

BOOKLET





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Research Paper

Effect of supplemented *Cordyceps militaris* extract on disease resistance and survival rate of striped catfish (*Pangasius hypophthalmus*) fingerlings

Lan T.T., Hang N.T.T., Hanh N.T.B., Mai V.P., Vy P.N.T., Nhi N.H.Y.

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DOI: <https://dx.doi.org/10.51227/ojafr.2026.1>

Abstract

The effects of supplemented *Cordyceps militaris* extract in diets has been studied on the survival and immune response of striped catfish (*Pangasius hypophthalmus*) fingerlings with bacterial challenge conditions. The experiment was conducted for 60 days with six treatments and three replicates, including negative and positive controls with four dietary supplementation levels of *C. militaris* extract (0.18%, 0.24%, 0.36%, and 0.47%). After the feeding trial, striped catfish were challenged with *Edwardsiella ictaluri* at a dose of 10^5 CFU/mL. Results showed that survival rates of striped catfish increased significantly with higher extract levels, reaching the highest value (70%) at 0.47% supplementation compared with only 16.67% in the positive control group. Hematological indices such as red blood cell (RBC) and white blood cell (WBC) including lymphocytes, monocytes, and neutrophils increased proportionally with extract concentration, which can indicate enhanced immune responses. In conclusion, the findings suggest that dietary supplementation of *C. militaris* extract at 0.47% improves the disease resistance and health status of striped catfish fingerlings against *E. ictaluri* infection. This result highlights the potential application of *C. militaris* extract as a natural immunostimulants to enhance survival and reduce antibiotic use in catfish aquaculture.

Keywords: *Cordyceps militaris*, *Edwardsiella ictaluri*, Immune response, *Pangasius hypophthalmus*, Survival rate.

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Research Paper

Comparison of the serum minerals that promote bone healing in healthy and clinically sick goats in Bangladesh

Yadav SK, Yadav S, Ahaduzzaman Md, Pallab MS, Sutradhar BC, and Chandra Das B.

Online J. Anim. Feed Res., 16(1): 10-15, 2026; pii: S222877012600002-16

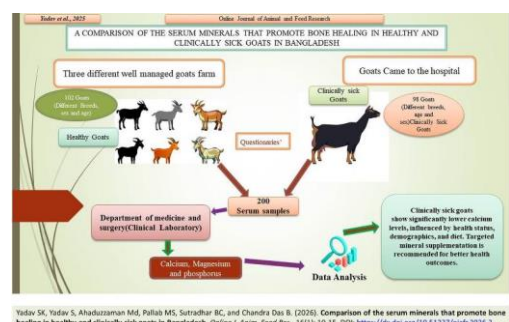
DOI: <https://dx.doi.org/10.51227/ojafr.2026.2>

Abstract

Serum minerals such as calcium, phosphorous, and magnesium are essential for the skeletal system during bone formation and remodeling as well as fracture repair. Present study was intended to assess the serum biochemical parameters of three of the most important macro minerals (calcium, phosphorous, and magnesium), which are key minerals for bone healing or development in healthy and clinical sick goats. Serum samples were collected from a total of 200 goats (healthy=102, clinically sick=98) from a veterinary hospital and three farms of Chattogram, Bangladesh. The overall mean serum calcium level of healthy goats was 8.47 ± 0.11 mg/dl and in sick goats it was 7.63 ± 0.12 mg/dl, and the value was much lower than the reference value in sick goats. The serum phosphorus and magnesium levels did not vary between the two groups and were within (phosphorus) or close to (magnesium) the range of reference values. In healthy goats, their serum calcium level varied significantly considering age, body weight, sex, feeding type, and owner's income. However, the serum phosphorus and magnesium levels did not vary significantly across the different parameters tested, except for sex and owner's income in the case of phosphorus. Three sick goats had hypocalcemia, which means that their calcium and magnesium levels were lower than the standard values of 5.9 ± 0.5 and 1.9 ± 0.2 mg/dl, respectively. However, their phosphorus levels were normal across all groups of sick goats. In conclusion, the value of an important mineral for bone healing is lower in both sick and healthy goats, which is a frightening sign for bone problems. Most goats have low levels of calcium and magnesium but average levels of phosphorous. Based on these findings, sufficient calcium and magnesium can be added to feed and given orally to further prevent bone fracture.

Keywords: Calcium, Clinically sick animals, Feeding type, Indigestion problems, Magnesium, South Asian region.

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Yadav SK, Yadav S, Ahaduzzaman Md, Pallab MS, Sutradhar BC, and Chandra Das B. (2026). Comparison of the serum minerals that promote bone healing in healthy and clinically sick goats in Bangladesh. *Online J. Anim. Feed Res.*, 16(1): 10-15. DOI: <https://dx.doi.org/10.51227/ojafr.2026.2>

Analysis of demand for water and its impact on broiler production in Imo state of Nigeria

Anyiam KH, Igwe KC, Amusa TA, Obinna-Nwandikom CO, Enoch OC, Isaiah IG, Obasi AC, Okoro FN, Iwezor-Magnus DN, Anene HU, Nnorom EI, Olumba UM, Igwenagu MO, Onu DO, Ozor MU.

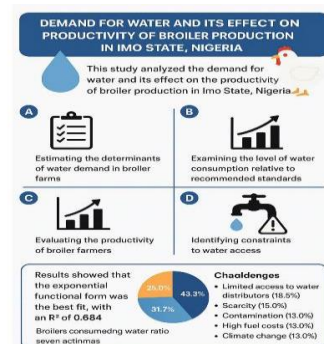
Online J. Anim. Feed Res., 16(1): 16-23, 2026; pii: S222877012600003-16
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Abstract

This study analyzed the demand for water and its effect on the productivity of broiler production in Imo State, Nigeria. Specifically, the objectives were to A) estimating the determinants of water demand in broiler farms, B) examining the level of water consumption relative to recommended standards, C) evaluating the productivity of broiler farmers, and D) identifying constraints to water access. Primary data were collected from 120 broiler farmers using a structured questionnaire and analyzed with descriptive statistics, the broiler water consumption ratio (BWCR), productivity index, and multiple regression models. Results showed that the exponential functional form was the best fit, with an R^2 of 0.684, indicating that 68.4% of the variation in water demand was explained by farm and bird characteristics. Age of birds ($P < 0.05$), stock size ($P < 0.01$), and bird weight ($P < 0.10$) significantly influenced water demand. Based on the BWCR, 43.3% of broilers consumed water below optimal levels, 25.0% consumed excessively, and only 31.7% were within the recommended range. Farmers demonstrated high productivity with a ratio of 3.30, suggesting efficient input use; however, sustainability is threatened by persistent water constraints. The most pressing challenges were limited access to water distributors (18.5%), scarcity (15.0%), contamination (13.0%), high fuel costs (13.0%), and climate change (13.0%). The findings underscore the need for policy interventions to strengthen rural water infrastructure, promote solar-powered pumping systems, and improve extension services on water-use efficiency. Addressing these issues will enhance both the productivity and resilience of broiler production in water-scarce environments.

Keywords: Broiler productivity, Poultry requirements, Water consumption ratio, Water demand, Nigeria

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Anyiam KH, Igwe KC, Amusa TA, Obinna-Nwandikom CO, Enoch OC, Isaiah IG, Obasi AC, Okoro FN, Iwezor-Magnus DN, Anene HU, Nnorom EI, Olumba UM, Igwenagu MO, Onu DO, Ozor MU (2026). Analysis of demand for water and its impact on broiler production in Imo state of Nigeria. Online J. Anim. Feed Res., 16(1): 16-23. DOI: <https://dx.doi.org/10.51227/ojafr.2026.3>

Research Paper

Optimization of the growth and nutritional quality of lesser mealworm (*Alphitobius diaperinus*) using the protein content of the medium

Nuraini N, Mirzah M, Nur YS, Azim A, Fathinah D, Wahyudi R.

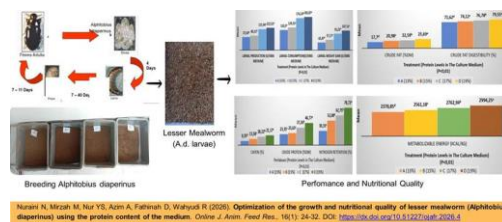
Online J. Anim. Feed Res., 16(1): 24-32, 2026; pii: S222877012600004-16

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Abstract

This study aims to examine the performance and nutritional quality of lesser mealworms (*Alphitobius diaperinus*) reared with different protein levels in the culture medium. The research method used was a completely randomized design (CRD) with 4 treatments and 5 replications. The treatments involved providing different protein levels in the culture medium: A (13%), B (15%), C (17%), and D (19%). The variables observed were larval consumption (g/500 g medium), larval production (g/500 g medium), larval body weight gain (g/500 g medium), chitin content (%), crude protein (%DM), nitrogen retention (%), crude fat (%DM), crude fat digestibility (%), and metabolizable energy (kcal/kg) of lesser mealworms. The result of this study showed that differences in protein levels in the culture medium had a very significant effect ($P < 0.01$) on larval consumption, larval production, larval body weight gain, chitin content, crude protein, nitrogen retention, crude fat, crude fat digestibility, and metabolizable energy of lesser mealworms. This study concluded that 19% protein culture medium was the optimal treatment for lesser mealworm rearing, yielding larval consumption of 181.87 g/500 g medium, larval production of 122.52 g/ 500 g medium, larval body weight gain of 107.51 g/500 g medium, chitin content of 21.17%, crude protein of 46.77% (DM), crude fat of 25.69% (DM), nitrogen retention of 78.72%, crude fat digestibility of 79.55%, and metabolizable energy of 2994.25 kcal/kg. The findings indicate that smaller mealworms hold considerable potential as an alternative source of animal protein for poultry feed.

Keywords: Feedstuff, Lesser Mealworm, Nutritional Quality, Performance, Protein Medium.



Nuraini N, Mirzah M, Nur YS, Azim A, Fathinah D, Wahyudi R (2026). Optimization of the growth and nutritional quality of lesser mealworm (*Alphitobius diaperinus*) using the protein content of the medium. Online J. Anim. Feed Res., 16(1): 24-32. DOI: <https://dx.doi.org/10.51227/ojafr.2026.4>

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Research Paper

Beneficial effects of *Canavalia gladiata* seed meal fermentation on proximate composition, bioactive compounds, fatty acid profile and amino acid profile

Kone GA, Koko NASB, Koutouan FP, N'guessan KR, and Kouba M.

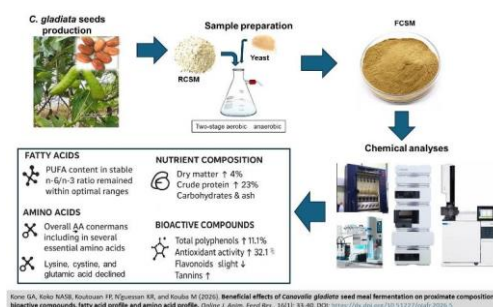
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Abstract

Soybean meal is the dominant protein source in animal feed, but rising costs have encouraged the search for alternatives. *Canavalia gladiata*, a tropical legume with good yield potential and resistance to pests, contains about 26% protein. However, its use in non-ruminant diets is constrained by anti-nutritional factors. Fermentation with *Saccharomyces cerevisiae* has been shown to enhance protein content and reduce anti-nutrients in various feed ingredients, making it a promising strategy for improving *C. gladiata* seed. The aim of this study was to evaluate the effect of yeast fermentation on *C. gladiata* seed meal proximate composition, bioactive compounds, fatty acid profile and amino acid profile. In this study, raw seed meal (RCSM) was prepared from *C. gladiata* seed grown in Côte d'Ivoire, while fermented seed meal (FCSM) was obtained through a two-step aerobic and anaerobic fermentation with yeast. Proximate composition, bioactive compounds, antioxidant activity, fatty acid and amino acid profiles were determined using standard analytical methods. Fermentation increased dry matter by 4% and crude protein by 23%, while reducing carbohydrates and ash. It also enhanced total polyphenols by 11.11% and antioxidant activity by 32.14%, though flavonoids decreased slightly and tannin levels increased. Fatty acid profiles showed stability of polyunsaturated fatty acid (PUFA) and n-6/n-3 ratios within optimal ranges. Amino acids increased overall, with notable rises in several essential amino acids, though lysine, cystine, and glutamic acid declined. Overall, yeast fermentation significantly improved the nutritional and bioactive properties of *C. gladiata* seed meal, supporting its potential as an affordable alternative protein source for monogastric animal diets.

Keywords: Bioactive compounds, *Canavalia gladiata*, Fermentation, Nutritional value, Yeast.

