



ISSN 2228-7701

Online Journal of Animal and Feed Research



BOOKLET

Online Journal of Animal and Feed Research

An international peer-reviewed journal which publishes in electronic format (online)

Online J. Anim. Feed Res., 15 (6): 310-386; November 2025

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Volume 15 (6); November 2025 [Booklet] [EndNote XML for Agris]

Research Paper

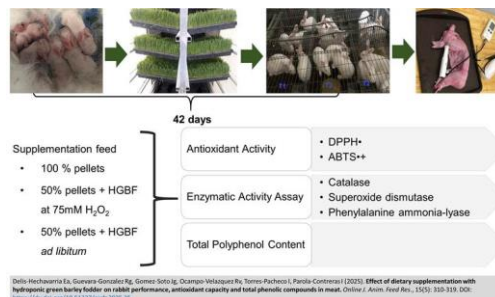
Effect of dietary supplementation with hydroponic green barley fodder on rabbit performance, antioxidant capacity and total phenolic compounds in meat

Delis-Hechavarria Ea, Guevara-Gonzalez Rg, Gomez-Soto Jg, Ocampo-Velazquez Rv, Torres-Pacheco I, Parola-Contreras I.

Online J. Anim. Feed Res., 15(6): 310-319, 2025; pii: S222877012500035-15
DOI: <https://dx.doi.org/10.51227/ojafnr.2025.35>

Abstract

Rabbit meat stands out as a nutritious low-fat protein source rich in essential amino acids, vitamins, and minerals, yet its production faces challenges from high feed costs and environmental impacts. Hydroponic green barley fodder (HGBF) emerges as a promising sustainable alternative, offering rapid growth, enhanced nutrient profiles including antioxidants, and reduced resource demands. This study aimed to evaluate the effects of dietary HGBF supplementation, with or without hydrogen peroxide (H₂O₂) seed pre-treatment, on rabbit performance, meat antioxidant capacity, and total phenolic compounds. Sixty-three post-weaned New Zealand rabbits (initial weight: 606 g) were divided into three groups for 42 days: T1 (100% pellets, control), T2 (50% pellets + 50% HGBF at 75 mM H₂O₂ pre-treatment), and T3 (50% pellets + 50% HGBF), All treatments were ad libitum. Carcass yields were assessed post-slaughter, while meat samples underwent 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) assays for antioxidant activity, enzymatic evaluations (catalase, superoxide dismutase, phenylalanine ammonia-lyase), and Folin-Ciocalteu method for phenolics. Results revealed no significant differences in hot or cold carcass weights across treatments, averaging below export standards due to the rabbits' young age. However, T2 diet significantly boosted total phenolic content (P < 0.05) and antioxidant capacity via DPPH (97.52% inhibition) and ABTS^{•+} (84.07% inhibition) compared to T1 and T3 groups with lower catalase activity indicating reduced oxidative stress. Enzymatic activities for superoxide dismutase and phenylalanine ammonia-lyase remained unaffected. These findings underscore HGBF with H₂O₂ pre-treatment as an effective strategy to enhance meat quality through elevated phenolics and antioxidants, fostering oxidative stability and nutritional value while promoting eco-friendly rabbit farming practices for broader livestock sustainability.



Delis-Hechavarria Ea, Guevara-Gonzalez Rg, Gomez-Soto Jg, Ocampo-Velazquez Rv, Torres-Pacheco I, Parola-Contreras I (2025). Effect of dietary supplementation with hydroponic green barley fodder on rabbit performance, antioxidant capacity and total phenolic compounds in meat. Online J. Anim. Feed Res., 15(6): 310-319. DOI: <https://dx.doi.org/10.51227/ojafnr.2025.35>

Keywords: Antioxidants, Carcass yield, Hydroponic green barley fodder, Oxidative stability, Rabbit meat, Total phenolic compounds.

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Research Paper

Evaluation of *Wedelia trilobata* (L.) Hitchc. as a forage for reproductive rabbits does in a tropical environment

Mo D and Chau NTV.

Online J. Anim. Feed Res., 15(6): 320-327, 2025; pii: S222877012500036-15
DOI: <https://dx.doi.org/10.51227/ojafnr.2025.36>

Abstract

In this study, *Wedelia trilobata* (L.) Hitchc. was evaluated as forage for reproductive rabbit does in a tropical environment. Eighteen crossbred does (New Zealand White × Local) were fed six dietary treatments in a Completely Randomized Design (CRD) with three replications. Six diets were designated as W0, W25, W40, W50, W60, and Wa, corresponding to *W. trilobata* levels of 0, 25, 40, 50, and 60% of concentrate intake (dry matter, DM, basis), ad libitum. The trial lasted nine months, covering three reproductive cycles. Feed and nutrient intake, reproductive performance, and milk yield were measured. *W. trilobata* contained 9.25% crude protein (CP), similar to *Brachiaria mutica*, but had higher metabolizable energy (ME: 10.3 MJ/kg DM) and lower fiber (CF: 18.9%, NDF: 40.4%, and ADF: 27.9%). Nutrient intake peaked at moderate inclusion (W40-W60), with DM intake of 124-132 g/day compared with 113 g in the W0 and 103 g in the Wa (P < 0.05). The W50 group had the highest CP intake (23.7 g/day), while the greatest ME intake was 1.52 MJ/day. Rabbit does in the moderate groups were heavier before kindling (2957-2958 g) than the control (2736 g) and produced more milk (86.9-88.2 g/day) than the W25 group (74.7 g/day) (P < 0.05). Litter size and weights did not differ significantly (P > 0.05) among treatments. In conclusion, including *W. trilobata* at 40-60% of concentrate intake improved doe condition and milk yield



Mo D and Chau NTV (2025). Evaluation of *Wedelia trilobata* (L.) Hitchc. as a forage for reproductive rabbits does in a tropical environment. Online J. Anim. Feed Res., 15(6): 320-327. DOI: <https://dx.doi.org/10.51227/ojafnr.2025.36>

without affecting litter size or weight. Its abundance makes it practical forage for smallholder rabbit production in tropical climates.

Keywords: Alternative forage, Rabbit does, Reproductive performance, Sustainable livestock breeding, Tropical climate, *Wedelia trilobata*.

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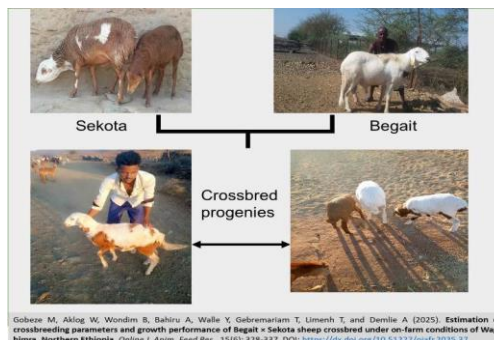
Estimation of crossbreeding parameters and growth performance of Begait × Sekota sheep crossbred under on-farm conditions of Wag-himra, Northern Ethiopia

Gobeze M, Aklog W, Wondim B, Bahiru A, Walle Y, Gebremariam T, Limenh T, and Demlie A.

Online J. Anim. Feed Res., 15(6): 328-337, 2025; pii: S222877012500037-15
DOI: <https://dx.doi.org/10.51227/ojaf.2025.37>

Abstract

Crossbreeding programs using either exotic or indigenous sires are an appropriate and rapid way to improve productivity within a shorter generation interval. Therefore, this study was designed to evaluate the growth performance traits of individual crossbreeding parameters of Sekota x Begait sheep crossbred under on-farm conditions in the lowland areas of Wag-himra, Northern Ethiopia. The cross-breeding program was conducted from 2018 to 2022 in Kasayred village of Abergele district on 699 animals. Least-squares means for weight at different ages and average daily weight gains were obtained by fitting the general linear model procedure in the SAS software. Crossbreeding parameters, including additive, heterosis, and recombination loss, were derived by fitting them as fixed covariates. The overall least square means for birth weight (BWt), three-month weight (TMWt), six-month weight (SMWt), nine-month weight (NMWt), and yearling weight (YWt) of evaluated progenies were 2.49±0.02, 11.61±0.09, 15.66±0.17, 19.27±0.18, and 23.46±0.23 kg, respectively. The overall least square means for average daily weight gain up to weaning (ADG1), six-month (ADG2), nine-month (ADG3), and yearling (ADG4) were 101.03±1.00, 45.30±1.38, 40.93±1.20, and 46.07±0.90 g/day. Only the additive effect of BWt for the Begait sheep breed was significant and it was found as 0.35±0.13 kg. Heterosis contributed 27-39 % live body weight improvement for Sekota sheep at yearling and six-month weights. In general, this pilot crossbreeding program resulted in 24% to 53% yearling weight improvement of pure Sekota breed (50%, 75%, and 25% blood levels, respectively), and participating farmers in the program witnessed that it was important in terms of improvement in phenotypic traits, growth rates, and market values. Based on the result of this study, we recommend establishing a 50% crossbred ram multiplication center in the area for long-term operation and further evaluating other blood levels by incorporating carcass and reproductive performance traits.



Gobeze M, Aklog W, Wondim B, Bahiru A, Walle Y, Gebremariam T, Limenh T, and Demlie A (2025) Estimation of crossbreeding parameters and growth performance of Begait × Sekota sheep crossbred under on-farm conditions of Wag-himra, Northern Ethiopia. *Online J. Anim. Feed Res.*, 15(6): 328-337. DOI: <https://dx.doi.org/10.51227/ojaf.2025.37>

Keywords: Begait sheep, Crossbreeding, Farmers management practice, Phenotypic traits, Sekota sheep

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Research Paper

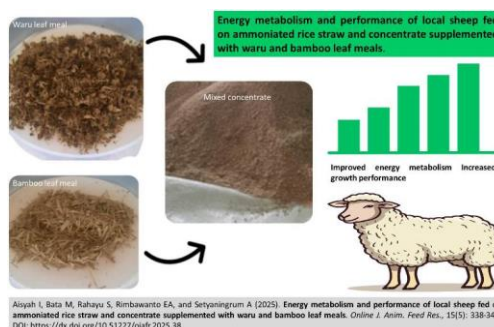
Energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate supplemented with waru and bamboo leaf meals

Aisyah I, Bata M, Rahayu S, Rimbawanto EA, and Setyaningrum A.

Online J. Anim. Feed Res., 15(6): 338-344, 2025; pii: S222877012500038-15
DOI: <https://dx.doi.org/10.51227/ojaf.2025.38>

Abstract

This study evaluated the effects of waru (*Hibiscus tiliaceus*) leaf meal (WLM) and bamboo (*Gigantochloa apus*) leaf meal (BLM) supplementation on energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate. Sixteen male local sheep aged 12–18 months with initial average body weight of 27.1 ± 2.11 kg (7.78% coefficient of variation) were completely randomized according to a Completely Randomized Design (CRD) to receive one of the following treatments: T0 (ammoniated rice straw + concentrate feed); T1 (T0 + 2.49 g WLM); T2 (T0 + 1.87 g WLM + 0.33 g BLM); and T3 (T0 + 1.25 g WLM + 0.65 g BLM). The concentrate was provided at 3% of live weight, and ammoniated rice straw was available on a controlled *ad libitum*. Analysis of variance indicated that WLM and BLM supplementation significantly increased digestible energy (DE), metabolizable energy (ME), energy retention (ER), ER to EI efficiency, ER to DE efficiency, average daily gain (ADG), and consumption rate (CR), while significantly decreasing the feed conversion ratio (FCR) (P<0.01). However, the treatments had no significant effect (P>0.05) on dry matter intake (DMI) and energy intake (EI). In conclusion, dietary



supplementation with 1.87 g of WLM and 0.33 g of BLM per kg concentrate was more effective than the other combinations in improving energy metabolism and performance of local sheep.

Keywords: Energy, Feed conversion, Flavonoid, Metabolism, Sheep nutrition.

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Research Paper

Prevalence, epidemiology and histopathology of *Ascaridia galli* infections in backyard chickens in Gazipur district, Bangladesh

Khatun F, Al Mahmud MA, Akhi IA, Sadi RS, Das ZC, Islam MS, Islam MT.

Online J. Anim. Feed Res., 15(6): 345-353, 2025; pii: S222877012500039-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.39>

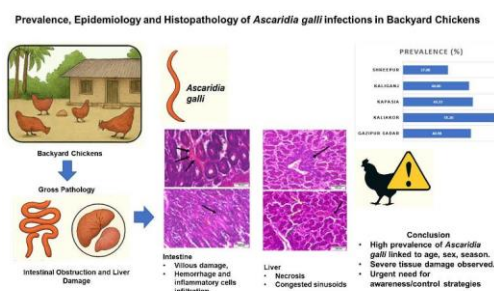
Abstract

Ascaridia galli is responsible for weight loss, emaciation, anemia, decreased egg production, and in severe cases, intestinal obstruction and death in chickens, resulting in significant economic losses for the poultry industry. This study aimed to assess the present status of *Ascaridia galli* in backyard chickens in Gazipur district. Additionally, the study examined histopathological changes in the intestines and livers of chickens heavily infested with this nematode. Postmortem examination, helminth collection and identification were conducted on 237 backyard chickens from March 2023 to February 2024. The overall prevalence was found to be 40.51%, with Kaliakair upazila showing the highest prevalence at 55.26%, while Sreepur upazila had the lowest at 27.08%.

Factors such as season, sex, and age significantly influenced the incidence of ascariasis in backyard chickens. Adults exhibited the highest occurrence rate at 44.88%, with males (44.19%) showing a greater prevalence than females (36.22%). The highest incidence was recorded in the summer at 57.14%. In cases of severe infection, *Ascaridia galli* obstructs the small intestine, leading to inflamed, hemorrhagic intestines that produce excess mucus. The livers of the infected chickens appeared swollen, enlarged, and pale, with signs of congestion, hemorrhage, and inflammation. Histopathological analysis revealed hemorrhage and inflammatory cell infiltration in the intestines, along with damaged and lacerated intestinal tissues. The livers showed congestion, ruptured blood vessels, inflammation, hemorrhage, and necrosis. In conclusion, this study identifies potential risk factors associated with *Ascaridia galli* infection in backyard chickens in Gazipur district, offering insights that could aid in controlling and preventing such infections.

Keywords: *Ascaridia galli*, Domestic birds, Epidemiology, Histopathology, Poultry health.

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Khatun F, Al Mahmud MA, Akhi IA, Sadi RS, Das ZC, Islam MS, Islam MT. (2025). Prevalence, epidemiology and histopathology of *Ascaridia galli* infections in backyard chickens in Gazipur district, Bangladesh. Online J. Anim. Feed Res., 15(6): 345-353. DOI: <https://dx.doi.org/10.51227/ojafr.2025.39>

Research Paper

Chemical composition of soybean meal and its effects on nutrient density, egg production, and blood parameters in Bovans Brown layers

Yimer Y, Demeke S. and Tegegne F.

Online J. Anim. Feed Res., 15(6): 354-362, 2025; pii: S222877012500040-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.40>

Abstract

Soybean meal (SBM) is an essential protein source in poultry diets. However, data on the variability of its chemical composition from Ethiopian processing plants and its optimal inclusion levels in layer diets are limited. This study aimed to assess the nutritional quality of locally sourced SBM from the Amhara Region and evaluate the effects of different levels of SBM inclusion on production performance, egg quality, and blood parameters in laying hens. The chemical composition of SBM samples from 11 regional processing plants was initially analyzed. Subsequently, a 12-week feeding trial was conducted using 120, 34-week-old Bovans Brown layers. The hens were randomly assigned to one of five dietary treatments in a completely randomized design (CRD). The treatments consisted of a commercial control diet and four experimental diets containing 15%, 20%, 25%, and 30% SBM. Each treatment was replicated three times, with eight hens per replicate. Significant variation ($P < 0.05$) was observed in the chemical composition of SBM samples from different processors. Crude protein ranged from 38.42 to 43.52%, ether extract (1.23 - 5.27%), crude fiber (5.58 - 7.35%), and metabolizable energy (3348.3 - 3594.8 kcal/kg DM). Dietary crude protein concentration increased with higher SBM inclusion from 14.74% to 17.56%. An inclusion level of 20% SBM was identified as optimal, resulting in the highest hen-day egg production (89.72%) and net return (446.63 ETB), with no adverse effects on egg quality and



Yimer Y, Demeke S and Tegegne F. (2025). Chemical composition of soybean meal and its effects on nutrient density, egg production, and blood parameters in Bovans Brown layers. Online J. Anim. Feed Res., 15(6): 354-362. DOI: <https://dx.doi.org/10.51227/ojafr.2025.40>

hematological parameters remaining within normal ranges. This study concludes that there is significant variation in the chemical composition of locally processed SBMs, highlighting the need for pre-use nutritional evaluation. Under the experimental conditions, a 20% SBM inclusion level is regarded as acceptable and optimal for mid-laying hens.

Keywords: Chemical composition, Egg production, Egg quality, Nutrient density, Soybean meal.

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Impact of pellet binder type on the quality and performance of pellet mills with different levels of oil and fat powder

Emadi M, Sephri Moghadam H, and Salehan M.

Online J. Anim. Feed Res., 15(6): 363-370, 2025; pii: S222877012500041-15
DOI: <https://dx.doi.org/10.51227/ojaf.2025.41>

Abstract

The current study aimed to assess the effects of fat powder levels, oil levels, and two types of pellet binders, including bentonite and gum Tragacanth on the physical quality and pellet durability index (PDI) over 75 days. The experiment was conducted using a completely randomized design with a factorial arrangement. Experimental treatments included 5 to 10 kilograms of fat powder, 7 to 11 kilograms of oil, and pellet binders. The pellet binders consisted of bentonite, plant-based binders such as gum Tragacanth, and their combination. The present results indicated that using fat powder and oil levels, with pellet binders, significantly increased PDI in the starter period. The concentration and type of pellet binder were effective parameters in treatments during the experiment. Fat powder significantly improved the physical quality of the pellet on days 15, 30, 60, and 75, while oil notably improved the effects of the pellet on all days except day 0. Fat powder, oil, and pellet binder played crucial roles in determining the physical quality of pellets during the finisher period across all days. The interaction between fat powder and oil substantially affected finisher pellet quality on all days except days 15 and 45. Oil levels did not have impacts on the physical quality of the finisher pellets. Gum Tragacanth binder demonstrated the highest average physical pellet quality on days 0, 45, 60, and 75, while bentonite exhibited the best performance on days 15 and 30. The utilization of pellet binders combined with adding oil and fat powder to the diet, improve the physical qualities of the produced pellets.

Keywords: Pellet binder, Pellet durability index, Pellet hardness, Fat powder, Oil

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Research Paper

Influence of dietary supplemented glutamate on growth performance, haematological and serum indices of broiler chickens in hot tropical environment

Adeyemo AA, Abdulahi UO, Adeyemo AJ, Iyanda AI, Adekola TO and Adekunle EO.

Online J. Anim. Feed Res., 15(6): 371-377, 2025; pii: S222877012500042-15
DOI: <https://dx.doi.org/10.51227/ojaf.2025.42>

Abstract

This study investigated the impact of glutamate on growth, haematological and serum indices of broiler chickens in hot tropical environment (> 30°C). One-hundred-and-sixty-day old Arbor acre male broiler chicks were randomly allotted into four dietary treatment groups of four replicates having 10 chicks per replicate in a completely randomized design. Dietary treatments groups contain different inclusion levels of glutamate (basal diet + 0%, 0.3%, 0.6% and 0.9% glutamate (G), in an open sided pen). The experiment lasted for seven weeks during which data were collected on growth, haematological and serum indices. Result showed birds fed diet containing 0.9% G at the starter phase had ($P < 0.05$) increased feed intake compared to other treatment groups. Inclusion of 0.6% G at the finisher phase ($P < 0.05$) improved final weight and weight gain in birds compared to 0.3% G group. Best feed conversion ratio ($P < 0.05$) was obtained from birds fed diet containing 0.6% G compared to birds on 0.3% and 0.9% inclusion. Inclusion of 0.6% G at the starter phase ($P < 0.05$) improved total protein. Increased mean corpuscular haemoglobin ($P < 0.05$) was obtained from birds on 0.6% G compared to lower mean values obtained from birds on 0.3% G. The study concluded that inclusion of glutamate in the diet of broiler chickens at 0.6% improved final weight, weight gain and feed conversion ratio without causing any adverse effect on blood indices of broiler chickens as glutamate increased serum protein and mean corpuscular haemoglobin of broiler chickens.

Keywords: Blood profile, Broiler chickens, Essential nutrients, Glutamic acid, Growth response.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]



Adeyemo AA, Abdulahi UO, Adeyemo AJ, Iyanda AI, Adekola TO and Adekunle EO (2025). Influence of dietary supplemented glutamate on growth performance, haematological and serum indices of broiler chickens in hot tropical environment. *Online J. Anim. Feed Res.*, 15(6): 371-377. DOI: <https://dx.doi.org/10.51227/ojaf.2025.42>

The addition of pumpkin seed oil to common carp diets affects several physiological and productive characteristics

Alhamadani ASA-A, Negaud KZ, Fazaa HA, and Sayed-Lafi RM.

Online J. Anim. Feed Res., 15(6): 378-386, 2025; pii: S222877012500043-15

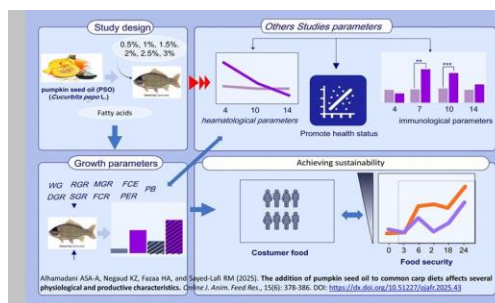
DOI: <https://dx.doi.org/10.51227/ojaf.2025.43>

Abstract

This study aimed to evaluate the effect of adding different levels of pumpkin seed oil (PSO) (*Cucurbita pepo* L.) on specific growth and immune blood parameters in common carp (*Cyprinus carpio* L.). Six oil concentrations were tested, representing six treatments: PSO at 0% (control), 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%. All treatments were incorporated into a diet containing 29.14% protein. A total of 147 fish were divided into 21 cages, with three replicates per treatment, averaging 32±0.72 g in weight. The fish were fed at 3% of biomass per day, split into two meals; rations were adjusted biweekly according to measured biomass for 82 days. The 2% PSO treatment showed significantly better results ($P < 0.05$) across all growth parameters studied, including Final Weight (FW), Weight Gain (WG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Specific Growth Rate (SGR), Food Intake (FI), Metabolic Growth Rate (MGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Efficiency Ratio (PER). Followed by the PSO 1.5% treatment. In blood parameters, PSO 2% also performed best in all measured aspects—Red Blood Cells (RBCs), Hemoglobin (Hp), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)—with 1.5% PSO closely behind. In immunological parameters, 2% PSO increased the value of WBCs, TPS, and IgM, with 1.5% PSO just behind. Blood performance, which assesses overall blood health as a single measure, showed the highest performance in the 2% PSO treatment, significantly surpassing other treatments ($P < 0.05$). Overall, the results indicated that adding different levels of PSO to carp diets has positive effects at a rate not exceeding 2%.

Keywords: Common carp, *Cucurbita pepo*, Fatty acids, Fish nutrition, Pumpkin.

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Online Journal of Animal and Feed Research



ISSN 2228-7701

ISSN: 2228-7701

Frequency: Bimonthly

Current Issue: 2025, Vol: 15, No: 6 (November)

DOI Prefix: [10.51227](https://doi.org/10.51227)

Publisher: [SCIENCELINE](https://www.science-line.com)

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EFFECT OF DIETARY SUPPLEMENTATION WITH HYDROPONIC GREEN BARLEY FODDER ON RABBIT PERFORMANCE, ANTIOXIDANT CAPACITY, AND TOTAL PHENOLIC COMPOUNDS IN MEAT

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➤ Supporting Information



ABSTRACT: Rabbit meat stands out as a nutritious low-fat protein source rich in essential amino acids, vitamins, and minerals, yet its production faces challenges from high feed costs and environmental impacts. Hydroponic green barley fodder (HGBF) emerges as a promising sustainable alternative, offering rapid growth, enhanced nutrient profiles including antioxidants, and reduced resource demands. This study aimed to evaluate the effects of dietary HGBF supplementation, with or without hydrogen peroxide (H₂O₂) seed pre-treatment, on rabbit performance, meat antioxidant capacity, and total phenolic compounds. Sixty-three post-weaned New Zealand rabbits (initial weight: 606 g) were divided into three groups for 42 days: T1 (100% pellets, control), T2 (50% pellets + 50% HGBF at 75 mM H₂O₂ pre-treatment), and T3 (50% pellets + 50% HGBF). All treatments were ad libitum. Carcass yields were assessed post-slaughter, while meat samples underwent 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) assays for antioxidant activity, enzymatic evaluations (catalase, superoxide dismutase, phenylalanine ammonia-lyase), and Folin-Ciocalteu method for phenolics. Results revealed no significant differences in hot or cold carcass weights across treatments, averaging below export standards due to the rabbits' young age. However, T2 diet significantly boosted total phenolic content ($P < 0.05$) and antioxidant capacity via DPPH (97.52% inhibition) and ABTS^{•+} (84.07% inhibition) compared to T1 and T3 groups with lower catalase activity indicating reduced oxidative stress. Enzymatic activities for superoxide dismutase and phenylalanine ammonia-lyase remained unaffected. These findings underscore HGBF with H₂O₂ pre-treatment as an effective strategy to enhance meat quality through elevated phenolics and antioxidants, fostering oxidative stability and nutritional value while promoting eco-friendly rabbit farming practices for broader livestock sustainability.

Keywords: Antioxidants, Carcass yield, Hydroponic green barley fodder, Oxidative stability, Rabbit meat, Total phenolic compounds.

INTRODUCTION

Rabbit meat is consumed all over the world, being delicious, appetizing and high quality. Its composition is mainly lipids, proteins and water, with high levels of micronutrients, essential amino acids, vitamins and minerals. Additionally, it has low uric acid, purines, and fat (Dalle Zotte and Szendro, 2011). However, it has been studied that the composition of the lipids depends on the type of rabbits feeding, being reflected in carcass composition (Oo et al., 2018). Furthermore, rabbit production is economically relevant for their sporting utility and the manufacture of skins that are used in multiple products (Pelletier et al., 2019). Furthermore, this species, the New Zealand rabbit, is commonly utilized in biological experiments within educational institutions and hospitals, serving as a valuable model for nutritional and antioxidant research (Song et al., 2020). Rising rabbits demand large volume of raw plant material to fattening (Wang et al., 2020). Fattening rabbits requires substantial plant-based feed, including tubers, cereals, fruits, and vegetables. Wild rabbits mainly feed of herbaceous and grasses, which contain considerable quantities of cellulose (Grioui et al., 2021).

In 2015, global cereal production reached 10 million tons; about 60% of world consumption of coarse grains (barley, maize, oats, quinoa, rye, sorghum, tef, and millet) used for livestock feed (FAO, 2021). Cereals also are produced for fodder livestock, since they can be converted directly into protein usable by animals, lowering the cost of feed, accelerating around 25% of growing and diminish time of fattening (Miah et al., 2020). Barley, constituting 65% of

RESEARCH ARTICLE
 PII: S222877012500035-15
 Received: June 28, 2025
 Revised: October 30, 2025
 Accepted: November 02, 2025

livestock feed. Whole grain barley includes flavonoids, folates, lignans, phenolic acids, phytosterols and tocols, with the predominant vitamin E (tocol), flavonoids, phenols, sterols and vitamins. These phytochemicals offer strong antioxidant capacity, useful for reducing free radicals. In addition, this specie is rich in dietary fiber made up of soluble and insoluble fibers (Abouelezz et al., 2019). Hydroponic Green Barley Fodder (HGBF) yields 7–10 kg per kg of grain in 8–15 days, with increased crude protein, and calcium content; additionally, acid detergent fiber, neutral detergent fiber, calcium content and crude protein are increased (Singh et al., 2019).

Furthermore, rabbit meat production requires diets that mixture between 100 to 200 g of HGBF and a daily average of 50 g of concentrates, per animal, for optimal production (Miah et al., 2020). Furthermore, HGBF includes great fiber content, that it is necessary for rabbits' digestion and nutrition (Delis-Hechavarría et al., 2021). Besides, Selim et al. (2021) obtained a final live weight gain (1,533 g) with a supplementation of 15 g/kg with *Moringa oleifera*. Additionally, Abouelezz et al. (2019) had the highest body weight in male rabbits using concentrated plus HGBF and the feed intake was similar in all treatments. Indeed, the mixture of two feeds allowed the absorption of nutrients for growth, which could have a benign effect on the characteristic of rabbit meat (Dalle Zotte et al., 2018). Meat is susceptible of losing its nutritional properties due to the action of the oxidation process on lipids to rather than the degradation process by bacteria (Chen et al., 2017). This deterioration is observed in the discoloration of the meat. For example, Beefs are most susceptible to this oxidation process, reducing quality and shelf life (Bouzaida et al., 2021). The chemical processes involved in this process are the water retention capacity, rancid odors due to the formation of aldehydes and ketones, reduced solubility of proteins (Kim et al., 2019). However, studies on the concentrated diet plus HGBF are very scarce. Therefore, in this manuscript the effect of two combinations of barley fodder one with seeds pre germination on 75 mM hydrogen peroxide (H₂O₂) and barley fodder with water supplemented plus 50% pellet, were evaluated on yield carcass and rabbit meat antioxidant capacity.

MATERIALS AND METHODS

Plant material and growth conditions

Hydroponic Green Barley Fodder (HGBF) was cultivated at Innovation and development Green (IDGREEN) farm, La Norita, Queretaro, México (20°32'58.6"N -100°30'10.6"W; altitude 1823 m) in January-February 2021. Barley seeds (*Hordeum vulgare* L var. Cantabria) were soaked in water and 75 mM hydrogen peroxide concentration for 24 h. It drained and placed on trays of 54 × 35 × 7 cm. The trays were stored in a chamber fodder for 11 days at 25 °C, 14 h light:10 h dark photoperiod, and relative humidity of 80%. Fodder was watered only with water, six times daily for two min each one. Rabbits were fed with HGBF (leaves, roots and seeds) immediately after harvesting, including (Morales et al., 2010).

Animal and materials

Sixty-three post-weaned New Zealand rabbits (average initial body weight (BW): 0.606 ± 0.01 kg) were housed in four wire cages (76 × 48 × 30 cm) containing three rabbits for each treatment. The temperature in the rabbitry was 15 ± 2 °C, 41.10 ± 10.68% relative air humidity and 12-h light: 12-h dark photoperiod. Rabbits had free access to clean water. The 42 days study included three fed diets: T1 (100 % pellets), T2 (50% pellets + HGBF at 75mM H₂O₂), T3 (50% pellets + HGBF *ad libitum*). All treatments were *ad libitum*. Rabbits were weighed and tranquilized with azaperone (2 mg/kg BW), anaesthetized with isoflurane (inhaled) and euthanized using a cardiac overdose of sodium pentobarbital (50 mg/kg BW) (Gómez Soto et al., 2018).

Ethical regulations

The study was performed at DGREEN farm. The protocol was reviewed and approved by the Autonomous University of Queretaro's Bioethics Committee (authorization: 90-FI-2017) of the Engineering Science Faculty's. The handling of the experimental animals was carried out conforming to the Mexican Official Norm 'NOM-062-ZOO-1999' guidelines (Norma Oficial Mexicana, 1999) and the International Guiding Principles for Biomedical Research Involving Animals (CIOMS/ICLAS 2012).

Sampling

Analyses were carried out at the Animal Nutrition Laboratory, Faculty of Natural Sciences, Autonomous University of Queretaro, Queretaro, Mexico. The stomach, jejunum, ileum, cecum, and colon were dissected, and emptied contents measured a potentiometer (Model PH211, Hanna Instruments®). Jejunum-ileum, liver, gallbladder, stomach, colon-rectum and cecum sections were collected, washed with saline solution, weighed, and preserved in 10% neutral formalin solution for further analyses (Gómez Soto et al., 2018).

Antioxidant activity (AOx)

Antioxidant properties of rabbit meat from each treatment were assessed using the 1,1-diphenyl-2-picrylhydrazyl

(DPPH•) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) assays.

DPPH• Radical Scavenging Activity (DRSA) Assay

The DPPH• assay was adapted from [Chen et al. \(2012\)](#) with modifications. A 0.5 mL sample solution's aliquot (600 µg/mL) was combined with 0.5 mL of 0.1 mM DPPH• in methanol. The mixture was vigorously mixed, incubated in darkness at ambient temperature for 30 min, and absorbance was recorded at 515 nm. Methanol replaced DPPH• for the blank, and deionized water replaced the sample for the control. The DRSA percentage was calculated using the equation (1): $DRSA(\%) = [1 - (A_{\text{sample}} - A_{\text{blank}} / A_{\text{control}})] \times 100$ (1)

where A_{sample} is the sample absorbance, A_{blank} is the blank absorbance, and A_{control} is the control absorbance.

ABTS^{•+} radical scavenging activity (ARSA) assay

The ARSA assay was conducted following [Re et al. \(1999\)](#) with adjustments. ABTS^{•+} radicals were produced by reacting 7 mM ABTS^{•+} with 2.45 mM potassium persulfate, incubating in darkness for 12 h at room temperature. The ABTS^{•+} solution was diluted in 0.15 M phosphate-buffered saline (PBS, pH 7.4) to achieve an absorbance of ~0.7 at 734 nm. Then, 3 mL of diluted ABTS^{•+} was mixed with 150 µL of sample (600 µg/mL), and absorbance was measured under dark conditions. PBS replaced ABTS^{•+} for the blank, and deionized water served as the control. ARSA was calculated as (2): $ARSA(\%) = [1 - (A_{\text{sample}} - A_{\text{blank}} / A_{\text{control}})] \times 100$ (2)

where A_{sample} is the sample absorbance, A_{blank} is the blank absorbance, and A_{control} is the control absorbance.

Enzyme activity assays

Enzyme activities in rabbit meat were evaluated to assess oxidative stress responses, focusing on catalase (CAT), superoxide dismutase (SOD), and phenylalanine ammonia-lyase (PAL).

Samples preparation of enzymatic assays

Lyophilized rabbit meat samples (0.5 g) were homogenized in 1 mL of chilled extraction buffer and centrifuged at 13,000 rpm for 20 min at 4°C. The resulting supernatant was used for enzyme activity measurements. Protein content was quantified using the [Bradford \(1976\)](#) method, with bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as the standard.

Catalase activity assay

Catalase (CAT; EC 1.11.1.6) activity was measured spectrophotometrically by monitoring the rate of H₂O₂ decomposition at 240 nm, following [Afiyanti and Chen \(2014\)](#). The reaction mixture contained 0.95 mL of 50 mM potassium phosphate buffer (pH 8.0), 0.05 mL of enzymatic extract, and 0.1 mL of 100 mM H₂O₂. Absorbance changes were recorded for 1 min at 25°C. One CAT unit decomposes 1 µmol of H₂O₂ per minute at pH 8.0. Results were expressed as U/mg protein.

Superoxide dismutase activity assay

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by measuring the inhibition of nitro blue tetrazolium (NBT) photochemical reduction, as described by [Hayat et al. \(2018\)](#). The reaction mixture included 1.5 mL of 50 mM potassium phosphate buffer (pH 7.8), 0.3 mL of 0.1 mM EDTA, 0.3 mL of 0.13 M methionine, 0.3 mL of 0.02 mM riboflavin, 0.05 mL of enzymatic extract, and 0.25 mL of distilled water. The mixture was exposed to fluorescent light (86.86 µmol/m²·s) for 20 min, and absorbance was measured at 560 nm. One SOD unit inhibits 50% of NBT reduction at pH 7.8 and 25°C. Results were reported as U/mg protein.

Phenylalanine ammonia-lyase (PAL) activity

PAL activity (EC 4.3.1.5) was assessed using the method of [Toscano et al. \(2018\)](#). The assay measured cinnamic acid production from L-phenylalanine at 290 nm. The reaction mixture consisted of 2.3 mL of 0.1 M borate buffer (pH 8.8) with 10 mM L-phenylalanine and 0.2 mL of enzymatic extract. After incubation at 40°C for 1 h, the reaction was stopped with 0.5 mL of 5 N HCl. Absorbance was recorded at 290 nm. One PAL unit releases 1 µmol of cinnamic acid per minute at pH 8.8 and 40°C. Results were expressed as U/mg protein.

Total polyphenol content (TPC)

Lyophilized rabbit meat samples (1 g) were pulverized in liquid nitrogen and extracted with a methanol:water mixture (1:10 w/v). The suspension was sonicated (Branson M2800-CPX-HE, Emerson, Ferguson, MO, USA) for 30 min, then centrifuged at 10,000 rpm for 15 min at 4°C. Supernatants were stored at 4°C in dark vials. Total phenols were quantified using the Folin-Ciocalteu method ([Vergara-Castañeda et al., 2010](#)) with gallic acid as the standard. A 140 µL extract was mixed with 460 µL of distilled water and 250 µL of 1 N Folin-Ciocalteu reagent. After 5 min, 1250 µL of 20% (w/v) sodium carbonate was added. The mixture was vortexed, incubated in darkness for 2 h, and absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Statistical analysis

Rabbits were randomly divided into three dietary treatment groups, 21 animals being assigned to each one of the treatments (T1, T2, and T3, described in Animal and materials section) and each group having three replications with seven rabbits in each replication. Data were analyzed as a randomized complete block design. Data were subjected to analysis of variance (ANOVA) and Tukey tests ($\alpha < 0.05$) were using the Statgraphics® Centurion XVI statistical software (StatPoint Technologies Inc., Bedford, MA, USA, 2010).

RESULTS AND DISCUSSION

Productive behavior of rabbit meat

The study determinates the productive performance of rabbit meat across three dietary treatments—100% pellets, 50% pellets + 50% HGBF at 75 mM H₂O₂, and 50% pellets + 50% HGBF—over a 42-day period, with results presented in Table 1. Live weight no was significantly different compared to the HGBF-supplemented groups, indicating a potential influence of pellet-based diets on growth. Stomach pH and organ weight showed no significant differences across treatments. Jejunum pH and length exhibited slight variations, though not statistically significant ($P > 0.05$). Cecum pH and length, colon-rectum pH, length, and liver relative weight remained consistent across groups, with no significant treatment effects ($P > 0.05$), suggesting that HGBF supplementation maintains gastrointestinal and organometric stability.

Table 1 – Productive behavior of rabbit meat ($\bar{x} \pm SD$, n=21).

