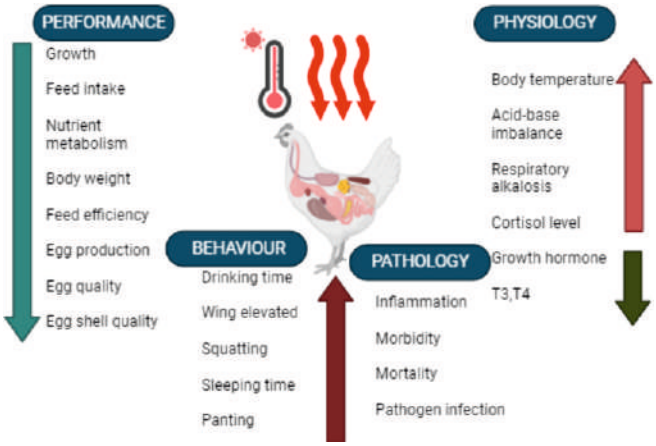

Figure 2. Impact of HS on poultry growth and productivity

Influence of Heat Stress on Heat Shock Protein

HS affects immune efficiency in poultry by increasing production of heat shock proteins (HSPs). HSPs are a group of proteins produced by organisms when they are exposed to extreme heat. These proteins work as molecular chaperones, helping maintain cellular stability by recognizing proteins that have become misfolded or damaged and guiding them either towards proper folding or degradation (Balakrishnan et al., 2023). Repeated cyclic chronic HS generally increases the activity of HSPs and antioxidant enzymes. However, acute or continuous long term HS weakens antioxidant defense system, lowers mitochondrial function and enzyme activity, increases protein breakdown and disrupts normal cellular process (Ma et al., 2021; Madkour et al., 2022)

Cytochrome P450 (CYP450), heme oxygenase-1 (HO-1) and nuclear factor erythroid 2 related factor (Nrf-2) are key components of the cellular defence system that detoxify reactive species and mitigate oxidative stress. In HS condition, Nrf-2 activates antioxidant pathways and up regulates HO-1, enhancing cellular protection (Men et al., 2021; Miao et al., 2020). Acute HS in broilers (26, 29, 32 and 35 °C for 6 hours increases hepatic Nrf-2 and HO-1 expression (Miao et al., 2020), where as constant chronic HS suppress their levels (Yilmaz and Gul, 2023a).

CYPP450 enzyme, which function as a heme dependant mono oxygenases, also respond variably- generally increasing under chronic HS, but decreasing during acute exposure (Li et al., 2023; Yilmaz & Gul, 2023b). HS furthers alters poultry behaviour, such as increased resting, moving away from other birds in the flock, extending and dropping their wings enhance heat dissipation. As HS intensifies, birds rely on more pronounced thermoregulatory behavior like panting and gular fluttering to dissipate excess heat (Li et al., 2015). The primary physiological response to HS is the activation of heat shock factors which trigger HSP formation in different tissues. These proteins are highly conserved and can also be induced by various non thermal conditions such as hormonal changes, restricted feed intake, hormonal regulation, tissue growth and immunological challenges including bacterial infection, inflammation and fever (El-Hack et al., 2018).

Based on molecular weight, HSPs are categorized into HSP 100, HSP 90, HSP 70, HSP 60, HSP 40 and small HSPs. Among these, HSP70 is widely regarded as a reliable indicator of thermal stress, particularly when body temperature exceeds 38.6°C (Berger et al., 2016). It enhances cytoprotection, supports proper protein folding and contributes significantly to maintaining epithelial integrity by strengthening tight junctions and improving gut barrier function under high temperature exposure (Chauhan et al., 2014). HSP70 expression is markedly up regulated across multiple tissues during thermal stress in a range of poultry species including quail, turkey and chickens (Kennedy et al., 2022).

Nutritional interventions can modulate these stress response as evidenced by reduced HSP70 expression in broilers supplemented with vitamin E and selenium. The lowest HSP70 expression were observed in birds ssupplemented with vitamin E (100 mg/L) combined with selenium (0.2 mg/L) and vitamin E (200 mg/L) combined with vitamin C (200 mg/L), suggesting improved cellular protection and better thermo tolerance under HS condition (Singh et al., 2023). The major effect of HS on poultry has been given in Table 1.

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Table 1: Symptoms of heat stress in various species of poultry

Species	Temperature & Humidity	Symptoms of heat stress	Reference
Broiler (42days age)	34 °C	Decrease in feed intake (16%) and body weight gain (29 %).	Alaqil et al., 2022
Broiler chicken	30 °C	Decrease in body weight, reduced insulin-like growth factor 1 (IGF-1), rise in cholesterol and glucose level, reduced villus surface area, height, crypt depth and high FCR.	Abdelqader et al., 2020
Laying Hen (32 weeks Hen)	33 °C and RH - 66 %	Lower in egg production (11%), Decrease in feed intake (30%).	Kim et al., 2024
Jinding duck	12 hour of HS at 40°C	Prolonged HS caused an initial rise followed by a decline in antioxidant activity and HSP expression. After 12 hrs of HS, increased gut permeability and compromised barrier integrity were shown. Digestive enzyme activity markedly reduced and there was significant decline in intestinal immune cell population.	Liu et al., 2025
Turkey (35 days)	35 °C and 75 % RH and control group in 32 °C and 60 % RH	No significant change occurred in feed intake and body weight gain in HS birds compared to control. But respiratory alkalosis and panting observed in HS birds.	Crespo and Grimes, 2024

Nutritional Management to Mitigate Heat Stress

Vitamins such as A, B- complex, C, D, are widely used to enhance immune function and antioxidant defense in poultry exposed to HS (Akinyemi and Adewole, 2021). Evidence suggests that combining vitamins is more effective than supplementing them individually in mitigating HS. In particular, the synergistic action of viatamin C and E improved feed efficiency, growth performance and immune response in heat stressed chickens (Attia et al., 2018). Similarly dietary inclusion vitamin E with organic selenium in the diet has been reported to lower mortality while enhancing growth and carcass traits in broilers under HS (Calik et al., 2022). Furthermore, dietary supplementation of vitamin E and selenium significantly improved haematological and biochemical parameters, liver enzyme activity, serum chloride levels, antioxidant status and heat shock protein response in broilers compared to the control group ($P<0.05$) (Singh et al., 2023). In addition, broiler chicken meat can be effectively enriched by supplementing the diet with 0.30 mg/kg selenium and 200 mg/kg vitamin E without adversely affecting bird performance while also, enhancing immune response (Sridhar et al., 2022).

Moreover, zinc, manganese, chromium and selenium, along with other trace elements such as calcium, phosphorus, copper, iron, sodium, potassium, magnesium and iodine, have been extensively studied and shown to play a beneficial role in mitigating the adverse effects of HS in poultry (Mir et al., 2018).

Plant-based supplements further extend these protective effects. For example, dietary rosemary extract may enhance HSP70 and CRYAB gene expression, protecting birds before and during heat exposure (Tang et al., 2018). Boldo leaf extract improves growth, blood health and antioxidant activity under HS (Abo Ghanima et al., 2019). Other supplements, such as *Morinda citrifolia* (Noni), also offer protective effects (Flees et al., 2017), while resveratrol supports intestinal health by strengthening gut morphology, increasing goblet cells and regulating tight junction genes (Zhang et al., 2017).

Oxidative stress and poor gut health affect broilers critically especially under HS in tropical and sub tropical regions (Tripathi et al., 2025). Traditionally, antibiotic growth promoters were used to manage these issues, but concerns over antibiotic resistance and antibiotic free poultry products have driven the shift to phytochemicals (Moustafa et al., 2021). Quercetin, a plant derived flavonoid has shown strong anti-inflammatory and antioxidant properties. It helps in neutralizing free radicals, increase antioxidant enzyme and reduces oxidative stress, ultimately supporting better growth and overall health in broilers (Tan et al., 2022). A recent bibliometric study indicates that compounds such as flavonoids, probiotics, curcumin, resveratrol, essential oils and plant extracts can alleviate HS in poultry (Uyanga et al., 2023). Bioactive compounds such as curcumin, resveratrol, quercetin have been reported to stimulate vitagene expression and modulate the antioxidant defence system particularly through activation of the Nrf2 signaling pathway, thereby reducing oxidative damage induced by HS (Madkour et al., 2022). Consistent with these findings, dietary supplementation of resveratrol has been shown to enhance growth performance, reduce hepatic injury and improve antioxidant status by increasing the activities of the enzyme Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) along with regulating the Nrf 2 keap 1 pathway in broiler chickens (Ding et al., 2023). Furthermore, polyphenols, possess strong free radical scavenging properties, limit lipid peroxidation, regulate antioxidant enzyme activity and alleviate oxidative stress making them sustainable nutritional approach for managing HS in poultry (Mas et al., 2023).

Probiotics supplementation

It was also observed that, inclusion of probiotic, especially with mannan-oligosaccharides and *Lactobacillus* strains has been found to increase serum tri iodo thyronine (T3) and thyroxine (T4) levels in broilers suffered from HS (Sohail et al., 2010). As thyroid hormones helps in the synthesis of essential proteins, enzymes and hormones, so, an increase in their levels due to probiotic supplementation may contribute to better digestion and metabolic activity in poultry under HS (Aluwong et al., 2013). Lowering down of corticosterone level may be one primary mechanism behind this effect. Given the sensitivity of probiotics to environmental conditions, their inclusion in poultry diet is helpful in ensuring their survival and activity within the digestive tract, thereby maximizing their positive effects (El-Moneim et al., 2020).

Sahin et al. (2018) observed that, in laying hens, exposure to acute stress can significantly affect gut health by altering villus morphology and intestinal epithelial integrity. Likewise, HS also hamper gut integrity and suppress immune function in broiler birds leading to decreased overall performance (Abdelqader et al., 2020).

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In poultry production, supplementation of probiotic in broilers subjected to chronic HS helped to mitigate the adverse effects of oxidative stress, thereby improving semen quality and increasing breast meat yield (Cramer et al., 2018). Dietary inclusion of *Bacillus subtilis* in poultry feed; inhibit the growth of pathogenic microorganisms, thereby enhancing dietary protein utilization making it a valuable probiotic option for enhancing health and production capacity of poultry (El-Moneim et al., 2020).

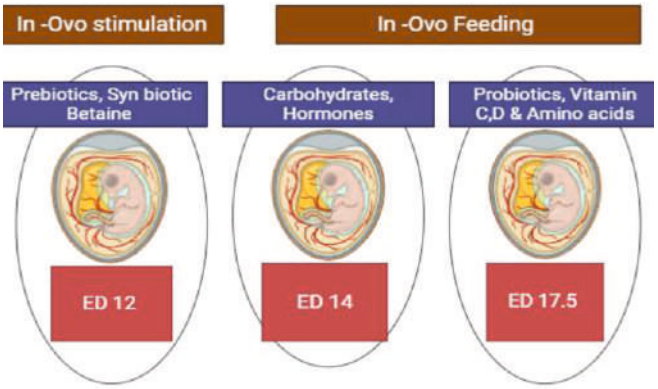
Furthermore, *Bacillus licheniformis*, improves gut function by enhancing nutrient absorption and promoting the growth of beneficial microorganisms such as *Aspergilus awamori*, *Lactobacillus* and *Bifidobacterium* making it valuable in poultry diet (Abdelqader et al., 2020). Researchers also observed that probiotic bacterium supplementation helps sustain goblet cell numbers in the ileum and caecum of heat stressed hens (Deng et al., 2012). This contributes to better gut health and overall resilience against environmental challenges.

Probiotics improve feed digestibility by breaking down complex compounds and producing enzymes like phytases, lipases, amylases and proteases, while also stimulating the bird's own enzyme production, which support better nutrient absorption and feed utilization (Flint et al., 2012).

Probiotics, beyond enzyme production also support the synthesis of antioxidants, vitamins, exopolysaccharides, thereby improving the overall nutritional profile of the feed. Cholesterol metabolism is also regulated by some probiotic strains contributing towards better health outcomes in poultry (Wealleans et al., 2017). Moreover, efficient utilization of nutrient is important because the accumulation of undigested feed in the gut can disturb microbial balance and increase the risk of digestive disorders (LeBlanc et al., 2017).

In-ovo feeding in poultry (IOF)

Embryonic interventions such as temperature adjustments during incubation and in ovo feeding (IOF) have shown promising results in developing heat-tolerant birds (Al-Zghoul et al., 2019). In IOF, nutrient and growth promoting substances are administered to the poultry embryo highlighting that nutrition before hatching is as critical as provided after hatch. More number of studies is being conducted in IOF as; incubation period significantly affects embryonic growth, hatchability and performance of chick post hatch. Recently in-ovo administration of amino acid has been explored to access their impact on HS in chickens. Studies have shown that supplementing embryo with L-Leu or methionine can enhance the thermo tolerance in chickens by regulating body temperature, increasing feed intake, enhancing the expression of immunity-related genes and positively influencing antioxidant status in chicken (Elnesr et al., 2019).



(Figure. 3: The optimal time for in-vivo inoculation varies depending on type of bio-active compound being administered. For in ovo stimulation bioactive additives such as prebiotics, synbiotics and betaine are generally administered around embryonic day 12 (ED-12) whereas, in ovo feeding carbohydrates, hormones and similar substances are introduced in ED-14 and probiotics, vitamins, amino acids are preferably administered on ED-17.5)

Zhu et al. (2020) observed that, Vitamin C plays a crucial role in enhancing DNA and histone demethylation, thereby influencing liver epigenetics by regulating gene expression. The same researchers also claimed that *in vivo* feeding of Vitamin–C at embryonic day 11 led to increased expression of genes HSP-60, Pyruvate Dehydrogenase Kinase 4 – (PDK-4) and secreted frizzled related protein 1 (SFRP-1) which contribute to embryonic protection against HS. However, finding from other studies have not consistently supported this epigenetic adaptation in response to HS during embryonic development (Sgavioli et al, 2019).

Other nutritional management practices

Feed restriction and feed withdrawal

Withholding feed for at least six hours during HS has been found to reduce body temperature, decrease mortality rates and lower the heterophil-to-lymphocyte ratio suggesting a mitigation of HS effects (Lozano et al., 2006). However, finding regarding growth performance of broilers have been inconsistent, with some studies reporting improvements (Mohamed et al., 2019) while others observed reduction in growth of birds (Lozano et al., 2006), likely influenced by timing and severity of feed restriction (Özkan et al., 2003). Removing feed a few hours before peak heat exposure may help prevent increased heat production caused by anticipatory feeding behaviour, as seen in birds undergoing intermittent fasting (Fondevila et al., 2020).

Wet feeding

In heat-stressed broilers, when feed was mixed with an equal amount of water enhanced dry matter intake (DMI), body weight gain (BWG), carcass weight and protein deposition. However, this approach also led to greater abdominal fat and higher lipid content relative to carcass weight, alongside a reduction in dry matter conversion efficiency (DM intake/BWG) (Kutlu., 2001). Despite some benefits, practical application of wet feeding remains limited, primarily due to the heightened risk of fungal contamination and mycotoxin exposure, which can negatively impact bird health (Wasti et al., 2020).

Form of feed (mash/crumble/pellets) and feed particle size

It has been observed that, under thermo neutral condition, pelleted form of feed enhanced body weight gain and feed intake along with improving digestibility in chicken (Massuquetto et al., 2019). Further, adding pelleted feed to chicken ration led to 8.3% rise in BWG, 10% increase in feed intake and improved crude protein (CP) digestibility by 2.3 % (Cardoso et al., 2022).

Feeding broilers, pelleted diets under HS improves intestinal morphology, increasing villus length and the villus-to-crypt depth ratio in the jejunum (Hosseini and Afshar, 2017b). Pelleted feed also lowers breast HSP70 and creatine kinase levels, reduces mRNA expression and decreases the heterophil-to-lymphocyte ratio, collectively mitigating HS (Hosseini and Afshar, 2017a). Additionally, feed particle size affects HS responses: broilers and laying hens consuming coarser corn particles show higher panting, elevated body temperature and reduced eggshell quality compared to finer particles (Santos et al., 2019).

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Lipid supplementation through feeding

In animals, exposed to HS, incorporating fats and oils into diet helps improve feed intake, supports better productivity and reduces overall heat load (Attia et al., 2021). The type of dietary fat significantly affects broiler performance under HS. Broilers fed isocaloric diets with coconut oil or beef tallow showed better body weight gain (BWG) and feed conversion ratio (FCR) than those receiving olive or soybean oil, likely due to their shorter-chain saturated fatty acids, which are rapidly absorbed and produce less heat during digestion (Seifi et al., 2020). Short and medium chain fatty acid (SCFAs and MCFAs) have likewise been reported to aid mitochondrial function and support the electron transport chain, often impaired under HS (Hecker et al., 2021; Akbarian et al., 2016). To compensate for reduced energy intake during HS, higher metabolizable energy (ME) diets are commonly used, which can improve BWG by up to 17%, FCR by 10% and lower skin and rectal temperatures (Attia et al., 2011, 2018; Wasti et al., 2020).

Supplementation of individual amino acids

Methionine is the primary limiting amino acid (AA) in poultry and along with cysteine (Cys), plays a crucial role in meeting the sulphur AA requirements of birds. Research indicates that Met supplementation can influence inflammation related gene expression in the liver of broilers exposed to high temperatures (Liu et al., 2019).

Lysine (Lys) is the second limiting amino acid in corn and soybean meal based broiler diets is crucial for muscle protein deposition (Ishii et al., 2019). However, studies suggest that providing Lys above the requirements for thermo neutral conditions does not prevent the growth reduction associated HS. Interestingly, when Lys was supplemented alongside Met in a reduced CP diet, broilers exhibited growth performance and carcass traits similar to those fed higher CP diets in hot climates (Attia et al., 2021).

In laying hens, increasing diatary threonine (Thr) from 0.43% to 0.66% did not improve performance but resulted in reduced HSP 70 expression in the ileum (Azzam et al., 2019) and increased superoxide dismutase (SOD) levels in serum and liver (Azzam et al., 2012), indicating potential antioxidant benefits of Thr under HS conditions.

Unlike mammals, poultry have limited ability to synthesize arginine (Arg) due to reduced activity in the de novo synthesis pathway (Castro and Kim, 2020). Increasing dietary Arg has been linked to improved growth, reproduction, immunity, antioxidant capacity and maternal antibody transmission in quails (Kalvandi et al., 2022). Arg also serves as the sole nitrogen donor for nitric oxide production, which supports vasodilatation and may aid in thermoregulation during HS (Uyanga et al., 2021).

Recently, researchers are more interested in citrulline (Cit), which is formed during catabolism of Arg and synthesis of nitric acid. Supplementation of Cit may be more effective than supplementation of L-Arg in increasing systemic Arg levels (Agarwal et al., 2017). Furthermore, as temperature changes, Cit concentration in blood also fluctuates (Chowdhury, 2019), and supplementation of this Cit could help to increase the synthesis of nitric acid which will provide anti-inflammatory effect and aid in regulation of central body temperature (Uyanga et al., 2021, 2022).

When Tryptophan (Trp) was supplemented in high dietary concentration in both broiler (Badakhshan et al., 2021) and layer (Dong et al., 2012) it did not show any improvement in performance. However, Trp supplementation found to decrease rectal temperature and alleviate corticosterone response to broilers affected in HS (Badakhshan et al., 2021).

Among non-essential AAs, glycine (Gly) has shown promise in improving FCR under both normal and HS conditions when included in low CP diets (Awad et al., 2018). Emerging research indicates that Gly and serine (Ser), often evaluated together as Gly equivalents, may be co-limiting or even more limiting than some branched-chain AAs (BCAA) in low CP diets under thermo neutral conditions (Chrystal et al., 2020). This suggests that Gly equivalents could play a critical role in alleviating the negative effects of HS-induced reductions in feed intake.

Effect of dietary protein level in diet

To meet digestible Amino Acid (AA) requirements while minimizing heat production from AA oxidation, reduced CP diets are often supplemented with feed grade AAs. Unlike intact protein sources, feed grade AAs does not require enzymatic digestion, thus preventing additional heat production during nutrient metabolism. However, the decline in performance seen with reduced crude protein diets are consistent with findings that broilers fed a high CP diet (220 g/kg) exhibit high heat production compared to those fed a low CP diet (160 g/kg) under cyclic HS condition (Soares et al., 2020).

Some studies suggest that broilers exposed to HS can achieve better performance when fed diets with both high metabolizable energy (ME) and CP levels (Attia and Hassan, 2017). However, in an experiment where broilers were reared under either thermo neutral or cyclic HS conditions from day 19 to 42 and provided with diets containing either 3,152 kcal/kg ME with 194.8 g/kg CP or 3,253 kcal/kg ME with 210.3 g/kg CP, no performance benefits were observed with the higher density diet. Furthermore, economic evaluations indicated reduced profitability with the increased nutrient density (Cardoso et al., 2022).

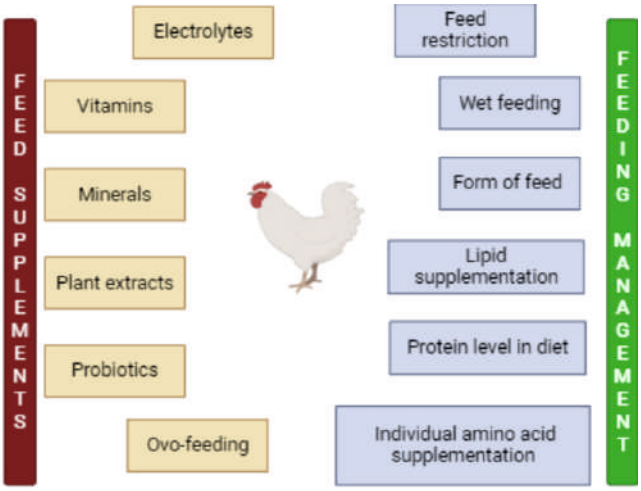

Figure-4: Dietary supplementation of feed additives and other feeding management practices to reduce heat stress in poultry birds

Conclusion

Birds naturally reduce feed intake to lower heat production, but this also limits growth and productivity. Nutrition offers a practical way to alleviate these effects. Although some feeding strategies can be difficult in intensive systems, targeted dietary adjustments show promise. Further research on nutrient partitioning and dietary optimization under heat stress is needed to develop cost-effective strategies that support performance, health and welfare in hot climates.

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References

Abdelqader, A., Abuajamieh, M., Hayajneh, F. and Al-Fataftah, A. R. 2020. Probiotic bacteria maintain normal growth mechanisms of heat stressed broiler chickens. J. Therm. Biol. 92: 1-8, 102654.

Abo Ghanima, M. M., Bin-Jumah, M., Abdel-Moneim, A. M. E., Khafaga, A. F., Abd El-Hack, M. E., Allam, A. A. and El-Kasrawy, N. I. 2019. Impacts of strain variation on response to heat stress and boldo extract supplementation to broiler chickens. Animals. 10(1): 1-18, 24.

Agarwal, U., Didelija, I. C., Yuan, Y., Wang, X. and Marini, J. C. 2017. Supplemental citrulline is more efficient than arginine in increasing systemic arginine availability in mice. J. Nutr. 147: 596–602.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A. and De Smet, S. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J. Anim. Sci. Biotechnol. 7: 1-14, 37.

Akinyemi, F. and Adewole, D. 2021. Environmental stress in chickens and the potential effectiveness of dietary vitamin supplementation. Front Anim Sci. 2: 1-21, 775311.

Alaqil, A. A., Abd El-Atty, H. K. and Abbas, A. O. 2022. Intermittent lighting program relieves the deleterious effect of heat stress on growth, stress biomarkers, physiological status, and immune response of broiler chickens. Animals. 12: 1-12, 1834.

Aluwong, T., Hassan, F., Dzenda, T., Kawu, M. and Ayo, J. 2013. Effect of different levels of supplemental yeast on body weight, thyroid hormone metabolism and lipid profile of broiler chickens. J. Vet. Med. Sci. 75: 291–298.

Al-Zghoul, M. B., Saleh, K. M., and Ababneh, M. M. K. 2019. Effects of pre-hatch thermal manipulation and post-hatch acute heat stress on the mRNA expression of interleukin-6 and genes involved in its induction pathways in 2 broiler chicken breeds. Poultry Science. 98(4): 1805–1819.

Attia, Y. A. and Hassan, S. S. 2017. Broiler tolerance to heat stress at various dietary protein/energy levels. Eur. Poult. Sci. 81: 1–15.

Attia, Y. A., Al-Harthi, M. A. and Elnaggar, Sh. A. 2018. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. Ital. J. Anim. Sci. 17: 686–697.

Attia, Y. A., Hassan, R. A., Tag El-Din, A. E. and Abou-Shehema, B. M. 2011. Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. J. Anim. Physiol. Anim. Nutr. 95: 744–755.

Attia, Y., Al-Harthi, M. and Hassan, S. 2021. Responses of broiler chicken to different oil levels within constant energy levels from 20 to 40 days of age under hot weather conditions. Italian J Anim Sci. 20: 664–76.

Awad, E. A., Idrus, Z., Soleimani Farjam, A., Bello, A. U. and Jahromi, M. F. 2018. Growth performance, duodenal morphology and the caecal microbial population in female broiler chickens fed glycine-fortified low protein diets under heat stress conditions. Br. Poult. Sci. 59: 340–348.

Awad, E. A., Najaa, M., Zulaikha, Z. A., Zulkifli, I. and Soleimani, A. F. 2019. Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora and meat quality in two broiler strains. Asian-Australasian Journal of Animal Sciences. 33(5): 778–787.

Azzam, M. M. M., Dong, X. Y., Xie, P. and Zou, X. T. 2012. Influence of L–threonine supplementation on goblet cell numbers, histological structure and antioxidant enzyme activities of laying hens reared in a hot and humid climate. Br. Poult. Sci. 53: 640–645.

Azzam, M. M., Alhotan, R., Al-Abdullatif, A., Al-Mufarrej, S., Mabkhot, M. and Alhidary, I. A. 2019. Threonine requirements in dietary low crude protein for laying hens under high-temperature environmental climate. Animals. 9: 1-12, 586.

Badakhshan, Y., Emadi, L., Esmaeili-Mahani, S. and Nazifi, S. 2021. The effect of L-tryptophan on the food intake, rectal temperature, and blood metabolic parameters of 7-day-old chicks during feeding, fasting, and acute heat stress. Iran. J. Vet. Res. 22: 55–64.

Balakrishnan, K. N., Ramiah, S. K. and Zulkifli, I. 2023. Heat shock protein response to stress in poultry: A review. Animals. 13(2): 1-28, 317.

Berger, E., Rath, E., Yuan, D., Waldschmitt, N., Khaloian, S., Allgäuer, M., Staszewski, O., Lobner, E.M., Schöttl, T. and Giesbertz, P. 2016. Mitochondrial function controls intestinal epithelial stemness and proliferation. Nat. Commun. 7: 1–17.

Bonato, M., Malecki, I.A., Rybnik-Trzaskowska, P.K., Cornwallis, C.K. and Cloete, S.W. 2014. Predicting ejaculate quality and libido in male ostriches: Effect of season and age. Anim. Reprod. Sci. 151: 49–55.

Calik, A., Emami, N. K., White, M. B., Walsh, M. C., Romero, L. F. and Dalloul, R. A. 2022. Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, part I: growth performance, body composition and intestinal nutrient transporters. Poult Sci. 101 (6): 1-10, 101857.

Cardoso, D. M., Cardeal, P. C., Soares, K. R., Sousa, L. S., Castro, F. L. S., Araújo, I. C. S. and Lara, L. J. C. 2022. Feed form and nutritional level for rearing growing broilers in thermoneutral or heat stress environments. Journal of thermal biology. 103: 103159.

Castro, F. L. S. and Kim,W. K. 2020. Secondary functions of arginine and sulphur amino acids in poultry health: Review. Animals. 10 (11): 1-15, 2106.

Chauhan, S.S., Celi, P., Ponnampalam, E.N., Leury, B.J., Liu, F. and Dunshea, F.R. 2014. Antioxidant dynamics in the live animal and implications for ruminant health and product (meat/milk) quality: Role of vitamin E and selenium. Anim. Prod. Sci. 54: 1525–1536.

Chowdhury, V. S. 2019. Heat Stress biomarker amino acids and neuropeptide afford thermotolerance in chicks. J. Poult. Sci. 56: 1–11.

Chrystal, P. V., Moss, A. F., Yin, D., Khoddami, A., Naranjo, V. D., Selle, P. H. and Liu, S. Y. 2020. Glycine equivalent and threonine inclusions in reduced-crude protein, maize-based diets impact on growth performance, fat deposition, starch-protein digestive dynamics and amino acid metabolism in broiler chickens. Animal Feed Science and Technology. 261: 1-14, 114387.

Cramer, T., Kim, H., Chao, Y., Wang, W., Cheng, H. and Kim, Y. 2018. Effects of probiotic (Bacillus subtilis) supplementation on meat quality characteristics of breast muscle from broilers exposed to chronic heat stress. Poult. Sci. 97: 3358–3368.

Crespo, R. and Grimes, J. 2024. Effect of brooding conditions on the blood chemistry and performance of Turkey poults. J Appl Poult Res. 33 (2): 1-12, 100408.

doi.org/10.56093/ijan.v43i2.176245

Deng, W., Dong, X., Tong, J. and Zhang, Q. 2012. The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poult. Sci. 91: 575–582.

Ding K. N., Lu, M. H., Guo, Y. N., Liang, S. S., Mou, R. W., He, Y. M. and Tang, L. P. 2023. Resveratrol relieves chronic heat stress-induced liver oxidative damage in broilers by activating the Nrf2-Keap1 signaling pathway. Ecotoxicology and Environmental Safety. 249: 1-8, 114411.

Dong, X. Y., Azzam, M. M. M., Rao, W., Yu, D. Y. and Zou, X. T. 2012. Evaluating the impact of excess dietary tryptophan on laying performance and immune function of laying hens reared under hot and humid summer conditions. Br. Poult. Sci. 53: 491–496.

El-Hack, A., Mohamed, E., Abdelnour, S.A., Swelum, A.A. and Arif, M. 2018. The application of gene marker-assisted selection and proteomics for the best meat quality criteria and body measurements in Qinchuan cattle breed. Mol. Biol. Rep. 45: 1445–1456.

El-Moneim, A.E.-M.E.A., El-Wardany, I., Abu-Taleb, A.M., Wakwak, M.M., Ebeid, T.A. and Saleh, A.A. 2020. Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics Antimicrob. Proteins. 12: 439–450.

Elnesr, S. S., Elwan, H. A. M., Xu, Q. Q., Xie, C., Dong, X. Y. and Zou, X. T. 2019. Effects of in ovo injection of sulfur-containing amino acids on heat shock protein 70, corticosterone hormone, antioxidant indices, and lipid profile of newly hatched broiler chicks exposed to heat stress during incubation. Poultry Science. 98(5): 2290–2298.

Farag, M. R. and Alagawany, M. 2018. Physiological alterations of poultry to the high environmental temperature. Journal of thermal biology. 76: 101-106.

Fernandes, E., Raymundo, A., Martins, L. L., Lordelo, M. and de Almeida, A. M. 2023. The naked neck gene in the domestic chicken: a genetic strategy to mitigate the impact of heat stress in poultry production—a review. Animals. 13(6): 1-15, 1007.

Flees, J., Rajaei-Sharifabadi, H., Greene, E., Beer, L., Hargis, B. M., Ellestad, L. and Dridi, S. 2017. Effect of Morinda citrifolia (Noni)-enriched diet on hepatic heat shock protein and lipid metabolism-related genes in heat stressed broiler chickens. Frontiers in Physiology. 8: 1-15, 919.

Flint, H.J., Scott, K.P., Duncan, S.H., Louis, P. and Forano, E. 2012. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 3: 289–306.

Fondevila, G., Archs, J. L., Cámara, L., de Juan, A. F. and Mateos, G. G. 2020. The length of the feed restriction period affects eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract of young broilers. Poult. Sci. 99: 1010–1018.

Hecker, M., Sommer, N. and Mayer, K. 2021. Assessment of short- and medium-chain fatty acids on mitochondrial function in severe inflammation. In: Mitochondrial medicine methods in molecular biology. Eds. Weissig, V., Edeas, M. and Humana New York, NY, p 125–132.

Hosseini, S. M. and Afshar, M. 2017a. Effect of diet form and enzyme supplementation on stress indicators and bone mineralisation in heat-challenged broilers fed wheat-soybean diet. Ital. J. Anim. Sci. 16: 616–623.

Hosseini, S. M. and Afshar, M. 2017b. Effects of feed form and xylanase supplementation on performance and ileal nutrients digestibility of heat-stressed broilers fed wheat–soybean diet. J. Appl. Anim. Res. 45: 550–556.

Ishii, T., Shibata, K., Kai, S., Noguchi, K., Hendawy, A. O., Fujimura, S. and Sato, K. 2019. Dietary supplementation with lysine and threonine modulates the performance and plasma metabolites of broiler chicken. The journal of poultry science. 56(3): 204-211.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M. and Espeisse, O. 2018. The value of anticoccidials for sustainable global poultry production. Int. J. Antimicrob. Agents. 51: 304–310.

Kala, M., Shaikh, M.V. and Nivsarkar, M. 2017. Equilibrium between anti-oxidants and reactive oxygen species: A requisite for oocyte development and maturation. Reprod. Med. 16: 28–35.

Kalvandi, O., Sadeghi, A. and Karimi, A. 2022. Arginine supplementation improves reproductive performance, antioxidant status, immunity and maternal antibody transmission in breeder Japanese quail under heat stress conditions. Ital. J. Anim. Sci. 21: 8–17.

Kennedy, G. M., Kuria, S. N., Panyako, P. M., Lichoti, J. K., Ommeh, S. C. 2022. Polymorphism of the heat shock protein 70 gene in indigenous chickens from different agro-climatic zones in Kenya. Afr J Biotechnol. 21: 73–82.

Kim, H. R., Ryu, C., Lee, S. D., Cho, J. H. and Kang, H. 2024. Effects of heat stress on the laying performance, egg quality, and physiological response of laying hens. Animals. 14 (7): 1-12, 1076.

Kumari, K. N. R. and Nath, D. N. 2018. Ameliorative measures to counter heat stress in poultry. World's poultry science journal. 74(1): 117-130.

Kutlu, H. R. 2001. Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature. Arch. Tierernahr. 54: 127–139.

LeBlanc, J.G., Chain, F., Martín, R., Bermúdez-Humarán, L.G., Courau, S. and Langella, P. 2017. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb. Cell Fact. 16: 1–10.

Li, M., Wu, J. and Chen, Z. 2015. Effects of heat stress on the daily behavior of wenchang chickens. Revista Brasileira de Ciência Avícola. 17(4): 559–566.

Li, X., Ma, W. and Jiang, Y. 2023. Expression patterns of heat shock protein genes and antioxidase genes in Apis cerana cerana (Hymenoptera: Apidae) under heat stress. Journal of Entomological Science. 58(1): 95–103.

Liu, G., Magnuson, A. D., Sun, T., Tolba, S. A., Starkey, C., Whelan, R. and Lei, X. G. 2019. Supplemental methionine exerted chemical form-dependent effects on antioxidant status, inflammation-related gene expression, and fatty acid profiles of broiler chicks raised at high ambient temperature. Journal of animal science. 97(12): 4883-4894.

Liu, X., Li, L., Miao, Z., Xin, Q., Zhang, L., Cai, Q., Shi, W., Zhao, B., Zheng, N. and Zhu, Z. 2025. Effect of acute heat stress on intestinal immune response of Jinding ducks. Poultry Science. 104(8): 1-10, 105273.

doi.org/10.56093/ijan.v43i2.176245

Lozano, C., De Basilio, V., Oliveros, I., Alvarez, R., Colina, I., Bastianelli, D., Yahav, S. and Picard, M. 2006. Is sequential feeding a suitable technique to compensate for the negative effects of a tropical climate in finishing broilers?. Animal Research. 55(1): 71-76.

Ma, B., Zhang, L., Li, J., Xing, T., Jiang, Y. and Gao, F. 2021. Dietary taurine supplementation ameliorates muscle loss in chronic heat stressed broilers via suppressing the perk signaling and reversing endoplasmic reticulum stress induced apoptosis. Journal of the Science of Food and Agriculture. 101(5): 2125–2134.

Madkour, M., Salman, F. M., ElWardany, I., AbdelFattah, S. A., Alagawany, M., Hashem, N. M., Abdelnour, S. A., ElKholy, M. S. and Dhama, K. 2022. Mitigating the detrimental effects of heat stress in poultry through thermal conditioning and nutritional manipulation. Journal of Thermal Biology. 103: 1-11, 103169.

Mas, A. L., Bonansea, R. I., Fernandez, M. E., Kembro, J. M., Labaque, M. C., Wunderlin, D. A. and Baroni, M. V. 2023. Dietary supplementation with chia polyphenols alleviates oxidative stress and improves egg nutritional quality in Japanese quails under heat stress. Journal of Thermal Biology. 111: 1-11, 103421.

Massuquetto, A., Panisson, J. C., Marx, F. O., Surek, D., Krabbe, E. L. and Maiorka, A. 2019. Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. Poult. Sci. 98: 5497–5503.

Men, H., Cai, H., Cheng, Q., Zhou, W., Wang, X., Huang, S., Zheng, Y. and Cai, L. 2021. The regulatory roles of p53 in cardiovascular health and disease. Cellular and Molecular Life Sciences. 78(5): 2001–2018.

Miao, Q., Si, X., Xie, Y., Chen, L., Liu, Z., Liu, L., Tang, X. and Zhang, H. 2020. Effects of acute heat stress at different ambient temperature on hepatic redox status in broilers. Poultry Science. 99(9): 4113–4122.

Mir, .S, Pal, R., Mani, V., Malik, T., Ojha, L. and Yadav, S. 2018. Role of dietary minerals in heat-stressed poultry: a review. J entomol Zool. 6: 820–6.

Mohamed, A. S. A., Lozovskiy, A. R. and Ali, A. M. A. 2019. Strategies to combat the deleterious impacts of heat stress through feed restrictions and dietary supplementation (vitamins, minerals) in broilers. J. Indones. Trop. Anim. Agric. 44: 155–166.

Moustafa, E. S., W. F. Alsanie, A. Gaber, N. Kamel,A. Alaqil, and A. O. Abbas. 2021. Blue-green algae (Spirulina platensis) alleviates the negative impact of heat stress on broilerproduction performance and redox status. Animals. 11: 1–12.

Nawaz, A.H., Amoah, K., Leng, Q.Y., Zheng, J.H., Zhang, W.L. and Zhang, L. 2021. Poultry response to heat stress: Its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Frontiers in veterinary science. 8: 1-16, 699081.

Özkan, S., Akbaş, Y., Altan, Ö., Altan, A., Ayhan, V. and ÖZkan, K. 2003. The effect of short-term fasting on performance traits and rectal temperature of broilers during the summer season. Br. Poult. Sci. 44: 88–95.

Sahin, N., Hayirli, A., Orhan, C. E. M. A. L., Tuzcu, M. E. H. M. E. T., Komorowski, J. R. and Sahin, K. 2018. Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poultry Science. 97(4): 1298-1305.

Santos, M. M., Souza-Junior, J. B. F., Queiroz, J. P. A. F., Costa, M. K. O., Lima, H. F. F., Arruda, A. M. V. and Costa, L. L. M. 2019. Broilers' behavioural adjustments when submitted to natural heat stress and fed different maize particle sizes in the diet. The Journal of Agricultural Science. 157(9-10): 743-748.

Seifi, K., Rezaei, M., Yansari, A. T., Zamiri,M. J., Riazi, G. H. and Heidari, R. 2020. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. J. Therm. Biol. 89: 1-8, 102520.

Sgavioli, S., De Almeida, V.R., Matos Júnior, J.B., Zanirato, G.L., Borges, L.L. and Boleli, I.C. 2019. In ovo injection of ascorbic acid and higher incubation temperature modulate blood parameters in response to heat exposure in broilers. Br Poult Sci. 60: 279–87.

Singh, C. D., Nath, S., Gohain, A. K., Tamuly, S. and Bhuyan, R. 2023. Effect of Vitamin E, Vitamin C and Selenium on Blood Haematological, Biochemi-cal, Liver Enzyme, Serum Electrolyte, Antioxidant Profile, Heat Shock Protein (HSP70) and Carcass Quality of Broiler Chickens During Heat Stress. Indian Journal of Animal Nutrition. 40(1): 63-75.

Soares, K.R., Lara, L.J.C., da Silva Martins, N.R., e Silva, R.R., Pereira, L.F.P., Cardeal, P.C. and Teixeira, M.D.P.F. 2020. Protein diets for growing broilers created under a thermoneutral environment or heat stress. Animal Feed Science and Technology. 259: 1-9, 114332.

Sohail, M. U., Ijaz, A., Yousaf, M. S., Ashraf, K., Zaneb, H., Aleem, M. and Rehman, H. 2010. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poultry science. 89(9): 1934-1938.

Sridhar, K., Kumari, N. N., Narasimha, J., Panda, A. K. and Sushma, K. 2022. Effect of Supplementation of Different Levels and Combinations of Selenium And Vitamin E on Broiler Chicken Performance, Immune Response, Meat Selenium And Vitamin E Concentration. Indian Journal of Animal Nutrition. 39(3): 314-324.

Tan, X., Xian W., Li X.-R., Chen Y., Geng J., Wang Q., Gao Q., Bi-Tang E., Wang H. and Kang P. 2022. Mechanisms of quercetinagainst atrial fibrillation explored by networkpharmacology combined with moleculardocking and experimental validation. Scientific Reports. 12 (1): 1-15, 9777.

Tang, S., Zhou, S.,Yin, B., Xu, J., Di, L., Zhang, J. and Bao, E. 2018. Heat stress-induced renal damage in poultry and the protective effects of HSP60 and HSP47. Cell Stress Chaperones. 23: 1033–1040.

Tripathi, U. K., Kumar, A., Kumari, A., Pathak, S., Kumar, M. and Maurya, V. 2025. Dietary Quercetin for Enhanced Broiler Performance-A Review. Indian Journal of Animal Nutrition. 42(3): 315-328.

Türk, G., Çeriba¸sı, A.O., ¸Sim¸sek, Ü.G., Çeriba¸sı, S., Güvenç, M., Kaya, ¸S.Ö., Çiftçi, M., Sönmez, M., Yüce, A. and Bayrakdar, A. 2016. Dietary rosemary oil alleviates heat stress-induced structural and functional damage through lipid peroxidation in the testes of growing Japanese quail. Anim. Reprod. Sci. 164: 133–143.

Uyanga, V. A., Liu, L., Zhao, J., Wang, X., Jiao, H. and Lin, H. 2022. Central and peripheral effects of L-citrulline on thermal physiology and nitric oxide regeneration in broilers. Poult. Sci. 101 (3): 1-15, 101669.

doi.org/10.56093/ijan.v43i2.176245

Uyanga, V.A., Musa, T.H., Oke, O.E., Zhao, J., Wang, X., Jiao, H., Onagbesan, O.M. and Lin, H. 2023. Global trends and research frontiers on heat stress in poultry from 2000 to 2021: A bibliometric analysis. Frontiers in Physiology. 14: 1123582.

Uyanga, V.A., Wang, M., Tong, T., Zhao, J., Wang, X., Jiao, H., Onagbesan, O.M. and Lin, H. 2021. L-citrulline influences the body temperature, heat shock response and nitric oxide regeneration of broilers under thermoneutral and heat stress condition. Frontiers in Physiology: 12:671691.

Wasti, S., Sah, N. and Mishra, B. 2020. Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals. 10: 1266.

Wasti, S., Sah, N., Singh, A.K., Lee, C.N., Jha, R. and Mishra, B. 2021. Dietary supplementation of dried plum: A novel strategy to mitigate heat stress in broiler chickens. Journal of Animal Science and Biotechnology. 12(1) :58.

Wealleans, A., Walsh, M., Romero, L. and Ravindran, V. 2017. Comparative effects of two multi-enzyme combinations and a Bacillus probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides, and gut microflora in broiler chickens. Poult. Sci. 96: 4287–4297.

Yilmaz, E. and Gul, M. 2023a. Effects of dietary supplementation of cumin (*Cuminum cyminum* L.) essential oil on expression of genes related to antioxidant, apoptosis, detoxification, and heat shock mechanism in heat-stressed broiler chickens. Animal Biotechnology. 34(7): 2766–2777.

Yilmaz, E. and Gul, M. 2023b. Effects of cumin (*Cuminum cyminum* L.) essential oil and chronic heat stress on growth performance, carcass characteristics,serum biochemistry, antioxidant enzyme activity, and intestinal microbiology in broiler chickens. Veterinary Research Communications. 47(2): 861-875.

Yousaf, A., Jabbar, A. and Ditta, Y. 2017. Effect of pre-warming on broiler breeder eggs hatchability and post-hatch performance. J. Anim. Health Prod. 5: 1–4.

Zhang, C., Zhao, X. H., Yang, L., Chen, X. Y., Jiang, R. S., Jin, S. H. and Geng, Z. Y. 2017. Resveratrol alleviates heat stress-induced impairment of intestinal morphology, microflora, and barrier integrity in broilers. Poultry Science. 96(12): 4325–4332.

Zhu, Y. F., Bodinga, M. B., Zhou, J. H., Zhu, L. Q., Cao, Y. L., Ren, Z. Z. and Yang, X. J. 2020. Effects of in ovo injection of vitamin C on heat shock protein and metabolic genes expression. Animal. 14(2): 360-367.

Review Article

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Agricultural Waste Biochar as a Novel Feed Additive for Livestock and Poultry: A Review

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Biochar, a carbonaceous material produced by pyrolysis from various types of biomass in low-to-no oxygen thermal process at temperature ranging from 350 to 1000 °C, predominantly used as toxin binder due to its high adsorption capacity. Studies conducted so far involving different Biochar have shown significant desirable biological responses in different animal models i.e. growth promotion, improved immunity, reduction in harmful microbiota in faecal matter and litter, meat quality attributes in poultry etc. Similarly, Biochar has modulated the rumen microbial population more towards desirable ecosystem thus benefiting the livestock productivity. Biochar can adsorbs a range of toxic compounds, including plant and animal-derived toxins, endogenous toxins, and gases such as methane and thus, use of biochar in aflatoxin contaminated feed stuffs can be a measure to lessen the feed wastage. Furthermore, addition of biochar increased nutrient digestibility, nitrogen retention and also resulted in increased fat and protein content of the milk. Likewise, biochar addition also resulted in decreased oxidative stress, improved blood biochemical indices and inflammation following immunization, which is an indicative of improvement in animal health. Addition of Biochar also tend to augment the intestinal histo-architecture in animals. However, though the studies done so far on Biochar have shown a lot of promise as feed additive for livestock and poultry, further in-depth research is essential to validate its efficacy and safety across livestock and poultry production systems in short time and long term addition in the ration.

Keywords: Agricultural waste; Biochar; Livestock Performance; Feed Additive; Poultry

Introduction

Since time immemorial, the animal husbandry is one of the major activities for human civilization in India, which serves as food for human consumption, provides animal energy for different agricultural activities and many more. Therefore, attempts are always made to increase the livestock production and augment in animal health by improving the animal feeding management (Naveena and Kiran, 2014). With ever increasing industry standards and consumer awareness, the demand for animal origin healthy food products keep the researchers on toes to find out novel and natural feed additives free from any residual and/or adverse effects. According to European Commission (EC) feed additives are substances, microorganisms or preparations other than feed materials or premixtures which are intentionally added to feed or water in particular to fulfil specific functions in animal body. Animals with high growth performance need to maintain a high health status, which requires the prime use of feed additives. In recent years ban on the use of antibiotic growth promoter, high cost of conventional feed additives and increased awareness about harmful residual effect of non-natural feed additives necessitates the emergence and search of natural, non-residual feed additive to gain importance in sustainable livestock production (Pandey et al., 2019). The natural and non-residual alternatives of conventionally used feed additives, biochar produced from various agricultural biomass has become increasingly popular in past few years. Biochar is a carbonaceous material produced by pyrolysis from various types of biomass in no oxygen thermal process at temperature ranging from 350 to1000C (European Biochar Foundation, 2012; International Biochar Initiative, 2015). Globally, wide range of biomass sources like animal manure, crop residues, agro-industrial byproducts and forestry waste are used (Weber and Quicker, 2018). All the biomass sources have the common characteristics like cost effectiveness, environment friendly and also its ability to enhance the recycling of organic wastes generated from agricultural, forestry and processing industries (Tang et al., 2013). In recent times, use of biochar as a regular feed supplement by livestock farmers is gaining attention thanks to its beneficial attributes towards animal health, nutrient intake efficiency resulting in enhanced productivity in livestock and poultry. Though in early days, biochar addition in livestock ration showcased its beneficial effects in terms animal health and production. In this section we will discuss regarding beneficial effects documented so far in different animal models, their probable mode of action, and way forward.

Production and Characterization of Biochar

Biomass is converted into biochar by the thermochemical conversion technique using heat and chemical catalyst, where pyrolysis is one of the most promising thermochemical conversion technique used to produce the biochar (Tripathi et al., 2016).There are six major types of pyrolysis technologies – fast pyrolysis, flash pyrolysis, slow pyrolysis, hydro pyrolysis, vacuum pyrolysis and microwave pyrolysis for the production of biochar (Sohi et al., 2009; Ippolito et al., 2020; Liu et al., 2021), bio-oil and gases which are the end products of thermochemical conversion technique but out of it, slow pyrolysis and microwave pyrolysis are the promising

technologies for production of biochar (Ippolito et al., 2020; Liu et al., 2021). Slow pyrolysis is thermal decomposition of biomass in the absence or partial absence of oxygen at moderate temperature ranges from 400-800 °C (Tripathi et al., 2016). In case of biochar, the physicochemical properties are governed by many factors such as composition of feedstock temperature of pyrolysis, duration of pyrolytic reaction, rate of heating, particle size of biomass etc. (Akhtar and Amin, 2012). With the increasing pyrolysis temperature, yield of biochar decreases due to the higher volatilization of the biomass which leads to decrease in moisture content, ash and fixed carbon (Gai et al., 2014). Shorter the residence time, higher the yield of biochar (Pecha and Garcia-Perez, 2020). Biochar can be produced from any biomass which is mostly considered as waste starting from, wood, leaves, straw, maize cob, etc. Report suggests that, the raw materials have a profound influence on biochar properties (Ippolito et al., 2020). For instance, in case of bood based biochar, the specific surface area is the greatest, which in combination with pyrolysis temperature could likely promote greater changes in soil physical characteristics than biochar produced from other biomass. Similarly, biochar produced from crops and grass have higher cation exchange capacities than other biochars, which in combination with pyrolysis temperature could potentially lead to longer-term changes in soil dynamics (Ippolito et al., 2020). However, the use of agricultural wastes for biochar making is well practised for soil fortification and soil nutrient management, and use as a potential feed additive may open up new possibilities and opportunities in the field of animal nutrition.

The systematic characterization of biochar includes chemical (pH, elemental composition), physical (surface area, pore structure) and structural (functional groups) properties, which is found important to understand the various functions of biochar including its use as a novel feed additive in livestock feeding (Table.1). Presence of functional groups like carboxylic, hydroxyl, amide, amine and lactonic groups at the surface of biochar increase its adsorption capacity, which depends on the biomass used for biochar production and temperature (Li et al., 2017). Determination of surface characteristics of biochar can be done by Fourier Transform Infrared (FTIR) and Raman spectroscopies, Scanning Electron Microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX) and X-ray photoelectron spectroscopy images (Chia et al., 2012). According to the international union of pure and applied chemistry the pores present in biochar may be micro (<2nm), meso (2-50nm) and micro (>50nm) which are described in detail by SEM. SEM and EDX are utilized for the examination of elemental composition on surface of biochar but it is not suitable for the organic contaminants (Yaashikaa et al., 2020). Brunauer-Emmett-Teller Analysis (BET) can be used to examine the surface area of biochar, which is an important property of biochar mainly responsible for its adsorption capacity (Yaashikaa et al., 2020).

Table.1. Comparison of composition of Biochar produced from various crop straws (Sun et al., 2014)

Sample	Carbon	Oxygen	Nitrogen	Silica	Potassium	Calcium
Rice straw biochar	78.5%	13.39%	0.70%	3.92%	1.56%	0.49%
Cotton straw biochar	74.8%	17.64%	0.69%	-	4.08%	1.17%
Wheat straw biochar	72.9%	17.2%	0.81%	3.44%	1.82%	2.08%
Corn straw biochar	72%	18.3%	1.09%	1.13%	4.57%	0.52%

Among the different biomass used for biochar production few commonly used sources are rice husks (Leng et al. 2012a, b; Phongpanith et al. 2013), pinewood chips (Saleem et al. 2018), jarrah wood (Joseph et al. 2015), bamboo, coconut, etc. (Rajpoot et al. 2024). However, for animal feeding purposes, there is a need to study and standardize the different variables affecting the production and nature of Biochar. However, still the science of Biochar as a feed additive in livestock and poultry feeding is at its infancy, but early studies have shown glimpses of an illustrious future.

Use of biochar as a feed additive in farm animals

Biochar, a carbon-rich product obtained through the pyrolysis process (Willson et al., 2019), has been associated with neutralizing the Gastro Intestinal Tract (GIT) toxins (Prasai et al., 2016), improving feed efficiency and overall bird (Gerlach and Schmidt, 2012; Kumar, 2025) and other livestock (Rajpoot et al. 2024) performances by acting as a good source of mineral and as an alternative to the antibiotic growth promotors as well. The adsorption capacity and mineral nutrient content make the use of biochar a versatile and multifunctional material for livestock and poultry production. Activated biochar has gained popularity as a universal adsorbent material with the capacity to selectively adsorb and neutralize ingested toxins and other contaminants in feed while supplying some essential minerals to animals (Schmidt et al., 2019). Early reports on biochar as a non-nutritional feed additive in poultry feed have shown promising effects (Fig.1), and can replace antibiotic growth promotors (Prasai et al., 2016; Willson et al., 2019), toxin binders (Gerlach and Schmidt, 2012) and improve growth performance (Kalus et al., 2019; Ruttanavut et al., 2009). Different studies have indicated multifarious beneficial effects of addition of biochar in the animal and poultry ration (Table. 2) such as promotes bone mineralization (Evans et al., 2017), enhancement in growth performance (El-kelawy et al., 2024) and litter quality (Kalus et al., 2020a) without harming broiler performance (Linhoss et al., 2019) alteration in rumen fermentation (Rajpoot et al. 2024). The detailed biological responses are discussed in hereafter. However, the studies conducted so far are highly diverse and difficult to draw any conclusions. Consequently, further in-depth research is essential to validate its efficacy and safety across poultry production systems in short time and long term addition in the ration.