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Kone GA, Koko NASB, Koutouan FP, N'guessan KR, and Kouba M (2026). Beneficial effects of *Canavalia gladiata* seed meal fermentation on proximate composition, bioactive compounds, fatty acid profile and amino acid profile. Online J. Anim. Feed Res., 16(1): 33-40. DOI: <https://dx.doi.org/10.51227/ojafr.2026.5>

Research Paper

The performance and hematological indices of growing rabbit fed sessilis joyweed (*Alternanthera sessilis*) and tridax daisy (*Tridax procumbens*)

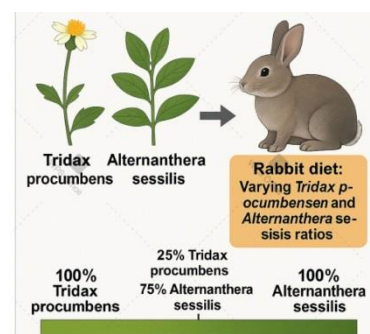
Adeyanju T, Alabi O, Adeleye B, Okunlola O, Oyewumi S, Oyelami B, Oguntunji A, Oladejo O, Afolabi C, and Adewumi A.

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Abstract

The performance and blood constituents of growing rabbits fed varying ratios of *Alternanthera sessilis* (Sessilis joyweed; AS) and *Tridax procumbens* (Tridax Daisy; TP) were evaluated. Thirty growing male rabbits with initial body weight ranging from 617.50g to 657.50g were randomly allotted to five dietary treatments, each comprising six animals housed individually in a completely randomized design. The experimental diets were as follows: T1 (100%AS), T2 (75%AS+25%TP), T3 (50%AS+50%TP), T4 (25% AS+75%TP) and T5 (100%TP). The experiment lasted for seven weeks, during which data on feed intake, body weight gain, hematological indices and serum biochemical parameters were collected and analyzed using one-way analysis of variance. The results indicated that dietary treatments had no significant effect ($P>0.05$) on performance parameters, except for average daily feed intake (ADFI). Rabbits fed T1 and T4 recorded the highest ADFI (96.49 and 96.01 g/day respectively), whereas those on T3 and T5 showed the lowest values. The best (lowest) feed conversion ratio (5.79) was observed in rabbits fed T4. Rabbits on T4 exhibited the highest packed cell volume (32.00%), hemoglobin concentration (10.20 g/dL), red blood cell count ($5.64 \times 10^6/\mu\text{L}$), platelet count ($140.00 \times 10^3/\mu\text{L}$), and white blood cell count ($32.50 \times 10^2/\mu\text{L}$). In addition, significantly higher ($P<0.05$) serum total protein (7.00 g/dL), albumin (2.90 g/dL) and globulin (4.10 g/dL) levels were recorded in rabbits fed T4 compared with other treatments. It can be concluded that the dietary combination of 25%AS+75%TP produced the most favorable performance and blood profile in growing rabbits. This suggests that *Alternanthera sessilis* can serve as a viable alternative forage resource *Tridax procumbens* in rabbit nutrition.

Keywords: Blood, Forage, Rabbit, Performance, Sessilis joyweed, Tridax daisy.



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THE PERFORMANCE AND HEMATOLOGICAL INDICES OF GROWING RABBIT FED SESSILIS JOYWEED (*Alternanthera sessilis*) AND TRIDAX DAISY (*Tridax procumbens*)

Taiwo ADEYANJU^{1,2} , Olufemi ALABI¹  , Bobola ADELEYE¹ , Oladayo OKUNLOLA^{1,3} , Samuel OYEWUMI^{1,4} , Busayo OYELAMI^{1,5} , Abel OGUNTUNJI¹ , Opeyemi OLADEJO¹ , Christiana AFOLABI¹ , and Adebayo ADEWUMI⁶ 

¹Agriculture Programme Bowen University, Iwo, 232001, Nigeria

²Animal Production Research Unit of National Centre for Genetics Resources and Biotechnology (NAGGRAB) Moor, Ibadan, Nigeria

³Department of Agricultural Education, Oyo-State College of Education, Lanlate, Nigeria

⁴Department of Agricultural Education, Emmanuel Alayande University of Education, Oyo, Nigeria

⁵Federal College of Forestry, Ibadan, Nigeria

⁶Department of Wildlife, Osun State University, Ejigbo Campus, Nigeria

 Email: olufemi.alabi@bowen.edu.ng

 Supporting Information



ABSTRACT: The performance and blood constituents of growing rabbits fed varying ratios of *Alternanthera sessilis* (Sessilis joyweed; AS) and *Tridax procumbens* (Tridax Daisy; TP) were evaluated. Thirty growing male rabbits with initial body weight ranging from 617.50g to 657.50g were randomly allotted to five dietary treatments, each comprising six animals housed individually in a completely randomized design. The experimental diets were as follows: T1 (100%AS), T2 (75%AS+25%TP), T3 (50%AS+50%TP), T4 (25%AS+75%TP) and T5 (100%TP). The experiment lasted for seven weeks, during which data on feed intake, body weight gain, hematological indices and serum biochemical parameters were collected and analyzed using one-way analysis of variance. The results indicated that dietary treatments had no significant effect ($P>0.05$) on performance parameters, except for average daily feed intake (ADFI). Rabbits fed T1 and T4 recorded the highest ADFI (96.49 and 96.01 g/day respectively), whereas those on T3 and T5 showed the lowest values. The best (lowest) feed conversion ratio (5.79) was observed in rabbits fed T4. Rabbits on T4 exhibited the highest packed cell volume (32.00%), hemoglobin concentration (10.20 g/dL), red blood cell count ($5.64 \times 10^6/\mu\text{L}$), platelet count ($140.00 \times 10^3/\mu\text{L}$), and white blood cell count ($32.50 \times 10^2/\mu\text{L}$). In addition, significantly higher ($P<0.05$) serum total protein (7.00 g/dL), albumin (2.90 g/dL) and globulin (4.10 g/dL) levels were recorded in rabbits fed T4 compared with other treatments. It can be concluded that the dietary combination of 25%AS+75%TP produced the most favorable performance and blood profile in growing rabbits. This suggests that *Alternanthera sessilis* can serve as a viable alternative forage resource *Tridax procumbens* in rabbit nutrition.

Keywords: Blood, Forage, Rabbit, Performance, Sessilis joyweed, Tridax daisy.

INTRODUCTION

The growing global population suggests the necessity of food security and a sustainable supply of dietary protein. According to FAOSTAT (2022), a substantial reduction in protein consumption has been observed in parts of Africa and Asia. In Nigeria, the average daily protein intake is estimated at 45.40 g per capita which is below the recommended level of 53.80 g per day set by the Food and Agriculture Organization (FAO) and also lower than the global average of 64.00 g (Akerele et al., 2017; Protein Challenge, 2020). Furthermore, Adetunji and Adepoju (2011) emphasized that approximately 35.00 g of daily protein intake should be derived from animal sources. However, a declining trend in animal protein consumption has been reported in Nigeria, despite the country's population being projected to increase from about 201 million in 2019 to approximately 401 million by 2050.

This rapid population growth, coupled with the increasing demand for animal protein highlights the urgent need to explore unconventional livestock species characterized by fast growth rates and short gestation periods (Sonea et al., 2023; Miassi and Dossa, 2023). Rabbit (*Oryctolagus cuniculus*) production represents a promising option, as rabbits are relatively inexpensive to raise and exhibit high meat production efficiency. Rabbits possess several advantageous characteristics, including rapid growth rate, early attainment of puberty, short gestation period, high reproductive potential, efficient feed conversion, ability to utilize forages, and ease of housing and handling (Arijeaniwa et al., 2002; Chipo et al., 2019; Khalid et al., 2020; Oladimeji et al., 2021). Despite these favorable attributes, rabbit production remains underutilized in tropical regions. This is largely due to the scarcity and high cost of conventional feed ingredients, which constrain large-scale rabbit production, particularly in Nigeria.

In order to tackle these challenges, research efforts have increasingly focused on identifying feed ingredients and additives that are readily available and relatively inexpensive (Oluremi et al., 2018; Aderemi and Alabi, 2023). Agro-industrial by-products, forages, and meals derived from various plant leaves and seeds represent promising alternatives to conventional feed resources (Aderemi et al., 2015; Alabi et al., 2017; Vastolo et al., 2022; Amos et al., 2025). Among the available forages, *Alternanthera sessilis* and *Tridax procumbens* are particularly noteworthy because of their nutritional and medicinal properties (Fahey, 2005). These plants therefore have the potential to partially or completely replace conventional protein sources in rabbit diets, thereby improving feed efficiency and growth performance.

Alternanthera sessilis (Sessilis Joyweed) is perennial forage belonging to the family *Amaranthaceae* and is commonly utilized for ruminant nutrition (Singla et al., 2022). In addition to its use as a leafy vegetable and edible herb, it possesses a pleasant aroma and contains desirable levels of dietary fibre, vitamins, flavonoids, minerals and carotenoids (Michael, 2011; Shehzad et al., 2018; Alabi et al., 2024a). According to Shehzad et al. (2018), *Alternanthera sessilis* provides approximately 251.00 KJ/mol of energy and 4.70 g/100 g of crude protein, indicating its potential value as a feed resource.

Similarly, *Tridax procumbens* (Tridax daisy) is characterized by excellent regrowth ability, resilient, and suitability as a forage crop for livestock. Its nutritional profile, including levels of protein, minerals, vitamins, tocopherols, phenolic compounds and β -carotene, has been well documented (Anwar et al., 2007; Samantha et al., 2018; Sarkar et al., 2022). These nutrients play vital roles in growth and reproductive development of the animals, making *T. procumbens* useful substitute for conventional protein sources such as soybean meal and fish meal in monogastric animal nutrition.

Given these nutritional qualities, *Alternanthera sessilis* and *Tridax procumbens* may enhance feed utilization and growth performance in growing rabbits. Therefore, the objective of this study was to evaluate the effect of graded dietary inclusion levels of these two forages on the performance of growing rabbits. Specifically, the study assessed feed intake, body weight gain, and feed conversion efficiency, as well as the effects of the diets on haematological and serum biochemical indices. The overall aim was to determine the suitability of these forages as alternative feed resources for rabbits and to identify the optimal combination level for maximizing growth performance and physiological health.

MATERIALS AND METHODS

Ethical consideration

Ethical approval for the study was obtained from the Institutional Research Ethics Committee.

Study location and duration

The experiment was carried out at the rabbit Unit of the Animal Production Research Faculty National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria.

Forage sourcing and processing

The two experimental forages used in the feeding trial were *Alternanthera sessilis* (AS) and *Tridax procumbens* (TP), both harvested locally. Fresh samples of the forages were collected from areas within the vicinity of the study site. Whole plants, including stems and leaves, were harvested at approximately 3 cm above ground level during the mid-vegetative to early flowering phase. The harvested material were rinsed with clean water to remove soil and debris, drained, and air-dried for 24 hours on a concrete surface while ensuring retention of green coloration. The air-dried forages were weighed using a Salter electronic scale and subsequently offered to the rabbits according to the assigned dietary treatments.

Experimental diets

Five dietary treatments were formulated based on varying proportions of AS (*Alternanthera sessilis*) and TP (*Tridax procumbens*) as follows:

T1: 100% AS; T2: 75% AS + 25% TP; T3: 50% AS + 50% TP; T4: 25% AS + 75% TP; T5: 100% TP

Animal selection and husbandry

Thirty weaned male Large White rabbits, aged eight weeks and weighing between 617 and 657 g, were sourced from the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan. Prior to the arrival of animals, all housing units, feeders, drinkers, and the hutch area were thoroughly cleaned and disinfected. Upon arrival, the rabbits were allowed a seven-day acclimatization period, during which they were managed under conditions similar to those of the experimental period. Each rabbit was individually housed in a metal battery cage measuring 23 × 18 × 15 inches. Feed and clean drinking water were provided *ad libitum* throughout the experiment.

The rabbits were allowed to stabilize on the experimental diets for four days before the data collection commenced. Feeding was carried out twice daily at 08:00 and 16:00 h and maintained consistently throughout the study. Equal quantities of moistened concentrate feed were provided daily as supplements. The proximate composition of the concentrate diet is presented in Table 1.

Table 1 - Proximate composition of the experimental diets.

Parameters (%)	T ₁ (100%AS)	T ₂ (75%AS+25%TP)	T ₃ (50%AS+50%TP)	T ₄ (25%AS+75%TP)	T ₅ (100%TP)
Dry matter	72.40	73.05	73.7	73.88	75
Crude Protein	26.16	24.47	22.78	21.25	19.4
Crude fibre	8.8	8.03	7.25	6.8	5.7
Ether extract	3.87	6.03	8.185	10.05	12.5
Ash	8.5	7.45	6.4	5.75	4.3
NFE	45.07	47.08	49.09	56.15	53.1

NFE: Nitrogen free extract; *Metabolizable energy of the experimental diets: T₁ = 2862.24, T₂ = 3046.44, T₃ = 3250.54, T₄ = 3573.59, T₅ = 3598.80 KCal/Kg.

Experimental design

The experiment was arranged in a completely randomized design. Thirty rabbits were randomly allocated to five dietary treatments, with six replicates per treatment and one rabbit constituting each replicate.

Nutrient composition analysis

The chemical composition of the experimental diets was determined using standard procedures of the Association of Official Analytical Chemists (AOAC, 2000). Parameters analyzed included dry matter, crude protein, crude fiber, ether extract and ash content while nitrogen-free extract (NFE) was calculated by difference. The Metabolizable Energy (Kcal/Kg¹) content of the experimental diets was estimated using the following formula:

ME (Kcal/Kg¹) = 3.50 x crude protein + 8.46 x acid ether extract + 3.50 x NFE as recommended by the National Research Council-NRC (Ohshima et al., 1995).