Parameters	Pellets 100%	Pellets 50% + 50% HGBF at 75 mM H ₂ O ₂	Pellets 50% + 50% HGBF
Alive weight (kg)	1.71 ± 0.09 ^a	1.63 ± 0.16 ^a	1.66 ± 0.15 ^a
Stomach pH	1.59 ± 0.34 ^a	2.07 ± 1.22 ^a	1.91 ± 0.37 ^a
Stomach organ empty weight (g)	19.58 ± 1.87 ^a	20.45 ± 0.82 ^a	20.85 ± 1.82 ^a
Jejunum-ileum pH	7.33 ± 0.21 ^a	7.91 ± 1.07 ^a	7.43 ± 0.36 ^a
Jejunum-ileum organ empty weight (g)	34.50 ± 5.66 ^a	34.73 ± 1.66 ^a	38.27 ± 2.17 ^a
Jejunum-ileum length (cm)	205.50 ± 46.75 ^a	225.50 ± 16.05 ^a	207.00 ± 49.28 ^a
Cecum pH	5.85 ± 0.08 ^a	6.02 ± 0.18 ^a	5.96 ± 0.14 ^a
Cecum organ empty weight (g)	24.60 ± 4.47 ^a	21.30 ± 2.85 ^a	25.13 ± 4.02 ^a
Cecum length (cm)	41.25 ± 3.03 ^a	40.25 ± 2.86 ^a	39.25 ± 0.83 ^a
Colon-rectum pH	6.41 ± 0.27 ^a	6.57 ± 0.14 ^a	6.46 ± 0.24 ^a
Colon-rectum organ empty weight (g)	28.93 ± 5.36 ^a	27.85 ± 1.12 ^a	28.95 ± 2.49 ^a
Colon-rectum length (cm)	95.25 ± 3.34 ^a	95.75 ± 6.26 ^a	96.50 ± 4.56 ^a
Liver relative weight (g)	63.00 ± 11.42 ^a	64.15 ± 11.23 ^a	62.83 ± 14.48 ^a
Gallbladder weight (g)	0.53 ± 0.13 ^a	0.70 ± 0.27 ^a	0.65 ± 0.32 ^a

SD=Standard deviation. ^a, Means within a column with different superscripts differ significantly ($P < 0.05$).

In recent years, interest in the consumption of nutraceutical products has increased in the market (Cesari et al., 2018). Meat is considered a functional food with many bioactive compounds; specifically, rabbit meat has important nutritional and dietary properties to improve and support health (Dal Bosco et al., 2018). Figure 1 illustrates the hot and cold carcass weights and yields for the three treatments.

No significant differences were observed in hot or cold carcass weights across the treatments (T1, T2, T3). Average carcass weights were below typical export standards, attributed to the rabbits' young age at slaughter (initial age: 35 days; final age: ~77 days). This suggests that partial substitution of pellets with HGBF (with or without H₂O₂ pre-treatment) maintains carcass yield without compromising growth performance, despite the shift to a more sustainable feed option. It has been seen that alternative rabbit feeds variations with alternative products such as herbs, spices, barley, corn, alfalfa, vegetables, among others, produce lower yields in the meat of different species for meat production (Dalle Zotte et al., 2014).

Studies such as those of Mattioli et al. (2016) reported higher carcass performance in rabbit meat with supplementation with Digestarom® or 15% Gliiricidia leaf meal with plus multi-enzymes (1 g/Kg) in the hot carcass weight in the diet of the 35-day-old rabbits (Oloruntola et al., 2018). El-Kady et al. (2021) achieved a carcass weight of 1,591.67 ± 49.50 g in white New Zealand rabbit with the diet of 33% of corn stalks treated with *Trichoderma reesei* fungi. Diets supplemented with organic products waste have also been evaluated, as is the case of Dabbou et al. (2017); they evaluated the cranberry waste as an alternative resource to the rabbit diet; carcass performance was not affected by the inclusion of this diet. While, Grioui et al. (2021) showed no significant effect of dried tomato waste in the feeding of growing rabbits was evaluated on the characteristics of the carcass and the meat quality. In addition, they determined that the weight of the carcass was between 1.27 and 1.185 Kg with the rabbits fed with 10 and 20% of the dried tomato

waste, and obtaining a higher percentage of yield (62.21%) with the treatment of 10% of the hot runner while in the cold runner the yield was 59.31% with the same treatment.

However, it should be noted that these supplemented diets types provide 2% more crude protein than diets based on concentrates (Mattioli et al., 2016). Likewise, it has been studied that factors such as ionization, acidification, and land use directly influence the weight of the carcass of rabbit meat production (Cesari et al., 2018). Some studies indicate that the meat 'softness' is associated with multiple factors involving chemical composition and muscle changes, stress from protein breakdown during slaughter, processing time, diet and refrigeration conditions of the meat canal. As well as, it varies between species and races, the different muscles and the cuts distances due to the change in temperature between them when the muscle becomes meat. Additionally, after cooking, the mechanical evolution of the meat is the result of the relative contribution of two components (myofibrillar and conjunctiva) in addition to their interaction (Grioui et al., 2021).

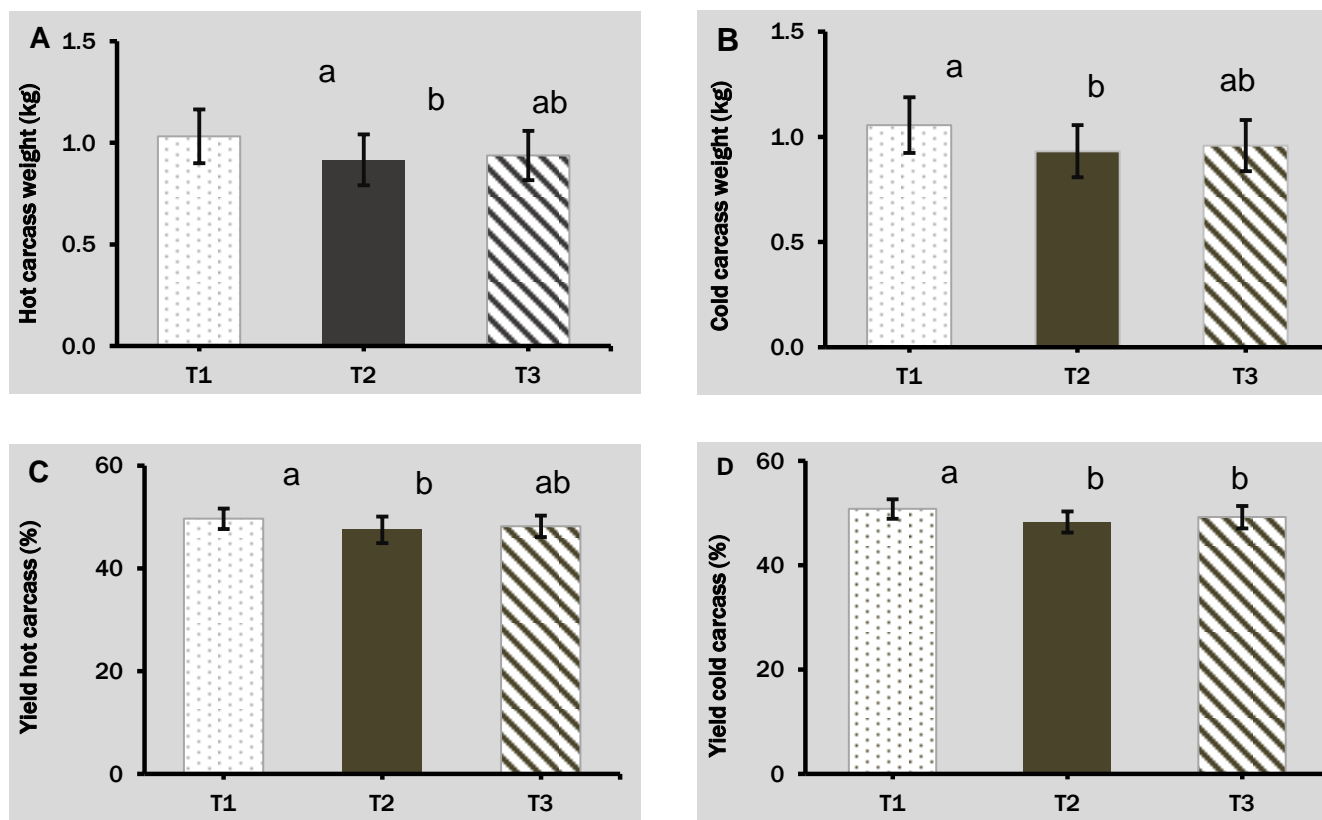


Figure 1 - The hot and cold carcass weights and yields for the three treatments. All treatments were ad libitum. Different letters indicate significant difference according to ANOVA and Tukey test (α = 0.05).

Antioxidant capacity of rabbit meat fed with three different treatments

Bioactive compounds present in functional foods are absorbed by animals enhance tissue antioxidant capacity (Mattioli et al., 2016). Similarly, the antioxidant capacity of plants and spices are derived from phenolic compounds (flavonoids, tannins, phenolic acids) and some vitamins such as E, C and A can prevent lipooxidation through trapping free radicals (Oloruntola et al., 2018). Table 2 presents the result of the antioxidant capacity of the rabbit meat evaluated in this study.

No significant differences were observed between treatments, suggesting consistent antioxidant activity. Some phytochemicals in plants improve the antioxidant, antimicrobial capacity, taste and palatability of foods, which could result in higher feed consumption and performance in animals (Hashem et al., 2017; Oloruntola et al., 2018). Phytochemicals in HGBF improve antioxidant and antimicrobial properties, potentially increasing feed intake. The antioxidant activity benefits are observed in the tissue's oxidation status, the intensification of the protein's metabolism, fats and carbohydrates; as well as better health and immunity of the animal fed with diets supplemented with nutraceutical characteristics (Mattioli et al., 2016). Hashem et al. (2017) in their study with supplementation of vitamin E (150 mg vitamin E/Kg dry matter), dried roots of *Moringa oleifera* (150 or 300 mg roots of moringa /kg dry matter) and propolis (150 or 300 mg propolis /Kg matter seca) obtained a greater significant difference (1.93 - 1.99 mM/L) compared to the diet without supplementation. For their part, Dal Bosco et al. (2015) in their study with 30-day-old rabbits, supplemented the diet with 20 g/d with alfalfa and flax sprouts, they found the presence of γ-tocopherol and lutein plus Zeaxanthin that was not present in the meat of rabbits fed with the control diet. Additionally, El-Desoky et al. (2017) orally

applied 50 mg/Kg of the ethanolic extract of *Moringa oleifera* leaves in seven-month-old rabbits; this dose increased serum albumin concentration, total antioxidant capacity (132%) and testosterone. Dal Bosco et al. (2018) modified the environment in addition to the inclusion of 3% linseed oil, once stored they found that lipoxydation was reduced with this treatment after 10 days. Including the study by Mattioli et al. (2016), in which the inclusion of Digestarom® at 300 mg/kg increased the levels of α -tocopherol that were absorbed by the animals, preventing lipid peroxidation through the extinction of free radicals.

Study findings support the inclusion of HGBF in improving the oxidative state of growing rabbits. Based on the HGBF chemical constitution, these effects could be attributed to the antioxidant capacity that is similar to cereals such as wheat and corn (Narwal et al., 2016), and to its content of phenolic acids, flavonoids, lignans, tocoles (vitamin E), phytosterols and folates. Most of these active metabolites have a strong antioxidant capacity (Abouelezz et al., 2019). Reports on the effects of this food category additives on rabbit growth performance, antioxidant and antibacterial activity (Narwal et al., 2016), meat quality, blood biochemical parameters (Shanti et al., 2017) and reproductive performance (El-Desoky et al., 2017) were compared with this study.

Table 2 - Quantification of the inhibitory activities of DPPH• and ABTS•+ in equivalent moles of Trolox/mg MS, and the inhibition ratio.

Rabbit meat treatments	ARSA		DRSA	
	Inhibition (%)	Polyphenols (trolox/mg)	Inhibition (%)	Polyphenols (trolox/mg)
Pellets 100%	80.47±11.84	1,986.84±266.92 ^a	37.51±17.24	1.13±0.52
Pellets 50% + 50% HGBF at 75 mM H ₂ O ₂	79.76±10.37	1,789.80±212.27 ^b	40.77±18.52	1.12±0.51
Pellets 50% + 50% HGBF	79.48±9.90	1,858.95±211.23 ^{ab}	44.04±16.69	1.25±0.48

DM: dry matter, HGBF: Barley Hydroponic Green Fodder. a,b,c, Means within a column with different superscripts differ significantly (P<0.05).

Tests of the enzymatic activity of rabbit meat fed with three different treatments

Similarly, the plants and spices antioxidant capacities are derived from phenolic compounds (flavonoids, tannins, phenolic acids) and some vitamins such as vitamin E, C and A, which mitigate lipoxydation through trapping free radicals or from the antioxidant enzymes activation such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Mattioli et al., 2016; Selim et al., 2021). Similarly, these enzymes can prevent oxidation by stabilizing initiated free radicals such as superoxide and hydrogen peroxide, one of the most reactive free radicals in vivo. Figure 2 shows the quantification of the stress-related enzymes and the enzyme of the phenylpropanoid pathway.

The enzymatic activity related to CAT HGBF-supplemented groups (T2 and T3) exhibited lower catalase activity compared to T1, suggesting reduced oxidative stress as the body relied less on CAT to neutralize reactive oxygen species, likely due to the antioxidant-rich HGBF diet. In addition, no significant differences were observed in SOD and PAL activities across all treatments (T1, T2, and T3). This stability indicates that HGBF supplementation did not disrupt these enzymatic defenses, maintaining baseline oxidative stress management, which distribute and retained in the tissues. However, this bioavailability has not yet been directly demonstrated due to the lack of equipment and the analytical technique developed (Mattioli et al., 2016). Oloruntola et al. (2018) studied the superoxides degradation in rabbit meat in diets supplemented with 15% *Gliricidia* leaf flour plus multi-enzymes (1 g/Kg). They promoted the elimination of the superoxide ion by increasing the activity of SOD. This trend is not reflected in the results of Figures 2A and 2B. In the study done by Vizzari et al. (2021) polysaccharides was used from brown algae (*Laminaria digitate* and *hyperborea*, ratio 1:1 at 0.6%) in the diet, increased SOD and glutathione peroxidase. A benefit of the use of food supplements rich in antioxidant enzymes is the ideal animal development since these enzymes block oxidative stress, delay or prevents the oxidation of proteins, lipids, DNA and carbohydrates. The PAL enzymatic activity (Figure 2C) did not show a significant difference between treatments. Therefore, it can be deduced that the phenylpropanoid pathway was not affected by the inclusion of the rabbit's diet variants in this study. Mattioli et al. (2016) found with the supplementation of Digestarom® (300 mg/kg) that lipid peroxidation was reduced by activating antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, because these components were distributed in the muscles through blood.

In mammals, the antioxidant defense system includes enzymes (superoxide dismutase, glutathione peroxidases and catalase), extracellular proteins that bind to iron and copper (albumin, transferrin, lactoferrin, haptoglobin and ceruloplasmin), antioxidant vitamins (C, E and β -carotene) and other cellular compounds (quinones, glutathione, uric acid and bilirubin) (Jiang and Xiong, 2016). In addition, several exogenous phenolic compounds derived from fruits, vegetables, legumes or supplementary ingredients of the diet added to food, such as spices and herbs, contribute to the antioxidants reserve (Loganayaki et al., 2013; Narwal et al., 2016; Castrica et al., 2019). These antioxidants dietary sources are essential when the body is exposed to a high degree of radical stress. Likewise, recent advances in antioxidant studies have allowed the possibility of mitigating chemical toxins in meat products through different strategies; such as,

moderate thermal processing conditions to reduce toxin formation, bioaccessibility restriction technology, and antioxidant interventions (Jiang and Xiong, 2016).

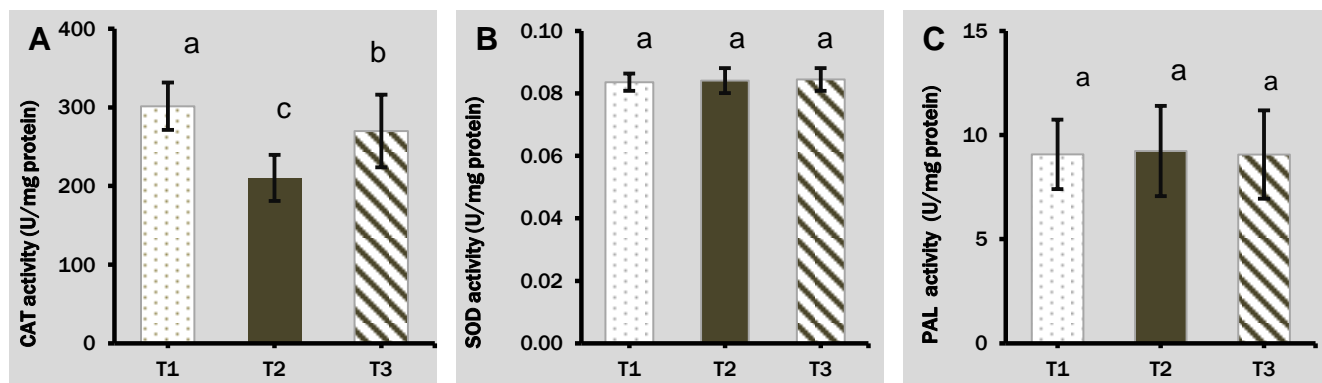


Figure 2 - Quantification of enzymatic activity in rabbit meat fed: T1- 100% Pellets, T2- 50% Pellets + 50% HGBF at 75 mM H₂O₂, T3- 50% Pellets + 50% HGBF. HGBF: Hydroponic Green Barley Fodder. SOD= Superoxide dismutase, CAT= Catalase and PAL= Phenylalanine ammonia-lyase. Different letters indicate significant differences according to the ANOVA and Tukey tests ($\alpha = 0.05$).

Total phenol content of rabbit meat fed with three different treatments

Phenolic compounds are key antioxidants role (Alagbe and Akintayo-Balogun, 2020). The content of phenols quantified in rabbit meat supplemented in the diet with HGBF is shown in Figure 3. The phenols presence in rabbit meat is an important indicator of antioxidant capacity, with fat-soluble antioxidants being those with the highest presence in tissues (Mattioli et al., 2020). Phenols have antimicrobial effects in general (Branciari et al., 2021). Vizzari et al. (2021) supplemented with 0.6% polysaccharides from brown algae (*Laminaria digitate* and *hyperborea*, 1: 1 ratio), finding an important effect on the growth, health and development of animals in general. Branciari et al. (2021) supplemented with 280 mg/Kg of the olive residue polyphenol extract in the rabbit diet. As expected, polyphenol sulfate metabolites were detected in the rabbits meat, in addition to the reduction of *Pseudomonas* spp. in meat during storage (Branciari et al., 2021). However, the 20% introduction of grape wastes that were incorporated into the diet; there was no significant difference in total phenolic content, antioxidant capacity, antioxidant inhibition power and lipid oxidation in meat (Bouzaida et al., 2021). Selim et al. (2021) fed rabbits for 42 days with *Moringa oleifera* leaves, and related the low presence of cholesterol in the longissimus lumborum muscles with the presence of phenols in the moringa leaves, since they act as a natural hypocholesterolemic agent. In addition, this diet can contribute to a high level of components such as phenols, tannins and phytate in meat (Selim et al., 2021). Menchetti et al. (2020) supplemented the New Zealand rabbits diet with Goji berries (3%) increased the phenolic content (24.96 mg GAE/100 g) and the antioxidant properties in the muscle (Castrica et al., 2020; Menchetti et al., 2020). The both radical systems antioxidant activity (DPPH• and ABTS^{•+}) and total phenolic compounds were influenced by the genotype and the environment. Likewise, the inclusion of foods high in phenolic compounds and antioxidant capacity for livestock feeding can modulate the content of phenolic compounds in the muscles and that the food rich in phenols can increase the level of antioxidants in the tissues (Menchetti et al., 2020). Therefore, both the genotype and the environment play an important role in determining the antioxidant activity and phenolic content of rabbit meat (Dal Bosco et al., 2018).

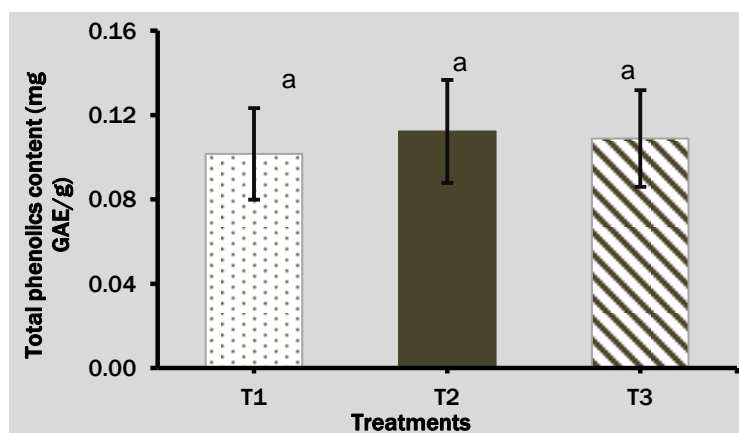


Figure 3- Total phenol content (CFT, mg GAE/g) of methanolic extracts from rabbit meat fed with: T1= Pellets 100%, T2= Pellets 50% + 50% HGBF at 75 mM H₂O₂, T3= Pellets 50% + 50% HGBF. GAE: Gallic Acid Equivalents, HGBF: Hydroponic Green Barley Fodder. Results plotted as means. Different letters indicate significant differences ($\alpha = 0.05$).

CONCLUSION

This study demonstrates that dietary supplementation with Hydroponic Green Barley Fodder (HGBF), particularly with hydrogen peroxide seed pre-treatment (T2), significantly enhances the antioxidant capacity and total phenolic content of rabbit meat after 42 days of fattening. These improvements, driven by HGBF's rich profile of bioactive compounds (e.g., polyphenols, flavonoids, and vitamins), contribute to superior meat quality and oxidative stability. Additionally, HGBF's sustainable production reduces greenhouse gas emissions and feed costs, offering an environmentally friendly alternative to conventional feeds. These findings support the adoption of HGBF in rabbit production to improve meat functionality and promote sustainable livestock systems, with potential applications in broader animal nutrition strategies.

DECLARATIONS

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Ethics approval

The authors followed all applicable international, national, and institutional guidelines for the care and use of rabbits. The authors complied with the ARRIVE guidelines.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

All authors contributed equally to the study.

Acknowledgements

We would like to acknowledge the Secretaría de Ciencia, Humanidades, Tecnología e Innovación [grant numbers 807820].

Consent to publish

All authors have reviewed and approved the final manuscript for publication.

Funding

This research was funded by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación [grant numbers 807820].

Competing interests

The authors declare no competing interests in this research and publication.

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EVALUATION OF *Wedelia trilobata* (L.) Hitchc. AS A FORAGE FOR REPRODUCTIVE RABBITS DOES IN A TROPICAL ENVIRONMENT

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➤Supporting Information



ABSTRACT: In this study, *Wedelia trilobata* (L.) Hitchc. was evaluated as forage for reproductive rabbit does in a tropical environment. Eighteen crossbred does (New Zealand White × Local) were fed six dietary treatments in a Completely Randomized Design (CRD) with three replications. Six diets were designated as W0, W25, W40, W50, W60, and Wa, corresponding to *W. trilobata* levels of 0, 25, 40, 50, and 60% of concentrate intake (dry matter, DM, basis), *ad libitum*. The trial lasted nine months, covering three reproductive cycles. Feed and nutrient intake, reproductive performance, and milk yield were measured. *W. trilobata* contained 9.25% crude protein (CP), similar to *Brachiaria mutica*, but had higher metabolizable energy (ME: 10.3 MJ/kg DM) and lower fiber (CF: 18.9%, NDF: 40.4%, and ADF: 27.9%). Nutrient intake peaked at moderate inclusion (W40-W60), with DM intake of 124-132 g/day compared with 113 g in the W0 and 103 g in the Wa ($P < 0.05$). The W50 group had the highest CP intake (23.7 g/day), while the greatest ME intake was 1.52 MJ/day. Rabbit does in the moderate groups were heavier before kindling (2957-2958 g) than the control (2736 g) and produced more milk (86.9-88.2 g/day) than the W25 group (74.7 g/day) ($P < 0.05$). Litter size and weights did not differ significantly ($P > 0.05$) among treatments. In conclusion, including *W. trilobata* at 40-60% of concentrate intake improved doe condition and milk yield without affecting litter size or weight. Its abundance makes it practical forage for smallholder rabbit production in tropical climates.

Keywords: Alternative forage, Rabbit does, Reproductive performance, Sustainable livestock breeding, Tropical climate, *Wedelia trilobata*.

INTRODUCTION

The rising demand for affordable animal protein continues to challenge sustainable livestock production, especially in tropical and subtropical regions. Rabbits are well suited to this context because they reproduce quickly, grow fast, and can use diets rich in roughage and low in grains (Lan et al., 2022; Christopher et al., 2023). This makes rabbit farming attractive for smallholder farmers, who often depend on locally available feeds rather than costly commercial concentrates. However, high temperatures and humidity can reduce feed intake, weaken immunity, and lower reproductive efficiency (Liang et al., 2022; Liu et al., 2022; Abdelsalam and Fathi, 2023; Trung et al., 2024).

The Mekong Delta, a low-lying alluvial plain with high temperatures, humidity, long sunshine hours, and heavy rainfall, has much land that is more competitive for rice and tropical fruits than for permanent pastures (Mo, 2024). These agro-ecological features constrain conventional forage availability for on-farm use and reinforce the need for locally abundant cut-and-carry feeds. In 2023, Vietnam's rabbit population reached ~1.172 million head with ~5.58 thousand tons of carcass output; distribution is concentrated in the Red River Delta (~39%), followed by the Northern midlands and mountains (~31%), while the Mekong Delta accounts for ~105 thousand head (~9%) and ~1.13 thousand tons (National Statistics Office, 2024). In practice, rabbit production remains largely smallholder-based with modest inputs, conditions under which low-cost forages are essential (Lan et al., 2022).

With restrictions on antibiotic use in animal production, natural and readily available feed alternatives are increasingly needed to support both productivity and animal health (Abdelsalam and Fathi, 2023). One promising option is *Wedelia trilobata* (L.) Hitchc. (Figure 1), commonly called creeping oxeye or Singapore daisy. Native to Central and South America, it now grows widely in wet tropical regions without the need for cultivation (Balekar et al., 2014; Khan et al., 2023; Mo, 2024).



Figure 1 - Image of *Wedelia trilobata* (L.) Hitchc.

Although sometimes viewed as invasive, the plant adapts well to poor soils, drought, and changing light conditions (Zhang et al., 2020; Mo, 2024; Dai et al., 2024). This resilience makes it a readily available forage source where grasses are scarce. *Wedelia trilobata* is a prostrate, creeping perennial in Asteraceae that forms a dense ground cover typically 15–30 cm tall, occasionally approaching ~70 cm. Stolon-like stems may reach ~2 m and root at the nodes, supporting rapid clonal spread and quick regrowth after cutting (Balekar et al., 2014). Leaves are opposite, glossy, slightly succulent, 2–9 × 2–5 cm, usually trilobed with serrate margins, and are sessile or borne on very short petioles (Balekar et al., 2014). Inflorescences are terminal/axillary capitula with 8–13 yellow ray florets (6–15 mm) surrounding numerous tubular disc florets; flowering occurs year-round, supporting a steady supply of leafy biomass for cut-and-carry feeding (Balekar et al., 2014).

Nutritionally, *W. trilobata* contains about 10–18% crude protein (CP) in dry matter (DM), similar to or higher than many tropical grasses, and provides more metabolizable energy (ME) (Ibok et al., 2023; Mo, 2024). It also supplies non-fiber carbohydrate (NFC), ether extract (EE), and essential minerals, while its lower fiber content may improve energy availability (Mo, 2024). In addition, *W. trilobata* contains tannins, saponins, flavonoids, and phenolic compounds linked to improved health and fertility in traditional medicine (Balekar et al., 2014; Zhao et al., 2019). Traditionally, it has been used to support reproductive health, suggesting benefits for breeding animals (Balekar et al., 2014; Christopher et al., 2023). Earlier studies on growing rabbits and goats reported good results for feed intake, nutrient digestibility, and growth when *W. trilobata* was included in the diet (Truong and Trung, 2023; Mo, 2024). Although secondary compounds might affect digestibility, rabbits consume it readily without apparent negative effects on palatability or nutrient use (María et al., 2021; Mo, 2024). Forages with such properties can also help reduce heat stress and support reproduction in tropical conditions (Miah et al., 2020; Abdelsalam and Fathi, 2023; Trung et al., 2024; El-Ratel et al., 2025). Based on available reports, the evidence on its effects on the reproductive performance of rabbit does, remains limited, especially under tropical smallholder systems (El-Gindy et al., 2022; Christopher et al. 2023). This study, therefore, examined the feeding value of *Wedelia trilobata*, focusing on nutrient intake and reproductive performance of rabbit does under tropical conditions in the Mekong Delta.

MATERIALS AND METHODS

Study area and time

The study was conducted at the private farm (10° 01'56.4"N; 105° 45'57.5"E), located in the Mekong Delta at a mean altitude of 0.8 meters, which experiences a tropical climate characterized by high temperatures, high humidity, abundant sunshine, and heavy rainfall. The experimental period spanned from spring to winter, lasting approximately 9 months, covering three consecutive reproductive cycles (litters 1 to 3). This study complied with the provisions of Article 72 of the Vietnamese Law on Animal Husbandry (National Assembly of Vietnam, 2018), which regulates the humane treatment of livestock in scientific research and related activities. Kien Giang College, as the institutional authority, reviewed and approved issues concerning rabbit ethics and welfare in this study.

Animals

A total of 18 reproductive female crossbred rabbits (New Zealand White x Local) were utilized in this study. These rabbits, approximately 5 months of age, newly mature and ready for being mated, with an average initial body weight of approximately 2486±50.2 g, were sourced from local farms in the Mekong Delta. Moreover, six male rabbits (New Zealand White x Local) of approximately 12 months of age were randomly used to mate with all female rabbits in the experiment. Before the commencement of the experiment, all experimental rabbits were vaccinated against coccidiosis, pasteurellosis, and internal parasites. An adaptation period of several days was provided to allow the animals to acclimate to the experimental conditions. Rabbits were housed in individual cages of 0.5 x 0.5 m in size. Each cage was equipped with feeders and water troughs.

Experimental design

The experiment was structured as a Completely Randomized Design, encompassing six dietary treatments, each with three replicates, where each experimental unit consisted of one reproductive female crossbred rabbit (New Zealand White x Local). These rabbits were carefully monitored through three consecutive reproductive cycles (litter 1 to 3), spanning an approximate duration of 9 months. The six dietary treatments were designated as W0, W25, W40, W50, W60, and Wa. For W0, W25, W40, W50, and W60, *Wedelia trilobata* was offered at levels corresponding to 0, 25, 40, 50, and 60% of the concentrate intake (DM basis), respectively, while the Wa treatment provided *Wedelia* with *ad libitum*. In all treatments, the concentrate was supplied consistently at an average of 75 g/rabbit/day, which constituted 55% of the total diet. Para grass (*Brachiaria mutica*) was offered *ad libitum* to all treatments except Wa throughout the experimental period. Rabbit does were fed diets as per the experimental design, while the rabbit bucks used for mating received a diet consistent with the W0 treatment. The W0 was also given to experimental female rabbits during the gilt stage.

Feeds and feeding

Two primary natural forages were provided daily were *Brachiaria mutica* and *Wedelia trilobata*. Both forages were harvested daily from natural sources around the farms. The forages were cut in the afternoon, thoroughly washed, drained, and then offered to the rabbits the following morning. Commercial concentrate feed was purchased from a livestock feed store. Rabbits were fed three times daily (at 8:00 AM, 11:00 AM, and 5:00 PM). The quantities of feed offered to each rabbit were weighed daily. Any leftover feed was collected and weighed the following morning to accurately determine the daily feed intake. Fresh, clean water was provided *ad libitum* daily.

Measurements and data collection

Daily feed intake was determined by recording the amount of feed offered and the amount of feed refused each morning. Samples of both offered feed and refusals were collected once a week in the morning. These samples were then chopped, dried at 60 °C for 12 hours, and ground through a 1 mm sieve. Subsequently, the chemical composition of the feed and refusals was analyzed to determine the daily intake of various nutrients. The following nutrient intake parameters were calculated per rabbit per day: DM, organic matter (OM), CP, EE, NFE, NFC, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ME. The reproductive performance of the female rabbits was monitored across three consecutive reproductive cycles (litter 1 to 3), as outlined in the experimental design. Key indicators included litter size recorded as the number of kits per litter at birth, alive, and the number of kits at weaning; litter weight measured as the total weight of the litter after birth, alive, and at weaning; and milk yield was determined daily for each doe by a weigh-suckle-weigh method. Kits were weighed immediately before and after suckling to ascertain the amount of milk consumed. This process commenced at birth, where kits were weighed immediately after being dried and before their first suckling, and continued with daily weighing once in the morning until weaned.

Chemical analysis

All feed samples (offered and refused), as well as any other samples collected for nutrient analysis, were processed as follows: samples were chopped, dried in an oven at 60 °C for 12 hours, and then finely ground using a 1 mm sieve. The analysis of nutrient composition, including DM (method 930.15), OM (method 942.05), CP (calculated as $N \times 6.25$, method 990.03), EE (method 920.39), and CF (method 962.09) content, was conducted according to standard procedures, such as those outlined by AOAC (1990). Concentrations of NDF and ADF were determined following the methods described by van Soest et al. (1991). NFE (= $OM - CP - EE - CF$) and NFC (= $OM - CP - EE - NDF$) were calculated by difference. ME was estimated based on the determined nutrient composition as suggested by Maertens et al. (2002), as $ME \text{ (MJ/kg DM)} = DE \text{ (MJ/kg DM)} \times ME/DE$, where $DE \text{ (MJ/kg DM)} = 13.932 - 0.196 \text{ CF (\%DM)}$, $ME/DE = 0.995 - 0.0048 \times DP \text{ (g/kg DM)}/DE \text{ (MJ/kg DM)}$, $DP \text{ (g/kg DM)} = CP \text{ (g/kg DM)} \times CPD/100$, and $CPD = 78.7 - 0.69 \times CF \text{ (\% DM)}$.

Statistical analysis

All quantitative data collected were subjected to analysis of variance following the model: $Y_{ij} = \mu + C_i + T_j + e_{ij}$, where Y_{ij} is the dependent variable, μ is the overall mean, C_i is the effect of reproductive cycles, T_j is the effect of treatments, and e_{ij} is the random residual error. Statistical analyses were performed using a recognized statistical software package (e.g., Minitab 21.4, 2023). When the F-test indicated significant differences among treatment means ($P < 0.05$), post-hoc comparisons were conducted using Tukey's HSD test to identify specific differences between pairs of means.

RESULTS AND DISCUSSIONS

Feed nutrients

The chemical composition analysis of the feed ingredients utilized in the feeding experiment, which included *Wedelia trilobata*, grass, and concentrate, is presented in Table 1. Grass exhibited a DM content of 16.1%, which was higher than that of *W. trilobata* at 12.3%. Regarding CP content, *Brachiaria mutica* had 11.8%, higher than *Wedelia* at 9.25%. EE content was found to be higher on *W. trilobata* at 6.34% compared to *Brachiaria mutica* at 5.53%. A difference was observed in NDF content, with *W. trilobata* determining 40.4%, lower than *Brachiaria mutica* at 69.1%. Similarly, the ADF content of *W. trilobata* was 27.9%, lower than grass at 37.7%. As anticipated, the concentrate served as a rich source of protein and fat. The concentrate contained 22.8% CP and 7.45% EE. Regarding carbohydrate content, the concentrate had the highest NFE at 53.7% and NFC at 39.3%. *W. trilobata* contained 47.4% NFE and 25.9% NFC, while *Brachiaria mutica* had the lowest values with 36.9% NFE and a very low 1.46% NFC. In terms of ME, the concentrate was the most energy-dense with 14.3 MJ/kgDM. *W. trilobata* provided 10.3 MJ/kgDM which was higher than grass at 6.85 MJ/kgDM.

The analysis of feed composition showed that *W. trilobata* had lower DM and CP than para grass but offered higher energy and less fiber. This may explain why rabbits readily consumed it when included in their diets. Grass generally had higher fiber fractions, while *W. trilobata* provided more EE and a better energy concentration. Such differences are important, as rabbits need both adequate fiber for gut health and enough energy to support reproduction. Earlier studies

also reported that *W. trilobata* contains less NDF and ADF than common grasses, while still offering moderate protein and higher energy (Mo, 2024). This means it can act as a useful energy source among tropical forages. However, rabbits usually perform best with diets containing around 18% CP (Christopher et al., 2023; Trung et al., 2024), so *W. trilobata* alone may not fully meet protein requirements unless balanced with concentrate.

Table 1 - Nutrient composition (%DM, excluding DM, ME) of feeds

Feeds	DM	OM	CP	EE	NFE	NFC	CF	NDF	ADF	ME
<i>Wedelia trilobata</i>	12.3	81.8	9.25	6.34	47.4	25.9	18.9	40.4	27.9	10.3
<i>Brachiaria mutica</i>	16.1	87.9	11.8	5.53	36.9	1.46	33.7	69.1	37.7	6.85
Concentrate	89.3	90.4	22.8	7.45	53.7	39.3	6.45	20.8	12.6	14.3

DM (%): dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFE: nitrogen-free extract, NFC: non-fiber carbohydrate, ME (MJ/kg DM): metabolizable energy.

Feeds and nutrients intake

The present study evaluated the feed, nutrient, and energy intake of reproductive rabbit does under varying proportions of *Wedelia trilobata* in their diets (Table 2). The experimental treatments, denoted as W0, W25, W40, W50, W60, and Wa, represent *Wedelia trilobata* proportions at 0, 25, 40, 50, 60% of concentrate, and *ad libitum*, respectively.