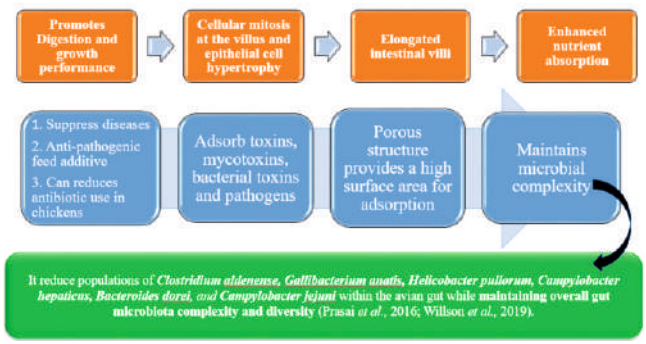

Fig.1 An overview of the different biological responses of biochar used as feed additive in the ration of livestock and poultry

Effect of biochar feeding on growth and performance of animal

Inclusion of various types of biochar in feeding ration at 1-3% have been studied to improve health conditions and performances of farm animals such as weight gain, immunity response, feed intake, feed conversion rates, carcass characteristics (Table 2) and overall quality of animal products (Lao and Mbega, 2020). Rajpoot et al (2024) reviewed the biological responses of different biochars used as feed additive in ruminants and reported that biochar at 0.5–3% inclusion level in ruminant ration have resulted in superior feed intake, body weight gain, feed conversion ratio, immune response, carcass characteristics . Similarly, cattle supplemented with 1% biochar made from cassava foliage, cassava roots in the diet resulted in growth increment by 20% (Leng et al., 2012c). Likewise, addition of bamboo biochar at 1.0% of feed DM in goat resulted in significant increase of DM and CP digestibility and also the nitrogen retention (Silivong and Preston, 2016). Biochar can adsorb organic molecules like amino acids, fatty acids, proteins and urea as well as mineral compounds such as ammonium, ammonia and nitrate very efficiently (Schmidt et al., 2019), which may lead to increased fat and protein content of the milk . Van et al (2006) studied the 0.6% biochar supplementation in feed resulted in growth enhancement in young goats by 17%. Similar growth promotion effect of biochar was also reported by Kana et al. (2010) in ducks and Ruttanvat et al. (2009) in broilers. Choi et al. (2012) conducted the study of supplementation of biochar in finishing pig feeding at the rate of 0, 0.3% and 0.6% of the DM of the feed and observed a significant increase in feed conversion rate, the carcass characteristics, live weight gain and immune response at 0.3% supplementation compared to the other treatments. Likewise, Lee et al. (2011) observed significant enhancement in meat marbling, meat colour traits as well as the tenderness of the meat when cooked due to use of biochar in their ration. Ohanaka et al. (2025) reported an improved (P<0.05) live body weight and body weight gain during 0-42 days with supplementation of activated charcoal at 0.05% level in the diet of male Arbor Acres broiler chicks, whereas average daily feed intake was higher in birds fed with 1.00% activated charcoal. Prasai et al. (2018) taken layer pullet of age 18 weeks and supplemented their feed with mixture of biochar, zeolite and bentonite at the rate of 0, 1.0%, 2.0% and 5.0% for 25 weeks and reported the best improved production performance traits of egg yield and FCR at 2.0% supplementation. Ohanaka et al. (2025) also reported significantly better FCR in the birds fed with 1.00% activated charcoal signifying that biochar and charcoal can influence the growth, feed intake in poultry birds. Similarly, El-kelawy et al. (2024) reported that Supplementation of 2% biochar in broiler diets improved body weight, weight gain, FCR, and production index along with improved CP and EE digestibility, augmented dressing percentage. This indicates the holistic welfare of the poultry birds by supplementation of biochar in their ration. Raz et al. (2025) reported that *Prosopis farcta* biochar supplementation significantly improved growth performance, immune function, liver health, and serum lipid profile in quails exposed to AFB1 ($P < 0.05$).

Biochar has the ability to modulate the pH levels in the digestive tract and maintain the stable pH which is very important for optimum enzyme activity and nutrient absorption by adsorbing excess acids or bases. This stable pH also less efficient for the growth of pathogenic microorganisms which further support the gut health (Wang et al., 2017; Viaene et al., 2024). Ruttanavut et al. (2009) demonstrated that supplementing Aigamos duck feed with a bamboo charcoal powder and bamboo vinegar solution resulted in increased body weight, attributed to enhanced nutrient absorption via elongated intestinal villi. Feeding of 0.3% and 0.6% bamboo biochar improved the quality of marketable meat and composition of pig fat with increase in unsaturated fat and decrease in saturated fat (Chu et al., 2013a). Likewise, Kumar (2025) also reported non-significant change in growth, feed intake and FCR in broiler chicken by adding either corn cob waste biochar or paddy straw biochar (at 1% and 2 % levels). However, still a lot to be explored as far as the biological responses of addition of different biochar in livestock and poultry ration is concerned.

Table 2. Biological responses of addition of different biochar to the poultry ration

biochar preparation / Type of biochar used	Scientific Name	Species of Bird	Salient Findings	Reference
Corn Cob Biochar	*Zea mays*	Quails	Corn cob biochar, at 2% level • Improved FCR, body weight gain • Supported intestinal integrity, immune function	Usman et al. (2025)
Pine Biochar	*Pinus spp.*	Broiler Chicken	• Biochar-amended litter (30 % vol/vol surface applied) did not reduce NH_3 levels or affect bird performance. • Significantly lowered litter moisture, making it beneficial for moisture control in commercial broiler houses.	Mohammadi-Aragh et al. (2025)
Non-Specific Biochar	NA	Broiler Chicken	Supplementation of 2% biochar in broiler diets • Improved body weight, weight gain, FCR, and production index • Improved CP and EE digestibility, dressing percentage, and immune parameters (IgA, IgM, IgG, LTT, phagocytic activity) • Increased RBCs, Hb, PCV, WBCs, lymphocytes, total protein, globulins, and thyroid hormones; reduced abdominal fat, serum lipids, oxidative stress markers • Decreased harmful gut bacteria and increasing beneficial microbes.	El-Kelawy et al. (2024)
Wet litter biochar (WLB)	NA	Broiler Chicken	Inclusion of 0.5% and 1% WLB in broiler diets under cold stress • Improved body weight gain, ileal and jejunal villous length and surface area • Reduced FCR	Hasanvand et al. (2023)

Poultry Litter Biochar (PLB)	NA	Broiler Chicken	• 5 g/kg feed PLB effectively mitigated the harmful effects of AFB1 (0.5 mg/kg feed) on broiler performance, biochemical indices and immunity.	Rashidi et al. (2020)
Rice husk biochar	*Oryza sativa*	Local chicken	Inclusion of 1% biochar in poultry feed or litter • No effect on feed intake, weight gain, or FCR • Did not alter RBC, WBC count, or Hb levels • Reduced plasma triglycerides, total coliform in litter and E. coli in faces.	Hien et al. (2018)
Bamboo Charcoal	NA	Broiler chicken	Dietary supplementation with 0.5% Bamboo Charcoal vs 0% charcoal • Higher final body weight, greater weight gain, and improved feed conversion efficiency • Slightly lower mortality rate and better meat quality, with reduced shear force, lower fat content, and a tendency for a higher unsaturated fatty acid ratio • Sensory evaluation indicated slightly higher overall acceptability in chickens fed with 0.5% Bamboo Charcoal. • No changes in haematological and serum biochemical parameters between the Charcoal and control groups.	Kim et al. (2011)
Charcoal	NA	Broiler Chicken	0.3% Charcoal was added to diets at the feed mill vs 0% charcoal group (control) • Increased final body weight by 3.5% • Improved FCR by 2.0% compared to control • No significant effects on carcass dressing percentage or muscle proportion	Majewska et al. (2011)
Charcoal	NA	Turkey	Diet supplemented with 0.3% charcoal vs 0% Charcoal (control) • Lowest mortality rate at 4.4%, which is half of that observed in the 0% charcoal diet. • Body weight increased by 3.9%, and the rearing efficiency index improved by 9.7%.	Majewska et al. (2009)

Effect of biochar feeding on Blood biochemistry, oxidative stress and immunity in farm animals

Though in early days, addition of agricultural waste biochar have indicated better responses in livestock and poultry when included in the ration. The better biological responses may be attributed to their better health condition. Mohsen et al. (2025) in Sprague Dowley rats observed that rats supplemented with 5.0% and 7.5% rice straw biochar resulted in decreased oxidative stress and inflammation following immunization, which is an indicative of improved antioxidant enzyme activity and reduced proinflammatory cytokines level. Similarly, superior ($P<0.05$) total antioxidant capacity, glutathione peroxidase and superoxide dismutase levels were observed in 1.0% Pistachios by-product Biochar added in the ration of Japanese quail (Zahed et al. 2025). Furthermore, Zahed et al. (2025) also reported highest lymphocyte percentage and lowest heterophil to lymphocyte ratio in Japanese quails fed with 1.0% Pistachios by-product Biochar indicating the impact of biochar on the health and wellbeing in Japanese quails. El-kelawy et al. (2024) reported superior immunity in terms of superior IgA, IgM, IgG, LTT, phagocytic activity and reduced serum lipids and oxidative stress marker by supplementation of commercial biochar at 2% level in broiler chicken. Zahed et al. (2025) fed Pistachios by-product biochar to Japanese quail at the rate of 0.35%, 0.65% and 1.0% but failed to observe any impact on humoral immunity due to addition of biochar in Japanese quail ration.

The blood biochemical indices of an animal are a true representative of the animal health status and thus during animal experimentations, keen attention is given towards the blood biochemistry. Emam et al. (2026) supplemented 1.5% and 3.0% biochar prepared from mango tree branches and wood as a feed additive to broiler chicks (*Arbor acres*), and reported significant decrease in serum AST, uric acid, creatinine values and blood ammonia content in biochar group chicken than that of control group. Emam et al. (2026) also observed higher total protein and albumin level in chicks supplemented with 1.5% biochar as compared to the 3.0% biochar supplementation. Likewise, Ohanaka et al. (2025) reported improved ($P<0.05$) important haematology and blood biochemical indices namely, Packed cell volume, Red blood cell count, Mean cell volume, Mean cell haemoglobin, Mean cell haemoglobin concentration, Blood clotting time, White blood cell count in male Arbor Acres broiler chicks by supplementing biochar at 0.5 and 1 % dose for 42 days, indicating the impact of biochar addition in broiler ration may aid in better health in poultry birds. Similarly, El-kelawy et al. (2024) observed improvement in the haematology in terms of increased RBCs, Hb, PCV, WBCs, lymphocytes, total protein, globulins, thyroid hormones profile in broiler chicken by supplementation of biochar at 2% level. Nevertheless, Emam et al. (2026) observed a significant increase in the blood calcium and phosphorus level in biochar supplemented chicks compared to the control, which might be due the presence of calcium and phosphorus from biochar. This indicates that, while including biochar as a feed additive, the mineral content of the total ration needs to be revisited to ensure the mineral balance of the ration. Similarly, Ohanaka et al. (2025) also found that, addition of 1% biochar in chicken feed significantly improved the total serum protein, albumin/globulin ratio, Alanine amino transferase, Aspartate amino transferase as compared to the birds fed diet devoid of biochar. Chu et al. (2013b) reported that in pigs, supplementation of bamboo biochar resulted in superior total protein, albumin, cholesterol, HDL-CH and LDL cholesterol level in blood plasma.

Effect of biochar feeding as a toxin adsorbent

Biochar is one of the low cost adsorbent which is highly effective for removing the various kinds of toxins like heavy metals and organic

compounds due to its unique physicochemical properties developed during the pyrolysis process, viz high specific surface area, porous structure, surface functional groups, cation exchange capacity, surface charge, hydrophobicity etc. Biochar also helps to suppress diseases because it can adsorb toxins such as mycotoxins (Gerlach and Schmidt, 2012) and other pathogen and their toxins (Kalus et al., 2019). Its porous structure provides a high surface area, crucial for pathogen binding. Furthermore, it adsorbs a range of toxic compounds, including plant and animal-derived toxins, endogenous toxins, and gases such as methane (Man et al., 2021). The adsorbent nature of the biochar has been studied using one of the most common mycotoxin that is aflatoxin as a model drug (Galvano et al., 1996), where addition of activated biochar at 2.0% level to animal feed lowered the concentration of extractable aflatoxins by 74% and at the same time, decreased aflatoxin concentration in milk by up to 45% (Galvano et al., 1996). Similarly, Prosopis farcta biochar demonstrates the highest efficiency in aflatoxin removal, reducing aflatoxin levels by 95.88% after 6 h of incubation (Raz et al., 2025). the preservation of microbial complexity may be attributed to the preferential adsorption of pathogens over native intestinal microflora (Watarai and Tana, 2005). *Prosopis farcta* biochar are containing higher surface area and porosity, which attributes towards its efficient adsorbent capability for various pollutants, including heavy metals and organic compounds (Raz et al., 2025; Khan et al., 2023; Reyhanitabar et al., 2020). This effect contributes to diminishing antibiotic dependence in chickens (Prasai et al., 2016; Willson et al., 2019). When biochar given with the silage as a feed additive, it can bind with the pesticides, reduce the formation of mycotoxins and also it has ability to enhance the lactic acid bacteria population (Calvelo Pereira et al., 2014). Chicken suffering from the aflatoxicosis when fed with the biochar made from the poultry litter at the rate of 5gm/kg, body weight of bird is maintained and the performance of the birds found to increase (Rashidi et al., 2020), which is an indicative of the effectiveness of the biochar in nullifying the adverse effect of mycotoxins in animal feeds. Nair et al. (2023) also documented that supplementation of biochar can adsorb harmful substances like mycotoxins, ammonia and heavy metals in the digestive tract and diminish their harmful effects on animal health.

Effect of biochar feeding on gut microorganisms

Gut health of livestock and poultry is an indicative of their overall well-being, which influences their nutrient utilization, growth rate, and disease resistance (Naeem and Bourassa, 2025). Biochar can be a potent anti-pathogenic feed additive; which has been found effective to reduce populations of *Clostridium aldenense, Gallibacterium anatis, Helicobacter pullorum, Campylobacter hepaticus, Bacteroides dorei*, and *Campylobacter jejuni* within the avian gut while maintaining overall gut microbiota complexity and diversity (Prasai et al., 2016; Willson et al., 2019). Leng et al. (2013) reported in cattle that, addition of rice hull biochar at 0.6% of feed dry matter reduced the enteric methane emission, which is an indicative of the alteration of rumen fermentation in ruminants by addition of biochar in their ration. Methane is produced by the methanogenic bacteria and consumed by the methanotrophic bacteria so, biochar containing soil amendments encourage the growth of the methanotrophs which reduce the emission of the intestinal methane by providing the habitat for the oxidation of the methane in the stomach (Leng et al., 2012a). Han *et al.* (2018) conducted experiment of feeding oral Rice Straw Biochar at the rate of 1120 mg/kg of body weight to rats for 5 weeks to evaluate the effect on caecal microbial community, where Firmicutes and Bacteroidetes were found to be prevalent and increased ratio of relative abundance (P<0.05) in biochar fed rats. In quails, Raz et al. (2025) found that oral addition of *Prosopis farcta* biochar increased the prevalence of useful gut microbiota, simultaneously reducing detrimental bacteria population. Addition of 2.0% biochar in layer diet significantly reduce the pathogenic bacteria and alter the intestinal microbiota positively and this effect demonstrate its potential as alternative to antibiotic in poultry industry (Willson et al., 2019). Feeding chestnut biochar at low concentration exhibit prebiotic effect by stimulating growth of *Lactiplantibacillus plantarum* and inhibiting the growth of pathogenic *E. coli* strains (Reggi et al., 2025). Furthermore, when biochar is combined with sea tangle (*Laminaria japonica*; Islam et al., 2014) or aged palm sap (Ohanaka et al., 2025) offers a viable antibiotic growth promoter alternative in poultry feed. Biochar prepared from *Prosopis farcta* can be fed to the poultry birds which is reported to be an eco-friendly, cost-effective, and promising strategy for mitigating aflatoxicosis in poultry production (Raz et al., 2025). The mechanisms of actions of superior biological activities of biochar addition in livestock and poultry ration may be corroborated with the enhanced adsorption abilities in detoxifying mycotoxins in feed raw materials, plant-produced toxins, pollutants, and simultaneously improving the populations of beneficial gut microorganisms (Rajpoot et al., 2024).

Reports indicated that biochar may reduce the production of ruminal CH4 emissions both in vitro (Hansen et al., 2012; Leng et al., 2012a,b) and *in vivo* (Leng et al., 2012c) conditions. Saleem et al. (2018) found that pine-based biochar could improve *in vitro* ruminal fermentation, nutrient disappearance, and microbial protein synthesis and reduce enteric methane (CH_4) production. In another study, inclusion of plant-based biochar at 81 g/kg DM reduced the digestibility of forage, however, encouraged volatile fatty acid production (McFarlane et al. 2017). Saleem et al. (2018) studied the effect of pine biochar supplementation in graded doses (0, 0.5, 1, and 2%) on rumen fermentation and digestibility of nutrients by Rumen simulation technique and found that disappearance of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) is proportional to the dose of biochar supplementation and enhanced ($P < 0.01$) production of total Volatile Fatty Acid (VFA), acetate, propionate, branch-chained VFAs, increase NH3- N, increase in Microbial protein synthesis and reducing the overall CH_4 production as compared with the control. However, long term supplementation and *in vivo* studies are essential before concluding and establishing the use of biochars in ruminant rations on regular basis.

Effect on Organ architecture, Carcass Characteristics and egg production

Biochar addition in the ration of poultry birds consistently indicated improvement in the meat quality in broiler birds. For instance, El-kelawy et al. (2024) observed an improvement in dressing percentage, reduction in abdominal fat, serum lipids, and oxidative stress markers in broiler chicken by supplementation of biochar at 2% level by feeding them biochar for 35 days. Kutlu et al. (2001) observed a decrease in the cracked egg production by supplementation of wood charcoal in hen's diet and he postulated that the decrease in the cracked egg production might be due to improved mineral intake from biochar, which contained high levels of ash, calcium, potassium and magnesium. Likewise, Kumar (2025) observed significant reduction (P<0.05) in giblet, liver, and heart weights in corn cob and paddy straw biochar-fed groups. Paddy straw and corncob waste biochar increased meat lightness in 2% paddy straw biochar and 2% corncob biochar fed broiler birds whereas, higher redness of meat was observed in 1% paddy straw biochar groups. Kumar (2025) also reported significantly

lower Free fatty in acids biochar-fed birds, particularly in 2% paddy straw biochar group birds, indicating improved lipid stability. However, biochar produced from paddy straw or corn cob at 1% or 2% level could not affect the meat pH and water-holding capacity (Kumar, 2025).

Ruttanavut et al. (2009) demonstrated that supplementing Aigamos duck feed with a bamboo charcoal powder and bamboo vinegar solution resulted in increased body weight, attributed to enhanced nutrient absorption via elongated intestinal villi. Ruttanavut et al. (2009) also reported active cellular mitosis at the villus, which further indicated improved intestinal functionality and suggests that Biochar supplements may induce villus and epithelial cell hypertrophy. Mohsen et al. (2025) also reported increased intestinal villi length and surface area in Biochar added treatment groups . Likewise, Usman et al. (2025) reported that corn cob Biochar supplementation in Japanese quails improved intestinal histomorphology and postulated that the alterations might be due to Biochar's detoxifying and adsorption capabilities. Mohsen et al. (2025) reported hepato and renal protective effect of rice straw Biochar in Sprague Dowley rats where, they observed hepatocyte architecture and renal tubular integrity preserved in rats with 5% and 7% rice straw Biochar treatment groups as compared to the control.

Litter management

Biochar is a porous material with a high surface area which is capable to absorb moisture and may mitigate NH3 volatilization (Mohammadi-Aragh et al., 2025). Reports advocate positive response by dietary supplementation of biochar improved air and litter quality in poultry houses through reduced volatilization from poultry faeces (Sha et al., 2019; Kalus et al., 2020a). Mohammadi-Aragh et al. (2025) used Biochar from *Pinus spp.* As a litter amendment agent and reported that Biochar-amended litter (30 % v/v surface applied) failed to reduce NH_3 levels or affect bird performance but significantly lowered litter moisture, making it beneficial for moisture control in commercial broiler houses. Similarly, Linhoss et al. (2019) reported beneficial effects of biochar used for litter amendment, which increased water-holding capacity and reduced NH3 volatilization in broiler litter without negatively impacting overall bird performances. If biochar is added in the poultry feed, the amount of biochar used for litter amendment can be reduced accordingly as poultry manure is more degraded microbiologically (Gerlach and Schmidt, 2012). Moreover, application of biochar in feeds and/ or in the litter materials in poultry husbandry tend to rises the marketing potential of poultry manure thanks to the fertilizer quality of the poultry manure which rises strongly due to addition of biochar (Gerlach and Schmidt, 2012). It is observed that when biochar was combined with bedding materials like straw or sawdust for ruminants at 5–10%, there is a decline in the incidences of hoof diseases (O'Toole et al. 2016) which is an indicator of the welfare of animal health (Rajpoot et al., 2024). Reports suggest that treatment of litter with adequate biochar may reduce the odour pollution as well (Gerlach and Schmidt, 2012).

Conclusion

Inclusion of biochar as a novel feed additive for livestock and poultry is capable of augmenting the animal health by optimizing the feed efficiency, adsorbing the toxins present in feed or toxins produced by the gut pathogenic microorganisms and altering the gut microbiota, thus influences overall wellbeing of the animals and poultry. The inclusion of Biochar in livestock and poultry ration has shown growth promotion, immunomodulation which are desirable attributes for an animal enterprise. Now it is essential to investigate the long term effects and disadvantages (if any) of biochar as a feed additive in animal feeding. Even, Biochar addition can be an effective measure to use the mycotoxin contaminated feeds for animal feeding. However, the level of inclusion of biochar in animal ration need to study further to establish its effectiveness before routine use in animal husbandry practices. The results obtained so far has open an window to try and test different biochars produced from different biomasses, their inclusion level in different animal models and molecular level effects for better understanding of its mechanism of action.

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References

Akhtar, J. and Amin, N.S. 2012. A review on operating parameters for optimum liquid oil yield in biomass pyrolysis. Renewable and Sustainable Energy Reviews. 16(7):5101-5109.

Calvelo Pereira, R., Muetzel, S., Arbestain, M. C., Bishop, P., Hina, K. and Hedley, M. 2014. Assessment of the influence of biochar on rumen and silage fermentation: A laboratory-scale experiment. Animal Feed Science and Technology. 196: 22-31.

Chia, H.H., Gong, B., Joseph, S.D., Marjo, C.E., Munroe, P. and Rich, A.M. 2012. Imaging of mineral-enriched biochar by FTIR, Raman and SEM-EDX. Vibrational Spectroscopy. 62:248-257.

Choi, J.S., Jung, D.S., Lee, J.H., Choi, Y.I. and Lee, J.J. 2012. Growth performance, immune response and carcass characteristics of finishing pigs by feeding stevia and charcoal. Korean J. Food Sci. Anim. Resour. 32 (2): 228–233.

Chu, G.M., Kim, J.H., Kang, S.N. and Song, Y.M. 2013a. Effects of dietary bamboo charcoal on the carcass characteristics and meat quality of fattening pigs. Korean Journal for Food Science of Animal Resources. 33(3): 348-355.

Chu, G.M., Kim, J.H., Kim, H.Y., Ha, J.H., Jung, M.S., Song, Y., Cho, J.H., Lee, S.J., Ibrahim, R.I.H. et al. 2013b. Effects of bamboo charcoal on the growth performance, blood characteristics and noxious gas emission in fattening pigs. Journal of Applied Animal Research. 41(1): 48-55.

El-kelawy, M.I., Elnaggar, A.S. and Enass, A.E.K. 2024. The influence of supplementing broiler chickens with humic acid or biochar as natural growth promoters on their productive performance, nutrient digestibility, and physiological performance. Egyptian Poultry Science Journal. 44(1): 123-142.

Emam, A.M., Elnesr, S.S., Mahmoud, B.Y., El-Full, E.A., Abdel-Kader, I.A. and Semida, D.A. 2026. Effect of dietary biochar supplementation on growth, blood constituents, digestive enzymes, intestinal microbiota and viscosity, antioxidant indices, and carcass traits of broiler chicks. Tropical Animal Health and Production, 58(1): 34-43.

European Biochar Foundation. 2012. European Biochar certificate - guidelines for a sustainable production of biochar. European Biochar Foundation (EBC), Arbaz, Switzerland. https://www.european-biochar.org/media/doc/2/version_en_10_4.pdf

Evans, A.M., Boney, J.W. and Moritz, J.S. 2017. The effect of poultry litter biochar on pellet quality, one to 21 d broiler performance, digesta viscosity, bone mineralization, and apparent ileal amino acid digestibility. Journal of Applied Poultry Research. 26(1): 89-98.

Gai, X., Wang, H., Liu, J., Zhai, L., Liu, S., Ren, T. and Liu, H. 2014. Effects of feedstock and pyrolysis temperature on Biochar adsorption of ammonium and nitrate. PLoS One. 9(12): 113888-113906.

Galvano, F., Galofaro, V. and Galvano, G. 1996. Occurrence and stability of aflatoxin M in milk and milk products: A worldwide review. Journal of Food Protection. 59(10): 1079-1090.

Gerlach, H. and Schmidt, H.P. 2012. Biochar in poultry farming. Ithaka Journal. 1: 262-264.

Han, J., Meng, J., Chen, S., Li, C. and Wang, S. 2018. Rice straw biochar as a novel niche for improved alterations to the cecal microbial community in rats. Scientific Reports. 8(1): 16426-16435.

Hansen, H.H., Storm, I.D. and Sell, A.M. 2012. Effect of biochar on in vitro rumen methane production. Acta Agriculturae Scandinavica, Section A - Animal Science. 62(4): 305-309.

Hasanvand, S., Khatibjoo, A., Shizadi, H., Mohamadi, Y., Karimi Torshizi, M.A. and Rahimhi, D. 2023. Effect of wet litter biochar, probiotic and zeolite on performance, blood metabolites and small intestine morphology of broiler chickens reared under cold stress. Journal of Animal Production. 25(3): 325-341.

Hien, N.N., Dung, N.N.X., Manh, L.H. and Le Minh, B.T. 2018. Effects of biochar inclusion in feed and chicken litter on growth performance, plasma lipids and fecal bacteria count of Noi lai chicken, Livestock Research for Rural Development. 30(7): 131.

International Biochar Initiative. 2015. Standardized product definition and product testing guidelines for biochar that is used in soil. IBI Biochar Standards. International Biochar Initiative. https://biochar-international.org/wp-content/uploads/2020/06/IBI_Biochar_Standards_V2.1_Final2.pdf

Ippolito, J.A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J.M., Fuertes-Mendizabal, T., Cayuela, M.L., Sigua, G., Novak, J., Spokas, K. and Borchard, N. 2020. Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. Biochar. 2: 421-438.

Islam, M.M., Ahmed, S.T., Kim, Y.J., Mun, H.S. and Yang, C.J. 2014. Effect of sea tangle (Laminaria japonica) and charcoal supplementation as alternatives to antibiotics on growth performance and meat quality of ducks. Asian-Australasian Journal of Animal Sciences. 27(2): 217-224.

Joseph, S., Doug, P.O.W., Dawson, K., Mitchell, D.R., Rawal, A., James, H and Solaiman, Z.M. 2015. Feeding biochar to cows: an innovative solution for improving soil fertility and farm productivity. Pedosphere. 25(5):666–679

Kalus, K., Konkol, D., Korczyński, M., Koziel, J.A. and Opaliński, S. 2020a. Effect of biochar diet supplementation on chicken broilers performance, nh3 and odor emissions and meat consumer acceptance. Animals. 10(9): 1539-1551.

Kalus, K., Koziel, J.A. and Opaliński, S. 2019. A review of biochar properties and their utilization in crop agriculture and livestock production. Applied Sciences. 9(17): 3494-3509.

Kana, J.R., Teguia, A., Mungfu, B.M. and Tchoumboue, J. 2010. Growth performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of charcoal from maize cob or seed of Canarium schweinfurthii Engl. Tropical Animal Health and Production. 43(1):51–56.

Khan, I.U., Qi, S.S., Gul, F., Manan, S., Rono, J.K., Naz, M., Shi, X.N., Zhang, H., Dai, Z.C. and Du, D.L. 2023. A green approach used for heavy metals phytoremediation via invasive plant species to mitigate environmental pollution: a review. Plants. 12(4): 725.

Kim, S.H., Lee, I.C., Kang, S.S., Moon, C.J., Kim, S.H., Shin, D.H., Kim, H.C., Yoo, J.C. and Kim, J.C. 2011. Effects of bamboo charcoal and bamboo leaf supplementation on performance and meat quality in chickens, Journal of Life Science. 21(6): 805-810.

Kumar, R. 2025. Effect of corn cob and paddy straw biochar as novel feed additives on the performance and health status of broiler chicken. M.V.Sc. Thesis, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India.

Kutlu, H.R., Ünsal, I. and Görgülü, M. 2001. Effects of providing dietary wood (oak) charcoal to broiler chicks and laying hens. Animal Feed Science and Technology. 90(3-4): 213-226.

Lao, E.J. and Mbega, E.R. 2020. Biochar as a feed additive for improving the performance of farm animals. Malaysian Journal of Sustainable Agriculture. 4(2): 86-93.

Lee, J.J., Park, S.H., Jung, D.S., Choi, Y.I. and Choi, J.S. 2011. Meat quality and storage characteristics of finishing pigs by feeding stevia and charcoal. Korean J. Food Sci. Anim. Resour. 31 (2): 296–303.

Leng, R.A., Inthapanya, S. and Preston, T.R. 2012a. Biochar lowers net methane production from rumen fluid in vitro. Livestock Research for Rural Development. 24(6): 103.

Leng, R.A., Inthapanya, S. and Preston, T.R. 2012b. Methane production is reduced in an in vitro incubation when the rumen fluid is taken from cattle that previously received biochar in their diet. Livestock Research for Rural Development. 24(11): 211.

Leng, R.A., Inthapanya, S. and Preston, T.R. 2013. All biochars are not equal in lowering methane production in in vitro rumen incubations. Livestock Research for Rural Development, 25(6): 106.

Leng, R.A., Preston, T.R. and Inthapanya, S. 2012c. Biochar reduces enteric methane and improves growth and feed conversion in local yellow cattle fed cassava root chips and fresh cassava foliage. Livestock Research for Rural Development. 24(11): 199.

Li, H., Dong, X., da Silva, E.B., de Oliveira, L.M., Chen, Y. and Ma, L.Q. 2017. Mechanisms of metal sorption by biochars: biochar characteristics and modifications. Chemosphere. 178: 466-478.

Linhoss, J.E., Purswell, J.L., Street, J.T. and Rowland, M.R. 2019. Evaluation of biochar as a litter amendment for commercial broiler production. Journal of Applied Poultry Research. 28(4): 1089-1098.

Liu, C., Liu, X., He, Y., An, X., Fan, D. and Wu, Z. 2021. Microwave-assisted catalytic pyrolysis of apple wood to produce biochar: co-pyrolysis behavior, pyrolysis kinetics analysis and

evaluation of microbial carriers. Bioresource Technology. 320: 124345-124353.

Majewska, T., Mikulski, D. and Siwik, T. 2009. Silica grit, charcoal and hardwood ash in turkey nutrition. Journal of Elementology. 14(3): 489-500.

Majewska, T., Pudyszak, K. and Kozłowski, K. 2011. The effect of charcoal addition to diets for broilers on performance and carcass parameters. Veterinarija ir Zootechnika (Vet Med Zoot). 55(77): 30-32.

Man, K.Y., Chow, K.L., Man, Y.B., Mo, W.Y. and Wong, M.H. 2021. Use of biochar as feed supplements for animal farming. Critical Reviews in Environmental Science and Technology. 51(2): 187-217.

McFarlane, Z.D., Myer, P.R., Cope, E.R., Evans, N.D., Bone, T.C., Bliss, B.E. and Mulliniks, J.T. 2017. Effect of biochar type and size on in vitro rumen fermentation of orchard grass hay. Agricultural Sciences. 8: 316-325.

Mohammadi-Aragh, M.K., Norris, K.L., Chesser, G.D., Lowe, J.W., Evans, J.D., Purswell, J.L. and Linhoss, J.E. 2025. Comparison of biochar and poultry litter treatment (PLT) amendments on broiler litter quality and bird performance. Journal of Applied Poultry Research. 34(1):100499-100505.

Mohsen, S., Reda, M., Waleed, F., Waleed, Y., Tarek, M., Saber, S., Farid, A. and Safwat, G. 2025. Evaluation of the effect of biochar on the metabolic status, oxidative stress, and immune response of Sprague Dawley rats. Research in Veterinary Science. 193: 105778-105791.

Naeem, M. and Bourassa, D. 2025. Probiotics in poultry: unlocking productivity through microbiome modulation and gut health. Microorganisms. 13(2): 257-277.

Nair, P.S., Sivani, M.P., Suresh, S., Sreekanth, A.J., Sivasabari, K., Adithya, K.S., Anuranj, P.R., Nayana, K., Parvathy, S., Chakraborty, S. and Chopra, H. 2023. Beneficial impacts of biochar as a potential feed additive in animal husbandry. Journal of Experimental Biology and Agricultural Sciences. 11(3) : 479-499.

Naveena, B.M. and Kiran, M. 2014. Buffalo meat quality, composition, and processing characteristics: contribution to the global economy and nutritional security. Animal Frontiers. 4(4):18-24.

Ohanaka, A.U.C., Ohanaka, J.N., Nwogu, C.M., Ogbuewu, I.P., Etuk, I.F. and Okoli, I.C. 2025. Growth promoting and health enhancing effects of aged palm sap-enriched activated biochar in broiler nutrition. Journal of Agriculture and Rural Development in the Tropics and Subtropics. 126(1):13-24.

O'Toole, A., Andersson, D., Gerlach, A., Glaser, B., Kammann, C., Kern, J., Kuoppamäki, K., Mumme, J., Schmidt, H.P., Schulze, M. and Srocke, F. 2016. Current and future applications for biochar. In: biochar in european soils and agriculture. Routledge, Routledge, p 253-280.

Pandey, A.K., Kumar, P. and Saxena, M.J. 2019. Feed additives in animal health. In: Nutraceuticals in Veterinary Medicine. Eds. Gupta, R.C., Srivastava, A. and Lall, R. Springer International Publishing, Cham, Switzerland, p 345-362.

Pecha, M.B. and Garcia-Perez, M. 2020. Pyrolysis of lignocellulosic biomass: oil, char, and gas. In: Bioenergy. Elsevier, p 581-619.

Phongpanith, S., Inthapanya, S. and Preston, T.R. 2013. Effect on feed intake, digestibility and N balance in goats of supplementing a basal diet of Muntingia foliage with biochar and water spinach (Ipomoea aquatica). Livest Res Rural Dev. 25(2):72

Prasai, T.P., Walsh, K.B., Bhattarai, S.P., Midmore, D.J., Rohman, T.H. and Moore, R.J. 2018. Manure from biochar, bentonite and zeolite feed supplemented poultry. Journal of Cleaner Production. 186: 1–11.

Prasai, T.P., Walsh, K.B., Bhattarai, S.P., Midmore, D.J., Van, T.T., Moore, R.J. and Stanley, D. 2016. Biochar, bentonite and zeolite supplemented feeding of layer chickens alters intestinal microbiota and reduces Campylobacter load. PLoS One. 11(4): 0154061-0154073.

Rajpoot, S.K., Sharma, P.R., Singh, J., Kumar, A., Vijayakumar, S., Chaudhary, R. and Kumar, D. 2024. Biochar as a novel feed additive for ruminants. In: Feed Additives and Supplements For Ruminants. Springer Nature Singapore, Singapore. pp 423-435.

Rashidi, N., Khatibjoo, A., Taherpour, K., Akbari-Gharaei, M. and Shirzadi, H. 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. Poultry Science. 99(11): 5896-5906.

Raz, M., Bagherzadeh-Kasmani, F., Karimi-Torshizi, M.A., Ghazaghi, M., Mokhtarpour, A. and Mehri, M. 2025. Prosopis farcta biochar neutralizes aflatoxin b1 and enhances health and productivity in quails. Discover Animals. 2(1):12-23.

Reggi, S., Frazzini, S., Pedrazzi, S., Ghidoli, M., Torresani, M.C., Puglia, M., Morselli, N., Guagliano, M., Cristiani, C., Pilu, S.R. and Onelli, E. 2025. Metabolomic insights into the potential of chestnut biochar as a functional feed ingredient. Applied Sciences. 15(3): 1084-1099.

Reyhanitabar, A., Frahadi, E., Ramezanzadeh, H. and Oustan, S. 2020. Effect of pyrolysis temperature and feedstock sources on physicochemical characteristics of biochar. Journal of Agricultural Science and Technology. 22(2): 547-561.

Ruttanavut, J., Yamauchi, K., Goto, H. and Erikawa, T. 2009. Effects of dietary bamboo charcoal powder including vinegar liquid on growth performance and histological intestinal change in Aigamo ducks. International Journal of Poultry Science. 8(3): 229-236.

Saleem, A.M., Ribeiro, G.O., Yang, W.Z., Ran, T., Beauchemin, K.A., McGeough, E.J., Ominski, K.H., Okine, E.K. and McAllister, T.A. 2018. Effect of engineered biocarbon on rumen fermentation, microbial protein synthesis, and methane production in an artificial rumen (RUSITEK) fed a high forage diet. Journal of Animal Science. 96(8):3121-3130.

Schmidt, H.P., Hagemann, N., Draper, K. and Kammann, C. 2019. The use of biochar in animal feeding. PeerJ. 7: 7373-7426.

Sha, Z., Li, Q., Lv, T., Misselbrook, T. and Liu, X. 2019. Response of ammonia volatilization to biochar addition: a meta-analysis. Science of the Total Environment. 655: 1387-1396.

Silivong, P. and Preston, T.R. 2016. Supplements of water spinach (Ipomoea aquatica) and biochar improved feed intake, digestibility, N retention and growth performance of goats fed foliage of Bauhinia accuminata as the basal diet. Livestock Research for Rural Development. 28(5): 78-79.

Sohi, S., Lopez-Capel, E., Krull, E. and Bol, R. 2009. Biochar, climate change and soil: a review to guide future research. CSIRO Land Water Sci Rep 5:17–31.

Sun, J., Lian, F., Liu, Z., Zhu, L. and Song, Z. 2014. Biochars derived from various crop straws: Characterization and Cd(II) removal potential. Ecotoxicology and Environmental Safety. 106: 226-231.

Tang, J., Zhu, W., Kookana, R. and Katayama, A. 2013. Characteristics of biochar and its application in remediation of contaminated soil. Journal of Bioscience and Bioengineering. 116(6): 653-659.

Tripathi, M., Sahu, J.N. and Ganesan, P. 2016. Effect of process parameters on production of biochar from biomass waste through pyrolysis: a review. Renewable and Sustainable Energy Reviews. 55: 467-481.

Usman, M., Qureshi, A.S., Shahid, R.U. and Deeba, F. 2025. Impact of biochar and organic acid-enriched diet on growth performance and intestinal histomorphometry in japanese quail (Coturnix Coturnix Japonica). Pakistan Veterinary Journal. 45(1): 352-359.

Van, D.T.T., Mui, N.T. and Ledin, I. 2006. Effect of method of processing foliage of Acacia mangium and inclusion of bamboo charcoal in the diet on performance of growing goats. Animal Feed Science and Technology. 130: 242–56.

Viaene, J., Peiren, N., Vandamme, D., Lataf, A., Cuypers, A., Debeer, L. and Vandecasteele, B. 2024. Application of biochar to anaerobic digestion versus digestate: effects on N emissions and C stability. Science of the Total Environment. 915: 170124-170131.

Wang, N., Chang, Z.Z., Xue, X.M., Yu, J.G., Shi, X.X., Ma, L.Q. and Li, H.B. 2017. Biochar decreases nitrogen oxide and enhances methane emissions via altering microbial community composition of anaerobic paddy soil. Science of the Total Environment. 581: 689-696.

Watarai, S. and Tana. 2005. Eliminating the carriage of Salmonella enterica serovar enteritidis in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid (Nekka-rich). Poultry Science. 84(4): 515-521.

Weber, K. and Quicker, P. 2018. Properties of Biochar. Fuel. 217: 240-261.

Willson, N.L., Van, T.H., Bhattarai, S.P. 2019. Feed supplementation with biochar may reduce poultry pathogens, including Campylobacter hepaticus, the causative agent of spotty liver disease. PLoS One. 14: 1-16.

Yaashikaa, P.R., Senthil Kumar, P., Varjani, S. and Saravanan, A. 2020. A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy. Biotechnology Reports. 28: 00570.

Zahed, O., Vakili, R. and Mokhtarpour, A. 2025. Pistachio by-product biochar as an antibiotic alternative: promoting growth and gut health in Japanese quails (Coturnix japonica). Tropical Animal Health and Production. 57: 249-257.

Review Article

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Water quality and its multidimensional impact on broiler chicken production: growth performance, health, immunity, and carcass characteristics - A comprehensive review

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Water is one of the most important nutrients for physiological functions but is frequently undervalued in commercial broiler production and influences digestion, metabolism, immunity and performance. This review compiles evidence on the effect of critical water quality parameters such as pH, alkalinity, salinity, total dissolved solids (TDS), hardness, mineral composition, and microbiological contamination on the broiler growth, feed efficiency, gut health, immune status, carcass traits, and mortality. Elevated alkalinity, primarily due to bicarbonate, carbonate, and hydroxide ions, disrupts gastric acidification, reducing pepsin activity and impairing protein digestion while promoting pathogenic microbial proliferation. Similarly, high TDS (>3,000 mg/L) and salinity are associated with reduced body weight gain, poor feed conversion ratio, osmotic diarrhoea and increased mortality. Alternatively, mild acidification of water (pH 5.5-6.5) increases the enzymatic efficiency, calcium and phosphorus utilization and promotes a favourable gut microbiota by encouraging beneficial microorganisms such as Bacillota and Bacteroidetes and suppressing pathogens such as *Salmonella* and *Escherichia coli*, thus improving immune responses. Poor water quality also negatively impacts carcass yield, dressing percentage and breast muscle development due to osmotic stress, inefficient nutrient utilization and increased protein catabolism. Overall, the findings highlight that systematic, source-specific water quality monitoring and evidence-based interventions including acidification, mineral management and microbiological treatment are key elements of precision poultry nutrition to optimize gut health, disease resistance, carcass quality and economic sustainability.

Keywords: Alkalinity, Broiler chickens, feed conversion ratio, gut health, immune function.

Introduction

Poultry production, especially the commercial broiler systems, is the central, growing component of global food security. The world population is expected to reach 9.7 billion by 2050 (United Nations, 2022), which will increase the demand for affordable and high-quality animal protein. Broiler meat is now the meat of choice with global output increasing from about 70 million tonnes in 2000 to an estimated 103.5 million tonnes in 2023 (USDA, 2023). Projections suggest continued growth to about 145 million tonnes by 2030, with the fastest growth expected in South and Southeast Asia, sub-Saharan Africa and Latin America (OECD FAO, 2024). India is projected to lead the growth with a compound annual growth rate (CAGR) of around 5.5% due to rising incomes, urbanisation and the shift to poultry as the main protein in the diet.

Even with the size and sophistication of the sector, production efficiency is still subjected to seemingly routine factors. Of these, water quality is arguably the most consequential and least carefully managed variable in commercial broiler operations. Water constitutes about 65-70% of the body weight of a broiler (Leeson and Summers, 2009) and is involved in almost all physiological processes such as thermoregulation, nutrient absorption and transport, metabolic reactions, waste excretion and immune function. Broilers drink water at a rate of approximately twice the amount of feed consumed (water-to-feed ratio ≈1.6-2.0:1 under thermoneutral conditions) and therefore the quality of drinking water has a direct and continuous effect on bird physiology from day zero to market age.

However, historically water has been regarded as a passive medium rather than an active nutrient with specified quality requirements. The consequences are profound and so significantly affect the farm economics and production. Poor water quality has been linked to reduced average daily gain (ADG), worsened Feed Conversion Ratio (FCR), increased mortality, compromised immune organ development, higher susceptibility to enteric pathogens, and poor carcass quality, leading to measurable economic losses (Augusto et al., 2022; Biswas et al., 2024).

This review addresses a structured, scientifically supported, comprehensive coverage of importance of water quality as a whole and its impacts on broiler production outcomes. The specific objectives are to: (i) describe the major physicochemical and microbiological water quality parameters relevant in broiler production; (ii) review the mechanistic and empirical evidence linking each parameter to growth performance, gut health, immune function, and carcass characteristics; (iii) examine the influence of water source on quality variability; and (iv) outline evidence-based water quality management and treatment strategies.

Water: The Quintessential but Overlooked Nutrient

Precision nutrition in commercial broiler production has evolved into complex scientific research that demands a careful and balanced formulation of macronutrients and micronutrients. Water, on the other hand, is the fundamental and systematically neglected nutrient. Water is much more than a simple solvent, it plays a role in physiological homeostasis in thermoregulation, in the absorption and transport of nutrients, and in the many metabolic reactions that are essential for cellular function (Leeson and Summers, 2009).

Broilers are especially susceptible to heat stress because they lack sweat glands and depend on evaporative cooling by panting to regulate their body temperature, which can lead to them consuming 4–8 fold more water than they usually do (Borges et al., 2003). Water is the solvent for enzymatic catalysis at the cellular level and even mild dehydration (1–2% of body weight) obviously reduces metabolic efficiency and feed intake. Modern poultry farming now treats water as a main nutrient with strict quality standards, rather than just an extra resource. If water quality is poor, bird body weight gain can drop by 5% to 20%, and FCR can worsen by 0.1 to 0.3 points (Augusto et al., 2022)

Water Intake Patterns and Feed-to-Water Ratio

Water intake (WI) patterns of broiler chicken depend on age-related patterns, environmental effects, dietary effects, and genetic and strain effects. Age related: Broiler WI increases linearly with age, a long-established trend confirmed in recent measurements; the average bird consumes ~7.8 L between 14-42 d with substantial individual variation (Aggrey et al., 2023), and earlier commercial data also show WI is a strong linear function of age (Pesti et al., 1985).

The water to feed intake ratio of broiler chickens is a dynamic and diagnostically significant indicator of flock health. Under thermoneutral conditions this ratio is usually between 1.6:1 to 2.0:1 but varies widely with age, temperature, diet composition, genetic strain and management practices (Pesti et al., 1985; May and Lott, 1992). The ratio changes from about 1.93:1 on day 11 to 1.72:1 on day 46, showing the changing metabolic needs (Williams et al., 2013). Environmental effects: heat stress increases WI and the WI/FI ratio as birds depend on evaporative cooling via panting and decreases Feed Intake (FI) (de Baets et al., 2026; Oluwagbenga & Fraley, 2023; Donkoh, 1989). Birds under heat stress have higher WI during hot periods even when corrected for light hours (de Baets et al., 2026). Environmental temperature is perhaps the most acute modulator, with water intake increasing by approximately 7% for each 1°C rise above the thermoneutral zone. Water temperature effects are less consistent, though cold water may improve comfort in heat conditions (Erensoy et al., 2020). Dietary effects: moisture-rich feed ingredients (e.g., live Black Soldier Fly larvae) reduce WI from drinkers (Dorper et al., 2024); dietary sodium, a potent osmotic stimulus, increases water intake proportionally; several water-delivered supplements increase WI especially under heat stress or via osmoregulatory effects (Fathima et al., 2025; Hamdi et al., 2026) and high-salinity drinking water stimulates thirst at low levels but may reduce WI at high concentrations (Biswas et al., 2024). Genetic and strain effects: males drink more than females, and strains differ significantly in WI patterns and in water conversion ratio (WCR) performance, though differences do not align strictly with “fast-growing” versus “high-yielding” types (Hiltz et al., 2025). Monitoring the water-to-feed ratio daily provides a sensitive, non-invasive early-warning indicator of flock health; a sustained ratio below 1.5:1 may signal water quality problems reducing palatability, while a ratio persistently above 2.5:1 may indicate heat stress, high dietary salt, diarrhoea, or renal disease (Pesti et al., 1985; May and Lott, 1992).

Water-to-feed intake ratio (WI/FI) in broiler chickens shows consistent baseline values and predictable responses to environmental, dietary, and genetic factors. Baseline ratios for conventional broiler diets cluster around 1.75–1.77 g/g (Pesti et al., 1985; Edge et al., 2025), and historical flock-level studies report comparable averages of 1.77 g/g (Edge et al., 2025). Factors that increase WI/FI include heat stress, which raises WI while depressing FI (de Baets et al., 2026; Oluwagbenga & Fraley, 2023), and certain supplements—most notably *Pluchea indica* extract at 6 mL/L—reported to raise WI/FI to 2.77 (Hamdi et al., 2026). Unrestricted water access during rearing increased breeder pullet WI/FI to 2.3 versus 1.8 under restricted access (Van Emous, 2024). Factors that decrease WI/FI include diets with high-moisture inputs such as live black soldier fly larvae, which supply water from feed and reduce drinker intake (Dorper et al., 2024), and water deprivation, which alters dynamics because FI often falls faster than WI (Mhmoud et al., 2023; Ndlela et al., 2020). Water conversion ratio (WCR = water consumed/weight gained) varies among strains but shows no sex × strain interaction (Hiltz et al., 2025), with higher-performing strains generally exhibiting lower (more efficient) WCR. Practically, monitoring WI provides a sensitive early indicator of flock health because sudden drops signal problems (Edge et al., 2025); heat mitigation (cooler water, additives, ventilation) is essential to stabilize WI/FI, diet formulations should be adjusted when WI/FI deviates markedly from baseline, and breeding for water efficiency is feasible since selection lines differ in WCR and neuroendocrine profiles (Roach et al., 2025). In conclusion, convergent evidence indicates broilers maintain a typical WI/FI near 1.75–1.77 under standard conditions; environmental stressors—especially heat—are the strongest drivers of elevated WI and WI/FI, while diet composition and management can modulate the ratio, and strain/sex contribute additional variation (Pesti et al., 1985; Dorper et al., 2024; de Baets et al., 2026; Hiltz et al., 2025).

Commercial poultry operations typically rely on inline water meters on drinking lines as the standard method for monitoring flock water intake (Edge et al., 2025). All poultry houses in developed countries are reported to be equipped with such water meters, which often provide electronic pulse outputs that integrate with house controllers to enable automated daily or hourly logging of WI per line or per whole house (Edge et al., 2025). These water intake patterns serve as real-time health diagnostics because disease produces sharp drops in WI and dietary salt problems cause elevated WI (Edge et al., 2025). Large-scale flow meters installed on commercial-style houses have been used successfully to monitor WI over the life of the flock, validating the method for both industry and scientific analyses (Edge et al., 2025). Where farms lack flow meters, some studies estimated water demand indirectly from feed consumption (e.g., 1 kg feed ≈ 2 L water), a suboptimal approach that underscores the importance of meter installation (Williams et al., 2013).

Automated systems that integrate meter pulse outputs with environmental controllers allow logging by row or entire house and support early detection of problems such as disease, salt toxicity, or leaking drinkers (Xin & Liu, 2017; Edge et al., 2025). While specialized research tools—novel low-flow monitoring systems, pressure-transducer drinking stations integrated with balances, and isotope dilution techniques—can measure individual bird water turnover, they are costly and impractical for routine commercial use (Puma et al., 2001; Riek et al., 2008; Hiltz et al., 2021). Similarly, RFID-linked stations and low-flow meters have been developed for research applications but are not used widely on commercial farms (Hiltz et al., 2021; Puma et al., 2001).

AI and precision-livestock technologies (computer vision, video action recognition, and behavior-detection systems) increasingly complement water monitoring by identifying drinking behavior, but they do not measure water volume directly and therefore depend on flow meters for quantitative intake data (Demmers et al., 2022; Wu et al., 2022; Nasiri et al., 2024). Reviews and recent field studies confirm that water meters remain the simplest, most reliable way to

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measure drinking performance in commercial operations (Bao & Xie, 2022; Edge et al., 2025). Daily meter-based measurements expressed as WI per house or per 1,000 birds are widely used for benchmarking and provide sensitive early warning of health and management issues, making meter installation and integration with data logging a practical priority for commercial broiler and layer farms (Williams et al., 2013; Edge et al., 2025).

Sources of Drinking Water on Poultry Farms

Municipal (Treated) Water

Municipal water sources assure the most consistent baseline quality. Residual chlorine (0.2–0.5 mg/L) can interfere with live vaccines, some medications, and probiotics administered through the water line (Kalita et al., 2020). pH fluctuations associated with treatment may reduce solubility of water-soluble vitamins and medications. The significant water quality parameters with their acceptable ranges for broilers and various characteristic challenges faced by different sources available has been shown in Table 1.

Groundwater (Well Water)

Groundwater is the primary source for the majority of global poultry operations. Its quality is highly variable and mostly depends on the geographical location of the farm. TDS frequently exceed 3,000 mg/L in well water from arid and semi-arid regions, and elevated sodium, sulphate, calcium, magnesium, and iron concentrations are common (Augusto et al., 2022; Raut et al., 2025). Nitrate contamination from agricultural runoff also represents an increasing problem for the only available drinking water source for the poultry farms.

Surface Water

Surface water sources are presented as the water sources with the most susceptible microbiological and chemical risks. Surveys indicate that up to 87% of stream samples in agricultural catchments exceed E. coli limits for poultry use (Derose et al., 2020). Surface water should never be supplied to broilers without comprehensive treatment.