Performance metrics

Feed Intake and growth performance

The experimental diets were offered to the rabbits in known quantities at designated feeding times. Feed refusals were collected and weighed after 24 hours to determine actual feed intake. Initial live body weights were recorded at the commencement of data collection, prior to feeding, and subsequently measured on a weekly basis throughout the experimental period. Average daily weight gain (ADG) was calculated by dividing the total weight gain by the duration of the experiment (49 days). Average daily feed intake (ADFI) and Feed Conversion Ratio (FCR) were computed using standard procedures.

Feed intake (kg) = Feed offered-Remnant

Average daily feed intake (kg) = $\frac{\text{Feed intake (g)}}{\text{Number of days of the experiment}}$

Weight Gain (kg) = Final Weight Gain - Initial Weight Gain

Average daily Weight gain (kg) = $\frac{\text{Weight Gain (g)}}{\text{Number of days of the experiment}}$

FCR = $\frac{\text{Average daily feed intake}}{\text{Average daily weight gain}}$

Blood sample collection and analysis

On the 49th day of the experiment, blood samples (5 ml) were collected from three rabbits randomly selected from each treatment group via the lateral saphenous vein of the hind limb, as described by Parasuraman et al. (2017). The collection site was shaved and sterilized prior to sampling. Of the 5 ml collected from each rabbit, 3 ml was dispensed into tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological analysis. The samples were gently mixed to prevent clot formation and analyzed within one hour of collection. Hematological parameters determined included red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, and packed cell volume (PCV), following the methods described by Dauce and Lewis (1991). The remaining 2 mL of blood was transferred into plain tubes without anticoagulant and centrifuged at 500 rpm for 3 minutes to obtain clear serum. Serum samples were analyzed using a spectrophotometry at a wavelength of 500 nm with commercial assay kits (Sentinel, Italy). Serum biochemical indices, including total cholesterol, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin were determined using diagnostic kits supplied by Randox Laboratories Ltd. (U.K).

Statistical analysis

All data generated were subjected to statistical analysis using one-way analysis of variance (ANOVA) in SAS software version 9.1 (SAS, 2005). Where significant differences were detected, treatment means were separated using Duncan's Multiple Range Test at a 5% level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

Growth performance of the experimental rabbits

Table 2 presents the growth performance indices of the rabbits fed graded levels of *Alternanthera sessilis* and *Tridax procumbens*. The results indicated that the dietary treatments had no significant effect ($P < 0.05$) on most performance parameters, except for average daily feed intake (ADFI), which differed significantly among the treatment groups. Rabbits fed diets containing 100%AS and 75%AS+25%TP recorded the highest ADFI values of 96.49 g/day and 96.01 g/day, respectively, with no significant difference between the two treatments. In contrast, rabbits fed 50%AS+50%TP (79.35 g/day) and 25%AS+75%TP (66.14 g/day) exhibited statistically similar ADFI values, which were significantly lower than those observed in the other treatment groups. The feed intake values recorded in the present study were higher than the 41.20 g/day previously reported by Adeniji and Ehiemerem (2003). Similarly, average daily weight gain observed in this experiment exceeded the range of 6.78 - 8.64 g/day earlier reported by Omoikhoje et al. (2006) and Odeyinka et al. (2008) for rabbits raised under tropical climates. Furthermore, the feed conversion ratios (FCR) values obtained in this study were higher than those previously documented by Mufwa et al. (2011) and Okorie (2003) for growing rabbit.

Table 2 - Performance characteristics of the experimental rabbits.

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	P-value
Parameters	(100%AS)	(75%AS+25%TP)	(50%AS+50%TP)	(25%AS+75%TP)	(100%TP)		
Initial weight (g)	620.33 ^b	617.5 ^b	657 ^a	625.33 ^b	618 ^b	18.61	.001
Final weight (g)	1247.3 ^b	1232 ^c	1225.7 ^c	1194.7 ^d	1286.3 ^a	30.45	.002
Weight gain (g)	626.97 ^b	614.5 ^c	568.7 ^d	569.37 ^d	668.3 ^a	32.18	.001
ADWG (g/d)	12.8 ^b	12.54 ^c	11.61 ^d	11.62 ^d	13.64 ^a	0.66	.004
TFI (g)	4728 ^a	4704.3 ^a	3888 ^b	3241 ^b	3966.3 ^{ab}	173.94	.002
ADFI (g/d)	96.49 ^a	96.01 ^a	79.35 ^b	66.14 ^b	80.95 ^{ab}	3.55	.001
FCR	7.86 ^a	7.84 ^a	6.83 ^b	5.79 ^c	6.11 ^b	0.36	.0001

^{a,b} Means along the same row with different superscripts are significantly different ($P < 0.05$). SEM: The standard error of the mean. ADWG: Average daily weight gain, TFI: Total feed intake, ADFI: Average daily feed intake, FCR: feed conversion ratio. T1: 100% *Alternanthera sessilis*, T2: 75% *Alternanthera sessilis* and 25% *Tridax procumbens*, T3: 50% *Alternanthera sessilis* and 50% *Tridax procumbens*, T4: 25% *Alternanthera sessilis* and 75% *Tridax procumbens* and T5: 100% *Tridax procumbens*

Hematological parameters

Blood analysis is a reliable tool for assessing the health status as well as the nutritional, physiological, and pathological conditions of animals (Alabi et al., 2024b). Hematological indices are particularly valuable for detecting potential dietary toxicity and evaluating the physiological responses of animals to experimental diets (Oyawoye and Ogunkunle, 1998). Table 3 presents the effects of the dietary treatments on the hematological parameters of the experimental rabbits. These indices serve as critical indicators of the animals' physiological and immunological status and provide insight into how dietary interventions influence systemic function and overall welfare.

Packed cell volume (PCV), hemoglobin concentration (Hb) and red blood cell (RBC) count

Rabbits fed diet in T₄ (25%AS+75%TP) recorded the highest packed cell volume (32.00%), hemoglobin concentration (10.20 g/dl), and red blood cell count ($5.26 \times 10^6/\mu\text{L}$), values that were significantly ($P < 0.05$) higher than those observed in the other treatment groups. These findings suggest that this dietary combination supported enhanced erythropoiesis, possibly due to a balanced supply of essential micronutrients such as iron, folic acid, and vitamin B₁₂, which are required for red blood cell synthesis (Jain, 1993). In contrast, rabbits fed T₅ (100% TP) exhibited the lowest PCV (21.00%), Hb concentration (6.90 g/dL), and RBC count ($3.41 \times 10^6/\mu\text{L}$), indicating a possible reduction in haematopoietic efficiency or a tendency toward anaemia when *Tridax procumbens* was offered as the sole forage source. Although *T. procumbens* possesses documented phytotherapeutic properties, these results suggest that it may not provide adequate nutritional support for optimal erythropoiesis when used alone, highlighting the importance of dietary complementarity with *Alternanthera sessilis*. Notwithstanding these variations, the hematological values obtained across treatments fell within the established reference ranges for healthy rabbits (Obinnea et al., 2013; Abdullahi et al., 2021), supporting earlier findings by Obinnea et al. (2013), who reported improved blood indices in rabbits fed *Tridax procumbens*. The higher inclusion level of *Tridax procumbens* particularly at 75%, appeared to enhance hematological parameters, likely due to its

favorable nutrient composition. The values of the packed cell volume recorded in this study were slightly lower than the range of 34.59 - 36.65% reported by Ayandiran et al. (2020) in rabbits fed sorghum brewers' dried grain.

Table 3 - Haematological indices of the experimental rabbits.

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	P-value
Parameters	(100%AS)	(75%AS+25%TP)	(50%AS+50%TP)	(25%AS+75%TP)	(100%TP)		
PCV (%)	29 ^b	26 ^c	31 ^a	32 ^a	21 ^d	1.06	.002
Haemoglobin (g/dL)	9.3 ^b	8.7 ^c	10.1 ^a	10.20 ^a	6.9 ^d	0.12	.002
Red blood cell ($\times 10^3$)	4.32 ^b	4.23 ^b	5.2 ^a	5.26 ^a	3.41 ^c	0.18	.001
Platelet ($\times 10^3$)	85 ^a	83 ^a	86 ^a	88 ^a	70 ^b	6.49	.002
WBC ($\times 10^2$)	23.5 ^c	21 ^d	26 ^b	32.5 ^a	15.34 ^e	2.90	.002
Lymphocytes (%)	39 ^c	35 ^d	46 ^b	51 ^a	3 ^e	2.01	.001
Neutrophils (%)	57 ^c	62 ^b	50 ^d	46 ^e	69 ^a	2.20	.001
Monocytes (%)	3 ^a	1 ^c	2 ^b	2 ^b	1 ^c	0.20	.001
Eosinophils (%)	1 ^b	2 ^a	2 ^a	1 ^b	0 ^c	0.20	.002

^{a,b} Means along the same row with different superscripts are significantly different ($P < 0.05$). SEM: The standard error of the mean. WBC: white blood cell. T₁: 100% *Alternanthera sessilis*, T₂: 75% *Alternanthera sessilis* and 25% *Tridax procumbens*, T₃: 50% *Alternanthera sessilis* and 50% *Tridax procumbens*, T₄: 25% *Alternanthera sessilis* and 75% *Tridax procumbens* and T₅: 100% *Tridax procumbens*

White blood cell (WBC) and platelet counts

White blood cell counts are widely used indicators of immunological competence and immune responsiveness. As presented in Table 3, rabbits in treatment T₄ recorded the highest WBC count ($32.50 \times 10^2/\mu\text{L}$), which was significantly higher ($P < 0.05$) than the values observed in the other treatment groups. In contrast, rabbits fed T₅ exhibited the lowest WBC count ($15.34 \times 10^2/\mu\text{L}$). This pattern suggests that a mixed forage diet, particularly the T₄ combination, may provide bioactive compounds capable of enhancing immune function. Similarly, platelet count was significantly higher ($P < 0.05$) in rabbits fed T₄ ($140 \times 10^3/\mu\text{L}$) compared with those in the other treatments. This increase may indicate enhanced thrombopoiesis. Beyond their primary role in hemostasis, platelets also contribute to inflammatory processes and host immune defense mechanisms (Semple et al., 2011).

Lymphocytes and neutrophils

Lymphocyte percentages were highest in rabbits fed T₄ (51.00%), further suggesting that partial substitution of AS with TP may stimulate adaptive immune responses. By comparison, rabbits fed the control diet (T₁; 100%AS) recorded a lower lymphocyte proportion (39%), while the lowest value was observed in rabbits fed T₅ (3%), indicating a potential compromise in immune competence under a diet composed solely of *T. procumbens*. Neutrophil percentages, which are often associated with stress and inflammatory responses, were significantly higher ($P < 0.05$) in rabbits fed T₅ (69%) and T₂ (62%). This elevation may reflect increased physiological stress or subclinical inflammatory conditions in these treatment groups. Conversely, rabbits fed T₄ (46%) and T₃ (50%) exhibited significantly lower neutrophil counts, which may be indicative of reduced stress levels and a more balanced immune status (Minka and Ayo, 2013).

Monocytes and eosinophils

Rabbits fed T₁ (100% AS) exhibited the highest monocytes percentage (30.00%) which was significantly greater ($P < 0.05$) than those recorded in T₂ (75%AS+25%TP) and T₅ (100%TP). Although, monocytes play crucial roles in phagocytosis and inflammation, excessive levels may indicate the presence of chronic inflammatory processes. Eosinophil percentages were highest in T₂ and T₃ (2%) and lowest in T₅ (0%). Elevated eosinophil levels may sometimes reflect allergic responses or parasitic infections; however, in this study, all values remained within the acceptable physiological range for healthy rabbits. Overall, the results suggest that T₄ (25%AS+75%TP) optimally supports a balanced hematological profile and immune status in rabbits. Inclusion of *T. procumbens* at higher proportions appears to enhance both red and white blood cell parameters, possibly due to synergistic interactions between its bioactive compounds and the nutritional composition of *A. sessilis*. In contrast, feeding *T. procumbens* exclusively, as in T₅, consistently resulted in suboptimal hematological outcomes, which may negatively affect productivity, immunity, and overall welfare.

Serum biochemical parameters

The effects of the dietary treatments on serum biochemical indices are presented in Table 4. Significant differences ($P < 0.05$) were observed among the treatment groups for several parameters. Blood urea nitrogen (BUN) values were

highest in T5 (32 mg/dL) and lowest in T1 (21 mg/dL). Conversely, rabbits fed T1 exhibited the highest levels of alanine aminotransferase (ALT; 61 μ /L) and alkaline phosphatase (ALP; 14 μ /L), in agreement with Bhuyan et al. (2018), who reported that inclusion of *A. sessilis* in rat diets may influence liver function.