Brachiaria mutica offer significantly diminished as the *W. trilobata* inclusion level increased, starting at 96.9 g for W0 and falling to 48.3 g for W60, with statistical significance ($P=0.001$). In stark contrast, the offer of *W. trilobata* saw a marked rise, from 16.0 g (W25) to 76.0 g (Wa), also highly significant ($P=0.001$). *Brachiaria mutica* refusal mirrored the trend of grass offer, being highest in W0 at 50.3 g and lowest in W60 at 25.5 g, indicating significant differences across treatments ($P=0.001$). *Wedelia trilobata* refusal was absent in treatments W25, W40, and W50. However, it became noticeable at W60 (12.2 g) and was highest in the *ad libitum* group, Wa (38.6 g), demonstrating significant variability ($P=0.001$). Concentrate intake exhibited remarkable consistency across all treatments, ranging narrowly from 65.4 g to 68.8 g, with no significant statistical difference ($P=0.473$). *Brachiaria mutica* intake was most prominent in the W0 group at 46.6 g and declined to 22.7 g in the W60 group, a statistically significant trend ($P=0.001$). Conversely, *Wedelia trilobata* intake showed a significant upward trajectory, from 16.0 g (W25) to 37.1 g (W60) and peaking at 37.4 g (Wa), with high statistical significance ($P=0.001$).

Table 2 - Feed, nutrients, and energy intake of does.

Variables	W0	W25	W40	W50	W60	Wa	SEM	P-value
<i>Brachiaria mutica</i> offer, g DM/doe/day	96.9 ^a	80.6 ^b	64.6 ^c	64.6 ^c	48.3 ^d	-	0.020	0.001
<i>Wedelia</i> offer, g DM/doe/day	-	16.0 ^e	24.7 ^d	37.0 ^c	49.3 ^b	76.0 ^a	0.229	0.001
<i>Brachiaria mutica</i> refuse, g DM/doe/day	50.3 ^a	41.4 ^{ab}	30.9 ^{abc}	35.0 ^{bc}	25.5 ^c	-	3.25	0.001
<i>Wedelia trilobata</i> refuse, g DM/doe/day	-	-	-	2.89 ^b	12.2 ^b	38.6 ^a	2.31	0.001
<i>Wedelia trilobata</i> intake, g DM/doe/day	-	16.0 ^c	24.6 ^{bc}	34.1 ^{ab}	37.1 ^a	37.4 ^a	2.36	0.001
<i>Brachiaria mutica</i> intake, g DM/doe/day	46.6 ^a	39.2 ^{ab}	33.7 ^{abc}	29.6 ^{bc}	22.7 ^c	-	3.25	0.001
Concentrate intake, g DM/doe/day	66.9	66.9	65.5	68.8	65.4	65.6	1.33	0.473
DM, g/doe/day	113 ^{ab}	122 ^{ab}	124 ^a	132 ^a	125 ^a	103 ^b	4.26	0.001
OM, g/doe/day	101 ^{ab}	108 ^a	109 ^a	116 ^a	110 ^a	90.7 ^b	3.60	0.001
CP, g/doe/day	22.1 ^{ab}	22.5 ^{ab}	22.6 ^{ab}	23.7 ^a	21.6 ^b	17.7 ^c	0.419	0.001
EE, g/doe/day	8.84 ^a	9.47 ^a	9.12 ^a	9.81 ^a	9.08 ^a	7.43 ^b	0.214	0.001
NFE, g/doe/day	50.5	55.8	56.6	59.8	58.9	52.6	2.07	0.054
NFC, g/doe/day	27.4 ^c	31.2 ^b	31.7 ^b	36.0 ^a	35.5 ^a	36.5 ^a	0.570	0.001
CF, g/doe/day	20.1 ^s	20.5 ^s	20.7 ^s	22.6 ^s	20.1 ^s	13.0 ^b	1.31	0.004
NDF, g/doe/day	43.2 ^{ab}	45.1 ^a	45.5 ^a	46.4 ^a	43.5 ^a	29.1 ^b	2.97	0.012
ADF, g/doe/day	26.5 ^a	27.9 ^a	26.7 ^a	27.7 ^a	26.9 ^a	18.6 ^b	1.62	0.013
ME, MJ/doe/day	1.30 ^b	1.42 ^{ab}	1.43 ^{ab}	1.52 ^a	1.46 ^{ab}	1.28 ^b	0.038	0.001

W0, W25, W40, W50, and Wa: proportions of *W. trilobata* at 0, 25, 40, 50, and 60% of concentrate, and *ad libitum*, respectively; DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFE: nitrogen-free extract, NFC: non-fiber carbohydrate, ME: metabolizable energy; SEM: standard error of means; P: = significant level; ^{a-e}: means within a row with different superscripts differ significantly ($P<0.05$).

DM intake differed significantly (P=0.001), with the highest values observed in treatments W40, W50, and W60, ranging from 124 g to 132 g. Notably, W0 (113 g) and Wa (103 g) exhibited lower DM intakes. OM intake followed a similar pattern to DM intake, with W40, W50, and W60 showing higher intakes (ranging from 109 g to 116 g) compared to W0 (101 g) and Wa (90.7 g) (P=0.001). CP intake was significantly highest in W50 (23.7 g), whereas W60 (21.6 g) and Wa (17.7 g) demonstrated significantly lower CP intakes compared to W50 (P=0.001). EE intake remained high across treatments W0 to W60 (ranging from 8.84 g to 9.81 g), but was significantly lower in Wa (7.43 g) (P=0.001). NFE intake did not display statistically significant differences among the treatments (P=0.054). NFC intake was significantly elevated in treatments W50, W60, and Wa (ranging from 35.5 g to 36.5 g) when contrasted with W0 (27.4 g) (P=0.001). CF intake did not show significant differences among W0 through W60, but was significantly lower in Wa (13.0 g) (P=0.004). NDF intake was highest in treatments W25, W40, W50, and W60 (ranging from 43.2 g to 46.4 g), and significantly lower in Wa (29.1 g) (P=0.012). ADF intake followed a similar trend to NDF, being highest in W0 through W60 (ranging from 26.5 g to 27.9 g), and significantly lower in Wa (18.6 g) (P=0.013). ME intake was significantly higher in W50 (1.52 MJ), and in other *Wedelia*-supplemented groups (W25, W40, W60), compared to W0 (1.30 MJ) and Wa (1.28 MJ) (P=0.001).

As *W. trilobata* levels increased, rabbits ate less grass but more *W. trilobata*, showing a clear substitution effect. Concentrate intake stayed almost the same across treatments, since the amount offered was fixed. This indicates that rabbits first consumed their concentrate and then adjusted their forage intake, a pattern also reported by Sánchez-Laiño et al. (2018) and Christopher et al. (2023). An important observation was that no *W. trilobata* was left uneaten in groups up to W40 or W50 group, while refusals appeared only at the W60 and Wa. These results showed that *W. trilobata* was well accepted at moderate proportions but became less palatable when it dominated the diet. Similar patterns of forage refusal at high inclusion levels have been noted in rabbit feeding trials with unconventional forages (Safwat et al., 2014; Okpakpor et al., 2022; Yaa et al., 2023).

DM and ME intake were highest when *W. trilobata* made up 40-60% of the concentrate intake equivalent. At these levels, rabbits also reached the best CP intake. Similar findings were observed by Mo (2024), who reported improved feed intake and energy supply with moderate *W. trilobata* inclusion. In contrast, the *ad libitum* *Wedelia* group ate less overall DM and CP, suggesting that too much *W. trilobata* alone reduced diet balance or palatability. Fiber intake was lowest when *W. trilobata* was fed *ad libitum*, which could be a concern. Rabbits need enough fiber to keep their digestion healthy and prevent disorders (Rommers et al., 1999; Maertens et al., 2002). Although *W. trilobata* has lower fiber than grass, combining it with some grass and concentrate gave a better balance. These results confirm that moderate *W. trilobata* inclusion supports both higher intake and better nutrient use, especially under tropical conditions where heat stress often reduces appetite (Abdelsalam and Fathi, 2023; Trung et al., 2024). In practice, this could help farmers who depend mainly on forages gathered locally, as *W. trilobata* grows abundantly without requiring cultivation or extra inputs.

Reproductive performance

The reproductive performance of the female rabbit does, including doe weight changes, milk yield, and litter characteristics, was monitored across six dietary treatments over three consecutive reproductive cycles (Table 3). The treatments, W0, W25, W40, W50, W60, and Wa, correspond to *W. trilobata* inclusions at 0, 25, 40, 50, and 60% of concentrate DM, and *ad libitum*, respectively.

Table 3 - Doe weight change and milk, and the number and weight of kits

Traits	W0	W25	W40	W50	W60	Wa	SEM	P-value
Doe W before mating, g/doe	2460	2534	2594	2544	2599	2583	62.9	0.502
Doe W before kindling, g/doe	2736 ^b	2836 ^{ab}	2957 ^a	2948 ^{ab}	2958 ^{ab}	2904 ^{ab}	56.4	0.045
Doe W change, g/doe/cycle	276	303	363	404	359	321	36.4	0.261
Milk yield, g/doe	84.1 ^{ab}	74.7 ^b	88.2 ^a	86.9 ^{ab}	87.8 ^{ab}	78.1 ^{ab}	3.46	0.034
No. kits born	6.89	6.44	7.00	6.44	7.00	6.78	0.504	0.932
No. kits alive at birth	6.67	6.00	7.00	6.44	7.00	6.11	0.477	0.547
No. weaned kits	6.44	5.67	6.11	5.89	6.78	5.78	0.507	0.618
Litter W at birth, g/litter	340	278	343	329	308	310	18.9	0.156
Litter W at 21 days old, g/litter	1519	1336	1335	1402	1519	1217	86.2	0.118
Litter W at weaning, g/litter	2188	1980	2143	2185	2389	2028	144	0.431

W0, W25, W40, W50, and Wa: proportions of *W. trilobata* at 0, 25, 40, 50, and 60% of concentrate, and *ad libitum*, respectively; W: live weight; SEM: standard error of means; P: = significant level; ^{a-e}: means within a row with different superscripts differ significantly (P<0.05).

Doe weight before mating did not show statistically significant differences among the treatment groups (P>0.05), with weights ranging from 2460 g (W0) to 2599 g (W60). However, doe weight before kindling was significantly influenced by the dietary treatments (P=0.045). The highest weights before kindling were observed in W40 and W60

groups (2957 g and 2958 g, respectively), and these were significantly higher than W0 (2736 g). Other groups (W25, W50, Wa) showed intermediate, non-significantly ($P>0.05$) different values. The change in doe weight during pregnancy (Doe W change) did not exhibit significant differences across treatments ($P>0.05$), ranging from 276 g (W0) to 404 g (W50). Milk yield was significantly affected by the dietary treatments ($P=0.034$). The highest milk yield was recorded in W40 (88.2 g/day), closely followed by W60 (87.8 g/day) and W50 (86.9 g/day), which were significantly ($P=0.034$) higher than W25 (74.7 g/day). W0 and Wa groups showed intermediate milk yields (84.1 g/day and 78.1 g/day, respectively). Number of kits born, number of kits alive at birth, and number of weaned kits did not show statistically significant differences among any of the treatments ($P>0.05$). The number of kits born ranged from 6.44 (W25, W50) to 7.00 (W40, W60). Similarly, litter weight at birth, litter weight at 21 days old, and litter weight at weaning showed no significant differences across the experimental groups ($P>0.05$).

Doe weight before mating did not differ among treatments, but weights before kindling were higher in groups with 40-60% *W. trilobata* compared to the control. This indicates that moderate *W. trilobata* inclusion helps build up better body reserves for pregnancy, a factor closely linked with their ability to sustain lactation (Quevedo et al., 2006; Pascual et al., 2013). Although overall weight gain during pregnancy was not different, the higher weights at kindling reflect improved nutritional status (Prieto-Huecas et al., 2023). Milk yield was higher in does fed *W. trilobata* at 40-60% than in those given 25%. This is important, since milk output is one of the strongest determinants of kit survival and growth in the early weeks (El-Gindy et al., 2022; Miah et al., 2020). Similar improvements in milk yield with dietary supplements have been reported in rabbits under tropical conditions (El-Speiy et al., 2024; Habeeb et al., 2025; Zhao et al., 2025). On the other hand, litter size and weights at birth or weaning did not differ significantly. This suggests that the basal diet, even in the control group, provided enough nutrients for basic reproductive outcomes. Genetic potential and environmental stressors, especially heat stress, may also have limited further improvements in litter traits (Lan et al., 2022; Abdelsalam and Fathi, 2023). Still, the higher doe weights and milk yields with moderate *W. trilobata* inclusion point to better maternal condition, which indirectly benefits kit survival and health. In tropical regions such as the Mekong Delta, heat stress often reduces feed intake and reproductive performance (Abdelsalam and Fathi, 2023; Trung et al., 2024). The ability of *W. trilobata* to support higher intake and sustain milk production shows its value as a resilient forage resource. It is easy, therefore, to provide a simple feeding option for household rabbit production in hot-humid regions.

CONCLUSIONS

The results show that *Wedelia trilobata* can be valuable forage for reproductive rabbit does in tropical conditions. Feeding *W. trilobata* at moderate levels of 40–60% of concentrate intake improved doe body weight before kindling and increased milk yield while maintaining good feed intake. Litter size and weights did not differ significantly among treatments, suggesting that the basal diet already supported essential reproductive outcomes. However, the lower fiber intake observed when *W. trilobata* was given *ad libitum* indicates that excessive use should be avoided. Because it grows easily without intensive cultivation, *W. trilobata* can help smallholder farmers reduce reliance on conventional forages and maintain productivity in hot, humid climates. Further studies should examine its long-term effects on health and reproductive success across multiple cycles, its potential use for growing rabbits, and its economic feasibility in smallholder systems.

DECLARATIONS

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Ethics committee approval

All procedures involving animals complied with Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14) and institutional guidelines. The research protocol was reviewed and approved by the institutional authority at Kien Giang College, An Giang, Viet Nam.

Acknowledgement

The authors would like to thank the staff and farmers who supported the feeding trial and animal care. We also appreciate the assistance of colleagues for their guidance during data collection and analysis.

Authors' contributions

NTV Chau conceived, designed, and collected the data for the experiment. D Mo analyzed and interpreted the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Data availability

The authors will provide the analysed datasets on reasonable request to the corresponding author.

Funding

This research received no external funding. All costs were self-funded by the authors.

Competing interests

The authors declare that there is no conflict of interest.

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ESTIMATION OF CROSSBREEDING PARAMETERS AND GROWTH PERFORMANCE OF BEGAI × SEKOTA SHEEP CROSSBRED UNDER ON-FARM CONDITIONS OF WAG-HIMRA, NORTHERN ETHIOPIA

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Supporting Information

ABSTRACT: Crossbreeding programs using either exotic or indigenous sires are an appropriate and rapid way to improve productivity within a shorter generation interval. Therefore, this study was designed to evaluate the growth performance traits of individual crossbreeding parameters of Sekota x Begait sheep crossbred under on-farm conditions in the lowland areas of Wag-himra, Northern Ethiopia. The cross-breeding program was conducted from 2018 to 2022 in Kasayred village of Abergele district on 699 animals. Least-squares means for weight at different ages and average daily weight gains were obtained by fitting the general linear model procedure in the SAS software. Crossbreeding parameters, including additive, heterosis, and recombination loss, were derived by fitting them as fixed covariates. The overall least square means for birth weight (BWt), three-month weight (TMWt), six-month weight (SMWt), nine-month weight (NMWt), and yearling weight (YWt) of evaluated progenies were 2.49±0.02, 11.61±0.09, 15.66±0.17, 19.27±0.18, and 23.46±0.23 kg, respectively. The overall least square means for average daily weight gain up to weaning (ADG1), six-month (ADG2), nine-month (ADG3), and yearling (ADG4) were 101.03±1.00, 45.30±1.38, 40.93±1.20, and 46.07±0.90 g/day. Only the additive effect of BWt for the Begait sheep breed was significant and it was found as 0.35±0.13 kg. Heterosis contributed 27-39 % live body weight improvement for Sekota sheep at yearling and six-month weights. In general, this pilot crossbreeding program resulted in 24% to 53% yearling weight improvement of pure Sekota breed (50%, 75%, and 25% blood levels, respectively), and participating farmers in the program witnessed that it was important in terms of improvement in phenotypic traits, growth rates, and market values. Based on the result of this study, we recommend establishing a 50% crossbred ram multiplication center in the area for long-term operation and further evaluating other blood levels by incorporating carcass and reproductive performance traits.

Keywords: Begait sheep, Crossbreeding, Farmers management practice, Phenotypic traits, Sekota sheep.

INTRODUCTION

Small ruminant production has proven to be an important component of food security and the economic and social livelihoods of the rural poor in all farming systems of Ethiopia, and more specifically in the Wag-himra zone (Yiheyes et al., 2012). In this area, sheep are raised as a major source of meat and immediate sources of cash income, even though the breeds are low producers. The Sekota sheep breed, with an estimated population over 200000 (CSA, 2021), is among Ethiopia's 14 breeds (Gizaw et al., 2008) found in all agroecological zones of Wag-Himra. The breed is characterized by low productivity in terms of growth, meat production, and reproductive performance but is highly resistant to feed scarcity and drought and can thrive with less available and poor-quality feed sources (Lemma et al., 2012; Yiheyes et al., 2012). In addition, Gizaw et al. (2008) described the country's sheep populations based on their threat status, population size, and economic, cultural, and ecological values, and recommended possible genetic improvement methods. With this, Sekota sheep have an average breed merit of 0.23, which is the lowest value from the characterized breeds, exceedingly only the Adilo breed (0.17). The value suggested the implementation of selective breeding in highland areas and controlled crossbreeding programs, especially in the lowlands, by considering the infrastructure, capacity, and breeding goals of smallholder producers.

Crossbreeding is a genetic improvement method that enables the utilization of the non-genetic effects of breed genetic variations and is an appropriate and rapid way to improve productivity within a shorter generation interval (Sheridan, 1981; Vanvanhossou et al., 2025). Crossbreeding of local sheep with exotic sire breeds in the country as a whole has been adopted for the last five decades as a major breeding strategy to improve the productivity performance of local sheep breeds (Gizaw et al., 2014). This strategy was not successful in Ethiopia, except for the Awassi crossbreeding program in limited areas, owing to the high cost of importing, multiplying, and maintaining exotic sheep breeds stemming from resource limitations. In addition, exotic genotypes were not well compatible with low-input system environments.

Crossbreeding programs using potential indigenous sheep breeds (Bonga, Washera, Farta, and Menz breeds) have been tested in the subalpine and Western parts of the Amhara region and have demonstrated several advantages over the use of exotic sires (Abebe and Alemayehu, 2019). Crossbreeding between indigenous breeds is suitable for farming communities in developing countries, such as Ethiopia, which share various sociocultural characteristics and geographical proximity. The limitation of using such indigenous breeds is the smaller performance variation, which results in the performance of crossbred progenies being parallel to that of the parents.

In the northern lowlands of Ethiopia, the Begait sheep breed, known for its superior growth, is a valuable resource for crossbreeding. Its higher growth performance suggests that it has been instrumental in improving smaller sheep breeds within its geographical range. The breed is characterized by a muscular body, higher adaptation to drought, and higher prolificacy (Amare et al., 2012). The adult weight of Begait rams can reach more than 80 kg under semi-intensive management and more than 45 kg under extensive systems (Amare et al., 2019). This breed could be a potential sire line to improve the productivity and phenotypic characteristics of Sekota sheep because of environmental and food source proximity. Robison et al., (1981) described; even though considerable research has evaluated crossbreeding in farm animals, the analyses and reporting of the results have often been inadequate, especially in quantifying the individual crossbreeding parameters. Therefore, this study was designed to evaluate the growth performance and estimate individual crossbreeding parameters of Sekota × Begait sheep crossbred levels under farmer management practices in the lowland areas of Wag-Himra, Northern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Kasayerd village in the Abergele district of the Wag-himra Zone, Amhara region, Ethiopia from 2018-2022. The area is located geographically at 13° 01'37"E 38° 58'36"N with an altitude of 1270m above sea level. The average minimum and maximum temperatures ranged between 16 and 36°C. The mean annual rainfall (ten-year average) in the area is reported as 620 mm with shorter intervals (mid-July to late August) and erratic distribution. The major feed sources in the study location were natural pastures, crop residues, rangeland shrubs, and acacia tree species. Small ruminant production is the mainstay of the livelihood in the area. In the highlands, producers maintain all herds together with their cropping activities, while in the lowlands, market-oriented sheep production is commonly practiced with a continuous supply of sheep to local markets.

Description of experimental animals and their management

The Sekota sheep breed is a short fat-tailed with, predominantly fine hair coat, smaller to medium size, and rudimentary ears. It is distributed in the Tekeze Valley of Amhara and some parts of the Tigray regional states and is reared by the Agew, Amhara, and Tigray communities (Gizaw, 2008; Gizaw et al., 2014). The breed is further characterized by a smaller mature body weight, longer reproductive performance, lower prolificacy, and resistance to drought and feed shortages. The Begait sheep breed is categorized under the long thin-tailed breed groups with convex facial profile, polled horn type, long and laterally dropping ear shape, produced for both meat and milk production, large-sized, prolific (frequent twinning), distributed in the northern parts of Ethiopia, and reared by Amhara and Tigray communities (Amare et al., 2019). Pure Begait rams brought from Humera were separately herded for a month before mating. Rams were provided with different vaccinations and treatments against the major diseases and parasites in the area. Experimental animals are managed under an extensive system, arranged in ranges throughout the year. Farmers practiced crop residue supplementation of pure Begait rams during the critical feed shortage season. Acacia pods and leaves, sorghum stalks, shrubs, and cowpea haulms are the major feed sources in the area. All experimental animals were vaccinated against common diseases in the area and were treated regularly. All experimental techniques, procedures, and animal care were properly applied considering ethical standards, and animal nutrition and health researchers of Sekota Dry-land Agricultural Research Center confirmed this during the annual review forum. In addition, the Center Ethical Clearance Committee approved that all the experimental procedures of this activity were conducted according to the institutional animal care guidelines of the Amhara Region Agricultural Research Institute.

Structure of the crossbreeding program

The Sekota × Begait crossbreeding program was implemented by mating pure Begait rams with pure Sekota ewes as dams for the production of 50% crossbred blood levels, as described in Figure 1. At the beginning of the crossbreeding program, 33 pure Begait rams were used as a sire line to mate a base population of 500 local Sekota ewes from 18 participating households with a 1:15 mating ratio from November 2017 to 2018 for two years. Pure Sekota rams were culled or castrated before the Begait ram distribution after an agreed consensus with participating farmers through discussion and training. Subsequently, the distributed pure Begait rams were utilized for two successive mating seasons or births within a single household before being transferred to the next household.

Begait rams were rotated by considering their previous mating history to avoid inbreeding. A total of 196 first filial (F1) generation crossbred lambs were produced during the first two years of the study. From these F1 progenies, fourteen 50% crossbred rams were selected for the production of next generation progenies. Then, F2 generations of 25% were produced from 50% crossbred rams and pure Sekota ewes, and 75% blood levels produced from pure Begait rams and 50% crossbred ewes, whereas 50% blood levels in this generation were produced from the inter se mating of 50% crossbreds. Pure Sekota lambs raised and monitored in the adjacent village with local practices served as the base population for pure Sekota sheep. The production of all blood levels for evaluation was critically managed by proper ram rotation and culling of unselected young lambs. The production of all three blood levels was conducted by considering the availability of the required reproductive ewes or rams in that household that would be appropriate for the production of the desired blood level. During the selection period of these rams, unselected ones were culled from the population by castration or selling to the local market. Breeding sire rotations were performed based on the drafted bylaw, considering the previous mating history of the pure Begait rams and the purpose of producing the desired blood levels. Subsequently, 28 pure Begait rams (an initial 33 minus 5 that died) and 14 crossbred (50%) rams were distributed among the participating households. These rams, both purebred and 50% crossbred, were used to sire the second-generation (F2) progeny. The resulting F2 generation, exhibiting 50%, 25%, and 75% Begait blood levels, was born within the targeted participating households. This variation in blood levels arose from mating the purebred and 50% crossbred rams with the existing first-generation (F1) ewes, which were all 50% crossbred ewes.

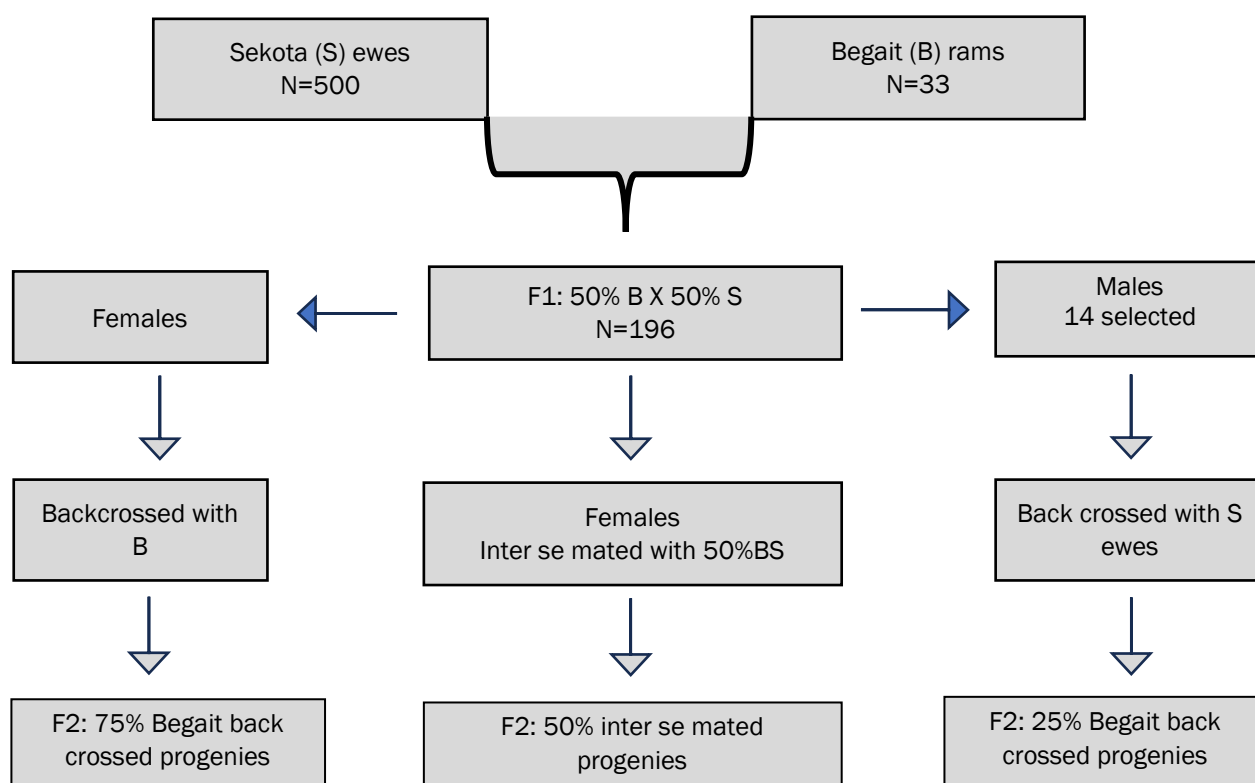


Figure 1 - Schematic presentation of Sekota X Begait sheep crossbreeding scheme in farmer flocks

Study design, data management and analysis

A cross-sectional study involving the purposive selection of completeness of study traits and records was conducted. From the implementation of the crossbreeding program, data were recorded on the first ram distribution date, lambing dates, ram rotation, breeding ram survival, body weight of lambs at different ages, sex, birth type, and other biological performances by trained enumerators. All animals were identified using plastic ear tags at the start of the program. A total of 699 births (231 pure Sekota sheep) were monitored and recorded for the evaluation of progeny growth performance. The number of animals was significantly reduced during the nine month and yearling stages due to the practice of selling young animals in these stages. The growth performance of the pure Sekota breed at different age groups was monitored and collected from adjacent villages with zero crossbreeding threat. All the collected biological data were checked for normality, and data structures not suitable for the analysis were corrected if possible or discarded when incomplete and inconsistent.

Continuous data, such as weight at different ages and average daily weight gains of crossbred blood levels, were analyzed using the general linear model procedures of Statistical Analysis Software (SAS, 2002). Mean separation was

conducted using Tukey's HSD test for significant fixed effects. Linear correlation analysis using Pearson's method was employed to evaluate the relatedness of progeny weights at different age groups. The fixed effects fitted in the evaluation model included genotype (Pure Sekota, 25%, 50%, and 75% crossbreeds), sex of the lamb (male, female), birth type (single, twin), and season of birth (wet from June to November and dry from December to May based on feed availability in the area). It was difficult to estimate parity for all pure Sekota ewes at the start of the crossbreeding program by asking the owners; therefore, it was excluded from the analysis. The statistical model fitted for the least squares estimation of various growth performance traits is as follows:

$$Y_{ijklm} = \mu + G_i + S_j + B_k + T_l + e_{ijklm} \quad (1)$$

Where Y_{ijklm} is the dependent variable (weight at different ages, average daily weight gains), G_i is the effect of i^{th} genotype, S_j is the effect of j^{th} sex of lamb, B_k is the effect of k^{th} birth type, T_l is the effect of l^{th} season of birth and e_{ijklm} is the residual error term.

The coefficient of individual crossbreeding parameters including breed additive (g_i), heterozygosity (h_{ij}), and recombination (r_{ij}) were analyzed using a multiple regression procedure developed by (Robison et al., 1982) by fitting them as fixed covariates instead of breed/genotypes. The expected coefficients used for crossbreeding parameters estimation for growth performance traits (Table 1) were derived using the formula:

$$g_i = 1/2(GB_s + GB_d) \quad (2)$$

$$h_{ij} = GB_s * GS_d + GS_s * GB_d \quad (3)$$

$$r_{ij} = 4 g_B * g_B - h_{ij} \quad (4)$$

The coefficients of individual crossbreeding parameters, including breed additive (g_i), heterozygosity (h_{ij}), and recombination (r_{ij}), were analyzed using a multiple regression procedure developed by Robison et al. (1982) by fitting them as fixed covariates instead of breed/genotypes. The expected coefficients used for crossbreeding parameter estimation for growth performance traits (Table 1) were derived using the following formula:

Table 1 - Genetic coefficients of Begait breed used for the estimation of cross-breeding parameters of growth traits in Sekota X Begait sheep breed

Genetic group	Blood level/Generation	Number of progenies born	g_c	h_c	r_c
SXS	Pure Sekota	231	0	0	0
BXS	F1 (50%)	311	0.5	1	0
BXBS	Back cross (75%)	68	0.75	0.5	0.25
BSXS	Back cross (25%)	89	0.25	0.5	0.25

Where; S= Sekota sheep; B= Begait sheep; BS= Sekota × Begait first filial generation (50%); BXBS= 50% females back cross with pure Begait ram producing 75% Begait; BSXS= pure Sekota ewes back cross with 50% crossbred rams to produce 25% Begait blood level; and the sires' breed is mentioned first; g_c = coefficient of breed additive; h_c = coefficient of heterosis and r_c = coefficient of recombination loss effects

RESULT AND DISCUSSION

Growth performances of Sekota sheep and its crosses with Begait breed

The overall least square means for birth weight (BWt), three-month weight (TMWt), six-month weight (SMWt), nine-month weight (NMWt), and yearling weight (YWt) of evaluated progenies were 2.49±0.02, 11.61±0.09, 15.66±0.17, 19.27±0.18, and 23.46±0.23 kg, respectively, as presented in Table 2. The pre-weaning growth performance obtained in this study was slightly higher than the values reported by Mekuriaw et al. (2013) for Washera × Farta, by Lemma et al. (2012) for Washera × Menz and Bonga × Menz crossbred lambs, and by Abebe et al. (2016) for Dorper × Afar crossbreds under station management, but lower than the performances of Dorper × Wollo crossbred sheep (Lakew and Haile-Melekot, 2014), Dorper × Tikur crossbreds in the highlands of North Wollo (Tilahun et al., 2016) and for Dorper × Menz sheep crossbreds in the cool highlands of North Shewa (Abebe et al., 2016). This is in agreement with the results reported by Habtegiorgis et al. (2025), who reported that the growth performance of Dorper × Local sheep crossbred in the southern region at birth and weaning age were 2.43±0.03 and 11.59±0.49 kg, respectively. The growth performance of crossbred lambs during the post-weaning period was also greater in this study than that reported in previous studies.

Blood level exerted a significant effect ($P < 0.0001$) on the growth performance of lambs at all growth stages, in which crossbred lambs were heavier than local counterparts (from birth to yearling weight). Half-cross crossbred lambs (50%) were heavier than the other blood levels up to weaning age and then beaten by 25% and 75% blood levels. In this study, 25% of the crossbred lambs outperformed their 50% and 75% counterparts at all post-weaning ages (from SMWt to YWt). Crossbred lambs with 25%, 50%, and 75% blood levels were 29.09, 31.23%, and 30.26% heavier than pure Sekota lambs at weaning age, respectively.

Table 2 - Least square means (\pm SE) for growth performances of Sekota X Begait crossbred sheep (kg) at different growth stages

Variables	N	BWt LSM \pm SE	N	TMWt LSM \pm SE	N	SMWt LSM \pm SE	N	NMWt LSM \pm SE	N	YWt LSM \pm SE
Overall mean	699	2.49 \pm 0.02	588	11.61 \pm 0.09	508	15.66 \pm 0.17	443	19.27 \pm 0.18	344	23.46 \pm 0.23
CV		15.73		13.58		10.74		9.85		10.99
Blood level		***		***		***		***		***
Pure Sekota	231	2.15 \pm 0.02 ^c	151	8.48 \pm 0.03 ^b	144	10.57 \pm 0.03 ^b	134	14.14 \pm 0.08 ^c	120	19.25 \pm 0.15 ^c
25% Begait	89	2.35 \pm 0.04 ^b	88	11.96 \pm 0.17 ^a	55	17.97 \pm 0.31 ^a	30	23.32 \pm 0.48 ^a	21	29.6 \pm 0.34 ^a
50% Begait	311	2.64 \pm 0.03 ^a	287	12.33 \pm 0.11 ^a	250	17.66 \pm 0.12 ^a	233	20.67 \pm 0.13 ^b	176	25.16 \pm 0.23 ^b
75% Begait	68	2.55 \pm 0.06 ^a	62	12.16 \pm 0.19 ^a	59	18.39 \pm 0.29 ^a	46	22.53 \pm 0.38 ^a	27	28.14 \pm 0.62 ^a
Lamb sex		ns		ns		ns		ns		ns
Male	334	2.41 \pm 0.03	285	11.27 \pm 0.13	247	16.16 \pm 0.23	217	20.12 \pm 0.24	165	25.49 \pm 0.32
Female	365	2.43 \pm 0.02	303	11.19 \pm 0.13	261	16.14 \pm 0.24	226	20.21 \pm 0.26	179	25.64 \pm 0.32
Birth type		ns		*		ns		ns		ns
Single	682	2.47 \pm 0.02	575	11.56 \pm 0.09 ^a	495	16.07 \pm 0.17	432	20.35 \pm 0.18	335	25.34 \pm 0.23
Twin	17	2.37 \pm 0.13	13	10.91 \pm 0.62 ^b	13	16.24 \pm 1.05	11	19.98 \pm 0.98	9	25.75 \pm 0.22
Season of birth		***		**		*		ns		ns
Dry	269	2.34 \pm 0.03	233	11.05 \pm 0.13 ^b	212	16.32 \pm 0.23 ^b	183	20.00 \pm 0.27	128	25.58 \pm 0.41 ^a
Wet	430	2.50 \pm 0.02	355	11.41 \pm 0.13 ^a	296	15.98 \pm 0.22 ^a	260	20.34 \pm 0.23	216	25.73 \pm 0.26 ^b

Where; BWt= Birth weight; TMWt= Three-month weight; SMWt= Six-month weight; NMWt= Nine-month weight; YWt= Yearling weight; LSM= least squares mean; SE= standard error and CV= coefficient of Variation; ns= P \geq 0.05; * = P < 0.05; ** = P < 0.01; and *** = P < 0.001

In addition, 25% and 75% of crossbred lambs had superior yearling weights than 50% and local counterparts. The reason for the higher post-weaning weight of all blood level crossbred lambs compared to the pure Sekota lambs could be connected with the positive effects of the crossbreeding program. This crossbreeding program also demonstrated that when the blood levels of pure Begait are reduced, the growth performance of produced progenies gets higher due to the compatibility of these genotypes to the existing environment which is in line with the report of Getachew et al., (2016) who reported the superiority of crossbreds in various aspects such as growth and market price.

The growth performances of crossbred progenies were not significantly affected $P > 0.05$ by the sex of lambs at all growth stages in this study. The non-significant effect of sex on the growth performance of traits in different growth stages was reported by Habtegiorgis et al. (2025) and Tilahun et al. (2016) whereas Abebe et al. (2016) and Lakew and Haile-Melekot (2014) reported the significant effect of sex. This variation could be connected with environmental, management, population size, and breed variations under investigation. In addition, this phenomenon could also be connected with breed specificity, uniform management practice, nutrient equivalence, and age of measurement variations. The birth type of lambs did not exert a significant effect, except at weaning age. The non-significance of birth type in this study was in contrast to other reports due to the extreme sample size variations observed in this study. The proportion of twin kids in this study were very small (2.43%) and that could affect the significance of type of birth in this study. On the other hand, the season of birth significantly affected the growth performance of lambs up to six-month weight then after, season did not affect nine month and yearling weight growth performance.

The observed growth performance differences in this study are in agreement with farmers' opinions regarding the crossbreeding program gathered during field visits and researcher observations at individual households. With this, Farmers are very interested in the performance of the overall program. Phenotypically they preferred 25% crossbred lambs due to increased ears size and short twisted tails. They are also interested in the meat quality of the crossbreds. In addition, the market price of crossbred lambs was maximum at six-month age whereas the local lambs were not reached for the market even at yearling age. And also, the program drawn project interests in the area and they are willing to scale out the technology to other villages.