Table 1: Key water quality parameters-acceptable ranges for broilers and characteristic challenges by source type

Parameter	Acceptable Range (Broilers)	Municipal	Well Water	Surface Water
pH	6.5-8.5	Variable	Often alkaline	Variable; seasonal
TDS (mg/L)	<1,500 (<3 weeks); <3,000 (>3 weeks)	Generally OK	Often >3,000	Moderate
Total Hardness (mg/L $CaCO_3$)	60–300	Variable	Often very high	Variable
Sodium (mg/L)	<200	Generally low	Often elevated	Usually, OK
Iron (mg/L)	<0.3	Usually, <0.1	Frequently elevated	
Elevated after runoff				
Nitrate (mg/L)	<15	Usually, low	Variable	Agricultural runoff risk
Total Coliforms (CFU/mL)	<100	Low (treated)	Contamination risk	High risk
E. coli (CFU/mL)	Absent (<1)	Low (treated)	Risk of contamination	Frequently positive
Turbidity (NTU)	<5	Low	Usually, low	Often elevated

(Do Amaral, 2004; Augusto et al., 2022; Raut et al., 2025; Munster and Kemper, 2024)

Key Physicochemical Water Quality Parameters and Their Effects on Broiler Production

Total Dissolved Solids (TDS) and Salinity

Total dissolved solids (TDS) is a comprehensive measure of all dissolved inorganic and organic substances in water (mg/L or ppm). Salinity refers specifically to the concentration of dissolved salts, predominantly sodium chloride, and forms a component of TDS. Both parameters are operationally measured via electrical conductivity (EC), with 1 µS/cm approximately equivalent to 0.5-0.7 mg/L TDS (Mirsalimi and Julian, 1993).

The physiological consequences of elevated TDS and salinity are dose-dependent. High osmotic load induces increased water consumption at low to moderate salinity as an attempt to dilute excess ions, followed by paradoxical reduction in water intake at high salinity as palatability deteriorates (Biswas et al., 2024). Experimental exposure of broilers to saline water at 0, 5, 10, and 15 g/L demonstrated a significant inverse relationship between salinity concentration and feed intake and BWG (Biswas et al., 2024; Mushtaq et al., 2013). Increasing drinking-water salt level from 0 to 15 g/L produced progressive negative effects: compared with the control (0 g/L), T1 (5 g/L) showed reduced body weight a moderate decline in antibody titers, a rise in *E. coli* antibiotic resistance to 47.22%, and minimal organ damage; T2 (10 g/L) caused further reductions in weight and intake, significant antibody decline, 50.00% *E. coli* resistance and fibrotic changes; T3 (15 g/L) produced the lowest body weight and feed intake, severe antibody decline, 55.56% *E. coli* resistance, and severe fibrosis-indicating a dose-dependent deterioration in production, immunity, and organ health associated with higher water salinity (Biswas et al., 2024). FCR worsens proportionally with increasing salinity. At the renal level, elevated dietary sodium imposes a significant excretory burden, and histopathological evidence of renal stress has been documented in experimental birds exposed to saline water (Mirsalimi et al., 1993). Salt-induced pulmonary hypertension and right ventricular hypertrophy-precursors to ascites syndrome are particularly prominent in rapidly growing broilers under three weeks of age (Mirsalimi and Julian, 1993).

Figure 1 represents graphical synthesis of the dose-response relationship between Total Dissolved Solids (TDS) and key production outcomes (BWG and FCR) across a range of published experimental values while Table 2 is depicting the general classification of drinking water for broiler chicken as per the TDS and showing its implications on health and performance.

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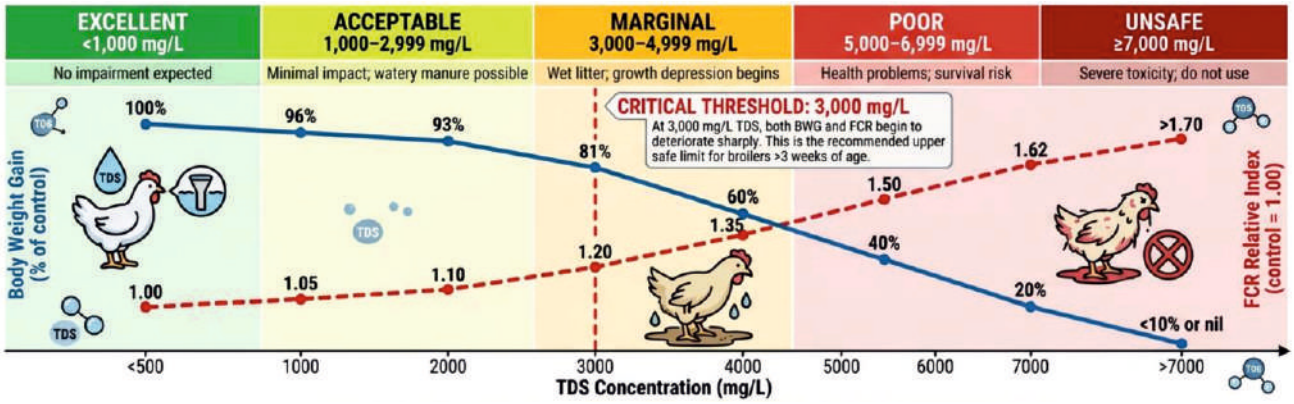

Figure 1: Dose-response relationship between drinking water total dissolved solids (TDS) concentration and broiler production outcomes. Body weight gain (BWG) is expressed as percentage of control (<500 mg/L TDS = 100%); feed conversion ratio (FCR) is expressed as a relative index with control=1.00. Data points represent means synthesised from published experimental and field studies. The critical threshold at 3,000 mg/L TDS marks the point beyond which both BWG and FCR deteriorate sharply. Zone classifications follow Hess and Macklin (2025).

Table 2: Classification of drinking water by TDS and associated health and performance implications for broiler chickens

TDS (mg/L)	Classification	Expected Effects on Broilers	Recommended Action
<1,000	Excellent	No health or performance impairment expected	No treatment required
1,000-2,999	Acceptable	Watery manure possible at upper range; no significant growth effect	Monitor closely; test regularly
3,000-4,999	Marginal	Wet litter, increased mortality risk, stunted growth	Treatment advised; limit to mature birds
5,000-6,999	Poor	Health problems, poor growth, survival concerns near upper limit	Treatment required; avoid for young chicks
7,000-10,000	Unsafe for Poultry	Severe dehydration, salt toxicity, organ failure	Do not use without full desalination
>10,000	Unsuitable	Lethal for poultry; unsuitable for most livestock	Must not be used

(Hess and Macklin, 2025; Mirsalimi and Julian, 1993; Biswas et al., 2024)

Drinking Water pH: Acidity, Neutrality, and Alkalinity

Water pH is among the most consequential and practically modifiable parameters in broiler water management. The gastrointestinal tract of broilers requires an acidic microenvironment-particularly in the proventriculus (pH 2.5-3.5) and gizzard (pH 2.0-3.5) to activate pepsinogen to pepsin, initiate protein denaturation, and create bacteriostatic conditions (Yadav et al., 2010). When drinking water pH is elevated, it contributes to buffering of gastric acid, raising luminal pH and impairing these critical early digestive processes.

Numerous studies have demonstrated that water acidification to pH 5.0-6.5 improves broiler BWG, ADG, and FCR relative to birds receiving neutral or alkaline water (Chaveerach et al., 2002; Hamid et al., 2018; Samudovská et al., 2018). The performance-enhancing effects of acidifiers are attributed to stimulation of pancreatic enzyme secretion (Dibner et al., 2002), enhanced mineral solubility and absorption, improved protein digestibility, and direct antimicrobial effects on enteric pathogens. The antimicrobial mechanism involves undissociated acid molecules penetrating bacterial cell membranes and dissociating intracellularly, releasing protons that acidify the cytoplasm and inhibit acid-sensitive metabolic enzymes (Davidson et al., 2012; Carpenter and Broadbent, 2009).

The claim that "alkaline water = health" for humans does not hold reliably for poultry. Raising drinking-water pH alone generally does not improve growth, feed or water intake, FCR, or amino-acid digestibility (Bassi et al., 2022; Raut et al., 2025), and highly alkaline water (pH > 8) can impair pepsin activity, protein digestion, and palatability (Azizi et al., 2026). Reported benefits typically come from ionized/electrolyzed reduced waters that alter ORP, TDS, and have mild antimicrobial effects properties that, not pH per se, seem responsible for improvements in weight or antioxidant markers (Khalil et al., 2025; El-Sabrout et al., 2026) yet these benefits are inconsistent under commercial stressors such as cyclic heat (de Baets et al., 2026). Mildly acidified water more consistently supports performance and gut health as depicted in Figure 2 (Hamid et al., 2018; Mahmoud et al., 2024). Practically, maintain drinking water at pH ~5.5 to 7 with low TDS, good hygiene, and balanced minerals rather than pursuing alkalinity (Bassi et al., 2022; Raut et al., 2025; Azizi et al., 2026).

On the other hand, highly alkaline water (pH >8.5), especially when induced by sodium bicarbonate supplementation, consistently affects the health and productivity of broilers, resulting in poor productive development, increased ascites incidence, higher mortality, and negative impacts on immune organ weights and caecal pH (Hamid et al., 2018). In practice, the pH of drinking water should be aimed at between 6.0 and 7.0, which represents the best compromise between digestive efficiency, mineral solubility, antimicrobial activity and palatability.

Water Alkalinity

Alkalinity frequently gets equated with pH but these are two different parameters. pH is a measure of the instantaneous concentration of hydrogen ions, while alkalinity is the ability of the water to buffer or resist changes in pH when acids are added. The buffering capacity is mainly provided by bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and hydroxide (OH^-) ions expressed in mg/L as calcium carbonate ($CaCO_3$). Operationally, alkalinity defines the

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practical difficulty and expense of pH adjustment with acidifiers: it takes significantly more acid to bring the pH of high-alkalinity water down to 6.0 than of low-alkalinity water, with the same initial pH (Hamid et al., 2018).

Broiler drinking water high in alkalinity has effects other than increasing pH. An excess of bicarbonate and carbonate ions buffers gastric acid in the crop, proventriculus, and gizzard, inhibiting the activation of pepsinogen to pepsin. This impairment reduces the efficiency of protein digestion and absorption of amino acids downstream with direct consequences on muscle adherence and growth performance. Furthermore, a high luminal pH favours the growth and colonisation of acid-sensitive pathogens such as *Clostridium perfringens* and *Campylobacter spp*. (van Bunnik et al., 2012).

At the microbiome level, acidified water induces a compositional change in favour of beneficial microbial communities. Gao et al., (2021) observed an increase in the relative abundance of *Firmicutes* and *Bacteroidetes* and a decrease in *Proteobacteria* in acidifier-treated broilers. These birds showed decreased intestinal pro-inflammatory cytokines, Tumor Necrosis Factor-α and Interleukin-1β (TNF-α, IL-1β) and increased serum immunoglobulins (IgG, IgA, IgM). Morphologically, acidified water leads to a significant increase in intestinal villus height and villus-to-crypt ratio, both of which indicate an increase in absorptive surface area (Gao et al., 2021). Acidifiers, used alone or in combination with essential oils, consistently reduce viable counts of *E. coli* and *Salmonella* in caecal microbiota while enhancing proliferation of beneficial *Lactobacillus* populations (Dong et al., 2024).

Water Hardness

Water hardness is defined by the combined dissolved concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, expressed in mg/L as calcium carbonate ($CaCO_3$) or German degrees of hardness (°dH). Hard water (>14°dH or >250 mg/L $CaCO_3$) promotes scale deposition in water distribution lines, nipple drinkers, filters, and evaporative cooling systems as shown in Table 3 (Munster and Kemper, 2024). The resulting blockages reduce water flow rates and create stagnant zones harbouring biofilm-forming bacteria. Scale deposits also significantly reduce the solubility and antimicrobial efficacy of disinfectants and impair the dissolution of water-soluble medications and vitamins.

Magnesium levels above 125 mg/L in drinking water can have a strong laxative effect by increasing osmotic activity in the intestine, resulting in wet litter and related problems such as footpad dermatitis (Munster and Kemper, 2024). Even lower magnesium concentrations, around 50 mg/L, may cause similar effects when sulphate or chloride levels are also high. Water hardness caused by excess magnesium can be reduced through chemical precipitation methods, but ion-exchange systems are generally more effective. However, when sodium levels in water are already high, potassium-based softeners or reverse osmosis (RO) systems are considered better alternatives.

Table 3: Water hardness classification and practical management implications for poultry operations

Hardness	Classification	Practical Implications	Management
<8.4°dH (<150 mg/L $CaCO_3$)	Soft	Minimal risk; suitable for most applications	No treatment required
8.4-14°dH (150-250 mg/L $CaCO_3$)	Moderately Hard	Moderate scale risk; monitor drug solubility	Periodic acidic flushing
>14°dH (>250 mg/L $CaCO_3$)	Hard	High scale/clogging risk; Mg laxative effects; reduced disinfectant/drug efficacy	Ion exchange or RO softening strongly advised

°dH = German degrees of hardness (1°dH = 17.8 mg/L $CaCO_3$). (Munster and Kemper, 2024.)

Specific Minerals and Trace Elements

Apart from general water quality measures such as TDS and hardness, specific minerals and trace elements can independently influence broiler physiology through different biological mechanisms. Table 4 summarises their acceptable limits, biological effects, interactions with equipment, and suitable treatment approaches.

Sodium and chloride require special attention because of their synergistic effects on bird health. High sodium levels (>300 mg/L), especially when accompanied by chloride or sulphate, can lead to wet droppings due to their osmotic laxative action and may also encourage the growth of *Enterococcus* in water lines.

Iron is another important concern in poultry drinking water. At concentrations above 0.3 mg/L, it supports the growth of biofilm-forming bacteria in the water system, gives water a metallic taste that can reduce voluntary intake, and may interfere with vaccine stability and the effectiveness of medications.

Nitrate contamination (>15 mg/L) is also becoming a growing global issue. In young chicks, intestinal bacteria can convert nitrate into nitrite, which changes haemoglobin into methaemoglobin and reduces the blood's oxygen-carrying capacity. This condition, known as methemoglobinemia, may appear clinically as dullness along with a bluish-purple discoloration of the combs and wattles.

Table 4: Key minerals and elements in drinking water-recommended thresholds, biological effects, and treatment strategies for broiler operations

Mineral/Parameter	Threshold (mg/L)	Biological/Equipment Effects	Treatment Options
Sodium (Na)	<200	Wet manure (laxative); >600: eggshell issues; promotes *Enterococcus*	Reverse osmosis; blend with low-Na water
Chloride (Cl^-)	<250	Laxative (synergistic with Na, SO_4^{2-}); wet manure; reduced feed intake	Reverse osmosis; address Na concurrently
Sulfate (So_4^{2-})	<200	Laxative; 'rotten egg' odour (H_2S from bacteria); reduced performance	Aeration; chlorine shock treatment; RO

Iron (Fe)	<0.3	Metallic taste; biofilm formation; impairs vaccines/medications	Oxidation (Cl_2, ClO_2, ozone) + aeration + filtration
Manganese (Mn)	<0.05	Black deposits in drinkers/filters; slow to remove	Extended Cl_2 contact + filtration; greensand filter
Magnesium (Mg)	<125	Laxative at >125 mg/L (>50 mg/L with high SO_4^{2-} or Cl^-)	Ion exchange; RO; blend with low-Mg water
Nitrate (No_3^-)	<15	Methemoglobinaemia; apathy; reduced weight gain (young chicks)	Reverse osmosis; anion exchange
Nitrite (No_2^-)	<1	Highly toxic; severe oxygen transport impairment	Reverse osmosis; ion exchange
Copper (Cu)	<0.6	Pipe corrosion source; oral/gizzard lesions; taste alteration	Filtration; replace corroded plumbing
Fluoride (F^-)	<2	Skeletal fluorosis; impaired bone mineralisation at high levels	Activated alumina; bone char; RO
Alkalinity	<100 mg/L $CaCO_3$	Buffering reduces acid efficacy; bitter taste; pipe corrosion	Acidification (target pH <6.5); anion exchange
TDS (Total)	<1,500 (<3 wks) <3,000 (>3 wks)	Osmotic diarrhoea; reduced growth; mortality at >5,000 mg/L	Filtration; RO for high TDS

(Munster and Kemper, 2024; Hess and Macklin, 2025; Mirsalimi and Julian, 1993) RO = reverse osmosis.

Microbiological Water Quality

Microbiological contamination of drinking water is a major, yet often overlooked, aspect of water quality management in broiler production. Contaminated water can easily spread enteric pathogens within and between flocks, leading to higher disease incidence, mortality, antibiotic usage, and increased food safety concerns (Di Martino et al., 2018; Derose et al., 2020). The most commonly implicated waterborne pathogens include *E. coli, Salmonella spp., Campylobacter spp., Clostridium perfringens,* and *Pseudomonas aeruginosa* (Di Martino et al., 2018). Surveys conducted on commercial poultry farms indicate that water contamination is a common concern. For example, Osei et al. (2019) found that the total *coliforms* and faecal *Enterococci* were present in 97% and 56% samples respectively. Along with these, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and non-coagulase *Staphylococci* were also isolated from four samples an found that 58% of water samples collected from small-scale layer farms exceeded recommended chemical limits, while 47.4% failed to meet microbiological quality standards.

Biofilm proliferation within water distribution systems presents a particularly insidious challenge to microbiological control. These structured communities of microorganisms reside within a self-produced extracellular polymeric matrix that confers substantial resistance to conventional disinfection protocols. Consequently, the biofilm functions as a persistent reservoir, continually seeding the water flow with planktonic bacteria (Zhu et al., 2021). To achieve effective biofilm management, a rigorous remediation regimen is required; this entails periodic line cleaning with alkaline detergents to disrupt the organic matrix, followed by acidic descaling, and culminating in terminal disinfection with chlorine dioxide, peracetic acid, or hydrogen peroxide-based products (Demkina et al., 2023).

While systemic biofilm management is critical, the continuous disinfection of drinking water remains the cornerstone of microbiological quality management. Chlorination is the most widely practiced method, typically maintaining a free residual chlorine concentration of 0.5–3.0 mg/L at the drinker. However, the biocidal efficacy of this practice is highly contingent upon water chemistry, being fundamentally pH-dependent and significantly reduced by the presence of organic matter (Gani et al., 2024; Jiang et al., 2024).

Effects of Water Quality on Carcass Characteristics and Meat Quality

Dressing Percentage and Carcass Yield

Poor water quality over a long period reduces dressing percentage by lowering body weight gain and muscle growth. Broilers exposed to high TDS or high-salinity water exhibit reduced live weights at any given age, and their carcasses tend to show proportionally lower yields of breast and thigh meat due to the diversion of metabolic resources from anabolic processes to osmotic regulation and immune activation (Biswas et al., 2024; Mushtaq et al., 2013). Experimental comparisons between control and saline-water groups have reported reductions in dressing percentage of 2-5 percentage points, with proportionally greater reductions in breast muscle yield.

Immune Organ Weights

Water quality profoundly affects the relative weights of immune organs particularly the spleen, bursa of Fabricius, and thymus. Exposure to strongly alkaline water or high-TDS water has been associated with reduced relative spleen and bursa weights, indicating impaired primary lymphoid tissue development (Hamid et al., 2018). In contrast, water acidification consistently improves immune organ development, increases antibody levels, and enhances circulating immunoglobulin concentrations (Gao et al., 2021; Dong et al., 2024).

Meat Quality Parameters

Meat quality attributes such as water holding capacity (WHC), ultimate pH (pH 24h), colour indicator as L*, a*, and b*, where L* determines the lightness of the meat surface (0 = black to 100 = white) and is used to distinguish pale from dark meat (Chmiel et al., 2018); a* denoting the redness closely related to myoglobin state while b* represents the yellowness, which increases with oxidation or bacterial growth during storage; tenderness (Sellimi et al., 2017); and oxidative stability are indirectly affected by water quality through its impact on ante-mortem physiology, muscle glycogen reserves, and oxidative stress status. Saline water induces osmotic stress in broilers and increases plasma corticosterone concentrations, which promotes protein catabolism and reduces muscle glycogen, resulting in the change of muscle fiber composition, ultimately leading to impaired WHC and increased

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drip loss of the processed carcass (Mushtaq et al., 2013). High dietary sodium and chloride from poor quality water may also contribute to pre-slaughter electrolyte imbalances that accelerate post-mortem glycolysis, lower ultimate muscle pH, and produce pale, soft, exudative (PSE)-like meat characteristics.

Water Quality Monitoring and Management Strategies

Routine Monitoring Protocols

Water chemistry (pH, hardness, TDS, salts, metals) changes slowly but has major, cumulative effects on bird health, so chemical testing twice yearly is justified. Broad panels capture corrosion, underground shifts and seasonal trends, and long-term surveys show chemical exceeding levels are common (Augusto et al., 2022; Djeffal et al., 2025; Mohebbi et al., 2023; Münster & Kemper, 2024).

Microbiological monitoring quarterly is appropriate, as microbial loads change rapidly due to temperature, season, distribution conditions and hygiene. Biofilms and contamination re-establish themselves within a single cycle, so more frequent checks are useful for early detection of problems (Mustedanagic et al., 2023; Schroeder et al., 2024; Raut et al., 2024). Collecting water samples from the source, storage tank/header, and drinker points provides a realistic assessment of water quality throughout the supply system. Source samples indicate the baseline water quality, tank or header samples help identify any deterioration during storage and distribution, while drinker-end samples often reflect the highest level of contamination that birds are actually exposed to (Augusto et al., 2022; Kamal et al., 2024; Raut et al., 2024).

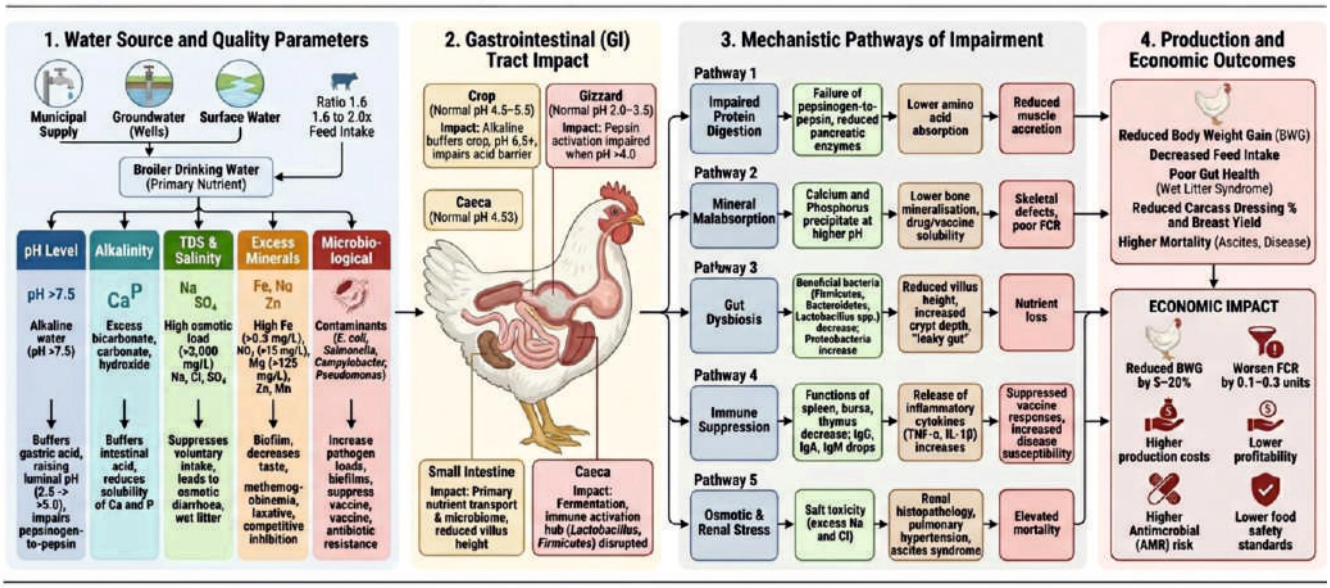

Figure 2. Integrated mechanisms through which drinking water quality affects broiler gastrointestinal function, nutrient utilisation, immune competence, and production performance.

(ADG, average daily gain; AMR, antimicrobial resistance; BWG, body weight gain; Ca, calcium; FCR, feed conversion ratio; Fe, iron; IgA/G/M, immunoglobulins; IL-1β, interleukin-1 beta; P, phosphorus; TDS, total dissolved solids; TNF-α, tumour necrosis factor alpha)

Knowledge Gaps and Future Research Directions

Carcass and meat quality research: The direct impact of various drinking water quality parameters on broiler carcass composition, meat quality traits such as pH, colour, water-holding capacity, tenderness, shelf life, and food safety has not been studied as extensively as their effects on growth performance. Therefore, well-designed controlled studies using standardised carcass evaluation methods are urgently required to better understand these relationships.

Interactive effects of multiple parameters: Mostly the experimental studies have focused to examine single water quality parameters in isolation, but in field conditions invariably involve complex combinations and situation during poultry production. Studies employing factorial or response surface methodology designs to examine interactive effects would substantially improve applicability to real-world and field level production systems.

Age-dependent sensitivity: Young chicks (0 to14 days) are extremely sensitive to osmotic, ionic, and microbiological water quality challenges. Systematic research demarcating the age-dependent thresholds and critical developmental strategies would enable the age-targeted management strategies.

Climate change and water quality: Rising temperatures and changing rainfall patterns are expected to influence the quality of drinking water by altering groundwater mineral concentration, increasing the risk of contamination in surface water sources, and promoting the accumulation of total dissolved solids (TDS). These effects are likely to be more pronounced in semi-arid livestock production regions, including large parts of India.

Economic modelling: A comprehensive cost-benefit analysis quantifying the return on investment for water quality testing, treatment infrastructure, and ongoing management programmes relative to losses from poor water quality is lacking in the literature and research trials in poultry production.

Conclusion

Water quality is a precision nutrition variable that needs to be systematically managed for productive and sustainable broiler production. The review presented here shows clearly, that pH, alkalinity, total dissolved solids, mineral composition and microbiological status have each mechanistically different effects on gut physiology, nutrient utilization, immune competence and

carcass quality, effects that are comparable in magnitude quantitatively to those of dietary formulation decisions. This understanding needs to be translated to the field with the non-negotiable components of flock management being routine, source-specific water testing, alkalinity-matched acidification to maintain drinker pH in the 6.0–7.0 range, and a continuous, evidence-based microbiological sanitation program.

But these growing bodies of evidence have three critical gaps that limit the full integration of water quality into poultry nutrition science. Firstly, the interaction between drinking water mineral profiles, in particular calcium, magnesium, sulphate and bicarbonate, and the bioavailability of key dietary nutrients such as phosphorus, amino acids and fat soluble vitamins is poorly characterised. Current feed formulation models do not take into account water-derived mineral contributions, which may result in systematic errors in nutrient balance calculations and affect the precision of diet formulation. Secondly, age-stratified water quality thresholds for the critical 0–14 day brooding period are largely absent from the literature. This developmental window, characterized by intestinal immaturity and increased osmotic vulnerability, requires dedicated dose-response research to establish evidence-based guidelines for young chick management that are currently lacking. Thirdly, there are no validated economic models that measure the return on investment for water quality monitoring, treatment infrastructure and programme management relative to production losses from compromised water, leaving nutritionists and producers without the decision-support tools needed to prioritise water quality interventions within integrated flock management programmes.

By addressing these gaps with controlled factorial studies on nutrient-water interactions, age-specific threshold trials in the brooding phase, and rigorous cost-benefit modelling, water quality will become a core, quantifiable pillar of evidence-based poultry nutrition with direct implications for feed efficiency, bird welfare, food safety, and the long-term sustainability of global broiler production.

References

Aggrey SE, Ghareeb AF, Milfort MC, Ariyo OW, Aryal B, Hartono E, Kwakye J, Sovi S, Hipple SA, Stevenson C, Fuller AL. Quantitative and molecular aspects of water intake in meat-type chickens. Poultry science. 2023 Nov 1;102(11):102973. https://doi.org/10.1016 /j.psj.2023.102973

Amaral LD. Drinking water as a risk factor to poultry health. Brazilian Journal of Poultry Science. 2004 Dec;6(4):191-9. https://doi.org/10.1590/S1516-635X2004000400001

Augusto E, Aleixo J, Chilala FD, Chilundo AG, Gaspar B, Bila CG. Physical, chemical and microbiological assessments of drinking water of small-layer farms. Onderstepoort Journal of Veterinary Research. 2022 Nov 28;89(1):2067.https://doi.org/10.4102/ojvr.v89i1.2067

Azizi, M.N., Aminullah, N., Alam, S., Danish, F. 2026. Poultry gut health: industry drivers, management determinants, and emerging research directions. Veterinary and Animal Science. https://doi.org/10.1016/j.vas.2026.100566

Bao J, Xie Q. Artificial intelligence in animal farming: A systematic literature review. Journal of Cleaner Production. 2022 Jan 10;331:129956. https://doi.org/10.1016/j.jclepro.2021.129956

Bassi LS, Teixeira LV, Sens RF, Sonálio KC, Santos MC, Kuritza LN, Maiorka A. Effect of phytase supplementation and drinking water pH for turkey poults. Livestock Science. 2022 Nov 1;265:105069.. https://doi.org/10.1016/j.livsci.2022.105069

Biswas S, Masum MA, Sarkar SK, Saud B, Akter R, Islam KS, Karim SJ, Rahman MM, Golbar HM, Alam ME, Islam MA. Salinity negatively correlates with the production and immunity of chicken: A molecular insight for food security and safety issues. Heliyon. 2024 Aug 15;10(15). https://doi.org/10.1016/j.heliyon.2024.e34819

Borges SA, Da Silva AF, Ariki J, Hooge DM, Cummings KR. Dietary electrolyte balance for broiler chickens under moderately high ambient temperatures and relative humidities. Poultry Science. 2003 Feb 1;82(2):301-8. https://doi.org/10.1093/PS/82.2.301

Bujňák L. Effect of water acidification on some morphological, digestive and production traits in broiler chickens. Bulgarian Journal of Veterinary Medicine. 2018 Jan 1. 21(3), 269–278. https://doi.org/10.15547/BJVM.1065

Carpenter CE, Broadbent JR. External concentration of organic acid anions and pH: key independent variables for studying how organic acids inhibit growth of bacteria in mildly acidic foods. Journal of food science. 2009 Jan;74(1):R12-5. https://doi.org/10.1111/J.1750-3841.2008.00994.X

Chaveerach P, Keuzenkamp DA, Urlings HA, Lipman LJ, Van Knapen F. In vitro study on the effect of organic acids on Campylobacter jejuni/coli populations in mixtures of water and feed. Poultry science. 2002 May 1;81(5):621-8. https://doi.org/10.1093/PS/81.5.621

Chmiel M, Hać-Szymańczuk E, Adamczak L, Pietrzak D, Florowski T, Cegiełka A. Quality changes of chicken breast meat packaged in a normal and in a modified atmosphere. Journal of Applied Poultry Research. 2018 Sep 1;27(3):349-62. https://doi.org/10.3382/JAPR/PFY004

Davidson PM, Taylor TM, Schmidt SE. Chemical preservatives and natural antimicrobial compounds. Food microbiology: fundamentals and frontiers. 2012 Dec 28:765-801.

De Baets R, Van Nerom S, Buyse K, Antonissen G, Degroote J, Delezie E. Comparing different water supplementation in cyclic heat stressed broilers. The effect of selected antioxidant mixtures, betaine, functional amino acids and electrolyzed reduced water. Poultry Science. 2025 Dec 19:106322. https://doi.org/10.1016/j.psj.2025.106322

Demkina EV, Ilicheva EA, El-Registan GI, Pankratov TA, Yushina YK, Semenova AA, Nikolaev YA. New approach to improving the efficiency of disinfectants against biofilms. Coatings. 2023 Mar 7;13(3):582. https://doi.org/10.3390/coatings13030582

Demmers TG, Green S, Rizzoli A. Can AI imaging technology be used for monitoring specific behaviours in broiler flocks?. InProceedings of the 10th European Conference of Precision Livestock Farming ECLF2024 2022 Aug (pp. 128-131). https://www.scopus.com/pages/publications/85172311463

Derose KL, Roche LM, Lile DF, Eastburn DJ, Tate KW. Microbial water quality conditions associated with livestock grazing, recreation, and rural residences in mixed-use landscapes. Sustainability. 2020 Jun 26;12(12):5207. https://doi.org/10.3390/SU12125207

Di Martino G, Piccirillo A, Giacomelli M, Comin D, Gallina A, Capello K, Buniolo F, Montesissa C, Bonfanti L. Microbiological, chemical and physical quality of drinking water for commercial turkeys: a cross-sectional study. Poultry science. 2018 Aug 1;97(8):2880-6. https://doi.org/10.3382/PS/PEY130

Dibner JJ, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of

applied poultry research. 2002 Dec 1;11(4):453-63. https://doi.org/10.1093/JAPR/11.4.453

Dibner JJ, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of applied poultry research. 2002 Dec 1;11(4):453-63. https://doi.org/10.32598/IJVM.19.1.1005481

Dong Y, Gao X, Qiao C, Han M, Miao Z, Liu C, Yan L, Li J. Effects of mixed organic acids and essential oils in drinking water on growth performance, intestinal digestive capacity, and immune status in broiler chickens. Animals. 2024 Jul 24;14(15):2160.

Donkoh A. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology. 1989 Dec;33(4):259-65.https://doi.org/10.1007/BF01051087

Dörper A, Berman HM, Gort G, van Harn J, Dicke M, Veldkamp T. Effects of different black soldier fly larvae products on slow-growing broiler performance and carcass characteristics. Poultry Science. 2024 Apr 1;103(4):103481. https://doi.org/10.1016/j.psj.2024.103481

Edge CM, Davis JD, Purswell JL, Campbell JC, Batchelor WD, Linhoss JE. Water consumption trends for commercial broilers grown to nine weeks. Journal of Applied Poultry Research. 2025 Jun 1;34(2):100511.https://doi.org/10.1016/j.japr.2024.100511

El-Sabrout K, Buonaiuto G, Cavallini D, Mishra B. Recent advancements to improve drinking water quality in poultry farms. Poultry Science. 2026 Jan 27:106523.https://doi.org/10.1016/j.psj.2026.106523

Erensoy K, Noubandiguim M, Sarıca M, Aslan R. The effect of intermittent feeding and cold water on performance and carcass traits of broilers reared under daily heat stress. Asian-Australasian Journal of Animal Sciences. 2020 Mar 12;33(12):2031. https://doi.org/10.5713/ajas.19.0980

Fathima SN, Rajendran RM, Balaji S, Mani R, Vyas SR, Chandrasekar S. Impact of algal β-glucan on stress, humoral immunity, plasma biomarkers, and carcass traits of broiler chickens reared during hot season. Journal of Thermal Biology. 2025 Aug 16:104227. https://doi.org/10.1016/j.jtherbio.2025.104227

Gani A, Singh M, Pathak S, Hussain A. Recent advancements in chlorine applications for water quality control. InDrinking water disinfection by-products: Sources, fate and remediation 2024 Feb 15 (pp. 35-58). Cham: Springer Nature Switzerland.. https://doi.org/10.1007/978-3-031-49047-7_3

Gao CQ, Shi HQ, Xie WY, Zhao LH, Zhang JY, Ji C, Ma QG. Dietary supplementation with acidifiers improves the growth performance, meat quality and intestinal health of broiler chickens. Animal Nutrition. 2021 Sep 1;7(3):762-9.https://doi.org/10.1016/J.ANINU.2021.01.005

Hamdi Z, Ashour EA, Nour-Eldeen AE, Sabike I, Osman AO, Mahgoub SA, Kamal M, El-Mekkawy MM, Aljumaa MA, Alwutayd KM, Khan MM. Influence of Pluchea indica (L.) less extract supplementation on performance, physiological responses, meat quality, and microbial load in broiler chickens. Journal of Applied Poultry Research. 2026 Sep 1;35(3):100702.https://doi.org/10.1016/j.japr.2026.100702

Hamid H, Shi HQ, Ma GY, Fan Y, Li WX, Zhao LH, Zhang JY, Ji C, Ma QG. Influence of acidified drinking water on growth performance and gastrointestinal function of broilers. Poultry Science. 2018 Oct 1;97(10):3601-9.. https://doi.org/10.3382/PS/PEY212

Hess, J.P. and Macklin, K.S. Evaluating Water Quality for Poultry. Alabama Cooperative Extension System. 2025. [Accessed on May, 2026; https://www.aces.edu/blog/topics/farming/evaluating-water-quality-for-poultry/

Hiltz JZ, Maynard CW, Tabler TW, Maqueda MA, Shafer KM, Nelson KB, Kidd MT, Anthony NB, Orlowski-Workman SK. Characterization of water intake and water conversion ratio for male and female broilers from four commercial broiler lines reared to eight weeks of age. Journal of Applied Poultry Research. 2025 Aug 5:100587. https://doi.org/10.1016/j.japr.2025.100587

Hiltz JZ, Orlowski SK, Harrington LN, Maynard CW, Tabler TW, Anthony NB. Applied research note: Development of a novel low flow water monitoring system in poultry/agriculture systems. Journal of Applied Poultry Research. 2021 Jun 1;30(2):100151. https://doi.org/10.1016/j.japr.2021.100151

Jiang Y, Qiao Y, Jin R, Jia M, Liu J, He Z, Liu Z. Application of chlorine dioxide and its disinfection mechanism. Archives of microbiology. 2024 Oct;206(10):400. https://doi.org/10.1007/s00203-024-04137-7

Kalita S, Mahanta JD, Sapcota D, Kalita N, Saikia R. Effect of different sources of drinking water on the haematological and biochemical parameters of commercial broiler chicken. IJCS. 2020;8(1):2943-5. https://doi.org/10.22271/CHEMI.2020.V8.I1AS.8715

Kamal MA, Alhotan RA, Al Sulaiman AR, Hussein EO, Galik B, Saleh AA. From source to house: unraveling the seasonal effect of water distribution system on drinking water quality of poultry farms under Egyptian environmental condition. Environmental Science and Pollution Research. 2024 Feb;31(9):12966-77. https://doi.org/10.1007/s11356-024-31911-3

Khalil M, Soliman F, El-Sabrout K, Aggag S. Impact of using ionized drinking water on growth performance and DNA damage of broiler chicks. Tropical Animal Health and Production. 2025 Dec;57(9):497. https://doi.org/10.1007/s11250-025-04715-x

Leeson, S. and Summers, J.D. Commercial Poultry Nutrition. 3rd Ed. 2009. University Books, Guelph.

Mahmoud UT, El-Reda GA, Ali FA, Mahmoud MA, Abd-Allah SM, El-Hossary FM, Khalil NS. Plasma activated water effects on behavior, performance, carcass quality, biochemical changes, and histopathological alterations in quail. BMC Veterinary Research. 2024 Sep 4;20(1):391. https://doi.org/10.1186/s12917-024-04231-4

May JD, Lott BD. Feed and water consumption patterns of broilers at high environmental temperatures. Poultry science. 1992 Feb 1;71(2):331-6. https://doi.org/10.3382/PS.0710331

Mhmoud A, Mkwanazi MV, Ndlela SZ, Moyo M, Chimonyo M. Responses of broiler chickens to incremental levels of water deprivation: Growth performance, carcass characteristics, and relative organ weights. Open Agriculture. 2023 Sep 6;8(1):20220184. https://doi.org/10.1515/opag-2022-0184

Mirsalimi SM, Julian RJ. Saline drinking water in broiler and Leghorn chicks and the effect in broilers of increasing levels and age at time of exposure. The Canadian Veterinary Journal. 1993 Jul;34(7):413. https://www.ncbi.nlm.nih.gov/pubmed/17424251

Mirsalimi SM, O'Brien PJ, Julian RJ. Blood volume increase in salt-induced pulmonary hypertension, heart failure and ascites in

broiler and white leghorn chickens. Canadian Journal of Veterinary Research. 1993 Apr;57(2):110.

Mohebbi F, Akbari M, Moosavi SG, Mostafaii G, Aboosaedi Z, Miranzadeh MB. The study of water quality in poultry farms in Ardestan, Iran. Journal of Environmental Health and Sustainable Development. 2023 Sep 10;8(3):2070-7. https://doi.org/10.18502/jehsd.v8i3.13705

Münster P, Kemper N. Long-term analysis of drinking water quality in poultry and pig farms in Northwest Germany. Frontiers in animal science. 2024 Sep 18;5:1467287. https://doi.org/10.3389/fanim.2024.1467287

Mushtaq MM, Pasha TN, Akram M, Mushtaq T, Parvin R, Farooq U, Mehmood S, Iqbal KJ, Hwangbo J. Growth performance, carcass traits and serum mineral chemistry as affected by dietary sodium and sodium salts fed to broiler chickens reared under phase feeding system. Asian-Australasian Journal of Animal Sciences. 2013 Dec;26(12):1742. https://doi.org/10.5713/AJAS.2013.13266

Mustedanagic A, Matt M, Weyermair K, Schrattenecker A, Kubitza I, Firth CL, Loncaric I, Wagner M, Stessl B. Assessment of microbial quality in poultry drinking water on farms in Austria. Frontiers in Veterinary Science. 2023 Nov 22;10:1254442. https://doi.org/10.3389/fvets.2023.1254442

Nasiri A, Amirivojdan A, Zhao Y, Gan H. An automated video action recognition-based system for drinking time estimation of individual broilers. Smart Agricultural Technology. 2024 Mar 1;7:100409. https://doi.org/10.1016/j.atech.2024.100409

Ndlela SZ, Moyo M, Mkwanazi MV, Chimonyo M. Responses of gut morphology, serum biochemistry, and quality of breast meat to water deprivation in broilers. Canadian Journal of Animal Science. 2019 Sep 5;100(1):59-68. https://doi.org/10.1139/cjas-2018-0221

OECD-FAO. Agricultural Outlook 2024–2033. 2024. OECD Publishing, Paris.

Oluwagbenga EM, Fraley GS. Heat stress and poultry production: a comprehensive review. Poultry Science. 2023 Dec 1;102(12):103141. https://doi.org/10.1016/j.psj.2023.103141

Osei FB, Boamah VE, Agyare C, Abaidoo RC. Physicochemical properties and microbial quality of water used in selected poultry farms in the ashanti region of ghana. The Open Microbiology Journal. 2019 May 31;13(1). https://doi.org/10.2174/1874285801913010121

Pesti GM, Amato SV, Minear LR. Water consumption of broiler chickens under commercial conditions. Poultry science. 1985 May 1;64(5):803-8. https://doi.org/10.3382/ps.0640803

Puma MC, Xin H, Gates RS, Burnham DJ. An instrumentation system for studying feeding and drinking behavior of individual poultry. Applied Engineering in Agriculture. 2001;17(3):365. https://www.scopus.com/pages/publications/0035351756

Raut R, Biswas BK, Taylor-Bowden T, Kilonzo-Nthenge A, Pokharel B, Tabler T, Nahashon SN, Maharjan P. Effects of chronic exposure of specific water quality parameters in poultry drinking water on dietary amino acid digestibility and early broiler performance. Poultry Science. 2025 Feb 1;104(2):104835.

Raut R, Kilonzo-Nthenge A, Aniume T, Basnet A, Watkins S, Maharjan P. Impacts of on-farm water sanitation practices on microbial hygiene in poultry waterlines and efficacy of sodium hypochlorite-based product on foodborne pathogens. Journal of Applied Poultry Research. 2024 Sep 1;33(3):100425.

Riek A, Gerken M, Werner C, Gonde A. Deuterium for estimating total body water and turnover rates in turkeys exposed to different incubation treatments. Poultry science. 2008 Dec 1;87(12):2624-8. https://doi.org/10.3382/ps.2008-00249

Roach B, Greene ES, Dridi S. Central regulation of drinking water in divergently selected high-water-efficient young broiler chickens: A minireview. Journal of Neuroendocrinology. 2025 Jun;37(6):e13488. https://doi.org/10.1111/jne.13488

Schroeder MA, Yfran Elvira MM, Bóbeda G, Rodríguez SC, De Asmundis CL. Water quality used for poultry drinking in farms in San Roque (Corrientes). Revista veterinaria. 2024 Dec;35(2):93-9. https://doi.org/10.30972/vet.3527869

Sellimi S, Ksouda G, Benslima A, Nasri R, Rinaudo M, Nasri M, Hajji M. Enhancing colour and oxidative stabilities of reduced-nitrite turkey meat sausages during refrigerated storage using fucoxanthin purified from the Tunisian seaweed Cystoseira barbata. Food and Chemical Toxicology. 2017 Sep 1;107:620-9. https://doi.org/10.1016/j.fct.2017.04.001.

United Nations, 2022, FAO. How to feed the world in 2050. Food and Agriculture Organization of the United Nations. 2009. Retrieved from https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf

USDA (2023). Livestock and Poultry: World Markets and Trade. Foreign Agricultural Service, October 2023.

Van Bunnik BA, Katsma WE, Wagenaar JA, Jacobs-Reitsma WF, De Jong MC. Acidification of drinking water inhibits indirect transmission, but not direct transmission of Campylobacter between broilers. Preventive veterinary medicine. 2012 Aug 1;105(4):315-9. https://dspace.library.uu.nl/handle/1874/274854

van Emous RA. Effects of water access time and unlimited access to alfalfa straw on litter quality, performance, and behavior of breeder pullets. Poultry science. 2024 Jun 1;103(6):103773. https://doi.org/10.1016/j.psj.2024.103773

Williams CL, Tabler GT, Watkins SE. Comparison of broiler flock daily water consumption and water-to-feed ratios for flocks grown in 1991, 2000–2001, and 2010–2011. Journal of applied poultry research. 2013 Dec 1;22(4):934-41. https://doi.org/10.3382/JAPR.2013-00767

Wu D, Cui D, Zhou M, Ying Y. Information perception in modern poultry farming: A review. Computers and Electronics in Agriculture. 2022 Aug 1;199:107131. https://doi.org/10.1016/j.compag.2022.107131

Xin H, Liu K. Precision livestock farming in egg production. Animal frontiers. 2017 Jan 1;7(1):24-31. https://doi.org/10.2527/af.2017.0105

Yadav GB, Kadam AS, Pachpande AM, Lambate SB, Lonkar VD, Maini S, Ravikanth K. Post hatch histo-morphological studies of small intestinal development in chicks fed with herbal early chick nutritional supplement. International Journal of Poultry Science. 2010 Aug 15;9(9):851-5. https://doi.org/10.3923/IJPS.2010.851.855

Zhu Z, Shan L, Zhang X, Hu F, Zhong D, Yuan Y, Zhang J. Effects of bacterial community composition and structure in drinking water distribution systems on biofilm formation and chlorine resistance. Chemosphere. 2021 Feb 1;264:128410. https://doi.org/10.1016/j.chemosphere.2020.128410.

Research Article

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Chemical characteristics, In-vitro digestibility and Rumen fermentation of durum and bread wheat straw varieties as potential feed for ruminants

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Wheat straw is one of the abundant agricultural residues used on large scale for ruminant fodder. To supply farmers with high-quality straws, the current study was carried out to evaluate durum and bread wheat straw varieties with regard to their chemical composition, in vitro digestibility, and rumen fermentation parameters. The samples were subjected to the estimation of protein, total ash, crude fiber, and fiber fractions. The mineral content was analyzed by using an atomic absorption spectrophotometer. In vitro digestibility and rumen fermentation parameters were also assessed using standard methods. Results showed that HI-8759, a bio-fortified durum variety released for the central zone, has significantly higher CP (6.66 ± 0.09) than HI-8713 (3.43± 1.11 and less NDF, ADF, and cellulose compared to HI-8713, HI-8737, and HI-8663. HI 8777, another bio-fortified durum wheat variety found for the peninsular zone, contains higher IVDMD and IVOMD than UAS 428. Similarly, WB 02, a bio-fortified bread wheat variety has higher CP (4.63±0.13) levels than HI 1633 (3.26±0.03) and HI 1605 (2.86 ±0.14). Furthermore, it contains more IVDMD, IVOMD, and ME than HI 1544. Overall, the cellulose content is higher (P<0.01) in durum compared to bread wheat straw. *In vitro* digestibility revealed that durum wheat straw varieties have significantly higher TGP, IVDMD, IVOMD, and ME than bread wheat varieties, indicating that durum wheat is more digestible. 1000 grain weight is positively correlated to straw NDF, cellulose, total gas production, ME, acetate, propionate, butyrate and TVFA concentrations.

Keywords: Wheat Straw; Bio-fortification; *In vitro* evaluation; Rumen fermentation and digestibility

Introduction

Wheat straw is an important renewable bioresource for producing ruminant feed and bioenergy due to its high production and high carbohydrate content (Battaglia et al., 2020, van Kuijk et al., 2015a). Wheat straw, rice straw, and corn stover are the most abundant lingocellulosic biomasses among agricultural residues in the world, used mainly as feed for livestock or agricultural supplements. Wheat straw consists mainly of cellulose (28%–39%), hemicelluloses (23%–24%), lignin (16%–25%), and fewer contents of ash and protein (Alvarez et al., 2021). Wheat straw is the fibrous crop residue left after removing the grain through threshing. Due to the higher content of indigestible fiber, these are low in energy and protein, and hence the nutritive value is low, which limits their effective utilization. To overcome these limitations, farmers in North India fed wheat straw, which is commonly fed to the cattle and buffaloes in the form of Sani (a wet mixture of grain, cakes, chopped green fodder, and wheat straw soaked overnight). Erickson et al. (1982) found that there was a significant difference between straw from different barley genotypes. Rao et al. (2022) evaluated rice straw of different bio-fortified varieties and landraces and found differences in chemical composition, in vitro dry matter digestibility, and fermentation metabolites. Keeping in mind the effect of varieties on the energy value of straw, farmers currently prefer to adopt cultivars that produce superior quality straw as well as high grain yield.

Wheat (Triticum aestivum) is grown in India under irrigation conditions to the tune of 31.6 million hectares with a production of 107.86 million tons in 2021 (Agricultural Statistics at a Glance, 2022). With a grain-to-straw ratio of 1:1.23 (Giridhar et al. 2013), the potential availability of wheat straw is 132.7 million tons. Practically wheat straw alone can provide the roughage needs of 72 million cattle and buffaloes (assuming 5 kg of wheat straw per head), which are quite substantial. The majority of wheat cultivated is used to make Chapathi (Bread). However, for pasta purposes, another important wheat species grown in Central India is i.e., durum wheat (Triticum durum). Several Indian durum varieties are of high nutritive value with higher protein, yellow pigment, and essential micronutrients like iron and zinc. The yellow pigment imparts a yellow hue to the pasta made from durum semolina, and which imparts good human health due to the antioxidant properties of the carotenoids present. Many wheat varieties (bread and durum) having potential yield and nutritional quality parameters (more protein, iron, and zinc) were released for peninsular, central, and rain-fed zones with limited irrigation and late-sown conditions of central India (Prasad and Prakasha, 2020). In the present study, twelve released varieties of wheat straw (six durum and six bread) obtained from IARI Regional Station in Indore (M.P) were analyzed for chemical composition, in vitro evaluation, fermentation metabolites.

Materials and Methods

Description of the plant material

Twelve released varieties of wheat straw obtained from IARI Regional Station in Indore (M.P) were used. They comprised of six durum wheat (*Triticum durum*) varieties (HI8713, HI8737, HI8663, HI8759, UAS 428, HI 8777) and six bread wheat (*Triticum aestivum*) varieties (HI1544, HI1634, HI1531, HI1605, WB02 and HI1633).

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HI8737, UAS428 and HI 1633 are recommended for North Eastern plain and Peninsular zone of India. WB02- BFV NWPZ is for North Western plain zone and the remaining verities are recommended for the central zone of the country.

Evaluation of chemical Composition

The samples were ground in Cyclotec Mill (Foss) using 1 mm sieve. The samples were analyzed for crude Protein (990.03: AOAC, 2012), total ash (942.05: AOAC, 2012) and crude fiber (962.09: AOAC, 2012) and fibre fractions like NDF, ADF, hemi-cellulose, cellulose and lignin (Van Soest *et al.* 1991).

Mineral estimation

About 1 g of feed sample was digested by 9 ml of tri-acid mixture (HNO_3: $HCLO_4$:H_2SO_4 - 3:2:1) in a 100 ml Kjeldahl flask containing glass beads to avoid bumping at very low heat initially and then at high temperature till the contents clear and perchloric acid fumes cease to come out. Then the digested sample was diluted with distilled water and filtered through Whatman filter paper No 42 and the final volume was made up to 25 ml (AOAC, 2005). The contents of different minerals in the samples were analyzed by using an atomic absorption spectrophotometer (Shimadzu-7880). A stock solution containing 1000 ppm of Zn mineral was purchased from Merck Chemicals. The operating parameters for estimation of Zn were: Zn hollow cathode lamp: Wavelength, 213.9 nm: slit width, 0.7 nm: lamp current, 8.0 mA: vapor type, air/acetylene.

Evaluation of *in vitro* gas production

The rumen liquor was collected from the fistulated crossbred cattle before the offering of feed and water in the morning at the experimental livestock unit of the institute in a double-walled (thermos) flask flushed with CO_2 and maintained at 39°C in the incubator. The rumen liquor was then strained with four-layered muslin cloth and added (440 ml) to the buffer. The buffer was prepared by mixing 486 ml of distilled water, 0.13 ml of micro mineral solution, 244 ml of rumen buffer solution, 244 ml of macro mineral solution, 1.26 ml of Resazurin solution, 2.13 ml of 1N NaOH, 0.293 g of Sodium sulfide, and 49.3 ml of distilled water (prepared fresh and added just before incubation). The medium mixture solution was pre-warmed to 39ºC and bubbled with CO_2 just before addition to rumen liquor. Air-equilibrated feed samples (200±10 mg) were incubated in 100 ml calibrated syringes at 39°C for 24 h with 30 ml mixed rumen suspension containing rumen buffer and rumen liquor in a ratio 2:1 (Menke and Steingass 1988) with parallel incubation of a blank without a feed sample. The syringes were shaken every 30 minutes till 2 h and thereafter every 2 h up to 8 h to initiate proper mixing of substrate with inoculum. Each sample was incubated in ten tubes over two independent runs. The total incubation period of 24 h was given, and at the end of incubation, the level of the piston was again recorded. The total gas production during the incubation period was calculated by subtracting the initial reading from the final reading and correcting for corresponding blanks.