Variations in serum metabolites indicate that dietary composition affects metabolic activity and nutrient utilization. Unlike the findings of Omoikhoje et al. (2006), who observed no significant effects from *Syndrella nodiflora*-based diets, this study demonstrated notable dietary influence on serum total protein, albumin, and globulin levels particularly in rabbits fed T4 (25%AS+75%TP). Enhanced protein fractions likely reflect improved nutrient assimilation and protein utilization, supporting the observations of Iheukwumere and Okoli (2002) that blood chemistry is a direct indicator of an animal's physiological response to diet and environment. Notably, all protein fractions in this study aligned with the physiological reference range for normal rabbits: total protein (5.4–7.5 g/dL), albumin (2.5–4.5 g/dL), and globulin (1.9–3.5 g/dL) (Eshar et al., 2021).

Table 4 - Serum biochemical indices of the experimental rabbits

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	SEM	P-value
Parameters	(100%AS)	(75%AS+25%TP)	(50%AS+50%TP)	(25%AS+75%TP)	(100%TP)		
Total protein (g/dL)	5.9 ^c	6.1 ^b	6 ^b	7 ^a	6.2 ^b	0.11	.002
Albumin (g/dL)	2.5 ^c	2.6 ^b	2.5 ^c	2.90 ^a	2.6 ^b	0.04	.001
Globulin (g/dL)	3.4 ^d	3.5 ^c	3.5 ^c	4.10 ^a	3.6 ^b	0.07	.001
A/G	0.74 ^a	0.74 ^a	0.71 ^b	0.72 ^b	0.71 ^b	0.02	.001
BUN (mg/dL)	21 ^e	25 ^c	22 ^d	27 ^b	32 ^a	1.05	.002
Creatinine (mg/dL)	0.6 ^c	0.7 ^b	0.5 ^d	0.7 ^b	0.8 ^a	0.03	.0001
AST (μ /L)	71 ^a	66 ^b	54 ^c	67 ^b	69 ^a	9.41	.003
ALT (μ /L)	45 ^e	53 ^c	49 ^d	55 ^b	61 ^a	1.45	.003
ALP (μ /L)	12 ^c	13 ^b	13 ^b	12 ^c	14 ^a	0.20	.001

^{a,b} Means along the same row with different superscripts are significantly different (P<0.05). SEM: The standard error of the mean. A/G: Albumin/ Globulin ratio, BUN: Blood Urea Nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, T1: 100% *Alternanthera sessilis*, T2 : 75% *Alternanthera sessilis* and 25% *Tridax procumbens*, T3: 50% *Alternanthera sessilis* and 50% *Tridax procumbens*, T4: 25% *Alternanthera sessilis* and 75% *Tridax procumbens* and T5: 100% *Tridax procumbens*

CONCLUSION

The findings of this study indicate that a dietary combination of 25% *Alternanthera sessilis* and 75% *Tridax procumbens* yields the most favorable outcomes in terms of feed efficiency, hematological indices and serum biochemical parameters. This dietary blend supported optimal physiological performance without adverse effects, suggesting its suitability for inclusion in growing rabbit diets to enhance health, immunity and productivity.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Olufemi ALABI; E-mail: olufemi.alabi@bowen.edu.ng; ORCID: 0000-0002-4434-7968

Ethical approval

All procedures involving animals in this study were conducted in accordance with the ethical standards of the Bowen University Research Ethics Committee .All authors complied with the ARRIVE guidelines for the reporting of animal research.

Authors' contribution

Conceptualization and formal analysis: T. Adeyanju , O. Alabi, A.Oguntunji

Data curation: O.Oguntunji, O.Oladejo, T.Adeyanju, B.Adeleye

Investigation: T.Adeyanju, O.Okunlola, S.Oyewumi, B.Oyelami, O.Alabi

Methodology: T.Adeyanju, O.Alabi, A. Adewumi, A.Oguntunji

Validation: O.Alabi, A.Oguntunji, T.Adeyanju

Writing – original draft: T.Adeyanju, O.Alabi

Writing – review & editing: O.Alabi, A.Oguntunji, B. Adeleye

Consent to publish

All authors agreed to the publication of this manuscript.

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Data availability

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

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Competing interests

The authors declared that there is no competing interests before, during and after the data collection and preparation of the manuscript.

REFERENCES

- Abdullahi U, Yusuf HB, Wafar RJ, Mijinyawa A, Abubakar A, Shuaibu US, et al (2021). Growth performance, carcass characteristics and internal organ weights of weaner rabbits fed replacement levels of sun-dried soyabean milk residue. *Nigerian Journal of Animal Production*, 48(3):93-99. DOI: <https://doi.org/10.51791/njap.v48i3.2948>
- Adeniji AA, and Ehiermere S (2003). Effects of replacing maize with sorghum offal in the diets of weaner rabbits. *Journal of Agricultural Research and Development*, 2(1): 1-6. DOI: <https://doi.org/10.4314/jard.v2i1.42193>
- Aderemi FA and Alabi OM (2023). Turmeric (*Curcuma longa*): an alternative to antibiotics in poultry nutrition, *Translational Animal Science*, 7(1): txad133, DOI: <https://doi.org/10.1093/tas/txad133>
- Aderemi F, Alabi OM and Awe A (2015). Evaluation of biodegraded and undegraded plantain peels as replacement to wheat offal in broiler production NSTI: *Advanced Materials - TechConnect Briefs*, 3: 338–341. <https://briefs.techconnect.org/papers/evaluation-of-biodegraded-and->
- Adetunji MO and Adepoju AA (2011). Evaluation of household protein consumption pattern in Orire Local Government Area of Oyo state. *International Journal of Agricultural Economics and Rural Development*, 4(2):72-82. <https://api.semanticscholar.org/CorpusID:3736072>
- Arijeaniwa A, Bratte L, Oteku IT, and Ikchimioya I (2002). Performance of weaner rabbits fed varying levels of dietary raw lima beans (*Phaseolus luteus*). In *Proceedings of the 2002 Annual Conference of Animal Science Association of Nigeria (ASAN)*, pp: 182-185.
- Akererele D, Sanusi RA, Fadare OA and Ashaolu OF (2017): Factors influencing nutritional adequacy among rural households in Nigeria: How Does Dietary Diversity Stand among Influencers? *Ecology of Food and Nutrition*. 56: 187–203. DOI: <https://doi.org/10.1080/03670244.2017.1281127>
- Alabi OM, Ayoola MO and Akinoso SO (2017): .Performance characteristics and physiological response of broiler chickens at finisher stage to oral supplementation with fluted pumpkin, *Telfairia occidentalis* leaf extract. *Journal of Central European Agriculture*, 18(3): 646-656. DOI: <https://doi.org/10.5513/JCEA01/18.3.1938>
- Alabi OM, Olagunju SO, Aderemi FA, Lawal TE, Oguntunji AO, Ayoola MO, et al. (2024a). Effect of litter management systems on incidence and severity of footpad dermatitis among broilers at finisher stage, *Translational Animal Science*, 8: txad145. DOI: <https://doi.org/10.1093/tas/txad145>
- Alabi OM, Aworinde HO, Adebayo S, Akinwunmi AO, Ayandiji A and Tatar A (2024b). Data analytics-based evaluation of blood indices and adaptation of medicated and non-medicated broiler chickens under humid tropical conditions, *Translational Animal Science*, Volume, 8: txae040. DOI: <https://doi.org/10.1093/tas/txae040>
- Amos AT, Kareem DU, Adeleye TM, Adeyeye EA, Abatan MO, Ayorinde OS, et al. (2025). The effects of solid-state Fermentation by *Aspergillus* spp. on the nutritional profile of selected agro-industrial by-products as potential feedstuffs for weaner rabbits. *Fermentation*, 11(6):356. DOI: <https://doi.org/10.3390/fermentation11060356>
- Anwar F, Latif S, Ashraf M and Gidani AH (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*, 21(1): 17-25. DOI: <https://doi.org/10.1002/ptr.2023>
- Association of Official Analytical Chemists – AOAC (2000). *Official methods of analysis of the Association of Official Analytical Chemists: method 984.13* (17th ed.). Washington: AOAC
- Ayandiran SK, Odeyinka SM, Adedokun MA, Adekunle I, Kazeem AA and Okunlola NA (2020). Serum and hematological parameters of growing rabbits fed sorghum brewers dried grain as replacement for maize. *Agricultural Science Research Journal*. 10 (9): 254– 257. <https://www.researchgate.net/publication/365156614>
- Bhuyan B, Baishya K, and Rajak P (2018). Effects of *Alternanthera sessilis* on liver function in carbon tetra chloride induced hepatotoxicity in Wister rat model. *Indian Journal of Clinical Biochemistry*, 33(2):190-195. DOI: <https://doi.org/10.1007/s12291-017-0666-1>
- Chipo MM, Lawrence M, Tapiwa KA and Lovemore M (2019). Challenges and Opportunities to Rabbit (*Oryctolagus cuniculus*) Production and Marketing (A Case of Mazowe District, Zimbabwe). *International Journal of Agriculture and Agribusiness*, 5:37–44. <https://api.semanticscholar.org/CorpusID:222522214>
- Device JU and Lewis SM (1991). *Practical haematology*, 8th Edition. Longman Ltd., London, U.K. pp: 22-28
- Eshar D, Solotoff KL and Beaufriere H (2021). Comparison of plasma total solids concentration as measured by refractometry and plasma total protein concentration as measured by biuret assay in pet rabbits and ferrets. *Journal of the American Veterinary Medical Association*, 258(9): 977-982. DOI: <https://doi.org/10.2460/javma.258.9.977>
- Fahey JW (2005). *M. oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. *Trees for Life Journal*, 1(5):12-19. http://formad-environnement.org/Fahey_medicament_moringa_2005.pdf

- FAOSTAT (2022). Food Security and Nutrition in the World. <https://www.wfpusa.org/wp-content/uploads/2025/07/SOFI-Report-2022.pdf>
- Iheukwumere FC and Okoli IC (2002). Preliminary studies on raw *Napoleana imperialis* as feed ingredient. 1: Performance and blood chemistry of weaner rabbits. Tropical Animal Production, 5: 100-110.
- Jain NC (1993). Essentials of Veterinary Hematology. Lea and Febingers, Philadelphia, pp. 365-372.
- Khalid AR, Yasoob TB, Zhang Z, Yu D, Feng J, Zhu X and Hang S (2020). Supplementation of *Moringa oleifera* leaf powder orally improved productive performance by enhancing the intestinal health in rabbits under chronic heat stress. Journal of Thermal Biology, 93:102680. DOI: <https://doi.org/10.1016/j.jtherbio.2020.102680>
- Miassi YE, and Dossa KF (2023). Forecasting animal protein supply in Asia and Europe in light of climate change, population growth and land pressure. Tropical Plants, 2(1): 22. DOI: <https://doi.org/10.48130/TP-2023-0022>
- Michael JM (2011). Understanding clinical pathology in Rabbits. Proceedings of the North America Veterinary Conference Orlando, Florida.
- Minka NS and Ayo JO (2013). Physiological and behavioral responses of goats to 12-hour road transportation, lairage and grazing periods, and the modulatory role of ascorbic acid. Journal of Veterinary Behavior, 8: 349-356. DOI: <https://doi.org/10.1016/j.jveb.2013.01.001>
- Mufwa BJ, Kibon A, Mafindi M and Yakubu B (2011). Effect of graded levels of brewers dried grain on the performance of growing rabbits: Growth performance and economy of production. Journal of Sciences and Multidisciplinary Research, 3: 47-54. <https://www.cenresinjournal.com/wp-content/uploads/2020/03/page-47-54.pdf>
- Obinnea JI, Mmereoleb FUC and Okonkwo JC (2013). Growth response and hematological parameters of juvenile rabbits fed concentrate diets supplemented with varying levels of *Tridax procumbens*. Scientific Journal of Animal Science, 2(11): 310-315. DOI: <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20133426411>
- Odeyinka SM, Oyedele OJ, Adeleke TO and Odedire JA (2008). Reproductive performance of rabbits fed *Moringa oleifera* as a replacement for *Centrosema pubescens*. In: 9th World Rabbit Congress, June 10-13, Verona, Italy. DOI: <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20113318274>
- Ohshima S, Fukuma Y, Suzuki T, Funaba M and Abe M (1995). Validity of NRC method for estimating metabolizable energy value of laboratory dry canine diets. Experimental Animals, 44(1), 37-41. DOI: <https://doi.org/10.1538/expanim.44.37>
- Okorie KC (2003). The effect of palmitic acid fortified maize wet milling by-product on the performance of weaner rabbits. Czech Journal of Animal Science, 48(9): 365-370. <https://agris.fao.org/search/en/providers/122644/records/64723f5b53aa8c8963033b44>
- Oladimeji AM, Johnson TG, Metwally K, Farghly MFA and El-Hack MEA. (2021). Environmental heat stress in rabbits: implications and amelioration. International Journal of Biometeorology, 66(1): 1-11. DOI: <https://doi.org/10.1007/s00484-021-02191-0>
- Oluremi OIA, Gabriel OS, Ipirakwagh EN, Ikwue CO and Afolabi ET (2018). Performance and blood profile of rabbits fed biodegraded-sweet orange (*Citrus sinensis*) peel based diets. Nigerian Journal of Animal Science, 20(3), 287-294. Retrieved from <https://njas.org.ng/index.php/php/article/view/1105>
- Omoikhoje SCA, Bamgbose M, Aruna ME and Animashum RA (2006). Response of weaner rabbits to concentrate diets supplemented with varying levels of *Syndrella nodiflora* forage. Pakistan Journal of Nutrition; 5 (6): 577-579. DOI: <https://doi.org/10.3923/PJN.2006.577.579>
- Oyawoye EO and Ogunkunle M (1998). Physiological and biochemical effects of raw jack beans on broilers. Proceedings of annual Conference of Nigerian Society of Animal Production, 23: 141-142.
- Parasuraman S, Raveendran R and Kesavan A (2017). Blood sample collection in small Laboratory animal. Journal of pharmacology & pharmacotherapeutics 1(2): 87. DOI: <https://doi.org/10.4103/0976-500X.72350>
- Protein Challenge (2020). Nigeria Protein Deficiency Survey Report 2019. <https://proteinchallengeng.com/protein-deficiency-report-2019/>
- Samantha B, Heather, M, Toma T, Esli-Armando C and Olga RK (2018). A Review of medicinal uses and pharmacological activities of *Tridax procumbens* (L.). Journal of Plant Studies, 7(1): 19-35. DOI: <https://doi.org/10.5539/jps.v7n1p19>
- Sarkar T, Salaududdin M, Roy S, Chakraborty R, Rebezov M, Shariati MA, et al. (2022). Underutilized green leafy vegetables: frontier in fortified food development and nutrition. Critical Reviews in Food Science and Nutrition, 63(33): 11679-733. DOI: <https://doi.org/10.1080/10408398.2022.2095555>
- SAS (2005): Statistical Analysis Systems (SAS) 2005. Statistical Analysis. SAS, Cary.
- Semple JW, Italiano JE, and Freedman J (2011). Platelets and the immune continuum. Nature Reviews Immunology, 11(4): 264-274. DOI: <https://doi.org/10.1038/nri2956>
- Shehzad A, Qayyum A, Rehman R and Shehzad MR (2018). A review of bioactivity guided medicinal uses and therapeutic potentials of noxious weed (*Alternanthera sessilis*). International Journal of chemical and biochemical sciences, 14: 95-103. <https://www.ijscitific.org/wp-content/uploads/2020/05/11-IJCBS-18-14-11.pdf>
- Singla RK, Dhir V, Madaan R, Kumar D, Singh BS, Bansal M, et al. (2022). The genus alternanthera: phytochemical and ethnopharmacological perspectives. Frontiers in Pharmacology, 13: 769111. DOI: <https://doi.org/10.3389/fphar.2022.769111>
- Sonea C, Gheorghe-Irimia RA, Tăpăloagă D, and Tăpăloagă PR (2023). Nutrition and animal agriculture in the 21st century: A review of future prospects. Annals of the University of Craiova-Agriculture Montanology Cadastre Series, 53(1):303-312. DOI: <https://doi.org/10.52846/aamc.v53i1.1482>
- Vastolo A, Calabrò S, and Cutrignelli MI (2022). A review on the use of agro-industrial CO-products in animals' diets. Italian Journal of Animal Science, 21(1):577-594. DOI: <https://doi.org/10.1080/1828051X.2022.2039562>