Growth rate (daily weight gains) of lambs

The overall least square means for average daily weight gain from birth to three-month (ADG1), from three-month to six-month (ADG2), from six-month to nine-month (ADG3), and from nine-month to yearling weight (ADG4) of different blood levels were 101.03 ± 1.00 , 45.30 ± 1.38 , 40.93 ± 1.20 , and 46.07 ± 0.90 g, respectively (Table 3). Pre-weaning and post-weaning average daily gain performances of lambs were significantly ($P < 0.0001$) affected by blood level. Lamb sex and birth type did not show significant effect ($P > 0.05$) at all stages even though male and twin lambs exhibit higher values. Season of birth was not significant at all stages of growth except in ADG2. During the pre-weaning growth phase, crossbred lambs exhibited higher average daily weight gain compared to pure local breeds, with this increase positively correlated to their body weight. However, during the post-weaning period, 50% crossbred lambs showed lower ADG even underperforming local lambs—likely due to the pronounced influence of environmental factors on their growth performance. The pre-weaning daily weight gain of this study is in accordance with the report of Tilahun et al. (2016) for Awassi X Tikur crossbred sheep, by report of Habtegiorgis et al. (2025) for 50% Dorper × Indigenous sheep in the southern Ethiopia who reported 98.16 ± 13.4 g/day, that the lower ADG of 50% crossbreds is also reported in Awassi X Wollo sheep crossbreeding program (Tesema et al., 2020). The pre-weaning average daily gain values obtained in this study are higher than the reports of Lemma et al. (2012) for pure Menz, 50% Washera × Menz and 50% Bonga X Menz breeding groups which were 65 ± 1.8 , 66 ± 1.7 , and 70 ± 1.7 g, respectively.

Table 3 - Phenotypic correlation values (with significant difference level) of weight at different age in Sekota X Begait sheep crossbreeding program.

	BWt	TMWt	SMWt	NMWt	Ywt
BWt	1	0.27 (***)	0.27 (***)	0.30 (***)	0.24 (***)
TMWt		1	0.74 (***)	0.64 (***)	0.52 (***)
SMWt			1	0.82 (***)	0.70 (***)
NMWt				1	0.82 (***)
Ywt					1

Where; BWt= birth weight; TMWt= three-month weight; SMWt= six-month weight; NMWt= nine-month weight; YWt= yearling weight; ns= $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; and *** = $P < 0.001$

Phenotypic correlation of growth performance traits of crossbreds

Understanding the magnitude of genetic and phenotypic correlations is important for multiple trait evaluation, particularly when predicting correlated responses to selection. Phenotypic correlations are better at evaluating the effect of both genetic and environmental variations in populations. The phenotypic correlation of growth performance traits for

crossbred lambs at different weights is presented in Figure 2. Phenotypic correlations of BWT with later growth stages were moderate and inline when compared to other various studies in sheep and goats. On the other hand, correlation values for TMWT-SMWT, SMWT-NMWT and NMWT-YWT are higher and positive which are comparable with the results of various studies in small ruminants. These higher phenotypic correlation values suggest that heavier weight of kids at weaning tend to attain heavier weights at later stages of growth.

Estimation of crossbreeding parameters

The estimates of additive and non-additive genetic (heterosis and recombination) effects are presented in Table 4. Only the additive effect of BWT for Begait sheep breed was significant and it was found 0.35±0.13 kg in this study. Weights of the crossbred lambs producing in this crossbreeding program other than birth weight are small and non-significant. Lower additive genetic effects in this study given the clear genetic distance between the two breeds might be related to the smaller sample size and the adverse effects of the experimental environment. However, all the additive effects of Begait breed were not significantly different for all the average daily weight gains and even for the later growth stages the additive genetic effect from the larger breed was negative. This could be explained by smaller to non-significant additive effects for most of the growth performance traits confirmed the lower level of breed difference between indigenous breeds compared to using exotic sire sources and indigenous breeds are more adapted to the locality than other breeds out of the area. However, higher and significant individual additive effects were reported in milk, growth and reproduction performance improvement of dairy cattle in Central Ethiopia (Birhanu et al., 2015).

The effect of heterosis for most of the growth performance and daily weight gain traits were positive and significant except for ADG3 and ADG4 which exerted negative values. These positive and highly significant heterosis values imply that heterosis contributed a positive effect on the improvement of the pure Sekota sheep breed. For instance, heterosis contributed 27-39 % live body weight improvement for Sekota sheep which is parallel to 7.20 and 6.77 kg improvement for yearling and six-month weights, respectively. Other positive effects for heterosis are also presented in various livestock crossbreeding studies as described by Tesema et al. (2023).

In this study, the estimates of recombination showed a positive and significant effect on most of the growth and average daily weight gain traits except for BWT. This result is in argument with the empirical principle that this effect should be negative and small rather. The reason for this result might be connected with the smaller data size in this study but the limitation here could be the difficulty to discuss these results in the existing condition. However, other scholars reported that recombination loss's effect was negative in most of the growth and reproductive traits of livestock species.

Table 4 - Least square means (±SE) for average daily weight gains (g) of Sekota X Begait crossbred sheep at different growth stages

Variables	N	ADG1 LSM±SE	N	ADG2 LSM±SE	N	ADG3 LSM±SE	N	ADG4 LSM±SE
Overall mean	588	101.03±1.00	508	45.30±1.38	443	40.93±1.20	344	46.07±0.90
CV		18.04		54.55		60.87		36.90
Blood level		***		***		**		***
Pure Sekota	151	70.19±0.38 ^b	144	23.72±0.37 ^c	134	42.42±0.89 ^a	120	50.66±0.83 ^{ab}
25% Begait	88	106.65±2.00 ^a	55	67.42±4.77 ^{ab}	30	49.90±4.72 ^a	21	59.05±2.95 ^a
50% Begait	287	107.66±1.32 ^a	250	58.43±1.73 ^b	233	34.87±1.86 ^b	176	43.93±1.52 ^b
75% Begait	62	106.64±2.15 ^a	59	69.37±4.10 ^a	46	46.97±5.06 ^a	27	55.24±4.38 ^a
Lamb sex		ns		ns		ns		ns
Male	285	98.29±1.45	247	54.36±2.03	217	42.82±1.70	165	51.84±1.32
Female	303	97.28±1.38	261	54.91±1.86	226	44.27±1.70	179	52.60±1.35
Birth type		ns		ns		ns		ns
Single	575	100.98±1.01	495	49.70±1.39	432	44.65±1.23	335	49.90±0.96
Twine	13	94.60±6.34	13	59.58±9.29	11	42.44±4.56	9	54.54±5.01
Season of birth		ns		**		ns		ns
Dry	233	96.75±1.45	212	58.62±2.27 ^a	183	41.06±2.19	128	51.58±1.94
Wet	355	98.82±1.35	296	50.66±1.57 ^b	260	46.03±1.35	216	52.86±6.97

ADG1= average daily weight gain from birth to three-month weight; ADG2= daily weight gain from three-month to six-month weights; ADG3= daily weight gain from six-month to nine-month weight; ADG4= weight gain from nine-month to yearling weights; Min= minimum; Max= maximum; ns= not significant/P >= 0.05; * = P < 0.05; ** = P < 0.01; and *** = P < 0.0001

Table 5 - Estimates of individual crossbreeding effects with standard errors of Sekota and Begait sheep breeds

Traits	N	Sekota means/Intercept	Additive	Heterosis	Recombination
BWt	699	2.24±0.03	0.35±0.13***	0.28±0.07**	-0.28±0.18 ^{ns}
TMWt	588	8.87±0.13	0.30±0.53 ^{ns}	3.65±0.31***	6.06±0.75***
SMWt	508	10.39±0.14	0.78±0.63 ^{ns}	6.77±0.36***	15.98±0.97***
NMWt	443	14.43±0.16	-1.41±0.89 ^{ns}	7.15±0.49***	22.72±1.45***
YWt	344	19.19±0.24	-2.77±1.50 ^{ns}	7.20±0.81***	28.55±2.33***
ADG1	588	73.60±1.48	-0.55±6.05 ^{ns}	37.61±3.54***	70.19±8.59***
ADG2	508	16.92±2.08	2.56±9.35 ^{ns}	34.42±5.36***	118.69±14.43***
ADG3	443	45.10±2.18	-3.32±11.71 ^{ns}	-7.06±6.45 ^{ns}	31.10±19.00 ^{ns}
ADG4	344	48.97±1.55	-7.35±9.87 ^{ns}	-3.44±5.33 ^{ns}	43.75±15.34**

Where; BWt= birth weight; TMWt= three-month weight; SMWt= six-month weight; NMWt= nine-month weight; YWt= yearling weight; ADG1= average daily gain from birth to three-month weight; ADG2= average daily gain from three-month to six-month weight; ADG3= average daily gain from six-month to nine-month weight; ADG4= average daily gain from nine-month to yearling weight; ns= not significant; **= P < 0.001; and ***= P < 0.0001

CONCLUSION AND RECOMMENDATIONS

Based on the findings of this study, it can be concluded that all crossbred types demonstrated improved growth rates compared to the local sheep breeds in the area. Specifically, the crossbred types with 25% and 75% blood levels showed superior performance compared to the F1 (50%) crossbred level. However, it is important to note that significant variation in number was observed in the 25% and 75% crossbred types, which might introduce bias to these results. However, it is crucial to acknowledge the existing gaps for further research. In addition to growth, overall productivity and benefits, it is important to consider other factors such as lamb survival and reproductive performances. These aspects should be taken into account when assessing the overall success and viability of crossbreeding programs. Further studies should aim to address these areas of concern and provide a comprehensive understanding of the potential benefits and limitations of crossbreeding in terms of lamb survival and reproductive performance. It is also essential that future recommendations build upon the findings of this study while acknowledging the need for additional research to bridge existing knowledge gaps and provide a more comprehensive understanding of the impact of crossbreeding on various aspects of sheep productivity.

Individual crossbreeding parameters especially the heterosis effects were higher in this study which enables the utilization of non-additive genes from breed combination. The households involved in this program showed keen interest in crossbreeding programs when they found it beneficial to them. Thus, from the pilot results obtained in this study, we recommend; the distribution of 50% crossbred rams by establishing a 50% crossbred multiplication site. In addition, further research needs to be done on the performance evaluation of other blood levels including carcass, lamb survival and reproductive performance traits addressing site-specific farmer interests.

DECLARATIONS

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

Sekota Dry-land Agricultural Research Center Ethical Clearance Team confirmed that all the experimental procedures of this activity were conducted by the institutional animal care guidelines of the Amhara Region Agricultural Research Institute under SDARC/EEC/2019/01-minute number.

Authors' contributions

Conceptualization: Mulatu Gobeze and Bekahegn Wondim; **Data Curation:** Mulatu Gobeze, Wubneh Aklog, Bekahegn Wondim, Adane Bahiru, Yeshiwas Walle, Tigabu Limenh, Alemu Demlie and Tesfaye G/Mariam; **Data analysis and Writing-original draft:** Mulatu Gobeze; **Writing – review & editing:** Mulatu Gobeze, Wubneh Aklog, Bekahegn Wondim, Adane Bahiru, Yeshiwas Walle, Tigabu Limenh, Alemu Demlie and Tesfaye Gebremariam

Acknowledgements

The authors are thankful to the Amhara Agricultural Research Institute (ARARI) for the financial support. The community members involved in this study are acknowledged for cooperation during data collection and their participation in the whole phase of design and implementation of the crossbreeding program.

Funding

This work received financial support from the Amhara Agricultural Research Institute (ARARI).

Competing interests

The authors declare no competing interests in this research and publication.

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ENERGY METABOLISM AND PERFORMANCE OF LOCAL SHEEP FED ON AMMONIATED RICE STRAW AND CONCENTRATE SUPPLEMENTED WITH WARU AND BAMBOO LEAF MEALS

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ABSTRACT: This study evaluated the effects of waru (*Hibiscus tiliaceus*) leaf meal (WLM) and bamboo (*Gigantochloa apus*) leaf meal (BLM) supplementation on energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate. Sixteen male local sheep aged 12–18 months with initial average body weight of 27.1 ± 2.11 kg (7.78% coefficient of variation) were completely randomized according to a Completely Randomized Design (CRD) to receive one of the following treatments: T0 (ammoniated rice straw + concentrate feed); T1 (T0 + 2.49 g WLM); T2 (T0 + 1.87 g WLM + 0.33 g BLM); and T3 (T0 + 1.25 g WLM + 0.65 g BLM). The concentrate was provided at 3% of live weight, and ammoniated rice straw was available on a controlled *ad libitum*. Analysis of variance indicated that WLM and BLM supplementation significantly increased digestible energy (DE), metabolizable energy (ME), energy retention (ER), ER to EI efficiency, ER to DE efficiency, average daily gain (ADG), and consumption rate (CR), while significantly decreasing the feed conversion ratio (FCR) ($P < 0.01$). However, the treatments had no significant effect ($P > 0.05$) on dry matter intake (DMI) and energy intake (EI). In conclusion, dietary supplementation with 1.87 g of WLM and 0.33 g of BLM per kg concentrate was more effective than the other combinations in improving energy metabolism and performance of local sheep.

Keywords: Energy, Feed conversion, Flavonoid, Metabolism, Sheep nutrition.

INTRODUCTION

The year-round availability of rice straw makes it a highly potential feed resource, particularly during the dry season. However, the low nutritional value of rice straw, due to its high silica content, low ruminal carbohydrate degradability, and low nitrogen content, limits its utilization (Peripolli et al., 2016). A widely adopted method to enhance its quality is urea ammoniation. An *in vitro* study by Ma et al. (2020) demonstrates that ammoniated rice straw can significantly increase acetate, butyrate, and volatile fatty acid (VFA) production.

The use of concentrate in the feedlot industry aims to increase productivity in a short period. A higher allocation of concentrates can improve energy intake and overall performance by increasing energy density (Schmitz et al., 2018), but a total ration made up of 60% to 80% concentrate can decrease ruminal pH to 6.0 and 5.6 at 6 hours post-feeding, respectively (Wanapat et al., 2014). The declining ruminal pH is due to lactate accumulation from starch fermentation, which reduces cellulolytic bacterial activity, decreases cellulase enzyme function, and lowers fiber digestibility as an energy source (Li et al., 2021). These disturbances negatively affect energy efficiency and animal performance. Given that smallholder farmers in the region predominantly raise local sheep breeds, addressing such nutritional challenges is crucial to sustain their production systems.

Rumen stability can be maintained by supplementing livestock diet with flavonoids. Flavonoids help modulate rumen conditions by stabilizing pH, reducing methane, increasing propionate proportions, and promoting the growth of lactate-utilizing bacteria, such as *Streptococcus ruminantium* and *Megasphaera elsdenii* (Balcells et al., 2012; Seradj et al., 2018). Flavonoids serve as buffers in cattle fed on high-concentrate diets (Bata et al., 2016). By maintaining rumen pH stability, flavonoids support cellulolytic bacterial activity, improve fiber digestion efficiency, and enhance energy availability for livestock growth.

Several studies have shown that waru (*Hibiscus tiliaceus*) and bamboo (*Gigantochloa apus*) leaves contain flavonoid compounds. Nasution et al. (2021) reported that the inclusion of 0.24% or 2.4 g/kg DM waru leaf meal was optimal for improving microbial protein synthesis efficiency in local sheep. Bata et al. (2016) demonstrated that supplementation with WLM increased body weight gain and improved feed efficiency in Sumba Ongole cattle. Li et al. (2021) reported that the supplementation of bamboo leaf extract at 0.13% or 1.3 g/kg DM enhanced milk production, rumen fermentation, and rumen microbial populations. It seems that, the combination of waru and bamboo leaves has the potential to produce synergistic effects in supporting rumen fermentation and improving feed digestion efficiency. Waru leaves

RESEARCH ARTICLE
 PII: S222877012500038-15
 Received: May 15, 2025
 Revised: November 18, 2025
 Accepted: November 21, 2025

contribute by enhancing microbial protein synthesis and improving feed utilization efficiency, while bamboo leaves contribute by maintaining gastrointestinal health through their antibacterial properties that inhibit pathogenic bacteria, as well as their antioxidant and anti-inflammatory compounds.

This study aimed to evaluate the potential synergistic effects of waru leaf meal (WLM) and bamboo leaf meal (BLM) supplementation on energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate.

MATERIALS AND METHODS

This study was conducted in accordance with the provisions set forth in the Government Regulation of the Republic of Indonesia No. 95/2012 on Veterinary Public Health and Animal Welfare, particularly those related to the humane treatment and welfare of animals used in research. All procedures involving animals were performed with due regard to minimizing pain, suffering, and stress, and ensuring appropriate care throughout the research period. The research also followed internal guidelines established by Universitas Jenderal Soedirman for animal-based research activities.

This study was conducted from March 1 to June 15, 2024, at Amanah Farm, Datar Village, Sumbang District, Banyumas Regency. The Proximate analysis was done at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Jenderal Soedirman University. The gross energy analysis was conducted at the Ruminant Instrument Standard Testing Laboratory, Pasuruan, and the Tropical Livestock Research Center Laboratory, Gadjah Mada University, Yogyakarta. Rice straw was collected from various rice varieties in Banyumas Regency and surrounding areas. It was chopped and treated with a mixture of 10% water, 4% urea, and 2% cassava pulp, then ensiled in sealed plastic drums for 14 days. After fermentation, the ammoniated straw was air-dried and sprayed with molasses before feeding. The nutrient composition of the feed is presented in Table 1. Waru leaf meal (WLM) and bamboo leaf meal (BLM) was prepared from mature and young leaves. Both types of leaves were oven-dried until constant weight was achieved, then pulverized and incorporated into the concentrate according to the treatment dosage.

Table 1 - Nutrient composition of experimental feed

Feed	DM (%)*	CP (% DM)**	CF (% DM)*	Ash (% DM)*	Energy (Mcal)**
Ammoniated rice straw	36.33	8.64	26.97	23.68	3.47
Concentrate T0	84.81	14.20	17.72	11.15	3.88
Concentrate T1	84.22	13.97	17.60	11.34	4.03
Concentrate T2	85.22	13.51	17.13	11.12	4.01
Concentrate T3	85.67	13.47	17.74	11.35	3.94

DM= Dry Matter; CP= Crude Protein; CF= Crude Fiber. * Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Jenderal Soedirman University, 2024. ** Laboratory of Large Ruminant Standard Instrument Testing, Pasuruan.

A total of sixteen male local sheep, aged 12–18 months with an average initial body weight of 27.1 ± 2.11 kg (coefficient of variation: 7.78%), were used. The sheep were dewormed prior to the experiments and housed individually in 50 cm × 100 cm pens. The sheep were completely randomized according to a Completely Randomized Design (CRD) to receive one of the following treatments: T0: Ammoniated rice straw + concentrate (control); T1: T0 + 2.49 g/kg concentrate WLM; T2: T0 + 1.87 g/kg concentrate WLM + 0.33 g/kg concentrate BLM; T3: T0 + 1.25 g/kg concentrate WLM + 0.65 g/kg concentrate BLM.

Each treatment was replicated four times. Concentrate was offered at 3% of body weight (on dry matter basis), ammoniated rice straw on a controlled ad libitum basis, and free access to drinking water. The concentrate was given twice daily (07:00 AM and 03:00 PM), followed by ammoniated rice straw. The experimental period was divided into three phases: adaptation (7 days), preliminary (14 days), and feeding trial (60 days). The 7-day adaptation phase allowed the animals to acclimate to the housing environment and basal diet, thereby minimizing stress-related variation before data collection. The subsequent 14-day preliminary phase was conducted to stabilize rumen fermentation patterns and feed intake under the experimental diet, ensuring that any transient effects from dietary change were eliminated prior to the main trial. The 60-day feeding trial was selected to cover a sufficient growth period for detecting differences in performance, feed efficiency, and nutrient utilization. Feed (consumed and residue), feces, and urine were collected during a five-day sampling period, in the middle of the feeding trial, using the total collection method (Cole and Ronning, 1974). A 100-gram feed sample was oven-dried at 60 °C for 48 hours. Feces were collected using trays placed beneath the pens, sprayed every four hours with 10% formalin to prevent decomposition, then weighed, recorded, and sampled by 100 g to be oven-dried. Urine was collected buckets placed beneath the pens and pre-filled with sulfuric acid to maintain pH below 3 to prevent nitrogen volatilization; a 10-ml sample was frozen at -20 °C for analysis. Proximate composition of feed, residues, and feces was analyzed using the AOAC (1990) method. Gross energy was determined using a bomb calorimeter (Dittmann et al., 2014).

Energy metabolism variables were calculated as follows: Energy intake (EI) = DMI × Feed energy ; Digestible energy (DE) = EI – Fecal energy; Methane energy (Hales et al., 2022) = 0.2433 × DMI; Metabolizable energy (ME) = DE – (Urinary energy + Methane energy); Energy retention (ER) = DE – Urinary energy; Efficiency of ER to EI (%) = (ER ÷ EI) × 100%; Efficiency of ER to DE (%) = (ER ÷ DE) × 100%

Sheep performance variables were calculated using the following formulas: Dry matter intake (DMI) = DM consumed – DM residue; Average daily gain (ADG) = (Final body weight – Initial body weight) ÷ Days of rearing; Feed conversion ratio (FCR) = DMI ÷ ADG; Consumption rate (CR) = Feed intake ÷ Feeding duration

The collected data were presented as mean ± standard deviation (SD) and were analyzed using IBM SPSS Statistics (version 30). One-way ANOVA was used to test treatment effects (model: $Y_{ij} = \mu + T_i + e_{ij}$). Significant differences ($\alpha = 0.05$) were further examined by Tukey’s HSD.

RESULTS

Energy metabolism for each treatment is presented in Table 2. Analysis of variance showed that the treatment had a highly significant effect ($P < 0.01$) on digestible energy (DE), urinary energy, metabolizable energy (ME), energy retention (ER), ER to EI efficiency, and ER to DE efficiency. However, the treatment had no significant effect ($P > 0.05$) on energy intake (EI), fecal energy, or methane energy. Digestible energy (DE), metabolizable energy (EM), and energy retention (ER) in T2 were similar to those in T3, but higher than those in T0 (control) and T1. The efficiency of ER to EI in T2 was higher than in T0, T1, and T3. The efficiency of ER to DE in T3 was higher than in T0, T1, and T2. The lowest urinary energy was observed in T3, followed by T1, T0, and T2.

The performance of sheep under each treatment is presented in Table 3. Analysis of variance showed that the treatment significantly affected body weight gain, average daily gain (ADG), and feed conversion ratio (FCR) ($P < 0.05$), as well as consumption rate (CR) ($P < 0.01$). However, the treatment had no significant effect ($P > 0.05$) on dry matter intake (DMI) or final body weight. Body weight gain, average daily gain (ADG), and consumption rate (CR) of sheep in T2 were higher than those in T0 (control), T1, and T3. The feed conversion ratio (FCR) of sheep in T2 was similar to T1 but lower than T0 and T3.

Table 2 - Energy metabolism of local sheep supplemented with waru leaf meal (WLM) and bamboo leaf meal (BLM).

Treatment	T0 (control)	T1 (2.49 g WLM)	T2 (1.87 g WLM + 0.33 g BLM)	T3 (1.25 g WLM + 0.65 g BLM)	P value
Energy intake (Mcal)	4.68±0.20	4.62±0.16	5.49±0.65	5.03±0.52	0.057
Fecal energy (Mcal)	1.51±0.08	1.45±0.02	1.37±0.10	1.52±0.25	0.452
Digestible energy (Mcal)	3.17±0.16 ^b	3.17±0.16 ^b	4.12±0.56 ^a	3.51±0.34 ^{ab}	0.007
Urinary energy (Mcal)	0.07±0.01 ^b	0.07±0.01 ^b	0.08 ±0.02 ^a	0.04±0.01 ^c	0.001
Methane energy (Mcal)*	0.31±0.02	0.29±0.01	0.35±0.04	0.31±0.05	0.152
Metabolizable energy (Mcal)	2.79±0.15 ^b	2.82±0.09 ^b	3.69±0.50 ^a	3.16±0.30 ^{ab}	0.005
Energy retention (Mcal)	3.10±0.16 ^b	3.11±0.16 ^b	4.03±0.54 ^a	3.47±0.34 ^{ab}	0.006
Efficiency ER to EI (%)	66.25±1.27 ^b	67.24±1.04 ^b	73.57±1.36 ^a	68.96±2.96 ^b	0.001
Efficiency ER to DE (%)	97.69±0.17 ^b	97.77±0.08 ^b	98.00±0.20 ^b	98.72±0.26 ^a	0.001
Microbial protein synthesis (mgN/day)	1298±716	1389±733	1734±691	480±145	0.142

a,b,c; Means within a column with different superscripts differ significantly ($P < 0.01$). *Based on methane energy estimation formula by Hales et al. (2022)

Table 3 - Performance of local sheep supplemented with waru leaf meal (WLM) and bamboo leaf meal (BLM).

Treatment	T0 (Control)	T1 (2.49 g WLM)	T2 (1.87 g WLM + 0.33 g BLM)	T3 (1.25 g WLM + 0.65 g BLM)	P value
Dry matter intake (DMI) (kg/day)	1.19±0.10	1.11±0.05	1.27±0.33	1.20±0.21	0.734
Ratio of rice straw intake to concentrate intake	22:78	20:80	25:75	21:79	
Initial body weight (kg)	27.00±1.78	25.95±1.53	27.25±3.13	28.18±2.45	0.607
Final body weight (kg)	37.55±2.91	36.70±1.39	41.78±5.45	39.28±5.47	0.370
Body weight gain (kg) in 45 days	6.18±0.89 ^b	6.78±0.45 ^{ab}	9.30±1.94 ^a	6.06±1.31 ^b	0.018
Average daily gain (ADG) (kg/day)	0.14±0.02 ^b	0.15±0.01 ^{ab}	0.21±0.04 ^a	0.15±0.03 ^b	0.019
Feed conversion ratio (FCR)	8.80±1.06 ^a	7.38±0.47 ^{ab}	6.16±1.15 ^b	8.26±0.94 ^a	0.010
Consumption rate (kg/hour)	0.44±0.05 ^b	0.39±0.05 ^b	0.95±0.17 ^a	0.43±0.14 ^b	0.001

a,b,c; Means within a column with different superscripts differ significantly ($P < 0.05$)

DISCUSSION

Energy metabolism

The analysis results indicated that the WLM and BLM supplementation did not affect ($P > 0.05$) dry matter intake (DMI), energy intake (EI), and, by extension, feed palatability. According to Parakkasi (1999), factors influencing feed intake include palatability, animal condition, feed type, and environmental factors. In this study, energy content was similar across treatments (Table 2), reflecting no differences in feed intake. Tahuk et al. (2021), stated that dietary energy content is one of the key factors influencing feed intake levels. The primary purpose of feed consumption in livestock is to meet energy requirements. When dietary energy is low, animals tend to consume more feed to fulfill their energy needs, but when dietary energy is high, feed intake tends to decline (Parakkasi, 1999)

Digestible energy in T1 was similar to T0, because ammoniated rice straw in T1, created an alkaline rumen environment, which moderated the significant impact of flavonoids derived solely from WLM. Ammonia derived from ammoniated rice straw acts as a buffer in the rumen and helps maintain microbial population balance, thereby supporting adequate fiber fermentation. A study by Utami (2020) reported that supplementing 0.24% Waru leaf meal (on a DM basis) in the concentrate was less effective in improving fiber digestibility in sheep fed ammoniated rice straw and concentrate. In contrast, the combined supplementation of WLM and BLM demonstrated higher energy digestibility. This improvement may be attributed to the higher flavonoid content of both ingredients, along with the ability of bamboo leaves to support digestive tract health, thereby optimizing digestion and nutrient absorption. According to a study by Bata and Rahayu (2017), the flavonoid content in bamboo leaves is 2.04 mg/mL, which is higher than that in waru leaves, at only 0.48 mg/mL. Flavonoids contribute to modulating rumen conditions, such as maintaining rumen pH, acting as anti-inflammatory agents, reducing methane production, increasing propionate proportion, and enhancing the population of lactate-utilizing bacteria in the rumen (Balcells et al., 2012). These conditions create a more efficient rumen environment, thereby supporting the fermentation process. Li et al. (2021) reported that the inclusion of bamboo leaf extract in the diet can improve rumen fermentation parameters, such as increasing total VFA, stabilizing rumen pH, and reducing the Firmicutes-to-Bacteroidetes ratio. Do et al. (2018) stated that a lower Firmicutes/Bacteroidetes ratio in the rumen is associated with more efficient fiber digestion, particularly of lignin. Additionally, flavonoids and phenolic acids in bamboo leaves exhibit high antioxidant activity (Shen et al., 2024). Antioxidants in the rumen can reduce protozoa populations, suppress pathogenic bacteria, and alter fermentation patterns by increasing the propionate-to-total VFA ratio, where propionate serves as a major energy source for ruminants through the process of gluconeogenesis (Wu et al., 2022).

Digestible energy in T3 (0.65 g BLM) showed a declining trend compared to T2 (0.33 g BLM), which may be related to the increased dosage of BLM. While supplementation with 0.33 g BLM (T2) had a positive effect on rumen fermentation, a higher dose of 0.65 g (T3) resulted in reduced fermentation performance. This effect is likely due to the antibacterial compounds present in bamboo leaves, which may have disrupted the microbial balance in the rumen. Bamboo leaves are rich in flavone C-glycosides such as orientin, isoorientin, vitexin, isovitexin, tricetin, luteolin, and luteolin-6-C-arabinoside (Zhang et al., 2008; Su et al., 2019), with orientin, vitexin, and tricetin known to exhibit antibacterial activity. These antibacterial compounds may inhibit carbohydrate-fermenting bacteria, thereby contributing to the reduction in total VFA (Crespo et al., 2012). Urinary energy reflects the amount of energy lost due to nitrogen excretion in the form of urea and other metabolites. Analysis of variance showed that the treatment had a highly significant effect on urinary energy ($P = 0.001$), with the lowest value observed in T3, which was significantly different from T0, T1, and T2. The higher urinary energy values in T0, T1, and T2 may indicate an asynchrony between energy and nitrogen availability in the rumen. Nitrogen derived from dietary protein that is not efficiently utilized due to limited fermentable energy is converted into ammonia (NH_3). Unused ammonia is absorbed through the reticulorumen villi into the bloodstream and subsequently converted into urea by the liver. A portion of this urea returns to the rumen via saliva as recycled nitrogen, but the majority is excreted through urine (Getahun et al., 2019). In contrast, the reduction in urinary energy observed in T3 suggests better synchronization between energy and nitrogen, allowing more nitrogen to be utilized for microbial protein synthesis and tissue formation, with less being lost through urinary excretion. Metabolizable energy in T2 was similar to that of T3, but higher than T0 and T1, indicating that the combined supplementation of WLM and BLM enhanced energy utilization efficiency. Metabolizable energy reflects the amount of energy available to livestock to support production processes and tissue maintenance. According to Weiss (2019) stated that metabolizable energy is obtained by subtracting the energy lost through urine and methane gas from the amount of digestible energy. Although urinary energy loss in T2 was higher than in the control, and methane energy losses were relatively similar across all treatments, the digestible energy values in T2 and T3 was higher compared to T0 and T1. Energy retention in T2 was similar to that in T3 but higher than in T0 and T1. High energy retention indicates that sheep can efficiently utilize the consumed energy for productive purposes, such as body weight gain. This increase is consistent with the higher values of digestible energy and metabolizable energy observed in T2 and T3. In contrast, T0 and T1 treatments showed lower energy retention, indicating that much of the consumed energy was not efficiently utilized, but rather lost through feces, urine, or methane gas. Ahmad et al. (2023) reported that incorporating 1% DM flavonoids in complete rumen modifier (CRM) improved energy retention in sheep.

The efficiency of ER to EI in T0, T1, and T3 were similar results but increased in T2. This indicates that, in this treatment, the digestion and rumen fermentation processes were optimal, allowing most of the consumed energy to be utilized effectively for growth. However, the efficiency of ER to DE was highest in treatment T3, even though total digestible energy in T3 tended to be lower compared to T2. This condition suggests that, although rumen digestibility in T3 was not as high as in T2, post-rumen digestion in T3 was more efficient, allowing the energy that had been digested to be utilized optimally by the body and stored as body energy. This efficiency is supported by the lower energy loss through urine and methane in T3, as well as the antioxidant and anti-inflammatory compounds in bamboo leaves, which is contributing in maintaining intestinal mucosal integrity and supporting nutrient absorption in the post-rumen digestive tract.

Sheep performance

The energy produced from metabolic processes in livestock is harnessed for maintenance, and any energy excess will fuel livestock growth and production. This study found that the increased energy digestibility, metabolizable energy, energy retention, and energy efficiency in T2 sheep positively impacted their average daily gain (ADG). The supplementation of 1.87 g of WLM and 0.33 g of BLM enhanced the sheep's ability to utilize energy more efficiently, leading to optimal growth and a significant increase in body weight. The increase in body weight gain in T2 is consistent with the high energy retention, indicating that more of the consumed energy was retained as body tissue. This efficiency is supported by the presence of flavonoids, which enhance rumen fermentation efficiency and microbial protein synthesis, both of which were higher in T2, with microbial protein synthesis at 1734 mgN. The increase in body weight with flavonoid supplementation has been reported in several studies. Wang et al. (2018) found that body weight gain in calves fed on mulberry leaf flavonoids was higher than in those without supplementation. Du et al. (2019) reported that supplementing finisher lambs with 3.40 g and 10 g of *A. mongolicum* Regel flavonoid extract improved body weight gain. According to Dorantes-Iturbide et al. (2022), supplementation with a low-dose polyherbal additive (1 g/kg DM) in finisher sheep enhanced feed energy utilization efficiency for body weight gain by up to 23%. The supplementation of flavonoid-rich plants has enhanced muscle protein synthesis in sheep (Qin et al., 2020). Different results were reported by Balcells et al. (2012) that supplementing flavonoid-enriched plant extract into a high-concentrate diet did not affect the ADG of cattle but improved rumen fermentation and reduced acidosis effectively by increasing the population of lactate-utilizing bacteria. The body weight gain of sheep in T3 was not significantly different from that in T0. This may be attributed to the lower energy retention in T3 compared to T2, as well as the low microbial protein synthesis, which was only 480 gN. The low microbial protein synthesis indicates suboptimal rumen microbial activity, likely due to the high concentration of antibacterial compounds resulting from the increased dose of bamboo leaf meal. As a consequence, the availability of fermentation end-products for animal growth was limited. This became one of the main factors contributing to the poor growth performance observed in T3, despite the fact that urinary energy loss was the lowest (0.04 Mcal), indicating relatively efficient nitrogen utilization. This finding is in line with (Paixão et al., 2022), who reported that the addition of flavonoids in the form of red propolis extract could enhance average daily gain up to an optimal dose, but exceeding that dose led to a decline in growth performance.

The feed conversion ratio (FCR) in T2 was similar to that in T1 but lower than in T0 and T3. A lower FCR indicates higher feed efficiency. Supplementation with WLM and BLM has enhanced feed efficiency because the flavonoids in both supplements modulate rumen conditions, improving rumen fermentation and positively affecting feed efficiency. Similarly, incorporating mulberry leaf flavonoids in calves' feed has reportedly improved feed efficiency (Kong et al., 2019). Studies have demonstrated the positive effects of flavonoids and phenolic compounds on livestock productivity and health, including enhanced rumen fermentation and mitigated nutritional stress, such as bloating and acidosis, which ultimately promote animal performance (Kalantar, 2018). The consumption rate of sheep in T2 was higher than in T0, T1, and T3. This result is closely related to the rate of digesta flow in the rumen, as explained by Mulyana et al. (2022), who stated that feed consumption is influenced by how quickly digesta passes through the rumen. A higher level of digestibility accelerates the outflow of digesta, resulting in faster rumen emptying and increased feed intake due to greater stimulation of hunger. These findings suggest that the sheep in T2 had a faster digesta flow rate than those in other treatments. Similarly, Fita et al. (2024) reported that supplementation with WLM at certain levels can increase feed consumption, supporting the results observed in the present study.

CONCLUSION

This study concluded the effectiveness of supplementing 1.87 g waru leaf meal (WLM) and 0.33 g bamboo leaf meal (BLM) into feed to improve energy metabolism and the performance of local sheep. The combination of these leaf meals enhanced digestible and metabolizable energy, increased energy retention, and supported better growth performance. Improvements in feed utilization efficiency were also observed, indicating better metabolic function and nutrient absorption. Incorporating WLM and BLM into smallholder feeding systems may serve as a low-cost and locally available strategy to improve sheep productivity.

DECLARATIONS

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Data availability

The dataset and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical approval

All procedures involving animals in this study were carried out humanely and without causing suffering, in accordance with the Government Regulation of the Republic of Indonesia No. 95/2012 on Veterinary Public Health and Animal Welfare, and complied with the internal ethical guidelines of Universitas Jenderal Soedirman for animal-based research.

Authors' contribution

I. Aisyah was responsible for data collection, statistical analysis, result interpretation, and drafting the manuscript. M. Bata and S. Rahayu designed the study, determined the methodology, interpreted the results, and finalized the manuscript. E. A. Rimbawanto and A. Setyaningrum reviewed the manuscript and provided critical feedback.

Acknowledgements

The authors sincerely appreciate Amanah Farm, Datar Village, Sumbang District, Banyumas Regency, for providing the necessary facilities and support throughout this research. The authors also gratefully acknowledge the financial support from the Ministry of Education, Culture, Research, and Technology under the Research and Community Service Program for the 2024 fiscal year (Contract No. 20.67/UN23.35.5/PT.01.00/VI/2024), which made this study possible.

Funding

This study was financially supported by the Ministry of Education, Culture, Research, and Technology under the Research and Community Service Program for the 2024 fiscal year (Contract No. 20.67/UN23.35.5/PT.01.00/VI/2024).

Competing interests

The authors declare no competing interests in this research and publication.

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PREVALENCE, EPIDEMIOLOGY AND HISTOPATHOLOGY OF *Ascaridia galli* INFECTIONS IN BACKYARD CHICKENS IN GAZIPUR DISTRICT, BANGLADESH

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Supporting Information



ABSTRACT: *Ascaridia galli* is responsible for weight loss, emaciation, anemia, decreased egg production, and in severe cases, intestinal obstruction and death in chickens, resulting in significant economic losses for the poultry industry. This study aimed to assess the present status of *Ascaridia galli* in backyard chickens in Gazipur district. Additionally, the study examined histopathological changes in the intestines and livers of chickens heavily infested with this nematode. Postmortem examination, helminth collection and identification were conducted on 237 backyard chickens from March 2023 to February 2024. The overall prevalence was found to be 40.51%, with Kaliakair upazila showing the highest prevalence at 55.26%, while Sreepur upazila had the lowest at 27.08%. Factors such as season, sex, and age significantly influenced the incidence of ascariasis in backyard chickens. Adults exhibited the highest occurrence rate at 44.88%, with males (44.19%) showing a greater prevalence than females (36.22%). The highest incidence was recorded in the summer at 57.14%. In cases of severe infection, *Ascaridia galli* obstructs the small intestine, leading to inflamed, hemorrhagic intestines that produce excess mucus. The livers of the infected chickens appeared swollen, enlarged, and pale, with signs of congestion, hemorrhage, and inflammation. Histopathological analysis revealed hemorrhage and inflammatory cell infiltration in the intestines, along with damaged and lacerated intestinal tissues. The livers showed congestion, ruptured blood vessels, inflammation, hemorrhage, and necrosis. In conclusion, this study identifies potential risk factors associated with *Ascaridia galli* infection in backyard chickens in Gazipur district, offering insights that could aid in controlling and preventing such infections.