Calculation:

Total Gas Production (ml/200mg)in 24 hours=(V-V0-G0)
V= Reading after 24hr; V0= Reading after 1st reading; Go= Gas produced in blank syringes

Evaluation of *in vitro* digestibility

To determine the substrate digestibility, contents of syringes after incubation were refluxed for 1 h with neutral detergent solution, filtered, and oven dried at 60°C for 48 h to determine the in vitro dry matter digestibility (IVDMD). Subsequently, the residue obtained after double-strength neutral detergent solution treatment was incinerated at 600°C for 3 h to estimate the in vitro true organic matter digestibility (IVOMD), and metabolizable energy (Menke and Steingass 1988) was calculated using formula.

ME=2.2+0.136 GP+0.057CP+0.0029EE

Where:

ME=Metabolizable Energy (MJ/Kg DM); GP=Net Gas Production ml/200 mg substrate in 24 hours; CP= Crude Protein (%); EE=Ether Extract (%)

Rumen fermentation parameters

The fatty acids like acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate in the incubation medium were estimated using the procedure described by Cottyn and Boucque, (1968), where a gas chromatograph (Agilent – 7890A) equipped with a flame ionization detector (FID) capillary column (Agilent J&W DB-WAX GC column 40mmX0.18mmX0.18µm) was used.

Statistical Analysis

The various parameters considered were compared between and within broad groups of durum and wheat using one way analysis of variance. Similarly, correlation co-efficients were calculated among all the parameters using Statistical Package for the Social Sciences software (PASW Statistics Release 18.0.0 (Jul 30 2009). The significance level of 0.05 was used to compare treatment means (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Chemical Composition

Durum

HI-8759, a bio-fortified variety released for the central zone has significantly higher CP than HI-8713 and comparable CP with HI-8737 and HI-8663. HI-8777, a bio-fortified variety found in the North Eastern Plain Zone and Peninsular zone has less CP than UAS 428. HI-8777 has lower EE than HI-8737 and HI-8663 and is comparable with HI-8713, whereas HI-8777 has similar EE to UAS-428. The total ash ranged from 7.29 in UAS 428 to 9.88 in HI 8663. HI-8759 has more Zn than HI-8713 and is comparable with HI-8737 and HI-8663. However, UAS 428 has more Zn than its bio-fortified counterpart (HI-8713) (Table 1a).

HI-8759 has less NDF and ADF compared to HI-8713, whereas HI8777 has more NDF, ADF, and cellulose compared to UAS428. Hemi-cellulose in HI8759 is higher than HI8737 and HI8663, whereas HI8777 and UAS428 have comparable hemi-cellulose content (Table 1b).

Table 1a. Proximate Composition (%) of different varieties of durum wheat straw

Variety	Dry Matter	Crude Protein	Ether Extract	Total Ash	Acid Insoluble	Ash Zinc (mg/kg)
HI 8759	93.22 ± 0.01	6.66^{a} ± 0.09	0.86^{bc}±0.08	9.50^{a}±0.28	4.09^{a} ± 0.11	18.0
HI 8713	91.39 ± 0.01	3.43^{bc} ± 1.11	0.68^{c} ± .02	8.25^{b}±0.18	3.90^{ab} ± 0.07	10.3
HI 8737	93.87 ± 0.01	4.50^{ab} ± 0.12	1.42^{a} ±0.04	9.88^{a}±0.13	3.50^{b} ± 0.07	14.3
HI 8663	93.22 ± 0.01	5.43^{ab} ± 0.18	1.20^{ab}±0.07	9.88^{a}±0.13	3.50^{a} ± 0.07	16.1
HI 8777	93.69 ± 0.01	1.36^{c} ± 0.17	0.52^{c} ±0.04	9.08^{a}±0.08	4.19^{a} ± 0.06	13.6
UAS 428	93.91 ± 0.01	6.70^{a} ± 0.17	0.79^{bc}±0.04	7.29^{c}±0.20	4.06^{a} ± 0.14	17.8
Significance	-	**	**	**	**	-
P Value	-	P<0.01	P<0.01	P<0.01	P<0.01	-

**- Highly Significant (P<0.01); Values bearing different superscripts a, b, c,in a column differ significantly at the indicated level.

Table 1b. Fiber components (% DMB) of different varieties of durum wheat straw

Variety	Neutral Detergent Fiber	Acid Detergent Fiber	Cellulose	Hemi cellulose	Acid Detergent Lignin
HI 8759	69.60^{b} ± 0.24	36.03^{c}±0.44	33.57^{a} ± 0.70	28.38^{d} ± 0.58	7.65±0.13
HI 87137	7.96^{a} ± 0.90	43.02^{a}±0.90	34.94^{a} ± 0.00	35.13^{ab} ± 0.78	7.89±0.13
HI 8737	69.51^{b} ± 1.07	45.03^{a} ± 0.69	24.48^{b} ± 1.77	37.01^{a} ± 0.62	8.02±0.07
HI 8663	68.24^{b} ± 0.25	40.25^{b} ± 0.50	27.99^{b} ± 0.76	32.16^{bc} ± 0.51	8.09±0.01
HI 8777	75.90^{a} ± 0.56	43.03^{a} ± 0.26	32.87^{a} ± 0.31	35.38^{a} ± 0.56	7.65±0.30
UAS 428	69.65^{b}±0.32	37.23^{c}± 0.29	32.42^{a} ± 0.03	29.20^{cd} ± 0.66	8.03±0.37
Significance	**	**	*	**	NS
P Value	P<0.01	P<0.01	P<0.05	0.01	0.52

NS-Not-Significant (P>0.05); **- Highly Significant (P<0.01); Values bearing different superscripts a, b, c, d in a column differ significantly at the indicated level.

Bread

WB-02, a bio-fortified bread wheat variety released for the Northwestern Plain Zones of the country, has comparable CP, lower EE, and Zn to its counterpart HI-1544 (Table 2a). However, HI-1633, a bio-fortified variety released for the late-sown conditions of the Peninsular zone of India has more Zn than its counterpart HI-1634. HI-1605, released for limited irrigation systems of Central India, has more iron and zinc with less CP than its counterpart, HI-1531.

WB-02 and HI-1544 bread wheat varieties have similar fiber fractions. HI-1634, identified for late-sown and irrigated conditions of Central India has high NDF, cellulose, hemicellulose, and low lignin. HI-1605 and HI-1531, identified for limited water systems have similar fiber fractions (Table 2b).

Table 2 a. Proximate Composition (%) of different varieties of bread type wheat straw

VarietyDry	MatterCrude	ProteinEther	ExtractTotal	AshAcid	Insoluble Ash	Zinc (mg/kg)
Wb02	94.09 ± 0.01	4.63^{b} ±0.13	0.93^{bc} ±0.14	9.99^{b}±0.23	5.91^{a}± 0.15	8.14
HI 1544	93.11 ± 0.01	4.20^{b} ±0.17	1.61^{a}±0.05	8.29^{d}±0.10	2.79^{c}±0.06	22.1
HI 1633	92.74 ± 0.01	3.26^{cd}±0.03	0.50^{d} ±0.03	10.52^{b}±0.06	6.19^{a}± 0.06	45.6
HI 1634	93.38 ± 0.01	3.76^{bc} ±0.28	0.65^{bcd} ±0.05	9.43^{c}±0.04	3.59^{b}± 0.01	13.7
HI 1605	91.94 ± 0.01	2.86^{d} ±0.14	0.96^{b} ±0.05	8.10^{d}±0.05	3.06^{c}± 0.01	14.2
HI 1531	93.86 ± 0.01	6.73^{a} ±0.23	0.58^{cd} ±0.06	11.07^{a}±0.05	3.53^{b}± 0.04	12.8
Significance	-	**	**	**	**	-
P Value	-	P<0.01	P<0.01	P<0.01	P<0.01	-

Table 2 b. Fiber components (% DMB) of different varieties of bread type wheat straw

Variety	Neutral Detergent	Fiber Acid Detergent Fiber	Cellulose	Hemi cellulose	Acid Detergent Lignin
Wb02	67.60^{ab} ± 1.43	41.97 ± 0.05	35.49^{ab} ± 0.10	25.63^{ab} ± 1.37	6.47^{b} ± 0.15
HI 1544	66.72^{b} ± 0.19	40.16 ± 1.03	33.39^{a} ± 1.40	26.55^{a} ± 0.83	6.77^{b} ± 0.37
HI 1633	66.34^{b} ± 0.43	43.53 ± 0.49	35.29^{ab} ± 0.53	22.80^{b} ± 0.06	8.23^{a} ± 0.03
HI 1634	72.82^{a} ± 0.58	44.81 ± 1.31	39.00^{a} ± 1.13	28.01^{a} ± 0.73	5.81^{b} ± 0.17
HI 1605	69.97^{ab} ± 2.15	43.47 ± 1.89	35.29^{ab} ± 1.35	26.50^{a} ± 0.25	8.17^{a} ± 0.54
HI 1531	69.22^{ab} ± 0.50	40.23 ± 0.26	31.26^{b} ± 0.31	28.99^{a} ± 0.24	8.96^{a} ± 0.04
Significance	**	NS	**	**	**
P Value	P<0.01	P=0.05	P<0.01	P<0.01	P<0.01

NS-Not-Significant (P>0.05); **- Highly Significant (P<0.01); Values bearing different superscripts a, b in a column differ significantly at the indicated level.

Comparisons among durum and wheat varieties

All the studied characteristics were compared between durum and bread varieties (Table 3). But were slightly higher (P>0.05) in durum wheat straw varieties than bread wheat straw. However, the cellulose content of straw was significantly (P<0.05) higher in durum wheat straw.

Table 3. Comparisons between durum and bread wheat straw

Parameter	Durum	Bread	Significance	P value
Dry Matter	93.21±0.38 (91.39-93.91)	93.18±0.32 (91.94-94.09)	NS	0.95
Crude Protein	4.68±0.48 (1.36-6.70)	4.24±0.56 (2.86-6.73)	NS	0.67
Ether Extract	0.91±0.13 (0.52-1.42)	0.87±0.16 (0.50-1.61)	NS	0.86

Total Ash	8.98±0.41 (7.29-9.88)	9.56±0.48 (8.10-11.07)	NS	0.38
Acid Insoluble Ash	3.87±0.12 (3.50-4.19)	4.18±0.60 (2.79-6.19)	NS	0.63
Neutral Detergent Fiber	71.81±1.65 (68.24-77.96)	68.77±0.99 (66.34-72.82)	NS	0.15
Acid Detergent Fiber	40.76±1.45 (36.03-45.03)	42.36±0.77 (40.16-44.81)	NS	0.36
Hemi Cellulose	31.05±1.62 (24.48-34.94)	34.95±1.04 (31.26-39.00)	NS	0.07
Cellulose	32.88±1.44 (28.38-37.01)	26.41±0.87 (22.80-28.99)	**	0.003
Acid Detergent Fiber	7.89±0.07 (7.65-8.09)	7.40±0.500 (5.81-9.05)	NS	0.36

NS-Not-Significant (P>0.05); **- Highly Significant (P<0.01)

In vitro digestibility parameters

HI-8759, a durum wheat straw has more ME and is lower (P<0.05) in IVDMD and IVOMD than HI-8713. HI 8777, a bio-fortified variety durum wheat is higher in IVDMD and IVOMD than UAS 428 with similar ME (Table 4

Table 4. *In vitro* digestibility parameters for durum wheat straw

Variety	TGP	IVDMD	IVOMD	ME
HI 8759	30.72 ± 0.96	34.08^{d} ± 0.87	40.99^{c} ± 1.88	6.75^{a} ± 0.13
HI 8713	27.72 ± 0.40	42.73^{b} ± 1.46	45.96^{b} ± 1.27	6.15^{bc} ± 0.07
HI 8737	27.38 ± 0.71	49.05^{a} ± 0.58	51.53^{a} ± 0.56	6.18^{bc} ± 0.09
HI 8663	28.50 ± 0.44	47.51^{a} ± 0.67	50.46^{a} ± 0.46	6.38^{b} ± 0.05
HI 8777	28.05 ± 0.48	48.07^{a} ± 0.57	51.02^{a} ± 0.48	6.09^{c} ± 0.06
UAS 428	25.05 ± 0.80	37.94^{c} ± 1.09	40.31^{c} ± 0.94	6.10^{c} ± 0.07
Significance	NS	**	**	**
P Value	0.12	P<0.01	P<0.01	P<0.01

NS-Not-Significant (P>0.05); **- Highly Significant (P<0.01); Values bearing different superscripts a, b, c in a column differ significantly at the indicated level. TGP- Total Gas Production ml/200mg substrate; IVDMD - In vitro dry matter digestibility; IVOMD - In vitro organic matter digestibility; ME - Metabolizable Energy.

WB-02 has more IVDMD, IVOMD and ME compared to HI 1544. HI-1633 and HI-1634 identified for late-sown irrigated conditions of Central India have similar IVDMD, IVOMD and ME. IVDMD and IVOMD were higher in HI-153 as compared to HI-1605 (Table 5). Almost all the characteristics of in vitro digestibility (TGP, IVOMD, ME) were significantly higher in durum wheat straw varieties compared to bread wheat varieties (Table 6).

Table 5. *In vitro* digestibility parameters for bread wheat straw

Variety	TGP	IVDMD	IVOMD	ME
Wb0	223.38 ± 0.73	43.57^{ab} ± 1.49	44.63^{ab} ± 0.65	5.64^{a} ± 0.74
HI 1544	22.83 ± 0.73	34.77^{d} ± 1.34	38.58^{c} ± 0.62	5.55a ± 0.60
HI 1633	20.16 ± 0.68	37.70^{cd} ± 1.75	41.21^{bc} ± 0.81	5.13^{b} ± 0.93
HI 1634	21.94 ± 0.48	39.97^{bc} ± 1.40	40.85^{bc} ± 0.81	5.40^{ab} ± 0.78
HI 1605	22.77 ± 2.80	16.25^{e} ± 1.55	25.86^{d} ± 1.06	5.46^{ab} ± 0.97
HI 1531	22.27 ± 0.33	44.87^{a} ± 1.40	46.82^{a} ± 1.40	5.62^{a} ± 0.27
Significance	NS	**	**	**
P Value	0.943	P<0.01	P<0.01	P<0.01

NS-Not-Significant (P>0.05); **- Highly Significant (P<0.01); Values bearing different superscripts a, b, c in a column differ significantly at the indicated level. TGP- Total Gas Production ml/200mg substrate; IVDMD - In vitro dry matter digestibility; IVOMD - In vitro organic matter digestibility; ME - Metabolizable Energy.

Table 6. Comparisons of *In vitro* digestibility parameters between durum and bread wheat varieties

Parameter	Durum	Bread	Significance	P value
TGP	27.90 ± 0.747 (25.05 - 30.72)	22.22 ± 0.459 (20.16 - 23.38)	**	0.001
IVDMD	43.23 ± 2.500 (34.08 - 49.05)	36.19 ± 4.266 (16.25 - 44.87)	NS	0.185
IVOMD	46.71 ± 2.082 (40.31 - 51.53)	39.66 ± 3.007 (25.86 - 46.82)	*	0.083
ME	6.27 ± 0.104 (6.09 - 6.75)	5.46 ± 0.771 (5.13 - 5.64)	**	0.001

NS-Not-Significant (P>0.05);*-Significant (P<0.05) **- Highly Significant (P<0.01); TGP- Total Gas Production ml/200mg substrate; IVDMD - In vitro dry matter digestibility; IVOMD - In vitro organic matter digestibility; ME - Metabolizable Energy.

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Rumen fermentation parameters

The volatile fatty acids were similar in all bread and durum varieties. TVFA ranged from 30.8 in HI-8737 to 35.6 in HI-8713 (durum wheat straw), and from 27.0 in HI-1605 to 35.1 in HI-1531 (bread wheat varieties (Table 7 and 8). Acetate, propionate (P<0.01), butyrate, and TVFA (P<0.05) are higher in durum wheat straw compared to bread wheat straw (Table 9).

Table 7. Volatile fatty acid proportions (mM/1000 ml incubation medium) in case of durum wheat straw varieties

Variety	Acetate	Propionate	Iso- butyrate	Butyrate	Iso-valerate	Valerate	Total
HI-8759	23.9±3.28	7.25±1.06	0.24±0.05	1.98±0.42	0.39 ± 0.10	0.78±0.16	34.5±4.50
HI-8713	24.7±2.87	7.45±0.85	0.26±0.07	1.97±0.43	0.43±0.12	0.85±0.24	35.6±4.55
HI-8737	21.4±2.24	6.70±0.68	0.19±0.05	1.53±0.19	0.30±0.08	0.66±0.14	30.8±3.04
HI-8663	24.5±0.97	7.74±0.22	0.17±0.03	1.71±0.11	0.27±0.06	0.68±0.08	35.0±1.46
HI-8777	24.9±1.13	7.45±0.27	0.16±0.03	1.69±0.13	0.26±0.06	0.68±0.09	35.1±1.71
UAS-428	21.6±0.68	6.37±0.35	0.19±0.04	1.49±0.04	0.34±0.08	0.63±0.05	30.6±0.75
Significance	NS	NS	NS	NS	NS	NS	NS
P value	0.73	0.67	0.60	0.68	0.68	0.87	0.75

NS-Not-Significant (P>0.05)

Table 8. Volatile fatty acid proportions (mM/1000 ml incubation medium) in case of durum wheat straw varieties

Variety	Acetate	Propionate	Iso- butyrate	Butyrate	Iso-valerate	Valerate	Total
WB-02	19.1±1.36	5.67±0.39	0.21±0.04	1.40±0.19	0.34±0.08	0.68±0.14	27.4±1.6
HI-1544	21.0±1.80	5.69±0.50	0.25±0.02	1.59±0.01	0.46±0.02	0.82±0.10	29.8±2.1
HI-1633	20.0±3.21	5.65±0.76	0.21±0.04	1.51±0.27	0.35±0.08	0.72±0.18	28.5±4.3
HI-1634	19.7±2.56	5.75±0.80	0.19±0.03	1.45±0.15	0.30±0.06	0.65±0.09	28.1±3.3
HI-1605	18.8±0.59	5.14±0.24	0.24±0.05	1.57±0.14	0.41±0.09	0.79±0.15	27.0±0.8
HI-1531	25.0±2.33	6.96±0.69	0.25±0.08	1.66±0.46	0.41±0.15	0.89±0.29	35.1±4
Significance	NS	NS	NS	NS	NS	NS	NS
P Value	0.41	0.44	0.93	0.98	0.82	0.91	0.44

NS-Not-Significant (P>0.05);

Table 9. Comparison in case of VFA concentrations (mM/1000 ml incubation medium) between bread and durum varieties of wheat straw

Parameter	Durum	Bread	Significance	P value
Acetate	23.49 ± 0.646 (21.40 - 24.90)	20.60 ± 0.934 (18.80 - 25.00)	**	0.002
Propionate	7.16 ± 0.212 (6.37 - 7.74)	5.18 ± 0.247 (5.14 - 6.96)	**	0.002
Isobutyrate	0.20 ± 0.162 (0.16 - 0.26)	0.22 ± 0.010 (0.19 - 0.25)	NS	0.252
Butyrate	1.78 ± 0.085 (1.49 - 1.98)	1.53 - 0.039 (1.40 - 1.66)	*	0.061
Isovalerate	0.33 ± 0.027 (0.26 - 0.43)	0.37 ± 0.023 (0.30 - 0.46)	NS	0.231
Valerate	0.71 ± 0.034 (0.63 - 0.85)	0.75 ± 0.037 (0.65 - 0.89)	NS	0.394
Total	33.60 ± 0.928 (30.60 - 35.60)	29.31 ± 1.222 (27.00 - 35.10)	*	0.02

NS-Not-Significant (P>0.05); *- Significant (P<0.05) **- Highly Significant (P<0.01);

Correlations between grain yield and straw quality

1000 grain weight is positively correlated to straw NDF, cellulose, total gas production, ME, acetate, propionate, butyrate, and TVFA concentrations (Table 10). Total grain yield is positively correlated straw yield and total biomass yield. Straw yield and total biomass yield further positively correlated with butyrate concentration

Table 10. Correlations between grain yield with straw quality parameters

Parameter	R^2	P value
1000 grain wt. X Straw NDF	0.57*	0.05
1000 grain wt. X Straw Cellulose	0.60*	0.04
1000 grain wt. X Total Gas Production	0.69**	0.01
1000 grain wt. X ME	0.61*	0.03
1000 grain wt. X Acetate	0.58*	0.04
1000 grain wt. X Propionate	0.59*	0.04
1000 grain wt. X Butyrate	0.58	*0.04
1000 grain wt. X Total VFA	0.59*	0.04

Discussion

To the best of our knowledge, we are reporting for the first time regarding comparative nutritional values of durum and bread wheat varieties. Very little information is available on these aspects. The chemical composition of proximate and VanSoest constituents reported in this study is somewhat similar to Vaswani et al. (2013), with some deviation in the case of CP, ADF, and hemicellulose. IVDMD and IVOMD values described in this study were lower than values recorded by Ali and Singh, (2003) and Vaswani et al. (2013). Vadiveloo et al. (1992) evaluated botanical fractions such as inflorescence, stem, leaf blade, and leaf sheath of four varieties of rice straw (MRl, MR71, MR84 and MR27) and discovered that IVD, crude protein, and acid insoluble ash are important traits to consider when comparing different varieties. The acid detergent lignin (ADL) content did not differ significantly among different varieties of durum and bread wheat varieties. Most papers reporting lignin contents in rice straw have used acid-detergent lignin by either the sulfuric acid or permanganate versions. There are undoubtedly soluble phenolics in rice straw that need investigation. Rao et al. (2022) evaluated 13 varieties of rice straw and found that straw CP was negatively correlated with CF of straw and positively correlated with iron and zinc content of brown rice and zinc content of polished rice. CF of rice straw was negatively correlated with important plant breeding parameters such as single plant dry weight, flag leaf length, iron content of brown rice, zinc content of both brown and polished rice. Improved bio-fortified breeding lines had more zinc than bio-fortified varieties indicating further scope of improvement of zinc content in straw. In vitro organic matter digestibility (IVOMD) ranged from 37.9 in DRR Dhan 45 to 49.6 in DRR Dhan49 with an average of 43.0 in different bio-fortified varieties.

Dai et al. (2016) suggested overall Harvest Index (HI) of 0.45 suggests that over half the above ground biomass is potentially available as feedstock annually. However, differences in straw yield and HI were detected among wheat classes and regions and HI is inversely proportional to straw yield. Durum wheat produced on an average 7.3 tons straw per hectare which is lower than soft red winter wheat and soft red spring wheat varieties. These variations are attributable to differences in agronomic conditions, rainfall distribution across these locations, and the genetic differences between entries in the individual trials.

The varieties evaluated in the present study are developed for their suitability for different climatic situations of Central Indian climate like late sowing and limited irrigation conditions. The variations observed in different nutritional parameters are due to the factors like altitude and intensity of irrigation (Bainton et al. 1991; Williams et al. 1996). It is likely that environmental effects dwarf the genetic effects (Schiere et al., 2004). Sohane and Singh (2000) have crossed low and high digestibility lines and indicate that selection for quality is possible. Keno et al. (2021) observed differences on feed intake, digestibility, body weight gain and feed-to-gain ratio among lambs fed straws from different barley varieties, pointing to the importance of genetic variation in the feeding value of barley straw. Wang et al. (2020) found that water-soluble carbohydrates, crude proteins, phosphorus, and nitrogen are closely associated attributes responsible for nutritional properties. Effort in plant breeding has been to develop short varieties with higher grain yield. This development has reduced straw quantity but not nutritive value (VanSoest, 2006). Tropical breeds of cattle show faster rate of passage compared to temperate breed (Mann et al. 1987) suggesting inherent capacity of indigenous cattle to digest poor quality roughages. Under these circumstances, crop residues will come to the rescue of farmers to exploit inherent digestive capability of Indigenous cattle.

The measure of VFA reflects fermentation potential of straw. Acetate is the end product of cellulose fermentation and propionate is the end product of soluble carbohydrate. Total VFA gives a measure of fermentation of complex carbohydrates present in the straw. In the present study, significant positive correlations were obtained between grain yield and straw quality and fermentation parameters. Similar to the findings, Joshi et al. (2019) found significant positive correlations with weak association between straw NDF and ADF in a wheat population of 287 diverse spring wheat lines. Earlier we (Rao et al. 2022) have evaluated different rice straw varieities and found similarities in fermentation pattern as indicated different volatile fatty acids with positive correlations ($P>0.05$) between plant quality parameters like single plant dry weight ($r=0.42$) and Iron content in Brown Rice ($r=0.42$).

CONCLUSION

Durum wheat straw varieties have significantly higher Cellulose, TGP, IVDMD, IVOMD and ME compared to bread wheat varieties. 1000 grain weight (g) is positively correlated to straw NDF, Cellulose, total gas production, ME, Acetate, Propionate, butyrate and TVFA.

References

Ali, N. and Singh, M. 2003. '*In sacco* nutritional evaluation of wheat straw varieties', *Animal Nutrition and Feed Technology*, 3, 67–74.

Álvarez, C., Mullen, A.M., Pojić, M., Dapčević Hadnađev, T.D. and Papageorgiou, M. 2021. 'Classification and target compounds', in Charis, M. and Galanakis, E. (eds.) *Food waste recovery – processing technologies, industrial techniques, and applications*. Academic Press, 21–49. doi:10.1016/B978-0-12-820563-1.00024-X.

AOAC (2005), AOAC Official methods of Analysis, 18th ed. Association of Official Analytical Chemists, Washinton, DC.

AOAC International. 2012. *Official methods of analysis*. 19th edn. Arlington, VA: Association of Official Analytical Chemists.

Cottyn, B.G. and Boucque, C.V. 1968. 'Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid', *Journal of Agricultural and Food Chemistry*, 16, 105–107.

Dai, J., Bean, B., Brown, B., Bruening, W., Edwards, J., Flowers, M., Karow, R., Lee, C., Morgan, G., Ottman, M., Ransom, J. and Wiersma, J. 2016. 'Harvest index and straw yield of five classes of wheat', *Biomass and Bioenergy*, 85, 223–227. doi:10.1016/j.biombioe.2015.12.023.

Department of Agriculture & Farmers Welfare. 2022. *Agricultural statistics at a glance, 2022. Wheat: Area, production and yield during 2020–21*. Directorate of Economics & Statistics, Ministry of Agriculture & Farmers Welfare, Krishi Bhavan, New Delhi, 53.

Giridhar, K. and Anandan, S. 2013. 'Remote sensing techniques for estimation of crop residues and biomass from grasslands', in Sampath, K.T., Ghosh, J. and Bhatta, R. (eds.) *Animal nutrition & reproductive physiology (recent concepts)*. Delhi: Satish Serial Publishing House, 547–556.

Joshi, A.K., Kumar, U., Mishra, V.K., Chand, R., Chatrath, R., Naik, R., Biradar, S., Singh, P.R., Budhlakoti, N., Devulapalli, R. and Blümmel, M. 2019. 'Variations in straw fodder quality and grain–straw relationships in a mapping population of 287 diverse spring wheat lines', *Field Crops Research*, 243, 107627.

Keno, M.T., Tolemariam, T., Demeke, S., Wamatu, J., Alkhtib, A. and Janssens, G.P.J. 2021. 'Effect of barley variety on feed intake, digestibility, body weight gain and carcass characteristics in fattening lambs', *Animals (Basel)*, 11(6), 1773.

Mann, D.L., Goode, L. and Pond, K.R. 1987. 'Voluntary intake, gain, digestibility, rate of passage and gastro-intestinal tract fill in tropical and temperate breeds of sheep', *Journal of Animal Science*, 64, 880–886.

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

Prasad, S.V.S. and Prakasha, T.L. 2020. 'Wheat varieties identified for release', *Madhya Bharat Wheat News*, 6(2), 1–9.

Rao, S.B.N., Suman, K., Chirravuri, C.N., Sundaram, R.M., Nalina, M., Chandrasekharaiah, M., Parthipan, S. and Soren, N.M. 2022. 'Nutritional evaluation of different rice (Oryza sativa) straw of bio-fortified varieties, bio-fortified breeding lines and breeding lines', *Indian Journal of Animal Science*, 92, 390–393.

Schiere, J.B., Joshi, A.L., Seetharam, A., Oosting, S.J., Goodchild, A.V., Deinum, B. and Van Keulen, H. 2004. 'Review paper: Grain and straw for whole plant value, implications for crop management and genetic improvement strategies', *Experimental Agriculture*, 40, 277–294.

Sohane, R.K. and Singh, M. 2000. 'Genetic variability and prediction of nutritive value of rice straw of different cultivars', *Indian Journal of Animal Nutrition*, 17, 271–278.

Vadiveloo, J. 1992. 'Varietal differences in the chemical composition and in vitro digestibility of rice straw', *Journal of Agricultural Science*, 119(1), 27–33. doi:10.1017/S0021859600071513.

Van Soest, P.J. 2006. 'Rice straw, the role of silica and treatments to improve quality – Review', *Animal Feed Science and Technology*, 130, 137–171.

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

Vaswani, S., Kumar, R., Roy, D. and Kumar, M. 2013. 'In vitro evaluation of wheat straw varieties for chemical composition, gas production and digestibility', *Indian Journal of Animal Research*, 47, 555–557.

Wang, X., Zengling, Y., Yang, Z., Liu, X., Huang, G., Xiao, W. and Han, L. 2020. 'The composition characteristics of different crop straw types and their multivariate analysis and comparison', *Waste Management*, 110, 87–97. doi:10.1016/j.wasman.2020.05.018.

Research Article

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Effect of Monensin Sodium Supplementation on Production Performance in Lactating Cattle

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The present study was undertaken to evaluate the effect of monensin sodium supplementation on nutrient utilization, metabolic response, blood biochemical profile, and production performance in lactating Sahiwal cattle. Eighteen Sahiwal cows of similar body weight and milk yield were randomly allocated into three dietary groups: Control (basal diet without monensin sodium: Ms0), Treatment-1 (basal diet with monensin sodium @ 150 mg/animal/day: MS_{150}), and Treatment-2 (basal diet with monensin sodium @ 300 mg/animal/day: MS_{300}). All animals were offered a uniform basal ration containing 60% concentrate and 40% wheat straw formulated to meet nutrient requirements. Daily dry matter intake and fortnightly body weight were recorded to assess changes in performance. Dry matter intake (kg/day and % BW) and metabolic body weight remained statistically similar among groups. Digestibility coefficients of Dry Matter (DM), Crude Fiber (CF), Ether Extract (EE), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were comparable across treatments with no significant difference in treatment groups; however, organic matter and crude protein digestibility were significantly enhanced in both monensin sodium supplemented groups. Haematological parameters remained within normal physiological ranges and were not impacted by supplementation of different levels of monensin sodium. Among blood metabolites, glucose and total protein showed a marked significant improvement while β-hydroxybutyrate (BHBA) levels showed a significant decrease. Plasma minerals (Ca, P, Cu, Zn, Mn) remained unaffected with supplementation of monensin sodium. Milk yield improved significantly in cows receiving 300 mg/day monensin sodium (MS_{300}), while composition parameters like milk fat, protein, lactose, and SNF remained unchanged with monensin sodium supplementation. It may be concluded that monensin sodium, at an optimal dose of 300 mg/animal/day (MS_{300}), improves nutrient digestibility, enhances metabolic efficiency and increases milk yield without adverse effects on animal health.

Keywords: Monensin, Ionophore, Sahiwal, Lactating, Milk production

Introduction

India possesses one of the largest livestock populations globally, and the livestock sector plays a vital role in improving rural livelihoods (DAHD, 2023). Although India is the world's leading milk producer, average milk yield per animal remains substantially lower than that of developed countries, mainly due to inefficient nutrient utilization (Ahvanooei et al., 2023). Inefficient rumen fermentation results in greater losses of feed energy as methane and nitrogen as ammonia, negatively affecting production efficiency and contributing to greenhouse gas emissions (Mammi et al., 2021). Consequently, nutritional strategies aimed at manipulating rumen microbial ecology have gained renewed attention in recent years as a sustainable approach to enhance productivity and reduce enteric methane emissions (Ahvanooei et al., 2023). Among feed additives, ionophore antibiotics remain one of the most effective tools for improving rumen efficiency. Ionophores such as monensin, lasalocid, laidlomycin, salinomycin, and narasin are commonly offered to ruminant animals to increase feed efficiency. Monensin is one of the most regularly utilised ionophores (Tyler et al., 1992). Monensin sodium, a polyether ionophore produced by *Streptomyces cinnamonensis*, selectively inhibits Gram-positive rumen bacteria, resulting in increased propionate production and reduced acetate, butyrate, methane, and ammonia synthesis (Trautmann et al., 2023). Monensin supplementation can reduce ruminal acetate to propionate ratio, enteric methane, and ammonia generation, while also improving feed efficiency and daily weight gain (Hemphill et al., 2018). Monensin reduces ammonia and lactate synthesis while increasing propionate production efficiency of energy metabolism, hence enhancing nitrogen metabolism by specifically inhibiting Gram-positive bacteria (Chalupa, 1980; McGuffey et al., 2001). Recent studies have demonstrated that monensin supplementation can improve milk yield, feed efficiency, and metabolic health in lactating cattle, with modest or no adverse effects on dry matter intake (DMI) when appropriately dosed (Gonzalez-Chappe et al., 2025).

Materials and Methods

The present study was conducted to evaluate the effect of monensin sodium supplementation on production performance, nutrient utilization, and blood biochemical parameters in lactating indigenous cattle.

Ethical Approval

The experiment was carried out at the Livestock Farm Complex (LFC), DUVASU, Mathura. Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), DUVASU, Mathura (Approval No. IAEC/25/59), constituted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

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Animals Detail

Eighteen lactating Sahiwal cows were selected from the institute herd and housed individually in a well-ventilated shed with separate arrangements for feeding and watering, ensuring no access to other animals' diets. The sheds were cleaned daily to maintain hygienic conditions. All animals were dewormed prior to the commencement of the experiment using fenbendazole and ivermectin, and were allowed weekly exercise. The experimental animals were randomly allotted into three groups of six animals each.

Feeding and Management

The control group (MS_0) was fed a basal diet without monensin sodium supplementation, whereas the treatment groups (MS_{150} & MS_{300}) received monensin sodium at 150 mg/animal/day/and 300 mg/animal/day, respectively. All animals were fed a basal ration consisting of wheat straw and a compounded concentrate mixture formulated to meet nutrient requirements as per NRC (2001). The concentrate mixture was prepared using maize grain, barley grain, wheat bran, gram chunni, mustard oil cake, cottonseed cake, and mineral mixture in fixed proportions. Monensin sodium was premixed thoroughly with the concentrate mixture and offered individually to animals according to their respective treatment groups. Ingredients and chemical composition (% DM basis) of total mixed ration (TMR) fed during experimental period are presented in Table 1 & 2. DMI was recorded daily by measuring the quantity of feed offered and the residue left. Body weight of experimental animals was recorded at the beginning of the experiment and subsequently at fortnightly intervals before feeding and watering.

Digestion Trial

Digestion trial of seven days was conducted at the end of the feeding experiment to assess nutrient utilization. During the digestion trial, weighed quantities of feed were offered, and total faeces voided over 24 hours were collected daily. Representative samples of feed, refusals and faeces were processed for chemical analysis, and apparent digestibility coefficients were calculated based on nutrient intake and faecal output.

Chemical Analysis

Feeds, residue and faecal samples were dried, ground, and analysed for dry matter, organic matter, crude protein, ether extract, crude fibre, and total ash following standard AOAC (2005) procedures. Fibre fractions including neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemicellulose were estimated as per the method described by Van Soest et al. (1991). Lactating cows were milked twice daily, and milk yield was recorded daily using a digital weighing balance. Milk samples were collected at fortnightly intervals during morning milking and analysed for fat, protein, lactose and solids-not-fat using a Lactoscan milk analyser MCC WS made by MILKOTRONIC LTD, Bulgaria.

Table 1. Ingredients composition (% DM basis) of total mixed ration (TMR) fed during experimental period

Ingredients (%)	Supplemental Monensin Sodium (mg/animal/day)		
	Ms_0	Ms_{150}	MS_{300}
Maize grain	21	21	21
Barley grain	16	16	16
Wheat bran	15	15	15
Gram chunni	16	16	16
Mustard oil cake	10	10	10
Mineral mixture	02	02	02
Cotton Seed cake	20.00	20.00	20.00
Wheat straw	40.00	40.00	40.00

Table 2. Chemical composition (% DM basis) of nutrients of total mixed ration (TMR) fed during experimental period

Ingredients (%)	Supplemental Monensin Sodium (mg/animal/day)		
	Ms_0	Ms_{150}	MS_{300}
Nutrients	Chemical composition (%)		
DM	91.80	91.80	91.80
OM	91.05	91.05	91.05
EE	3.15	3.15	3.15
CP	13.80	13.80	13.80
ASH	8.97	8.97	8.97
CF	23.31	23.31	23.31
NFE	50.17	50.17	50.17
NDF	61.99	61.99	61.99
ADF	29.88	29.88	29.88
ADL	1.70	1.70	1.70
Cellulose	28.19	28.19	28.19
Ca (%)	1.16	1.16	1.16
P (%)	0.56	0.56	0.56

DM=Dry Matter; OM=Organic Matter; EE=Ether Extract; CP=Crude Protein; CF=Crude Fiber; NFE=Nitrogen Free Extract; NDF=Neutral Detergent Fiber; ADF=Acide Detergent Fiber; ADL=Acid Detergent Lignin;

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Blood samples were collected at 0, 30, and 60 days before feeding and watering. Whole blood was used for haematological analysis, while plasma was separated and stored at −20 °C for biochemical estimations. Haematological attributes (Hb, PCV, RBCs, WBCs, MCV, MCHC and Platelets) in blood were analyzed by Celltac-á (MEK-6500K) auto haemoanalyser made by Nihon Kohden, Surat, India. The plasma concentration of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), bilirubin, creatinine, total protein, albumin and Plasma Urea Nitrogen (PUN), triacylglycerides, cholesterol and glucose were determined by using automated biochemical analyzer using commercial kits (Span Diagnostic Ltd. Surat, India). Plasma â-hydroxybutyric acid (BHBA) and insulin were determined in plasma by using bovine-specific ELISA kits (Bioassay Technologies, China). Plasma mineral concentrations were analysed following microwave digestion (Microwave digestion system: Multiwave 5000, Anton Paar, Virginia, USA) using inductively coupled plasma optical emission spectroscopy (ICP).

Statistical Analysis

The data for measured variables were subjected to analysis of variance (ANOVA) using the mixed model repeated measure procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). Individual animal was used as the experimental unit for all data. The statistical difference between the means was determined by using ''Tukey's honest significant difference (HSD) test.''

Results

Effect on intake and growth performance

Effect of monensin sodium supplementation on DMI and growth performance are depicted in Table 3. Daily DMI (kg/d) in MS_0, MS_{150} and MS_{300} groups during the experimental feeding was recorded and statistical analysis of data revealed that variation among the groups for DMI was not significantly different (P=0.635) between the groups and the mean daily DMI in the MS_0, MS_{150} and MS_{300} groups was 9.83, 9.39 and 9.45 kg/day. BW gain of Lactating cattle in 3 different groups measured at fortnightly intervals and found no effects of treatment on the fortnightly BW change. There was also no significant (P=0.971) effect of treatment on the fortnightly BW gain during the experimental period.

Table 3. Effect of monensin sodium supplementation on growth performance, intake and nutrients digestibility

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance
	Ms_0	Ms_{150}	MS_{300}		
BW change (kg)	350.98	353.88	353.36	5.13	0.971
BW change (kg $^{W0.75}$)	80.99	81.46	81.29	0.88	0.977
Daily DMI (kg/day)	9.83	9.39	9.45	0.19	0.635
Nutrient Digestibility (%)					
DM	54.14	55.06	56.19	0.60	0.388
OM	62.55^{a}	64.23^{ab}	65.89^{b}	0.54	0.044
CP	64.31^{a}	69.19^{b}	68.41^{b}	0.65	0.004
CF	52.60	50.31	53.63	0.83	0.407
EE	76.20	76.38	77.76	0.33	0.119
NFE	68.10	70.34	69.10	0.46	0.136
NDF	54.44	55.42	57.69	0.61	0.076
ADF	41.66	39.19	44.73	1.04	0.096

DM=Dry Matter; OM=Organic Matter; CP=Crude Protein; CF=Crude Fiber; EE=Ether Extract; NFE=Nitrogen Free Extract; NDF=Neutral Detergent Fiber; ADF=Acide Detergent Fiber. Means within a row with different superscript letters differ significantly (P<0.05).

Effect on nutrient utilization

Effect of monensin sodium supplementation on nutrients digestibility are depicted in Table 3. The digestibility coefficients of DM, EE, NFE were unaffected by different levels of monensin sodium supplementation. However organic matter and crude protein digestibility increased significantly in monensin-fed groups, particularly in 300 mg/day group.

Effect on milk production and milk composition

The effect of monensin sodium supplementation on milk yield (kg/d), milk fat (%), milk protein (%), milk lactose (%) and milk SNF (%) of experimental animals is presented in Table 4. During the experimental period, milk production showed a significant (P<0.05). improvement in the MS_{300} group compared to MS_0 and MS_{150}. The mean daily milk yield was 6.15, 6.18, and 6.57 kg/d for MS_0, MS_{150} and MS_{300} groups, respectively. The mean fat percentage across the study period was 4.24, 4.17, and 4.09% in MS_0, MS_{150} and MS_{300} groups, respectively. statistical analysis revealed no significant difference (P>0.05) among the groups. Across the experimental period, milk protein percentage remained relatively stable, with mean values of 3.29, 3.25, and 3.26% in respective groups. No significant variation (P>0.05) was detected among treatments, suggesting that inclusion of monensin sodium in the diet did not alter milk protein content The mean lactose percentage was 4.93, 4.92, and 4.92% for MS_0, MS_{150} and MS_{300} groups, respectively. Statistical analysis revealed no significant difference (P>0.05) among the groups. The mean SNF percentage for the overall experimental period was 8.99, 8.94, and 8.93% in the MS_0, MS_{150} and MS_{300} groups, respectively, showing a similar non-significant trend among all treatments.

Effect on blood biomarkers

Effect of monensin sodium supplementation on haematology profile is showed in Table 5. Hematological parameters RBC, WBC, Hb, PCV, MCV, MCHC and platelets showed no significant variation among groups in the present study.

Effect of monensin sodium supplementation on plasma mineral concentration is showed in Table 5. Based on the overall mean values, monensin sodium supplementation did not exert any significant influence on the plasma mineral profile of lactating Sahiwal cattle throughout the experimental period. Overall, the consistency of both macro and micro mineral concentrations within normal physiological ranges suggests that supplementation of

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monensin sodium at either 150 or 300 mg/animal/day did not disturb mineral metabolism, absorption, or homeostasis in lactating cattle under the conditions of the present study. Similarly, plasma Zn, Cu, and Mn concentrations showed no significant differences among groups in the present study, indicating that monensin did not alter trace-mineral status under the applied dietary conditions.

Effect of monensin sodium supplementation on blood biochemicals are presented in table 6. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma glucose concentration (P<0.05). The overall mean values across the 60-day study averaged 63.19, 68.03 and 72.07 mg/dL in MS_0, MS_{150} and MS_{300} groups, respectively. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma total protein concentration (P<0.05). The overall mean values across the 60-day study averaged 6.55, 6.69 and 6.81 g/dL in MS_0, MS_{150} and MS_{300} groups, respectively. In present study, plasma cholesterol, triglycerides, albumin and globulin concentration remained statistically similar among treatments. In the present study, â-hydroxybutyrate (BHB) concentration was significantly lower in monensin-supplemented groups, especially at 300 mg/day, indicating improved energy balance and reduced ketone body accumulation. The overall mean BHBA (â-hydroxybutyric acid) concentration was 0.77 and 0.82 in $MS_0$and MS_{150} groups, respectively, while a lower value of 0.52 was recorded in MS_{300} group indicating a noticeable reduction in BHBA levels in the monensin sodium supplemented groups. Liver enzymes (ALT, AST, ALP and Bilirubin) and renal indicators (creatinine, PUN) remained within normal physiological range in all treatment groups, exhibiting no negative response to monensin supplementation.

Discussion

Effect on Intake and Growth Performance

Statistical analysis of data revealed that variation among the groups for daily DMI was not significantly different (P=0.635) among the different groups (MS_0, MS_{150} and MS_{300}). A comparable non-significant (P=0.247) intake trend was reported by Duffield et al. (2000) in Holstein cows supplemented with monensin at 200 mg/day, wherein intake remained stable despite improved production efficiency. Similar observations were presented by Ahvanooei et al. (2023) in a study involving 48 early-lactation cows offered monensin through TMR, where DMI remained statistically unaffected. Similar to the current study Mullins et al. (2012) observed that DMI in pre and postpartum period was not affected. A variety of factors, including the number of animals in the trial, the lactation stage, and the duration of data collection, could account for the varying response to monensin supplementation in the DMI. Monensin improves rumen fermentation efficiency without causing measurable changes in intake, supporting the findings of the current study. The increased propionate production driven by monensin influences satiety and also DMI. Contrary to this, Navarro et al. (2019) conducted a 72-day study on 24 lactating Jersey cows supplemented with gradual monensin levels and recorded a significant decline in feed intake during peak lactation. BW gain of lactating cattle in 3 different groups measured at fortnightly intervals and found no effects of treatment on the fortnightly BW

Table 4: Effect of monensin sodium supplementation on milk yield and milk composition

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Milk yield (Kg/d)	6.15[a]	6.18[a]	6.57[b]	0.05	0.041	0.367	0.624
Milk fat (%)	4.24	4.17	4.09	0.05	0.263	0.984	0.961
Milk protein (%)	3.29	3.25	3.26	0.01	0.322	0.977	0.893
Milk lactose (%)	4.93	4.92	4.92	0.02	0.600	0.907	0.899
Milk SNF (%)	8.99	8.94	8.93	0.26	0.383	0.917	0.846

SNF=Solid Not Fat. Means within a row with different superscript letters differ significantly (P<0.05).

Table 5. Effect of monensin sodium supplementation on haematology profile and plasma mineral concentration

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Haematology Profile							
RBCs (10^6/µL)	5.51	5.95	5.37	0.10	0.601	0.854	0.890
WBCs (10^3/µL)	10.31	9.07	9.15	0.29	0.120	0.935	0.942
Hb (g/dL)	8.91	9.08	8.59	0.13	0.076	0.870	0.937
HCT (%)	25.73	26.81	24.04	0.54	0.301	0.888	0.910
MCV (fL)	46.72	45.53	45.54	0.66	0.496	0.996	0.997
MCHC (g/dL)	33.38	33.38	33.17	0.07	0.312	0.827	0.289
Platelets (10^3/µl)	211.61	243.61	249.55	8.97	0.082	0.828	0.386
Plasma mineral concentration							
Calcium (mg/dL)	9.73	9.45	9.62	0.17	0.802	0.853	0.561
Phosphorous (mg/dL)	5.59	5.51	5.60	0.03	0.207	0.851	0.538
Copper (mg/L)	1.16	1.12	1.09	0.02	0.805	0.079	0.256
Zinc (mg/L)	1.19	1.15	1.17	0.02	0.961	0.237	0.145
Manganese (µg/L)	2.56	2.17	2.50	0.03	0.943	0.205	0.992

HCT=Haematocrt Value; MCV=Mean Corpuscular Volume; MCHC=Mean Corpuscular Haemoglobin Concentration. Means within a row with different superscript letters differ significantly (P< 0.05).

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change. There was also no significant (P=0.971) effect of treatment on the fortnightly BW gain during the experimental period. There was no significant difference in BW observed on supplementation of different levels of monensin sodium in diet of cattle. Comparable outcomes were obtained by Ipharraguerre and Clark (2003) who supplemented (335 mg/cow/day) monensin in 60 lactating Holstein cows for 120 days with no significant change in BW. Similarly, Penner et al. (2014) evaluated monensin inclusion in 36 multiparous cows and reported no appreciable variation in body mass despite higher energy extraction. In contrast, Makwana et al. (2022) observed slight BW gain when 24 Gir cows were supplemented with monensin for 75 days. This improvement is attributed to the reduction in methane energy losses and the increased capture of dietary energy as metabolizable energy. Based on the potential of ionophores to increase the supply of glucogenic precursors such as propionate, the administration of monensin to dairy cows may increase the hepatic synthesis of glucose and, therefore, improve the energy balance.

Effect on nutrient utilization

Effect of monensin sodium supplementation on nutrients digestibility are depicted in Table 3. The digestibility coefficients of DM, EE, NFE were unaffected by different levels of monensin sodium supplementation. However organic matter and crude protein digestibility increased significantly in monensin-fed groups, particularly in 300 mg/day group. This agrees with McGuffey et al. (2001) who reported increased OM and CP utilization in dairy cattle receiving monensin-based diets. Additionally, Souri et al. (2022) evaluated monensin in 12 multiparous cows over 60 days and documented a similar rise in digestibility coefficients, supporting the findings in this study. The improvement of digestibility of organic matter in T2 collaborates with finding of Sengupta et al. (2017). While the improvement in crude protein digestibility was similar to findings of Ipharraguerre and Clark. (2003). This improvement can be attributed by effect of monensin sodium supplementation on gram positive bacteria which are sensitive to monensin and indirectly reduce rumen ammonia concentration. However, contrasting responses have been observed in some species. Saeed et al. (2020) supplemented monensin to 30 lambs over 90 days and reported numerical but non-significant improvements in OM and CP digestion. In another comparable trial, Tian et al. (2021) supplemented monensin to 36 buffaloes and reported significant improvements in CP degradation but no change in OM utilization.

Fiber fractions (NDF, ADF and CF) did not significantly differ among groups, matching results of Kononoff et al. (2006) in Holstein cows. Xia et al. (2020) in a 60-day study on lactating buffaloes also reported no improvement in cell-wall digestion with monensin. Together, these findings demonstrate consistency in fiber retention patterns and show that monensin primarily enhances fermentable nutrient digestion rather than structural carbohydrates. However, Ahvanooei et al. (2023) observed adding monensin enhanced fibre digestion without having any adverse effect on ADF.

Effect on milk production and milk composition

During the experimental period, milk production showed a significant (P<0.05). improvement in the MS_{300} group compared to MS_0 and MS_{150} (Table 4). Similar results were found by Ueda et al. (2003), reported increased yield in monensin-fed Holstein cows, aligning precisely with our results. Schultz et al. (2006) also recorded dose-responsive improvement in milk, closely reflecting the T2 response. Yadav et al. (2023) noted that monensin supplementation in early-lactating buffaloes (400 mg/day) reduced DMI but improved milk production and altered milk fat composition, milk yield increased by approximately 0.59 kg/day compared to control group. Phipps et al. (2000) examined the effects of monensin on milk output and feed intake in two studies with Holstein-Friesian cows given completely varied menus. In experiment 1. When compared to the control cows, the milk yield response of 2.8 and 2.5 kg/d that occurred when cows received 150 and 300 mg/d of monensin was significant (P<0.05). 1.5 kg/d increase in milk yield from the use of the highest dose rate (300 mg/d), there was no discernible difference between the control and the two lower dose rates. While experiment 2 observed monensin raised milk yield in T1 and T2 by 0.8 and 1.1 kg/d, respectively as compared to control. Similarly, Fernandes et al. (2020) supplemented monensin to 30 Murrah buffaloes for 56 days and recorded a 9-12% yield increment, which resembles the rise recorded in the present study in Sahiwal cows. Meanwhile, Konwar et al. (2019) observed no significant increase when monensin was fed to Gir × HF crossbreds, indicating that genetic background may govern response magnitude.

Across the experimental period, milk fat, protein, lactose and SNF percentage remained relatively stable, among the groups. No significant variation (P>0.05) was detected among treatments, suggesting that inclusion of monensin sodium in the diet did not alter the concentration of these components of milk. Statistical analysis revealed no significant difference (P>0.05) among the groups. It was similar to findings of Gupta et al. (2018) who evaluated effect of dietary monensin supplementation of 12 lactating buffaloes which was allocated to two groups one without monensin supplementation and other with monensin 24 mg/kg of DMI for 60 days, in this study it was evaluated that the average milk fat, milk protein, milk lactose and milk solid not fat were similar in both groups. Similarly, Ipharraguerre and Clark (2003) documented identical results in monensin-supplemented Holstein cows. Grainger et al. (2010) reported similar SNF and protein stability in monensin-based feeding trials. In a contrasting observation, Sadri et al. (2023) reported a decrease in milk fat in high-dose monensin cows, although total milk output improved.

Effect on blood biomarkers

Effect of monensin sodium supplementation on haematology profile is showed in Table 5. Hematological parameters RBC, WBC, Hb, PCV, MCV, MCHC and platelets showed no significant variation among groups in the present study. Similar outcomes were reported by Muthy et al. (2018) when monensin was supplemented in growing cattle. Likewise, Hidalgo et al. (2020) conducted a 10-week trial on 27 multiparous cows and reported no hematological changes under monensin inclusion. Conversely, Anwar et al. (2022) supplemented monensin sodium to 18 crossbred calves and observed elevated lymphocyte counts, suggesting additive-specific immune modulation. No such deviation occurred in the present study, indicating monensin's hematological stability.