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EFFECT OF SUPPLEMENTED *Cordyceps militaris* EXTRACT ON DISEASE RESISTANCE AND SURVIVAL RATE OF STRIPED CATFISH (*Pangasius hypophthalmus*) FINGERLINGS

Trinh Thi LAN^{1,3} , Nguyen Thi Thuy HANG^{1,3} , Nguyen Thi Bich HANH^{2,3} , Vo Phuong MAI^{2,3} , Phan Nguyen Thai VY^{1,3} , and Nguyen Huu Yen NHI^{1,3}  

¹Department of Aquaculture, An Giang University, An Giang, Vietnam

²Experimental – Practical Area, An Giang University, An Giang, Vietnam

³Vietnam National University Ho Chi Minh City (VNU-HCM), Ho Chi Minh City, Vietnam

✉ Email: nhynhi@agu.edu.vn

➤ Supporting Information



ABSTRACT: The effects of supplemented *Cordyceps militaris* extract in diets has been studied on the survival and immune response of striped catfish (*Pangasius hypophthalmus*) fingerlings with bacterial challenge conditions. The experiment was conducted for 60 days with six treatments and three replicates, including negative and positive controls with four dietary supplementation levels of *C. militaris* extract (0.18%, 0.24%, 0.36%, and 0.47%). After the feeding trial, striped catfish were challenged with *Edwardsiella ictaluri* at a dose of 10⁵ CFU/mL. Results showed that survival rates of striped catfish increased significantly with higher extract levels, reaching the highest value (70%) at 0.47% supplementation compared with only 16.67% in the positive control group. Hematological indices such as red blood cell (RBC) and white blood cell (WBC) including lymphocytes, monocytes, and neutrophils increased proportionally with extract concentration, which can indicate enhanced immune responses. In conclusion, the findings suggest that dietary supplementation of *C. militaris* extract at 0.47% improves the disease resistance and health status of striped catfish fingerlings against *E. ictaluri* infection. This result highlights the potential application of *C. militaris* extract as a natural immunostimulants to enhance survival and reduce antibiotic use in catfish aquaculture.

Keywords: *Cordyceps militaris*, *Edwardsiella ictaluri*, Immune response, *Pangasius hypophthalmus*, Survival rate.

INTRODUCTION

Cordyceps species are parasitic fungi that grow on insect larvae, pupae, or adult forms. The most well-known species, *Cordyceps sinensis*, was first identified in the high-altitude regions of the Tibetan Plateau, parasitizing larvae of moths (*Thitarodes* spp.) (Lo et al., 2013; Sheng and Gerasimova, 2024; Kumar and Sharma, 2025). Another related species, *Cordyceps militaris*, also parasitizes insects of the same genus and has attracted great attention for its rich bioactive compounds, particularly cordycepin and adenosine (Lo et al., 2013; Park, 2025; Jędrejko et al., 2021).

There is substantial evidence showing that *Cordyceps* fungi possess medicinal properties and contain a variety of bioactive compounds with therapeutic potential (Das et al., 2021). These compounds have been used in traditional medicine for treating malaria, palpitations, cancer, fever, diabetes, chronic kidney disease, and other disorders (Sharma et al., 2023). Beyond human medicine, the application of *Cordyceps militaris* as a dietary supplement has been extended to terrestrial animals such as pigs (Li et al., 2024; Cheng et al., 2016; Boontiam et al., 2020) and chickens (Wang et al., 2025; Barido et al., 2024; Lan et al., 2021), where it has shown positive effects on immune response and health status.

In aquaculture, *Cordyceps* supplementation has been investigated for several aquatic species (Tian et al., 2025; Sun et al., 2021; Lan et al., 2025; Da-oh et al., 2024; Choosong et al., 2025; Van Doan et al., 2018). Van Doan et al. (2017a); Van Doan et al. (2017b) demonstrated that adding powdered *C. militaris* at 10 g/kg feed improved the growth and health of Nile tilapia (*Oreochromis niloticus*). Similarly, Vietnamese aquaculture practitioners have reported beneficial effects of *Cordyceps* supplementation on shrimp hatchery performance, including improved post-larvae immunity, reduced stress in broodstock, and enhanced larval survival (Deng et al., 2015).

The striped catfish (*Pangasius hypophthalmus*) is one of Vietnam's most economically important aquaculture species, particularly in the Mekong Delta. However, its production faces major challenges from bacterial diseases such as bacillary necrosis of the kidney and liver (BNKL), caused by *Edwardsiella ictaluri*. This disease can lead to substantial mortality in nursery and grow-out stages, threatening farm productivity (Hoa et al., 2021; Tran et al., 2025). Therefore, the search for safe and natural immunostimulants to enhance disease resistance is critical for sustainable production.

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Given the proven bioactivity of *Cordyceps militaris* in promoting immunity and growth in terrestrial animals, this study aimed to evaluate the effects of dietary supplementation with *C. militaris* extract on the survival and immune responses of striped catfish fingerlings challenged with *Edwardsiella ictaluri*. The findings are expected to provide scientific evidence for the application of *Cordyceps* extracts as natural immunostimulants in catfish aquaculture.

MATERIALS AND METHODS

Experimental design

The experiment was conducted following a completely randomized design with six treatments and three replicates per treatment. Two control groups were used:

- Negative control: fish fed a commercial diet without extract and not challenged.
- Positive control: fish fed a commercial diet without extract and challenged with *E. ictaluri*.

Four other treatments included diets supplemented with *C. militaris* extract at 0.18%, 0.24%, 0.36%, and 0.47%, corresponding to 1.8 g, 2.4 g, 3.6 g, and 4.7 g extract per kg feed. Fish were reared in 500-L composite tanks at a density of 200 fish per tank for 60 days.

Feed preparation and feeding management

The *C. militaris* extract was dissolved in 100 mL distilled water and sprayed onto the feed to achieve the desired inclusion level for each treatment. The feed was then mixed thoroughly, air-dried at 60°C for 6 hours (mixed every 2 hours), and stored at -20°C until use. Fish were fed ad-libitum twice daily (07:30 and 17:00).

Bacterial challenge

After 60 days of feeding, fish were challenged with *Edwardsiella ictaluri* (isolated in An Giang University) by immersion in a 500 mL bacterial suspension at a dose of LD₅₀ 10⁵ CFU/mL (Hang et al., 2013; Hang and Phuong, 2020) for 30 minutes (except the negative control). After challenge exposure, fish were returned to their respective tanks for 14 days of observation. Mortality was recorded daily, and moribund fish were collected for bacterial re-isolation to confirm infection.

Hematological analysis

Ten fish per tank were randomly sampled before infection (0 h), 48 h post-infection, and 14 days post-infection for hematological analysis. Red blood cells (RBC), white blood cells (WBC), lymphocytes, monocytes, and neutrophils were counted using standard hemocytometer techniques following methods by Hrubec et al. (2000); Dung (2010) and Hang et al. (2025a).

Statistical analysis

Data were analyzed using the General Linear Model (GLM) in Minitab 16.0 (Minitab, 2010). One-way ANOVA was applied to compare mean differences among treatments, followed by Duncan's multiple range test at a significance level of $P < 0.05$. Results were expressed as mean $\pm$ standard error (SE).

RESULTS AND DISCUSSION

Survival rate

The survival rate of fingerlings significantly increased with higher levels of *C. militaris* extract ($P < 0.05$) (Table 1). The survival rate of *Pangasius* fingerlings was increased correspondingly with the increase in the rate of supplementing cordyceps extract. The highest survival (70%) was observed at 0.47% extract treatment, compared to 16.67% in the positive control treatment. Meanwhile, in the negative control treatment, the survival rate reached 100%, because the infection method and the setup conditions did not affect the experimental results. This proves that the diseased and dead fish in the remaining experiments were caused by *E. ictaluri* bacteria. Therefore, it can be concluded that 0.47% *Cordyceps* extract can be used to supplement the diet of *Pangasius* fry to help increase the survival rate when there is pathogenic *E. ictaluri* bacteria. The improvement in survival indicates that *C. militaris* extract enhances the host's resistance against *E. ictaluri*. Similar immunoprotective effects have been observed with inulin (Hang and Phuong, 2020) and *Lactobacillus plantarum* supplementation (Hang et al., 2022).

Research on *Cordyceps* extract on aquatic animals is still very new and can be said to be the first research at An Giang University and the Mekong Delta region. The results have shown that *Cordyceps* extract has great potential in the application to prevent diseases in *Pangasius* fry today. Compared with other studies on the effects of herbal extracts, probiotics, prebiotics or other immune stimulants, *Cordyceps* extract also has similar potential.

Many studies have demonstrated the effectiveness of supplementing beneficial biological substances and microorganisms to the diet to enhance resistance and reduce mortality in fish species when infected with bacteria.

Specifically, Adeshina et al. (2024) reported that supplementing alpha-lipoic acid at a dose of 1.5 to 2 g/kg of feed to the diet of catfish infected with *Edwardsiella tarda* significantly reduced mortality, to only 3.3% compared to 86.7% in the control group. In addition, the use of *Lactobacillus plantarum* at a density of 10^{12} CFU/kg of feed also showed positive effects, helping fish recover health after infection and achieving a survival rate of 56.7%.

Meanwhile, tests on *Pangasius* showed that inulin is a potential additive in improving growth and immunity. According to Hang and Phuong (2020) supplementing 1% inulin every two weeks helps fish grow well, while improving resistance to *Edwardsiella ictaluri* bacteria. Another study by Kattakdad et al. (2025) reported that supplementing 0.8% inulin in an effective functional feed additive for enhancing growth, immune defense, and tissue integrity in striped catfish culture. In addition, the survival rate of *Pangasius* fingerlings in this study was lower than the results of the trial of supplementing EPS to the diet of whiteleg shrimp (97.3%) and swamp eel fingerlings (96.67%) (Thanh et al., 2025; Hang et al., 2025a). Similarly, the study added 0.2% MOS and 0.6% MOS to the diet of carp (98.08%, 83.3%) (Staykov et al., 2005; Culjak et al., 2006).