Keywords: *Ascaridia galli*, Domestic birds, Epidemiology, Histopathology, Poultry health.

INTRODUCTION

Ascaridia galli is the large nematode that parasitize the small intestine of domestic and wild birds particularly chicken (Soulsby, 1982). *Ascaridia galli* is the most prevalent (22 to 84%) parasite in backyard chickens (Kaufmann et al., 2011; Thapa et al., 2017). The adult parasite resides in the lumen of the small intestine of their host, feeding primarily on intestinal contents (Soulsby 1982; Shohana et al., 2023). *Ascaridia galli* infections causes substantial damage by reducing growth rate, weight loss, reduction in production efficiency, pathological lesions and specifically economic losses in chicken, turkey, geese and some other birds (Das et al., 2012; Bazh, 2013). Studies indicated that *Ascaridia galli* infections in laying hens can significantly reduce egg production, with severe infestations resulting in up to 30% decrease and higher mortality (Rahul et al., 2023). Direct losses in poultry occurs through intestinal obstruction, damages to the intestinal mucosa, alteration of beneficial gut microflora, suppression of the immune system and increased susceptibility to concurrent infections. *Ascaridia galli* plays a potential role in transmission of other secondary pathogens such as *Escherichia coli* (Permin et al., 2006), and *Pasteurella maltocida* (Dahl et al., 2002). This nematode can serve as a vector for transmission of pathogens such as *Salmonella enterica* (Chadfield et al., 2001), and *Histomonas meleagridis* (Jinghui and McDougald, 2003), and also impair the development of immunity following vaccination against Newcastle disease virus (Pleidrup et al., 2013).

Heavy load with *Ascaridia galli* in the intestine changes the integrity of the intestinal villi (Permin et al., 2006), thickening of the intestinal walls, intestinal lumen becomes thicken due to enteritis, and mucosal walls also become velvety. These changes can result in impaired nutrient absorption and a reduction of liver lipid storage (Das et al., 2012; Torres et al., 2019; Feyera et al., 2022). Larvae deeply embedded in the intestinal mucosa may induce hemorrhage and

RESEARCH ARTICLE
 PII: S222877012500039-15
 Received: June 26, 2025
 Revised: November 24, 2025
 Accepted: November 25, 2025

extensive epithelial damage, increased mucus secreting cells and subsequent adhesion and atrophy of the villi (Shohana et al., 2023). The larvae's migration during the tissue phase of their life cycle significantly impacts the birds that are affected, causing chronic bleeding due to migrating larvae causing gastrointestinal damage including gastritis, enteritis and ulceration of the digestive tract (Ritu et al., 2024). The severity of intestinal damage is directly related to the worm burdens in the intestine. Traumatic and toxic effects induced by the migrating larvae causes inflammatory tracts, portal vein congestion containing fibrous tissue and inflammatory cells, disarrangement of hepatic architecture, hepatocellular shrinkage and dilation of sinusoids (Feroza et al., 2019).

In general, coprological analysis is used to estimate the prevalence of gastrointestinal helminths in animals and birds. Assessing the efficacy of this technique is limited, due to the lack of availability of adult worm burden. For accurate diagnosis, requires detailed knowledge of the parasite and precise localization within the hosts. Therefore, this study investigates the present prevalence of *Ascaridia galli* infection in backyard chickens in Gazipur district by worm count. This research also evaluated the risk factors and pathological changes associated with ascariidiosis.

MATERIALS AND METHODS

Study Area and Sampling

A cross-sectional study was conducted over one year from March 2023 to February 2024, to assess the prevalence of *Ascaridia galli* among backyard chickens in Gazipur district, Bangladesh. Gazipur district covers an area of 1806.36 square kilometers and is geographically positioned between latitudes 23°53 and 24°21 north and longitudes 90°09 and 92°39 east (Figure 1). A total of 237 apparently healthy chickens of both sexes (129 male, and 108 female) and varying ages were collected from various villages across five upazilas: Gazipur Sadar (61), Kaliganj (45), Kapasia (45), Kaliakair (38), and Sreepur (48). For sampling convenience, the chickens were categorized into two groups: growers and adults. The research was conducted over three distinct seasons: hot-dry summer (March to June), hot-wet rainy (July to October), and cool-dry winter (November to February) to account for regional climatic variations. The chickens were then caged and transported to the Parasitology Laboratory in the Department of Pathobiology at Gazipur Agricultural University, Gazipur, Bangladesh.

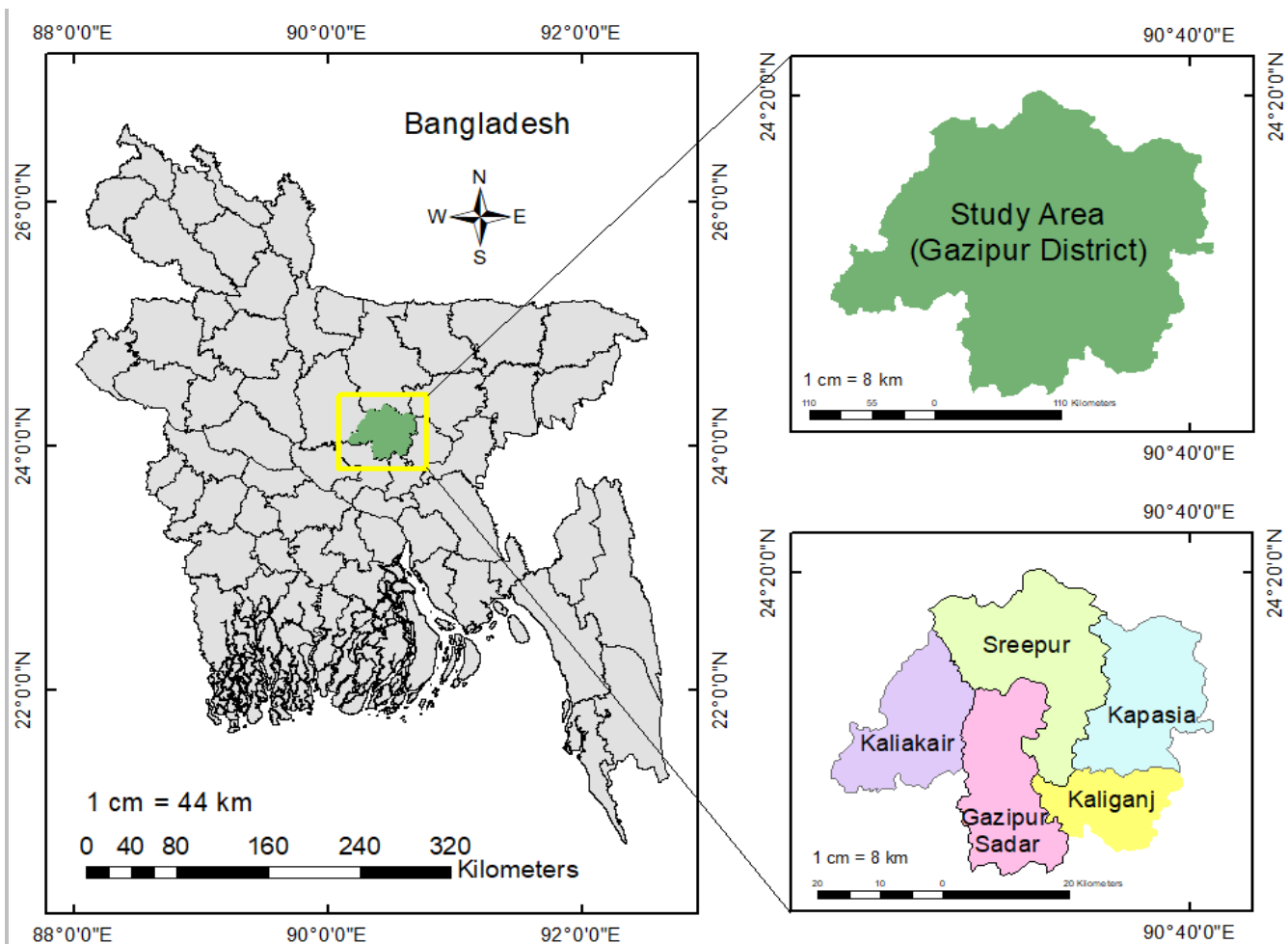


Figure 1 - Study area in Gazipur District, Bangladesh with its five upazilas.

Ethical approval

Throughout the course of this research, the authors maintained all possible ethical guidelines approved by the Animal Research Ethics Committee of Gazipur Agricultural University, Gazipur (FVMAS/AREC/2023/20).

Post-mortem examination, helminth collection and identification

After euthanasia the birds, the small intestine was separated and incised longitudinally, and the contents were emptied into the sieves. The intestinal tracts and contents were washed in normal saline and worms were collected by curved needle or forceps. The collected helminths were examined under a light microscope after preparing temporary slide using a drop of lactophenol and identified based on taxonomic keys described by Soulsby (1982).

Histopathological examination

The mucosal surface was cautiously inspected to detect helminths particularly *Ascaridia galli* nematodes. Parasite-affected mucosal region was observed to find any pathological changes. The affected parts showing gross abnormalities were collected and preserved in 10% formalin solution for fixation. Chickens infected exclusively with *Ascaridia galli* were chosen for histopathological studies, and the affected intestine and liver tissues were collected. For studying histopathological details, tissue samples were processed routinely and the paraffin embedded tissue sections were cut at 5 μ m and stained with Hematoxylin and Eosin (H&E) (Fischer et al., 2008). Control tissues taken from non-infected chickens, matched for age and sex, were used as the comparison group. At least three foci were evaluated from control and infected tissue samples, with three slides evaluated per sample in a blinded manner.

Statistical analysis

The collected data were stored in Microsoft Excel and subsequently analysed using the Statistical Package for Social Sciences (SPSS) version 25. To assess the association between *Ascaridia galli* incidence and various risk factors including age, sex, and seasonal comparison, Fisher's exact test was employed. Odds ratios (ORs) were calculated to evaluate risk within groups of identified risk factors, accompanied by 95% confidence intervals (CIs) to estimate the precision of the ORs. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

Overall prevalence of *Ascaridia galli* infections

Backyard chickens were collected in different rural areas of the Gazipur district. These samples were collected from the five sub-districts namely Gazipur Sadar, Kaliakair, Kapasia, Kaliganj and Sreepur. Each location has different occurrence percentage i.e. 40.98%, 55.26%, 42.22%, 40%, and 27.08%, respectively (Table 1). A total of 237 (40.51%) of the sampled chickens were infected with *Ascaridia galli*. There were no significant differences in total prevalence regarding sex in backyard chickens ($P > 0.05$). Males showed a higher prevalence of *Ascaridia galli* infection compared to females. In this study, out of 96 infected chicken males were 57 (44.19%), whereas females were 39 (36.22%) in Table 2. The current study showed a higher prevalence of *Ascaridia galli* in adult birds (44.88%, 57/127) compared to grower birds (35.45%, 39/110); however, the difference was not statistically significant ($P > 0.05$). No significant difference in infection rates was observed between grower and adult chickens ($P > 0.05$). The prevalence of parasites in chicken was found throughout the year, but the prevalence varied from season to season. This study showed a significant seasonal variation in the prevalence of *Ascaridia galli* infections in slaughtered backyard chickens, with the highest infection rates recorded during the summer season (57.14%) followed by 44.16% in the winter and 26.80% in the rainy season (Table 2).

Histopathology of *Ascaridia galli* infections in backyard chicken

The digestive organs, including the liver and intestine, were evaluated following the postmortem of the chickens. In heavy infection, the intestinal lumen is stacked with a large number of *Ascaridia galli* parasites (Figure 2). A sectional view of the lumen of the intestine may reveal a long, yellowish helminth, which is indicative of *Ascaridia galli*. It was also noted that the intestinal lesion was similar to a hemorrhagic lesion in the intestinal mucosa. The small intestine was noticeably inflamed and was secreting more mucus. The liver appeared pale, enlarged, and swollen. Additionally, there was inflammation of the liver, hemorrhage and congestion. Histopathological examination of duodenum sections revealed hemorrhage, inflammatory cell infiltration, and ruptured and lacerated tissue. The submucosal layer ruptured and showed signs of hemorrhage. There were noticeable inflammatory cells in the layer of muscularis mucosa (Figures 3A-F). In contrast to non-infected chicken characterized by no inflammatory cells, no hemorrhagic cells and no ruptured tissue in the intestinal sections (Figure 4). In the liver sections, the tissue of infected chickens had congestion, hemorrhage and inflammation of inflammatory cells. Congested sinusoids and blood vessels were present in the liver. Focal necrotic regions were visible in the liver portion. Necrotic zones had a high concentration of inflammatory cells (Figures 5A-D). In contrast, in the control chickens, there were no inflammatory cells, congestion in the central vein or any other lesions (Figure 6).

Table 1 - Prevalence of *Ascaridia galli* in Gazipur district.

Locality	No. examined	No. Infected	Overall prevalence (%)	χ^2 /Fisher's exact	P-value
Gazipur Sadar	61	25	40.98	7.09	0.13
Kaliakair	38	21	55.26		
Kapasias	45	19	42.22		
Kaliganj	45	18	40.00		
Sreepur	48	13	27.08		
Total	237	96	40.51		

Table 2 - Prevalence associated risk factors of *Ascaridia galli* infection in backyard chickens.

Factors	Categories	No. examined	No. Infected	Overall prevalence (%)	OR	95% CI	χ^2 /Fisher's exact	P-value
Age	Grower	110	39	35.45	1.48	0.877-2.504	2.17	0.1403
	Adult	127	57	44.88	1			
Sex	Male	129	57	44.19	1	0.829-2.366	1.59	0.2072
	Female	108	39	36.11	1.40			
Season	Summer (Mar-Jun)	63	36	57.14			15.22	0.0004
	Rainy (Jul-Oct)	97	26	26.80				
	Winter (Nov-Feb)	77	34	44.16				
Total		237	96	40.51				



Figure 2 - Intestinal lumen stacked with large number of *Ascaridia galli*.

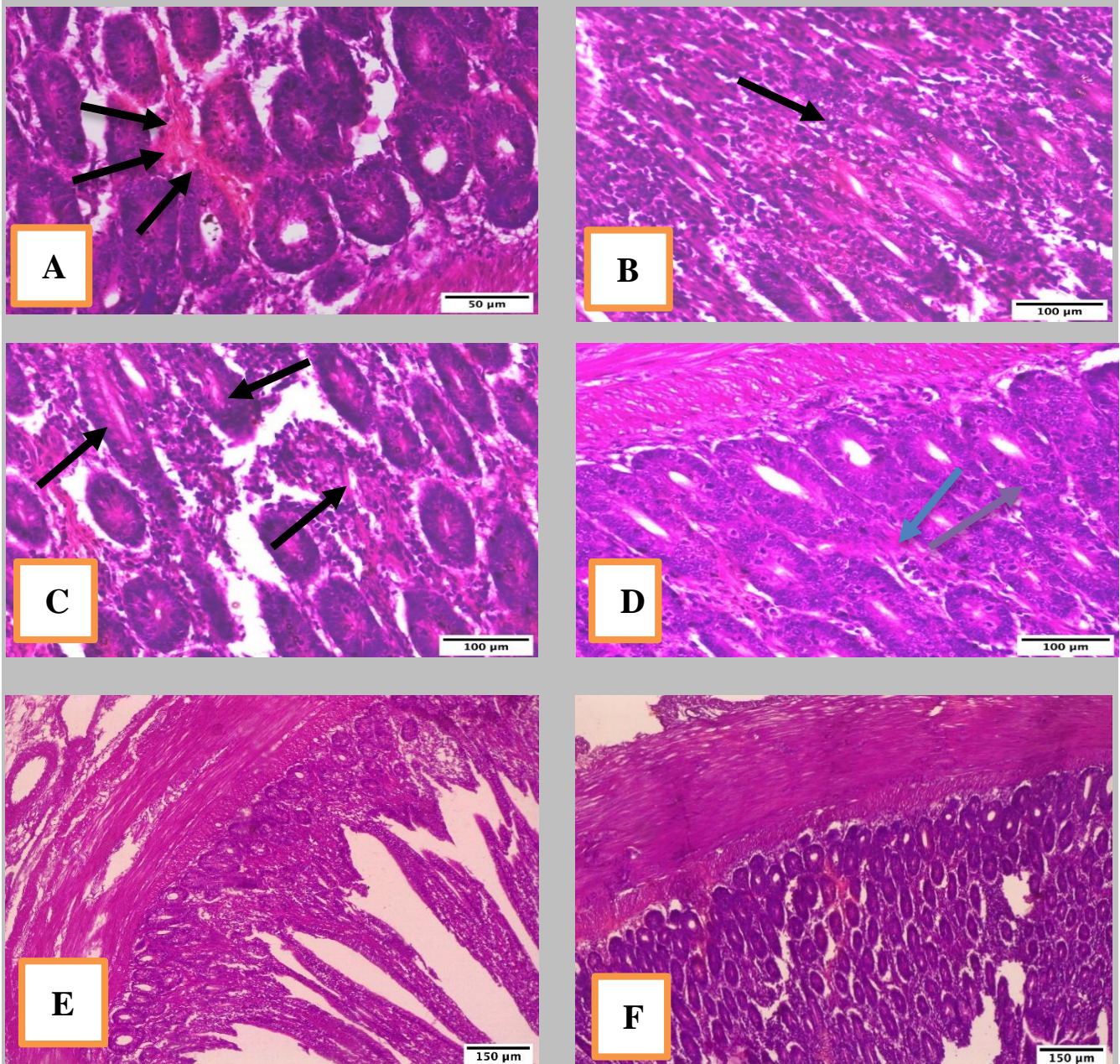


Figure 3 - Histopathology of intestine affected with *Ascaridia galli*. A) hemorrhage of the intestinal tissue (black arrow), B) inflammatory cell infiltration (black arrow), C) ruptured and lacerated tissue of intestine (black arrow) and D) hemorrhage (blue arrow) and inflammatory cells (yellow arrow), E) Desquamation of muscularis layer of intestine (black arrow) and desquamation of villi (blue arrow), F) Hemorrhage of intestine (black arrow).

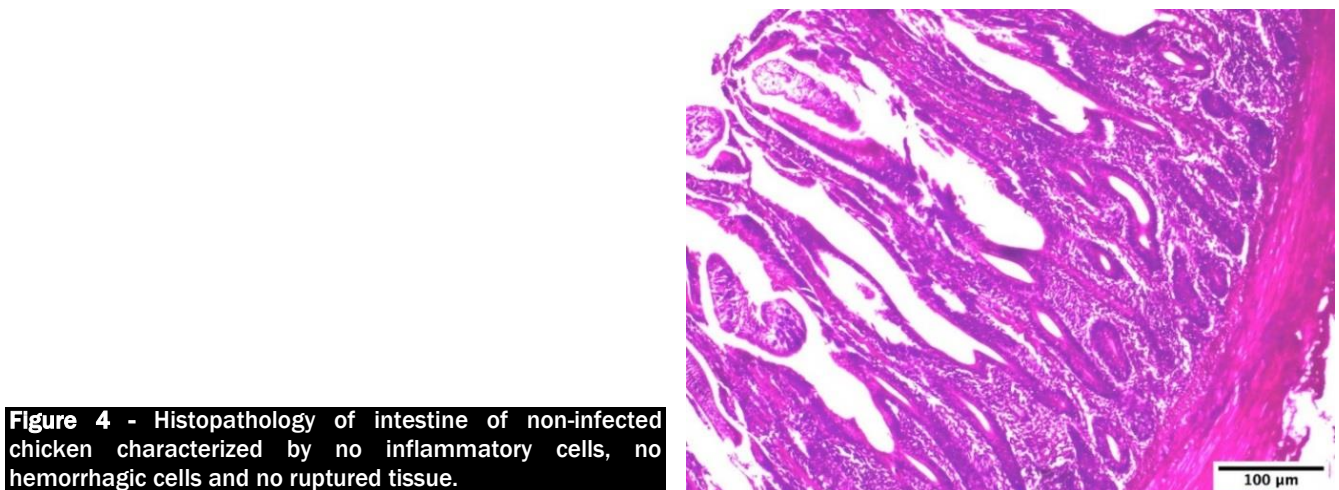


Figure 4 - Histopathology of intestine of non-infected chicken characterized by no inflammatory cells, no hemorrhagic cells and no ruptured tissue.

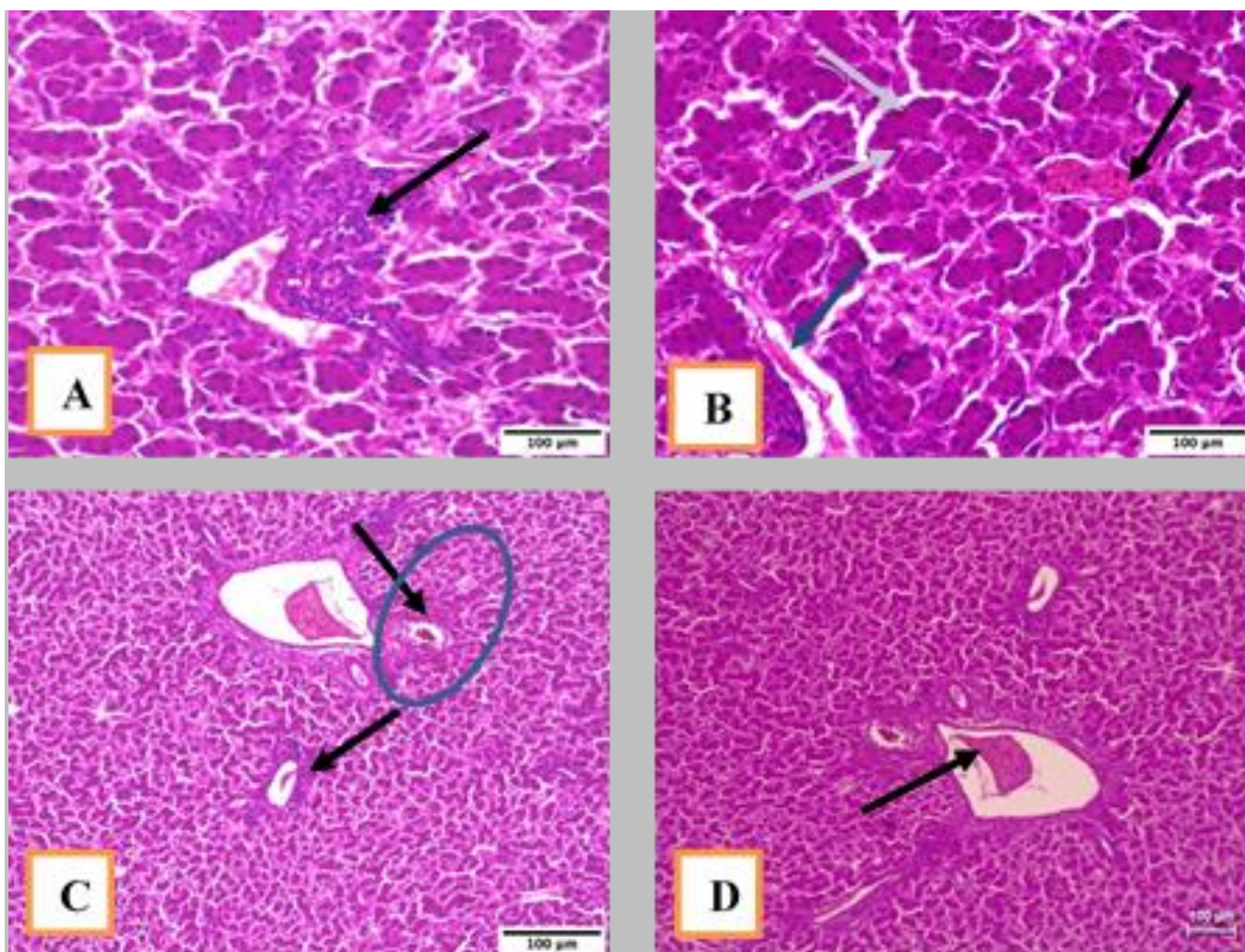


Figure 5 - Histopathological lesions in liver of affected chicken with *Ascaridia galli* A) infiltration of inflammatory cells (black arrow), B) hemorrhage (black) with congestion and ruptured tissue (yellow) and C) Necrotic zones (blue circle) concentration of inflammatory cells (black arrow), D) Congestion of tissue of liver (black arrow).

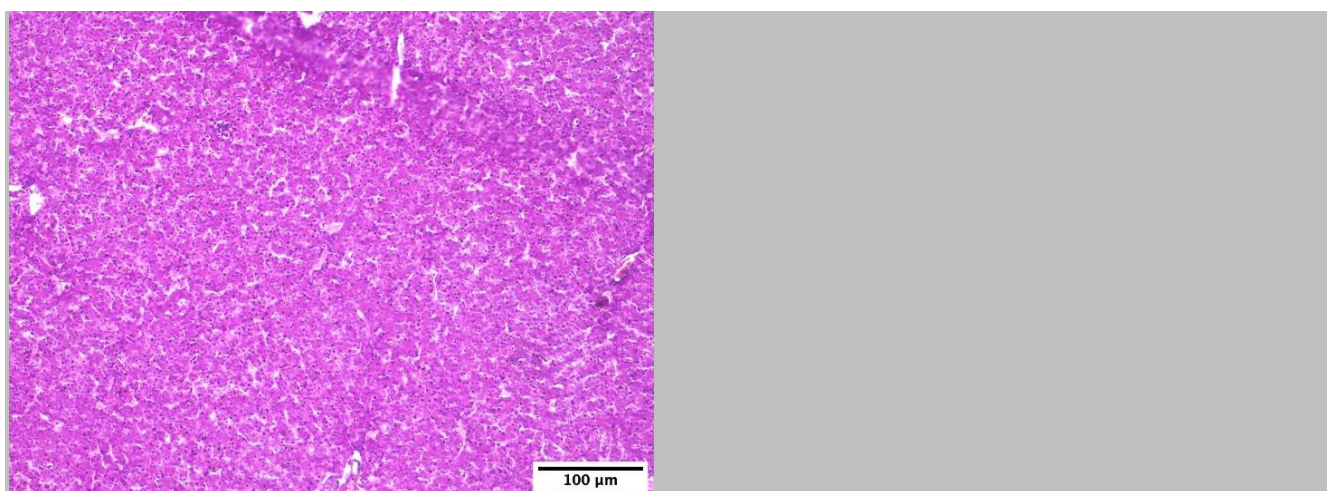


Figure 6 - Histopathology of liver of non-infected chicken characterized by no lesions of infection of *Ascaridia galli*.

DISCUSSION

The current study showed that backyard chickens naturally infected with *Ascaridia galli* reached 40.51%. This finding is consistent with another study performed in another region of Bangladesh recorded 45.6% prevalence of *Ascaridia galli* among gastrointestinal helminths in scavenging or semi-scavenging chicken (Ritu et al., 2024). However, other studies reported higher prevalence (70-85%) in Narsingdi district (Ferdushy et al., 2016), and (56%) in Dinajpur district (Trisha et al., 2021). Worldwide, the prevalence rate of *Ascaridia galli* was 32.97 % in India (Ara et al., 2021), 88% in Germany

(Kaufmann et al., 2011), 77.1% in Sweden (Jansson et al., 2010), 32.3% in Tanzania (Permin et al., 2006), and 30% in Ghana (Anane et al., 2022). This difference may reflect the geographical location, sample size and management practices that play a role in the transmission dynamics of helminth infections in poultry. The current study showed that the prevalence in five sub-districts of Gazipur were comparable. The high prevalence was observed in Kaliakair (55.26%) and lower in Sreepur (27.08%). This variation might be influenced by the scavenging behaviour of the local chickens, which frequently roam freely in search of foods, scavenging various arthropods and earthworms from the superficial layer of the soil that act as the intermediate and paratenic hosts for *Ascaridia galli*, facilitating the transmission of parasitic infections (Soulsby, 1982).

The present study showed males were more susceptible to *Ascaridia galli* infection compared to females, which is agreed with the earlier studies (Sarba et al., 2019; Ritu et al., 2024). High prevalence in male chickens is probably due to reduced immune responses, hormonal differences and feeding habits (Klein, 2004). The higher percentage was found in adult birds rather than the grower might be due to the cohort effect. Nevertheless, the increase in infestation rates with increasing host age may be attributed to prolonged exposure to environmental factors, which facilitate the chances of ingestion of infective larvae through contaminated food or polluted drinks. In terms of seasonal dynamics, the highest peak of infection was observed in summer, with a comparatively lower prevalence in winter and the rainy season. The heightened occurrence of intestinal infections during summer could be attributed to the increased abundance of insects and other invertebrates, which serve as food for birds that act as hosts for the intermediate stages of these helminths in hot climates (Ara et al., 2021).

In terms of histopathological changes, heavy infection with *Ascaridia galli* resulted small in intestinal obstruction, petechial hemorrhages in the small intestine, marked inflammation and secretion of more mucus. In some cases, we found desquamation of the muscularis mucosa and loss of villi in the intestine. Previous studies found that, *Ascaridia galli* mostly causes desquamation and adhesion of mucosal villi, petechial hemorrhages in the duodenum (Ritu et al., 2024), ulcerative ventriculitis (Gopal et al., 2017), intestinal wall damage and bleeding (Skallerup et al., 2005), necrotic patches of intestinal mucosa (Rajkovic et al., 2019), petechial hemorrhage in the intestinal mucosa, nodular haemorrhagic enteritis, and inflammation of the proventriculus (Permin and Ranving, 2001).

Microscopical examination of liver showed congestion of sinusoids, fatty degeneration with coagulation necrosis and necrotic zones with a high concentration of inflammatory cells due to migration of larvae during the tissue phase of life cycle (Sharma et al., 2018). Similar finding was partially agreed with the previous study (Sharma et al., 2018; Abdel Rahman et al., 2019) who observed necrosis of liver, with mononuclear cellular infiltration, congested blood vessels and fatty degeneration with coagulation necrosis.

CONCLUSION

This study revealed a high infection rate of *Ascaridia galli* in backyard chickens in the Gazipur district. It causes subclinical infections in chickens, which may result in invisible production and economic loss. Age, sex, and season were found to have a significant impact on the prevalence of these helminth infections. Histopathological findings demonstrated that heavily infected small intestines exhibited lesions characterized by hemorrhage, inflammatory cell infiltration, villous desquamation, and necrosis of the sub-mucosal glands. Additionally, the infected livers showed severe lesions. Therefore, awareness among poultry producers should be created about the transmission of poultry parasites and the importance of proper disposal of poultry droppings for prevention and control.

DECLARATIONS

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Acknowledgements

We express our sincere gratitude to the owners of different chicken farms of Gazipur District for providing chickens.

Ethical considerations

Throughout the course of this research, the authors maintained all possible ethical guidelines approved by the Animal Research Ethics Committee of Gazipur Agricultural University, Gazipur (FVMAS/AREC/2023/20).

Availability of data and materials

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

Author's contributions

F. Khatun conceptualized the study, managed the project, conducted the experimental protocols and wrote the manuscript. I. Akter Akhi performed the prevalence and histopathological study. R. Sani Sadi contributed in histopathological study. Md A. Al Mahmud contributed to the research and data analysis. Z. Chandra Das and Md S. Islam contributed in draft preparation. Md T. Islam contributed in final manuscript writing, edited the manuscript and supervised the research work. All authors reviewed and approved the final version of the manuscript.

Funding

This research was funded by the Research and Management Wing, Gazipur Agricultural University, with Grant No. RMW 016, 2022-2025 FY.

Competing interests

The authors report no competing interest in this work.

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CHEMICAL COMPOSITION OF SOYBEAN MEAL AND ITS EFFECTS ON NUTRIENT DENSITY, EGG PRODUCTION, AND BLOOD PARAMETERS IN BOVANS BROWN LAYERS

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↳ Supporting Information



ABSTRACT: Soybean meal (SBM) is an essential protein source in poultry diets. However, data on the variability of its chemical composition from Ethiopian processing plants and its optimal inclusion levels in layer diets are limited. This study aimed to assess the nutritional quality of locally sourced SBM from the Amhara Region and evaluate the effects of different levels of SBM inclusion on production performance, egg quality, and blood parameters in laying hens. The chemical composition of SBM samples from 11 regional processing plants was initially analyzed. Subsequently, a 12-week feeding trial was conducted using 120, 34-week-old Bovans Brown layers. The hens were randomly assigned to one of five dietary treatments in a completely randomized design (CRD). The treatments consisted of a commercial control diet and four experimental diets containing 15%, 20%, 25%, and 30% SBM. Each treatment was replicated three times, with eight hens per replicate. Significant variation ($P < 0.05$) was observed in the chemical composition of SBM samples from different processors. Crude protein ranged from 38.42 to 43.52%, ether extract (1.23 - 5.27%), crude fiber (5.58 - 7.35%), and metabolizable energy (3348.3 - 3594.8 kcal/kg DM). Dietary crude protein concentration increased with higher SBM inclusion from 14.74% to 17.56%. An inclusion level of 20% SBM was identified as optimal, resulting the highest hen-day egg production (89.72%) and net return (446.63 ETB), with no adverse effects on egg quality and hematological parameters remaining within normal ranges. This study concludes that there is significant variation in the chemical composition of locally processed SBMs, highlighting the need for pre-use nutritional evaluation. Under the experimental conditions, a 20% SBM inclusion level is regarded as acceptable and optimal for mid-laying hens.

Keywords: Chemical composition, Egg production, Egg quality, Nutrient density, Soybean meal.

INTRODUCTION

Ethiopia's poultry sector is undergoing a transitional phase, with efforts underway to shift from traditional backyard systems toward more intensive and commercialized production. This transition requires urgent attention to several key challenges, particularly the quality, consistency, and affordability of poultry feed (MoA, 2023). Among the major feed ingredients, soybean meal (SBM) plays a central role as a primary protein source. Recognizing this, the 2022 National Poultry Development Strategy outlined a ten-year plan emphasizing large-scale production of essential feed ingredients like soybeans (MoA, 2023). In response to rising demand for cooking oil and the expansion of multi-million-dollar edible oil processing industries, soybean cultivation has increased significantly, especially in the Amhara Region (Mussema et al., 2022). According to CASA (2023), the region accounted for 265,263 hectares of soybean cultivation and 753,600 metric tons of production, approximately 39% of the national output. As a result, Amhara is emerging as a major hub for both soybean production and oil processing, generating increasing volumes of SBM as a by-product.

Soybean meal (SBM) is a widely used protein ingredient in poultry feed formulation. Its high protein content (44–49% CP), balanced amino acid profile, and bioavailability make it a benchmark for other plant-based protein sources and a key complement to cereal grains in poultry diets (Banaszkiewicz, 2011). However, its nutrient profiles is highly variable, largely due differences in processing methods (Tangendjaja, 2020; Pope et al., 2023). Despite the growing availability of SBM, no studies in Ethiopia, particularly in the Amhara Region, have evaluated its chemical composition or optimal dietary inclusion levels in poultry feed. This gap limits the ability of producers to formulate cost-effective and nutritionally balanced rations based on locally available ingredients.

This study aimed to evaluate the chemical composition of SBMs in the Amhara region and assess the effects of varying inclusion levels on diet nutrient density, production performance, egg quality, blood hematology, and serum cholesterol in Bovans Brown layers.

RESEARCH ARTICLE
 PII: S222877012500040-15
 Received: June 24, 2025
 Revised: November 24, 2025
 Accepted: November 26, 2025

MATERIALS AND METHODS

Soybean meal samples collection

Soybean meal (SBM) samples were collected from 11 oil processing plants in the Amhara regional state. The plants were located in Bahir Dar (6), Enjbara (2), Bure (1), Debre Markos (1), and Debre Brehan (1). Each plant was visited once, and a representative sample was obtained by compositing multiple sub-samples from different bags. All sub-samples were collected and stored in sturdy, airtight plastic bags and each composite sample was then ground to a homogeneous particle size (1 mm sieve) for laboratory analysis.

Feed formulation

The SBM selected for the feeding trial was mechanically extracted and sourced from a processing plant chosen based on its demonstrated capacity for consistent, year-round production, established supply chain partnerships, and a market strategy focused on meeting local demand. Experimental diets were formulated with varying SBM inclusion levels (15%, 20%, 25%, and 30%), assuming a daily feed intake of 120 g per hen using Win Fed (2.8) computer software. A locally available commercial layer diet (from Mara animal feed processing industry) was used as a control treatment to serve as a practical benchmark for evaluating the performance and nutritional adequacy of the experimental diets. This approach allows for a direct comparison between standard feeding practices commonly used in the study region and scientifically formulated diets with varying levels of soybean meal inclusion. The composition and proportions of feed ingredients used in the experimental diets are detailed in Table 1.

Table 1 - Ingredient proportion of the different treatments (as-fed basis)

Feed Ingredient	15% SBM	20% SBM	25% SBM	30% SBM
White maize grain	55	54	50	50
Soybean meal	15	20	25	30
Noug seed cake	15	11	10	5
Bone meal	4	4	4	4
Limestone, ground	9	9	9	9
L-lysine-HCL	0.5	0.5	0.5	0.5
DL-Methionine	0.5	0.5	0.5	0.5
Vit. and Min Premix	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5

Laboratory chemical analysis

Prior to laboratory analysis, all soybean meal (SBM) and feed ingredient samples used in the feeding trial were prepared in triplicate per sample by oven-drying at 105 °C for 3 hours and grinding to pass through a 1 mm sieve. The samples were then analyzed for dry matter (DM), crude fiber (CF), total ash, ether extract (EE), and crude protein (CP) according to AOAC (2000) procedure. The Metabolizable energy (ME) content of the experimental diets was calculated by the equation adopted from Wiseman (1987): $ME \left(\frac{\text{Kcal}}{\text{kg DM}} \right) = 3951 + 54.4EE - 88.7CF - 40.8 \text{ ash}$

Management of experimental hens

This experiment was conducted at the Bahir Dar University poultry farm in Ethiopia. A total of 120 Bovans Brown hens, 34 weeks of age, were used. The hens were randomly assigned to one of five dietary treatment groups in a completely randomized design (CRD), containing 24 hens per treatment. Each treatment group was further divided into three replicates, each containing eight hens. Hens were housed in pens measuring 2 m², which were bedded with 5 cm of teff (*Eragrostis tef*) straw. Prior to the placement of the hens, all sanitary measures were applied to the experimental house. Feed was offered twice daily (morning at 8:00 AM and afternoon at 2:00 PM), and refusals were collected and weighed the following day, while clean drinking water was supplied *ad libitum* throughout the experimental period. Following placement, a one-week adaptation period was provided to allow the hens to acclimate to the experimental diets before formal data collection began. Data on production performance, egg quality, and blood hematology and chemistry parameters were collected over a 12-week period. During the trial, the average ambient temperature ranged from a minimum of 18.9 °C to a maximum of 32.36 °C.