Effect of monensin sodium supplementation on plasma mineral concentration is showed in Table 5. Based on the overall mean values, monensin sodium supplementation did not exert any significant influence on the plasma mineral profile of lactating Sahiwal cattle throughout the experimental period. Overall, the consistency of both macro and micro mineral concentrations within normal physiological ranges suggests that supplementation of monensin sodium at either 150 or 300 mg/animal/day did not disturb mineral metabolism, absorption, or homeostasis in lactating cattle under the conditions of the present study. Calcium and phosphorus concentrations remained statistically unchanged. Nagaraja et al. (1997) reported similar Ca-P stability in ionophore-fed cattle, reinforcing our findings. Smith et al. (2021) in dairy

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heifers also observed no significant fluctuations in mineral homeostasis under monensin, confirming that monensin does not alter mineral absorption pathways. Similarly, plasma Zn, Cu, and Mn concentrations showed no significant differences among groups in the present study, indicating that monensin did not alter trace-mineral status under the applied dietary conditions. Similar outcomes were noted by Beckett et al. (1998), who reported that monensin inclusion did not produce measurable changes in plasma Cu or Zn in dairy cows.

Effect of monensin sodium supplementation on blood biochemicals are presented in table 6. The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma glucose concentration (P<0.05). These findings align with Bauer et al. (1995) who reported enhanced metabolic glucose in cows receiving ionophore supplementation. Mammi et al. (2021) found that monensin supplementation with 300–400 mg/day of monensin boosts glucose availability by boosting propionate synthesis in the rumen, a key gluconeogenic precursor, monensin supplementation in the trial elevated blood glucose levels by 10-15% during early lactation phase. The increased plasma glucose can be due to increased production of propionate as higher propionate production results more conversion of propionate to methyl malonyl coA and further to succinyl Co-A which is intermediate metabolite of TCA cycle (Lean et al., 1992).

The feeding of a diet supplemented with monensin sodium observed significant treatment effect for plasma total protein concentration (P<0.05). A similar result was found in which total protein levels rose by around 1 g/dL, from 6.5 to 7.5 after monensin sodium feeding, indicating better amino acid absorption and liver efficiency (McGuffey et al., 2001). In present study, plasma cholesterol, triglycerides, albumin and globulin concentration remained statistically similar among treatments. Similar unaltered biochemical profile was stated by Oltenacu et al. (2023) in monensin-fed animals during lactation. Plasma cholesterol, triglycerides, total protein, albumin and globulin concentration remained statistically similar among treatments which follows the trend reported by Duffield et al. (2008) where serum protein and lipid status were unaffected by ionophore feeding in cows. Zahra et al. (2006) in a study found that monensin supplementation at 350 mg/day decreased free fatty acids concentrations by 20-25%, from 0.6 mEq/L to ~0.45 mEq/L. This indicates reduced lipolysis and improved energy balance. Mullins et al. (2012) also found a 20% reduction in liver triglycerides in cows given with 350 mg/day monensin.

Table 6. Effect of monensin sodium supplementation on blood biomarkers

Attributes	Supplemental Monensin Sodium (mg/animal/day)			SEM	Significance		
	Ms_0	Ms_{150}	MS_{300}		Treatment (T)	Period (P)	T×P
Glucose (mg/dL)	63.19[a]	68.03[a]	72.07[b]	1.61	0.028	0.501	0.346
Triacylglycerides (mg/dL)	25.97	26.55	23.78	0.74	0.744	0.942	0.921
Cholesterol (mg/dL)	146.00	144.27	146.11	1.16	0.784	0.080	0.997
Total protein (g/dL)	6.55[a]	6.69[a]	6.81[b]	0.06	0.047	0.022	0.009
Albumin (g/dL)	2.99	3.10	3.05	0.03	0.166	0.198	0.360
Globulin (g/dL)	3.36	3.47	3.72	0.07	0.131	0.590	0.302
BHBA (mmol/L)	0.77[a]	0.82[a]	0.52[b]	0.04	0.032	0.489	0.742
Insulin (mIU/L)	13.34	14.03	14.88	0.68	0.346	0.572	0.779
ALT (IU/L)	27.79	27.52	26.25	0.54	0.174	0.527	0.904
AST (IU/L)	91.70	84.83	87.45	1.50	0.437	0.752	0.932
ALP (IU/L)	90.92	79.32	83.46	2.38	0.435	0.832	0.986
Bilirubin (mg/dL)	0.40	0.44	0.41	0.00	0.756	0.184	0.173
PUN (mg/dL)	26.68	25.36	28.10	0.49	0.751	0.181	0.290
Creatinine (mg/dL)	1.12	1.14	1.07	0.01	0.534	0.134	0.186

BHBA=β-Hydroxy Butyric Acid; ALT=Alanine Aminotransferase; AST=Aspartat Aminotransferase; ALP=Alkaline Phosphatase; PUN=Plasma Urea Nitrogen. Means within a row with different superscript letters differ significantly (P<0.05).

In the present study, β-Hydroxy Butyric Acid (BHB) concentration was significantly lower in monensin-supplemented groups, especially at 300 mg/day, indicating improved energy balance and reduced ketone body accumulation. This finding is consistent with Duffield et al. (2008) who reported that monensin supplementation in transition cows reduced BHB levels and minimized the risk of subclinical ketosis. Similarly, McCarthy et al. (2013) observed a significant decline in circulating BHB and Non-esterified Fatty Acid (NEFA) values when monensin was fed to multiparous Holstein cows, reflecting greater glucose availability and reduced adipose mobilization. A comparable metabolic advantage was documented by Zeineldin et al. (2022) in early-lactation cows, where monensin administration improved ketone clearance and stabilized postpartum metabolic adaptation. The results of the present study therefore align with literature indicating that monensin promotes more efficient carbohydrate energy utilization, thereby decreasing reliance on fat-derived ketones. The reduction in BHB can be interpreted as a positive metabolic outcome as lower ketone levels reduce oxidative strain, support immune resilience and improve overall production energy efficiency in Sahiwal cows. Bovine insulin levels showed no significant variation among groups in the present study, indicating that monensin supplementation did not influence endocrine responses related to glucose metabolism. Comparable findings were reported by McGuffey et al. (2001), who observed stable insulin patterns in monensin-fed cattle. Similarly, Melendez et al. (2006) noted no alteration in insulin secretion in transition cows receiving monensin. Conversely, Grainger et al. (2010) documented reduced insulin concentrations under monensin supplementation.

Liver enzymes (ALT, AST, ALP and Bilirubin) and renal indicators (creatinine, PUN) remained within normal physiological range in all treatment groups, exhibiting no negative response to monensin supplementation. Similar observations were reported by Sanz-Fernandez et al. (2014) who supplemented monensin to multiparous dairy cows and noted no significant change in AST, ALT or creatinine despite improved metabolic efficiency. In

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agreement, Duffield et al. (2008) recorded normal liver and renal marker values in monensin-fed lactating cows, further confirming safety of the additive. In another controlled evaluation on early-lactation cows, O'Connor et al. (2018) also reported non-significant changes in ALT, AST and PUN upon monensin inclusion findings nearly identical to our results. The consistency across multiple independent studies strengthens the interpretation that monensin does not impose hepatic or renal stress when fed within recommended dosage, thus validating its metabolic safety in lactating Sahiwal cows.

Conclusion

The present study concludes that monensin sodium supplementation in lactating Sahiwal cattle did not affect dry matter intake, body weight gain, milk composition, most of blood biomarkers, mineral status, or overall health. However, improved organic matter and crude protein digestibility, increased milk yield, higher plasma glucose, total protein and reduced BHBA concentration particularly at the higher supplementation level indicate enhanced rumen fermentation and energy metabolism. Thus, monensin sodium supplementation appears to be a safe and effective nutritional strategy for improving milk production in lactating Sahiwal cattle without compromising animal health or milk quality.

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REFERENCES

Ahvanooei, M.R., Norouzian, M.A., Piray, A., Vahmani, P. and Ghaffari, M. 2023. Effects of monensin supplementation on lactation performance of dairy cows: A systematic review and dose-response meta-analysis. Scientific Reports. 13:1-13, 568.

Anwar, M., Khan, S., Ali, S. and Rehman, A. 2022. Hematological and immune responses of crossbred calves to monensin supplementation. Journal of Animal Physiology and Animal Nutrition. 106:1452–1461.

AOAC. 2005. Official methods of analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Bauer, M.L., Herbein, J.H. and Polan, C.E. 1995. Glucose dynamics and responses to ionophore feeding in dairy cows. Journal of Dairy Science. 78:1936–1947.

Beckett, S., Lean, I., Dyson, R., Tranter, W. and Wade, L. 1998. Effects of monensin on the reproduction, health, and milk production of dairy cows. Journal of Dairy Science. 81:1563–1573.

Chalupa, W., Corbett, W. and Brethour, J.R. 1980. Effects of monensin and amicloral on rumen fermentation. Journal of Animal Science. 51:170–179.

DAHD. 2023. Annual report 2022–2023 of the Department of Animal Husbandry and Dairying. Government of India. https://dahd.gov.in

Duffield, T.F., Merrill, J.K. and Bagg, R.N. 2008. Meta-analysis of the impact of monensin in lactating dairy cattle. Journal of Dairy Science. 91:1334–1346.

Duffield, T.F., Sandals, D., Leslie, K.E., Lissemore, K.D., Lumsden, J.H., Dick, P. and Bagg, R. 2000. Efficacy of monensin for the prevention of subclinical ketosis in dairy cows. Journal of Dairy Science. 83:412–425.

Fernandes, A.R., Mudgal, V. and Rao, S. 2020. Effect of monensin supplementation on milk yield and composition of Murrah buffaloes. Buffalo Bulletin. 39:45–52.

Gonzalez-Chappe, L., Bruni, M.A. and Dall-Orsoletta, A.C. 2025. Phytochemicals and monensin in dairy cows: Impact on productive performance and ruminal fermentation profile. Animals. 15(15):1-19, 2172.

Grainger, C., Williams, R. and Clarke, T. 2010. Supplementation of dairy cows with monensin and its effect on milk components. Australian Journal of Experimental Agriculture. 50:839–845.

Gupta, S., Mohini, M., Thakur, S.S. and Mondal, G. 2018. Effect of dietary monensin supplementation on nitrogen utilization and plasma metabolites in lactating Murrah buffaloes. International Journal of Current Microbiology and Applied Sciences. 7:3838–3845.

Hemphill, C.N., Wickersham, T.A., Sawyer, J.E., Brown-Brandl, T.M., Freetly, H.C. and Hales, K.E. 2018. Effects of feeding monensin to bred heifers fed in a dry lot on nutrient and energy balance. Journal of Animal Science. 96(3):1171–1180.

Hidalgo, L.G., de Souza, J., Morrison, S. and McDougall, S. 2020. Hematological and biochemical responses in monensin-supplemented dairy cows. Livestock Science. 231:1-13, 103883.

Ipharraguerre, I.R. and Clark, J.H. 2003. Usefulness of ionophores for lactating dairy cows: A review. Animal Feed Science and Technology. 106:39–57.

Kononoff, P.J., Ivan, S. and McLeod, K. 2006. Nutrient digestibility and monensin supplementation in Holstein cows. Journal of Dairy Science. 89:190–198.

Konwar, D., Gogoi, R. and Das, A. 2019. Effect of monensin feeding on milk production in crossbred cows. Indian Journal of Animal Research. 53:789–793.

Lean, I.J., Rogers, P. and Stockdale, C.R. 1992. Energy partitioning and metabolic effects of monensin in dairy cows. Australian Journal of Agricultural Research. 43:1581–1591.

Makwana, R.B., Patel, D.M. and Chaudhari, N. 2022. Monensin supplementation in Gir cows and its effects on growth and performance. Indian Journal of Dairy Science. 75:312–320.

Mammi, L.M.E., Guadagnini, M., Mechor, G., Cainzos, J.M., Fusaro, I., Palmonari, A. and Formigoni, A. 2021. The use of monensin for ketosis prevention in dairy cows during the transition period: A systematic review. Animals. 11(7):1-14, 1988.

McCarthy, M.M., Mann, S., Nydam, D.V. and Overton, T.R. 2013. NEFA, BHB and metabolic changes with monensin supplementation. Journal of Dairy Science. 96:566–579.

McGuffey, R.K., Richardson, L.F. and Wilkinson, J.I.D. 2001. Ionophores for dairy cattle: Current status and future outlook. Journal of Dairy Science. 84:194–203.

Melendez, P., Goff, J.P., Risco, C.A., Archbald, L.F., Littell, R.C. and Donovan, G.A. 2006. Effect of administration of a controlled-release monensin capsule on incidence of calving-related disorders, fertility, and milk yield in dairy cows. American Journal of Veterinary Research. 67(3):537–543.

doi.org/10.56093/ijan.v43i2.175226

Mullins, C.R., Mamedova, L.K., Brouk, M.J., Moore, C.E., Green, H.B. and Perfield, K.L. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. Journal of Dairy Science. 95:1323–1336.

Muthy, S., Reddy, P.S. and Naik, B.R. 2018. Effect of monensin on hematological parameters in cattle. Veterinary World. 11:1523–1527.

Nagaraja, T.G., Avery, T.B. and Galyean, M.L. 1997. Ionophores: Their effect on rumen fermentation. Journal of Animal Science. 75:2964–2986.

Navarro, G., Canaza-Cayo, A. and Carrión, F. 2019. Influence of monensin supplementation on intake and production in Jersey cows. Journal of Applied Animal Research. 47:211–217.

NRC. 2001. Nutrient requirements of dairy cattle. 7th revised ed. National Academy of Sciences, Washington, DC, USA.

O'Connor, A., Sordillo, L.M. and Hammon, D.S. 2018. Metabolic safety of monensin supplementation in early lactation dairy cows. Journal of Dairy Science. 101:4557–4569.

Oltenacu, P., Ekeberg, T. and Nilsen, K. 2023. Serum biochemical responses to monensin in lactating cows. Animals. 13:256.

Penner, G.B., Steele, M.A. and Aschenbach, J.R. 2014. Regulation of metabolic body weight in monensin-fed dairy cows. Journal of Dairy Science. 97:7154–7166.

Phipps, R.H., Weller, R.F. and Ellis, N.M. 2000. The effect of monensin on feed intake and milk production of dairy cows. Journal of Dairy Science. 83:2798–2804.

Sadri, H., Ghaffari, M. and Ahmadi, A. 2023. High-dose monensin reduces milk fat but enhances milk yield in dairy cows. Journal of Animal Science. 101:1-9, 41.

Saeed, M., Younas, M. and Sharif, M. 2020. Effect of monensin supplementation on growth performance and digestibility in lambs. Small Ruminant Research. 190:106–112.

Sanz-Fernandez, M.V., Johnson, J.S. and Abuajamieh, M. 2014. Heat stress and monensin: Metabolic response in dairy cows. Journal of Dairy Science. 97:7490–7502.

Schultz, A.J., Pickering, N.K. and Parkinson, T.J. 2006. The effect of monensin supplementation on milk yield in dairy cows. New Zealand Veterinary Journal. 54:145–150.

Sengupta, S., Ghosh, S. and Saha, S.K. 2017. Effect of monensin supplementation on nutrient digestibility in dairy cows. Indian Journal of Dairy Science. 70:362–368.

Smith, P., Gordon, J. and Wallace, R.J. 2021. Effects of ionophores on mineral metabolism and absorption in ruminants. Animals. 11:1-12, 2049.

Souri, M., Tahmasbi, A.M. and Naserian, A.A. 2022. Effects of monensin on nutrient digestibility and rumen fermentation in multiparous dairy cows. Journal of Animal Physiology and Animal Nutrition. 106:452–461.

Tian, H., Zhou, Z., Wang, C., Ding, L. and Gong, J. 2021. Effects of monensin supplementation on rumen fermentation and nutrient digestibility in buffaloes. Buffalo Bulletin. 40:221–231.

Trautmann, A., Müller, J., Fischer, S., Rehfeldt, C. and Kuhla, B. 2023. Monensin alters microbial ion transport and energy metabolism in the rumen ecosystem. Frontiers in Microbiology. 14:1-12, 1189204.

Tyler, J.W., Wolfe, D.F. and Maddox, R. 1992. Clinical indications for dietary ionophores in ruminants. Compendium on Continuing Education for the Practicing Veterinarian. 14:989–993.

Ueda, K., Ferlay, A., Chabrot, J., Faverdin, P. and Peyraud, J.L. 2003. Effect of monensin on milk production, methane emission and nutrient digestibility in Holstein cows. Animal Science Journal. 74:371–378.

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

Xia, Y., Niu, L., He, T., Guo, Y. and Liu, D. 2020. Fiber digestibility and rumen fermentation patterns in buffaloes supplemented with monensin. Tropical Animal Health and Production. 52:3211–3219.

Yadav, D.S., Kushwaha, R. and Ghosh, M.K. 2023. Effect of monensin on milk yield and fatty acid profile in lactating buffaloes. Indian Journal of Dairy Science. 76:125–132.

Zahra, L.C., Duffield, T.F., Leslie, K.E., Overton, T.R., Putnam, D. and LeBlanc, S.J. 2006. Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient dairy cows. Journal of Dairy Science. 89:4808–4818.

Zeineldin, M., Megahed, A. and Pula, B. 2022. Effect of monensin supplementation on metabolic response and ketosis in dairy cows. Animals. 12:1-13, 3251.

Statements & Declarations:

Ethical requirements & declaration: The experiment was carried out at the Livestock Farm Complex (LFC), DUVASU, Mathura. Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), DUVASU, Mathura (Approval No. IAEC/25/59), constituted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Data Availability statement: None of the data were deposited in an official repository and are available upon request.

AI policy statement: The authors carefully reviewed, revised, and verified all AI-generated content to ensure its accuracy, scientific integrity, and compliance with journal standards. The authors take full responsibility for the content of the manuscript, including all interpretations, analyses, conclusions, and any errors that may remain. No AI tool was involved in the generation, analysis, or interpretation of the research data unless explicitly stated elsewhere in the manuscript.

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Research Article

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Nutritional, Phytochemical and *In-vitro* Fermentation Characteristics of Pelleted Concentrate Feed Containing Moringa *(Moringa oleifera)* and Hedge lucerne *(Desmanthus virgatus)* Leaves on Camel *(Camelus dromedarius)* Calves

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This study investigated the potential of tree leaves as alternative feed resource by evaluating their nutritional composition, nitrogen fractions, phytochemical constituents, antioxidant activity and *in-vitro* fermentation characteristics of pelleted concentrate feeds containing Moringa and Hedge lucerne leaves. Three iso-nitrogenous pelleted diets were formulated: a control diet based on conventional roughages (T_1), diets containing 10% Moringa leaf incorporated pelleted meal (T_2) and 10% Hedge lucerne leaf incorporated pelleted meal (T_3). Samples were analyzed for proximate composition, fibre fractions, Neutral Detergent Insoluble Nitrogen (NDIN), Acid Detergent Insoluble Nitrogen (ADIN), polyphenolic fractions, saponins, and antioxidant activity. *In-vitro* gas production, methane emission, Metabolizable Energy (ME), Net Energy (NE) and short-chain fatty acid production were analyzed. Both Moringa and Hedge Lucerne leaf incorporated concentrated pelleted sources exhibited high crude protein content (25.06-26.30%) with favourable nitrogen fractions. Incorporation of tree leaves significantly enhanced total polyphenols, non-tannin polyphenols, flavonoids and saponins in pelleted feeds. The condensed tannins and other anti-nutritional factors were within safe limits. Moringa leaves showed higher total antioxidant capacity, hydroxyl and superoxide radical scavenging, and metal chelating activities, whereas Hedge lucerne leaves exhibited slightly higher 2,2-diphenyn-1-picrylhydrazyl (DPPH) radical scavenging activity. *In-vitro* total gas production, methane emission, ME, NE and Short Chain Fatty Acid (SCFA) concentrations were non-significant in all the treatments, although improvements in energy values and reduced methane in T_2 were observed. Overall, incorporation of Moringa and Hedge lucerne leaves at 10% in pelleted concentrate feeds improved protein quality, phyto-chemical enrichment and antioxidant potential which were sustainable and cost-effective alternative in livestock feeding.

Keywords: Phytochemicals; Antioxidant activity; Pelleted concentrate feed; Tree leaves

Introduction

The increasing cost and limited availability of conventional concentrate feed ingredients such as oilseed cakes and cereal grains pose major challenges to sustainable livestock production, particularly in developing countries where feed accounts for a major portion of total production cost (FAO, 2020; Smith and Jones, 2021). This has stimulated interest in exploring alternative, locally available and nutrient-rich feed resources (Makkar, 2022). Tree leaves and leguminous forages are promising options due to their high crude protein content, favourable nitrogen fractions and presence of bioactive compounds (Patra and Saxena, 2021). *Moringa oleifera* and *Desmanthus virgatus* leaves are known for their superior protein quality with moderate NDIN and low ADIN, which enhance ruminal degradability and protein utilization (Leitanthem *et al*., 2023). Additionally, these leaves contain phytochemicals such as polyphenols, tannins, flavonoids and saponins that improve rumen fermentation, reduce methane emission and enhance animal health (Jayanegara *et al*., 2022; Patra and Yu, 2021). Their antioxidant properties further contribute to reducing oxidative stress and improving metabolic efficiency (Abu El-Hamd *et al*., 2025). Pelleting improves feed handling, intake consistency and stability of bioactive compounds (Thomas *et al*., 2020). So, it will help the commercial feed industry to adopt these feeds and produce affordable, balanced, and health-supporting diets for livestock. Therefore, the present research was designed to evaluate and compare the nutritional composition, nitrogen fractions, phytochemical content and antioxidant activities of pelleted concentrate feeds containing Moringa and Hedge lucerne leaves on *in-vitro* total gas production and energy values. This work aims to provide scientific evidence supporting the use of leaves as sustainable, cost-effective alternatives in livestock feeding strategies.

Materials and Methods

The study was conducted at ICAR-National Research Centre on Camel (NRCC), located in the Jorbeer area of Bikaner city, Rajasthan, India. The Centre is situated at 28°01′ N latitude and 73°11′ E longitude, with a time zone of GMT +05:30 h. Ambient temperatures generally range from 30 to 48 °C during summer and from 4 to 28 °C during winter.

The protocol of experiment was sanctioned by Institutional Animal Ethical Committee (IAEC) at ICAR-NRCC/IAEC/2024/19/01. Animals were cared and managed as per protocol and directives of IAEC.

Three iso-nitrogenous pelleted concentrate feeds were formulated on a dry matter basis using conventional roughages (T_1), 10% *Moringa oleifera* leaf meal (T_2), and 10% Hedge lucerne (*Desmanthus virgatus*) leaf meal (T_3). The roughage component consisted of groundnut straw (*Arachis hypogaea*) and guar straw (*Cyamopsis tetragonoloba*) mixed in a 50:50 ratio. The concentrate mixture comprised groundnut cake, til cake, mustard cake, cottonseed cake, maize, moong churi, and bajra grains. Molasses was used as a binding agent during pelleting. The nutrient requirements of the experimental animals were formulated to meet ICAR (2013) standards. Fifteen healthy un-weaned female camel calves of similar age (3-4 months), body weight, and uniform conformation were randomly selected from the camel herd of ICAR-NRCC, Bikaner. The calves were randomly allotted to three

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treatment groups (n = 5 per group) for a feeding trial of 180 days. The experimental camel calves were allocated to three treatment groups (T_1, T_2, and T_3) following a randomized block design, with five calves in each group. Calves in the control group (T_1) were fed a pelleted concentrate without leaf inclusion, whereas calves in treatment groups T_2 and T_3 received pelleted concentrate mixtures containing 10% Moringa leaves and 10% Hedge lucerne leaves, respectively. All animals were fed individually throughout the experimental period.

Leaves of Moringa and Hedge lucerne were collected from the NRCC campus, air-dried, and ground into powder form. The leaf meals were incorporated with the concentrate mixture and processed into pellets using a pelleting machine. The dried feed samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and crude fiber (CF) following AOAC (2016) procedures. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to Van Soest *et al*. (1991).

Total polyphenols and polyphenol fractions were analyzed by the methods described by Hagerman *et al*. (2000). Determination of total phenols, total tannins and condensed tannins in the samples were carried out by following the procedure described by Makkar (2003). Total phenols and total tannins were assessed by a modified Folin Ciocalteu method using polyvinyl polypyrrolidone for separating non-tannin phenols from tannin phenols. Condensed tannins were analysed by the butanol-HCl-iron method (Porter *et al*., 1986). Hydrolysable tannins were estimated as the difference between total tannins and condensed tannins. Total phenols and total tannins were expressed as tannic acid equivalents, while CT as leucocyanidin equivalents. Total saponin was estimated as per procedure described by Makkar (2007). Total Flavonoids were estimated using $AlCl_3$ method. Oxalates and HCN were estimated by titration method ($KMnO_4$ and $AgNO_3$).

DPPH radical scavenging activity was determined by using DHPP assay according to Chang *et al*. (2001). Phosphomolybdate assay was evaluated by the method of Prieto (1999). Hydroxyl radical scavenging activity was determined according to (Halliwell and Gutteridge, 1981). Superoxide anion radical scavenging activity was evaluated according to Nishikimi *et al*. (1972). Metal chelating activity was performed according to Soler-Rivas *et al*. (2000).

Accurately weighed ground substrates (200 mg) for gas production kinetics were transferred into the syringes, and anaerobic culture medium. Syringes were incubated in an incubator maintained at 39 °C and shaken manually every two hr intervals. The gas production was recorded at 0, 2, 4, 6, 8 and 12 hr of incubation taking the reading from the graduated syringes. Net gas production was calculated by subtracting the volume of gas produced in blank from the volume of gas produced from incubated feeds. Incubations were terminated for total gas, methane production and fermentation variables. Metabolisable energy was determined using gas production and chemical analysis Crude Protein (CP) and Ether Extract (EE) data. All the determinations were carried out by using McDougall buffer and 10 ml SRL as described by Menke *et al*. (1979) in triplicate. Strained rumen liquor was used as inoculum for *in vitro* digestibility and gas production studies. The metabolizable energy (ME) was calculated as per Elghandour *et al*. (2015).

ME (MJ/kg DM) = 2.20+0.136 Gp+0.057 CP%, (R^2=0.94), where, CP is crude protein% and Gp is ml of net gas production from 200 mg dry sample.

The data generated during the experiment was subjected to statistical analysis using SPSS statistical software (2011) Version 20.0 and the data were statistically analyzed using one-way and two-way ANOVA procedures for difference between treatments and periods. The difference between means was declared significant at $P<0.05$ and $P<0.01$. Duncan's multiple range test was conducted for comparing among the averages.

Results and Discussion

The proximate composition and fibre fractions of the leaves and experimental feeds are presented in Table 1. The dry matter and crude protein contents of Moringa leaves were 23.22% and 25.06%, respectively, whereas the corresponding values for Hedge lucerne leaves were 25.03% and 26.30%.

In the present investigation, the total nitrogen content of Moringa and Hedge lucerne leaves was found to be 4.01% and 4.208%, respectively (Table 2). The total nitrogen content of the pelleted concentrate feeds (T_1, T_2, and T_3) was recorded as 4.34%, 4.51%, and 4.64%, respectively. The neutral detergent insoluble nitrogen content of Moringa and Hedge lucerne leaves was 1.21% and 0.90%, respectively. In comparison, the NDIN content of the pelleted concentrate feeds T_1, T_2, and T_3 was 3.46%, 2.64%, and 0.59%, respectively. The acid detergent insoluble nitrogen content recorded in Moringa and Hedge lucerne leaves was 0.38% and 0.43%, respectively. Similarly, ADIN values of 0.13%, 0.32%, and 0.39% were observed in the pelleted concentrate feeds T_1, T_2, and T_3, respectively. The ratio of acid detergent insoluble nitrogen to total nitrogen was observed to be 9.57% in Moringa leaves, 10.31% in Hedge lucerne leaves and 3.13%, 7.19%, and 8.52% in T_1, T_2, and T_3 pelleted concentrate feeds, respectively, as presented in Table 2. The polyphenolic fractions and saponin contents of the roughage sources and pelleted feeds offered to different dietary groups (% on DM basis) are presented in Tables 3 and 4.

Table 1. Chemical composition of experimental pelleted concentrate feeds (% DM basis)

Proximate	Moringa leaves	Hedge lucerne leaves	T_1	T_2	T_3
DM	23.22	25.03	96.76	95.99	96.08
OM	91.31	91.88	95.64	96.67	96.64
CP	25.06	26.30	17.14	18.24	18.02
EE	4.42	3.42	4.19	3.43	4.38
CF	17.35	14.45	9.55	6.70	9.71
NFE	35.77	39.59	50.40	54.96	49.56
TA	17.37	16.22	8.70	6.64	7.31
TCHO	61.81	62.15	66.38	64.99	62.93
NDF	39.53	42.40	26.62	26.56	28.33
ADF	32.24	34.60	15.30	15.34	20.70
Lignin	7.28	9.88	4.39	6.32	5.11
Cellulose	22.40	13.31	17.68	17.82	15.77
Hemicellulose	7.29	7.79	11.32	11.22	7.63

DM=Dry Matter; OM=Organic Matter; CP=Crude Protein; EE=Ether Extract; CF=Crude Fiber; NFE=Nitrogen Free Extract; TA=Total Ash; TCHO=Total Carbohydrate; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber.

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Table 2. Nitrogen fractions of tree leaves and pelleted concentrate feeds

Nitrogen Fractions	Moringa leaves	Hedge Lucerne leaves	T_1	T_2	T_3
Total N (%)	4.01±0.04	4.20 ±0.03	4.34±0.05	4.51±0.00	4.64±0.00
NDIN (%)	1.21±0.00	0.90±0.00	3.46±0.00	2.64±0.00	0.59±0.00
ADIN (%)	0.38±0.00	0.43±0.00	0.13±0.00	0.32±0.00	0.39±0.00
ADIN/TN (%)	9.57±0.12	10.31±0.07	3.13±0.06	7.19±0.03	8.52±0.04

Total N=Total Nitrogen; NDIN=Neutral Detergent Insoluble Nitrogen; ADIN=Acid Detergent Insoluble Nitrogen;

Table 3. Polyphenolic fractions and saponin in roughage sources used in pelleted feeds (% DM basis)

Phytochemicals	Groundnut straw	Guar straw	Moringa leaves	Hedge Lucerne leaves
Total Polyphenols	1.38	0.71	6.36	7.17
Total Tannin Polyphenols	0.96	0.35	1.65	2.79
Non- tannin Polyphenols	0.42	0.36	4.71	4.38
Condensed Tannin	0.33	0.04	1.16	1.02
Hydrolysable Tannin	0.41	0.25	0.92	0.78
Saponin	2.98	2.72	2.11	1.91
Flavonoids	0.33	1.36	1.43	0.38
Oxalate	0.98	1.26	0.31	0.12
Hydrogen cyanide	0.0010	0.0021	0.0003	0.0050

The antioxidant Assay measures the ability of antioxidants to scavenge and neutralize free radicals. Antioxidants help in protecting against oxidative stress and related disease. The antioxidant Assay of Moringa and Hedge lucerne leaves are presented in Table 5 and Fig.1. Hedge lucerne leaves have marginally higher DPPH radical scavenging activity compared to Moringa leaves.

Table 4. Polyphenolic fractions and saponin in pelleted concentrate feeds fed to different dietary groups (% DM basis)

Phytochemicals	T_1	T_2	T_3
Total Polyphenols	2.18±0.01	2.66±0.01	3.24±0.00
Total Tannin Polyphenols	0.90 ±0.00	0.36 ±0.00	0.38 ±0.00
Non- tannin Polyphenols	1.28 ±0.01	2.30 ±0.00	2.86±0.00
Condensed Tannin	0.38 ±0.01	0.31±0.00	0.44±0.00
Hydrolysable Tannin	0.43±0.00	0.22 ±0.00	0.18 ±0.00
Saponin	0.57±0.01	1.98 ±0.00	0.41±0.00
Flavonoids	1.00 ±0.03	2.01±0.00	1.11±0.12
Oxalate	0.02±0.00	0.04±0.00	0.01 ±0.00

Table 5. Antioxidant Assay in Moringa and Hedge Lucerne leaves

Attributes (%)	Moringa leaves	Hedge Lucerne leaves	P value
DPPH radical scavenging activity (% RSA)	80.88^{a}±0.01	83.58^{b} ±0.23	0.001
Phosphomolybdate assay (Total antioxidant capacity)	14.81^{b}±0.00	8.23^{a} ±0.01	0.001
Hydroxyl radical scavenging activity (% HRSA)	13.01^{b}±0.00	8.09^{a} ±0.01	0.001
Superoxide anion radical scavenging activity (SOD)	68.1^{b} ±0.01	46.01^{a} ±0.00	0.001
Metal chelating activity	60.63^{b}±0.01	48.42^{a} ±0.02	0.001

^{ab} Means with different superscripts in a row differ significantly (P<0.01)

The effect of leaves-incorporated pelleted concentrate feed on total gas production, methane emission, metabolizable energy (ME), net energy (NE) and short-chain fatty acids (SCFA) is summarized in Table 6 and Fig.2. No significant differences were observed among treatments for total gas production, methane production, ME, NE, or SCFA concentrations, although numerical variations were evident.

Table 6. Effect of leaves incorporated in pelleted concentrate feed on Total gas production, ME, NE and SCFA

Attributes	T_1	T_2	T_3	P value
Total gas production (ml)	22.88±12.34	28.00±7.15	22.00±4.48	0.306
Ch_4 production(ml)	8.78±1.92	8.08±2.64	7.08±1.98	0.506
ME (Mcal/kg DM)	1.49±0.20	1.68±0.26	1.49±0.21	0.297
NE (Mcal/kg DM)	1.00±0.13	1.12±0.18	1.02±0.13	0.409
SCFA (mM)	0.26±0.07	0.31±0.09	0.24±0.07	0.318

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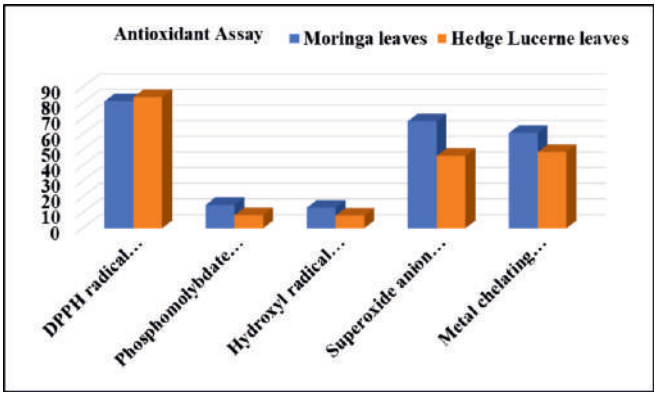

Fig.1. Graph denoting various antioxidant profile present in both the leaves namely Moringa and Hedge lucerne leaves

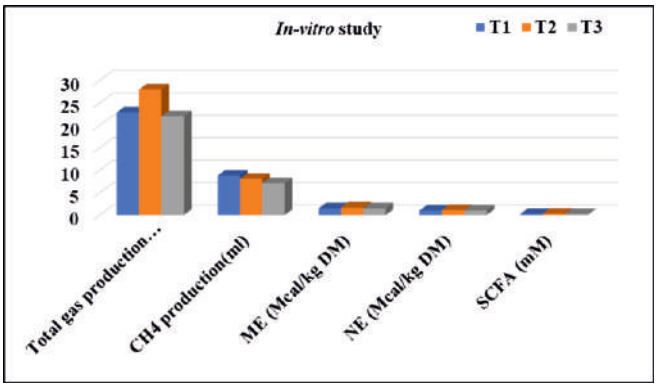

Fig.2. Graph showing total gas production, methane emission, metabolizable energy, net energy and short-chain fatty acids in different treatment groups

The results revealed variations among leaf sources and formulated diets due to differences in nutrient density and fibre fractions relevant to feed utilization and animal performance. Higher DM and OM contents in T_1, T_2, and T_3 indicate improved storage stability and formulation quality. Hedge lucerne (26.30%) and Moringa leaves (25.06%) showed higher CP levels, confirming their value as plant protein sources, whereas formulated diets contained moderate CP (17.14-18.24%), remaining adequate for maintenance and moderate production. EE was highest in T_3 (4.38%) whereas, CF, NDF, ADF and lignin were substantially higher in Hedge lucerne leaves, indicating greater structural carbohydrates. In contrast, reduced fibre fractions and lignin in T_1-T_3 suggest improved digestibility and feed quality. Higher NFE and total carbohydrates in the formulated diets, especially T_2, reflect enhanced availability of soluble carbohydrates for rumen fermentation. Overall, the formulated diets exhibited a more balanced nutrient and fibre profile compared to sole leaf sources, supporting improved digestibility and efficient nutrient utilization. The results of the present study are in agreement with the compiled information reported by Garg *et al*. (2012), Kholif *et al*. (2015), Jayaprakash *et al*. (2016), Thirumeignanam *et al*. (2019), Sonawane *et al*. (2019), Wankhede *et al*. (2022) and Masitlha *et al*. (2024). However, variations in the nutrient composition of leaves have been reported by other researchers, including Suksombat and Buakeeree (2006), Radhakrishanan *et al*. (2007), Hassan *et al*. (2020) and Mynavathi *et al*. (2021). The possible variation in crude protein content is the response of Moringa and Hedge lucerne leaves to environmental conditions, soil nutrients and time of collection which affect chemical composition.

The nitrogen fractionation revealed marked differences among Moringa leaves, Hedge lucerne leaves and pelleted concentrate feeds. Total nitrogen content was highest in Hedge lucerne leaves and increased progressively in pelleted feeds containing tree leaves, revealed improved crude protein supply. The lower ADIN and ADIN/total N ratio observed in Moringa leaves and T_2 diet denotes superior protein availability, as ADIN represents heat-damaged or indigestible protein fractions. Diets with lower ADIN values are associated with enhanced ruminal degradability and intestinal protein absorption. A higher NDIN value in Hedge lucerne leaves reflect a greater proportion of rumen slowly degradable protein, which can sustain microbial growth over time. Pelleting reduce NDIN content in concentrate feeds, likely due to structural disruption of cell walls therefore improving nitrogen accessibility. Therefore, it indicates incorporation of tree leaves improved protein quality without increasing indigestible nitrogen fractions. The nitrogen fractions of leaves have been reported by other researchers, including Bhatt *et al*. (2020).

Tree leaves exhibited higher concentrations of total polyphenols, tannins and flavonoids compared to conventional roughages. Hedge lucerne leaves showed the highest total polyphenols, while Moringa leaves contained greater non-tannin polyphenols and flavonoids. These compounds are known to have antioxidant, antimicrobial and rumen-modulatory effects. Total polyphenols and non-tannin polyphenols increased in T_2 and T_3 diets showing their antioxidant potential. These compounds are known to modulate rumen microbial populations, suppress oxidative stress and improve animal health. Saponin content was comparatively higher in groundnut and guar straw, whereas Moringa and Hedge lucerne leaves showed moderate levels. Saponins play a crucial role in reducing rumen protozoal population, thereby enhancing microbial protein synthesis and reducing methane production.

Condensed tannin levels remained within moderate ranges, which are considered beneficial for ruminant nutrition. Saponin content increased in T_2, therefore suppress rumen protozoa, enhancing microbial protein synthesis and nitrogen utilization. Flavonoid content was highest in Moringa leaves and guar straw, indicating their role as potent antioxidants. Low oxalate and HCN concentrations across all feeds confirm the safety of leaf inclusion at the tested level. Overall, the phytochemical profile of Moringa and Hedge lucerne leaves and leaf incorporated pelleted concentrate feeds indicates enriched diet with beneficial secondary metabolites.

Several researchers have reported the presence of polyphenols and saponins in Moringa and Hedge lucerne leaves. Studies by Alikwe *et al*. (2013), Aye *et al*. (2013), Okiki *et al*. (2015) and Shaikh *et al*. (2025) documented varying levels of tannins, phenolics, flavonoids, saponins, phytates, and other secondary metabolites in Moringa leaves. Similarly, Thirumeignanam *et al*. (2019) and Sarkar *et al*. (2022) reported appreciable concentrations of total phenolics, tannins, saponins, and flavonoids in hedge lucerne leaves, which corroborate the findings of the present study.

Reason behind the marginally higher DPPH radical scavenging activity in Hedge lucerne might be due to differences in polyphenolic composition and hydrogen-donating capacity, as plant phenolics effectively quench DPPH radicals via hydrogen atom and electron transfer mechanisms (Li *et al*., 2018; Shahidi and Ambigaipalan, 2020; Liu *et al*., 2021). Moreover, both leaf sources showed strong DPPH scavenging activity, reflecting their richness in phenolics, flavonoids and condensed tannins (Pérez-Jiménez *et al*., 2019; Koley *et al*., 2022).

Moringa oleifera leaves demonstrated significantly higher total antioxidant capacity, hydroxyl and superoxide radical scavenging activities and metal chelating ability than Hedge lucerne leaves, indicating a broader spectrum of water- and lipid-soluble

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antioxidants (Prieto *et al*., 2018; Vergara-Jimenez *et al*., 2019; Gullón *et al*., 2020; Saleem *et al*., 2021). These enhanced activities showed synergistic effects of flavonoids, non-tannin polyphenols and other redox-active compounds capable of electron donation, radical quenching, and transition-metal chelation (Perron and Brumaghim, 2019; Shahidi and Ambigaipalan, 2020; Santos-Sánchez *et al*., 2024).

Total gas production was numerically higher in T_2 compared with T_1 and T_3, which indicates enhanced fermentative activity of leaf inclusion. Though statistical non-significance but indicates that leaf incorporation did not adversely affect overall rumen fermentation kinetics. The higher *in-vitro* gas production might be due to higher limiting amino acids like lysine and methionine in the protein of moringa leaves (Henuk, 2018) and optimal composition of amino acids with higher digestible protein content (Babiker *et al*., 2017) which is essential for rumen microbial multiplication and growth. Methane production showed a decreasing numerical trend from T_1 to T_3, although differences were not statistically significant. This reduction may be associated with decreased protozoal populations, which are known to harbor methanogens and facilitate interspecies hydrogen transfer. The numerical decline in methane output aligns with the observed shift in VFA profiles and reduced acetate: propionate ratio. The ME and NE values were numerically highest in T_2, reflecting improved energy availability from the diet. This attributed to increased bacterial activity and improved starch and cellulose degradation. The lack of significant differences suggests that energy utilization efficiency was maintained across treatments. Similarly, SCFA concentrations did not differ significantly among treatments, indicating stable fermentation end-product synthesis despite changes in microbial populations and enzyme activities. These findings suggest that leaves-incorporated pelleted concentrate feed can be effectively used as a functional dietary strategy to improve rumen efficiency and potentially reduce environmental impact without compromising fermentation performance.

Several studies have demonstrated the methane-mitigating potential of *Moringa oleifera* in ruminant diets. Abdel-Raheem and Hassan (2021) reported that inclusion of 15% *Moringa oleifera* leaf meal reduced methane production in buffalo calves. Similarly, Badawi *et al*. (2022) observed the lowest CH_4 emission (mg/kg DMI) in Barki male lambs when dried *Moringa oleifera* leaves were supplemented at up to 200 mg/kg BW (≈5 g/day). Kholif *et al*. (2022) showed that replacing concentrate feed with *Moringa oleifera* leaf silage significantly reduced methane generation in ruminants. Lunagariya *et al*. (2022) reported that incorporation of *Moringa oleifera* meal at 5.0%, with incremental levels up to 22.5%, improved *in-vitro* gas production ($P<0.05$) and metabolizable energy content in total mixed rations for growing calves. Bhosale *et al*. (2024) further confirmed that supplementation of *Moringa oleifera* powder at 2% DM, alone or in combination with neem leaf powder and coconut oil, significantly ($P<0.05$) reduced methane emissions in calves.

Conclusion

Incorporation of *Moringa oleifera* and *Desmanthus virgatus* leaves at 10% in pelleted concentrate feeds improved crude protein quality, nitrogen fractions, phytochemical composition and antioxidant activity without adversely affecting *in-vitro* rumen fermentation parameters. Moringa-based diets showed comparatively higher antioxidant potential and numerically improved energy values with reduced methane emission. Thus, these leaves can be effectively utilized as sustainable and cost-effective alternative feed resources in camel nutrition.

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References

Abdel-Raheem, S.M. and Hassan, E.H. 2021. Effects of dietary inclusion of Moringa oleifera leaf meal on nutrient digestibility, rumen fermentation, ruminal enzyme activities and growth performance of buffalo calves. Saudi J. Biol. Sci. 28(8):4430–4436.

Abu El-Hamd, M.A., Elghandour, M.M.Y. and Salem, A.Z.M. 2025. Antioxidant-rich forage resources as modulators of rumen fermentation and oxidative stress in ruminants. *Anim. Feed Sci. Technol.* 316:115791.

Akintunde, A.O., Olorunnisomo, O.A. and Adu, O.A. 2024. Dietary antioxidants and metabolic efficiency in growing ruminants. *Trop. Anim. Health Prod.* 56 (2): 103-112.

Alikwe, P.C.N. and Omotosho, M.S. 2013. Evaluation of proximate and phytochemical composition of *Moringa oleifera* leaf meal as potential feedstuff. *Agri. Biol. J. N. Am.* 4(1):71–77.

AOAC. 2016. Official methods of analysis. 20th edn. Assoc. Off. Anal. Chem., Washington, DC, USA.

Aye, P.A. and Adegun, M.K. 2013. Chemical composition and functional properties of Moringa, Leucaena and Gliricidia leaf meals. *Agric. Biol. J. N. Am.* 4(1):71–77.

Babiker, E.E., Juhaimi, F.A. and Ghafoor, K. 2017. Amino acid composition and protein quality of *Moringa oleifera* leaves. *Food Chem.* 221:138–145.

Badawi, A.Y., Kholif, A.E. and Hassan, A.A. 2022. Mitigation of methane emissions using *Moringa oleifera* leaves in lamb diets. *Environ. Sci. Pollut. Res.* 29:38761–38771.

Bhatt, R.S., Sahoo, A. and Karim, S.A. 2020. Nitrogen fractionation and protein quality of tree leaves. *Indian J. Anim. Nutr.* 37(2):111–118.

Bhosale, T.R., Raypurkar, T.R., Gaikwad, U.S., Kamble, D.K. and Mandakmale, S.D. 2024. Effect of anti-nutritional factors on growth and methane emission in calves. *Asian J. Dairy Food Res.* 43(4):28–34.

Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. 2001. Estimation of total flavonoid content by colorimetric methods. *J. Food Drug Anal.* 10(3):178–182.

Elghandour, M.M.Y., Salem, A.Z.M. and Kholif, A.E. 2015. *In-vitro* gas production technique for feed evaluation. *Anim. Feed Sci. Technol.* 200:1–15.

FAO. 2020. Feed resources and livestock productivity in developing countries. FAO, Rome, Italy.https://www.fao.org/3/cb0891en/CBO891EN

Garg, D.D., Mehta, A.K. and Choubey, M. 2012. Nutritional evaluation of tree leaves for ruminants. Indian J. Anim. Sci. 82(3):276–279.

Gullón, B., Pintado, M.E., Pérez-Álvarez, J.A. and Viuda-Martos, M. 2020. Polyphenolic profile and antioxidant activity of plant extracts. Food Res. Int. 133:109142.

doi.org/10.56093/ijan.v43i2.178259

Hagerman, A.E., Harvey-Mueller, I. and Makkar, H.P.S. 2000. Quantification of tannins in tree foliage. J. Chem. Ecol. 26:1241–1255.

Hassan, F.U., Arshad, M.A., Li, M., Rehman, M.S.U., Loor, J.J. and Huang, J. 2020. Mulberry leaf flavonoids in ruminant production. Animals 10(11):2076.

Henuk, Y.L. 2018. Amino acid profile and rumen degradability of protein sources. Anim. Nutr. 4:87–94.

ICAR. 2013. Nutrient Requirements of Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi, India.

Jayanegara, A., Goel, G. and Makkar, H.P.S. 2022. Secondary plant metabolites as rumen modifiers. Anim. Feed Sci. Technol. 287:115275.

Jayaprakash, G. et al. 2016. Biomass yield and composition of forage species. Int. J. Sci. Environ. Technol. 5(4):2290–2295.

Kholif, A.E., Gouda, G.A., Morsy, T.A., Salem, A.Z.M., Lopez, S. and Kholif, A.M. 2015. Moringa leaf meal in lactating goat diets. Small Rumin. Res. 129(2):129–137.

Kholif, A.E., Hassan, A.A. and Matloup, O.H. 2022. Methane mitigation using Moringa silage. J. Clean. Prod. 332:130051.

Koley, T.K., Maurya, A., Tripathi, A., Singh, B. and Singh, M. 2022. Phenolic composition of leafy vegetables. J. Food Compos. Anal. 104:104204.

Leitanthem, S., Patra, A.K. and Saxena, J. 2023. Nitrogen fractions of tree leaves. Anim. Nutr. 13:115–124.

Li, Y., Zhang, J. and Liu, H. 2018. Antioxidant mechanisms of plant phenolics. Food Chem. 259:41–48.

Liu, Z., Ren, Z., Zhang, J. et al. 2021. Role of ROS and antioxidants in diseases. Front. Physiol. 12:1–14.

Lunagariya, P.M., Chaudhary, L.C. and Agarwal, N. 2022. In vitro evaluation of Moringa meal. Indian J. Anim. Sci. 92(4):453–459.

Makkar, H.P.S. 2003. Quantification of Tannins in Tree Foliage. FAO/IAEA, Vienna, Austria.

Makkar, H.P.S. 2007. Assays for plant secondary metabolites. Anim. Feed Sci. Technol. 133:3–14.

Makkar, H.P.S. 2022. Alternative feed resources for livestock. Anim. Feed Sci. Technol. 284:115193.

Masitlha, E.P., Seifu, E. and Teketay, D. 2024. Nutritional composition of Moringa leaves. Food Prod. Process. Nutr. 6(1):3–8.

Menke, K.H., Raab, L. and Salewski, A. 1979. Estimation of digestibility using gas production. J. Agric. Sci. 93:217–222.

Mynavathi, V.S., Valli, C. and Gunasekeran, S., 2021. Effect of harvesting interval and cutting height on forage yield and proximate composition of hedge lucerne (Desmanthus virgatus) in Cocus nucifera based hortipasture system. Range Management and Agroforestry, 42(2), pp.301-306.

Nishikimi, M., Appaji, N. and Yagi, K. 1972. Superoxide anion occurrence. Biochem. Biophys. Res. Commun. 46(2):849–854.

Okiki, P.A., Osibote, I.A., Balogun, O. et al. 2015. Composition of Moringa oleifera. Adv. Biol. Res. 9(6):436–443.

Patra, A.K. and Saxena, J. 2021. Dietary phytochemicals and rumen fermentation. Anim. Feed Sci. Technol. 269:114623.

Patra, A.K. and Yu, Z. 2021. Tannins and saponins in methane mitigation. Front. Microbiol. 12:617432.

Pérez-Jiménez, J., Neveu, V., Vos, F. and Scalbert, A. 2019. Polyphenols in foods database. J. Agric. Food Chem. 58(8):4959–4969.

Perron, N.R. and Brumaghim, J.L. 2019. Antioxidant mechanisms of polyphenols. Cell Biochem. Biophys. 53(2):75–100.

Porter, L.J., Hrstich, L.N. and Chan, B.G. 1986. Conversion of procyanidins. Phytochemistry 25(1):223–230.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269 (2):337-341.

Prieto, P., Pineda, M. and Aguilar, M. 2018. Antioxidant capacity assay. Anal. Biochem. 269(2):337–341.

Saleem, A., Saleem, M. and Akhtar, M.F. 2021. Bioactivities of Moringa leaf extracts. S. Afr. J. Bot. 129:245–253.

Sarkar, S., Bhatt, R.S. and Meena, R.L. 2022. Hedge lucerne as fodder. Livest. Nutr. 33(1):21–23.

Shahidi, F. and Ambigaipalan, P. 2020. Phenolics and antioxidant activity. J. Funct. Foods 71:104080.

Shaikh, S., Padmere, M., Khanum, H. et al. 2025. Physicochemical characterization of moringa mix. Indian J. Chem. Technol. 32(4):433–440.

Smith, J. and Jones, R. 2021. Economics of feed utilization. Livest. Res. Rural Dev. 33(6):45–52.

Soler-Rivas, C., Espín, J.C. and Wichers, H.J. 2000. Free radical scavenger capacity test. Phytochem. Anal. 11(5):330–338.

Sonawane, A.S., Deshpande, K.Y., Rathod, S.B. et al. 2019. Feeding hedge lucerne in goats. Indian J. Anim. Sci. 89(8):881–884.

Suksombat, W. and Buakeeree, K. 2006. Effect of cutting interval on hedge lucerne. Asian-Australas. J. Anim. Sci. 19(1):31–34.

Thirumeignanam, D., Chellapandian, M. and Arulnathan, N. 2019. Tanniferous feeds in silage. Anim. Nutr. Feed Technol. 20(1):51–60.

Thomas, M., van Zuilichem, D.J. and van der Poel, A.F.B. 2020. Physical quality of pelleted feeds. Anim. Feed Sci. Technol. 260:114344.

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

Vergara-Jimenez, M., Almatrafi, M.M. and Fernandez, M.L. 2019. Bioactive components in Moringa. Nutrients 11(2):263.

Wankhede, S.D., Dutta, N., Tambe, M.B. et al. 2022. Moringa foliage in goat nutrition. Emerg. Anim. Species 3(8):100–115.

Research Article

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Effect of energy fortification and functional nutrient multicomponent supplementation in peripartum HFX and Deoni cows on post partum performance

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This study examined whether prepartum close-up period energy fortification (+10% dietary gross energy via glycerol, jaggery, or maize) combined with a functional nutrient multicomponent supplement influenced colostrum immunoglobulin G (IgG) concentration, calf birth weight, fetal membrane expulsion time (FMET) and early lactation milk yield in HF-crossbred (HFX) and indigenous Deoni cows. Thirty-two prepartum cows (16 HFX, 16 Deoni; 3rd–5th parity; 35 ± 5 d pre-calving) were assigned to 2 (breed) × 2 (FNMS ± supplement) × 4 (energy source) factorial arrangement. Colostrum IgG was estimated from colostrometer-derived specific gravity. Day-0 colostrum IgG showed significant (P = 0.001) Energy × Breed and Energy × FNMS interactions; FNMS-supplemented HFX cows averaged 61.81 mg/mL compared to 56.30 mg/mL in unsupplemented Deoni cows. IgG concentration declined steeply by day 1 (44%) and no treatment differences were detectable from days 1–4. HFX calves at birth were heavier (49%) than Deoni calves (P < 0.001) and FNMS supplementation improved (P < 0.001) overall calf birth weight by 8% (12% in HFX, 5% in Deoni;). FMET was 38 min shorter (P < 0.001) in Deoni than HFX cows, with FNMS showing a borderline tendency to hasten expulsion (P = 0.08). Milk yield was overwhelmingly governed by breed genetics. No metabolic disease or calf mortality was recorded. These results suggest that FNMS supplementation during the close-up period enhanced colostrum quality and calf vigour in crossbred dairy cows.