Compared with the above results, the survival rate of *Pangasius* fingerlings in the present study reached 70%. This shows the obvious effectiveness of increasing the survival rate of the method of supplementing *Cordyceps sinensis* extract into the diet of *Pangasius* fingerlings. At the same time, it opens up new potential for practical application in *Pangasius* fingerlings.

Table 1 - The survival rate of catfish.

Treatments	Survival rate (%)
Negative control	100.00 ^a
Positive control	16.67 ^d
0.18%	56.67 ^c
0.24%	60.00 ^c
0.36%	60.00 ^c
0.47%	70.00 ^b
SEM	1.92
p-Value	0.000

^{a,b,c,d} Different letters following the mean values in the same column are statistically different ($p < 0.05$)

Red blood cells (RBC)

RBC counts showed no significant differences among treatments before infection with *Edwardsiella ictaluri* ($P > 0.05$). However, 48 hours and 14 days post-challenge, RBC counts were significantly higher in all extract-supplemented groups compared to the positive control, suggesting that *C. militaris* mitigated infection-induced anemia. The RBC concentration in fish fed 0.47% extract ($2.35\text{--}2.38 \times 10^6$ cells/mm³) was consistent with healthy catfish values reported by Dung (2010). The elevation in RBCs indicates better oxygen transport and physiological status, consistent with reports by Wilhelm Filho et al. (1992) and Hang and Hoa (2020).

Table 2 - Red blood cells of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^6 cell /mm³)

Treatments	RBC0H	RBC48H	RBC14D
Negative control	2.00	1.96 ^b	1.95 ^a
Positive control	2.27	1.16 ^b	1.32 ^b
0.18%	2.29	2.24 ^a	2.28 ^a
0.24%	2.29	2.19 ^a	2.24 ^a
0.36%	2.30	2.15 ^a	2.27 ^a
0.47%	2.31	2.35 ^a	2.38 ^a
SEM	0.08	0.08	0.12
P-value	0.135	0.000	0.001

RBC0H: red blood cell before infection; RBC48H: red blood cell after infection 48 hours; RBC14D: red blood cell after infection 14 days; ^{a,b,c,d} Different letters following the mean values in the same column are statistically different ($p < 0.05$).

Red blood cell (RBC) counts showed no significant differences among treatments before infection with *Edwardsiella ictaluri* ($P > 0.05$). This also shows that the red blood cell density before infection did not affect the experimental results. However, 48 hours and 14 days post-challenge with bacteria causing pyogenic liver and kidney disease, the red blood cell density results fluctuated and were different between the treatments, especially the positive control treatment ($P < 0.05$).

After 48 hours and 14 days of infection, the positive control treatment had the lowest red blood cell density, respectively 1.16×10^6 cells/mm³ and 1.32×10^6 cells/mm³, which was different from the remaining treatments. In particular, the experiments supplemented with *Cordyceps sinensis* extract showed that the red blood cell density did not differ between the treatments, but at the highest supplement concentration of 0.47%, the red blood cell density reached the highest level of 2.35×10^6 cells/mm³ and 2.38×10^6 cells/mm³. RBC counts were significantly higher in all extract-supplemented groups compared to the positive control, the red blood cell density increased correspondingly with the concentration of *Cordyceps sinensis* extract, suggesting that *C. militaris* mitigated infection-induced anemia. The RBC concentration in fish fed 0.47% extract ($2.35\text{--}2.38 \times 10^6$ cells/mm³) was consistent with healthy catfish values reported by Dung (2010).

Red blood cells play a very important role in the metabolism of fish, transporting oxygen and nutrients to nourish cells in the body and eliminating CO₂ from the fish body (Tu, 2000). The elevation in RBCs indicates better oxygen transport and physiological status, consistent with reports by Wilhelm Filho et al. (1992) and Hang and Hoa (2020). According to Wilhelm Filho et al. (1992), the red blood cell density of bony fish is determined to be 2.289×10^6 cells/mm³. Meanwhile, according to the research results of Hii et al. (2007) on freshwater eels (*Monopterus albus*), the red blood cell density is only about $1.05\text{--}1.10 \times 10^6$ cells/mm³. For healthy catfish, the red blood cell density is determined to be $1.79\text{--}2.75 \times 10^6$ cells/mm³ (Galagarza et al., 2017). At the same time, the number of red blood cells in the blood of freshwater fish varies greatly, depending on the stage of development, physiological status and activity of the fish, ranging from 1 - 3.5 million cells/mm³ (Tu, 2000).

Compared with other studies on supplementing products that help stimulate fish immunity, the results of red blood cell density in this study were also higher and still within the appropriate range. When *Pangasius* had white liver and white gill disease, the red blood cell density decreased to only 0.1×10^6 cells/mm³ (Hrubec et al., 2000). Compared with the study of adding 1% inulin to *Pangasius* feed, the red blood cell density only reached 1.99×10^6 cells/mm³ (Hang and Phuong, 2020). Supplementation vitamin C in feed on the hematology parameters of Mekong Giant Catfish (*Pangasianodon gigas*) got $2.34\text{--}2.64 \times 10^6$ cells/mm³ (Pimpimol et al., 2012). Or the study of adding 3% pomegranate extract to *Pangasius* feed, the red blood cell density reached 2.99×10^6 cells/mm³ (Hang and Hoa, 2020), which is equivalent to the results of adding cordyceps extract in this result. However, the red blood cell density when supplementing *Cordyceps sinensis* extract was lower than the study of supplementing probiotic *Lactobacillus acidophilus* on catfish fry at 3.10×10^6 cells/mm³. At the same time, it was lower than the research results of Hang et al. (2025b) when adding 8 g/kg EPS to the feed of swamp eel fingerling at 3.03×10^6 cells/mm³. Besides, the red blood cell results in this study were also much lower than the study of Hu et al. (2020) when adding vitamin C to the diet of eel, the red blood cell density reached 7.6×10^{12} cells/mm³.

White blood cells (WBC) and Immune Indices

White blood cells (WBC)

WBC counts increased significantly with extract supplementation after infection ($P < 0.05$). Fish fed 0.47% extract recorded WBC counts of 201.43×10^3 cells/mm³ at 48 hours and 644.35×10^3 cells/mm³ at 14 days, the highest among all treatments (Table 3).

Table 3 - White blood cells of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^3 cell/mm³).

Treatments	WBC0H	WBC48H	WBC14D
Negative control	68.32 ^c	67.96 ^c	78.32 ^c
Positive control	86.29 ^{bc}	65.33 ^c	100.48 ^c
0.18%	91.99 ^{ab}	141.83 ^b	303.57 ^b
0.24%	90.92 ^{ab}	136.67 ^b	369.19 ^b
0.36%	94.15 ^{ab}	152.34 ^b	525.46 ^a
0.47%	106.89 ^a	201.43 ^a	644.35 ^a
SEM	3.89	5.72	27.26
P-value	0.0001	0.0001	0.0001

WBC0H: white blood cell before infection; WBC48H: white blood cell after infection 48 hours; WBC14D: white blood cell after infection 14 days; a,b,c,d Different letters following the mean values in the same column are statistically different ($p < 0.05$).

Similar to the results of red blood cell density, white blood cell density also fluctuated when infected with bacteria causing pyogenic liver and kidney disease after 48 hours and 14 days. The results showed that the positive control experiment had the lowest white blood cell density and was different from the remaining experiments ($P < 0.05$). Meanwhile, the experiments supplemented with *Cordyceps* extract tended to increase gradually according to the concentration of the extract, so at a concentration of 0.48%, it reached the highest level after 48 hours and 14 days of bacterial infection, respectively (201.43×10^3 cells/mm³ and 644.35×10^3 cells/mm³). This result shows that adding *Cordyceps* extract to the diet of *Pangasius* fish at a concentration of 0.48% after only 48 hours of exposure to the

pathogen has been effective in preventing the disease. The function of white blood cells in fish blood is to destroy pathogens that attack the fish body and the number of white blood cells in fish blood is 10-100 times smaller than the number of red blood cells (Tu, 2000). According to Dung (2010), the density of white blood cells in the blood of healthy catfish is determined to be 1.0×10^6 cells/mm³. But when catfish have white liver and white gill disease, the white blood cell density is only 0.01×10^3 cells/mm³ (Hrubec et al., 2000). Compared with the results of the study on supplementing probiotic *Lactobacillus acidophilus* into the catfish diet, the white blood cell density is only 25×10^3 cells/mm³ (Al-Dohail et al., 2009), much lower than the addition of 0.48% Cordyceps extract in this study. But it is much lower than the result of adding 68.60 mg/kg of vitamin to the feed of freshwater eels, reaching 16.68×10^{10} cells/mm³ (Hu et al., 2020). In addition, the result of leukocyte density when adding 0.48% of cordyceps extract is also much lower than the result of the study adding 8 g/kg EPS to the diet of freshwater eels, reaching 1.4×10^6 cells/mm³ (Hang et al., 2025b). At the same time, it is also lower than the study of adding 1% inulin, reaching 232.45×10^3 cells/mm³ (Zhou et al., 2020). At the same time, the results of determining the white blood cell density of this study were also higher than the results of the study adding 3% pomegranate extract to *Pangasius* feed (Hang and Hoa, 2020) or the study adding 1.2% inulin in diets of striped catfish (Kattakdad et al., 2025). The results recorded in this study show that 0.48% of cordyceps extract can be used to supplement the diet of *Pangasius* to bring high disease prevention efficiency.

Immune indices

Lymphocytes, monocytes, and neutrophils followed similar trends of WBC, indicating enhanced cellular immunity (Table 4). The increase in lymphocytes implies a stronger adaptive immune response (Houston, 1997; Groff and Zinkl, 1999); , while elevated monocyte and neutrophil counts reflect activation of innate defenses (Yano, 1992; Magnadóttir, 2006). Comparable immune enhancements were reported in fish fed β -glucan (Hoang et al., 2024) and *Eclipta alba* extract (Harikrishnan et al., 2009).

Table 4 - Immune indices (lymphocytes, monocytes, and neutrophils) of *Pangasius* before and after infection with *Edwardsiella ictaluri* bacteria (10^3 cell /mm³)

Treatments	Negative control	Positive control	0.18%	0.24%	0.36%	0.47%	SEM	P-value
Immune indices								
<i>Lymphocytes</i>								
Lym0H	6.62 ^d	4.75 ^d	11.00 ^{cd}	23.9 ^{bc}	33.14 ^b	53.50 ^a	2.90	0.0001
Lym48H	8.89 ^d	8.61 ^d	32.50 ^c	36.29 ^c	53.53 ^b	81.95 ^a	3.17	0.0001
Lym14D	16.64 ^d	16.03 ^d	84.07 ^{cd}	117.40 ^c	223.60 ^b	315.50 ^a	18.61	0.0001
<i>Monocytes</i>								
Mono0H	6.45 ^{bc}	5.90 ^c	10.52 ^{bc}	10.21 ^{bc}	12.78 ^b	21.80 ^a	1.37	0.0001
Mono48H	8.74 ^c	7.33 ^c	16.96 ^b	17.79 ^b	21.11 ^b	33.44 ^a	1.40	0.0001
Mono14D	18.96 ^d	18.70 ^d	98.08 ^{cd}	136.97 ^c	260.87 ^b	368.08 ^a	21.71	0.0001
<i>Neutrophils</i>								
Neu0H	10.92 ^c	14.21 ^c	20.22 ^c	20.23 ^c	48.89 ^b	68.79 ^a	3.97	0.0001
Neu48H	12.93 ^d	9.65 ^d	36.40 ^c	40.64 ^c	59.96 ^b	91.79 ^a	3.53	0.0001
Neu14D	23.46 ^d	21.38 ^d	112.10 ^{cd}	156.50 ^c	298.10 ^b	420.70 ^a	24.81	0.0001

Lym0H: Lymphocytes before infection; Lym48H: Lymphocytes after infection 48 hours; Lym14D: Lymphocytes after infection 14 days; Mono0H: Monocytes before infection; Mono48H: Monocytes after infection 48 hours; Mono14D: Monocytes after infection 14 days; Neu0H: Neutrophils before infection; Neu48H: Neutrophils after infection 48 hours; Neu14D: Neutrophils after infection 14 days; ^{a,b,c,d} Different letters following the mean values in the same row are statistically different ($p < 0.05$).

Lymphocytes play an essential role in the immune defense mechanism of fish, especially in recognizing and destroying pathogens such as bacteria and parasites. They are one of the key cell types involved in specific immunity, contributing to stimulating macrophages to produce antibodies and enhancing the overall immune response (Houston, 1997). In some fish species, lymphocytes account for a high proportion of the total leukocytes, up to 85%, indicating their prominent role in the immune system of fish (Groff and Zinkl, 1999). The increase in the number of lymphocytes may be related to the ability to adapt and better respond to adverse environmental conditions. In addition, the increase in the number of lymphocytes after infection is a normal physiological phenomenon in fish species, demonstrating the core role of this cell in the specific immune mechanism (Magnadóttir, 2006).

The results from Table 4 show that the number of lymphocytes fluctuated in a significant increasing trend corresponding to the concentration of Cordyceps extract supplementation and gradually increased over time. In the positive control group, the number of lymphocytes remained low at 48 hours and 14 days after infection. In contrast, the treatment supplemented with 0.47% Cordyceps extract gave outstandingly high results, especially at the time after 12

days of infection, reaching the highest level of 315.5×10^3 cells/mm³, proving that this concentration strongly stimulates the immune system and has a high disease prevention effect.