Production performance parameters

Daily feed intake (FI) per replicate was calculated as the difference between the amount of feed offered and the amount refused. Body weight (BW) was measured individually at the start and end of the trial, and body weight change

(BWC) per replicate was determined by subtracting the initial weight from the final weight. Eggs were collected twice daily from each pen to monitor egg production. The hen-day egg production (HDEP) rate was calculated as the average daily percentage per replicate, using the formula (Hunton, 1995): % HDEP = $\frac{\text{No of eggs collected per day}}{\text{No of hens present that day}} \times 100$

Daily egg weight (EW) per replicate was measured immediately after collection. Average EW was obtained by dividing the total weight of eggs by the number of eggs laid. Egg mass (EM) per pen was calculated as follows (North, 1984):

$$EM = \%HDEP \times EW$$

Feed conversion ratio (FCR) was calculated per replicate as the total feed intake divided by the total egg mass produced during the experimental period.

Egg quality parameters

A total of 30 eggs (six per treatment, two per replication) were randomly sampled at two-week intervals for quality analysis, with replicate means calculated from sample averages for each parameter. Eggs were weighed (w), broken on a flat tray, and shells separated from membranes for weight measurement. Shell thickness was determined with a digital caliper, averaging measurements at three points (air cell, equator, and sharp end). Yolk color was evaluated using a Roche color fan, while albumen height (h) and yolk height were measured with a tripod micrometer. Haugh unit (HU) value for each replicate was calculated by using the formula given by Haugh (1937):

$$HU = 100 \log_{10} (h + 7.57 - 1.7w^{0.37})$$

Economic analysis

The economic viability of graded SBM inclusion in Bovans-Brown layer diets was calculated using a method developed by Upton (1979), considering feed formulation costs and egg prices. Total feed intake was multiplied by ingredient costs, while egg revenue was calculated using local market price of eggs in Bahir Dar, Ethiopia, during the study period. Net return was derived as total revenue minus feed costs.

Measurements of blood parameters

At the end of the experiment, approximately 3 mL of blood was collected from the wing veins of 15 randomly selected hens (three hens per treatment) between 8:00 AM and 11:00 AM after the night's fast. The hematology analysis was performed with an automated hematology analyzer (BC- 5800, Mindray, China) after blood samples were deposited in K3-EDTA heparinized tubes, serum samples were analyzed for total cholesterol (COL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) after being separated by centrifuging (3,000 rpm, 15 min).

Statistical analysis

All data were analyzed using the GLM procedure of SAS (9.4, Cary, NC). For SBM proximate composition, the main effect of the model was the fixed effect of the edible oil processing plant. The effect of feeding different levels of SBM was analyzed using the following model: $Y_{ij} = \mu + T_i + e_{ij}$. Where, Y_{ij} = represents the j^{th} observation (experimental unit) taken under treatment i ; μ = overall mean; T_i = feed effect; e_{ij} = random error. When the mean was significant, the least significant difference (LSD) test was used to make pairwise comparisons between treatment means. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of SBMs

The proximate composition of the 11 SBM samples varied significantly ($P < 0.05$) in all parameters (Table 2). Significant variations ($P < 0.05$) were noted among the processing plants in CP content, ranging from 38.42 to 43.52%. However, the CP content of the current study is relatively lower compared to the world's market standard, which ranges from 44 to 49% CP (Banaszkiewicz, 2011). This may be due to differences in both the origin and variety of soybean used to produce the SBM (Ibáñez, et al., 2020; Ateye et al., 2025), and the differences in processing conditions during the production of the SBM (Karr-Lilienthal et al., 2005). Typically, the soybean processing industries conduct crude fat analysis to determine the oil content of SBM. The solvent-extracted SBM samples contained significantly lower ($P < 0.05$) EE within the range of 1.23-1.87% compared to mechanically extracted SBM, which contained higher ($P < 0.05$) EE content within a range of 2.41- 5.27%, indicating more oil is extracted by solvent (hexane). Grieshop et al. (2003) also reported that SBM samples from mechanical extraction installation contained higher ($P < 0.05$) lipid content than SBM from solvent extraction plants as determined by both crude fat and acid-hydrolyzed fat analyses.

The analyzed CF values ranged from 5.58 to 7.35%, which indicated that the meals were non-dehulled. Depending upon the processing methods used, crushing and removal of the hulls decrease the fiber content of the meal. Therefore, hulled SBM is higher in CF content than dehulled soybean meal. Dehulled soybean meals normally contain a maximum of 3.5% CF, while non-dehulled soybean meals contain 6-7% CF (Tangendjaja, 2015). The metabolizable energy (ME) content

observed in this study ranged from 3348.3 to 3594.8 kcal/kg DM. These values are within the range reported by Li et al. (2015), who reported ME values between 3405.8 and 3840.8 kcal/kg. Notably, mechanically extracted SBM samples exhibited higher ME values than solvent-extracted SBMs, which can be attributed to residual oil content remaining after mechanical extraction, thereby increasing the energy value of the meal.

Table 2 - Chemical composition of SBMs from eleven edible oil processing plants

Processing plants	DM	CP	EE	CF	ASH	NFE	ME (Kcal/kg DM)
1	93.81 ^a	42.75 ^{bc}	3.65 ^c	7.08 ^b	1.85 ^{de}	38.47 ^{bc}	3446.1 ^d
2	89.29 ^d	41.03 ^e	2.86 ^{de}	5.58 ⁱ	1.6 ^e	38.21 ^c	3546.4 ^b
3	92.08 ^b	41.7 ^d	2.63 ^{ef}	7.35 ^a	2.29 ^{cd}	38.09 ^c	3348.3 ^f
4	93.45 ^a	43.52 ^a	4.93 ^a	6.42 ^c	2.65 ^{abc}	35.93 ^f	3541.6 ^b
5*	87.61 ^e	40.5 ^e	1.23 ^h	5.7 ^h	2.99 ^a	37.18 ^{de}	3390.3 ^e
6	93.31 ^a	42.9 ^b	2.41 ^f	6.3 ^{ed}	1.92 ^{de}	39.78 ^a	3444.9 ^d
7	90.77 ^c	43.31 ^{ab}	3.06 ^d	6.27 ^e	2.89 ^{ab}	35.24 ^f	3443.4 ^d
8*	87.11 ^e	38.42 ^f	1.87 ^g	5.68 ^{hi}	3.14 ^a	38.01 ^{cd}	3421.5 ^{de}
9	89.6 ^d	38.47 ^f	5.27 ^a	5.84 ^g	3.06 ^a	36.96 ^e	3594.8 ^a
10	92.83 ^{ab}	41.06 ^e	4.14 ^b	6.07 ^f	2.47 ^{bc}	39.09 ^{ab}	3537.1 ^b
11	92.13 ^b	42.27 ^{cd}	3.85 ^{bc}	6.37 ^{cd}	2.44 ^{bc}	37.21 ^{de}	3495.8 ^c
SEM	0.499	0.372	0.259	0.118	0.115	0.283	15.888
P value	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001

Means within the same column with different ^{a-i} superscripts are significantly different (P < 0.05). Whereas values with the same superscripts not differ significantly (P > 0.05). Processing plants with a [*] superscripts represent solvent extracted SBM whereas the others are mechanically extracted SBM. 1: Abay; 2: Nile; 3: Rafa; 4: Reach Land; 5: Emy; 6: Meheret; 7: Sky; 8: WA; 9: Unison; 10: TOP; 11: MSA.

Nutrient composition of treatment diets

Data in Table 3 shows that different soybean meal inclusion levels significantly (P < 0.05) affect the nutrient density of the formulated experimental diets (T2-T5). The nutritional concentration of feed is crucial in the poultry industry as it affects laying performance and profitability (Kim and Kang, 2022). Dietary ME and CP are key nutritional parameters in layer hen diets. In this study, CP increased proportionally with higher levels of SBM inclusion and the highest ME value (3265.42 kcal/kg DM) was recorded at the 30% SBM inclusion level. This increase is attributed to the nutritional richness of SBM, which is characterized by its high protein content, digestible oil, and carbohydrate components. In contrast, the commercial control diet CP content was lower than that of the experimental diets with 20%, 25%, and 30% SBM inclusion, while its ME was higher. This aligns with the findings of Negash (2020), who reported that commercial layer feeds in Ethiopia often have variable nutritional content, with CP levels ranging from 10.91% to 17.90% and ME values between 1364 and 2746 kcal/kg DM, frequently falling below recommended benchmarks.

Table 3 - Chemical composition of feed ingredients and experimental diets (on a dry matter basis)

Processing plants	DM	CP	CF	EE	ASH	NFE	ME (Kcal/kg DM)
T1 (Control)	88.73 ^c	15.01 ^c	3.65 ^e	1.94 ^c	4.96 ^d	63.16 ^a	3530.51 ^a
T2 (15%SBM)	91.14 ^b	14.74 ^d	6.24 ^a	4.17 ^a	10.59 ^c	55.39 ^c	3192.08 ^c
T3 (20%SBM)	91.04 ^b	16.03 ^b	5.06 ^c	1.29 ^e	11.07 ^b	57.58 ^b	3120.25 ^d
T4 (25%SBM)	91.51 ^{ab}	17.43 ^a	5.98 ^b	1.62 ^d	11.19 ^b	55.28 ^c	3051.56 ^e
T5 (30%SBM)	92.06 ^a	17.56 ^a	4.61 ^d	3.69 ^b	11.71 ^a	54.49 ^d	3265.42 ^b
SEM	0.384	0.393	0.314	0.387	0.833	1.055	55.161
P value	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means within the same column with different ^{a-e} superscripts are significantly different (P < 0.05). Whereas values with the same superscripts do not differ significantly (P > 0.05).

Egg production performance

This study indicated that the groups fed on the treatment containing 20%SBM (16%CP & 3120.25 of ME/kg of DM) had higher production performances than the groups fed on the control diet which had higher ME and low CP (3530.51 Kcal/kg DM ME and 15.01CP) and the groups fed on 30%SBM which had higher CP and ME (17.56 CP and 3265.42 Kcal/kg DM ME) Figure 1. The results indicate that a 20% SBM inclusion is sufficient to support egg production in mid-lay Bovans Brown layers (34-46 weeks), with higher levels providing no additional production benefit. This result is in agreement with Li et al. (2013), diets containing 16.0% balanced CP produced optimal egg performance for Lohmann Brown laying hens aged from 26 to 38 weeks.

The effect of different soybean meal inclusion levels on production performance is presented in Table 4. According to the results of the current study, an increase in nutrient density had no significant effect on the rate of egg production, egg mass, egg weight, feed intake, and feed conversion ratio of laying hens. The comparable egg production performance of all treatment rations might be related to the amount of nutrient density, in which all diets have an adequate amount of ME and variation in CP was supplemented by the most limiting amino acids (lysine and methionine). These results align with those of Panda et al. (2012) and Kim & Kang, (2022), who reported that an increase in nutrient density did not affect laying performance and egg quality.

However, increasing the SBM inclusion level significantly ($P < 0.05$) affected body weight; the highest BWC (400g) was recorded for a 25% SBM inclusion level, and the lowest BWC (167g) was recorded for the control diet. The significant increase in BWC of the groups fed increasing levels of SBM could be due to the increase in nutrient intake, which might have increased fat deposition. Ding et al. (2016) and Kim and Kang (2022) also support the hypothesis that an excess in nutrient intake results primarily in increases in body weight gain rather than in further increases in egg mass production.

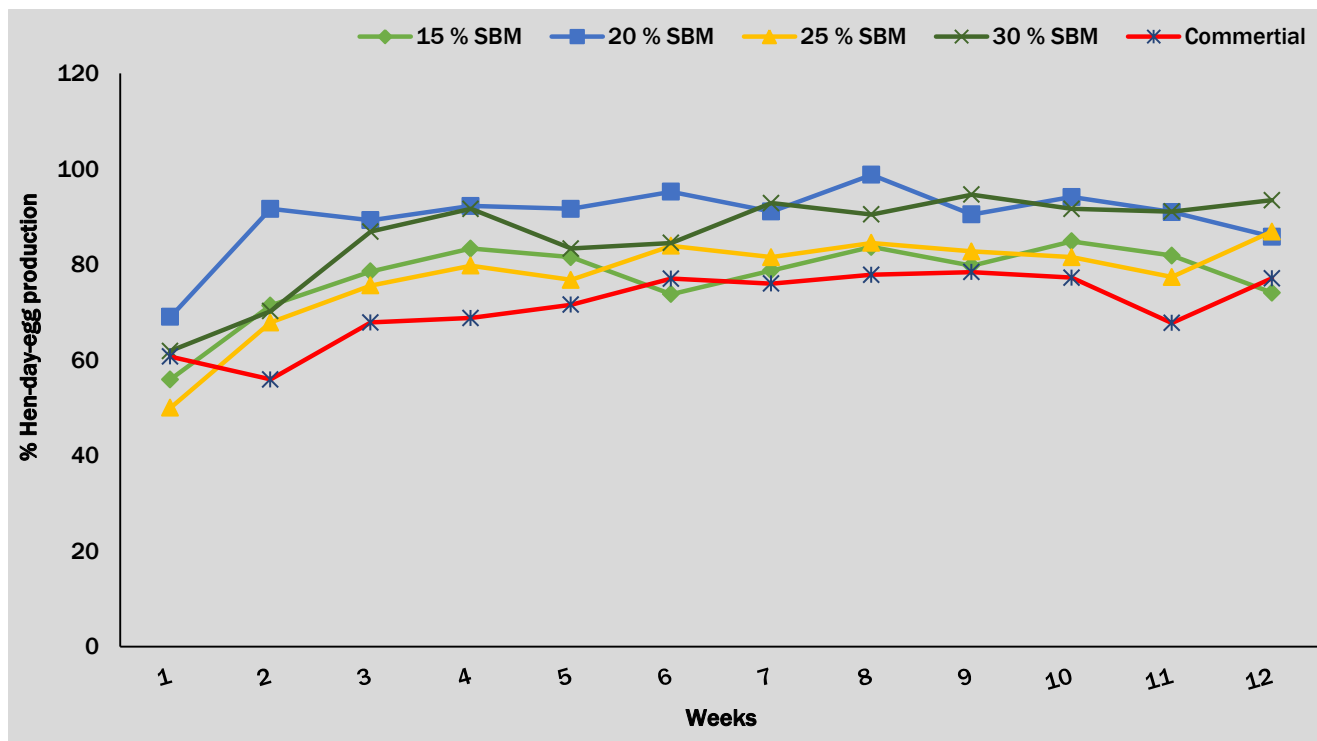


Figure 1 - Effect of SBM inclusion levels on % of hen day egg production in 12 weeks.

Table 4 - Effect of different levels of SBM inclusion on production performance of layer hens

Parameters	T1	T2	T3	T4	T5	SEM	P value
% HDEP	71.887	77.373	89.712	77.500	86.276	2.243	0.0693
Egg mass (g/hen/day)	43.440	45.865	54.280	47.342	53.607	1.482	0.0704
Egg weight (g)	60.447	60.447	60.482	61.082	62.120	0.404	0.3561
Final BW (kg)	1.635	1.601	1.743	1.827	1.759	0.031	0.0884
Initial BW (kg)	1.467	1.363	1.429	1.427	1.394	0.019	0.5804
BWC (kg)	0.167 ^c	0.238 ^{bc}	0.314 ^{abc}	0.400 ^a	0.365 ^{ab}	0.028	0.0351
FI (g/hen/day)	108.507	110.291	112.031	114.218	115.743	1.784	0.8246
CPI (g/hen/day)	16.283 ^b	16.261 ^b	17.959 ^{ab}	19.903 ^a	20.326 ^a	0.527	0.0110
MEI (kcal/hen/day)	383.09	352.06	349.57	348.54	377.95	6.755	0.4059
FCR	2.541	2.419	2.061	2.419	2.163	0.069	0.1960

Means within the same rows with different ^{a-c} superscripts are significantly different ($P < 0.05$). Whereas values with no superscripts do not differ significantly ($P > 0.05$). T1: Commercial layer diet, T2: 15%SBM, T3: 20%SBM, T4:25%SBM and T5:30%SBM inclusion level. HDEP: hen day egg production. BWC: Body Weight change. FI: Feed Intake. CPI; crude protein intake. MEI: metabolizable energy intake. FCR: Feed Conversion Ratio.

Egg quality

The results of the egg quality parameters are presented in Table 5. There were no significant ($P > 0.05$) differences between all the treatment groups in all egg quality parameters, except for yolk color. Similarly, [Junqueira et al. \(2006\)](#) did not find any effect on egg quality parameters of laying hens by comparing 3 metabolizable energy levels (2,850; 2,950, and 3,050 kcal of ME/kg) and 3 protein levels (16, 18, and 20% CP) and [Pérez-Bonilla et al. \(2012\)](#) also reported, that increasing the levels of CP in the diet from 16.5 to 18.5% does not affect the performance or egg quality of brown egg-laying hens. Significantly higher ($P < 0.0001$) value for egg yolk coloration was recorded from the groups placed on the control diet, which might be due to the addition of pigmenting additives in commercial diets.

Partial Budget Analysis

The economic returns from the perspective of partial budget analysis from egg sales and feed costs are presented in Table 6. Feed cost rose with increasing dietary SBM inclusion. The control diet had a significantly lower cost than all SBM-based diets ($P < 0.05$). Despite having a higher feed cost than the control diet, the diet with 20% SBM inclusion resulted a significantly ($P < 0.05$) higher net return compared to all other dietary treatments. Therefore, a 20% SBM inclusion is the most profitable and balanced option for mid-lay Bovans Brown layer hens. It generates higher egg production while maintaining a relatively lower feed cost than higher inclusion levels, which increase expense without a proportional return.

Blood Hematology and some serum chemistry

The effect of different SBM inclusion levels on hematology and serum cholesterol is presented in Table 7. There was no significant difference between the dietary treatments in WBC values ranging between $37.76-40.97 \times 10^3/\mu\text{L}$. However, the results found in this research were higher than the normal $12-30 \times 10^3/\mu\text{L}$ range ([Bounous and Stedman, 2000](#)). This elevation likely reflects the hens' exposure to tropical heat stress and may represent a compensatory response to counteract pathogen invasion, which is exacerbated by heat-induced compromised immunity ([Hassan et al., 2023](#)). All RBC values were within the normal range. The recommended normal range was for RBC: $2.5-3.5 \times 10^6/\mu\text{L}$, PCV: 22-35%, HGB: 7-13 g/dl, MCV: 90-140 fL, MCH: 33-47 pg/cell and MCHC: 26-35 g/dl ([Bounous and Stedman, 2000](#)). Cholesterol levels ranged from 94 to 131 mg/dL, with no significant differences among treatments. The HDL and LDL values ranged from 13.5-29.5 (mg/dL) and 33.5-49.50 (mg/dL), respectively. Similarly, there was no statistically significant difference between the dietary treatments. Similar to our result, [Hassan et al. \(2023\)](#) reported that the standard laying hen strain had lower plasma HDL, but higher plasma LDL.

Table 5 - Effect of different levels of SBM inclusion on egg quality parameters of layer hens

Parameters	T1	T2	T3	T4	T5	SEM	P value
Egg index	78.564	79.531	78.331	79.082	79.357	0.275	0.3062
Sample egg weight (g)	60.163	58.865	59.917	62.808	62.083	0.557	0.1446
Albumen height (mm)	9.196	9.104	8.446	8.9458	8.868	0.098	0.1656
Yolk height (mm)	18.587	17.811	18.402	18.725	18.552	0.185	0.6757
Yolk weight (g)	14.868	14.725	15.417	15.963	15.589	0.191	0.2759
Eggshell weight (g)	7.8438	8.1750	7.687	7.846	7.8500	0.077	0.5107
Eggshell thickness (mm)	0.359	0.359	0.357	0.355	0.365	0.003	0.8841
Albumen weight (g)	38.475	35.906	36.754	39.067	38.565	0.498	0.1522
Yolk index	0.455	0.452	0.462	0.462	0.465	0.004	0.9388
Haugh unit (%)	95.519	95.377	91.804	93.703	93.477	0.517	0.1472
Yolk color	9.333 ^a	1.00 ^b	1.00 ^b	1.00 ^b	1.00 ^b	0.893	<0.0001

Means within the same rows with no superscripts do not differ significantly ($P > 0.05$). T1 is a commercial diet, whereas the other diets contain T2 (15%), T3 (20%), T4 (25%), and T5 (30%) SBM inclusion levels.

Table 6 - Effect of dietary inclusion of SBM in the diet on economics during the experimental period

Parameters	T1	T2	T3	T4	T5	SEM	P value
Total egg (number/hen)	64.42	69.64	80.87	69.75	77.65	2.028	0.0587
Total feed consumed (kg/hen)	9.766	9.926	10.083	10.279	10.417	0.161	0.8246
Total feed cost per head (ETB)	419.93 ^b	510.07 ^a	523.86 ^a	535.938 ^a	550.38 ^a	14.257	0.0158
Total return (ETB)	773.06	835.67	970.48	837	931.84	24.334	0.0587
Net return (ETB)	353.14 ^{ab}	325.61 ^{ab}	446.63 ^a	301.06 ^b	381.45 ^{ab}	19.166	0.1604

T1 is a commercial diet, whereas the other diets containing T2 (15%), T3 (20%), T4 (25%), and T5 (30%) SBM inclusion levels. Means within the same rows with ^{a-b} superscripts are significantly different ($P < 0.05$). Whereas values with no superscripts do not differ significantly ($P > 0.05$). ETB - Ethiopian Birr.

Table 7 - The effect of different SBM inclusion on blood hematology, cholesterol, LDL, and HDL

Parameters	T1	T2	T3	T4	T5	SEM	P value
WBC ×10 ³ /μL	40.480	37.760	40.265	38.375	40.975	0.778	0.6163
HT ×10 ³ /μL	11.985	9.778	13.964	9.360	8.419	0.853	0.2613
LYM, ×10 ³ /μL	25.337	25.151	22.893	25.953	29.015	1.142	0.7447
MON ×10 ³ /μL	1.8927	2.1148	1.2074	2.0642	2.3827	0.166	0.1531
EO ×10 ³ /μL	1.266	0.697	2.201	0.978	1.158	0.343	0.7903
BAS ×10 ³ /μL	0.00	0.0187	0.00	0.0198	0.00	0.005	0.6833
RBC ×10 ⁶ /μL	2.46	2.485	2.57	2.51	2.45	0.026	0.7753
HGB, g/dL	9.050	9.000	8.850	9.350	10.550	0.327	0.5790
HCT, %	31.500	31.550	32.250	32.100	31.800	0.312	0.9569
MCV, fL	128.010	126.992	125.558	127.855	129.791	0.708	0.5139
MCH, pg	36.696	36.215	34.457	37.219	43.096	1.347	0.4013
MCHC, g/dL	28.593	28.524	27.443	29.104	33.209	0.929	0.4370
PLT ×10 ³ /μL	2.5	8.0	5.5	2.0	3.5	1.086	0.5061
HDL (mg/dL)	29.50	22.00	22.50	14.00	13.50	3.783	0.5908
LDL (mg/dL)	49.00	34.50	33.50	47.50	49.50	4.986	0.7409
COL (mg/dL)	119.50	94.00	131.00	123.00	118.00	4.817	0.1580

Means within the same rows with no superscripts not differ significantly (P > 0.05). T1 is a commercial diet, whereas the other diets containing T2 (15%), T3 (20%), T4 (25%), and T5 (30%) SBM inclusion levels. WBC = white blood cell count; HT = heterophil; LYM = lymphocytes; MON = monocytes; EO = eosinophils; BAS = basophil; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; HDL = high-density lipoprotein; LDL = low-density lipoprotein; COL = cholesterol.

CONCLUSION

This study demonstrated significant variation in the proximate composition of soybean meals (SBM) sourced from different processing plants in the Amhara Region of Ethiopia. These findings highlight the importance of routinely assessing the quality of processed SBM to ensure consistency and reliability in poultry feed formulation. Among the inclusion levels tested, a 20% SBM inclusion was found to be optimal for Bovans Brown layers during mid-production, providing the highest egg production, comparable egg quality, and improved economic returns. Overall, this research provides valuable guidance for formulating nutritionally and economically efficient layer diets while emphasizing the need for ongoing quality control and further study of local SBM sources.

DECLARATIONS

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval

The animals were handled and cared for following welfare standards and experimental protocols approved by the College of Agriculture and Environmental Sciences Animal and Research Ethics Committee of Bahir Dar University (Ref. No. 007-2025).

Authors' contribution

The authors declare that no funds, grants, or other support were received. All authors contributed to the study conception and design. Y. Yimer conducted material preparation, data collection, and analysis, and drafted the initial manuscript. F. Tegegne and S. Demeke collaborated on the critical review and editing. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to express their gratitude to Bahir Dar University for providing materials and experimental hens for this study. They also extend their appreciation to Andasa Livestock Research Center and the Amhara Regional Agricultural Research Institute for establishing the laboratory for egg quality analysis.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing Interests

The authors declare that they have no competing interests.

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IMPACT OF PELLET BINDER TYPE ON THE QUALITY AND PERFORMANCE OF PELLET MILLS WITH DIFFERENT LEVELS OF OIL AND FAT POWDER

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✉Supporting Information



ABSTRACT: The current study aimed to assess the effects of fat powder levels, oil levels, and two types of pellet binders, including bentonite and gum Tragacanth on the physical quality and pellet durability index (PDI) over 75 days. The experiment was conducted using a completely randomized design with a factorial arrangement. Experimental treatments included 5 to 10 kilograms of fat powder, 7 to 11 kilograms of oil, and pellet binders. The pellet binders consisted of bentonite, plant-based binders such as gum Tragacanth, and their combination. The present results indicated that using fat powder and oil levels, with pellet binders, significantly increased PDI in the starter period. The concentration and type of pellet binder were effective parameters in treatments during the experiment. Fat powder significantly improved the physical quality of the pellet on days 15, 30, 60, and 75, while oil notably improved the effects of the pellet on all days except day 0. Fat powder, oil, and pellet binder played crucial roles in determining the physical quality of pellets during the finisher period across all days. The interaction between fat powder and oil substantially affected finisher pellet quality on all days except days 15 and 45. Oil levels did not have impacts on the physical quality of the finisher pellets. Gum Tragacanth binder demonstrated the highest average physical pellet quality on days 0, 45, 60, and 75, while bentonite exhibited the best performance on days 15 and 30. The utilization of pellet binders combined with adding oil and fat powder to the diet, improve the physical qualities of the produced pellets.

Keywords: Pellet binder, Pellet durability index, Pellet hardness, Fat powder, Oil

INTRODUCTION

Pelletizing involves the process of compressing feed particles to form a solid unit into different shapes. Throughout the pelletizing process, applying the incremental pressure results in adhesion of the feed particles, thereby decreasing the void space between particles and increasing the density of the materials. Pelletizing the diet balances the protein-to-energy ratio and adjusts nutrient levels, which improves weight gain and breast meat yield by affecting feed and amino acid intake (Noll, 2002). The pellet durability index (PDI) is one of the primary parameters that assesses the physical quality of pellets. The PDI indicates the percentage of pellets that remain intact after transportation from the feed mill to poultry farms and while subjected to different mechanical forces (Mina-Boac et al, 2006; Löwe, 2007). Essentially, PDI is used to evaluate pellet abrasion and fracture into smaller particles, and the fineness of the fracture point. Pellets should be manufactured to be as robust and sturdy as possible to withstand heavy handling (Pourreza et al., 2014). Poor physical pellet quality (PPQ) results in more fine particles during feed transportation to poultry farms (Hancock, 2010). Several studies have demonstrated that increasing mixer fat level from 1 to 18.2% substantially reduces the PDI by 13%. Soybean oil and saturated fat powder, which consists of 65% palmitic acid (Acofeed), have distinct effects on PDI, indicating that each type of fat possesses unique factors influencing pellet quality (Loar et al, 2014). Increasing soybean oil levels reduces starch gelatinization (Behnke et al., 2006), while Muramatsu et al. (2014) indicated that increasing soybean oil up to a level of 3.5% results in a parabolic increase in starch gelatinization.

Moreover, utilizing pellet binders can influence pellet quality. Pellet binders are used to improve the strength, integrity, and stability of pellets, thereby preventing their breakage and abrasion during handling, transportation, and storage. There are different types of pellet binders, including colloidal binders, molasses, and fats, that have been used for many years, and their effects on PDI have been thoroughly documented (Young et al., 1962). Considering the importance of pellet quality, the impact of various factors, including the type and quantity of feed ingredients, the type and amount of fat incorporated into the mixture, the type and quantity of pellet binders, dicalcium phosphate, and processing parameters such as feed particle size, conditioner retention time, production rate, and die thickness, on the PDI has been investigated. In industrial poultry nutrition, high-fat diets are often necessary; however, increasing fat content in the diet is inversely related to pellet durability, thereby reducing pellet strength. Considering that pellets alone do not directly

RESEARCH ARTICLE
 PII: S222877012500041-15
 Received: August 16, 2025
 Revised: November 26, 2025
 Accepted: November 28, 2025

influence poultry performance, the physical quality of pellets, particularly their durability index, serves as a crucial factor of animal performance and production. Numerous studies have demonstrated that the type and quantity of fat are among the primary factors influencing pellet quality. One study found that increasing the level of bentonite as a pellet binder in a diet containing 1.5% soybean oil improved pellet durability and physical quality, while increasing bentonite in a diet with 1.5% calcium fat powder only enhanced physical quality. The same study demonstrated that increasing bentonite from 1 to 2% in diets containing 3% of soybean oil and 3% of calcium fat powder was not effective in preventing a reduction in pellet durability (Abadi et al., 2019). Additionally, utilizing 1.5% of bentonite in corn-soy-based diets improved PDI and physical pellet quality during the growth period (Attar et al., 2016). Therefore, the present study aimed to investigate the effects of different levels of fat powder and oil, along with pellet binders, on the quality of pelleted feed.

MATERIALS AND METHODS

The current experiment was conducted in 2022 utilizing a completely randomized design with a 4×2×2 factorial arrangement, encompassing two types of pellet binders (Sodium bentonite, plant-based compounds such as gum Tragacanth, and a combination of both), as well as two levels of oil, 7 and 14 kg, and two levels of fat powder, 5 and 10 kg. The experimental treatments are reported in Table 1. The present study evaluated qualitative pellet indices over 75 days at the Gohar daneh Shargh Livestock and Poultry feed mill, located in Khorasan Razavi, Iran.

Feeds were produced at the mill using high-capacity equipment across three growth phases. The horizontal ribbon mixer of the mill encompasses a production capacity of up to 2500 kg. The steam pressure in the DDC (Yemuack) model conditioner boiler was configured at 6-8 bar, subsequently decreased to 2-3 bar before entering the conditioner. Additionally, the cooking temperature for all feeds was consistently maintained at an appropriate level of 70°C. This conditioner enabled the control of temperature and feed retention time. The pellet mill used for feed production was manufactured by Feed Tech (Turkey) with a pellet diameter of 850 mm. To ensure precision in the production of experimental treatments, and considering the capacities of the mixer, conditioner, pellet mill, and cooler, a quantity of 1250 kg of feed was produced per treatment, resulting in a total of 20,000 kg. Starter and finisher diets were produced using dies with hole diameters of 2 mm and 4 mm, respectively. Pellet physical quality was determined using a hardness tester (Model K6432-0054, Kahl, Germany). To precisely evaluate the PDI (%) on days 0, 15, 30, 45, 60, and 75 post-production, four replicate samples of 1 kg each were collected from under the cooler after cooling. The samples were then transferred to the laboratory for PDI measurement. The Holmen NHP100 tester (Tekpro; Norfolk, UK) was used for durability testing. For each sample, 100 g of pellets without fines were weighed and placed in the Holmen tester. The feed was subjected to several shocks for 30, 60, 90, and 120 seconds. The resulting fines were collected and weighed, and the PDI was calculated using the following formula.

$$PDI (\%) = (\text{Weight of intact pellets after tumbling} / \text{Initial weight of pellets}) \times 100$$

Table 1 - Arrangement of experimental treatments

Treatment 1	7 kg oil + 5 kg fat powder
Treatment 2	7 kg oil + 10 kg fat powder
Treatment 3	14 kg oil + 5 kg fat powder
Treatment 4	14 kg oil + 10 kg fat powder
Treatment 5	1% Bentonite + 7 kg oil + 5 kg fat powder
Treatment 6	1% Bentonite + 7 kg oil + 10 kg fat powder
Treatment 7	1% Bentonite + 14 kg oil + 5 kg fat powder
Treatment 8	1% Bentonite + 14 kg oil + 10 kg fat powder
Treatment 9	1% Gum Tragacanth + 7 kg oil + 5 kg fat powder
Treatment 10	1% Gum Tragacanth + 7 kg oil + 10 kg fat powder
Treatment 11	1% Gum Tragacanth + 14 kg oil + 5 kg fat powder
Treatment 12	1% Gum Tragacanth + 14 kg oil + 10 kg fat powder
Treatment 13	1% Combination (Both) + 7 kg oil + 5 kg fat powder
Treatment 14	1% Combination (Both) + 7 kg oil + 10 kg fat powder
Treatment 15	1% Combination (Both) + 14 kg oil + 5 kg fat powder
Treatment 16	1% Combination (Both) + 14 kg oil + 10 kg fat powder

Statistical analysis

All data were analyzed using SAS software (2004, SAS 9.1, Cary, NC) with the Proc GLM procedure. Differences between treatments were examined using Tukey's test at a 5% significance level ($P < 0.05$). The statistical model used was: $Y_{ijk} = \mu + A_i + T_j + (A \times T)_{ij} + e_{ijk}$

Y_{ijk} is the observation, μ is the overall mean for each parameter, A_i is the effect of the i , T_j is the effect of the j , i means the pellet binder type, j means the fat level, $(A \times T)_{ij}$ is the interaction effect between the i and j , and e_{ijk} is the experimental error.

RESULTS

Pellet durability Index and physical quality in the starter and finisher periods

The present results regarding the effects of two levels of oil and fat powder, along with three types of pellet binders, on the PDI in the starter period are reported in Table 2. Utilizing fat powder and oil levels in combination with pellet binders significantly increased PDI in the starter period ($P < 0.05$). The effect of the concentration and type of pellet binder significantly influenced the treatments across all experimental days, while the effect of fat powder was significant on days 0, 60, and 75 ($P < 0.05$). The interaction effect of fat powder, oil, and pellet binder was significantly influenced by the experimental treatments on days 15 and 75 ($P < 0.05$). The highest PDI concerning the effect of fat powder on days 45, 60, and 75 was linked to a level of 5 kg/ton, and for the effect of oil, the highest value on days 15, 30, and 60 was associated with a level of 7 kg. Treatments without pellet binders (control) exhibited the lowest PDI values during the starter period among all experimental groups. From day 0 to 30, incorporating pellet binders led to the highest average PDI.

Table 2 - The effects of fat and oil powder levels, along with pellet binders, on the pellet durability index in the starter period.

Main effects		Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
Fat levels	5	89.66 ^a	88.4	88.15	87.96	85.84 ^a	84.64 ^a
	10	88.87 ^b	88.8	86.93	87.65	83.44 ^b	83.15 ^b
SEM		0.4	0.25	0.27	0.36	0.45	0.63
Oli levels	7	91.79 ^a	91.76 ^a	83.06 ^a	83.06 ^a	88.58 ^a	80.94
	14	86.76 ^b	85.5 ^b	80.07 ^b	80.07 ^b	80.70 ^b	77.03
SEM		0.49	0.25	0.82	0.82	0.45	0.02
Pellet binder	Control (no Pellet Binder)	87.38 ^a	87.1 ^a	86.25 ^a	84.93 ^a	83 ^a	81.94 ^a
	Bentonite	89.4 ^b	88.68 ^b	87.65 ^b	87.92 ^b	88.45 ^b	85.64 ^b
	Gum tragacanth	89.69 ^b	88.04 ^b	88.08 ^b	88.69 ^{bc}	84.63 ^b	84.98 ^{bc}
	Both	90.56 ^c	90.03 ^c	90.06 ^c	88.26 ^c	85.49 ^b	83.91 ^c
SEM		0.498	0.265	0.259	0.3	0.3	0.3
Interaction effect of fat powder + oil + pellet binder levels							
Treatment 1		90.47	89.64	89.52	87.42	87.2	85.15
Treatment 2		85.25	84.2	84.65	84.22	8345	81.97
Treatment 3		89.15	89.87	88.15	82.9	79.45	78.6
Treatment 4		84.65	84.65	82.7	85.17	81.9	82.05
Treatment 5		91.125	91.95	91.12	92.35	91.22	89.77
Treatment 6		85.42	84.6	83.92	83.17	79.7	80.05
Treatment 7		93.125	92.8	90.95	93.57	90.45	91.77
Treatment 8		88.1	85.4	84.62	82.6	80.27	80.27
Treatment 9		91.65	91.57	92.12	93.1	91.32	82.35
Treatment 10		87.4	85.4	83.75	84.92	81.05	80.8
Treatment 11		92.07	91.25	91.65	9165	87.4	87.77
Treatment 12		87.45	85.4	84.8	85.1	78.75	79.02
Treatment 13		92.6	93.17	93.15	94.12	90.97	89.6
Treatment 14		87.1	86.82	87.02	84.42	81.75	77.42
Treatment 15		94.17	93.85	93.62	93.95	90.55	89.07
Treatment 16		88.37	87.52	87.02	84.95	78.7	79.55
P value							
Effect of fat powder		0.031	0.031	0.1	0.6	0.01	0.0018
Effect of oil		0.001	0.0001	0.0001	0.0001	0.001	0.009
Effect of pellet binder		0.001	0.0001	0.0001	0.001	0.0001	0.007
Fat powder with oil		0.74	0.71	0.77	0.0006	0.3	0.6
Fat powder with binder pellet		0.011	0.01	0.52	0.0001	0.0001	0.0001
Oil with binder pellets		0.57	0.57	0.51	0.0001	0.04	0.22
Fat powder with oil and a type of pellet binder		0.92	0.9	0.9	0.001	0.0001	0.0005

Treatment 1: 7 kg oil + 5 kg fat powder; Treatment 2: 7 kg oil + 10 kg fat powder; Treatment 3: 14 kg oil + 5 kg fat powder; Treatment 4: 14 kg oil + 10 kg fat powder; Treatment 5: 1% bentonite + 7 kg oil + 5 kg fat powder; Treatment 6: 1% bentonite + 7 kg oil + 10 kg fat powder; Treatment 7: 1% bentonite + 14 kg oil + 5 kg fat powder; Treatment 8: 1% bentonite + 14 kg oil + 10 kg fat powder; Treatment 9: 1% gum Tragacanth + 7 kg oil + 5 kg fat powder; Treatment 10: 1% gum Tragacanth + 7 kg oil + 10 kg fat powder; Treatment 11: 1% gum Tragacanth + 14 kg oil + 5 kg fat powder; Treatment 12: 1% gum Tragacanth + 14 kg oil + 10 kg fat powder; Treatment 13: 1% both binders + 7 kg oil + 5 kg fat powder; Treatment 14: 1% both binders + 7 kg oil + 10 kg fat powder; Treatment 15: 1% both binders + 14 kg oil + 5 kg fat powder; Treatment 16: 1% both binders + 14 kg oil + 10 kg fat powder. In each column, values not sharing similar letters are significantly different ($P < 0.05$).