Keywords: Calf birth weight, Colostrum IgG, Functional nutrient multicomponent supplement, Prepartum nutrition, Transition cow.

Introduction

The transition period is three to four weeks before and after calving which is the most critical nutritional phase in dairy cattle management. As parturition approaches, dry matter intake (DMI) falls by 25–35%, precipitating negative energy balance (NEB) that predisposes cows to various metabolic conditions like ketosis, fatty liver, displaced abomasum, and retained placenta (Drackley et al., 2005). A concurrent biological imperative during this period is colostrogenesis, which is the immunoglobulin G (IgG)-enriched secretory process in the mammary gland, essentially completes by calving. Because calves are born immunologically naive owing to the synepitheliochorial placentation of cattle, first-milking colostrum IgG is a critical determinant of passive immunity transfer, neonatal survival and lifetime health trajectory (Godden, 2008; Barrington and Parish, 2001, Westhoff et al., 2024).

Augmenting close-up diet energy density with fermentable carbohydrate sources(glycerol, jaggery crude sugar or maize starch) has been proposed as a strategy to blunt NEB without demanding large increases in feed volume (McLeod and Baldwin, 2000; Trubenbach et al., 2019). These energy substrates differ markedly in their chemical nature: glycerol is a polyol requiring phosphorylation via the glpK or dhaK pathway before microbial utilization; jaggery provides rapidly fermentable monosaccharides (glucose and fructose); and maize starch is a complex polysaccharide with longer fermentation kinetics (Blötz and Stülke, 2017).

Complementary to energy fortification, a functional nutrient multicomponent supplement (FNMS) containing niacin, omega-3 fatty acids, selenium and key trace minerals addresses the periparturient immune shift through multiple converging mechanisms. Niacin modulates adipocyte lipolysis via G protein-coupled receptor 109A (GPR109A) activation; selenium and zinc underpin selenoprotein-mediated antioxidant defence, while long-chain omega-3 fatty acids from flaxseed attenuate pro-inflammatory eicosanoid synthesis, collectively reducing the oxidative and inflammatory burden at parturition (Bertoni et al., 2006; Moallem et al., 2013; Aragona et al., 2016, Hare et al., 2023).

Cross-breeding of Deoni cattle, an indigenous draft-cum-dairy breed native to the Deccan plateau with Holstein Friesian (HF) bulls to produce HFX (crossbreds) is a prevalent improvement strategy in peninsular India. These two genotypes differ considerably in body weight, milk production potential, metabolic resilience and presumably in the hormonal and immunological determinants of colostrogenesis, making them a biologically informative pair for studying prepartum nutritional interventions. The present study, therefore, was designed to evaluate the *in vivo* effects of close-up period energy fortification with or without FNMS on colostrum IgG, calf birth weight, fetal membrane expulsion time (FMET) and early lactation performance in HFX and Deoni cows.

Materials and Methods

2.1 Ethical Approval and Study Location

All experimental procedures were approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2019/No.10). The study was conducted at the Livestock Research

Centre, ICAR-NDRI Southern Regional Station, Bengaluru (12.947°N, 77.608°E; elevation 921 m amsl) between November 2019 and March 2020. The site experiences a tropical savanna climate (Köppen–Geiger: Aw) with mean ambient temperatures of 17–28°C and relative humidity of 51–71% across the study period. Heat index values recorded during the experimental period ranged from the normal to alert category for most months.

2.2 Animals, Housing, and Experimental Design

A total of thirty-two prepartum cows, 16 HFX (HFX; *Bos taurus* × *Bos indicus*) and 16 Deoni (*Bos indicus*) were enrolled at 35 ± 5 days before expected calving. All animals were in their 3rd to 4th parity with average body weights 476.69±16.78 kg in HFX and 350.06±8.55 kg in Deoni. Animals were individually housed in ventilated 6 × 6 ft calving pens with evaporative cooling, twice-daily cleaning, daily bathing and unrestricted access to clean drinking water.

A 2 (breed: HFX vs. Deoni) × 2 (FNMS: with or without) × 4 (energy source: control, glycerolor jaggery or maize) factorial arrangement was employed, resulting in 16 treatment cells with two animals per cell (N = 32). The basal diet was formulated to meet nutrient requirements ICAR (2013) for late-gestation cows and comprised of mixed green fodder (Para grass : Hybrid Napier : Maize at 25:25:50), finger millet straw (*Eleusine coracana*), and a commercial concentrate mixture (Karnataka Milk Federation, Bengaluru; with principal ingredients such as maize 38%, deoiled oilseed cakes, rice bran and molasses). Green fodder, straw, and concentrate were offered in 50:20:30 ratio (roughage-to-concentrate ratio 70:30). Isocaloric energy fortification at +10% of total dietary gross energy was provided as glycerol (GE: 3,994 kcal/kg), jaggery (GE: 3,860 kcal/kg), or ground maize grain (GE: 4,094 kcal/kg). Experimental feeding ran from 35 days prepartum to calving.

2.3 Functional Nutrient Multicomponent Supplement (FNMS) Composition

The FNS was a precision blend formulated to supplement micronutrients after accounting for dietary supply relative to target requirements (ICAR, 2013). The composition of FNMS used in the present study consisted of copper (Cu) at 20 mg/kg DM, zinc (Zn) at 60 mg/kg DM, cobalt (Co) at 0.11 mg/kg DM, selenium (Se) at 0.3 ppm DM, iodine (I) at 0.4 mg/kg DM, chromium (Cr) at 5 mg/d, niacin at 12 g/d, and omega-3 fatty acids at 40 g/d. Omega-3 fatty acids were supplied through whole flaxseed (*Linum usitatissimum*; 35.6% ether extract, 53% of total fatty acids as alpha-linolenic acid). The FNMS was offered once daily at 08:00 AM mixed with the concentrate mixture.

2.4 Measurements and Analytical Methods

Proximate composition of dietary ingredients and refusals was determined using AOAC (2000) procedures. Cell wall constituents (NDF, ADF, ADL, hemicellulose, cellulose) were analysed per Van Soest et al. (1991). Gross energy was measured using an adiabatic bomb calorimeter. Colostrum was collected aseptically at each of the first five milkings (0–4 days of post-calving). IgG concentration (mg/mL) was estimated from colostrometer-measured specific gravity using the conversion equation: IgG = 958 × SG – 969 (Mechor et al., 1992). Calf birth weight (kg) was recorded within 2 h of birth. The fetal membrane expulsion time (FMET) was measured as the duration between the time of calf birth and the time of complete expulsion of placental membranes. Milk yield was recorded daily from day 5 post-calving and aggregated to weekly totals for eight weeks and average was calculated.

2.5 Statistical Analysis

The *in vivo* data were analysed using a 2 × 2 × 4 factorial ANOVA with breed, FNMS and energy source as fixed effects. For colostrum IgG (repeated over days 0–4) and weekly milk yield (repeated over 8 weeks), a mixed model with repeated measures was fitted (PROC MIXED) using an autoregressive AR(1) covariance structure. Least-squares means ± SEM are reported; treatment means were separated by Duncan's Multiple Range Test. Statistical significance was declared at P ≤ 0.05 and tendencies at P ≤ 0.10.

Results and Discussion

3.1 Colostrum IgG Concentration

Estimated colostrum IgG concentrations across days 0–4 are presented in Fig 1. All day-0 group means (range 55.10–62.77 mg/mL) exceeded the 50 mg/mL threshold associated with adequate colostrum quality (Godden, 2008), which is encouraging from a herd management standpoint. The overall day-0 mean was 58.76 mg/mL. *Error bars omitted for clarity, Broken lines indicate*

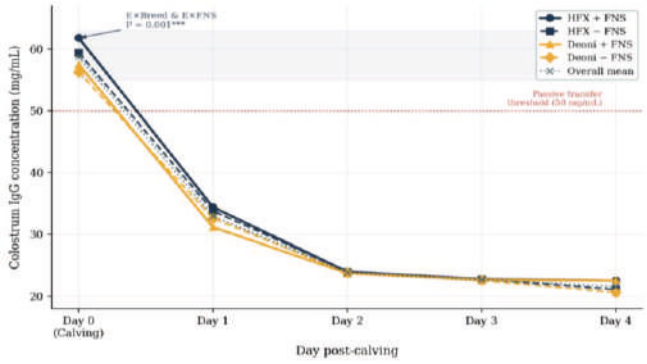

Figure 1. Estimated colostrum IgG concentration (mg/mL) across days 0–4 day post-calving by breed, energy and FNMS supplementation

without FNS and solid lines indicate with FNS. Dotted grey line is overall mean. Values are least-squares means from colostrometer-derived SG conversion. All day-0 group means exceeded the 50 mg/mL indicative threshold for adequate colostrum quality (broken red line). Treatment effects were confined to day 0; no significant differences were detected from days 1 to 4.

A significant (P = 0.001) Energy × Breed interaction was observed on day 0: HFX cows averaged 60.61 mg/mL as compared to 56.90 mg/mL in Deoni cows. The Energy × FNMS interaction was equally significant (P = 0.001), with FNMS groups averaging 59.66 mg/mL versus 57.86 mg/mL without supplementation. The highest (P=0.087) group mean was recorded in FNMS-supplemented HFX cows (61.81 mg/mL) and the lowest in unsupplemented Deoni cows (56.30 mg/mL). The three-way E × B × S interaction was non-significant (P = 0.310), suggesting that the FNMS benefit on day-0 IgG was consistent across energy substrates within the same breed.

The 3% improvement in day-0 IgG associated with FNMS is biologically plausible though more modest than the 18% gain reported by Aragona et al. (2016) using 48 g/d nicotinic acid. The FNMS in the present study incorporated only 12 g niacin/d, which may explain the attenuated response if dose-dependent GPR109A receptor activation is the primary mechanism. Selenium (0.3 ppm) and long-chain omega-3 fatty acids likely contributed via complementary antioxidant (selenoprotein activity) and anti-inflammatory (eicosanoid modulation) pathways that support lymphocyte function during colostrogenesis. Hough et al. (1990) and Westland et al. (2017) also reported similarly modest but directionally consistent improvements in colostrum IgG with

nutritional supplementation. The colostrum quality on day 0 measured as specific gravity in either HFX or Deoni cows was in agreement with Vaz et al. (2004), who reported total immunoglobulin levels >50 mg/dL in HF cows.From day 1 onwards, IgG concentrations diminishes sharply across all groups (day-1 mean: 32.98 mg/mL, a 44% decline) and stabilised between 22 and 24 mg/mL through day 4. No treatment differences were detectable on days 1–4 (P > 0.107 for all terms), consistent with the established biology of mammary tight-junction closure at parturition which terminates immunoglobulin transport into milk regardless of circulating IgG levels (McGrath et al., 2016). The quality of colostrum decreased by approximately 50% on day 1 itself and by day 4 was only one-third of the day-0 value, which is in agreement with Puppel et al. (2019).

3.2 Calf Birth Weight

Calf birth weight data are presented in Table 2. HFX calves were on average 10 kg or 49% heavier (P<0.001) at birth than Deoni calves (30.16 vs. 20.29 kg;), reflecting the well-established direct proportionality between dam body size and offspring birth weight (Paputungan and Makarechian, 2000). The metabolic BW of HFX calves was approximately 26% greater, mirroring the 20% difference in metabolic BW between HFX and Deoni dams. These values are consistent with birth weight differentials of 8–12 kg between crossbred and indigenous calves reported by Singh and Srinivas (2020) for peninsular India.

Table 2. Calf birth weight (kg) and metabolic BW (kg0.75) by energy source, breed, and FNS supplementation

	Control	Glycerol	Jaggery	Maize	P-value
Calf BW (kg)					
Breed					
HFX	27.95	32.19	29.48	31.03	0.001***
Deoni	19.93	19.48	21.37	20.38	
FNMS					
+	24.28	27.91	26.11	26.86	0.001***
–	23.60	23.75	24.74	24.54	
HFX × FNMS					
+	29.75	35.13	30.28	32.13	0.027*
–	26.15	29.25	28.69	29.93	
Deoni × FNMS					
+	18.80	20.70	21.95	21.60	NS
–	21.05	18.25	20.79	19.16	
(E×B×S)					0.049*

*SEM: 0.685 (BW) and 0.227 (MBW). ***P ≤ 0.001; *P ≤ 0.05; NS = not significant.*

Table 3. Effect of energy source, breed and FNMS supplementation on Fetal membrane expulsion time (min)

	Control	Glycerol	Jaggery	Maize	P-value
FMET (min)					
Breed					
HFX	206	211	194	191	0.001***
Deoni	175	154	159	163	
FNMS					
+	188	176	174	173	0.08†
–	194	189	179	181	
HFX × FNMS					
+	195	210	188	188	NS
–	218	213	200	195	
Deoni × FNMS					
+	180	143	160	158	NS
–	170	165	158	168	
(E×B×S)					0.099†

****P ≤ 0.001; †P ≤ 0.10 (tendency); NS = Not significant (P > 0.05).*
FMET = Fetal Membrane Expulsion Time. E×B×S = Energy × Breed × Supplement interaction.
No animal in any group experienced retained placenta (≥ 720 min threshold).

The FNMS supplementation was associated with an overall 8% improvement in calf birth weight (24.16 vs. 26.29 kg without and with FNMS, respectively; P < 0.001). The response was larger in HFX dams (12%; 28.50 vs. 31.82 kg) than in Deoni dams (5%; 19.81 vs. 20.76 kg). The three-way E × B × S interaction on birth weight reached significance (P = 0.049), driven primarily by calves born to dams receiving maize + FNMS being heavier than control calves. The approximate 2 kg absolute improvement in calf birth weight with FNMS (3 kg in HFX, 2 kg in Deoni) is biologically consistent with the functional roles of selenium in placental angiogenesis and cell proliferation, zinc in protein synthesis during the period of maximum fetal growth and omega-3 fatty acids in uteroplacental blood flow (Mee et al., 1995; ICAR, 2013). It is noteworthy that the last trimester accounts for more than 70% of calf birth weight, with approximately 30% accrued in the final month (ICAR, 2013), making the close-up nutritional window critically important for fetal development. Khan et al. (2004) reported 2.8 kg higher birth weight in cows fed 30% more than recommended energy during the last trimester, while Loerch (1996) reported 4.2 kg heavier calves from high-grain prepartum diets, contextualising the present 1–3 kg FNMS-associated improvement.

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3.3 Fetal membrane Expulsion Time

Results for FMET revealed that Deoni cows expelled fetal membranes significantly (P < 0.001) faster than HFX cows in Table 3. (163±5.4 vs 201±6.2 min). This breed difference most likely reflects differences in uterine musculature tone, oxytocin receptor density and the lower body weight of Deoni cows, which reduces mechanical resistance during placental separation. Importantly, all observed FMET values were well below the 720-min (12-h) threshold that defines retained placenta clinically, and no animal in any group experienced placental retention, a meaningful positive finding confirming the safety of the tested regimen.

FNMS supplementation showed a borderline tendency to hasten FMET (P = 0.08), possibly mediated by the selenium component (0.3 ppm): selenium is known to support prostaglandin F2α synthesis and myometrial contractility that facilitate placental separation and expulsion (Mee et al., 1995; Allison et al., 2000). Weiss and Gonzalo (2006) reported that Se supplementation at 0.3 ppm elevated plasma antioxidants and reduced immune-suppressive oxidative stress that is known to impair uterine contractility in the periparturient period. The Energy × Breed × FNMS interaction effect on FMET was significant at the 10% level (P = 0.099), though the absolute breed × FNMS difference was only 5–10 min, which has limited clinical significance.

3.4 Early Lactation Performance

The Table 4 presents weekly milk yield across the first eight weeks of lactation. The significant aspect of the data is the breed effect HFX cows produced between three and four times as much milk as Deoni cows every week without exception (P < 0.001 throughout), reflecting the fundamental difference in genetic milk production capacity between a Holstein-influenced crossbred and an indigenous draft-cum-dairy breed (Mohanavel and Srinivas 2016). These results show that neither prepartum energy source nor FNMS produced a statistically significant effect on weekly milk yield at any time point. In HFX cows, FNMS- animals produced numerically 5–7% more milk (Fig 2) in weeks 1 and 2 (18.04 vs. 15.47 kg/week in week 1 for the control group), but this difference was well within chance variation given the sample size (P = 0.64) (Fig 2). In Deoni cows, FNMS made no discernible difference at any point. The absence of a sustained milk yield response to prepartum FNS supplementation is consistent with DeFrain et al. (2004) and Osborne et al. (2009), and may simply reflect the relatively short carry-over duration of close-up nutritional interventions on lactation performance.

Table 4. Effect of energy source, breed and FNMS supplementation on early lactation (1–8 weeks) average milk yield (kg/cow/week)

Week	Breed	Control	Glycerol	Jaggery	Maize	P-value
I	HFX	15.19	14.38	14.82	14.71	0.001***
	Deoni	3.70	3.34	3.66	4.46	
II	HFX	18.29	17.50	15.96	15.91	0.001***
	Deoni	4.14	4.06	4.61	4.20	
III	HFX	18.48	16.66	16.32	16.30	0.001***
	Deoni	4.17	4.15	4.30	4.43	
IV	HFX	16.82	15.76	16.38	16.38	0.001***
	Deoni	4.52	4.05	4.51	4.53	
V	HFX	18.06	15.77	16.16	15.77	0.001***
	Deoni	4.60	4.07	4.61	4.65	
	VIHFX	18.70	16.21	15.95	15.77	0.001***
	Deoni	4.83	4.43	4.46	4.62	
VII	HFX	18.92	16.04	15.38	15.88	0.001***
	Deoni	4.88	4.56	4.19	4.56	
VIII	HFX	19.29	15.41	15.75	14.70	0.001***
	Deoni	4.66	4.50	4.49	4.56	
Avg	HFX	17.08	15.15	15.04	14.87	0.001***
	Deoni	4.22	3.93	4.14	4.28	

*SEM range: 0.885–1.252. Energy × FNMS and E × B × S interactions non-significant at all weeks (P = 0.161–0.978). ***P ≤ 0.001 for Energy × Breed at every weekly interval.*

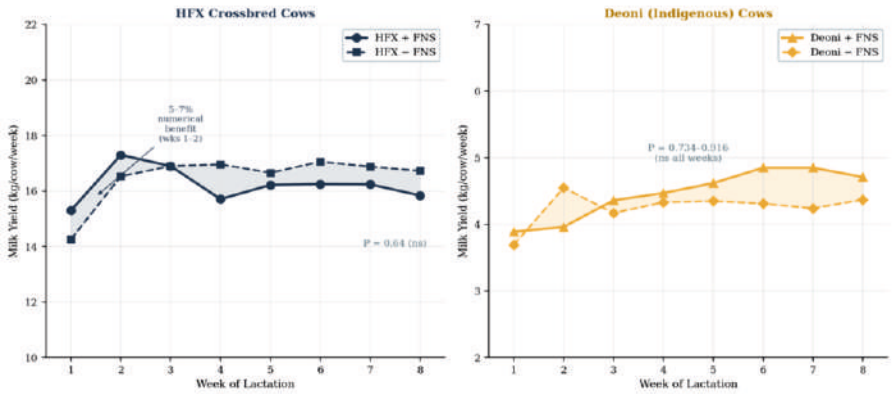

Figure 2: Early lactation weekly milk yield (weeks 1-8) with or without FNMS

Prepartum FNMS supplementation showed a non-significant (P=0.64) 5-7% advantage in HFX cows during weeks 1-2 only dissipating from week 3. Deoni cows showed no FNMS-related milk yield difference at any week. HFX produced 3.75X more than Deoni (P<0.001) throughout.

3.5 Animal Health and Welfare

There were no cases of milk fever, clinical ketosis, displaced abomasum, retained placenta, calf respiratory disease or calf mortality were recorded across all 32 dam-calf pairs through out the observation period. While the absence of metabolic disease is encouraging and confirms the effectiveness and safety of all tested supplementation levels, the clinical observation window was extended only up to 30 days of post-calving.

Conclusion

The FNMS supplementation during the close-up period significantly improved day-0 colostrum IgG in HFX cows (Energy × Breed, P = 0.001) and improved calf birth weight across both breeds (8%; P < 0.001), with a numerically larger response in HFX (12%) than Deoni (5%) dams. A borderline tendency for FNMS to hasten fetal membrane expulsion (P = 0.08) was observed, consistent with selenium-mediated enhancement of myometrial contractility. Early lactation milk yield was overwhelmingly determined by breed genetics and was not significantly influenced by prepartum nutritional interventions. The absence of metabolic disease or calf mortality across all groups confirms the safety of the tested close-up supplementation regime. These findings support the inclusion of a multicomponent FNMS in close-up prepartum diets, particularly in HF-crossbred herds under Southern Indian conditions, to improve colostrum quality and calf birth vigour. Larger, adequately powered trials with direct IgG measurement by radial immunodiffusion or ELISA and comprehensive metabolic monitoring are warranted to confirm and extend these observations.

Conflict of Interest

The authors declare no competing interests. This investigation formed part of a doctoral dissertation conducted at ICAR-NDRI, Southern Regional Station, Bengaluru. No external funding with conditions attached to outcomes was received.

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References

Allison, R. D. and Laven, R. A. 2000. Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. Veterinary Record. 147: 703–708.

AOAC. 2000. Official Methods of Analysis. 17th edn. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Aragona, K. M., Poindexter, M. B., Kweh, M. F., Zimpel, R., Zenobi, M. G., Nelson, C. D. and Staples, C. R. 2016. Prepartum nicotinic acid supplementation modifies immune function and impacts transition cow health and production. Journal of Dairy Science. 99(5): 3529-3538.

Barrington, G. M. and Parish, S. M. 2001. Bovine neonatal immunology. Veterinary Clinics of North America: Food Animal Practice. 17: 463–476.

Bertoni, G., Trevisi, E. and Lombardelli, R. 2006. Some new aspects of nutrition, health conditions and fertility of cows in the transition period. Italian Journal of Animal Science. 5: 213–240.

Blötz, C. and Stülke, J. 2017. Glycerol metabolism and its implication in virulence in Mycoplasma. FEMS Microbiology Reviews. 41: 640–652.

DeFrain, J. M., Hippen, A. R., Kalscheur, K. F. and Jardon, P. W. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. Journal of Dairy Science. 87: 4195–4206.

Drackley, J. K., Dann, H. M., Douglas, G. N., Janovick Guretzky, N. A., Litherland, N. B., Underwood, J. P. and Loor, J. J. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science. 4: 323–344.

Godden, S. 2008. Colostrum management for dairy calves. Veterinary Clinics of North America: Food Animal Practice. 24: 19–39.

Hare, K. S., Fischer-Tlustos, A. J., Wood, K. M., Cant, J. P. and Steele, M. A. 2023. Prepartum nutrient intake and colostrum yield and composition in ruminants. Animal Frontiers. 13: 24–36.

Hough, R. L., McCarthy, F. D., Kent, H. D., Eversole, D. E. and Wahlberg, M. L. 1990. Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. Journal of Animal Science. 68: 2622–2627.

ICAR. 2013. Nutrient Requirements of Animals: Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi.

Khan, R. U., Khan, S. and Chand, N. 2004. Effect of increasing energy levels during the last trimester of gestation on calf birth weight and early growth in beef cows. Pakistan Journal of Biological Sciences. 7: 1701–1703.

Loerch, S. C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. Journal of Animal Science. 74: 1211–1216.

McGrath, B. A., Fox, P. F., McSweeney, P. L. H. and Kelly, A. L. 2016. Composition and properties of bovine colostrum: a review. Dairy Science & Technology. 96: 133–158.

McLeod, K. R. and Baldwin, R. L. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. Journal of Animal Science. 78: 760–770.

Mechor, G. D., Gröhn, Y. T. and Van Saun, R. J. 1992. Effect of temperature on colostrometer readings for estimation of immunoglobulin concentration in bovine colostrum. Journal of Dairy Science. 75: 3461–3465.

Mee, J. F., Rogers, P. A. M. and Murphy, J. J. 1995. Effect of pre-partum administration of a freeze-dried selenium/vitamin E preparation on the retention of placenta and fertility in dairy cattle. Veterinary Record. 136: 241–244.

Moallem, U., Zachut, M., Yakoby, S., Gabai, G. and Zeron, Y. 2013. Increased milk production and energy balance in high-producing dairy cows supplemented with calcium salts of polyunsaturated fatty acids during the transition period. Journal of Dairy Science. 96: 1479–1490.

Mohanavel, K. S. and Srinivas, B. 2016. Comparative performance of crossbred and indigenous cattle for lactation traits. Indian Journal of Animal Sciences. 86: 1091–1094.

Osborne, V. R., Odongo, N. E., Cant, J. P., Swanson, K. C. and McBride, B. W. 2009. Evidence that propylene glycol and glycerol are equipotent precursors of glucose for postabsorptive cattle. Journal of Dairy Science. 92: 1399–1405.

Paputungan, U. and Makarechian, M. 2000. Influence of dam body weight and body condition on calf birth weight, dystocia and calf survival in beef cattle. Asian-Australasian Journal of Animal Sciences. 13: 1083–1087.

Puppel, K., Golebiewski, M., Grodkowski, G., Slósarz, J., Kunowska-Slosarz, M., Solarczyk, P., Kuczyńska, B., Budzińska, M. and Przysucha, T. 2019. Composition and factors affecting quality of bovine colostrum: a review. Animals. 9(12): 1070.

Singh, N. and Srinivas, B. 2020. Birth weight and early growth of calves in Indian livestock. Indian Journal of Dairy Science. 73: 114–119.

Trubenbach, L. A., Wickersham, T. A., Rodgers, J. C., Liao, S. F., Santos, J. E. P. and Loor, J. J. 2019. Effects of prepartum dietary energy intake on the hepatic transcriptome of Holstein cows during the periparturient period. Journal of Dairy Science. 102: 742–756.

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

Vaz, A. K., Roesch, M., Perreten, V., Blanco Cachao, P., Peel, J. E. and Peel, E. 2004. Variations in bovine colostralimmunoglobulins in relation to serum levels and their measurement. Revue de Médecine Vétérinaire. 155: 275–279.

Weiss, W. P. and Gonzalo, F. A. 2006. Vitamin and mineral nutrition for ruminants. Proceedings of the Mid-South Ruminant Nutrition Conference, Arlington, TX. 57–68.

Westhoff, T. A., Borchardt, S. and Mann, S. 2024. Invited review: Nutritional and management factors that influence colostrum production and composition in dairy cows. Journal of Dairy Science. 107: 4109–4128.

Westland, A., Martin, R., White, R., & Martin, J. H. 2017. Mannan oligosaccharide prepartum supplementation: effects on dairy cow colostrum quality and quantity. Animal, 11(10), 1779–1782.

Research Article

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Yeast fermented *Azolla pinnata* meal in diets of silver barb *Barbonymus gonionotus*: Growth performance, protein digestibility and muscle composition under mid-hill conditions of Meghalaya

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A 45-day feeding trial in 1000-litre cemented tanks under mid-hill conditions of Meghalaya (mean water temperature 20.8±0.9°C) evaluated the effect of graded replacement of unfermented *Azolla pinnata* meal (UAM) with yeast fermented *Azolla pinnata* meal (FAM) on growth performance, survival, condition factor, apparent protein digestibility and muscle proximate composition of juvenile silver barb (*Barbonymus gonionotus*). Five isonitrogenous diets with fish meal (23%), mustard oil cake (25%) and rice bran (30%) fixed identically were formulated with 0% (T1), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) FAM replacement of the 20% UAM component. Fish (initial weight 12.21 ± 1.31 g, 20/ tank, triplicate) were sampled on days 0, 7, 15, 30 and 45. Specific growth rate improved progressively from T1 (0.84 ± 0.07 % day^{-1}) to T4 (1.16 ±0.07 % day^{-1}), feed conversion ratio from 2.61 ± 0.21 to 2.08±0.14, and apparent protein digestibility from 72.14 ± 1.84% to 83.47 ± 1.62%, with T4 and T5 statistically comparable in all parameters (P > 0.05). Survival, condition factor and muscle crude protein followed the same trend. Results suggest yeast fermentation reduces antinutritional factors and improves protein digestibility of azolla, with 75% FAM replacement offering a practical optimum for silver barb under hill aquaculture conditions.

Keywords : Fermented azolla, *Barbonymus gonionotus,* Growth performance, Protein digestibility, Hill aquaculture

Introduction

Silver barb (*Barbonymus gonionotus* Bleeker, 1850) is a widely cultivated medium-sized carp in South and Southeast Asia, valued for its rapid growth, adaptability to plant-based diets and tolerance of variable environmental conditions (Jena et al., 2007). In the hill climate of Meghalaya, the species has shown considerable aquaculture potential, with ICAR-NEH Region trials recording satisfactory growth in pond and cage systems (Singh et al., 2024) and a polyculture yield of 1,350 kg/ha over six months, where *B. gonionotus* attained 189.12 g final weight with 81.25% survival (Das and Mahanta, 2025). In eastern India and Bangladesh, the species is commonly reared on farm-made feeds using rice bran, mustard oil cake and fish meal (Mondal et al., 2019; Mazid et al., 1997), and rising ingredient costs have generated interest in locally available, low-cost protein supplements that can be incorporated without compromising fish performance (Jahan et al., 2021).

Azolla pinnata, a free-floating aquatic pteridophyte growing abundantly in the paddy fields of Northeast India, offers considerable potential in this context. Through symbiosis with *Anabaena azollae*, it accumulates biomass rapidly and contains 22 to 29% crude protein on a dry matter basis, along with essential amino acids, minerals and vitamins (Swain et al., 2022; Datta et al., 2011). Dried *A. pinnata* can substitute up to 50% of mustard oil cake in silver barb fry diets without significant growth depression, with an optimal dietary inclusion of 47.8% established through regression analysis (Debnath, 2025). A recognised limitation, however, is the presence of antinutritional factors (ANFs), particularly tannins and phytic acid, which inhibit digestive enzyme activity and impair protein digestibility when unfermented azolla is included beyond the tolerance threshold of the target species (Bhadake et al., 2024; Mpwaga et al., 2023; Francis et al., 2001).

Yeast solid-state fermentation (SSF) offers a practical means of addressing this constraint by generating extracellular proteases, cellulases and phytases that degrade ANFs and improve protein solubility (Siddik et al., 2024; Agboola et al., 2021). Fermentation has improved growth and protein digestibility in rohu fed plant-based diets (Das et al., 2021), and growth benefits of fermented over unfermented azolla have been demonstrated in tilapia (Ismail et al., 2022; Refaey et al., 2023), yet no comparable study exists for *B. gonionotus* under hill aquaculture conditions. We hypothesised that progressive replacement of unfermented azolla meal with yeast fermented azolla meal would reduce dietary ANF load in a dose-dependent manner, thereby improving protein digestibility, growth performance and muscle composition of juvenile *B. gonionotus*. The present study therefore evaluated graded replacement at 0%, 25%, 50%, 75% and 100% within a fixed 20% dietary azolla inclusion under mid-hill conditions of Meghalaya.

Materials and methods

Study location and fish

The trial was conducted at Umiam, Meghalaya, northeastern India. A 45-day trial duration was considered adequate for preliminary screening of dietary treatments under controlled tank conditions, consistent with similar short-duration growth evaluations in *B. gonionotus* (Jahan et al., 2020) and *azolla*-based cyprinid feeding studies (Naji and Alzurfi, 2023). Juvenile fish with a mean initial body weight of 12.21 ± 1.31 g were obtained from ICAR farm and acclimatised for 7 days on a commercial diet (25 % CP) before the trial commenced.

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Cultivation and preparation of azolla meal

Azolla pinnata was collected from local paddy fields in Meghalaya and identified on the basis of its distinctly triangular pinnate plant form, green non-reddish colouration, acute leaf apices and 2 to 3 roots per node (Kumar et al., 2018; Lellinger et al., 1983). Authenticated material was subsequently cultivated in outdoor concrete beds fertilized weekly with diluted cow dung slurry and superphosphate. Harvested biomass was washed, shade-dried for 48 h and 60°C for 24 hrs. One portion was retained as UAM after grinding to 0.5 mm. For FAM, dried azolla powder was rewetted to 50 to 55% moisture, inoculated with fresh baker's yeast (*Saccharomyces cerevisiae*, procured from a local grocery shop, Barapani, Meghalaya) at 5 g/kg dry substrate (Manoppo and Kolopita, 2016), mixed uniformly and incubated under aerobic solid-state conditions at 30 ± 1°C for 48 hours following the SSF protocol of Siddik et al. (2024). The fermented material was re-dried at 60°C for 24 hrs, reground to 0.5 mm and stored at room temperature until use. Substrate pH was measured before inoculation and after 48 hours of incubation using a calibrated digital pH meter on a 1:5 substrate-to-distilled-water suspension, declining from 6.42 ± 0.11 to 4.87 ± 0.09. Organoleptic characteristics of UAM and FAM were recorded: UAM presented a dull olive-green colour, grassy odour and fibrous texture, whereas FAM showed a darker brownish hue, yeasty and mildly acidic odour, and more friable texture. Both UAM and FAM were assessed for proximate composition (AOAC, 2005), tannin content by the vanillin-HCl colorimetric method (Price et al., 1978) and phytic acid by the Wade reagent colorimetric method (Haug and Lantzsch, 1983) prior to diet preparation (Table 1).

Table 1. Proximate composition and anti-nutritional factor content of unfermented (UAM) and yeast fermented (FAM) *Azolla pinnata* meal (dry matter basis)

Parameter	UAM	FAM	% Change
Crude protein (%)	23.14 ± 0.42	27.86 ± 0.51	+20.39
Crude fat (%)	3.81 ± 0.24	4.12 ± 0.29	+8.14
Crude fibre (%)	15.24 ± 0.38	11.37 ± 0.31	−25.39
Total ash (%)	10.42 ± 0.31	9.84 ± 0.27	−5.57
Tannin (mg/g DM)	8.42 ± 0.61	4.91 ± 0.43	−41.69
Phytic acid (g/kg DM)	6.18 ± 0.47	2.84 ± 0.32	−54.05
Substrate pH	6.42 ± 0.11	4.87 ± 0.09	−24.14

Note: Values are mean ± SD of triplicate analyses. % change = [(FAM − UAM) / UAM] × 100

Experimental diets

Five iso-nitrogenous diets were formulated with fish meal (23%), MOC (25%), rice bran (30%) and vitamin-mineral premix (2%) fixed and identical across all treatments. The remaining 19.5% comprised UAM and FAM in varying proportions constituting 0%, 25%, 50%, 75% and 100% FAM replacement of UAM (T1 through T5, Table 2), with 0.5% chromic oxide included as an inert digestibility marker in all diets. The estimated crude protein ranged from 29.43% in T1 to 30.40% in T5, an increment of less than 1 percentage point inherent to the replacement design since FAM carries higher CP than UAM. Analysed dietary CP values did not differ significantly among treatments ($P>0.05$); the small progressive increment in estimated CP was inherent to the replacement design and nutritionally negligible at <1% across the full replacement range.

Table 2. Ingredient composition (g per 100 g diet, dry matter basis) and estimated crude protein of experimental diets

Ingredient	T_1	T_2	T_3	T_4	T_5
Fish meal	23.00	23.00	23.00	23.00	23.00
Mustard oil cake	25.00	25.00	25.00	25.00	25.00
Rice bran	30.00	30.00	30.00	30.00	30.00
UAM	19.50	14.63	9.75	4.88	0.00
FAM	0.00	4.87	9.75	14.62	19.50
Vitamin-mineral premix	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Estimated CP (%)	29.46 ± 0.36	29.70 ± 0.36	29.93 ± 0.36	30.15 ± 0.36	30.38 ± 0.36

UAM and FAM proportions reflect 0%, 25%, 50%, 75% and 100% FAM replacement of the 20% azolla component

Experimental management and protein digestibility

Fifteen 1000-litre cemented tanks filled with dechlorinated tap water were used, with five treatments in triplicate. Each tank was stocked with 20 fish after acclimatisation. Fish were fed twice daily at 09:00 and 15:00 h at 5% body weight, adjusted every 15 days. Uneaten feed and faeces were removed by siphoning on alternate days, and 1/3rd of the water column was exchanged weekly. Mortalities were recorded daily. Water quality parameters were monitored weekly using a portable dissolved oxygen meter (Lutron PDO-519, Taiwan) and a multiparameter water quality tester (Eutech Instruments PCSTestr 35, Singapore).

For protein digestibility, faecal samples were collected from each tank on days 30 and 45 by siphoning following the last daily feeding, centrifuged, freeze-dried and stored at -4°C. Chromic oxide in diets and faeces was determined by wet ash digestion spectrophotometry. Apparent digestibility coefficient for crude protein (ADC-CP) was calculated as:

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ADC-CP (%) = 100 × [1 – (Cr_2O_3 in diet / Cr_2O_3in faeces) × (CP in faeces / CP in diet)]

Sampling and growth indices

Fish were individually weighed and measured on days 0, 7, 15, 30 and 45. Weight gain (WG), percentage weight gain (% WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF = W/L^3 × 100) and survival were calculated by standard formulae. At day 45, five fish per tank (15 fish /treatment) were killed by immersion in ice slurry and dorsal muscle tissue was dissected and pooled at the tank level. Pooled samples from each tank (n = 3/ treatment) were analysed separately for crude protein, crude fat, moisture and total ash by AOAC (2005), so that the tank remained the experimental unit for muscle proximate composition.

Statistical analysis

Intermediate sampling data collected on days 7, 15 and 30 were used solely for feed ration adjustment and welfare monitoring and were not subjected to statistical analysis. Terminal day-45 data for all growth indices, feed utilisation parameters, condition factor, survival and ADC-CP were subjected to one-way ANOVA and treatment means compared by Duncan's multiple range test (DMRT) at P ≤ 0.05 using SPSS version 21.0. Values are expressed as mean ± SD of triplicate groups.

Results

Water quality

Water quality remained stable across all tanks throughout the trial: temperature 20.8 ± 0.9°C, dissolved oxygen 7.4 ± 0.5 mg/L, pH 7.1 ±0.3 and total ammonia nitrogen 0.02 ± 0.01 mg/L. Parameters did not differ significantly among tanks.

Proximate composition and ANF content of UAM and FAM

Yeast SSF altered the nutritional and antinutritional profile of *A. pinnata* meal (Table 1). Crude protein was higher in FAM than in UAM (27.86 vs. 23.14%), while crude fibre (11.37 vs. 15.24%), total ash (9.84 vs. 10.42%) and crude fat (4.12 vs. 3.81%) differed marginally between the two meals. Tannin concentration declined from 8.42 mg/g in UAM to 4.91 mg/g in FAM, a reduction of approximately 42%. Phytic acid decreased from 6.18 g/kg in UAM to 2.84 g/kg in FAM, representing a reduction of approximately 54%. Substrate pH declined from 6.42±0.11 before inoculation to 4.87± 0.09 after 48 hr. of SSF, and sensory assessment recorded a transition from grassy odour and fibrous texture of UAM to a yeasty, mildly acidic odour and more friable texture in FAM, providing qualitative confirmation of fermentation. Estimated dietary CP ranged from 29.46% in T1 to 30.38% in T5, with analysed values not differing significantly among treatments (P > 0.05).

Growth performance, survival and condition factor

Growth parameters improved progressively from T1 to T4, with T4 and T5 statistically comparable in most indices (P>0.05) (Table 3). Fish in T1 recorded the lowest final weight (17.84 ± 1.24 g), WG (5.63 ± 0.74 g), SGR (0.84 ± 0.07 % day^{-1}) and PER (1.31 ± 0.08), and the highest FCR (2.61 ± 0.21), all significantly inferior to T3, T4 and T5 (P<0.05). Fish in T4 achieved the highest SGR (1.16 ± 0.07 % day^{-1}), best FCR (2.08 ± 0.14), highest PER (1.57 ± 0.08) and condition factor (2.11 ± 0.09), with T5 recording comparable values (P>0.05). Survival ranged from 86.67 ± 2.89% in T1 to 93.33 ± 2.31% in T4.

Table 3. Growth performance, feed utilisation, survival, condition factor and apparent protein digestibility of *B. gonionotus* at day 45 (mean ± SD, n = 3 tanks)

Parameter	T_1 (0% FAM)	T_2 (25% FAM)	T_3 (50% FAM)	T_4 (75% FAM)	T_5 (100% FAM)
Initial weight (g)	12.22 ± 1.29^a	12.18 ± 1.34^a	12.24 ± 1.27^a	12.19 ± 1.33^a	12.21 ± 1.31^a
Final weight (g)	17.84 ± 1.24^c	18.62 ± 1.18^{bc}	19.47 ± 1.09^b	20.38 ± 1.14^a	20.21 ± 1.21^a
WG (g)	5.63 ± 0.74^c	6.41 ± 0.68^{bc}	7.26 ± 0.82^b	8.17 ± 0.79^a	8.00 ± 0.84^a
% WG	46.11 ± 5.83^c	52.54 ± 4.97^{bc}	59.52 ± 6.14^b	66.97 ± 6.42^a	65.57 ± 6.89^a
SGR (%/ day)	0.84 ± 0.07^c	0.96 ± 0.06^{bc}	1.07 ± 0.08^b	1.16 ± 0.07^a	1.14 ± 0.08^a
FCR	2.61 ± 0.21^a	2.43 ± 0.18^{ab}	2.24 ± 0.16^{bc}	2.08 ± 0.14^c	2.11 ± 0.17^c
PER	1.31 ± 0.08^c	1.38 ± 0.09^{bc}	1.46 ± 0.10^b	1.57 ± 0.08^a	1.54 ± 0.09^{ab}
Condition factor	1.87 ± 0.07^c	1.94 ± 0.08^{bc}	2.02 ± 0.07^b	2.11 ± 0.09^a	2.09 ± 0.08^a
Survival (%)	86.67 ± 2.89^b	88.33 ± 3.21^{ab}	90.00 ± 2.00^{ab}	93.33 ± 2.31^a	91.67 ± 2.89^a
ADC-CP (%)	72.14 ± 1.84^c	75.83 ± 1.47^{bc}	79.41 ± 1.63^b	83.47 ± 1.62^a	82.94 ± 1.71^a

Means in each row with different superscripts are significantly different (P<0.05)

Muscle proximate composition

Muscle crude protein and crude fat increased progressively from T1 to T4, with T4 and T5 recording significantly higher values than T1 and T2 (P < 0.05). Moisture declined correspondingly. Total ash did not differ significantly among treatments (Table 4).

Table 4. Muscle proximate composition (% wet weight) of *B. gonionotus* at day 45 (mean ± SD, n = 15 fish per treatment)

Parameter	T_1	T_2	T_3	T_4	T_5
Moisture (%)	73.42 ± 0.71^a	72.81 ± 0.68^{ab}	72.14 ± 0.74^{ab}	71.38 ± 0.62^b	71.62 ± 0.69^b
Crude protein (%)	14.87 ± 0.48^c	15.44 ± 0.42^{bc}	16.12 ± 0.39^b	16.91 ± 0.44^a	16.72 ± 0.41^{ab}
Crude fat (%)	4.61 ± 0.29^c	4.89 ± 0.27^{bc}	5.18 ± 0.31^{ab}	5.51 ± 0.28^a	5.39 ± 0.33^a
Total ash (%)	3.08 ± 0.18^a	3.11 ± 0.21^a	3.14 ± 0.19^a	3.17 ± 0.22^a	3.15 ± 0.20^a

Means in each row with different superscripts are significantly different (P<0.05)

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Discussion

The trial was conducted at a mean water temperature of 20.8 ± 0.9°C, characteristic of mid-hill conditions of Meghalaya. The SGR values recorded across treatments (0.84 to 1.16 % day^{-1}) are broadly consistent with growth performance documented for *B. gonionotus* under mid-hill pond conditions at Umiam (Das and Mahanta, 2025) and within the range reported for the same species in earthen ponds in Bangladesh (Mollah et al., 2011), though direct comparison remains limited by differences in fish size, culture system and diet composition. The small but progressive increment in estimated dietary CP from T1 (29.46 %) to T5 (30.38 %) was inherent to the replacement design and nutritionally negligible at less than 1 percentage point across the full replacement range, and growth and digestibility responses are therefore more plausibly attributed to progressive reduction in dietary ANF load than to differences in protein level per se. The relatively elevated dissolved oxygen (7.4 ± 0.5 mg/L) may have partially supported aerobic metabolic scope across all treatments, as dissolved oxygen availability has been shown to significantly influence oxygen consumption rate and aerobic metabolism in cyprinid fishes (Zhang et al., 2012).

The quality assessment of UAM and FAM (Table 1) confirmed that yeast SSF reduced the ANF content of *azolla*, with tannin declining by approximately 42 % and phytic acid by 54 % in FAM relative to UAM. Cruz Velasquez et al. (2011) reported comparable reductions in tannins and phytates in fermented *Azolla* species, with post-fermentation values falling below the critical dietary thresholds for cultured fish as described by Francis et al. (2001). The phytic acid content of UAM (6.18 g/kg) exceeded the 5 g/kg threshold identified by Francis et al. (2001) above which phytate impairs protein digestibility and mineral absorption in fish, while that of FAM (2.84 g/kg) fell below it. Tannins in UAM (8.42 mg/g) also exceeded the levels at which they inhibit digestive enzyme activity in fish (Francis et al., 2001), whereas FAM (4.91 mg/g) showed a meaningful reduction, though whether this reduction fully eliminates the inhibitory effect in *B. gonionotus* specifically cannot be confirmed from the present data alone. Mpwaga et al. (2023) noted that tannins and phytic acid in azolla are the primary contributors to its limited digestibility in fish at high inclusion levels.

The progressive improvement in ADC-CP from T1 (72.14 %) to T4 (83.47 %) is a central finding of this study and is consistent with the mechanistic explanation that increased FAM proportion progressively reduced the dietary ANF load, improving the accessibility of protein fractions to intestinal digestive enzymes. Siddik et al. (2024) reviewed that SSF improves protein digestibility through degradation of protein-ANF complexes and partial pre-hydrolysis of proteins by microbial enzymes. Das et al. (2021) demonstrated improved protein digestibility in rohu (*L. rohita*) fed yeast SSF plant-based diets compared to unfermented controls. Feng et al. (2024) similarly noted that yeast SSF of plant proteins improves *in vitro* digestibility through reduction of trypsin inhibitors and tannins. The comparable ADC-CP of T4 and T5 ($P \geq 0.05$) suggests that the digestibility benefit of fermentation approaches a plateau between 75 and 100% FAM replacement at this inclusion level, though whether this plateau holds at higher azolla inclusion levels or different fish sizes would require further investigation.

The progressive improvement in WG, SGR and PER from T1 to T4 is consistent with the improvement in ADC-CP, as higher protein digestibility would be expected to increase the proportion of dietary amino acids available for anabolism, supporting better growth and tissue deposition. Das et al. (2018) reported declining SGR in *B. gonionotus* fed increasing proportions of unfermented azolla, attributed to ANF-mediated impairment of digestion. Cruz Velásquez et al. (2015) reported improved FCR in tilapia fed fermented aquatic macrophytes compared to unfermented forms, and Jahan et al. (2021) recorded better FCR and PER in *B. gonionotus* fed fermented soybean meal over untreated meal. The present findings are directionally consistent with these studies, though the specific magnitude of response may differ across species, fermentation agents and rearing conditions. The statistical equivalence of T4 and T5 in most growth and digestibility parameters suggests that 75% FAM replacement captures most of the growth benefit achievable at this level, which may have practical relevance for reducing fermentation effort, though this inference should be tested across a wider range of conditions before practical guidance is drawn. The progressive improvement in FCR may also partly reflect enhanced diet palatability, as yeast SSF generates free amino acids and volatile organic acids recognised as feeding stimulants in cyprinid fish (Siddik et al., 2024; Agboola et al., 2021), though a formal feed preference trial was not conducted.

The improvement in condition factor from T1 (1.87) to T4 (2.11) indicates relatively better body robustness in higher FAM groups, consistent with improved nutrient retention (Farhad et al., 2023). The corresponding improvement in muscle crude protein and fat, with lower moisture in T4 compared to T1, supports the interpretation that better protein digestibility in higher FAM treatments translated into improved tissue synthesis. Similar trends in muscle or whole-body composition with fermented plant protein diets in cyprinids have been reported by Jahan et al. (2021) and Magouz et al. (2020), though direct comparison with these studies is limited by differences in species, diet composition and rearing conditions.

The lower survival in T1 (86.67%) compared to T4 (93.33%) may reflect the cumulative effect of a higher dietary ANF load over the 45-day period. Fiogbe et al. (2004) observed reduced survival in tilapia fed high-level unprocessed azolla, and Mpwaga et al. (2023) noted that prolonged ANF exposure may progressively impair antioxidant defence in fish. However, since water quality was maintained within acceptable ranges and no overt disease signs were recorded, differential mortality cannot be confirmed from the present data and should be interpreted cautiously. The yeast inoculation rate of 5 g/kg dry substrate and 48-hr incubation at 30°C represent practically feasible conditions for on-farm SSF in Meghalaya, though fermentation efficiency may vary with ambient temperature across seasons. On-farm yeast fermentation of locally cultivated *azolla* appears a potentially useful feed strategy for silver barb in Northeast Indian hill regions, and feed management strategies adapted to local conditions have been shown to substantially improve pond productivity in NE India (Debnath, 2025).

Limitations and future recommendations

As this study was limited to a 45-day cemented tank trial under a single seasonal thermal condition and did not include an economic analysis, the findings should be considered preliminary. The duration was considered adequate to detect treatment-level differences in growth and digestibility indices under controlled conditions, but is insufficient to support broad practical feeding recommendations without further validation under pond conditions across seasons and fish size classes. Faecal collection by siphoning may have resulted in some degree of nutrient leaching prior to recovery, potentially introducing a modest upward bias in the estimated ADC-CP values; future studies should consider stripping or settlement-column collection methods to minimise this source of error. The fermentation process was characterised through proximate, ANF, pH and qualitative sensory analyses;

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microbial enumeration, enzyme activity profiling and instrumental colour measurement were not conducted, and a formal feed palatability trial was not incorporated, which limits mechanistic interpretation of the observed responses. Future studies should evaluate graded FAM replacement under pond conditions across seasons, incorporate microbial and enzymatic characterisation of the SSF process, assess economic feasibility of on-farm yeast fermentation, and examine longer-term effects on fish health and reproductive performance at the farm level in Northeast India.

Conclusion

Yeast solid-state fermentation of *Azolla pinnata* meal reduced tannin by 42% and phytic acid by 54%, and graded replacement of unfermented azolla meal with fermented azolla meal progressively improved all measured parameters in juvenile *B. gonionotus* over 45 days under mid-hill conditions of Meghalaya. The 75% fermented azolla replacement level recorded the best SGR (1.16 ± 0.07 %/ day), ADC-CP (83.47 ± .62%), FCR (2.08 ± 0.14), survival (93.33 ± 2.31 %), condition factor (2.11 ± 0.09) and muscle crude protein (16.91 ± 0.44 %), performing at par with 100% fermented azolla. Complete unfermented azolla produced the poorest results across all parameters.

References

Agboola, J. O., Øverland, M., Skrede, A. and Hansen, J. Ø. 2021. Yeast as major protein-rich ingredient in aquafeeds: A review of the implications for aquaculture production. Reviews in Aquaculture. 13(2): 949–970.

AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC.

Bhadake, P. P., Mote, M. G., Gaikwad, U. S. and Tanpure, M. U. 2024. Effect of dietary supplementation of *Azolla* meal on feed intake and digestibility in Sangamneri kids. Indian Journal of Animal Nutrition. 41(2): 236-241.

Cruz Velasquez, Y., Kijora, C., Wedler, E., Danier, J. and Schulz, C. 2011. Fermentation properties and nutritional quality of selected aquatic macrophytes as alternative fish feed in rural areas of the Neotropics. Livestock Research for Rural Development. 23: 239–246.

Cruz Velásquez, Y., Kijora, C., Wuertz, S. and Schulz, C. 2015. Effect of fermented aquatic macrophytes supplementation on growth performance, feed efficiency and digestibility of Nile tilapia (*Oreochromis niloticus*) juveniles fed low fishmeal diets. Livestock Research for Rural Development. 27: Article 177: 1-8.

Das, K. C., Mohanty, S., Sahoo, P. K., Das, R., Sahoo, L. and Swain, P. 2021. Effect of solid-state fermented aquafeed on growth performance, digestive enzymes and innate immunity of rohu (*Labeo rohita*). Agricultural Science Digest. 41(3): 484–491.

Das, M., Rahim, F. I. and Hossain, M. A. 2018. Evaluation of fresh *Azolla pinnata* as a low-cost supplemental feed for Thai silver barb *Barbonymus gonionotus*. Fishes. 3(1): 15 : 1-11.

Das, S. K. and Mahanta, P. 2025. Short term polyculture of three cyprinid species under mid-hill conditions in Meghalaya, North-east India. Indian Journal of Fisheries. 72(1): 147–149.

Datta, S. N. 2011. Culture of azolla and its efficacy in diet of *Labeo rohita*. Aquaculture. 310(3-4): 376–379.

Debnath, C. 2025. Comparative assessment of feeding regimens on the performance of composite fish culture in tribal villages of Tripura. Indian Journal of Animal Nutrition. 42(3): 384-391.

Debnath, C. 2025. Evaluation of *Azolla pinnata* as a protein source in the diet of silver barb (*Barbonymus gonionotus*) during fry-to-fingerling rearing under hill conditions of Meghalaya. Animal Nutrition and Feed Technology. 25: 235–245.

Farhad, F. B., Hashem, S., Rana, K. M. S. and Salam, M. A. 2023. Growth performance and hematological responses of silver barb (*Barbonymus gonionotus* Bleeker, 1850) fingerlings to dietary blanched moringa (*Moringa oleifera* Lam.) leaf meal as a substitute of soybean meal. Heliyon. 9(2): e13552.