For healthy *Pangasius*, the number of lymphocytes is 81×10^3 cells/mm³, but in diseased *Pangasius* with white liver and white gills, the number was only 0.25×10^3 cells/mm³ (Dung, 2010). This result shows that the number of lymphocytes in healthy *Pangasius* fish will be equivalent to the number of lymphocytes in *Pangasius* fish supplemented with 0.47% cordyceps extract after only 48 hours of infection with bacteria causing pyogenic liver and kidney disease. However, the lymphocyte results in this study were much lower than the study of supplementing 8 g/kg EPS to the diet of young eels, reaching 2.5×10^6 cells/mm³. In addition, Oligochitosan (COS) and oligo- β -glucan (β OG) show strong potential as immunostimulants and growth-enhancing additives for *Pangasianodon hypophthalmus*, as they are capable of stimulating innate immune parameters, improving disease resistance, and promoting growth performance in aquaculture systems (Nguyen et al., 2017). At the same time, the results of this study are also consistent with the results of (Rattanachaiakunsopon and Phumkhachorn, 2009), when using *Andrographis paniculata* extract on tilapia (*Oreochromis niloticus*), also showed a significant increase in the number of lymphocytes in the blood of the fish. Similarly, the study by (Harikrishnan et al., 2009) on *Sparus aurata* fish when using *Eclipta alba* plant extract showed a sharp increase in the number of lymphocytes and enhanced antibody production, helping the fish to improve their ability to prevent diseases caused by *Vibrio harveyi*.

Thus, the results of lymphocytes in this study have opened up the potential for applying *Cordyceps* extract to stimulate immunity in *Pangasius* fish with the most effective addition concentration of 0.47%. The results in Table 4 showed that the number of monocytes increased significantly according to the concentration of supplementation after 48 hours and 12 days of infection. In which, the positive control group had the lowest number of monocytes and was different from the remaining treatments, while the 0.47% supplemented treatment reached the highest number at 48 hours and 12 days, respectively, at 33.44×10^3 cells/mm³ and increased sharply at 14 days after infection to 368.08×10^3 cells/mm³. This clearly reflects the stimulation of the fish's immune system. Because monocytes are white blood cells that play an important role in the fish's immune system when pathogens invade (Tu, 2000). When compared with other research results on aquatic species, the quantity and function of monocytes also tend to increase when there is a pathogen attack or when there is an addition of immunostimulants in the fish diet (Aly et al., 2008; Hang et al., 2025b; Misra et al., 2006). For example, according to the results of Aly et al. (2008), tilapia (*Oreochromis niloticus*) when supplemented with the immunostimulant β -glucan was recorded to not only increase the number of monocytes, but also increase the ability of macrophages. Similarly, Misra et al. (2006) reported that common carp (*Labeo rohita*) after infection with *Aeromonas hydrophila* had a significant increase in monocytes, reflecting the core role of this cell line in the cellular immune response. In addition, the results of (Harikrishnan et al., 2011) also showed that the use of plant extracts such as *Withania somnifera* in sea bream (*Sparus aurata*) increased the density of monocytes, thereby enhancing the ability to fight against pathogenic bacteria. This result was also consistent with the increasing trend of monocytes, especially in the treatments with high concentrations of 0.36% and 0.47%.

The results of neutrophil counts also showed a similar trend as monocytes, lowest in the positive control and highest in the 0.47% treatment (Table 4). This suggests that, when supplemented at a concentration of 0.47%, fish may have strongly mobilized the number of neutrophils. This is the first important cell type in detecting and destroying pathogens (Tu, 2000). This result is consistent with previous studies such as those of Reyes-Becerril et al. (2011), which recorded a significant increase in the number of neutrophils in sea bream (*Sparus aurata*) when injected with *Aeromonas hydrophila*, a component of Gram-negative bacteria. Similarly, Magnadóttir (2006) described the key role of neutrophils and macrophages in the acute inflammatory response in fish, especially the ability to phagocytose and secrete enzymes to destroy bacteria. Studies on grass carp (*Ctenopharyngodon idella*) supplemented with β -glucan also showed an increase in the number and activity of neutrophils, contributing to enhanced non-specific defense (Yano, 1992).

These findings suggest that *C. militaris* extract stimulates both specific and non-specific immune mechanisms, leading to improved disease resistance against *E. ictaluri*. The dose-dependent pattern also supports the hypothesis that bioactive compounds such as cordycepin and adenosine contribute to hematological and immunostimulatory effects.

CONCLUSION

The results of the study showed that the addition of *Cordyceps militaris* extract to the diet of *Pangasius* fingerlings significantly improved the survival rate and enhanced the immune response when the fish were infected with *Edwardsiella ictaluri* bacteria causing pyogenic liver and kidney disease. At the addition concentration of 0.47%, the fish achieved the highest survival rate (70%) and had significantly increased red blood cell, white blood cell, lymphocyte, monocyte and neutrophil indices compared to the positive control. This proves that *Cordyceps militaris* extract has the ability to stimulate the immune system and enhance the natural resistance of *Pangasius* fish. Therefore, it is recommended to use *Cordyceps militaris* extract at a rate of about 0.47% in the diet of *Pangasius* fingerlings to increase disease prevention and reduce dependence on antibiotics in aquaculture.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Nguyen Huu Yen Nhi; E-mail: nhynhi@agu.edu.vn; ORCID: 0000-0002-6527-9618

Data availability

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

Authors' contribution

Conceptualization, resources, supervision and funding acquisition: Lan T.T. and Nhi N.H.Y.;
Methodology, validation, and data curation: Lan T.T., Nhi N.H.Y., and Hang N.T.T.;
Formal analysis and investigation: Nhi N.H.Y., Lan T.T., Hanh N.T.B., Vy P.N.T., Mai V.P., and Hang N.T.T.;
Writing—original draft preparation, visualization: Nhi N.H.Y. and Hang N.T.T.;
Writing—review and editing: Nhi N.H.Y., Lan T.T. and Hang N.T.T.;
Project administration: Lan T.T.
All authors have read and agreed to the published version of the manuscript.

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Competing interests

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

REFERENCES

- Adeshina I, Paray BA, Bhat EA, Ibrahim AD and Tihami LO (2024). Stimulatory effect of dietary alpha-lipoic acid on growth performance, antioxidant capacity, liver enzymes, immunity and protection of African catfish, *Clarias gariepinus* (B.), Edwardsiella tarda infection. *Journal of Animal Physiology and Animal Nutrition* 108(1): 163-173. <https://doi.org/10.1111/jpn.13876>
- Al-Dohail MA, Hashim R and Aliyu-Paiko M (2009). Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquaculture Research* 40(14): 1642-1652. <https://doi.org/10.1111/j.1365-2109.2009.02265.x>
- Aly SM, Ahmed YA-G, Ghareeb AA-A and Mohamed MF (2008). Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. *Fish & shellfish immunology* 25(1-2): 128-136. <https://doi.org/10.1016/j.fsi.2008.03.013>
- Barido FH, Hertanto BS, Cahyadi M, Kartikasari LR, Sujiwo J, Kim J, Kim H-Y, Jang A and Lee SK (2024). Enzymatic hydrolyzation of *Cordyceps militaris* mushroom extracts and its effect on spent hen chicken. *Animal Bioscience* 37(7): 1277. <https://doi.org/10.5713/ab.23.0518>
- Boontiam W, Wachirapakorn C and Wattanachai S (2020). Growth performance and hematological changes in growing pigs treated with *Cordyceps militaris* spent mushroom substrate. *Veterinary world* 13(4): 768. <https://doi.org/10.14202/vetworld.2020.768-773>
- Cheng Y, Wen C, Dybus A and Proskura W (2016). Fermentation products of *Cordyceps militaris* enhance performance and modulate immune response of weaned piglets. *South African Journal of Animal Science* 46(2): 121-128. <https://doi.org/10.4314/sajas.v46i2.2>
- Choosong K, Kiriratnikom S, Pewhom A and Nitipan S (2025). Enhancing digestive and immune functions in Nile tilapia: Effects of *Pediococcus acidilactici* and *Cordyceps militaris* spent mushroom substrate extracts and their combination. *Aquaculture Reports* 45: 103093. <https://doi.org/10.1016/j.aqrep.2025.103093>
- Culjak V, Bogut I, Has-Schön E, Milaković Z and Canecki K (2006). Effect of Bio-Mos* on performance and health of juvenile carp. *Nutritional Biotechnology in the Feed and Food Industries*. 90-90. <https://www.croris.hr/crosbi/publikacija/prilog-skup/544728>
- Da-oh H, Kiriratnikom S, Choosong K and Nitipan S (2024). Evaluation of spent mushroom substrate extracts from *Cordyceps militaris*: Impact on growth Performance, Immunomodulation, and intestinal microbiota of Nile tilapia. *Egyptian Journal of Aquatic Research* 50(4): 577-584. <https://doi.org/10.1016/j.ejar.2024.11.001>
- Das G, Shin H-S, Leyva-Gómez G, Prado-Audelo MLD, Cortes H, Singh YD, Panda MK, Mishra AP, Nigam M and Saklani S (2021). *Cordyceps* spp.: A review on its immune-stimulatory and other biological potentials. *Frontiers in Pharmacology* 11: 602364. <https://doi.org/10.3389/fphar.2020.602364>
- Deng B, Wang Z, Tao W, Li W, Wang C, Wang M, Ye S, Du Y, Wu X and Wu D (2015). Effects of polysaccharides from mycelia of *Cordyceps sinensis* on growth performance, immunity and antioxidant indicators of the white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition* 21(2): 173-179. <https://doi.org/10.1111/anu.12147>
- Dung TT (2010). Research on hematology of striped catfish (*Pangasianodon hypophthalmus*) affected by white liver and white gill disease. *Can Tho University Journal of Science* (15b): 81-90. <https://ctujsvn.ctu.edu.vn/index.php/ctujsvn/article/view/818>