The results regarding the effects of two levels of oil and fat powder, along with three types of pellet binders, on the physical quality of pellets in the starter period are reported in Table 3. The effect of fat powder on days 15, 30, 60, and 75, and the effect of oil on all experimental days except day 0, significantly improved the physical quality of pellets ($P < 0.05$). The interaction effects of fat powder and oil on day 30 and fat powder and pellet binder on days 15 and 45 did not significantly affect physical pellet quality ($P > 0.05$). However, the three-way interaction of all factors (1% pellet binder levels, 5 and 10 kg of fat powder, and 7 and 14 kg of oil) significantly improved the physical quality of pellets during the starter period on all experimental days except days 15 and 30 ($P < 0.05$).

Table 3 - The effects of fat and oil powder levels, along with pellet binders, on pellet physical quality in the starter period.

Main effects		Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
Fat levels	5	4.17	7.28 ^a	4.36	4.28 ^a	4.23 ^a	4.34 ^a
	10	4.18	4.22 ^b	4.32	4.19 ^b	4.05 ^b	4.12 ^b
SEM		0.0048	0.0036	0.004	0.006	0.45	0.0097
Oli levels	7	4.17	4.26	4.28 ^a	4.11 ^a	4.12 ^a	4.15 ^a
	14	4.17	4.18	4.25 ^b	4.36 ^b	4.70 ^b	4.31 ^b
SEM		0.008	0.07	0.004	0.007	0.45	0.009
Pellet binder	Control (no Pellet Binder)	4.06 ^a	4.037 ^a	4.13 ^a	4.01 ^a	3.981 ^a	3.98 ^a
	Bentonite	4.25 ^b	4.36 ^b	4.21 ^b	4.41 ^b	4.250 ^b	4.2 ^b
	Gum Tragacanth	4.025 ^a	4.16 ^c	4.2 ^b	4.25 ^c	4.100 ^b	4.1 ^{ab}
	Both	4.27 ^b	4.23 ^b	4.42 ^c	4.27 ^c	4.200 ^b	4.64 ^c
SEM		0.035	0.030	0.004	0.0062	0.01	0.0097
Interaction effect of fat powder + oil + pellet binder levels							
Treatment 1		3.97	3.95	4.075	4.00	4.02	4.05
Treatment 2		4.02	4.17	4.125	3.85	3.95	3.97
Treatment 3		4.32	4.20	4.350	3.95	3.95	3.97
Treatment 4		3.95	4.30	3.975	4.25	4.00	3.95
Treatment 5		4.20	4.40	4.300	4.35	4.10	4.10
Treatment 6		4.4	4.30	4.370	4.35	4.07	4.25
Treatment 7		4.42	4.40	4.570	4.10	4.02	3.97
Treatment 8		4.37	4.37	5.150	4.85	4.65	4.50
Treatment 9		4.17	4.25	4.300	4.02	4.22	4.35
Treatment 10		4.07	4.22	4.500	4.80	4.52	4.12
Treatment 11		4.00	4.27	4.170	4.15	4.02	3.82
Treatment 12		3.85	3.90	4.00	4.05	4.35	4.00
Treatment 13		4.25	4.55	4.250	4.20	4.25	4.35
Treatment 14		4.32	4.45	4.900	4.72	4.70	5.42
Treatment 15		4.15	4.35	4.250	4.12	4.00	4.85
Treatment 16		4.37	4.45	4.300	4.05	4.55	3.95
P value							
Effect of fat powder		0.928	0.03	0.04	0.09	0.0001	0.006
Effect of oil		0.65	0.007	0.0001	0.0001	0.0001	0.0001
Effect of pellet binder		0.001	0.0001	0.0001	0.0001	0.001	0.0001
Fat powder with oil		0.04	0.007	0.50	0.01	0.012	0.002
Fat powder with binder pellet		0.005	0.16	0.02	0.07	0.0001	0.0001
Oil with binder pellets		0.005	0.17	0.01	0.0001	0.0001	0.0001
Fat powder with oil and a type of pellet binder		0.03	0.0001	0.40	0.001	0.0004	0.006
Treatment 1: 7 kg oil + 5 kg fat powder; Treatment 2: 7 kg oil + 10 kg fat powder; Treatment 3: 14 kg oil + 5 kg fat powder; Treatment 4: 14 kg oil + 10 kg fat powder; Treatment 5: 1% bentonite + 7 kg oil + 5 kg fat powder; Treatment 6: 1% bentonite + 7 kg oil + 10 kg fat powder; Treatment 7: 1% bentonite + 14 kg oil + 5 kg fat powder; Treatment 8: 1% bentonite + 14 kg oil + 10 kg fat powder; Treatment 9: 1% gum Tragacanth + 7 kg oil + 5 kg fat powder; Treatment 10: 1% gum Tragacanth + 7 kg oil + 10 kg fat powder; Treatment 11: 1% gum Tragacanth + 14 kg oil + 5 kg fat powder; Treatment 12: 1% gum Tragacanth + 14 kg oil + 10 kg fat powder; Treatment 13: 1% both binders + 7 kg oil + 5 kg fat powder; Treatment 14: 1% both binders + 7 kg oil + 10 kg fat powder; Treatment 15: 1% both binders + 14 kg oil + 5 kg fat powder; Treatment 16: 1% both binders + 14 kg oil + 10 kg fat powder. In each column, values not sharing similar letters are significantly different ($P < 0.05$).							

The influence of different levels of fat powder, oil, and pellet binders on the PDI during the finishing period is documented in Table 4. The current results indicated that the effects of fat powder, oil, and pellet binder significantly influenced the PDI in the finisher period on all experimental days ($P < 0.05$). The interaction effects of fat powder and oil significantly affected the PDI in the finisher period on all days except days 15 and 45. The interaction effects of fat powder with the pellet binder and oil with the pellet binder were significant only on day 0 and day 60, respectively, leading to an increase in the pellet durability index on those days ($P < 0.05$). The three-way interaction between fat powder with oil and pellet binder demonstrated statistically significant effects on days 0, 30, and 75 ($P < 0.05$). However, it had no noticeable effect on the PDI on days 15, 45, and 60 ($P > 0.05$). A significant difference was observed between the levels of 5 and 10 kg fat powder and the levels of 7 and 14 kg oil across all treatments. The 5 kg level of fat powder from days 0 to 45 and the 7 kg level of oil on days 0, 15, 30, 45, and 75 yielded the highest PDI. Among the pellet binders, the control treatment exhibited the lowest mean value.

The results from the analysis of the effects of fat powder and oil levels, along with pellet binders, on the physical quality index of pellets during the finisher period are reported in Table 5. The current findings indicated that oil levels did not affect the physical quality of finisher pellets ($P>0.05$). The levels of fat powder and the effects of pellet binders significantly influenced the physical quality of pellets on all experimental days except on days 0 and 75 ($P < 0.05$). The gum Tragacanth binder on days 0, 45, 60, and 75, as well as the bentonite binder on days 15 and 30, yielded the highest mean values (4.56 and 4.531, respectively), thereby enhancing the physical quality of the pellets. The 5 kg level of fat powder on day 30 indicated a lower mean value (4.12) in comparison to the 10 kg level (4.22). The Treatment 2 on days 45, 60, and 75, the Treatment 7 on days 15 and 30 (with mean values of 4.15 and 4.22, respectively), the Treatment 14 on day 45 (with a mean value of 5), and the Treatment 15 on day 0 (with a mean value of 4.85) demonstrated the highest physical pellet quality.

Table 4 - The effects of fat and oil powder levels, along with pellet binders, on the pellet durability index in the finisher period.

Fat levels		Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
Fat levels	5 10	84.25 ^a	84.42 ^a	84.45 ^a	83.08 ^a	81.46 ^a	81.08 ^a
SEM		81.19 ^b	82.29 ^b	80.22 ^b	80.06 ^b	87.15 ^b	77.66 ^b
Oli levels	7 14	0.052 84.7 ^a	1.8 84.48 ^a	0.67 83.19 ^a	0.822 83.06 ^a	0.71 81.22 ^a	0.64 80.94 ^a
SEM		81.49 ^b	82.22 ^b	80.51 ^b	80.07 ^b	87.29 ^b	77.80 ^b
Pellet binder	Control (no pellet binder)	0.528	1.803	0.677	0.822	0.718	0.648
Pellet binder	Bentonite	80.43 ^a	81.12 ^a	77.66 ^a	76.58 ^a	75.52 ^a	74.86 ^a
Pellet binder	Gum Tragacanth	83.20 ^b	84.01 ^b	81.97 ^b	80.42 ^b	78.65 ^b	78.63 ^b
SEM	Both	83.70 ^b	84.25 ^a	85.64 ^c	85.46 ^c	84.02 ^c	83.81 ^c
SEM		85.07 ^c	84.008 ^b	82.30 ^b	82.30 ^d	81.03 ^d	80.25 ^d
Fat levels	5	0.528	1.803	0.677	0.822	0.715	0.650
Interaction effect of fat powder + oil + pellet binder levels							
Treatment 1		83.72	84.07	80.80	80.15	78.20	77.50
Treatment 2		79.92	79.72	77.82	76.32	75.77	76.40
Treatment 3		80.45	81.40	77.70	77.05	75.80	75.00
Treatment 4		77.62	79.30	74.35	72.80	72.32	70.55
Treatment 5		87.20	86.20	84.95	82.05	80.05	80.00
Treatment 6		83.20	83.70	82.45	80.55	79.70	79.80
Treatment 7		82.70	84.45	82.20	81.05	80.15	80.25
Treatment 8		79.70	81.70	78.30	78.50	74.70	74.50
Treatment 9		85.80	87.20	89.55	90.05	88.02	87.50
Treatment 10		83.85	84.95	83.95	85.55	83.27	83.00
Treatment 11		84.20	83.77	83.67	87.30	84.52	84.25
Treatment 12		81.20	55.81	84.70	83.60	80.27	79.50
Treatment 13		88.95	86.65	85.70	86.05	83.77	83.25
Treatment 14		81.70	82.87	72.45	83.85	82.95	80.50
Treatment 15		84.70	82.12	80.90	80.80	79.30	79.75
Treatment 16		84.77	95.86	80.10	79.87	78.12	77.52
P value							
Effect of fat powder		0.0001	0.002	0.0001	0.001	0.0001	0.0002
Effect of oil		0.001	0.001	0.001	0.001	0.0001	0.001
Effect of pellet binder		0.0001	0.004	0.0001	0.0001	0.001	0.0001
Fat powder with oil		0.007	0.15	0.03	0.960	0.08	0.006
Fat powder with binder pellet		0.01	0.65	0.800	0.140	0.30	0.520
Oil with binder pellets		0.60	0.60	0.610	0.127	0.04	0.380
Fat powder with oil and a type of pellet binder		0.001	0.30	0.005	0.700	0.10	0.040
Treatment 1: 7 kg oil + 5 kg fat powder; Treatment 2: 7 kg oil + 10 kg fat powder; Treatment 3: 14 kg oil + 5 kg fat powder; Treatment 4: 14 kg oil + 10 kg fat powder; Treatment 5: 1% bentonite + 7 kg oil + 5 kg fat powder; Treatment 6: 1% bentonite + 7 kg oil + 10 kg fat powder; Treatment 7: 1% bentonite + 14 kg oil + 5 kg fat powder; Treatment 8: 1% bentonite + 14 kg oil + 10 kg fat powder; Treatment 9: 1% gum Tragacanth + 7 kg oil + 5 kg fat powder; Treatment 10: 1% gum Tragacanth + 7 kg oil + 10 kg fat powder; Treatment 11: 1% gum Tragacanth + 14 kg oil + 5 kg fat powder; Treatment 12: 1% gum Tragacanth + 14 kg oil + 10 kg fat powder; Treatment 13: 1% both binders + 7 kg oil + 5 kg fat powder; Treatment 14: 1% both binders + 7 kg oil + 10 kg fat powder; Treatment 15: 1% both binders + 14 kg oil + 5 kg fat powder; Treatment 16: 1% both binders + 14 kg oil + 10 kg fat powder. In each column, values not sharing similar letters are significantly different ($P < 0.05$).							

Table 5 - The effects of fat and oil powder levels, along with pellet binders, on the pellet physical quality in the finisher period

Main effects	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
Fat levels	5	4.25 ^a	4.28	4.12	4.44 ^a	4.28 ^a	4.26
	10	4.10 ^b	4.18	4.22	4.20 ^b	4.19 ^b	4.15
SEM		0.008	0.07	0.13	0.006	0.008	0.022
Oli levels	7	4.17	4.17	4.29	4.33	4.20	4.20
	14	4.15	4.21	4.24	4.32	4.21	4.10
SEM		0.092	0.08	0.13	0.062	4.008	0.023
Pellet binder	Control (no Pellet Binder)	4.03 ^a	3.96 ^a	4.24 ^{ab}	4.40 ^{ab}	4.33 ^a	4.331 ^a
	Bentonite	4.05 ^a	4.56 ^b	4.51 ^c	4.32 ^a	4.15 ^b	4.168 ^{ab}
	Gum Tragacanth	4.29 ^b	4.20 ^{ab}	4.39 ^{ba}	4.47 ^b	4.35 ^a	4.343 ^a
	Both	4.51 ^b	4.18 ^{ab}	4.10 ^b	4.11 ^c	4.13 ^b	3.993 ^b
SEM		0.008	0.071	0.12	0.006	0.008	0.91
Interaction effect of fat powder + oil + pellet binder levels							
Treatment 1		4.025	3.225	4.270	4.350	4.050	4.075
Treatment 2		4.100	4.325	4.550	5.000	5.000	4.850
Treatment 3		3.970	4.150	4.000	4.450	4.075	4.000
Treatment 4		4.050	4.160	4.150	3.800	4.200	4.400
Treatment 5		4.350	4.550	4.550	4.170	3.970	3.970
Treatment 6		4.370	4.850	4.670	4.520	4.300	4.850
Treatment 7		4.420	4.700	4.680	4.300	4.200	4.220
Treatment 8		4.020	4.150	4.220	4.300	4.100	4.050
Treatment 9		4.200	4.500	4.380	4.540	4.470	4.470
Treatment 10		4.140	4.370	4.720	5.000	4.470	4.550
Treatment 11		4.850	3.925	4.270	4.400	4.310	4.350
Treatment 12		4.000	4.075	4.200	4.360	4.100	4.000
Treatment 13		4.200	4.325	4.000	4.00	4.002	3.970
Treatment 14		4.570	4.150	4.150	4.100	4.980	3.800
Treatment 15		4.400	4.175	4.170	4.450	4.540	4.420
Treatment 16		4.100	4.075	4.078	3.990	3.980	3.770
P value							
Effect of fat powder		0.003	0.42	0.001	0.0001	0.04	0.14
Effect of oil		0.9	0.5	0.36	0.88	0.228	0.56
Effect of pellet binder		0.0001	0.02	0.0001	0.0001	0.001	0.005
Fat powder with oil		0.01	0.12	0.004	0.0001	0.0001	0.002
Fat powder with binder pellet		0.4	0.18	0.08	0.001	0.001	0.06
Oil with binder pellets		0.17	0.23	0.125	0.013	0.0001	0.0002
Fat powder with oil and a type of pellet binder		0.009	0.21	0.53	0.0005	0.12	0.94

Treatment 1: 7 kg oil + 5 kg fat powder; Treatment 2: 7 kg oil + 10 kg fat powder; Treatment 3: 14 kg oil + 5 kg fat powder; Treatment 4: 14 kg oil + 10 kg fat powder; Treatment 5: 1% bentonite + 7 kg oil + 5 kg fat powder; Treatment 6: 1% bentonite + 7 kg oil + 10 kg fat powder; Treatment 7: 1% bentonite + 14 kg oil + 5 kg fat powder; Treatment 8: 1% bentonite + 14 kg oil + 10 kg fat powder; Treatment 9: 1% gum Tragacanth + 7 kg oil + 5 kg fat powder; Treatment 10: 1% gum Tragacanth + 7 kg oil + 10 kg fat powder; Treatment 11: 1% gum Tragacanth + 14 kg oil + 5 kg fat powder; Treatment 12: 1% gum Tragacanth + 14 kg oil + 10 kg fat powder; Treatment 13: 1% both binders + 7 kg oil + 5 kg fat powder; Treatment 14: 1% both binders + 7 kg oil + 10 kg fat powder; Treatment 15: 1% both binders + 14 kg oil + 5 kg fat powder; Treatment 16: 1% both binders + 14 kg oil + 10 kg fat powder. In each column, values not sharing similar letters are significantly different (P < 0.05).

DISCUSSION

Based on the current results, it was found that utilizing two levels of fat powder (5 and 10 kg), two levels of oil (7 and 14 kg), and two types of pellet binders (Bentonite, gum Tragacanth, and both) significantly affected the PDI in both starter and finisher periods of poultry feed. In both periods, lower levels of fat powder (5 kg) and oil (7 kg) resulted in higher mean PDI and physical pellet quality. In all treatments with the highest concentrations of added fat powder and oil, such as Treatments 4, 8, 12, and 16, lower mean values of PDI and physical quality were observed in both the starter and finisher periods. During the starter phase, the bentonite pellet binder demonstrated superior performance, whereas in the final phase, the gum Tragacanth pellet binder exhibited the most advantageous outcomes.

It was found that adding vegetable oils through the mixer negatively affected the physical quality of feed pellets (-40; Thomas et al., 2001). Increasing soybean oil levels from 1.5% to 3% resulted in a 21% reduction in PDI (Pope, 2016). Similarly, increasing fat content from 1% to 18.2% in the mixer reduced PDI by up to 13% (Loar et al., 2014), which may be attributed to the reduction of force within the die holes caused by oil, resulting in lower quality pellets. Additionally, high oil levels form a coating on feed particles that prevents steam from penetrating and stops starch from gelatinizing (Lowe, 2005). The present findings are entirely consistent with the previous findings, as both conclusively demonstrated that increasing the fat or oil levels in feed formulations significantly reduces PDI, an adverse effect that persists even when using specialized binders

Based on the present study, fat powder demonstrated a more advantageous effect on pellet quality in comparison to oil. This observation aligns with the findings of Zimonja et al. (2007), who indicated that different types of fat exert differing impacts on pellet quality. Additionally, it was found that adding 0.5% of calcium lignosulfonate to treatments containing 3% soybean oil improved pellet quality (Abadi et al., 2019). Evaluating two types of fat, including soybean oil and saturated fat powder containing 65% palmitic acid, at levels of 2.5% and 5% on the PDI of broiler diets revealed that adding fat powder increased starch gelatinization and enhanced PDI outcomes compared to soybean oil. It has been demonstrated that soybean oil decreases starch granule gelatinization because calcium fat powder has a higher melting point than soybean oil, which allows superior steam penetration into feed components (Zimonja et al., 2007). The higher glycerol concentration in fat powder (approximately 10%), which acts as an emulsifier or surfactant, may improve PDI, consistent with the findings of Tavernari et al. (2013).

Based on the present findings, adding pellet binders to the treatments increased the PDI and physical quality. However, no significant difference was observed between the types of pellet binders across the tested treatments on different days. Although each treatment employed several methods on different experimental days, these differences were not statistically significant. Similar to the current findings, Abadi et al. (2019) have demonstrated that treatments containing 3% calcium fat powder without a pellet binder exhibited a reduction in PDI, aligning with the outcomes of the present investigation study. Additionally, adding 1.5% calcium fat powder along with 2% bentonite improved pellet quality indices (Abadi et al., 2019). Corey et al. (2014) indicated that adding fat along with calcium lignosulfonate as a pellet binder resulted in an increase in post-extrusion pellet temperature and an enhancement in pellet quality. The differential responses of each pellet binder to different ranges of oil and fat powder suggested that the selection of pellet binders should be based on the specific types and concentrations of mixed fats (Corey et al., 2014).

CONCLUSION

The utilization of 5 and 10 kg of fat powder and 7 and 14 kg of oil, along with three varieties of pellet binders, including bentonite, gum Tragacanth, and a combination of both, exerted a significant influence on the pellet durability index (PDI) and physical properties quality. Throughout all starter and finisher periods, the treatment devoid of any pellet binder demonstrated the lowest PDI and physical quality, highlighting the influence of pellet binders in treatments that include fat powder and oil. Treatments containing lower levels of fat powder and oil yielded higher-quality pellets. Furthermore, an increase in the quantity of fat powder and oil in the feed typically resulted in a decrease in the PDI and physical quality. During the starter phase, the bentonite pellet binder demonstrated optimal performance, whereas in the final phase, the gum Tragacanth pellet binder produced the most promising outcomes.

DECLARATION

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Acknowledgments

The authors extend the deepest gratitude to all those who assisted us in conducting this research, especially the Gohar daneh Shargh factory and Sepehri Moghadam Noavaraneh Sanat for their invaluable support and cooperation.

Authors' contribution

M. Emadi, H. Sepehri and M. Salehan contributed to the research, data analysis, and manuscript writing.

Conceptualization and data curation: M. Emadi, H. Sepehri and M. Salehan

Formal analysis, funding acquisition, investigation, project administration, software, writing original draft, review & editing: M. Emadi

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Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

The authors declare no competing interests in this research and publication.

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INFLUENCE OF DIETARY SUPPLEMENTED GLUTAMATE ON GROWTH PERFORMANCE, HAEMATOLOGICAL AND SERUM INDICES OF BROILER CHICKENS IN HOT TROPICAL ENVIRONMENT

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➤ Supporting Information



ABSTRACT: This study investigated the impact of glutamate on growth, haematological and serum indices of broiler chickens in hot tropical environments (> 30°C). One-hundred-and-sixty-day old Arbor acre male broiler chicks were randomly allotted into four dietary treatment groups of four replicates having 10 chicks per replicate in a completely randomized design. Dietary treatments groups contain different inclusion levels of glutamate (basal diet + 0%, 0.3%, 0.6% and 0.9% glutamate (G), in an open sided pen). The experiment lasted for seven weeks during which data were collected on growth, haematological and serum indices. Result showed birds fed diet containing 0.9% G at the starter phase had ($P < 0.05$) increased feed intake compared to other treatment groups. Inclusion of 0.6% G at the finisher phase ($P < 0.05$) improved final weight and weight gain in birds compared to 0.3% G group. Best feed conversion ratio ($P < 0.05$) was obtained from birds fed diet containing 0.6% G compared to birds on 0.3% and 0.9% inclusion. Inclusion of 0.6% G at the starter phase ($P < 0.05$) improved total protein. Increased mean corpuscular haemoglobin ($P < 0.05$) was obtained from birds on 0.6% G compared to lower mean values obtained from birds on 0.3% G. The study concluded that inclusion of glutamate in the diet of broiler chickens at 0.6% improved final weight, weight gain and feed conversion ratio without causing any adverse effect on blood indices of broiler chickens as glutamate increased serum protein and mean corpuscular haemoglobin of broiler chickens.

Keywords: Blood profile, Broiler chickens, Essential nutrients, Glutamic acid, Growth response.

INTRODUCTION

Feed additives used in poultry industry that provide essential nutrients, increase palatability of the feed, improve growth performance and ensure efficient feed utilization. Broiler chickens with high genetic potentials need to maintain good immune functions in order to achieve their genetic potentials. The use of suitable and quality feed additives is important in the poultry industry in order to enhance bird genetic potentials, maintain immune functions, mitigate effect of stress and ensure balance in gut environment (Adedokun and Olojede 2019).

With increasing industry standards and consumer awareness as well as demand for healthy food products of animal origin, there is an increased awareness for alternatives than the conventional feed additives used in animal feed products (Pandey et al., 2019). Higher feed efficiency and profitability are essential in broiler production. Heat-stressed broilers have higher glutamate requirement, hence supplementing with glutamate or its decarboxylated derivatives (γ -GABA) can enhance the intestinal morphology and survival rate in heat-stress conditions (Porto et al., 2015; Zhong et al., 2020). This has necessitated the search for additional feed additives that can enhance growth for livestock, reduce impact of different stressors and protect cells against oxidative damage in tropical environment. Monosodium glutamate, according to (Young and Ajami, 2000), is a feed additive that can improve growth rate and enhance immune system in broiler chickens. Glutamate functions as a constituent of proteins, a substrate in the synthesis of amino acids and a precursor to several nonessential amino acids which helps the metabolism. Monosodium glutamate is the sodium salt of glutamic acid and the main component of many proteins (Tawfik and AlBadr, 2012).

Inclusion of glutamate in the feed as feed additive was reported to improve performance by improving the quality of small intestine for better nutrient utilization and enhancing immune system function (Maslami et al., 2019). Glutamate in temperate environment has proven to mitigate heat stress and enhance oxidative defenses by protecting cells from oxidative damage which in turn enhance growth performance (Olubodun et al., 2015). Broiler production in tropics has been characterized by different stressors which restrict growth, result in economic losses and comprise immune status of the birds.

Therefore, there is need to investigate the use of glutamate in tropical environment where different stressors prevent broilers from achieving their genetic potentials. Thus, this study aims to evaluate the influence of glutamate on growth performance, haematological and serum indices of broiler chickens in tropical environment.

MATERIALS AND METHODS

The experiment was carried out at the poultry unit of the teaching and research Farms, Federal University of Agriculture, Abeokuta, Ogun State. It lies on Latitude 7 10' N and Longitude 3 2'E, it is 76 metres above sea level and located in the tropical rainforest vegetation zone with an average temperature of 34.7C and relative humidity of 82% (Google-Earth, 2023).

Source of test Ingredients

Glutamate was sourced from a market in Abeokuta metropolis Ogun State Nigeria. The brand name of the Glutamate was VEDAN. The monosodium glutamate was kept in an air-tight container before usage.

Experimental animals and management

A total number of 160 day-old Arbor acre broiler chicks were purchased from a reputable hatchery in Ibadan, Oyo State. Prior to the arrival of the chicks, the pens were cleaned and disinfected thoroughly with a disinfectant. The chicks were assigned to four dietary treatment groups of four replicates each having ten chicks per replicate in a completely randomized design. Dietary inclusion of the test ingredient (glutamate) commenced at the beginning of the experiment. Routine vaccination and medication were administered to the birds according to routine procedures while, feed and water were provided *ad libitum*

Experimental diet and design

Birds were fed with Commercial starter mash between day one to 28 days. The starter diet contains (2900 kcal/kg Metabolizable Energy, 23% crude protein while, the finisher diet was given from 29 days to the end of the experiment (2600 kcal/kg Metabolizable Energy, 20% crude protein. Both diets contain different inclusion levels of monosodium glutamate at (0%, 0.3%, 0.6% and 0.9%). The treatment groups were arranged thus, Treatment 1: Control diet without glutamate, treatment 2: Control diet + 0.3% Glutamate, treatment 3: Control diet + 0.6% Glutamate, treatment 4: Control diet + 0.9% Glutamate. The experiment lasted for a period of seven weeks.

Feed Intake

Feed intake was recorded weekly, the leftover of the feed was subtracted from total feed given to the birds and expressed as Feed intake (kg) = Total feed given (kg) – Feed leftover (kg)

$$\text{Average feed intake} = \frac{\text{Total feed intake}}{\text{Number of birds in the replicate}}$$

Weight gain

Birds were weighed on weekly basis throughout the experimental period. The body weight gain was calculated by subtracting initial body weight from the final body weight. Weight gain = Final body weight – Initial body weight.

Feed conversion ratio

This was calculated by the feed intake divided by weight gain.

$$\text{FCR} = \frac{\text{Feed intake} \left(\frac{\text{g}}{\text{day}} \right)}{\text{Weight gain} \left(\frac{\text{g}}{\text{day}} \right)}$$

Water intake

Water intake was recorded daily as Water intake = Total volume of water given (ml) – Volume of water left (ml).

Mortality

Mortality was recorded as number of dead birds divided by the number of birds stocked and expressed in percentage.

$$\text{Mortality (\%)} = \frac{\text{Total number of dead birds}}{\text{Total number of birds stocked}} \times 100$$

Blood collection

Blood samples were collected randomly from two birds across treatments groups making a total of 32 samples. Blood samples were collected on the 28th and 49th day of the experiment for haematological and serum biochemical

indices. Blood was collected through the brachial vein with a disposable syringe. 5 ml of blood was collected from each bird, 3ml was dispensed into well labeled sterile anticoagulant bottles for hematological indices while, 2ml of the blood was dispensed into well labeled plain bottle for serum biochemical indices.

Determination of haematological indices

The Packed cell Volume and Haemoglobin were determined using microhematocrit and cyanmethemoglobin methods respectively as outlined by Jain (1986). Red blood cells count was determined using haemocytometry method has outlined by Jain (1986). White blood cell was determined using Neubauer counting chamber according to Jain (1986). The Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration were calculated using standard formulae (Samour 2015).

Statistical analysis

All data collected were subjected to one-way Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using (SAS, 2012). Significant ($P < 0.05$) different means were separated using Duncan multiple range test of the same statistical package. The statistical model was: $Y_{ij} = \mu + T_i + \sum ij$

Where; Y_{ij} = Observed mean value; μ = Population mean; T_i = Effect of glutamate levels; $\sum ij$ = Random residual error.

RESULTS

Performance of broiler chickens fed diet containing glutamate at starter phase is shown in table 1. Inclusion of glutamate in the diet of broiler birds had no significant ($P > 0.05$) effect on all performance parameters measured except total feed intake and daily feed intake that was significantly ($P < 0.05$) influenced by the inclusion of glutamate in the diet. Significantly ($P < 0.05$) higher feed intake and daily feed intake was observed in birds fed diet containing 0.9% glutamate compared to lowest mean value recorded for the control groups. Numerically higher final weight and weight gain were observed in birds fed diet containing 0.3% glutamate than mean values obtained from birds across the dietary treatment groups. Performance of broiler chickens fed diet containing monosodium glutamate at finisher phase is shown in table 2. Inclusion of glutamate in diet of broiler chickens significantly ($P < 0.05$) influenced final weight, weight gain and feed conversion ratio. While, all other parameters measured are not significantly ($P > 0.05$) influenced. Highest final weight and weight gain was observed from groups 0.6% glutamate compared to lowest final weight and weight gain observed from groups fed 0.3% glutamate. Numerically higher feed intake was observed in groups fed 0.90% glutamate than lowest feed intake obtained in groups fed 0.3% glutamate. Best feed conversion ratio was observed in birds fed diet containing 0.6% glutamate than poorer and higher feed conversion obtained from birds fed diet containing 0.3% and 0.9%. The effect of glutamate on the hematological parameters of broiler chickens at the starter phase is shown in table 3. There were no significant ($P > 0.05$) differences obtained on all the parameters measured except eosinophil and lymphocyte. Significantly higher ($P < 0.05$) eosinophils and lymphocyte count was observed from groups fed diet containing 0.6% monosodium glutamate compared to significantly ($P < 0.05$) lowered eosinophils and lymphocytes count observed for the control groups. The effect of glutamate on serum biochemistry indices of broiler chickens at the starter phase is shown in table 4. There were no significant ($P > 0.05$) differences obtained on all the parameters measured except for total protein. Significantly ($P < 0.05$) higher total protein was observed in groups fed 0.6% monosodium glutamate than lowered mean values observed in groups fed 0.3% and 0.9% monosodium glutamate. The effect of glutamate on haematological parameters of broiler chickens at finisher phase is shown in Table 5. Significant differences ($P > 0.05$) were observed in all the parameters measured except for mean corpuscular haemoglobin that was significantly influenced by the inclusion of glutamate. Significantly ($P < 0.05$) higher mean corpuscular haemoglobin was observed in groups fed 0.6% compared to lowered mean values obtained from birds fed 0.3% glutamate. The effect of glutamate on serum biochemistry indices of broiler chickens at finisher phase is shown in table 6. There were no significant differences ($P > 0.05$) observed in all parameters measured except for Aspartate aminotransferase. Significantly ($P < 0.05$) higher aspartate aminotransferase was observed in the control groups compared to significantly lowered mean values observed from groups fed 0.3% and 0.6%, respectively.

Table 1 - Effect of glutamate on performance of broiler chickens at starter phase.

Parameters	Inclusion level				SEM	P-value
	0%	0.3%	0.6%	0.9%		
Initial weight (g)	45.55	44.45	45.60	48.27	0.79	0.40
Final weight (g)	1096.11	1172.50	1145.56	1155.83	20.63	0.64
Weight gain (g)	1050.56	1128.05	1099.56	1107.56	20.22	0.62
Total feed intake/bird (g)	1945.28 ^b	2025.50 ^{ab}	2078.63 ^{ab}	2195.63 ^a	36.54	0.04
Daily feed intake/bird/day (g)	69.47 ^b	73.30 ^{ab}	74.24 ^{ab}	78.42 ^a	1.31	0.01
Feed conversion ratio	1.87	1.82	1.89	1.98	0.04	0.21
Total water intake (l)	42.71	43.54	4.61	42.53	42.82	0.08
Water intake/bird (l)	4.38	4.35	4.61	4.63	63.96	0.16
Mortality rate	0.25	0	0.25	0.75	0.15	0.38

^{ab} Means values in the same row with different superscript differ significantly ($P < 0.05$); SEM: Standard Error of Mean

Table 2 - Effect of glutamate on performance of broiler chickens at finisher phase.

Parameters	Inclusion level	0%	0.3%	0.6%	0.9%	SEM	P-value
Initial weight (g)		1096.11	1172.50	1145.56	1155.83	20.63	0.64
Final weight (g)		2126.94 ^{ab}	2105.00 ^b	2286.11 ^a	2162.78 ^{ab}	41.46	0.04
Weight gain (g)		1030.84 ^{ab}	932.5 ^b	1140.55 ^a	1006.95 ^{ab}	30.44	0.01
Total feed intake/bird (g)		2995.50	2862.50	2998.33	3085.31	44.57	0.39
Daily feed intake/bird/day (g)		149.77	143.12	149.92	154.27	2.23	0.02
Feed conversion ratio		2.91 ^{ab}	3.06 ^a	2.65 ^b	3.06 ^a	0.07	0.02
Total water intake (l)		60.18	59.72	60.96	60.96	444.81	0.27
Water intake/bird (l)		6.18	5.97	6.26	6.36	83.47	0.44
Mortality rate		0	0	0	0	0	0

^{ab} Means values in the same row with different superscript differ significantly (P<0.05); SEM: Standard Error of Mean

Table 3 - Effect of Glutamate on the hematological parameters of broiler chickens at starter phase.

Parameters	Treatment	Control	0.3% G	0.6% G	0.9% G	SEM	P-value
Packed cell volume (%)		27.50	27.00	27.50	23.00	1.01	0.23
Haemoglobin (g/dl)		9.20	9.00	9.20	7.65	0.37	0.21
Red blood cells (x10 ⁹ /L)		3.41	4.28	4.11	2.42	0.36	0.11
White blood cells (x10 ⁹ /L)		14.76	24.00	16.25	0.02	54.98	0.22
Lymphocytes (%)		84.50	65.00	82.50	100.00	6.45	0.11
Neutrophil		29.50	30.50	30.50	32.00	0.46	0.11
Monocytes (%)		1.00	2.50	1.50	1.50	0.26	0.08
Eosinophil (%)		1.00 ^b	2.00 ^{ab}	2.50 ^a	2.00 ^{ab}	0.23	0.04
Lymphocytes (%)		2.83 ^b	3.56 ^{ab}	4.07 ^a	3.80 ^{ab}	0.20	0.03
Mean corpuscular volume (%)		94.10	93.90	92.90	92.15	0.44	0.19
Mean corpuscular hemoglobin (g/l)		29.07	21.01	27.74	32.95	0.24	0.14

^{abc}: Means values in the same row with different superscript differ significantly different at P<0.05.

Table 4 - Effect of glutamate on the serum biochemistry of broiler chickens at starter phase.

Parameters	Treatment	Control	0.3% G	0.6% G	0.9% G	SEM	P-value
Albumin (g/dl)		1.51	1.36	1.64	1.33	0.31	0.45
Cholesterol (mg/dl)		60.30	76.65	57.16	35.01	10.79	0.24
AST (U/L)		32.52	29.95	30.57	30.24	3.75	0.08
ALT (U/L)		7.54	11.07	12.85	12.05	0.94	0.07
Total protein (g/dl)		2.89 ^{ab}	2.41 ^b	3.88 ^a	2.73 ^b	0.23	0.04
Globulin (g/dl)		1.39	1.06	1.09	1.40	0.13	0.49
Glucose (mg/dl)		58.57	85.57	86.48	88.13	7.59	0.06
Creatinine		0.29	0.50	0.40	0.66	0.87	0.24

^{abc}: Means with different superscript are significantly different at P<0.05. AST:Aspartate aminotransferase; ALT: Alanine aminotransferase

Table 5 - Effect of Glutamate on the hematological parameters of broiler birds at finisher phase.