Feng, X., Ng, K., Ajlouni, S., Zhang, P. and Fang, Z. 2024. Effect of solid-state fermentation on plant-sourced proteins: A review. Food Reviews International. 40(9): 2580–2617.

Fiogbe, E. D., Micha, J. C. and Van Hove, C. 2004. Use of a natural aquatic fern, *Azolla microphylla*, as a main component in food for the omnivorous-phytoplanktonophagous tilapia, *Oreochromis niloticus* L. Journal of Applied Ichthyology. 20(6): 517–520.

Francis, G., Makkar, H. P. S. and Becker, K. 2001. Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199(3-4): 197–227.

Hastuti, S., Yuniarti, T., Harwanto, D. and Subandiyono, S. 2024. Growth performance of Java barb (*Barbonymus gonionotus*) in relation with water temperature. AACL Bioflux. 17(3): 949–959.

Haug, W. and Lantzsch, H. J. 1983. Sensitive method for the rapid determination of phytic acid in cereals and cereals products. Journal of the Science of Food and Agriculture. 34: 1423–1426.

Ismail, T., Hegazi, E., Nassef, E., Habotta, O. A. and Gewaily, M. S. 2022. The optimized inclusion level of *Azolla pinnata* fermented with *Bacillus subtilis* in Nile tilapia (*Oreochromis niloticus*) diets: Immunity, antioxidative status, intestinal digestive enzymes and histomorphometry, and disease resistance. Fish Physiology and Biochemistry. 48(3): 767–783.

Jahan, H., Ema, N. S., Hossain, M. S., Pervin, M. A., Akter, R. and Hossain, Z. 2020. Growth performance study of silver barb (*Barbonymus gonionotus*) by replacing fishmeal with soybean meal in the diet. Asian Journal of Medical and Biological Research. 6(2): 149–154.

Jahan, H., Tumpa, I. J., Qasem, W. A., Moniruzzaman, M., Pervin, M. A., Akter, R., Omri, A., Min, T. and Hossain, Z. 2021. Evaluation of the partial replacement of dietary fish meal with fermented or untreated soybean meal in juvenile silver barb, *Barbonymus gonionotus*. Frontiers in Nutrition. 8: 733402.

Jena, J., Das, P. C., Mondal, S. and Das, R. 2007. Compatibility of silver barb (*Puntius gonionotus* Bleeker) with Indian major carps in a grow-out polyculture. Aquaculture Research. 38(10): 1061–1065.

Lellinger, D. B., Lumpkin, T. A. and Plucknett, D. L. 1983. Azolla as a green manure: Use and management in crop production. American Fern Journal. 73: 96.

Magouz, F. I., Dawood, M. A. O. and Salem, M. F. I. 2020. The effects of fish feed supplemented with *Azolla* meal on the growth performance, digestive enzyme activity, and health condition of genetically-improved farmed tilapia (*Oreochromis niloticus*). Annals of Animal Science. 20(3) : 1029-1045.

Manoppo, H. and Kolopita, M. E. F. 2016. The use of baker's yeast to promote growth of carp (*Cyprinus carpio* L.). International Journal of PharmTech Research. 9(9): 415–420.

doi.org/10.56093/ijan.v43i2.178732

Mazid, M. A., Zaher, M., Begum, N. N., Ali, M. Z. and Nahar, F. 1997. Formulation of cost-effective feeds from locally available ingredients for carp polyculture system for increased production. Aquaculture. 151(1-4): 71–78.

Mollah, M. F. A., Moniruzzaman, M. and Rahman, M. M. 2011. Effects of stocking densities on growth and survival of Thai sharpunti (*Barbonymus gonionotus*) in earthen ponds. Journal of Bangladesh Agricultural University. 9(2): 327–338.

Mondal, D., Rahman, T., Paul, S., Islam, M. J. and Miah, I. 2019. Effects of probiotic supplementation on the growth performance of Thai silver barb (*Barbonymus gonionotus* [Bleeker, 1850]) fry. Journal of the Science of Food and Agriculture. 4(1): 1.

Mpwaga, A. Y., Gyan, W. R., Yang, Q. and Asare, D. 2023. A review on the use of Azolla meal as a feed ingredient in aquatic animals' diets. Aquaculture Research. 2023(7): 1–16.

Naji, M. H. and Alzurfi, S. K. L. 2023. Possibility of using *Azolla* meal as an alternative fish feed. In: Proceedings of the 2nd International Conference of Mathematics, Applied Sciences, Information and Communication Technology. AIP Conference Proceedings. 2834: 020005-1–020005-7.

Price, M. L., Van Scoyoc, S. and Butler, L. G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry. 26(5): 1214–1218.

Refaey, M. M., Mehrim, A. I., Zenhom, O. A., Areda, H. A., Ragaza, J. A. and Hassaan, M. S. 2023. Fresh *Azolla pinnata* as a complementary feed for *Oreochromis niloticus*: Growth, digestive enzymes, intestinal morphology, physiological responses, and flesh quality. Aquaculture Nutrition. 2023: 1403704.

Siddik, M. A. B., Julien, B. B., Islam, S. M. M. and Francis, D. S. 2024. Fermentation in aquafeed processing: Achieving sustainability in feeds for global aquaculture production. Reviews in Aquaculture. 16(3): 1244–1265.

Singh, S. G., Debnath, C., Mahanta, P. and Tayung, T. 2024. Silver barb: A promising candidate for aquaculture diversification in Meghalaya hills. Indian Farming. 74(08): 03–07.

Swain, B. K., Naik, P. K. and Beura, C. K. 2022. Nutritive value of *Azolla* as poultry feed: A review. Indian Journal of Animal Nutrition. 39(1): 1-11.

Zhang, W., Cao, Z. D. and Fu, S. J. 2012. The effects of dissolved oxygen levels on the metabolic interaction between digestion and locomotion in Cyprinid fishes with different locomotive and digestive performances. Journal of Comparative Physiology B. 182(5): 641–650.

Research Article

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Effect of dietary inclusion of rice distillers dried grains solubles (RDDGS) with or without enzyme on growth performance and cost economics in broiler chickens

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The present study was carried out to assess the effect of dietary inclusion of rice distillers dried grains solubles (RDDGS) with or without enzyme supplementation on growth performance and cost economics in broiler chickens. A total of two-hundred and forty (240), day-old commercial broiler chicks were randomly allocated into six dietary groups (T_1, T_2, T_3, T_4, T_5 and T_6), each with four replicates of 10 birds. The experimental diets included: T_1 (control diet with conventional feed ingredients), T_2 (control diet with enzyme), T_3 (12.5% RDDGS), T_4 (12.5% RDDGS with enzyme), T_5 (15% RDDGS) and T_6 (15% RDDGS with enzyme). The study was conducted over 42 days, with feed and water provided *ad libitum*. Results revealed that dietary inclusion of RDDGS with enzyme (T_4) significantly ($P<0.01$) improved body weight gain during the starter (530.15 g) and finisher (1717.10 g) phases as well as overall body weight gain (2335.91 g) compared to other treatments. Feed intake was lowest ($P < 0.01$) in birds fed 15% RDDGS, with or without enzyme, whereas feed conversion efficiency was better in birds fed 12.5% RDDGS with enzyme (T_4). Feed cost per kg live weight gain was significantly ($P<0.01$) reduced in T_4 (Rs. 58.44) compared to the control group (Rs. 62.36). Based on the findings, it can be concluded that incorporating 12.5% RDDGS with enzyme supplementation improves growth performance, nutrient utilization and cost efficiency in broiler chickens.

Keywords: Broiler chickens, RDDGS, growth performance, cost economics, protease.

Introduction

The poultry industry is one of the fastest-growing sectors of livestock production, driven by the increasing demand for affordable, high-quality animal protein. However, rising costs of conventional feed ingredients viz., maize and soybean meal pose a major challenge to the profitability of broiler production, necessitating the exploration of cost-effective alternative feed resources.

Distillers dried grains solubles (DDGS) a by-product of the ethanol industry, gained attention as a potential feed ingredient due to its appreciable content of crude protein, energy and digestible amino acids (Fries-Craft and Bobeck, 2019). In India, damaged and surplus grains unsuitable for human consumption are increasingly utilized for ethanol production, enhancing DDGS availability. Among various types, rice distiller dried grains solubles (RDDGS) is abundant in Asian countries due to high rice production, with India producing about 135.76 million tonnes during 2022–23 (Ministry of Fisheries, Animal Husbandry and Dairying, 2023). Rice DDGS is a by-product of ethanol industry, where starch is fermented, leaving behind a nutrient-rich residue containing around 42-50% crude protein along with moderate fiber and fat levels (Singh *et al.,* 2020, Dinani *et al.,* 2022). Despite its nutritional value, the use of RDDGS in broiler diets is limited by non-starch polysaccharides (NSPs) and protein-fibre complexes that reduce nutrient digestibility. Supplementation with protease may improve protein utilization and nutrient availability, thereby enhancing the feeding value of RDDGS. Therefore, the present study evaluated the effect of including RDDGS at 12.5 and 15%, with or without protease, on growth performance and cost economics of broiler chickens.

Materials and Methods

Experimental birds and diets

A total of two hundred and forty (240), day old commercial broiler chicks were randomly (CRD) allocated to six treatment groups (T_1, T_2, T_3, T_4, T_5 and T_6), with four replicates of each containing 10 birds. The experiment was carried out from day-old to 42 days of age in three phases (pre-starter, starter and finisher). Isocaloric and isonitrogenous experimental diets were formulated for broiler pre-starter (0-7 days), starter (08-21 days) and finisher (22-42 days) phases as per the nutrient requirements of broilers (BIS, 2007) shown in Table 1. The basal diet (maize and soybean meal) was control (T_1), while the other experimental diets consists of basal diet with protease enzyme (T_2), 12.5% RDDGS (T_3), 12.5% RDDGS with protease enzyme (T_4), 15% RDDGS (T_5) and 15% RDDGS with protease enzyme (T_6) respectively. The enzyme protease was procured from Kemin Industries South Asia, Pvt. Ltd., Chennai marketed as KEMZYME® PROMACH 5; it was supplemented at a dose of 300 mg/kg. The nutrient composition of the experimental diets is presented in Table 2.

Data collection

The individual body weights of the birds were recorded at weekly intervals up to 6 weeks of age. From this average weekly body weight gain per bird was calculated in all the replica of the six treatments. Feed intake was recorded daily on a replicate-wise basis for each treatment. The feed conversion ratio (FCR) was calculated based on total feed intake and total body weight gain of each replicate. Metabolism trial was conducted during the last five days of the finisher phase, wherein two birds from each replicate (total eight birds per treatment) were randomly selected and housed in metabolic cages equipped with feeding and watering facilities. Birds were fed their respective experimental diets for five

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consecutive days and daily records of feed offered, leftover feed and faeces voided were maintained. Representative faecal samples were pooled over five days and along with feed samples, analyzed for nutrient composition as per AOAC (2019) methods. Nutrient retention (%) of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and metabolizable energy (ME) was calculated. Cost economics of broilers up to six weeks of age fed RDDGS, with or without enzyme supplementation, was evaluated based on input costs, feed intake and body weight gain. Statistical analysis of the experimental data was performed using one-way ANOVA as described by Snedecor and Cochran (1994) and differences among treatment means were assessed using Duncan's multiple range test (Duncan, 1955) with SPSS Version 21.0 software.

Table 1: Ingredient composition (%) of broiler pre-starter, starter and finisher experimental rations

	Pres-starter						Starter						Finisher					
Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6
Maize	52.89	52.89	62.79	62.79	63.17	63.17	53.98	53.98	62.54	62.54	63.63	63.63	59.78	59.78	67.87	67.87	65.87	65.87
Soybean meal	40	40	18.71	18.71	15.8	15.8	37.9	37.9	17.7	17.7	13.6	13.6	31.5	31.5	11.6	11.6	11.2	11.2
RDDGS	-	-	12.5	12.5	15	15	-	-	12.5	12.5	15	15	-	-	12.5	12.5	15.0	15.0
Palm oil	2.5	2.5	1.2	1.2	1.0	1.0	3.8	3.8	2.5	2.5	2.75	2.75	4.535	4.535	3.5	3.5	3.5	3.5
Calcite	2.0	2.0	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.75	1.75	1.9	1.9	1.9	1.9
DCP	1.25	1.25	1.24	1.24	1.2	1.2	1.2	1.2	1.24	1.24	1.2	1.2	1.25	1.25	1.2	1.2	1.1	1.2
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine Hcl	0.2	0.2	0.6	0.6	0.67	0.67	0.1	0.1	0.5	0.5	0.6	0.6	0.1	0.1	0.35	0.35	0.35	0.35
DL-Methionine	0.2	0.2	0.1	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Toxin binder*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral**	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin Premix***	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat****	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Liver tonic	0.08	0.08	0.075	0.075	0.075	0.075	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
E care Se	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01
Protease	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03	-	0.03
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Feed cost/kg diet (Rs.)	37.78	37.79	36.86	36.87	39.35	39.36	37.58	37.59	37.2	37.21	38.21	38.22	36.95	36.96	36.01	36.02	37.27	37.28

Toxin binder (Each kg contains: Phylosilicates :800g, Surfactant: 100 g, Mycotoxin destroyer complex:100g) ** Trace Minerall (Manganese: 54 g, Zinc :52 g, Copper; 4g, Cobalt :0.1 g, Iron :30 g, Chromium :0.2 g, Iodine :1 g and Selenium :100 ppm) * Vitamin premix: (Each kg contains: Vit-A: 50MIU, Vit-D3: 10MIU, Vit-B1: 4g, Vit-B2: 20g, Vit-B6: 6g, Vit-B12: 0.08g, Niacin:48g, Calcium-D pantothenate: 32g, Vit-K3:6g, Vit-E: 20g, Folic acid: 4g) ****Coccidiostat (3-5, Dinitro-O-Toluamide: 25 per cent w/w).*

Table 2: Nutrient composition (% DM) of Pre-starter, starter and finisher experimental diets fed to broiler

	Pres-starter						Starter						Finisher					
Nutrient	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6
Dry matter	91.29	91.52	91.42	91.11	91.33	91.07	91.35	90.47	90.57	90.62	91.04	91.32	90.38	90.60	90.08	90.54	90.47	90.81
Organic matter	92.85	92.83	92.89	92.95	92.02	91.75	92.02	91.97	92.78	92.91	92.98	92.84	92.23	92.29	92.27	92.44	92.54	92.60
Crude protein	23.01	23.07	22.98	23.02	22.99	22.96	21.96	22.01	21.91	21.96	22.08	21.92	20.16	20.14	19.94	19.89	19.88	19.91
Ether extract	4.92	4.97	4.49	4.76	4.52	4.51	5.11	5.05	4.95	5.03	4.87	4.85	5.65	5.54	5.17	5.21	4.97	4.95
Crude fiber	4.31	4.33	4.20	4.17	4.26	4.21	4.79	4.76	4.49	4.59	4.48	4.56	5.02	4.99	5.02	5.01	5.12	5.13
Total ash	7.15	7.17	7.11	7.12	7.17	7.05	7.98	8.02	7.21	7.07	7.01	7.15	7.76	7.70	7.72	7.56	7.46	7.40
Acid insoluble ash	1.08	1.06	1.06	1.03	1.05	1.11	1.79	1.76	1.62	1.61	1.56	1.58	1.77	172	1.53	1.58	1.47	1.44
Nitrogen free extract	60.61	60.46	61.22	60.93	61.06	61.27	60.16	60.16	61.44	61.35	61.56	61.52	61.41	61.63	62.15	62.33	62.57	62.61
ME (kcal/kg) *	3006	3010	3013	3019	3020	3014	3100	3100	3100	3098	3097	3100	3200	3200	3203	3198	3199	3200
Calcium	1.02	1.01	1.01	1.02	1.02	1.01	1.01	1.03	0.99	0.97	0.98	0.99	0.98	0.97	0.94	0.93	0.93	0.95
Total phosphorus	0.69	0.68	0.70	0.70	0.72	0.72	0.58	0.58	0.56	0.57	0.60	0.60	0.58	0.60	0.56	0.56	0.60	0.60
Lysine (%) *	1.41	1.41	1.40	1.40	1.43	1.43	1.35	1.35	1.30	1.30	1.31	1.31	1.12	1.12	1.01	1.01	1.10	1.10
Methionine (%) *	0.59	0.59	0.56	0.56	0.79	0.79	0.51	0.51	0.56	0.56	0.77	0.77	0.45	0.45	0.52	0.52	0.74	0.74

*Calculated values

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Table 3: Effect of dietary inclusion of rice distillers dried grains solubles (RDDGS) with or without enzyme on growth performance in broilers

Parameter	Phases	Treatments					
		T1	T2	T3	T4	T5	T6
Body Weight Gain (g)	Pre-starter (0-7 days)	91.65±1.64	100.10±1.83	91.32±2.02	88.67±6.33	84.11±2.42	90.23±5.65
	Starter (8-21 days) **	506.57^{b}±1.12	515.48^{b}±1.25	504.92^{b}±2.79	530.15^{a}±5.22	508.50^{b}±4.17	507.24^{b}±6.03
	Finisher (22-42 days) **	1663.11^{b}±5.67	1664.06^{b}±3.66	1673.97^{b}±8.37	1717.10^{a}±7.45	1611.65^{c}±4.99	1618.55^{c}±3.30
	Overall (0-42 days) **	2261.33^{c}±5.54	2279.63^{b}±3.19	2270.21^{b}±6.38	2335.91^{a}±7.49	2204.25^{d}±3.98	2216.01^{d}±4.03
Feed Intake (g)	Pre-starter (0-7 days)	95.25±0.74	97.41±1.94	100.70±1.88	94.99±5.16	92.81±2.90	99.10±5.34
	Starter (8-21 days) **	705.99±1.30	711.92±4.20	688.53±7.94	701.96±10.15	703.55±3.85	681.38±11.46
	Finisher (22-42 days) **	2936.82^{b}±8.49	2970.86^{a}±7.09	2936.59^{b}±6.69	2977.72^{a}±10.14	2908.48^{c}±8.34	2940.57^{b}±7.38
	Overall (0-42 days) **	3738.05^{b}±8.54	3780.19^{a}±3.48	3725.88^{b}±14.70	3774.67^{a}±17.07	3704.84^{b}±10.16	3721.05^{b}±13.25
Feed Conversion Ratio	Pre-starter (0-7 days) **	1.04^{b}±0.014	0.97^{c}±0.026	1.10^{a}±0.004	1.08^{b}±0.022	1.10^{a}±0.017	1.10^{a}±0.024
	Starter (8-21 days) **	1.39^{a}±0.003	1.38^{a}±0.011	1.36^{a}±0.018	1.32^{c}±0.010	1.38^{a}±0.006	1.34^{b}±0.011
	Finisher (22-42 days) **	1.77^{cd}±0.002	1.79^{b}±0.008	1.75^{d}±0.012	1.73^{e}±0.002	1.80^{ab}±0.003	1.82^{a}±0.006
	Overall (0-42 days) **	1.65^{b}±0.001	1.66^{b}±0.003	1.64^{c}±0.010	1.62^{d}±0.005	1.68^{a}±0.001	1.67^{a}±0.004
Cost Economics	Cost of feed per bird (Rs.)**	141.01^{a}±0.14	139.03^{bc}±0.59	136.45^{d}±0.20	136.51^{d}±0.83	139.83^{ab}±0.19	138.07^{c}±0.26
	Feed cost/kg live weight gain (Rs.)**	62.36^{b}±0.10	60.99^{c}±0.18	60.11^{d}±0.25	58.44^{e}±0.18	63.44^{a}±0.10	62.31^{b}±0.05

^{abcde}Values bearing different superscripts in a same row differ significantly **(P < 0.01).

Feed Intake

Feed intake (Table 3) was highest (P<0.01) in broilers fed 12.5% RDDGS with enzyme (T_4) or control diet with enzyme (T_2), as compared to control diet without enzyme (T_1), 12.5% RDDGS without enzyme (T_3), 15% RDDGS with enzyme (T_6) and 15% RDDGS without enzyme (T_5). The present results are in agreement with the findings of Rao *et al.* (2016) who observed reduction in feed intake when 15% of RDDGS was fed to birds. The results obtained by Dinani *et al.* (2022) are in agreement with the present findings, where feed intake was not significantly affected by RDDGS inclusion at 7.5, 10 and 12.5% with or without enzyme. Kumar *et al.* (2023) noted that higher levels of RDDGS may result in nutrient imbalances, further suppressing feed intake, also the fine texture and powdery nature of RDDGS, coupled with its residual ethanol odor and bitter taste, may have negatively impacted palatability.

Feed Conversion Ratio

The present results (Table 3) indicated feed conversion ratio was better (P < 0.01) in birds fed 12.5% RDDGS with enzyme (T_4) followed by 12.5% RDDGS without enzyme (T_3) or control diet with enzyme (T_2) or control diet without enzyme (T_1) , while lowest in 15% RDDGS either with (T_6) or without enzyme (T_5). The present findings are consistent with Dinani *et al.* (2022), who reported poorer feed conversion efficiency at 15% RDDGS compared to 12.5%, while enzyme supplementation (xylanase, protease and multienzyme) significantly (P<0.01) improved feed conversion ratio. Rao *et al.* (2016) found that including up to 10% RDDGS in broiler diets did not affect feed efficiency, but higher levels (15%) significantly reduced feed conversion ratio. The reduced feed conversion efficiency at higher levels of RDDGS may be due to lower lysine availability (Xue *et al.*, 2012), over estimation of metabolizable energy and reduced bulk density of the diets (Wang *et al.*, 2008)

Nutrient Retention

Incorporation of RDDGS with or without enzyme had no significant effect on DM, OM, EE and CF retention (Table 4). However, CP retention was significantly higher (P<0.01) in enzyme-supplemented groups (T_2, T_4, T_6). Similarly, ME retention differed significantly (P<0.01), being highest in T_4 and T_2, moderate in T_6 and lowest in non-enzyme supplemented groups (T_1, T_3 and T_5). The present results are in agreement with Butle (2024), who reported no significant effect on DM, EE, CF except higher CP metabolizability with protease supplemented RDDGS diets. Similarly, Dinani *et al.* (2022) and Kwak *et al.* (2024) observed improved nitrogen retention and energy metabolizability with enzyme supplementation, irrespective of RDDGS levels.

Table 4: Effect of dietary inclusion of rice distillers dried grains solubles (RDDGS) with or without enzyme on nutrient retention (%) in broilers

Treatments	Parameter (%)					
	T1	T2	T3	T4	T5	T6
DM	71.60±0.56	72.59±0.97	71.42±0.77	73.27±0.44	70.68±059	71.18±0.47
OM	76.31±0.41	77.18±0.96	76.44±0.64	77.42±0.49	75.62±0.54	76.06±0.63
CP **	67.57^{c}±0.63	74.80^{a}±0.90	68.01^{c}±0.84	75.77^{a}±0.41	66.57^{c}±0.74	71.44^{b}±0.37
EE	78.36±0.64	77.80±0.93	76.97±0.62	77.39±0.63	76.02±0.39	76.14±0.38
CF	25.26±0.54	27.71±1.95	26.59±0.46	27.94±0.39	26.75±1.25	26.90±0.71
ME**	74.61^{c}±0.37	77.54^{a}±0.23	74.47^{c}±0.50	78.19^{a}±0.44	73.88^{c}±0.65	76.65^{b}±0.49

^{abc}Values bearing different superscripts in a same row differ significantly **(P < 0.01). DM=Dry Matter; OM=Organic Matter; CP=Crude Protein; EE=Ether Extract; CF=Crude Fiber; ME=Metabolizable Energy.

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Cost Economics

The feed cost per kg live weight gain (Table 1) ranged from Rs. 58.44 (T_4) to Rs. 63.44 (T_5). Broilers fed diets containing 12.5% RDDGS with enzyme (Rs.58.44 in T_4) had significantly ($P < 0.01$) lower feed cost per kg live weight gain, followed by 12.5% RDDGS without enzyme (Rs.60.11 in T_3), control diet with enzyme (60.99 in T_2), 15% RDDGS with enzyme (62.31 in T_6) or control diet (62.36 in T_1), while the highest cost was recorded in 15% RDDGS without enzyme (63.44 in T_5). Feed cost per kg live weight gain decreased by Rs. 0.88 and 2.35 in 12.5% RDDGS diets (with or without enzyme), while it increased by Rs. 2.45 and 1.32 in 15% RDDGS diets compared to control (T_1). These findings agree with Dinani *et al.* (2022), Gupta *et al.* (2019) and Khose *et al.* (2021), who also reported improved cost efficiency with RDDGS and enzyme supplementation. Based on the findings, it can be concluded that incorporating 12.5% RDDGS with enzyme supplementation improves growth performance, nutrient utilization and cost efficiency in broiler chickens.

Conclusion

Rice DDGS can be effectively used as a feed ingredient in broiler diets up to an inclusion level of 12.5% with enzyme supplementation without negatively affecting growth performance and nutrient utilization; however, increasing the inclusion level to 15% resulted in poor performance either with or without protease enzyme.

References

AOAC 2019.Official methods of analysis of AOAC International. 21st Edition, Washington DC.

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

Butle, G. A. 2024. Dietary Inclusion of Rice DDGS Supplemented with Protease Through Different Growth Phases In Broiler Chicken. *Thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur.*

Dinani, O. P., Tyagi, P. K., Mandal, A. B., Tyagi, P. K., Bhanja, S., K. and Dutta, N. 2022. Augmenting feeding value of rice distillers dried grain with solubles through dietary addition of enzymes in broilers. *Indian Journal of Animal Research*, 56(12): 1506-1512.

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

Fries-Craft, K. and Bobeck, E. A. 2019. Evaluation of a high-protein DDGS product in broiler chickens: performance, nitrogen-corrected apparent metabolisableenergyand standardised ileal amino acid digestibility. *British Poultry Science*, 60(6): 749–756.

Gupta, S. L., Tyagi, P. K., Mir, N. A., Dev, K., Begum, J., Mandal, A. B. and Tyagi, P. K. 2019. Feeding value of rice distiller's dried grains with solubles as protein supplement in diet of laying hens. *Tropical Animal Health and Production*, 52(3): 1229–1237.

Khose, K. K., Manwar, S. J., Joshi, M. V., Kuralkar, S. V., Gole, M. A. and Wade, M. R. 2021. Effect of dietary rice distillers dried grains with solubles and enzyme supplementation on growth performance and immune status in broiler chickens. *Indian Journal of Poultry Science,* 56(2): 115-121.

Kumar, A. Singh, D. K. Kumar, A. Fahim, A. Kumar, A. Ranjan, K.Thakur, V. Verma, A. and Kumar, A. 2023. Effect of dietary inclusion of rice distillers dried grains with solubles (rDDGS) on growth performance and production efficiency of Kuroiler chicken. *The Indian Journal of Animal Sciences*, 93(1): 72-76.

Kwak, M., Ha. D. Park, M. Eor., J. Whang K. and Kim, Y.2024. Comparison study between single enzyme and multienzyme complex in distiller's dried grains with soluble supplemented diet in broiler chicken. *Journal of Animal Science and Technology*, 66(2): 398-411.

Ministry of Fisheries, Animal Husbandry and Dairying2023.*Fourth advance estimates of production for 2022–23. Government of India.*

Rao, Rama S. V., Raju, M. V. L. N., Prakash B., Reddy, E. P. K. and Anusha, R.2016. Effect of dietary supplement of distillery dried grain soluble from rice on performance of commercial broilers and White Leghorn layers. In *Proc. XXV World's Poultry Congress, Beijing.pp-52*.

Singh, N., Dubey, M., Doneria, R.,Gendley, M. K., Dinani, O. P. and Ramteke, R. C.2020. Effect of feeding different levels of rice distillers dried grains with soluble (RDDGS) on performance of broiler. *Indian Journal of Animal Nutrition*, 37(2):172-178.

Snedecor, G. W. and Cochran, W.G. 1994. Statistical Methods 8thed Affiliated East West Press, New Delhi 13: 1467- 1473.

Wang, Z., Cerrate, S., Coto, C., Yan, F. and Waldroup, P. W. 2008. Evaluation of high levels of distillers dried grains with solubles (DDGS) in broiler diets. *International Journal of Poultry Science,* 7 (10): 990-996.

Xue, P. C., Dong, B., Zang, J. J. Zhu, Z. P. and Gong, L. M.2012. Energy and standardized ileal amino acid digestibilities of chinese distillers dried grains, produced from different regions and grains fed to growing pigs. *Asian-Australasian journal of animal sciences*, 25(1): 104-106.

Research Article

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Effects of Postbiotic and Organic Selenium supplementation on Immunity, Antioxidant Status and Cost economics in Heat-Stressed Broiler chicken

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Heat stress is a major constraint to broiler productivity, impairing immunity, antioxidant status and overall performance. The present study sought to elucidate the individual and combined effects of a *Saccharomyces cerevisiae*–derived postbiotic and organic selenium on humoral immunity, serum biochemical profile, antioxidant enzymes, cortisol and cost economics in broiler chickens reared during summer conditions. A total of 288 day-old broiler chicks (45.13 ± 3 g) were equally and randomly distributed into six dietary regimens with eight replicates of six birds each for 35 days: T_1 (basal diet), T_2 (basal diet + 0.3 mg organic selenium/kg), T_3 (basal diet + 500 mg postbiotic/kg), T_4 (basal diet + 1000 mg postbiotic/kg), T_5 (basal diet + 0.3 mg organic selenium + 500 mg postbiotic/kg) and T_6 (basal diet + 0.3 mg organic selenium + 1000 mg postbiotic/kg). Humoral immunity was assessed by Newcastle disease hemagglutination inhibition titres, while serum biochemical parameters (total protein, albumin, globulin, (AG) ratio and cholesterol levels), antioxidant indices (Glutathione peroxidase, Glutathione reductase, Superoxide dismutase, Malondialdehyde) and cortisol were evaluated on days 21 and 35.Dietary supplementation significantly ($P < 0.05$) enhanced ND antibody titres, increased serum total protein and albumin, reduced cholesterol levels, improved antioxidant enzyme activities, decreased lipid peroxidation and lowered cortisol levels compared with the control. Combined supplementation (T5 and T6) produced the most pronounced improvements. Economic analysis revealed higher net profit per bird in supplemented groups, with the greatest return observed in T6, followed by T5.In conclusion, dietary inclusion of postbiotic and organic selenium, particularly in combination, effectively improved immune response, antioxidant status, stress resilience and profitability in broiler chickens during heat stress, indicating their potential as practical nutritional strategies for enhancing productivity during hot climatic conditions.

Keywords : Postbiotic, Organic selenium, Humoral immunity, Antioxidant status, Cost economics

Efficient poultry production depends on the combined influence of high-quality genetics, optimal housing conditions and precisely balanced nutrition. In practical farming systems, broiler chickens are routinely subjected to multiple environmental challenges, among which heat stress (HS) has become one of the most serious concerns, particularly with the ongoing impacts of climate change (Abdel-Moneim *et al.,* 2021). Exposure to elevated temperatures compromises growth performance, physiological functions and metabolic processes in broilers by impairing gut integrity, inducing oxidative stress and promoting epithelial injury and inflammatory responses (Lian *et al.,* 2020). To counteract these adverse effects, several dietary interventions have been explored to improve bird adaptability and productivity under stressful conditions. Recently, postbiotics and organic selenium have attracted increasing interest owing to their enhanced bioavailability, strong antioxidant potential and ability to support birds during thermal stress.

The International Scientific Association of Probiotics and Prebiotics characterizes postbiotics as preparations comprising non-living microbial cells and/or their components that promote beneficial physiological effects in the host. (Salminen *et al.,* 2021). These compounds demonstrate diverse functional properties, such as antimicrobial, antioxidant, anti-inflammatory and immunomodulatory actions, along with hypocholesterolemic, antiproliferative, hepatoprotective and growth-enhancing effects, thereby supporting overall host wellbeing (Aguilar-Toala *et al.,* 2018).

Selenium (Se) is a vital micronutrient necessary for maintaining animal health and productivity, mainly through its role in antioxidant defense mechanisms that safeguard cells against free-radical-induced damage (Prasad *et al.,* 2023). Compared with inorganic forms, organic selenium exhibits superior absorption, higher tissue deposition and reduced toxicity (Liu *et al.,* 2022). Therefore, the present investigation was undertaken to evaluate the individual and combined effects of postbiotic derived from *Saccharomyces cerevisiae* and organic selenium on immunity, biochemical constituents, antioxidant status, stress marker cortisol and cost economics in heat stressed broiler chickens.

Materials and Methods

The present trial was carried out, using 288-day-old broiler chicks initially weighing 45.13 ± 3g, were randomly distributed into 6 experimental groups. Each group was housed in 8 replicate cages, with 6 birds accommodated in each cage. The feeding trial was undertaken for five weeks, encompassing 3 distinct phases: phase1

(d1-14), phase2 (d15-28) and phase3 (d29-35). Dietary treatments were formulated based on a basal diet, with supplementation levels of Postbiotic (Celmanax®, Arm and Hammer Co., Ltd (USA)) and Organic selenium (AJ Supplements Private Limited, HYD) *viz*.T_1-Control (Basal Diet), T_2 – BD + 0.3mg Organic selenium/kg diet, T_3– BD + 500mg Postbiotic/kg diet, T_4– BD + 1000mg Postbiotic/kg diet, T_5– BD + 0.3mg Organic selenium + 500mg Postbiotic/kg diet and T_6– BD + 0.3mg Organic selenium + 1000mg Postbiotic/kg diet. The experimental protocols and associated procedures were performed in accordance with established ethical guidelines and were reviewed and approved by the Institutional Animal Ethics Committee (17/30/C.V. Sc,Hyd, dated:11/06/2024) in accordance with the CPCSEA. All birds were housed in four-tier battery cages measuring 1.25 m × 0.80 m × 0.50 m (length × width × height). Throughout the experimental period, feed and fresh drinking water were provided *ad libitum*. The basal diets were formulated to meet the nutrient specifications recommended in the breeder management manual, thereby fulfilling the nutritional requirements of the birds.These diets were detailed in Table-1.

The research work was carried out in the Poultry Experimental Station, College of Veterinary Science, P. V. Narsimha Rao Telangana Veterinary University, Hyderabad, Telangana from 16th May 2025 to 20th June 2025. Throughout the study, the average temperature fluctuated between 23.2 to 38.2°C. Similarly, the relative humidity during the experiment ranged from 25 to 65.2%. The average Temperature Humidity Index (THI) noted was 78.53 during the experimental period.

Humoral Immunity: The influence of dietary supplementation with graded levels of postbiotic and organic selenium on the humoral immune response of broiler chickens was assessed by determining antibody titres against the Newcastle disease virus. Birds were immunized with the Lasota strain of Newcastle disease vaccine (ND Lasota Vac-1000; Ventri Biologicals, Pune, India) via the ocular route on days 7 and 28 of the experimental period. At 35 days of age, blood samples were collected, and serum was separated by centrifugation for immunological analysis. Serum antibody titres against NDV were quantified using the hemagglutination inhibition assay as described by Allan *et al.,* (1978), and the results were expressed as $\log_2$ HI titres.

Serum Analysis: Blood was aseptically collected from one bird per replicate on days 21 and 35 via the brachial vein. After clotting at room temperature, samples were centrifuged at 3000 rpm for 5 minutes, and serum was stored at -20°C for analysis of antioxidants (glutathione peroxidase (Cat. No. E0298Ch), glutathione reductase (Cat. No. E029Ch), superoxide dismutase (Cat. No. E0295Ch) Malondialdehyde (Cat. No. E0171Ch), and cortisol (Cat. No. E009cC) determined using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit, Bioassay Technology Laboratory, Jiaxing, Zhejiang, China. The biochemical parameters (total protein, albumin, globulin and cholesterol) were analysed according to Erba Mannheim kit protocol (TransAsia Bio-Medicals Limited, India).

Cost Economics: The economics of broiler production were evaluated using the following calculations:

Total feed cost per bird (Rs) = Total feed intake per bird (kg) × Feed cost (Rs/kg)

Total production cost per bird (Rs) = Total feed cost + Cost of day-old chick + Miscellaneous cost

Cost of production per kg live body weight (Rs/kg) = Total production cost per bird ÷ Final live body weight (kg)

Sale value per bird (Rs) = Final live body weight (kg) × Market price of live broiler (Rs/kg)

Net profit per bird (Rs) = Sale value per bird – Total production cost per bird

Net profit per kg live body weight (Rs/kg) = Net profit per bird ÷ Final live body weight (kg)

$$\text{Return on Investment \% (ROI\%)} = \frac{\text{Net Profit}}{\text{Total Cost of Production}} \times 100$$

$$\text{Benefit:Cost (B:C) ratio} = \frac{\text{Sale Value}}{\text{Total Cost of Production}}$$

Statistical Analysis: All experimental data were subjected to statistical analysis using the General Linear Model procedure of the Statistical Package for the Social Sciences (Version 27.0). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955). Biochemical and antioxidant variables were further analysed by two-way univariate analysis of variance following the procedures described by Snedecor and Cochran (1980). Differences among treatments were considered statistically significant at $P< 0.05$.

Results

Dietary supplementation of postbiotic and organic selenium significantly (P<0.05) enhanced the humoral immune response in broiler chickens, as evidenced by higher Newcastle Disease (ND) hemagglutination inhibition (HI) titres (Table 2). The supplemented groups exhibited significantly higher antibody titres compared to the control group, indicating improved immune response following ND vaccination.

Dietary treatments significantly (P<0.05) affected serum total protein and cholesterol concentrations during the experimental period (Table 3), whereas the sampling period and diet × period interaction had no significant effect. Serum albumin concentration was significantly influenced (P<0.05) by dietary treatment and sampling period. However, serum globulin concentration and albumin:globulin (A/G) ratio were not significantly affected (P>0.05) at either 21 or 35 days, although numerically higher A/G ratios were observed in the supplemented groups.

Dietary supplementation with postbiotic and organic selenium significantly (P<0.05) influenced antioxidant enzyme activities in broiler chickens (Table 4). Glutathione peroxidase (GPx) activity was significantly affected by dietary treatment, sampling period, and their interaction (P<0.05). Glutathione reductase (GR) and superoxide dismutase (SOD) activities were significantly affected by dietary treatment and sampling period (P < 0.05), while their diet × period interactions were not significant (P>0.05). Malondialdehyde levels were significantly reduced (P<0.05) in supplemented groups, whereas the effects of sampling period and diet × period interaction were not significant.

Dietary supplementation with postbiotic and organic selenium significantly (P<0.05) reduced serum cortisol concentrations in broiler chickens (Table 5). Sampling period also had a significant effect (P<0.05), whereas the diet × period interaction was not significant (P>0.05).

The economics of broiler production during the overall experimental period (0–35 days) are presented in Table 6. Supplementation with postbiotic and organic selenium improved net profit per bird compared with the control. The highest net profit was recorded in T6 (₹39.17), followed by T5 (₹37.35), T3 (₹36.71), T4 (₹35.44), T2 (₹33.65), while the lowest profit was observed in T1 (₹29.14).

Discussion

Newcastle Disease (ND) HI titre is a reliable indicator of humoral immune response and reflects the antibody production following vaccination. The higher antibody titres observed in the supplemented groups indicate enhanced immune competence due to dietary postbiotic and organic selenium supplementation.

These findings agree with those of Chang *et al.* (2022), Doski and Kareem (2023) and Soren *et al.* (2024) who also reported improved antibody responses following supplementation with postbiotics and organic selenium. The improvement may be attributed to enhanced immune cell function, improved intestinal health, and the antioxidant role of selenium, which protects immune cells against oxidative damage. However, Al-Sagheer *et al.* (2023) reported no significant effect of selenium, inactivated yeast, or their combination on immune function in heat-stressed rabbits, suggesting that responses may vary depending on animal species and experimental conditions.

The increased serum total protein and albumin concentrations observed in the supplemented groups suggest improved protein metabolism and liver function. The reduction in serum cholesterol may indicate improved lipid metabolism and enhanced physiological status. Similar observations were reported by Fang *et al.* (2024), Soren *et al.* (2024), and Monika *et al.* (2024), who found significant improvements in serum protein profile and reduced cholesterol concentrations following postbiotic supplementation. In contrast, Al-Sagheer *et al.* (2023) and Soren *et al*. (2024) observed no significant changes in serum total protein or albumin concentrations after supplementation with *Saccharomyces cerevisiae* fermentation products, indicating that responses may depend on dietary composition, environmental conditions, and supplementation level.

The improved GPx, GR, and SOD activities together with reduced lipid peroxidation demonstrate that dietary supplementation effectively enhanced the antioxidant defence system in broiler chickens. Organic selenium is an essential component of glutathione peroxidase, while postbiotics provide bioactive metabolites that enhance endogenous antioxidant capacity and reduce oxidative stress. These findings are consistent with those of Humam *et al*. (2021), Al-Sagheer *et al.* (2023), and Gul *et al.* (2023), who reported enhanced antioxidant enzyme activities in supplemented broilers exposed to heat stress. Conversely, Humam *et al*. (2020) reported no significant influence of postbiotic supplementation on SOD or GPx activity under heat stress, indicating that differences in postbiotic source, dosage, and environmental conditions may contribute to inconsistent responses.

The significant reduction in serum cortisol concentration observed in the supplemented groups indicates a lower physiological stress response. Selenium, through its antioxidant properties, and postbiotics, through modulation of gut health and immune function, likely reduced oxidative stress and activation of the hypothalamic-pituitary-adrenal axis during heat stress. Similar findings were reported by Sun *et al.* (2021) and Abbas *et al.* (2022), who demonstrated that selenium-enriched yeast supplementation significantly reduced cortisol levels in heat-stressed birds. In contrast, Forder *et al.* (2024) observed no effect of *Saccharomyces cerevisiae* extract supplementation on plasma cortisol concentration in breeder birds, suggesting that physiological responses may differ according to bird type, supplementation strategy, and production conditions.

The improved economic returns observed in the supplemented groups were primarily associated with enhanced growth performance, improved feed efficiency, better immune response, and reduced oxidative stress. The highest economic return recorded in the combined postbiotic and organic selenium treatment indicates that supplementation can be a cost-effective nutritional strategy for improving broiler productivity, particularly under heat stress conditions.

Conclusion

Dietary supplementation with postbiotic and organic selenium, particularly in combination, significantly enhanced immune response, antioxidant status and stress tolerance while improving economic returns in broiler chickens under heat stress, demonstrating their potential as effective nutritional strategies for sustainable broiler production.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Sagi Raju, J. Narasimha, Sarin. K. Kunnath and N. Nalini kumari contributed to the original idea and design of the study. Boini Sravanthi conducted the experiments and collected the data. All authors were involved in the manuscript preparation.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on request.

AI Policy

No generative AI tools were used in the experiment design, data collection, data analysis, interpretation of results or preparation of manuscript. All work presented is the original work of the authors.

References

Abbas, A. O., Alaqil, A. A., Mehaisen, G. M., & El Sabry, M. I. 2022. Effect of organic selenium-enriched yeast on relieving the deterioration of layer performance, immune function, and physiological indicators induced by heat stress. *Frontiers in veterinary science*, *9*:1-11, 880790.

Abdel-Moneim, A. M. E., Shehata, A. M., Khidr, R. E., Paswan, V. K., Ibrahim, N. S., El-Ghoul, A. A & Ebeid, T. A. 2021. Nutritional manipulation to combat heat stress in poultry–A comprehensive review. *Journal of Thermal Biology*, *98*:1-19, 102915.

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A. 2018. Postbiotics: An evolving term within the functional foods field. *Trends in food science & technology*, *75*, 105-114.

Al-Sagheer, A. A., Alagawany, M., Bassiony, S. S., Shehata, A. M., El-Metwally, A. E., & El-Kholy, M. S. 2023. Inactivated Saccharomyces cerevisiae and selenium as alternatives to antibiotic in rabbits reared under summer conditions: Effects on growth, nutrient utilization, cecal fermentation, blood components, and intestinal architecture. *Animal Feed Science and Technology*, *302*:1-12, 115688.

Allan, W. H., Lancaster, J. E., & Toth, B. 1978. *Newcastle disease vaccines, their production and use* (p. 163pp).

Bureau of Indian Standards (BIS). 2007. Indian standard poultry feeds specification. 5th ed. New Delhi: Bureau of Indian Standards.

Chang, H. M., Loh, T. C., Foo, H. L., & Lim, E. T. C. 2022. *Lactiplantibacillusplantarum* postbiotics: alternative of antibiotic growth promoter to ameliorate gut health in broiler chickens. *Frontiers in veterinary science*, *9*, 883324.

Doski, J. M. M., & Kareem, K. Y. 2023. Effect of different levels of postbiotic and phytobiotic combination as on growth performance, economic production, and immune response of broiler chickens. *Polytechnic Journal*, *14*(1):1-9.

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

Fang, S., Fan, X., Xu, S., Gao, S., Wang, T., Chen, Z., & Li, D. 2024. Effects of dietary supplementation of postbiotic derived from Bacillus subtilis ACCC 11025 on growth performance, meat yield, meat quality, excreta bacteria, and excreta ammonia emission of broiler chicks. *Poultry Science*, *103*(5):1-8, 103444.

Forder, R. E., Willson, N. L., Angove, J. A., Mcwhorter, T. J., Mcqueen, M. A., & Cadogan, D. J. 2024. Dietary inclusion of a Saccharomyces cerevisiae metabolite improved reproductive performance but did not affect intestinal permeability in two chicken meat breeder lines. *Poultry Science*, *103*(5):1-9, 103595.

Gul, F., Ahmad, B., Afzal, S., Ullah, A., Khan, S., Aman, K & Ahmad, L. 2021. Comparative analysis of various sources of selenium on the growth performance and antioxidant status in broilers under heat stress. *Brazilian Journal of Biology*, *83*:1-10, e251004.

Humam, A. M., Loh, T. C., Foo, H. L., Izuddin, W. I., Awad, E. A., Idrus, Z. & Mustapha, N. M. 2020. Dietary supplementation of postbiotics mitigates adverse impacts of heat stress on antioxidant enzyme activity, total antioxidant, lipid peroxidation, physiological stress indicators, lipid profile and meat quality in broilers. *Animals*, *10*(6):1-21, 982.

Humam, A. M., Loh, T. C., Foo, H. L., Izuddin, W. I., Zulkifli, I., Samsudin, A. A., & Mustapha, N. M. 2021. Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. *Poultry science*, *100*(3):1-14, 100908.

Lian, P., Braber, S., Garssen, J., Wichers, H. J., Folkerts, G., Fink-Gremmels, J., & Varasteh, S. 2020. Beyond heat stress: intestinal integrity disruption and mechanism-based intervention strategies. *Nutrients*, *12*(3):1-31, 734.

Liu, M., Cao, W., Gao, P., Zhao, J., Muhammad, U., Ni, S. & Zuo, S. 2022. Effects of two different selenium fertilizers on accumulation of selenium and heavy metals in rice grains in field trials. *Food Science and Technology*, *42*:1-9, e117521.

Prasad, M. V., Prakash, B., Narasimha, J., Rama Rao, S. V., Raju, M. V. L. N., Zeba, P., & Sreenivasa Reddy, C. 2023. Effect of dietary supplementation of organic and inorganic Se on performance and antioxidant response in commercial broiler chickens. *British Poultry Science*, *64*(1):1-6, 110-115.

Monika, M., Tyagi, J. S., Sonale, N., Biswas, A., Murali, D., Sky & Rokade, J. J. 2024. Evaluating the efficacy of Lactobacillus acidophilus derived postbiotics on growth metrics, Health, and Gut Integrity in broiler chickens. *Scientific Reports*, *14*(1):1-15, 24768.

Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. &Vinderola, G. 2021. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature reviews Gastroenterology & hepatology*, *18*(9):649-667.

Snedecor, G. W., & Cochran, W. G. 1980. Statistical Methods, Oxford and IBIT Public Co. *New York*.

Soren, S., Mandal, G. P., Mondal, S., Pradhan, S., Mukherjee, J., Banerjee, D., & Samanta, I. 2024. Efficacy of *Saccharomyces cerevisiae* fermentation product and probiotic supplementation on growth performance, gut microflora and immunity of broiler chickens. *Animals*, *14*(6):1-15, 866.

Sun, H., Zhao, L., Xu, Z. J., De Marco, M., Briens, M., Yan, X. H., & Sun, L. H. 2021. Hydroxy-selenomethionine improves the selenium status and helps to maintain broiler performances under a high stocking density and heat stress conditions through a better redox and immune response. *Antioxidants*, *10*(10):1-13, 1542.

Table 1 - Diet ingredients and chemical composition of the basal control diet

Ingredient	Pre-Starter (1-14 days)	Starter (15-28 days)	Finisher (29-35 days)
Maize	53.1	53.79	58.43
Oil-veg	2.74	4.30	5.00
Deoiled soybean meal	40.24	38.00	32.55
Salt	0.35	0.30	0.30
Dicalcium phosphate	1.8	1.90	2.00
Lime stone powder	1.2	1.20	1.20
DL-Methionine	0.15	0.15	0.13
L-lysine Hydrochloride	0.06	0.00	0.03
Choline chloride,75%	0.06	0.06	0.06
Toxin binder[1]	0.05	0.05	0.05
Trace mineral mixture[2]	0.15	0.15	0.15
Vitamin premix[3]	0.05	0.05	0.05
Coccidiostat[4]	0.05	0.05	0.05
Total	100	100	100
Nutrient composition DMB(%)			
Me[b], MJ/kg	12.49	12.95	13.32
Protein[a], (% g)	23.01	22.03	20.00
Lysine[b], (%)	1.31	1.20	1.10
Methionine[b](%)	0.50	0.49	0.45
Calcium[a], (%)	0.97	1.00	1.02
Available phosphorous[a], (%)	0.44	0.45	0.46

1. Five gram of toxin binder provided per kg diet: Acetic acid 2mg, Formic acid 2mg, propionic acid 2mg, citric acid 2mg, Benzoic acid 1mg, Sorbic acid 0.25 mg, HSCAS 9.1mg.
2. Each gram of trace mineral mixture provided per kg diet: Manganese 1.1 mg, Zinc 1mg, Iron 110mg, Copper 20mg, Iodine 2.5mg, Cobalt 1mg, Chromium 0.4mg and Selenium 1.5mg
3. Vitamin pre-mix provided per kg diet: Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg, Niacin 15mg, Vitamin A 20000 IU, Vitamin D3 3000 IU, Vitamin K 2mg
4. Five gram of coccidiostat provided per kg diet: Maduramicin 5 mg
DMB, dry matter basis [a]Analysed value, [b]calculated value

Table 2 -Individual and Combined Effects of dietary supplementation ofPostbiotic and Organic selenium on ND titration(log_2) in broiler chicken during summer.

Treatment	HI titre (log_2)
Standard diet	5.62[b]
OS-0.3mg/kg	7.00[a]
PB-500mg	6.50[ab]
PB-1000mg	6.87[a]
OS+500mg PB	7.13[a]
OS+1000mg PB	7.25[a]
N8SEM	0.15
P-value	0.02

[a,b]Means with different superscript in a column differ significantly: (*P < 0.05)
P- value: probability value. N: number of replicates (6 birds in each replicate); SEM: Standard Error Mean
SD: Standard diet; OS-0.3mg/kg: standard diet + 0.3mg organic selenium/kg diet; PB-500mg: standard diet + 500mg postbiotic/kg diet; PB-1000mg: standard diet + 1000mg postbiotic/kg diet; OS+500mg PB: standard diet + 0.3mg organic selenium/kg diet +500mg postbiotic/kg diet; OS+1000mg PB: standard diet + 0.3mg organic selenium/kg diet +1000mg postbiotic/kg diet.

Table 3 - Individual and Combined Effects of dietary supplementation of Postbiotic and Organic selenium on Biochemical constituents in broiler chicken during summer.

Parameters	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	AG ratio	Cholesterol (g/dL)
			Treatments		
Standard diet	4.29[c]	2.18[c]	2.11	1.04	141.00[a]
OS-0.3mg/kg	4.49[b]	2.27[bc]	2.22	1.07	135.89[ab]
PB-500mg/kg	4.46[bc]	2.40[ab]	2.06	1.18	130.62[abc]
PB-1000mg/kg	4.50[b]	2.42[ab]	2.08	1.25	127.78[bc]
OS+PB-500mg/kg	4.69[a]	2.51[a]	2.18	1.17	122.87[c]
OS+PB-1000mg/kg	4.77[a]	2.57[a]	2.21	1.20	123.39[c]
SEM	0.05	0.06	0.08	0.06	5.38
p- value	0.001	0.001	0.61	0.26	0.01
			Day		
21st day	4.51	2.31[y]	2.19	1.07	130.77
35th day	4.57	2.40[x]	2.09	1.23	130.05
SEM	0.07	0.09	0.11	0.07	2.32
p-value	0.29	0.001	0.09	0.09	0.77
Treatmentx Day	0.93	0.72	0.93	0.95	1.00

[a,b,c,x,y]Means with different superscript in a column differ significantly: (*P < 0.05)
P- value: probability value. N: number of replicates (6 birds in each replicate); SEM: Standard Error Mean
SD: Standard diet; OS-0.3mg/kg: standard diet + 0.3mg organic selenium/kg diet; PB-500mg: standard diet + 500mg postbiotic/kg diet; PB-1000mg: standard diet + 1000mg postbiotic/kg diet; OS+500mg PB: standard diet + 0.3mg organic selenium/kg diet +500mg postbiotic/kg diet; OS+1000mg PB: standard diet + 0.3mg organic selenium/kg diet +1000mg postbiotic/kg diet

Table 4 - Individual and Combined Effects of dietary supplementation of Postbiotic and Organic selenium on Antioxidant status in broiler chicken during summer.

Parameters	Glutathione peroxidase (ng/mL)	Glutathione reductase (ng/mL)	Superoxide dismutase (ng/mL)	Malondialdehyde (nmol/mL)
		Treatments		
Standard diet	2.94[c]	2.93[c]	1.59[c]	4.01[a]
OS-0.3mg/kg	5.44[ab]	3.91[ab]	2.96[ab]	2.29[b]
PB-500mg	4.69[b]	3.35[bc]	2.49[b]	2.52[b]
PB-1000mg	4.99[ab]	3.59[bc]	2.99[ab]	2.47[b]
OS+500mg PB	5.53[a]	4.15[ab]	3.33[a]	2.23[b]
OS+1000mg PB	5.73[a]	4.46[a]	3.46[a]	2.18[b]

SEM	0.28	1.04	0.20	0.17
p- value	0.001	0.001	0.001	0.001
Day				
21st day	4.63[y]	3.49[y]	2.45[y]	2.803
5th day	5.15[x]	4.29[x]	3.16[x]	1.92
SEM	0.21	0.15	0.14	0.22
p-value	0.02	0.001	0.001	0.06
TreatmentxDay	0.04	0.46	0.79	0.92

[a,b,c,x,y]Means with different superscript in a column differ significantly: (*P < 0.05)

P- value: probability value. N: number of replicates (6 birds in each replicate); SEM: Standard Error Mean

SD: Standard diet; OS-0.3mg/kg: standard diet + 0.3mg organic selenium/kg diet; PB-500mg: standard diet + 500mg postbiotic/kg diet; PB-1000mg: standard diet + 1000mg postbiotic/kg diet; OS+500mg PB: standard diet + 0.3mg organic selenium/kg diet +500mg postbiotic/kg diet; OS+1000mg PB: standard diet + 0.3mg organic selenium/kg diet +1000mg postbiotic/kg diet

Table 5 - Individual and Combined Effects of dietary supplementation of Postbiotic and Organic selenium on Cortisol levels in broiler chicken during summer.

Parameters	Cortisol(ng/mL)
Treatment	
Standard diet	8.25[a]
OS-0.3mg/kg	4.04[b]
PB-500mg	4.93[b]
PB-1000mg	4.33[b]
OS+500mg PB	3.83[b]
OS+1000mg PB	3.46[b]
SEM	0.48
p- value	0.001
Period	
21st day	5.25[x]
35th day	4.36[y]
SEM	0.37
p-value	0.04
TreatmentxDay	0.94

[a,b,c,x,y]Means with different superscript in a column differ significantly: (*P < 0.05)

P- value: probability value. N: number of replicates (6 birds in each replicate); SEM: Standard Error Mean

SD: Standard diet; OS-0.3mg/kg: standard diet + 0.3mg organic selenium/kg diet; PB-500mg: standard diet + 500mg postbiotic/kg diet; PB-1000mg: standard diet + 1000mg postbiotic/kg diet; OS+500mg PB: standard diet + 0.3mg organic selenium/kg diet +500mg postbiotic/kg diet; OS+1000mg PB: standard diet + 0.3mg organic selenium/kg diet +1000mg postbiotic/kg diet

Table 6 Cost of production during overall experimental period (0 – 35 days) of broiler chickens during summer.

Particulars	**Treatments**					
	Control	**0.3mg OS**	**500mg PB**	**1000mg PB**	**0.3mg OS + 500mg PB**	**0.3mg OS + 1000mg PB**
Live body weight (g)	1940	2076	2034	2057	2101	2127
Total FI/bird (g) (0-35 days)	2782	2909	2877	2875	2940	2944
Cost of feed/kg (Rs)	40	40.0001	40.15	40.30	40.1501	40.3301
Cost of day-old chick(Rs)	35	35	35	35	35	35
Miscellaneous cost/bird (Rs)	5	5	5	5	5	5
Total feed cost, Rs/bird	111.28	116.36	115.51	115.86	118.04	118.64
Total cost, Rs/bird	151.28	156.36	159.53	155.86	158.04	158.64
Total cost, Rs/Kg bird	77.97	75.317	76.46	75.77	75.22	74.59
Sale of each bird (Rs)	180.42	193.068	189.16	191.30	195.39	197.81
Net profit /bird (Rs)	29.14	36.708	33.65	35.44	37.35	39.17
Net profit/kg b. wt. (Rs)	15.02	17.68	16.54	17.23	17.78	18.41
ROI%	19.26	23.48	21.09	22.74	23.63	24.69
B:C Ratio	1.19	1.23	1.18	1.23	1.24	1.25

SD: Standard diet; OS-0.3mg/kg: standard diet + 0.3mg organic selenium/kg diet; PB-500mg: standard diet + 500mg postbiotic/kg diet; PB-1000mg: standard diet + 1000mg postbiotic/kg diet; OS+500mg PB: standard diet + 0.3mg organic selenium/kg diet +500mg postbiotic/kg diet; OS+1000mg PB: standard diet + 0.3mg organic selenium/kg diet +1000mg postbiotic/kg diet

Research Article

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Postprandial glycaemic dynamics response of cockerels to extruded sweetpotato-bran diets

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This study investigated extruded sweetpotato–bran energy concentrates in cockerels, emphasizing the effect of extrusion temperature and bran type on postprandial glycaemic dynamics. Sweetpotato roots were enriched with corn bran (SP-CB), rice bran (SP-RB), and wheat bran (SP-WB) and extruded at 80 °C, 100 °C, and 120 °C to create energy concentrates. Diets containing raw or extruded concentrates were fed alongside a reference diet to 8 cockerels each for 20 minutes, and blood glucose was obtained preprandial, plus 30, 60, 90, 120, 180, and 300 minutes postprandial per cockerel. Non-compartmental and one-compartment oral absorption models were applied to glucose concentration–time data obtained. The glucose absorption rate constant, Ka, absorption and elimination rate constant half-lives, t1/2Ka and t1/2K10, hydrolysis index, HI, and glycaemic index, GI, were recorded. Increasing extrusion temperature accelerated glucose absorption ($p < 0.05$) but, at the highest temperature (120 °C), tended to reduce total glucose exposure, AUC_o–t, in rice and corn-bran diets, suggesting retrogradation and restricted enzyme accessibility. Conversely, wheat-bran blends extruded at 120 °C exhibited elevated AUC_o–t, alluding to temperature-dependent modulation of fibre–starch interactions. Overall, rice bran enrichment produced the most consistent lowering of glycaemic indices. These results underscore the potential of extrusion and bran enrichment to control starch digestibility and glycaemic release in sweetpotato-based poultry diets and support the use of postprandial glycaemic response dynamics as a functional tool in poultry nutrition research.

Keywords: High-temperature short-time extrusion cooking, Glycaemic index, Resistant starch, Kinetic modeling, Bran enrichment

Introduction

Energy concentrates are feedstuffs of high calorific density and relatively low in other nutrients. While this term may refer to single-grain ingredients such as maize, barley, and wheat, it also describes energy blends and premixes offered as value-added products tailored to balance forage or protein feeds (Onche et al., 2025). Combined with commercial high-protein concentrates, it creates a complete feed (Adeleye and Ojeniyi, 2023). Calorie-dense feedstuffs such as cassava (*Manihot esculenta* L. Crantz) and sweet potato (*Ipomoea batatas*) tubers could also be harnessed to create energy concentrate blends. Despite the year-round availability of sweet potato and its comparable metabolizable energy to maize (Pandi et al., 2016; Woolfe, 1992), its use in poultry diets is constrained by high moisture content, which advances spoilage. Sweetpotato roots also contain trypsin inhibitors (Pandi et al., 2016), reducing sugars (Woolfe, 1992), soluble fibre, and ipomoein, its major soluble protein, all of which impair its digestibility and may elicit a laxative effect in chickens (Dominguez, 1991; Varon et al., 1989). These constraints to sweetpotato use in poultry feeding may be alleviated by extrusion processing.

The structural reorganization and physicochemical changes that occur in the process of extrusion cooking induce starch gelatinization, protein denaturation, fibre modification, and inactivation of anti-nutritional factors (Ali et al., 2024; Adeleye et al., 2020a, 2020b; Roye et al., 2020). The resultant effect of extrusion conditions on physicochemical and nutritional properties of foods is somewhat complex as intrinsic properties of the ingredient or blends, such as amylose-amylopectin ratio, fibre, protein, and lipid content, interact with processing variables- barrel temperature, moisture, screw speed, and pressure- to collectively affect outcomes (Ali et al., 2024). The characteristic composition of individual ingredients in blended products also influences how extrusion cooking conditions affect their nutritional and physicochemical indices. Blending cereals with legumes or other fibres often increases SDS and lowers *in vitro* estimated Glycemic Index (GI) after extrusion (Wani et al., 2021), while purely starchy ingredients show marked GI increases under similar conditions (Adeleye et al., 2020b). The GI-lowering ability of extruded cereal-legume or cereal-fibre blends could be due to physical entrapment of starch granules by dietary fibre (Zhang et al., 2022), formation of amylose-lipid and starch-protein complexes (Altan et al., 2009), and/or increasing soluble fibre fractions (Khalid et al., 2022) that slow gastric emptying and starch hydrolysis (Fujiwara, et al., 2017). These processes limit the accessibility of digestive enzymes to starch, reducing the rate at which glucose is released into the bloodstream.

Riding on these desirable benefits of extrusion, the potential of the extrusion process to modify "glycaemic response dynamics" is crucial in nutritional studies (Zhao et al., 2024). While three independent pathways; *prandial* intestinal absorption, glycogenolysis, and gluconeogenesis- contribute to blood glucose concentrations, *postprandial glycaemic* concentrations primarily reflect glucose absorption from recent meals (Khan, 2001; Zebda et al., 2018). Kinetic indices derived from modeling glucose concentration-time data also reveal how rapidly glucose becomes available, how much glucose is supplied, how sustained the supply is, and how glucose/glucose precursors may interact with other nutrients (Huang et al., 2025). These kinetic features are highly relevant for animal performance, metabolic health, and developing formulation strategies. In the present study, we investigate the extent to which extrusion cooking × cereal bran combinations will alter glycaemic response dynamics in cockerels fed sweet potato-bran concentrates.

Materials and methods

Development of sweet-potato-bran energy concentrates and diets

White-fleshed sweet potato (*Ipomoea batatas*) roots were obtained from the open market, and cereal brans- corn, rice, and wheat- from reputable feed stockists. Sweet potato roots were washed to remove sand and debris, chipped, and dried in a forced-air oven at 60°C until constant weight was attained. The sweet potato chips and brans were then milled to obtain a fine texture. Sweetpotato-bran energy concentrates were created by blending sweet potato with corn bran or rice bran at a ratio of 3:2, and with wheat bran at a ratio of 7:3 (Table 1). Extrudates were prepared as described by Adeleye and Ojeniyi (2023), with slight modifications. The sweet potato-bran blends were adjusted to 30% moisture content and fed into a single-screw industrial extruder. Extruder characteristics were screw length-to-diameter (L/D) ratio, 6:1, barrel length, 317.5mm, screw speed: 305rpm, die diameter: 8mm, feed rate: 33.6 kg/h, and extrusion temperatures were 80, 100 and 120 °C. The extrudates were again dried in a forced-air oven at 50 °C until constant weight was attained.

Table 1. Calculated and analyzed composition of the un-extruded sweet potato-bran energy concentrates: sweet potato-corn bran, (SP-CB) sweet potato-rice bran (SP-RB) and sweet potato-wheat bran (SP-WB).

	SP-CB	SP-RB	SP-WB
	3:2	3:2	7:3
Calculated nutrients			
Crude protein, %	6.17	8.44	7.30
Sugars, %	4.78	5.00	6.05
Starch + sugars, %	54.47	53.98	55.13
Crude fibre, %	5.56	5.70	4.89
Calcium, %	0.11	0.16	0.16
Phosphorus, %	0.27	0.78	0.43
Non-phytate phosphorus, %	0.08	0.31	0.19
Lysine, %	-	-	-
Digestible lysine, %	0.11	0.20	0.16
Methionine, %	-	-	-
Digestible methionine, %	0.08	0.11	0.07
Digestible methionine + cysteine, %	0.13	0.18	0.13
Digestible threonine, %	0.12	0.16	0.13
Analyzed composition			
Gross energy, kcal/g DM			
Dry matter, %	91.05	88.00	90.00
Crude protein, %	6.00	6.00	5.65
Ether extract, %	4.90	4.50	4.70
Crude fibre, %	3.80	11.50	11.45
Carbohydrates (calculated as nitrogen free extract, %)	66.85	59.08	64.80
Ash, %	9.50	7.50	3.40

SP-CB: sweet potato-corn bran, SP-RB: sweet potato-rice bran, SP-WB: sweet potato-wheat bran

Experimental Animals Management

In three different assays, cockerels (n=40) with a weight/bird of 2.15 ± 0.28 kg were obtained from a research flock and used to investigate *postprandial* glycaemic response to extrusion temperatures per sweetpotato + cereal bran combination. Cockerels were pre-tagged for individual identification and housed in metabolic cages, 120cm × 69cm × 85cm, 2 birds per cage and 4 cages per treatment. Cages were equipped with feeding troughs and nipple drinkers and placed in an open-sided house with natural ventilation and lighting (14L: 10D).

Diets and glycaemic response dynamics study

In three independent assays, combinations of sweet potato+ bran energy concentrates; sweet potato-corn bran (SP-CB), sweetpotato-rice bran (SP-RB), or sweet potato-wheat bran (SP-WB) (Table 2), were assessed. Feed was withdrawn 12 hours proceeding, with *ad libitum* water to ensure thorough emptying of the gastrointestinal tract. At commencement of the study, *preprandial* glucose values were obtained by glucometry (ACCUCHEK active, Roche Diagnostics GmbH, Mammheim, Germany) in a "prick glucose test" which involved applying a lancet to the wing vein and obtaining 20-50µL of blood via a capillary tube. Birds in four cages (n=8) were offered the diets for 20 minutes, and blood glucose values were again obtained at 30, 60, 90, 120, 180, and 300 minutes *postprandial*.

Calculations and Statistical Analysis

Concentration-time curves were analyzed by non-compartmental analysis and fitted with a one-compartment oral absorption model (Bateman function) using PK Solver 2.0 (Zhang et al., 2010).

The hydrolysis index, HI, of each sweet potato-bran energy concentrate was obtained per bird, calculated as the ratio of the area under the glucose concentration-time curve of the bird on the

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Table 2. Gross composition of experimental diets used in this study

	Reference diet	SP-CB diet				SP-RB diet				SP-WB diet			
		Raw	80°C	100°C	120°C	Raw	80°C	100°C	120°C	Raw	80°C	100°C	120°C
Ingredients													
Glucose monohydrate	48.50	-	-	-	-	-	-	-	-	-	-	-	-
Fibre concentrate, 66% CF	3.50	-	-	-	-	-	-	-	-	-	-	-	-
Fish meal, 72% CP	8.00	-	-	-	-	-	-	-	-	-	-	-	-
Soy oil	-	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Protein concentrate, 30% CP	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Sweet potato + cereal bran concentrate	-	57.00	57.00	57.00	57.00	57.00	57.00	57.00	57.00	57.00	57.00	57.00	57.00
	100	100	100	100	100	100	100	100	100	100	100	100	100

SP-CB: sweet potato-corn bran, SP-RB: sweet potato-rice bran, SP-WB: sweet potato-wheat bran

test diet to the area under the glucose concentration-time curve of the bird on the reference diet. The glycaemic index (GI) was calculated by the equation of Goñi et al. (1997).

"Glycaemic index (GI) = 39.71+(0.549 * HI)"

One-way analysis of variance, ANOVA, comparisons was conducted to assess the effect of extrusion cooking temperature within each sweet potato-bran energy concentrate using SPSS.

Results

Figure 1 shows the *postprandial* glucose concentration-time curves of matured cockerels fed sweet potato-corn bran (SP-CB), sweet potato-wheat bran (SP-WB), and sweet potato-rice bran (SP-RB), energy concentrates. Extrusion cooking had no significant effect on glucose concentrations of the cockerels from 30-300 minutes postprandial except for SP-RB at 30 minutes (p=0.08) and SP-WB at 300 minutes (p=0.01) where glucose concentration was higher in cockerels on the reference diet.

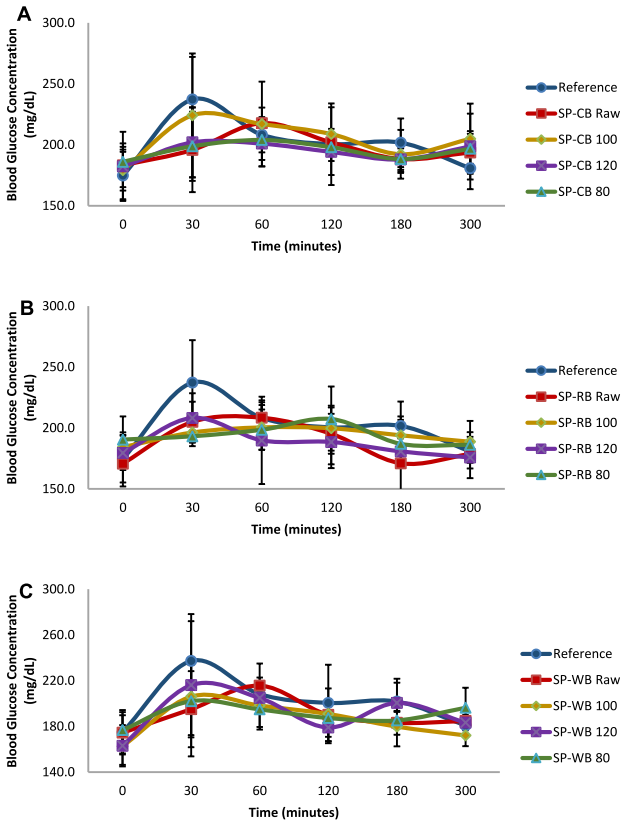

Figure 1(A-C). The effect of extrusion cooking temperature (80- 120 °C) on *postprandial* glucose concentration-time curves of matured cockerels fed the sweet potato-bran energy concentrates; SP-CB (sweet potato-corn bran), SP-RB (sweet potato-rice bran), SP-WB (sweet potato-wheat bran), Raw- unextruded sweet potato-bran treatments. n=8

Table 3 shows the effect of extrusion cooking temperatures on indices of glycaemic dynamics in matured cockerels fed sweet potato-corn bran (SP-CB), sweet potato-wheat bran (SP-WB) and sweet potato-rice bran (SP-RB), energy concentrates. Parameters of interest estimated from the compartmental analysis of the *postprandial* glucose concentration-time curve include the peak glucose concentration in the blood, C_{max}, time taken for blood concentration to reach the peak, T_{max}, and area under the glucose concentration-time curve, AUC_{0-t} which is considered the observed systemic glucose exposure over the study period, i.e. 300 mins *postprandial*. C_{max} peaked in cockerels offered the 100 °C extruded SP-CB diet, declined with increasing extrusion temperatures in cockerels on the extruded SP-RB diets ($p < 0.05$) while remaining unaffected by extrusion in cockerels fed the SP-WB diets. Conversely, T_{max} decreased with increasing extrusion temperatures ($p < 0.05$) in cockerels offered the 120°C extruded SP-CB (0.36h), SP-RB (0.40h), and SP-WB (0.35h) diets. The AUC_{0-t} estimate was not impacted by lower extrusion temperatures, i.e., 80 and 100 °C, in cockerels fed the SP-WB and SP-RB diets but significantly peaked in the cockerels fed the 120 °C SP-WB diet (9.69 mg/mL*h, $p < 0.05$). Conversely, AUC_{0-t} estimate was lowest in cockerels fed the 120 °C SP-RB diet (8.99 mg/mL*h, $p < 0.05$). Cockerels fed the raw, 80 °C, and 120 °C extrudate SP-CB diets showed similar AUC_{0-t} estimate with the highest AUC_{0-t} estimate recorded in cockerels on the 100 °C extrudate diet (10.29 mg/mL*h, $p < 0.05$).

The first-order rate constants, absorption rate constant (K_a) and the elimination rate constant (K_{10}) reflect how quickly glucose enters the systemic circulation of the cockerels after feeding extruded sweet potato–bran diets, and how it is eliminated from circulation through metabolic use, tissue uptake, or excretion. K_a estimates steadily increased with increasing extrusion temperature, peaking at 120°C for SP-CB. Similarly, K_a estimates for SP-RB and SP-WB peaked at 120°C ($p < 0.05$); however, K_a was unaffected by extrusion of SP-RB at 80 and 100°C and extrusion of SP-WB at 100°C. However, there were no significant effects of SP-RB extrusion at lower temperatures (i.e. 80 and 100°C) on K_a estimates, as they were similar to K_a estimates from cockerels fed the unextruded SP-RB diets. Cockerels fed the SP-WB diet with 120 °C extrudates also showed similar K_a estimates ($p > 0.05$) with the 80 °C extrudates group. The K_{10} remained unaffected by extrusion temperature in cockerels fed the SP-RB and SP-WB diets, while decreasing with increasing extrusion temperature ($p < 0.05$) in cockerels fed the SP-CB diets. Conversely, the absorption half-life ($_{t1/2}K_a$) and the elimination half-life ($_{t1/2}K_{10}$) represent the time required for half of the

orally administered dose of glucose or glucose precursors in the sweet potato-bran diets to be absorbed from the gastrointestinal tract into systemic circulation, and the time required for blood glucose concentration to decrease by half owing to metabolic utilization or excretion. Absorption half-life ($_{t1/2}K_a$) was unaffected by extrusion at lower temperatures, i.e., 80 and 100 °C in cockerels fed the SP-RB and SP-WB diets and was lowest in the 120 °C extrudate diet. However, $_{t1/2}K_a$ decreased progressively with increasing extrusion temperature (0.15 h > 0.09 h > 0.03 h, $p < 0.05$), indicating a faster glucose absorption rate at higher processing temperatures.

The peak elimination half-life ($_{t1/2}K_{10}$) was attained at 66.10h, 45.86h, and 31.31h in cockerels fed the SP-CB, SP-RB, and SP-WB diets containing 80 °C extrudates, while extrusion at 120 °C did not impact $_{t1/2}K_{10}$differently from the control ($p > 0.05$).

The hydrolysis index, HI, and glycaemic index (GI) values were highest in cockerels fed the diets containing SP-CB and SP-WB extruded at 100°C ($p < 0.05$). At the same time, extrusion cooking significantly lowered HI and GI in cockerels fed the SP-RB diets.

Table 3. The effect of extrusion cooking temperature on indices of glycaemic response kinetics in matured cockerels fed the sweet potato-bran energy concentrates

	Extrusion temperature				
Parameters	Raw	80°C	100°C	120°C	SEM
Sweet potato – corn bran, SP-CB					
T_{max}, h	0.99^{ab}	1.12^{a}	0.73^{b}	0.36^{c}	0.13
C_{max}, mg/ml	2.17^{ab}	2.07^{b}	2.29^{a}	2.07^{b}	0.04
AUC_{0-t}, mg/ml*h	9.73^{b}	9.52^{b}	10.29^{a}	9.43^{b}	0.15
K_a, 1/h	10.63^{c}	16.01^{b}	17.38^{b}	29.09^{a}	3.66
K_{10}, 1/h	0.07^{a}	0.03^{c}	0.04^{b}	0.03^{c}	0.00
$t_{1/2}k_a$, h	0.16^{a}	0.15^{a}	0.09^{b}	0.03^{c}	0.20
$t_{1/2}k_{10}$, h	16.14^{c}	66.10^{a}	39.46^{b}	14.29^{c}	10.24
HI	1.98^{b}	1.25^{b}	3.09^{a}	1.13^{b}	0.47
GI	40.80^{b}	40.40^{b}	41.41^{a}	40.33^{b}	0.26
Sweet potato – Rice bran, SP-RB					
T_{max}, h	0.65^{b}	0.81^{a}	0.72^{b}	0.40^{c}	0.09
C_{max}, mg/ml	2.10^{a}	2.03^{b}	2.06^{ab}	2.03^{b}	0.03
AUC_{0-t}, mg/ml*h	9.43^{a}	9.50^{a}	9.58^{a}	8.99^{b}	0.12
K_a, 1/h	15.46^{b}	13.56^{b}	13.48^{b}	32.58^{a}	3.63
K_{10}, 1/h	0.05	0.03	0.03	0.03	0.01
$t_{1/2}k_a$, h	0.09^{a}	0.11^{a}	0.09^{a}	0.04^{b}	0.01
$t_{1/2}k_{10}$, h	24.94^{b}	45.86^{a}	45.91^{a}	11.55^{b}	7.58
HI	1.05^{a}	1.04^{a}	0.46^{b}	0.67^{b}	0.20
GI	40.29^{a}	40.28^{a}	39.96^{c}	40.08^{b}	0.11
Sweet potato – Wheat bran, SP-WB					
T_{max}, h	0.78^{a}	0.53^{b}	0.76^{a}	0.35^{c}	0.11
C_{max}, mg/ml	2.12	2.04	2.08	2.30	0.59
AUC_{0-t}, mg/ml*h	9.51^{b}	9.37^{c}	9.38^{c}	9.69^{a}	0.12
K_a, 1/h	15.60^{c}	29.79^{b}	18.74^{c}	34.58^{a}	3.98
K_{10}, 1/h	0.04	0.03	0.04	0.03	0.01
$t_{1/2}k_a$, h	0.10^{a}	0.07^{a}	0.10^{a}	0.02^{b}	0.02
$t_{1/2}k_{10}$, h	22.27^{b}	31.31^{a}	24.64^{ab}	18.60^{b}	3.82
HI	1.51^{b}	1.27^{b}	1.92^{a}	1.37^{b}	0.31
GI	40.54^{b}	40.41^{b}	40.77^{a}	40.46^{b}	0.17

Means with different superscripts along the row differ significantly ($p<0.05$)
T_{max} = time taken for blood glucose concentration to reach the peak, C_{max} = peak glucose concentration in the blood, AUC_{0-t} = area under the glucose concentration-time curve, K_a = absorption rate constant, K_{10} = elimination rate constant, $t_{1/2}k_a$ = absorption rate half-life, $t_{1/2}k_{10}$ = elimination rate half-life

Discussion

Monogastric animal production benefits from extrusion technology because it improves feed texture, digestibility, and palatability; however, incorporating extruded high-starch ingredients at high levels in diets tends to accelerate glucose release, disrupting glucose and amino acid release patterns (Zhao et al., 2024). Non-starch feed components, particularly fibre, stall glycaemic and insulin spikes that arise from high dietary concentrations of rapidly digesting starch, RDS, which are often observed in extruded diets (Tu et al., 2025). This study aimed to develop value-added sweet potato-based energy concentrates to balance commercial protein

concentrates (often about 30-40% CP), creating balanced custom feed mixes for poultry. Extrusion in this study was necessitated by the need for textural modification to address the dusty and bulky nature of sweet potato tubers, while the choice of blending with brans was to mitigate the extent of starch damage and free glucose release during extrusion. In the past, extrusion of sole sweet potato tubers reportedly resulted in brittle, dense, and burnt extrudates due to their high sugar content (Khalil and Henry, 1997).

Sweet potato starch represents the main component of sweet potato tubers, up to 80% dry weight (Zhu et al., 2011); hence, the *postprandial* glycaemic response kinetics of these sweet potato-bran energy concentrates largely reflect the extent and rate of their starch digestibility *in vivo* (Butterworth et al., 2022) as affected by fibre dilutions and processing conditions. When investigating the influence of extrusion on the digestibility of starch in whole sweet potato flour, Waramboi et al. (2014) reported a significant increase in "very rapidly digesting starch" from 2.3-11.1 g/100g dry starch to 7.9-18.4g/100g dry starch. In contrast, bran enrichment dilutes the starch content of formulations, competes with fibre molecules for water, and favours the formation of amylose-lipid complexes, ultimately limiting the extent of gelatinization (Tyl et al., 2021), mitigating glucose release.

While there exists a paucity of studies that report the effect of extrusion temperatures x bran enrichment on plasma glycaemic response conducted in poultry, the combined effect of extrusion and bran enrichment on glycaemic responses is reportedly variable. An assessment of glycaemic responses of male Wistar rats to high-fibre cookies made from wheat flour and wheat + sweet potato flour, both enriched with corn and sorghum brans, showed the ability of brans to moderate blood glucose concentrations compared to a glucose reference group and a wheat flour cookie control group (Saha et al., 2025). A similar effect of wheat and oat bran enrichment of wheat-based rolls has been reported (Knudsen et al., 2000). According to Lewko et al. (2025), an increased absorption rate constant (ka) reflects faster glucose appearance in the plasma owing to enhanced starch gelatinization and increased enzymatic accessibility. Hence, our results allude to the ability of the selected brans to mitigate complete gelatinization in sweet potato-bran blends. Although the application of first-order kinetics to *in vivo* glycaemic response studies remains relatively underexplored—being more commonly employed in pharmaceutical research— the combined effects of starch gelatinization and molecular fragmentation induced by extrusion significantly enhance the initial rate of enzymatic hydrolysis and glucose release (Jiang et al., 2022). This acceleration corresponds to an increased absorption rate constant (K_a) and a shorter half-life of absorption, ($_{t1/2}K_a$). Experimental data also indicate that higher extrusion temperatures are associated with reduced area under the glucose concentration time curve (AUC) as seen in cockerels fed sweet potato–corn bran (SP-CB) and sweet potato–rice bran (SP-RB) diets extruded at 120 °C in the current study. This reduction in total glucose exposure may be attributed to partial dextrinization and retrogradation, both of which facilitate the gradual formation of resistant starch (Gulzar et al., 2021). High extrusion temperatures intensify the structural modifications that promote the formation of resistant starch, leading to a lower overall glycaemic response. Furthermore, physical encapsulation of starch granules by bran fibre, coupled with increased resistant starch, limits the availability of digestible starch, similar to resistant starch 1, RS1 (Han et al., 2023), collectively contributing to a diminished glycaemic response. High-temperature extrusion, 100-150°C, of rice bran-enriched corn-based snack and wheat bran-enriched cassava-defatted soy flour composite showed a significant reduction in starch digestion and estimated glycaemic index (Oladiran and Emmambux, 2017; Renoldi et al., 2021). This was attributed to an extrusion-induced conversion of insoluble arabinoxylans in wheat bran to soluble fibres, which increase viscosity and reduce postprandial glucose response (Renoldi et al., 2021). Also Yadav et al. (2009) described the low solubility of rice bran and its inability to achieve proper expansion and texture during extrusion as a plausible cause.

Conclusion

Postprandial glycaemic response kinetics in cockerels provide a dynamic and mechanistic measure of starch digestibility, reflecting how extrusion-induced modifications in the sweet potato–bran matrix alter glucose availability. The findings reveal that extrusion temperature and bran type modulate glucose absorption and clearance rates, with rice bran showing the greatest glycaemic moderation. Elevated extrusion temperatures accelerate starch gelatinization and glucose appearance but may simultaneously promote retrogradation and resistant starch formation, thereby reducing total glycaemic exposure. Integrating postprandial glycaemic kinetics into poultry nutrition and feed processing research offers a framework for a deeper understanding of how ingredient composition and processing conditions influence nutrient synchronization and metabolic energy utilization. Quantitative indices derived from kinetic modelling tools similar to PKSolver enable more precise synchronization of energy–amino acid metabolism to support feed efficiency in poultry.

Ethical requirements and declaration

The experimental protocols, adopted in this study, were reviewed and approved by the Department of Animal Science, University of Ibadan, Nigeria, according to the guidelines for ethical conduct and reporting of animal research (Jarvis et al., 2005; Kilkenny et al., 2010).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

Author's contribution

Adeleye, O.O-Conceptualization, Methodology, Data curation, Manuscript Writing, and Supervision; Osofisan, D.B- Investigation, Data curation, Formal analysis

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Conflict of interest statement

None of the authors have a conflict of interest to disclose

References

Adeleye, O. O., Awodiran, S.T., Ajayi, A.O., and Ogunmoyela, T.F (2020a). Effect of high-temperature, short-time cooking conditions on in vitro protein digestibility, enzyme inhibitor activity and amino acid profile of selected legume grains. Heliyon 6: e05419

Adeleye, O. O., Awodiran, S. T., Ajayi, A. O., and Ogunmoyela, T. F (2020b). Influence of extrusion cooking on physicochemical properties and starch digestion kinetics of Sphenostylis stenocarpa, Cajanus cajan, and Vigna subterranean grains. PloS One 15 (12): e0242697.

Adeleye, O.O., Ojeniyi, M.O., 2023. Evaluation of Metabolizable Energy Values in Extruded Sweet potato-cereal Bran blends as

energy concentrates for chickens. Tropical Animal Production Investigations 26, 6–11.

Ali, I.M., Forsido, S.F., Kuyu, C.G., Ahmed, E.H., Andersa, K.N., Chane, K.T., Regasa, T.K., 2024. Effects of extrusion process conditions on nutritional, anti-nutritional, physical, functional, and sensory properties of extruded snack: A review. Food Science and Nutrition 12, 8755–8761. https://doi.org/10.1002/fsn3.4472

Altan, A., McCarthy, K.L., Maskan, M., 2009. Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. J Food Sci 74. https://doi.org/10.1111/j.1750-3841.2009.01051.x

Butterworth, P.J., Bajka, B.H., Edwards, C.H., Warren, F.J., Ellis, P.R., 2022. Enzyme kinetic approach for mechanistic insight and predictions of in vivo starch digestibility and the glycaemic index of foods. Trends in Food Science and Technology 120, 254–264. https://doi.org/10.1016/j.tifs.2021.11.015

Dominguez, P.L., 1991. Feeding of sweet potato to monogastrics, in: Machin, D., Nyvold, S. (Eds.), Roots, Tubers, Plantains and Bananas in Animal Feeding. Proceedings of the FAO Expert Consultation Held in CIAT, Cali, Colombia. Food and Agriculture Organization of the United Nationst.

Fujiwara, N., Hall, C., Jenkins, A.L., 2017. Development of low glycemic index (GI) foods by incorporating pulse ingredients into cereal-based products : Use of in vitro screening and in vivo technologies. Cereal Chemistry. 94, 110–116.

Goñi, I., Garcia-Alonso, A., Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17, 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Gulzar, B., Hussain, S.Z., Naseer, B., Naik, H.R., 2021. Enhancement of resistant starch content in modified rice flour using extrusion technology. Cereal Chemistry. 98, 634–641. https://doi.org/10.1002/cche.10407

Han, J., Wu, J., Liu, X., Shi, J., Xu, J., 2023. Physiological effects of resistant starch and its applications in food: a review. Food Production, Processing and Nutrition 5. https://doi.org/10.1186/s43014-023-00156-x

Huang, M., Xue, L., Wu, Y., Sun, Q., Xu, Y., Li, J., Yu, X., Cao, Y., Huang, J., Zhang, Z., Zhao, J., Han, D., Li, D., Wang, J., 2025. Glucose release kinetics of different feed ingredients and their impact on short-term growth of pigs by influencing carbon-nitrogen supply synchronization. Journal of Animal Science and Biotechnology. 16, 1–19. https://doi.org/10.1186/S40104-025-01198-6/FIGURES/6

Jarvis, S.C., Day, J. E. L., Reed, B., 2005. Ethical Policy: British society of animal science ethical guidelines for research in animal science. Proceedings of the British Society of Animal Science, 247-253. https://www.cambridge.org/core/services/aop-file-manager/file/58948e84b2c37d2f06971df4/ANM-Ethical-Issues.pdf

Jiang, L., Qi, M., Deng, Y., Suo, W., Song, J., Zhang, M., Zheng, H., Zhang, D., Chen, S., Li, H., 2022. Extrusion-induced pre-gelatinization and hydrolyzation of rice adjunct contributed to the mashing performance. LWT 158, 113126. https://doi.org/10.1016/J.LWT.2022.113126

Khalid, W., Arshad, M.S., Jabeen, A., Muhammad Anjum, F., Qaisrani, T.B., Suleria, H.A.R., 2022. Fiber-enriched botanicals: A therapeutic tool against certain metabolic ailments. Food Science and Nutrition. 10, 3203-3218. https://doi.org/10.1002/fsn3.2920

Khalil, H.M., Henry, B.R., 1997. Feasibility of utilizing sweet cooking de batata. Food Science and Technology International 3, 171–174.

Khan, R., 2001. Postprandial Blood Glucose. Diabetes Care 24, 775–778. https://doi.org/10.2337/DIACARE.24.4.775

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., and Altman, D. G. 2010. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biology, 8(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Knudsen, K.E.B., Jorgensen, H., Canibe, N., 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: Model experiment with catheterised pigs fed on wheat- or oat-based rolls. British Journal of Nutrition 84, 449–458. https://doi.org/10.1017/s0007114500001756

Lewko, P., Wójtowicz, A., Rudaś, M., 2025. Effect of Processing Conditions of Enzymatic, Extrusion, and Hybrid Treatment Methods on Composition and Selected Technofunctional Properties of Developed Wheat Flour. International Journal of Food Science 2025. https://doi.org/10.1155/ijfo/3317924

Oladiran, D.A., Emmambux, N.M., 2017. Effects of extrusion cooking and wheat bran substitution on the functional, nutritional, and rheological properties of cassava-defatted toasted soy composite. Starch/Staerke 69, 1–9. https://doi.org/10.1002/star.201600183

Onche, E., Cho, H., Rangandang, A.D., Kang, N., Kim, S., Kim, H., Seo, S., 2025. Enhancing Feed Efficiency and Growth in Early-Fattening Hanwoo Steers Through High-Energy Concentrate Feeding. Animals 2025, Vol. 15, Page 490 15, 490. https://doi.org/10.3390/ANI15040490

Pandi, J., Glatz, P., Forder, R., Ayalew, W., Waramboi, J., Chousalkar, K., 2016. The use of sweet potato (Ipomoea batatas (L.) Lam) root as feed ingredient for broilerfinisher rations in Papua New Guinea. Animal Feed Science and Technology 214, 1–11. https://doi.org/10.1016/J.ANIFEEDSCI.2016.01.011

PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel

Renoldi, N., Peighambardoust, S.H., Peressini, D., 2021. The effect of rice bran on physicochemical, textural and glycaemic properties of ready-to-eat extruded corn snacks. Int J Food Sci Technol 56, 3235–3244. https://doi.org/10.1111/ijfs.14939

Roye, C., Henrion, M., Chanvrier, H., de Roeck, K., de Bondt, Y., Liberloo, I., King, R., Courtin, C.M., 2020. Extrusion-cooking modifies physicochemical and nutrition-related properties of wheat bran. Foods 9, 1–23. https://doi.org/10.3390/foods9060738

Saha, B.U.F., Choumessi, A.T., Teta, I., Javnyuy, N.Y., Mbassi, G.G.M., Eyili, N.J.K., Djeutsop, A.T., Navti, L.K., 2025. Effects of bran-enriched flour blends on the antioxidant properties, nutritional quality, and glycemic control of high-fiber biscuits. Exploration of Foods and Foodomics 3, 1–20. https://doi.org/10.37349/eff.2025.101076

Tu, J., Chen, Q., Zhou, J., Fan, Y., Li, Y., Ma, Y., Zeng, X., Qiao, S., Cai, S., 2025. Characteristics of Amino Acid and Glucose Digestion and Metabolism in Energy and Protein Feedstuffs for Pigs 1–14.

Tyl, C., Bresciani, A., Marti, A., 2021. Recent Progress on Improving the Quality of Bran-Enriched Extruded Snacks. Foods 10, 1–20.

Varon, D., Collins, W., Foegeding, E.A., 1989. Ipomoein is the Major Soluble Protein of Sweet Potato Storage Roots. Horticultural Science 24, 829–830. https://doi.org/10.21273/hortsci.24.5.829

Wani, S.A., Ganie, N.A., Kumar, P., 2021. Quality characteristics, fatty acid profile and glycemic index of extrusion processed snacks enriched with the multicomponent mixture of cereals and legumes. Legume Science 3, e76. https://doi.org/10.1002/LEG3.76

Waramboi, J.G., Gidley, M.J., Sopade, P.A., 2014. Influence of extrusion on expansion, functional and digestibility properties of whole sweet potato flour. LWT 59, 1136–1145. https://doi.org/10.1016/j.lwt.2014.06.016

Woolfe, J.A., 1992. Sweet potato: an untapped food resource. Cambridge University Press.

Yadav, B.S., Sharma, A., Yadav, R.B., 2009. Studies on effect of multiple heating/cooling cycles on the resistant starch formation in cereals, legumes and tubers. International Journal of Food Science and Nutrition 60 Suppl 4, 258–272. https://doi.org/10.1080/ 09637480902970975

Zebda, A., Alcaraz, J.P., Vadgama, P., Shleev, S., Minteer, S.D., Boucher, F., Cinquin, P., Martin, D.K., 2018. Challenges for successful implantation of biofuel cells. Bioelectrochemistry 124, 57–72. https://doi.org/10.1016/J.BIOELECHEM.2018.05.011

Zhang, Y., Huo, M., Zhou, J., Xie, S., 2010. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer Methods and Programs in Biomedicine 99, 306–314. https://doi.org/10.1016/j.cmpb.2010.01.007

Zhang, Z., Zhang, G., Zhang, S., Zhao, J., 2022. Effects of Extrusion on Energy Contents and Amino Acid Digestibility of Corn DDGS and Full-Fat Rice Bran in Growing Pigs. Animals 12. https://doi.org/10.3390/ani12050579

Zhao, Y., Dang, X., Du, H., Wang, D., Zhang, J., Liu, R., Ge, Z., Sun, Z., Zhong, Q., 2024. Understanding the Impact of Extrusion Treatment on Cereals: Insights from Alterations in Starch Physicochemical Properties and In Vitro Digestion Kinetics. Animals 14, 1–16. https://doi.org/10.3390/ani14213144

Zhu, F., Yang, X., Cai, Y.Z., Bertoft, E., Corke, H., 2011. Physicochemical properties of sweet potato starch. Starch/Staerke 63, 249–259. https://doi.org/10.1002/star.201000134

Short Communication

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Challenges for Rural Women in Nutritional Management of Dairy Animals

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The current study was carried out in four villages of Samrala Block, Ludhiana district under a project implemented by Plant clinic, PAU to encourage efficient dairy farming among rural women in order to address challenges of livelihood and nutritional security. Data were collected from 60 purposively selected rural women who were beneficiaries under the project, through personal interviews with a structured schedule. Data was analyzed using Garrett's ranking technique. The findings revealed that women faced multiple challenges in dairy nutrition management which included lack of access to land for cultivation of fodder crops, high cost of compounded feeds for dairy animals, limited access to technical knowledge, resources and markets, insufficient infrastructure at household level and lack of knowledge regarding balanced ration preparation, feed additives, bypass nutrients etc. In order to address these challenges it was necessary to hand hold them in terms of scientific training and guidance, easy approach to market for beneficial returns along with capacity building of these women in terms of skill development.

Keywords : Dairy farming, nutrition management, rural women, livelihood.

Introduction

Dairy farming serves as a vital income-generating activity, offering both employment opportunities and financial support to rural households. Livestock not only contributes to food and nutritional security for mankind but also plays an important role in utilization of non-edible agricultural byproducts. Milk production increased from 198.4 million tonnes in 2020 to 247.87 million tonnes during 2024-25 registering a growth of 3.58 % (BAHS 2025). Percapita availability of milk has sharply increased from 485 gm per day in 2025 (BAHS 2025). Sakthivel (2017) in a study reported that income from livestock rearing was the major contributor to rural household's income accounting for more than 50 % of the gross income for more than 60 percent of the households. Productive and reproductive performance of dairy cattle is mainly dependent on their nutritional status. The feeds alone contribute about 65 to 70% of the total cost of production. Animals are malnourished particularly with regards to micronutrients owing to lack of awareness among dairy farmers and lack of available information on micro and macro mineral status of soil, feed and dairy animals. A majority of the cattle owners do not prefer to feed commercial feeds because of higher cost. Due to variation in the dietary concentration owing to availability, season, location, the formulation of area wise mineral mixture becomes necessary. It is also observed that inspite of feeding liberally, the productivity is not achieved upto a desirable level. One of the reasons for this is the gap between availability of nutrients in the feedstuff and the requirements of nutrients by the animals.

Punjab is also a leading player in milk production in the country producing, 13.9 million tonnes of milk, of the total milk production in country which stands at 239.3 million tonnes (National Dairy Development Report 2023-2024). The scientific knowledge imparted through training, is crucial for the success of any enterprise. Dissemination of information on the latest developments in animal husbandry is necessary to support decision making capacity of the livestock farmers to improve the productivity of their stocks (Singh *et al*. 2016).

Women's participation in small-scale dairy farming is an important tool in reducing poverty and very vital to livestock development in rural area. Women constitute about 69% of workforce engaged in livestock sector (Anonymous 2013). The role and contribution of women members of every rural family to dairying is not documented although known too well in rural India as significant. About 75 million women as against 15 million men are engaged in dairying in India (Thakur and Chander 2006). Women increasingly play an important role in fostering inclusive development by contributing to household income and promoting community well-being. In developing economies, even small- scale ventures offer women a pathway to socio-economic empowerment, improved household welfare, and greater participation in decision-making (Gupta 2025). Despite societal barriers, rural women continue to demonstrate resilience and innovation through micro-enterprises that integrate livelihood generation with household responsibilities. However, widespread challenges persist, addressing these barriers through training, infrastructure support, and market linkages is essential to unlock the full potential of women- led dairy enterprises.

Against this backdrop, the present study was conducted under the Project “Interventions of low cost technologies for upliftment of rural women in Punjab” to examine the farm nutritional challenges faced by rural women engaged in dairy farming and to document them. By capturing women's lived experiences the study seeks to inform targeted interventions that strengthen dairy farming as a sustainable livelihood option.

Data Collection

The study was conducted in four villages Bharthala, Gehlewal, Rahaun, Bamb of Samrala block, Ludhiana district, Punjab, where the Project “Interventions of low cost technologies for upliftment of

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rural women in Punjab" was implemented from January, 2025 to January 2026. A purposive sample of 60 beneficiary rural women was drawn, comprising 15 respondents from each selected village. Data were collected through personal interviews using a structured interview schedule, supplemented with direct observations to ensure accuracy and reliability of responses.

Experimental Design

To examine the relative importance of various challenges faced by rural women engaged in dairy farming, Garrett's ranking technique was used for analysis of tabulated data.

According to Garrett's ranking technique by Garrett and Woodworth (1969), the respondents were asked to enlist and give ranks to different problems or constraints. This ranking helped in prioritizing the constraints based on their perceived importance. The orders of level as given by the interviewee were assigned ranks, with the help of following formula:

Percent position = $[100 (R_{ij} - 0.50)]/N_j$

Where,

R_{ij} = Rank given for i^{th} problem by j^{th} individual.

N = Number of problems ranked by the j^{th} individual.

After the respondents assigned ranks to the different problems, the percent position of each and every rank was transformed into scores by the use of Garrett's table. The counts for each and every responder were then totaled and divided by the total number of responders. This resulted in mean scores for each problem. Finally, the mean scores were arranged in descending order, and ranks were allotted to sort the constraints.

Statistical analysis

A perusal of Table 1 shows that limited scientific knowledge regarding various feeding technologies, lack of easy access to markets in addition to the high cost of compounded feeds for dairy animals was also impacting the day to day animal rearing activities by rural women. These problems necessitate supportive infrastructure, capacity building and resources tailored to their needs. Table 2 represent ranking pattern of responses of respondent women farmers for different constraints in Garrett's ranking technique. Table 3 represents Garrett value calculation and ranking of constraints perceived by rural women and enumerates the various challenges/problems faced by rural women in nutritional management of dairy animals. A significant number of respondents reported high cost of compounded feeds for dairy animals, had insufficient infrastructure at household level and had limited access to technical knowledge, resources. These were the major limiting factors. It was also seen that many rural women who were beneficiaries were also working with MNREGA scheme and hence could not manage to pay constant attention to care for animals and had to depend on other family members or relatives for the same. For some women (35 %), this support was also lacking and they were unable to provide the minimal resources required for dairy animals in some cases. This impacted their work efficiency and ability to make important decisions. Addressing these personal factors is crucial to ensure the wellbeing and productivity of women in rural areas. These findings agreed with those of many other researchers (Santosh *et al.,* 2021). Therefore, regular training and awareness programs on animal health management are essential to support rural farmers. Sharma and Singh (2008), Kumar *et al.*, (2019) and Pandey *et al.*, (2024) also reported similarly.

Table 1. Various challenges/problems faced by rural women (n=60)

Sr.No.	Challenges/problems	Percentage of respondents (n=60)
1	Lack of knowledge regarding balanced ration preparation	62.0
2	Lack of knowledge regarding area specific mineral mixture	71.0
3	Lack of knowledge regarding bypass nutrients and other feed additives	92.0
4	Lack of year round fodder availability	58.0
5	Unable to pay constant attention	76.0
6	Lack of support from family member	48.0
7	High cost of compounded feeds for dairy animals	84.0
8	Lack of access to land for cultivation of fodder crops	63.0
9	Insufficient infrastructure at household level	36.0
10	Lack of gender specific extension support	82.0
11	No preparation of silage and hay	44.0
12	Limited access to technical knowledge, resources	85.0
13	Limited access to markets and mass media	78.0
14	Awareness of feed additives, anti-nutritional factors	61.0

Table 2. Ranking pattern of responses from beneficiary rural women (n=60) for different challenges in Garrett's ranking technique

Challenges/ problems	1st	2nd	3rd	4th	5th	6th
Lack of knowledge regarding balanced ration preparation	21	16	10	7	4	2
Lack of knowledge regarding area specific mineral mixture	22	11	10	95	3	
Lack of knowledge regarding bypass nutrients and other feed additives	14	20	16	4	3	3
Lack of year round fodder availability	78	13	8	10	1	4
Unable to pay constant attention	5	7	11	11	9	17

Lack of support from family member	6	6	12	11	10	15
High cost of compounded feeds for dairy animals	32	11	5	5	4	3
Lack of access to land for cultivation of fodder crops	14	20	16	4	3	3
Insufficient infrastructure at household level	24	14	10	8	3	1
Lack of gender specific extension support	22	14	9	7	5	3
No preparation of silage and hay	7	9	14	16	7	7
Limited access to technical knowledge, resources	22	11	10	9	5	3
Limited access to markets and mass media	14	20	16	4	3	3
Awareness of feed additives, anti-nutritional factors	16	14	10	6	7	7

Table 3. Calculation of Garret Value and Ranking by beneficiary women (n=60)

Challenges/ problems	Rank order						Total score	Average score	Rank
	1st	2nd	3rd	4th	5th	6th			
Lack of knowledge regarding balanced ration preparation	1785	1184	680	441	236	112	4438	73.97	III
Lack of knowledge regarding area specific mineral mixture	1870	814	680	567	295	168	4394	73.23	V
Lack of knowledge regarding bypass nutrients and other feed additives	1190	1480	1088	252	177	168	4355	72.58	VI
Lack of year round fodder availability	595	592	884	504	590	784	3949	65.82	IX
Unable to pay constant attention	425	518	748	693	531	952	3867	64.45	XI
Lack of support from family member	510	444	816	693	590	840	3893	64.88	X
High cost of compounded feeds for dairy animals	2720	814	340	315	236	168	4593	76.55	I
Lack of access to land for cultivation of fodder crops	1190	1480	1088	252	177	168	4355	72.58	VI
Insufficient infrastructure at household level	2040	1036	680	504	177	56	4493	74.88	II
Lack of gender specific extension support	1870	1036	612	441	295	168	4422	73.7	IV
No preparation of silage and hay	595	666	952	1008	413	392	4026	67.1	VIII
Limited access to technical knowledge, resources	1870	814	680	567	295	168	4394	73.23	V
Limited access to markets and mass media	1190	1480	1088	252	177	168	4355	72.58	VI
Awareness of feed additives, anti-nutritional factors	1360	1036	680	378	413	392	4259	70.98	VII

Conclusion

Women participation in managing livestock was significantly higher than men however the constraints limited the realization of their potential. Poor access to training, preoccupation with other household chores, lack of participation in decision making, costly inputs like feed, additives, veterinary services etc. for higher animal productivity were the major constraints faced by them. Therefore, women need to be provided with technical training on scientific livestock farming in order to strengthen and encourage their participation in livestock sector. These findings emphasize the need for targeted policy implementation and gender specific extension support to strengthen dairy farming as a sustainable and profitable enterprise for rural women.

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References

Anonymous. 2013. National Livestock Policy. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, GoI, New Delhi.

BAHS (Basic Animal Husbandry and Statistics). 2025. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India, New Delhi.

Garrett, H.E. and Woodworth, R.S. 1969. Statistics in psychology and education. Vakils, Feffer and Simons Pvt. Ltd. Bombay. pp. 329.

Gupta, A. Assessing women's time allocation and empowerment in rural India. arXiv [Preprint]. 2025. Available from: https://arxiv.org/abs/2504.03202

Kumar, R., Sharma, M.C., Gurjar, M.L. and Kumar, R. 2019. Socio-economic characteristic of tribal goat keepers of Dungarpur district of Rajasthan state. *Journal of Entomology and Zoology Studies* 7 (4): 1101-4.

National Dairy Development Report 2016-17 https://www.nddb.coop/information/ stats/milkprodstate

Pandey, S., Mishra, S., Jha, A. K. and Pandey, H. 2024. Women in Indian dairy farming business: Significance, challenges, and way forward. International Journal of Veterinary Sciences and Animal Husbandry 2024; SP-9(1): 701-708.

Sakthivel, K. M. 2017. Role and contribution of livestock in the livelihood of marginal and landless livestock farmers in rural Tamil Nadu, India. Indian Research Journal of Extension Education (Special issue on Veterinary Research & Extension): 20-25.

Santosh, S. P., Singh, B. P., Chander, M., Bardhan, D., Kannadhasan, M.S. and Khode, N. V. 2021. Perceived constraints and suggestions in adoption of animal husbandry technologies: A study in semi-arid zone of Maharashtra. Haryana Veterinarian, 60(1): 8-12.

Sharma, K. and Singh, S.P. 2008. Dairy farmer's knowledge about buffalo feeding practices. Haryana Veterinarian, 47: 68-71.

Singh, A., Sidhu, S., Hundal, J.S. and Chahal, U.S. 2016. Information sources for Indian livestock farmers. Journal of Livestock Science, 7: 150-156

Thakur, D. and Chandar, M. 2006. Gender based differential access to inform among livestock owners and it's impact on house hold milk production in Kangra district of Himachal Pradesh. Indian Journal of Dairy Science 59(6): 401- 404.