Parameters	Treatment	Control	0.3% G	0.6% G	0.9% G	SEM	P-value
Packed cell volume (%)		31.50	31.00	30.00	31.00	0.72	0.59
Haemoglobin (g/dl)		10.30	10.00	10.10	10.10	0.20	0.71
Red blood cells (x10 ⁹ /L)		3.13	3.11	3.02	3.12	0.81	0.73
White blood cells (x10 ⁹ /L)		15.77	15.50	15.50	14.02	0.85	0.29
Lymphocytes		65.50	68.00	69.00	66.00	0.74	0.15
Neutrophil		27.00	25.00	25.00	28.00	0.75	0.24
Monocytes (%)		3.50	3.00	1.50	3.00	0.13	0.09
Eosinophil (%)		2.50	3.50	4.00	3.00	0.56	0.48
Basophil		0.50	0	0	0	0.13	0.24
Mean corpuscular haemoglobin (g/l)		33.15 ^{ab}	30.30 ^b	33.78 ^a	32.41 ^{ab}	0.58	0.04

^{abc}: Means with different superscript are significantly different at P<0.05.

Table 6 - Effect of glutamate on the serum biochemistry of broiler chickens at finisher phase

Parameters	Control	0.3% G	0.6% G	0.9% G	SEM	P-value
Albumin (g/dl)	1.42	1.48	1.44	1.38	0.08	0.74
Cholesterol (mg/dl)	38.03	34.42	38.11	30.32	3.69	0.58
AST(U/L)	50.75 ^a	32.10 ^b	33.03 ^b	43.56 ^{ab}	3.36	0.03
ALT (U/L)	2.08	2.24	3.92	7.04	0.96	0.10
Total Protein(g/dl)	2.71	2.54	2.60	2.45	0.06	0.22
Globulin (g/dl)	1.18	1.01	1.17	1.07	0.09	0.65
Glucose (mg/dl)	152.38	132.61	134.44	193.48	0.2	0.42
Creatinine	0.75	0.90	0.71	0.85	0.52	0.31

^{abc}: Means with different superscript are significantly different at $p < 0.05$. AST:Aspartate aminotransferase; ALT: Alanine aminotransferase

DISCUSSION

Higher feed intake observed at the starter phase in groups fed 0.9% glutamate could be attributed to flavor enhancing attributes of glutamate as it has been reported that glutamate have flavoring enhancing attributes which influences feed intake, this result affirms the attributes of glutamate as a food enhancer. This result agrees with the findings of [Khadiga et al. \(2009\)](#) that reported increased feed intake in chicks fed 1% monosodium glutamate. This result is also in tandem with the findings of [Maslami et al. \(2019\)](#) who reported significant difference in feed intake of broiler chickens fed dietary monosodium glutamate inclusion. Research findings by [Gbore et al. \(2016\)](#) also aligns with this studies that increased feed intake in response to an increased level of monosodium glutamate among female rabbits showed that monosodium glutamate is capable of enhancing feed palatability which in turn influences appetite positively and induces weight gain, which has been linked with stimulation of the sensory receptors ([Moore, 2003](#)).

Highest final weight observed at the finisher phase in groups fed 0.6% glutamate might be attributed to better nutrient absorption and utilization at this inclusion level of the additive as a result of improved feed intake, since glutamate has capability of improving intestinal conditions by increasing the villi length and increasing the intestinal integrity ([Reeds et al., 2000](#); [Newsholme et al., 2003](#)). This result corroborates the findings of [Olarotimi and Adu \(2022\)](#) that reported heavier final weight in broiler chickens fed diet containing 0.5% monosodium glutamate. Highest weight gain observed in groups fed 0.6% glutamate might be attributed to better nutrient absorption and improved digestion that improved weight gain as a result of glutamate inclusion as glutamate is a precursor to other non- essential amino acids such as arginine, glutamine and proline ([Blachier et al., 2009](#)). This result corroborates the findings of [Maslami et al. \(2019\)](#) that reported significant difference in the body weight of broiler chickens supplemented monosodium glutamate at 0.6 - 0.8% inclusion. Best feed conversion ratio was observed in groups fed 0.6% glutamate, this might be attributed to better nutrient absorption and utilization as a result of glutamate inclusion because as body weight increases as a result of increased feed nutrient absorption improved feed conversion ratio is achieved. This result agrees with the findings of ([Maslami et al., 2019](#); [Olarotimi and Adu 2022](#)) that reported significant and best feed conversion in broiler chickens fed dietary monosodium glutamate at 0.5 g/kg and between 0.4-0.8% inclusions respectively.

Result observed on packed cell volume, hemoglobin, red blood cell, Neutrophils and mean corpuscular hemoglobin at the starter phase corroborates the findings of [Olarotimi and Adu \(2022\)](#) who reported no significant difference when monosodium glutamate was fed to broiler chickens. Higher eosinophils count at starter phase was observed in group fed diet 0.6% glutamate. This result might be attributed to ability of glutamate to stimulate body eosinophils to protect body cells from parasites, allergies and foreign bacteria within and outside the birds thereby acting as immune booster. This result is contrary to the findings of [Olarotimi and Adu \(2022\)](#) who reported no significant difference in eosinophils of broiler chickens fed monosodium glutamate. Higher lymphocytes count was observed in groups fed 0.6 % glutamate; this result could be attributed to ability of glutamate to stimulate the immune system to prevent against foreign bacteria and organism within the system of the birds. This result is contrary to the findings of [Zanfirescu et al. \(2019\)](#) who reported monosodium glutamate to decrease the level of lymphocytes in the blood without interfering with the basal phagocytes of Neutrophils. Similarly, this result also disagree with the findings of [Olarotimi and Adu \(2022\)](#) that reported no significant difference in the lymphocytes of broiler chickens fed monosodium glutamate. Result observed on serum indices at the starter phase showed significantly higher total protein was observed in groups fed 0.6% glutamate. This might be attributed to inclusion of glutamate acting as precursor for other amino acids and increasing the serum protein content. Similarly, glutamate has been reported to act as a transcriptional promoter and enhancer used for control of gene expression that promoted the ability of ribonucleic acid polymerase to identify the nucleotide at the initiation stage thereby improving protein synthesis. This result is in agreement with the findings of [Azine et al. \(2018\)](#) and [Obochi et al.](#)

(2009) that reported significant difference and increase in the serum protein of broilers and rats respectively when monosodium glutamate was fed to the animals but contrary to the findings of Okediran et al. (2014) that reported decrease in the serum protein of rats supplemented with diet containing monosodium glutamate.

Mean corpuscular hemoglobin was significantly influenced by dietary inclusion of glutamate. Groups fed 0.6% glutamate had higher 33.78g/l corpuscular hemoglobin than groups on 0.3% glutamate. The significant effect observed on mean corpuscular haemoglobin signified enough presence of oxygenated blood within the system of the birds thereby preventing anaemic condition. This result agrees with the findings of Olarotimi and Adu (2022) that reported significant differences in the mean corpuscular volume of broiler chickens fed dietary monosodium glutamate.

Result observed on serum indices at the finisher phase showed AST was significantly influenced by dietary inclusion of glutamate. Higher AST was observed in the control group compared to lower mean values observed from the treatment groups. The significantly lower AST observed from the treatment groups showed that glutamate does not have any negative impact on the liver cells of the birds as the values obtained were within the range for healthy chicken. This result is contrary to the findings of Azine et al. (2018) and Prabakar et al. (2023) that reported no significant difference in the AST of broiler chickens fed diets containing jumbo cube as source of monosodium glutamate and L-glutamate in the diets of broilers chickens respectively.

CONCLUSION

In conclusion, dietary inclusion of glutamate improved growth performance of broiler chickens at the finisher phase with no detrimental effect on haematological, liver and kidney functions in hot tropical environment. Also, glutamate improved total protein, while maintaining higher mean corpuscular haemoglobin in broiler chickens. As a practical suggestion, glutamate could be added up to 0.9% in the diet of broiler chickens.

DECLARATIONS

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Ethical approval

The authors complied with the ARRIVE guidelines and Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee. Guidelines of the Animal Ethical Ethics committee (FUNAAB, 2020) of the Federal University of Agriculture, Abeokuta, was also strictly adhered to ensure the welfare of the birds was not compromised.

Authors' contribution

A.A ADEYEMO: Conception, design and final approval, U. O ABDULAH: Drafting, Field Study and Monitoring, A.J ADEYEMO: Design and Proof reading, A.I IYANDA: Coordination and revising, T.O ADEKOLA: Acquisition of data, E.O ADEKUNLE: statistical analysis. All authors read and approved the final manuscript

Availability of data and materials

The data sheet generated during and /or analysed during this study are not publicly available but are available from the corresponding author based on reasonable request.

Funding

The authors declare that no funds, grants, or other support were received during the project and preparation or publication of this manuscript.

Competing interests

The authors declared they have no competing interest with respect to research, authorship and publication of this article.

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THE ADDITION OF PUMPKIN SEED OIL TO COMMON CARP DIETS AFFECTS SEVERAL PHYSIOLOGICAL AND PRODUCTIVE CHARACTERISTICS

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➤ Supporting Information



ABSTRACT: This study aimed to evaluate the effect of adding different levels of pumpkin seed oil (PSO) (*Cucurbita pepo* L.) on specific growth and immune blood parameters in common carp (*Cyprinus carpio* L.). Six oil concentrations were tested, representing six treatments: PSO at 0% (control), 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%. All treatments were incorporated into a diet containing 29.14% protein. A total of 147 fish were divided into 21 cages, with three replicates per treatment, averaging 32±0.72 g in weight. The fish were fed at 3% of biomass per day, split into two meals; rations were adjusted biweekly according to measured biomass for 82 days. The 2% PSO treatment showed significantly better results ($P<0.05$) across all growth parameters studied, including Final Weight (FW), Weight Gain (WG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Specific Growth Rate (SGR), Food Intake (FI), Metabolic Growth Rate (MGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Efficiency Ratio (PER). Followed by the PSO 1.5% treatment. In blood parameters, PSO 2% also performed best in all measured aspects—Red Blood Cells (RBCs), Hemoglobin (Hp), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)—with 1.5% PSO closely behind. In immunological parameters, 2% PSO increased the value of WBCs, TPS, and IgM, with 1.5% PSO just behind. Blood performance, which assesses overall blood health as a single measure, showed the highest performance in the 2% PSO treatment, significantly surpassing other treatments ($P<0.05$). Overall, the results indicated that adding different levels of PSO to carp diets has positive effects at a rate not exceeding 2%.

Keywords: Common carp, *Cucurbita pepo*, Fatty acids, Fish nutrition, Pumpkin.

INTRODUCTION

Dietary vegetable oils have various effects on the physiological characteristics of fish (Sutuli et al., 2018). These oils are sources of metabolic energy (ATP) and are the most energy-dense nutrients (FAO, 2018). In addition, they provide essential organic acids (Ng and Koh, 2011). The addition of oils must be within proper proportions and tailored to the needs of the fish. Previous studies confirmed that using pumpkin seed oil (PSO) can act as a natural antibiotic, antioxidant, and indicator of normal fish growth, while also improved key feed properties such as taste, digestibility, and binding ability (Mahendra et al., 2019). Adequate supplementation with oils rich in polyunsaturated fatty acids has boosted the immune response of fish (Mesa-Rodriguez et al., 2017). A deficiency of these polyunsaturated fatty acids may lead to health issues in fish, such as altered intestinal morphology (Torrecillas et al., 2017), reduced bacterial resistance (Montero et al., 2010; Ferreira et al., 2015), and lowered immune parameters (Montero et al., 2003; Conde-Sieira, 2018). Increasing the ω 3 PUFA content in the lipid profile of farmed fish is therefore crucial. These modifications also have significant effects on the quality of fish products, which may influence public perception of farmed fish (Sealey and Gatlin, 1999; Hixson, 2014, Mesa-Rodriguez et al., 2017).

Generally, pumpkin plants are propagated by seed and distributed worldwide. Their seeds are considered more durable and less prone to spoilage. These characteristics are reflected in the quality of the oil extracted from their seeds (Yadav et al., 2010). PSO contains the essential fatty acids linoleic acid and oleic acid. Additionally, the predominant fatty acids are stearic acid and palmitic acid, which make up about 98% of the total amount of fatty acids in pumpkin oil (Murkovic et al., 1996; Ardabili et al., 2011). The oil is extracted by cold pressing roasted or unroasted pumpkin seeds (Paris, 1989), with sensory differences in taste, color, and aroma, between the two oil types (Kim et al., 2012). Many previous studies analyzing the components of PSOs extracted from roasted seeds have shown that these oils are rich in monounsaturated and polyunsaturated fatty acids, minerals, vitamins, pigments, phytosterols, phenolic compounds, and pyrazine derivatives (Aktaş et al., 2018). Recently, much attention has been given to the essential fatty acid requirements of fish. It has also been proven that they need unsaturated fatty acids for normal growth. Fish fed diets lacking certain acids tend to have slower growth and show signs of essential fatty acid deficiency, indicating changes in cell membrane

RESEARCH ARTICLE
 PII: S222877012500043-15
 Received: August 07, 2025
 Revised: November 28, 2025
 Accepted: November 28, 2025

structure (Mesa-Rodriguez et al., 2017). Therefore, essential fatty acids must be included in their diet. Based on the background discussed above, the present study aims to examine the effect of adding different levels of PSO, which contains various types of fatty acids, on certain productive and immune traits. It also seeks to identify the optimal inclusion level in the feed and its effects on common carp *Cyprinus carpio* L.

MATERIALS AND METHODS

Experiment location and fish

The study was conducted at the First Agricultural Research and Experiments Station, affiliated with the College of Agriculture at Al-Muthanna University, located north of Samawah city, near the end of the Al-Atshan River, one of the branches of the Euphrates River, approximately 4 km before it joins the Al-Sabil branch. Water from this river was used as a water source for the experiment. Common carp fingerlings were obtained from a local hatchery; 147 fingerlings with an average weight of 32 ± 0.72 g was transported in a specialized fish transport vehicle. The fish were placed in a 3% saline solution under observation and then placed in a specially prepared tank. After resting for 24 hours, they were distributed into the experimental cages at a rate of seven fish per cage as experimental units. The fish used in the experiment were acclimated for two weeks to the experimental conditions.

Experimental diets

During the experiment, the fish received a diet made up of several ingredients listed in (Table 1). The diet was given twice daily, in the morning and evening, at 3% of biomass per day, split into two meals; rations were adjusted biweekly according to the measured biomass. The ingredients were sourced locally, mixed thoroughly, and then pelletized using a heat press (Table 1). Pumpkin seed oil (PSO) was added at six levels: 0.5%, 1%, 1.5%, 2.0%, 2.5%, and 3.0%, along with a control group. Each treatment was replicated three times. The experiment used 21 cylindrical plastic cages (three cages for each treatment), each measuring 50 cm in diameter and 65 cm deep.

Table 1 - Ingredients of the experimental diet.

Ingredients	Percentage in the diet (%)
Soybean meal	40
AL-Wafi protein concentration*	20
Wheat bran	15
Corn	15
Barley	5
Wheat flour	3
Premix	1
Oil	1
Total	100
Chemical composition of diet	
Component	(%) Result
Moisture	5.03
Dry matter	94.97
Crude protein	29.14
Crude fat	1.74
Crude fiber	4.48
Nitrogen-free extract (NEF)	51.1
Ash	8.51
Gross energy (Kcal g ⁻¹) **	396.726
Digestible energy (Kcal g ⁻¹) ***	297.5445
Metabolizable energy (Kcal g ⁻¹) ****	323.7654
Protein: Calorie ratio ****	979.34

*locally manufactured protein **Gross energy (Kcal g⁻¹) was calculated according to NRC (1993) by using factors 5.65, 9.45, and 4.22 Kcal g⁻¹ of protein, lipid, and carbohydrate, respectively. *** Digestible energy (Kcal g⁻¹) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to NRC (1993). **** Metabolizable energy (Kcal g⁻¹) was calculated using a value of 4.5 Kcal g⁻¹ proteins, 8.51 Kcal g⁻¹ fat, and 3.48 Kcal g⁻¹ carbohydrates according to Hephher et al. (1983). **** Protein: Calorie ratio it was calculated using by below equation: $P: E = \text{crude protein} \times 10000 / \text{digestible energy}$, according to Jauncey and Ross (1982).

Studied parameters

Weight gain (WG) = FW-IW (g); Daily growth rate (DGR) = $\frac{FW-IW}{\Delta T}$; Relative Growth Rate (RGR) = $\frac{FW-IW}{IW}$; Specific growth rate (SGR) = $\frac{\ln(FW)-\ln(IW)}{\Delta T}$; Metabolic growth Rate (MGR) = $\frac{((WG/((WI/1000)^{0.8}+(WF/1000)^{0.8}))^2)\Delta T}{(Dabrowski et al., 1986)}$; Feed Conversion Ratio (FCR) = (Food intake (g))/WG; Feed conversion efficiency (FCE) = $\frac{WG}{\text{food intake (g)}} \times 1000$; Protein Efficiency Ratio (PER) = $\frac{WG}{\text{protein intake}}$; Blood Performance (BP) = $\ln(Hb) + \ln(Ht) + \ln(RBC) + \ln(WBC) + \ln(TP)$ (Esmaeili, 2021). Where FW is the final body weight, IW is the initial body

weight, ΔT is the period time, Hb is the hemoglobin, RBC is the red blood cells, WBC is the white blood cells, and TP is the total protein.

Chemical analysis

The PSO sample was analyzed in the Iraqi Ministry of Science and Technology laboratories. The chemical analysis results showed that the oil contains the fatty acids shown in Table 2.

Table 2 - Fatty acid content of local pumpkin seed oil (PSO) (mean \pm standard error)

Fatty acid	Content (%)
Gadoleic acid	1.04 \pm 0.21
Linolenic acid	0.88 \pm 0.24
Palmitoleic acid	1.38 \pm 0.11
Linoleic acid	38.74 \pm 0.52
Oleic acid	37.33 \pm 0.41
Stearic acid	7.95 \pm 0.57
Palmitic acid	12.68 \pm 0.32

Statistical analysis

The data were presented as mean \pm SD. The results were subjected to a one-way analysis of variance (ANOVA) to determine the impact of PSO inclusion on fish performance. IBM SPSS Statistics version 26 (IBM Corp.) was used to analyze the data. We looked at differences among means at the significance level ($P < 0.05$) using the least significant difference (LSD) multiple range test.

RESULTS

Environmental water measurements

The temperature ranged from 16.5 °C at the start of the experiment to 30 °C at the end. The pH was between 7 and 8.1. The water's salinity level varied from 4.981 to 6.730 g L⁻¹, due to water scarcity and the suspension of water releases into the river as a result of Iraq's ongoing water shortage. The amount of dissolved oxygen (DO) in the water ranged from 7.8 mg L⁻¹ at the beginning to 7.2 mg L⁻¹ at the end of the experiment.

Growth criteria

The current results in Table 3 show the most important growth parameters studied, which are Final Weight (FW), Weight Gain (WG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Specific Growth Rate (SGR), Food Intake (FI), Metabolic Growth Rate (MGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Efficiency Ratio (PER). The results revealed significant differences among all treatments, with treatment T4 performing the best in all parameters, followed by treatment T3, which did not differ significantly ($P \geq 0.05$) from T4 in feed intake (FI). Then, treatments T2 and T5, respectively, showed no significant difference from each other in most of the parameters measured. Meanwhile, treatment T1 had the lowest values ($P \geq 0.05$) and did not differ significantly from the control group. All treatments significantly outperformed the control ($P < 0.05$), which showed the lowest values across all parameters.

Hematological parameters

The current results, as shown in Table 4, indicated that T4 was significantly different ($P < 0.05$) in all hematological parameters studied, namely: MCV, PCV, RBCs, Hb, MCH, and MCHC, followed in order by T3. T2 and T5 did not differ significantly from one another in any measured parameter. Likewise, treatments T1 and T6 did not differ significantly from each other ($P > 0.05$). Although all treatments showed significant variations, the control group exhibited the lowest values among all treatments.

Immunological parameters

Treatment 4 demonstrated a significant effect ($P < 0.05$) on all immune parameters, as shown in Table 5, where IgM, STP, and WBC were significantly affected by T3 across all studied parameters. Treatment 2 and T5 did not differ significantly from each other. Treatment 1 and T6 did not differ significantly ($P > 0.05$) in STP, but they showed significant differences in WBC and IgM.

Blood performance

As shown in Table 5, there were significant differences in blood performance among the treatments. The best blood performance was observed in T4 (13.19 \pm 0.08), followed ($P < 0.05$) by T3, which showed no significant difference ($P > 0.05$) from T4. The remaining treatments varied considerably from each other with respect to this parameter.

Table 3 - Some of the studied growth parameters (mean \pm standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	IW (g)	FW (g)	WG (g)	DGR (g day ⁻¹)	RGR (%)	SGR (% day ⁻¹)	FI (g Fish ⁻¹)	MGR (g Kg ⁻¹ day ⁻¹)	FCR	FCE	PER (%)
Control	32.87 \pm 0.08	138.46 \pm 0.33 ^e	105.59 \pm 0.30 ^e	1.27 \pm 0.00 ^e	334.44 \pm 1.08 ^f	1.75 \pm 0.00 ^f	248.21 \pm 0.18 ^f	9.43 \pm 0.01 ^f	2.33 \pm 0.01 ^a	42.94 \pm 0.09 ^e	1.48 \pm 0.00 ^e
T1	32.00 \pm 0.09	173.25 \pm 1.82 ^d	141.26 \pm 1.74 ^d	1.68 \pm 0.02 ^d	441.46 \pm 4.19 ^d	2.01 \pm 0.01 ^d	301.12 \pm 0.46 ^d	10.86 \pm 0.06 ^d	2.13 \pm 0.03 ^b	46.91 \pm 0.60 ^d	1.61 \pm 0.02 ^d
T2	32.00 \pm 0.06	185.61 \pm 0.31 ^c	153.61 \pm 0.30 ^c	1.83 \pm 0.00 ^c	480.03 \pm 1.22 ^c	2.09 \pm 0.00 ^c	321.53 \pm 0.55 ^b	11.30 \pm 0.01 ^c	2.09 \pm 0.01 ^c	47.78 \pm 0.14 ^c	1.64 \pm 0.01 ^c
T3	32.12 \pm 0.08	194.23 \pm 0.57 ^b	162.11 \pm 0.49 ^b	1.93 \pm 0.01 ^b	504.68 \pm 0.82 ^b	2.14 \pm 0.00 ^b	330.40 \pm 0.57 ^a	11.58 \pm 0.01 ^b	2.04 \pm 0.00 ^d	49.06 \pm 0.13 ^b	1.68 \pm 0.00 ^b
T4	32.28 \pm 0.16	214.81 \pm 0.54 ^a	182.53 \pm 0.59 ^a	2.17 \pm 0.01 ^a	565.60 \pm 3.95 ^a	2.26 \pm 0.01 ^a	331.53 \pm 0.42 ^a	12.20 \pm 0.03 ^a	1.81 \pm 0.01 ^e	55.06 \pm 0.23 ^a	1.89 \pm 0.01 ^a
T5	32.11 \pm 0.08	185.08 \pm 0.81 ^c	152.98 \pm 0.75 ^c	1.82 \pm 0.01 ^c	476.48 \pm 1.72 ^c	2.09 \pm 0.00 ^c	315.23 \pm 0.90 ^c	11.27 \pm 0.02 ^c	2.06 \pm 0.01 ^c ^d	48.53 \pm 0.14 ^b ^c	1.67 \pm 0.00 ^b ^c
T6	32.36 \pm 0.11	170.89 \pm 1.50 ^d	138.53 \pm 1.54 ^d	1.65 \pm 0.02 ^d	428.09 \pm 5.50 ^e	1.98 \pm 0.02 ^e	287.90 \pm 2.96 ^e	10.72 \pm 0.06 ^e	2.08 \pm 0.00 ^c	48.12 \pm 0.08 ^c	1.65 \pm 0.00 ^c

Data with different superscripts are significantly different ($P < 0.05$). IW: Initial weight; FW: Final weight (g); WG: Weight gain (g); DGR: Daily Growth Rate (g day⁻¹); RGR: Relative Growth Rate (%); SGR: Specific Growth Rate (% day⁻¹); FI: Food Intake (g Fish⁻¹); MGR: Metabolic Growth Rate (g Kg⁻¹ day⁻¹); FCR: Feed Conversion Ratio; FCE: Feed Conversion Efficiency; PER: Protein Efficiency Ratio (%).

Table 4 - Some heamatological parameters (mean ± standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	Concentration	RBC (106 mm ³)	Hb (%)	PCV (%)	MCV (µm ³)	MCH (pg) ³	MCHC (g dl ⁻¹) ³
Control	PO 0.0 %	1.05±0.04 ^e	7.09±0.06 ^f	20.56±0.34 ^e	164.34±1.94 ^e	81.70±0.40 ^e	33.72±0.40 ^e
T1	PO 0.5 %	1.23±0.01 ^d	9.43±0.06 ^e	23.00±0.70 ^d	176.17±3.68 ^d	87.05±1.35 ^d	35.73±0.41 ^{cd}
T2	PO 1.0 %	1.35±0.01 ^c	10.20±0.07 ^{cd}	26.46±1.15 ^{bc}	193.92±1.80 ^c	91.200.10 ^c	37.21±0.04 ^c
T3	PO 1.5 %	1.580.01 ^b	11.85±0.09 ^b	28.18±0.04 ^b	215.55±1.65 ^b	94.80±0.60 ^b	40.48±0.27 ^b
T4	PO 2.0 %	1.77±0.05 ^a	12.97±0.06 ^a	30.82±0.37 ^a	250.81±2.71 ^a	99.30±0.60 ^a	44.34±0.43 ^a
T5	PO 2.5 %	1.44±0.06 ^c	10.63±0.32 ^c	24.96±0.75 ^{cd}	199.20±0.95 ^c	90.25±0.35 ^c	37.04±0.18 ^c
T6	PO 3.0 %	1.22±0.01 ^d	9.72±0.18 ^{d^e}	22.82±0.70 ^d	182.60±1.00 ^d	85.10±0.20 ^d	36.49±2.04 ^{cd}

Data with different superscripts are significantly different (P < 0.05). RBC: Red blood cell; Hb: Hemoglobin (%); PCV: Packed Cell Volume (%); MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Table 5 - Some immunological parameters (mean ± standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	Concentration	WBC (10 ³ mm ³)	STP	BP	Ig M (µg ml ⁻¹) ⁵
Control	PO 0.0 %	189.71±3.32 ^a	2.00±0.07 ^d	10.97±0.03 ^e	0.0023±0.00005 ^f
T1	PO 0.5 %	200.75±0.53 ^d	2.37±0.05 ^c	11.75±0.03 ^d	0.0032±0.00004 ^e
T2	PO 1.0 %	206.72±0.44 ^{cd}	2.60±0.01 ^{bc}	12.44±0.09 ^b	0.0047±0.00004 ^c
T3	PO 1.5 %	215.39±1.77 ^b	2.71±0.04 ^b	12.63±0.02 ^b	0.0051±0.00007 ^b
T4	PO 2.0 %	225.89±3.18 ^a	3.36±0.15 ^a	13.19±0.08 ^a	0.0064±0.00007 ^a
T5	PO 2.5 %	209.85±1.41 ^{bc}	2.48±0.07 ^{bc}	12.19±0.04 ^c	0.0051±0.00010 ^b
T6	PO 3.0 %	209.00±1.87 ^{bc}	2.40±0.06 ^c	11.81±0.08 ^d	0.0040±0.00016 ^d

Data with different superscripts are significantly different (P < 0.05). WBC: White Blood Cell; STP: Scientist Training Programme; BP: Blood Pressure; Ig M: Immunoglobulin M

DISCUSSION

Growth standards

Previous studies that examined various types of fatty acids have shown that it is better to combine these acids rather than use a single type. This is because they work together to reduce or inhibit the growth of pathogenic bacteria and lower the pH, especially in the front part of the intestine (Ng and Koh, 2017; Huang et al., 2022). Additionally, the synergistic effect of different fatty acids among each other may enhance their effectiveness and benefits for animals (Abdel-Tawwab et al., 2019; Pearlin et al., 2020). The present study was based on this concept. The results indicated that the group of fatty acids in PSO affected all the growth characteristics studied. In WG and FW, all treatments showed significant differences from the control, and treatment T4 achieved the greatest differences compared to the control and the other treatments. Treatment T3 also produced results similar to T4. This suggests that fatty acids like linoleic, oleic, stearic, and palmitic significantly influenced all the growth traits studied, particularly FW, WG, DGR, RGR, and SGR. Overall, dietary lipid serves as a more important energy source than carbohydrate for feeding carnivorous fish and has a sparing function for dietary protein (Lee et al., 2002). On the other hand, growth performance may have improved because PSO, containing these acids, reduced Gram-negative bacteria populations or because of a suitable concentration that favored the growth of Gram-positive bacteria, in addition to maintaining the optimal pH for digestive enzyme activity. Combining organic acids creates a diverse activity within the intestine, counteracting many types of bacteria, especially pathogenic ones. This allows beneficial bacteria to proliferate more freely by reducing competition within the lower intestine (Banerjee and Ray, 2017). In this study, it was observed that higher oil concentrations led to a decrease in growth performance. This aligns with our previous research on the synergistic effects of butyric and propionic acids in common carp diets, where combined supplementation yielded greater benefits than individual additions (Alhamadany et al., 2023). Additionally, there was a notable increase in the metabolic growth factor across all parameters. This could be because fatty acids lower the pH in the front part of the intestine, providing a larger area for enzyme activity (Lückstädt, 2008), or because these oils are absorbed directly without requiring digestion, as demonstrated in a study on Atlantic salmon fed graded triglyceride levels, which showed decreased carbohydrate digestion and increased fat absorption (Nordrum et al., 2003). The reduced growth performance observed in treatments T5 and T6 might be due to excessively high concentrations that reduced palatability due to the oil's strong taste. Excessive fatty acids can decrease palatability, reducing food intake due to the unpleasant smell or taste (Xie et al., 2003).

From Table 3, it is observed that a high concentration of PSO slightly increased the protein efficiency ratio. This confirms the findings of Zhang et al. (2021), who used different levels of complex acidifying substances and an extensive range of acidifiers to study the growth performance and intestinal health of young fish. In the American eel, *Anguilla rostrata*, these compounds improved the PER values when low levels of acidifying materials were used. However, at

higher concentrations, the measured parameters, including the protein efficiency ratio, began to decline. This indicates that using high levels of acidifying agents may negatively affect some performance indices.

Blood standards

Many studies have documented an increase in the number of RBCs when feeding fish with some functional additives, including studies by Zarei et al. (2021), Raissy et al. (2022), and Mohamed et al. (2022). These studies confirmed that acids and functional additives boost the number of RBCs, hemoglobin, and other blood parameters. The present results align with this, as MCV, PCV, RBCs, Hb, MCH, and MCHC increased across all treatments, showing a significant difference ($P < 0.05$) compared to the control. This can be explained by hematopoietic activity that increases blood volume in fish, which may result from the synergy of various nutritional factors. These include vitamins such as B12, B6, K, and D; fatty acids and other nutrients; and minerals like iron, zinc, and copper (Choi and Kim, 2005). The role of acids in enhancing blood activity is clear, as fatty acids can improve nutrient digestion and stimulate digestive enzyme activity, leading to increased growth as a natural consequence of heightened metabolism. Consequently, fish require larger blood volumes to support greater metabolic demand. Increased metabolic activity and blood volume can thus contribute to increases in body mass. A study by Hongyan et al. (2023), which investigated the relationship between body mass and blood indicators in young yellowfin tuna *Thunnus albacares* concluded that larger body mass is generally linked to higher metabolism and correlates with increased blood volume. This study confirms that the rise in metabolic rate was accompanied by higher levels of PSO, which in turn increased the fatty acids present in the diet.

Immunological standards

The synergistic mixture of acids found in PSO, resulting from various types of fatty acids, appears to have a definite effect on the values of immune parameters IgM, STP, and WBCs. Heshmatfar et al. (2023) demonstrated that the synergistic effect of fatty acids can lead to improved results, as they observed an increase in total protein and immunoglobulin during their experiment investigating the effects of combined or single administration of formic acid and *Pediococcus acidilactici* on stress resistance, growth performance, immune responses, and gene expression in fish. This was also supported by Hamed et al. (2023), who studied the effect of certain fatty acids on enzymatic parameters and immune responses in common carp, finding noticeable improvements in immune parameters. Immunoglobulin (IgM) is also considered the first line of defense for fish against diseases, helping to identify and weaken pathogenic bacteria and viruses (Giri et al., 2012). The results in Table 5 demonstrated the effectiveness and superiority of the T4 treatment on blood globulin levels (Zarei et al., 2021). Their study also confirmed that adding certain fatty acids improved the performance of fingerlings in yellowfin sea bream (*Acanthopagrus latus*). The total immunoglobulin content and lysozyme activity in skin mucus increased as the amount of fatty acid supplemented increased. It was also observed in our study that fish had a high mucus content on their skin, which serves as the body's first defense mechanism against many pathogens, such as fungi and bacteria that infect fish (Hussain and Ghosh, 2023).

Blood performance

The mathematical formula for the blood performance standard is based on the idea that any component of the blood - Hb, RBCs, WBCs, or total serum protein - cannot always serve as a reliable biomarker for fish growth or health. For this reason, blood performance may be a better choice because it considers all these variables within one composite formula. The blood performance parameter is reliable and suitable for comparing parameters within each experiment, but it is not ideal for comparing results across separate experiments. A high value of this parameter indicates better growth or overall fish health. The values of this criterion ranged between 10.68 and 18.24, based on analyzing 441 samples, with an average of 14.43 as reported in the study by Esmaeili (2021). It was also confirmed that blood serum proteins and WBCs have the greatest impact on blood performance, while hemoglobin has a lesser effect. Additionally, these five parameters account for 95% of the influence on blood performance. Generally, when we observe differences in these five parameters across treatments, this formula becomes more meaningful and reliable, as it integrates all the components that collectively explain the fish's physiological response to dietary treatments. The natural logarithm (Ln) is added to the formula to reduce the variance among variables. The present results were within the range reported by Esmaeili (2021), corresponding to the normal blood performance standard. This can be considered an indicator of fish health and is presented in Table 5.

The blood performance standard can be viewed in two ways

First, consider the relationship between blood performance and growth. After absorbing energy from feed, this energy contributes to three main physiological components: growth, respiration, and energy loss through feces and nitrogenous excretory products (Jobling, 1994). In other words, increases in growth parameters are associated with higher blood performance values. Although glucose serves as an essential energy source, it appears to be less important for fish metabolism. The physiological underpinnings of fish's apparent glucose intolerance have not yet been thoroughly

investigated. The relative inability of fish to effectively utilize dietary glucose may be explained by their distinct regulation of hepatic glucose production (gluconeogenesis) and glucose utilization (glycolysis) (Enes et al., 2009). It is also known that fatty acids contain a large amount of energy. For example, wheat provides five times less energy than propionic acid (Freitag, 2007; You et al., 2023). The high energy content in PSO contributed to the increase in RBC count, based on the findings. Accordingly, the observed rise in RBC count corresponded with an overall increase in blood performance.

Second, the relationship between blood performance and the immune system: The results indicate an increase in blood performance with higher levels of WBC, IgM, and STP. This was confirmed by Esmaeili (2021), who showed a close connection between the blood performance standard and the studied immunological markers, particularly WBCs, total blood proteins, and globulin. These findings align with those of Zarei et al. (2021) and a study by Hassaan et al. (2018). All these studies confirm that fatty acids and functional additives boost the number of RBCs, hemoglobin, and other blood characteristics, as well as enhance immune parameters.

CONCLUSION

Adding oils to diets significantly boosts the growth and overall health of common carp, especially oils rich in diverse fatty acids. Combining these acids provides the most effective approach for maximizing physiological and immune performance. However, aquaculture professionals are advised to use these PSOs at a rate not exceeding 2% to achieve optimal growth.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

A.S.A-A. Alhamadany and H.A. Fazaa contributed in formal analysis, methodology; investigation, writing, preparation of original draft, writing, review & editing. K.Z. Negaud performed conceptualization, formal analysis, acquisition of funding, research, and methodology; and R. M. Sayed-Lafi performed writing, review & editing.

Ethical regulations

All parts of this research were conducted in accordance with the regulations and instructions of the Research Ethics Committee at the College of Agriculture, Al-Muthanna University, and in accordance with the Iraqi Animal Health Law No. 23 of 3013. All necessary measures were taken to minimize pain and stress to the experimental fish and to minimize the measurements made for this research.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

The authors declare no competing interests in this research and publication.

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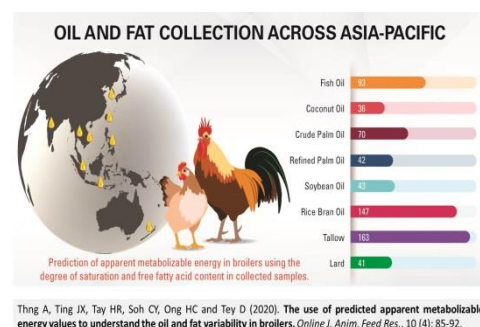
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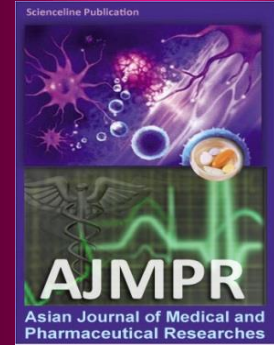
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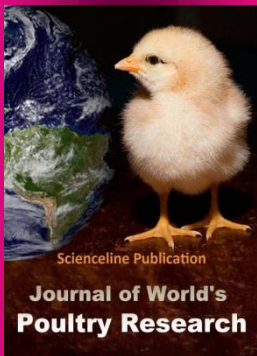
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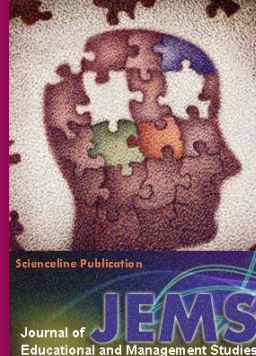
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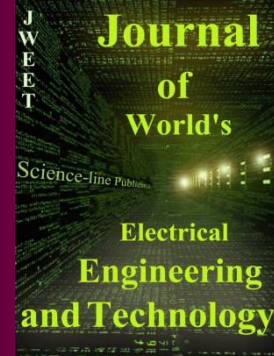
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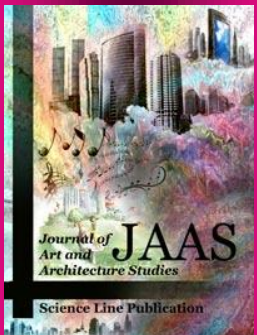
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