- Galagarza OA, Kuhn DD, Smith SA and Hrubec TC (2017). Hematologic and plasma chemistry RIs for cultured striped catfish (*Pangasius hypophthalmus*) in recirculating aquaculture systems. *Veterinary Clinical Pathology* 46(3): 457-465. <https://doi.org/10.1111/vcp.12513>
- Groff JM and Zinkl JG (1999). Hematology and clinical chemistry of cyprinid fish: common carp and goldfish. *Veterinary clinics of North America: Exotic animal practice* 2(3): 741-776. [https://doi.org/10.1016/S1094-9194\(17\)30120-2](https://doi.org/10.1016/S1094-9194(17)30120-2)
- Hang BT, Balami S and Phuong NT (2022). Effect of *Lactobacillus plantarum* on growth performance, immune responses, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture, Aquarium, Conservation & Legislation* 15(1): 174-187. <https://bioflux.com.ro/docs/2022.174-187.pdf>
- Hang BTB and Hoa TTT (2020). Effects of Pomegranate (*Punica granatum*) extract on growth performance and immune responses of striped catfish (*Pangasianodon hypophthalmus*). *CTU Journal of Science* 56(Special issue on Aquaculture and Fisheries): 161-169. <https://doi.org/10.22144/ctu.jsi.2020.018>
- Hang BTB, Milla S, Gillardin V, Phuong NT and Kestemont P (2013). In vivo effects of *Escherichia coli* lipopolysaccharide on regulation of immune response and protein expression in striped catfish (*Pangasianodon hypophthalmus*). *Fish & shellfish immunology* 34(1): 339-347. <https://doi.org/10.1016/j.fsi.2012.11.025>
- Hang BTB and Phuong NT (2020). Effects of dietary inulin supplementary duration on immune responses of striped catfish (*Pangasianodon hypophthalmus*). *CTU Journal of Science* 56(2): 100-109. <https://doi.org/10.22144/ctu.jvn.2020.036>
- Hang NTT, Lan TT and Nhi NHY (2025a). Evaluation of the effect of extracellular polymeric substance (EPS) from *Lactobacillus plantarum* (LP) on the disease resistance of rice field eel (*Monopterus albus*) fingerlings. *Journal of Global Innovations in Agricultural Sciences*: 1095-1101. <https://doi.org/10.22194/JGIAS/25.1700>
- Hang NTT, Lan TT, Nhi NHY, Hanh NTB and Thanh NH (2025b). Evaluation of the effects of extracellular polymeric substance (EPS) produced from *Lactobacillus plantarum* on growth performance and survival rate of ricefield eel (*Monopterus albus*) fingerlings. *Egyptian Journal of Aquatic Biology & Fisheries* 29 (4)(4). <https://doi.org/10.21608/ejabf.2025.445041>
- Harikrishnan R, Balasundaram C and Heo M-S (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture* 317(1-4): 1-15. <https://doi.org/10.1016/j.aquaculture.2011.03.039>
- Harikrishnan R, Balasundaram C, Kim M-C, Kim J-S, Han Y-J and Heo M-S (2009). Innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. *Fish & shellfish immunology* 27(3): 508-515. <https://doi.org/10.1016/j.fsi.2009.07.004>
- Hii YS, Lee MY and Chuah TS (2007). Acute toxicity of organochlorine insecticide endosulfan and its effect on behaviour and some hematological parameters of Asian swamp eel (*Monopterus albus*, Zuiew). *Pesticide biochemistry and physiology* 89(1): 46-53. <https://doi.org/10.1016/j.pestbp.2007.02.009>
- Hoa TTT, Boerlage AS, Duyen TTM, Thy DTM, Hang NTT, Humphry RW and Phuong NT (2021). Nursing stages of striped catfish (*Pangasianodon hypophthalmus*) in Vietnam: Pathogens, diseases and husbandry practices. *Aquaculture* 533: 736114. <https://doi.org/10.1016/j.aquaculture.2020.736114>
- Hoang D-H, Thuy NTT and Ky PX (2024). A synergistic effect of dietary β -glucan and mannan oligosaccharide on growth performance, haematology, body composition, nutrient utilisation, and intestinal morphology in pompano, *Trachinotus ovatus*. *Regional Studies in Marine Science* 73: 103494. <https://doi.org/10.1016/j.rsma.2024.103494>
- Houston AH (1997). Are the classical hematological variables acceptable indicators of fish health? *Transactions of the American Fisheries Society* 126(6): 879-894. [https://doi.org/10.1577/1548-8659\(1997\)126%3C0879:RATCHV%3E2.3.CO;2](https://doi.org/10.1577/1548-8659(1997)126%3C0879:RATCHV%3E2.3.CO;2)
- Hrubec TC, Cardinale JL and Smith SA (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Veterinary clinical pathology* 29(1): 7-12. <https://doi.org/10.1111/j.1939-165X.2000.tb00389.x>
- Hu Y, Zhang J, He L, Hu Y, Zhong L, Dai Z and Zhou D (2020). Effects of dietary vitamin C on growth, antioxidant activity, and immunity in ricefield eel (*Monopterus albus*). *Journal of the World Aquaculture Society* 51(1): 159-170. <https://doi.org/10.1111/jwas.12636>
- Jędrejko KJ, Lazur J and Muszyńska B (2021). *Cordyceps militaris*: An overview of its chemical constituents in relation to biological activity. *Foods* 10(11): 2634. <https://doi.org/10.3390/foods10112634>
- Kattakdad S, Phungam N, Pongket U, Muangmala W, Udduang S, Aripin SA and Yuangsoi B (2025). Microencapsulated inulin as a prebiotic: Enhancing growth, digestive enzyme activity, and immune response in striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture Reports* 43: 102986. <https://doi.org/10.1016/j.aqrep.2025.102986>
- Kumar G and Sharma A (2025). The Parasitic Power of *Cordyceps*. https://vigyanvarta.in/adminpanel/upload_doc/VV_0225_47.pdf
- Lan J, Chen G, Cao G, Tang J, Li Q, Zhang B and Yang C (2021). Effects of α -glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens. *Poultry Science* 100(3): 100875. <https://doi.org/10.1016/j.psj.2020.11.052>
- Lan TT, Hang NTT, Hanh NTB, Dang ND, Mai VP and Nhi NHY (2025). Improving the growth performance of striped catfish (*Pangasius hypophthalmus*) fingerlings through supplementation with *Cordyceps militaris* extract. *Livestock Research for Rural Development* 37 (3) 2025. <https://lrrd.cipav.org.co/lrrd37/3/37311tla.html>
- Li Y, Lu Y, Yu B, Huang Z, Luo Y, Zheng P, Mao X, Yu J, Luo J and Yan H (2024). Effect of *Cordyceps militaris* on growth performance, antioxidant capacity, and intestinal epithelium functions in weaned pigs. *Journal of Animal Science* 102: skae194. <https://doi.org/10.1093/jas/skae194>
- Lo H-C, Hsieh C, Lin F-Y and Hsu T-H (2013). A systematic review of the mysterious caterpillar fungus *ophiocordyceps sinensis* in DongChongXiaCao (冬蟲夏草 *dōng chōng xià cǎo*) and related bioactive ingredients. *Journal of traditional and complementary medicine* 3(1): 16-32. [https://doi.org/10.1016/S2225-4110\(16\)30164-X](https://doi.org/10.1016/S2225-4110(16)30164-X)
- Magnadóttir B (2006). Innate immunity of fish (overview). *Fish & shellfish immunology* 20(2): 137-151. <https://doi.org/10.1016/j.fsi.2004.09.006>
- Minitab (2010). Minitab 16 Statistical Software 16.2.0. <https://www.minitab.com/en-us/products/minitab/>
- Misra CK, Das BK, Mukherjee SC and Pattnaik P (2006). Effect of multiple injections of β -glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. *Fish & shellfish immunology* 20(3): 305-319. <https://doi.org/10.1016/j.fsi.2005.05.007>
- Nguyen ND, Van Dang P, Le AQ, Nguyen TKL, Pham DH, Van Nguyen N and Nguyen QH (2017). Effect of oligochitosan and oligo- β -glucan supplementation on growth, innate immunity, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*). *Biotechnology and applied biochemistry* 64(4): 564-571. <https://doi.org/10.1002/bab.1513>

- Park H-J (2025). Influence of Culture Conditions on Bioactive Compounds in *Cordyceps militaris*: A Comprehensive Review. *Foods* 14(19): 3408. <https://doi.org/10.3390/foods14193408>
- Pimpimol T, Phoosamran K and Chitmanat C (2012). Effect of dietary Vitamin C supplementation on the blood parameters of Mekong Giant Catfish (*Pangasianodon gigas*). *International Journal of Agriculture & Biology* 14(2). <https://www.cabidigitallibrary.org/doi/full/10.5555/20123099879>
- Rattanachaikunsopon P and Phumkhachorn P (2009). Prophylactic effect of *Andrographis paniculata* extracts against *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*). *Journal of bioscience and bioengineering* 107(5): 579-582. <https://doi.org/10.1016/j.jbiosc.2009.01.024>
- Reyes-Becerril M, López-Medina T, Ascencio-Valle F and Esteban MÁ (2011). Immune response of gilthead seabream (*Sparus aurata*) following experimental infection with *Aeromonas hydrophila*. *Fish & Shellfish Immunology* 31(4): 564-570. <https://doi.org/10.1016/j.fsi.2011.07.006>
- Sharma H, Sharma N and An SSA (2023). Unique bioactives from zombie fungus (*Cordyceps*) as promising multitargeted neuroprotective agents. *Nutrients* 16(1): 102. <https://doi.org/10.3390/nu16010102>
- Sheng J and Gerasimova K (2024). *Sustainable Management of Cordyceps: Supply Chains and Resource Management Policies*: Taylor & Francis. <https://www.routledge.com/Sustainable-Management-of-Cordyceps-Supply-Chains-and-Resource-Management-Policies/Sheng-Gerasimova/p/book/9781032548159>
- Staykov Y, Spring P and Denev S (2005). Influence of dietary Bio-Mos® on growth, survival and immune status of rainbow trout (*Salmo gairdneri* irideus G.) and common carp (*Cyprinus carpio* L.), 333-343.. <https://www.cabidigitallibrary.org/doi/full/10.5555/20063210034>
- Sun Y, Rabbi MH, Ma S, Wen Z, Li X, Mi R, Meng N, Li Y, Wang Q and Du X (2021). Effect of dietary *Cordyceps* polysaccharide supplementation on intestinal microflora and immune response of *Apostichopus japonicus*. *Aquaculture Research* 52(11): 5198-5212. <https://doi.org/10.1111/are.15389>
- Thanh NH, Lan TT, Hang NTT, Nhi NHY and Tho NP (2025). Effect of dietary Extracellular polymeric substances from Lactic acid bacteria on disease resistance and immune response in the whiteleg shrimp (*Litopenaeus vannamei*). *Veterinary Integrative Sciences* 23(3): 1-16. <https://doi.org/10.12982/VIS.2025.061>
- Tian F, Li S, Xiao H, Li W, Wang H, Wang Y, Han L, Zhao C, Chang Y and Ding J (2025). The effects of dietary *Cordyceps militaris* substrate on growth, immunity, gonadal quality, and gut microbiota of the sea urchin *Strongylocentrotus intermedius*. *Animal Feed Science and Technology*: 116428. <https://doi.org/10.1016/j.prevetmed.2025.106527>
- Tran VL, Barnes AC, Samsing F, Vu UN and Wiley K (2025). Striped catfish (*Pangasianodon hypophthalmus*) farmers' perspectives on challenges and health management practices in the Mekong Delta, Vietnam: A qualitative study. *Preventive Veterinary Medicine* 239: 106527. <https://doi.org/10.1016/j.prevetmed.2025.106527>
- Tu NV (2000). Textbook of Aquatic Animal Physiology. <https://caf.ctu.edu.vn/en/images/upload/AUN2022/AQ211.pdf>
- Van Doan H, Hoseinifar SH, Dawood MA, Chitmanat C and Tayyath K (2017a). Effects of *Cordyceps militaris* spent mushroom substrate and *Lactobacillus plantarum* on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). *Fish & shellfish immunology* 70: 87-94. <https://doi.org/10.1016/j.fsi.2017.09.002>
- Van Doan H, Hoseinifar SH, Tapingkae W, Chitmanat C and Mekchay S (2017b). Effects of *Cordyceps militaris* spent mushroom substrate on mucosal and serum immune parameters, disease resistance and growth performance of Nile tilapia, (*Oreochromis niloticus*). *Fish & shellfish immunology* 67: 78-85. <https://doi.org/10.1016/j.fsi.2017.05.062>
- Van Doan H, Mekchay S and Chitmanat APDC (2018). The use of prebiotics (from corncobs and *Cordyceps militaris* mushroom) and probiotics as alternative strategies for antibiotics in culturing of tilapia, *Oreochromis niloticus*. *Thailand Science Research and Innovation*. https://elibrary.tsri.or.th/fullIP/MRG5980127/MRG5980127_full.pdf
- Wang X, Zhang J, Zhang K, Xu G, Guo Z, Gu X, Ahmad S, Huang L, Liu S and Li J (2025). Effects of *Cordyceps militaris* solid medium on the growth performance, immunity parameters and intestinal health of broilers. *Animal Bioscience* 38(9): 1959. <https://doi.org/10.5713/ab.24.0791>
- Wilhelm Filho D, Eble GJ, Kassner G, Caprario FX, Dafré AL and Ohira M (1992). Comparative hematology in marine fish. *Comparative Biochemistry and Physiology Part A: Physiology* 102(2): 311-321. [https://doi.org/10.1016/0300-9629\(92\)90141-C](https://doi.org/10.1016/0300-9629(92)90141-C)
- Yano T (1992). Assay of hemolytic complement activity. *Techniques in fish immunology*: 131-141. <https://cir.nii.ac.jp/crid/1574231875535834880>
- Zhou L, Li H, Qin JG, Wang X, Chen L, Xu C and Li E (2020). Dietary prebiotic inulin benefits on growth performance, antioxidant capacity, immune response and intestinal microbiota in Pacific white shrimp (*Litopenaeus vannamei*) at low salinity. *Aquaculture* 518: 734847. <https://doi.org/10.1016/j.aquaculture.2019.734847>

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COMPARISON OF THE SERUM MINERALS THAT PROMOTE BONE HEALING IN HEALTHY AND CLINICALLY SICK GOATS IN BANGLADESH

Saroj Kumar YADAV^{1,2}  , Sunil YADAV³ , Md. AHADUZZAMAN¹ , Monoar Sayeed PALLAB¹ , Bibek Chandra SUTRADHAR¹ , and Bhajan CHANDRA DAS¹ 

¹Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Chattogram-4225, Bangladesh

²Raaz Veterinary Hospital and Research Center, JanakpurDham, 13, Nepal

³Department of Anatomy and Histology, Gazipur Agricultural University (GAU), Bangladesh

✉ Email: shirfraaz@gmail.com

➤ Supporting Information



ABSTRACT: Serum minerals such as calcium, phosphorous, and magnesium are essential for the skeletal system during bone formation and remodeling as well as fracture repair. Present study was intended to assess the serum biochemical parameters of three of the most important macro minerals (calcium, phosphorus, and magnesium), which are key minerals for bone healing or development in healthy and clinical sick goats. Serum samples were collected from a total of 200 goats (healthy=102, clinically sick=98) from a veterinary hospital and three farms of Chattogram, Bangladesh. The overall mean serum calcium level of healthy goats was 8.47 ± 0.11 mg/dl and in sick goats it was 7.63 ± 0.12 mg/dl, and the value was much lower than the reference value in sick goats. The serum phosphorus and magnesium levels did not vary between the two groups and were within (phosphorus) or close to (magnesium) the range of reference values. In healthy goats, their serum calcium level varied significantly considering age, body weight, sex, feeding type, and owner's income. However, the serum phosphorus and magnesium levels did not vary significantly across the different parameters tested, except for sex and owner's income in the case of phosphorus. Three sick goats had hypocalcemia, which means that their calcium and magnesium levels were lower than the standard values of 5.9 ± 0.5 and 1.9 ± 0.2 mg/dl, respectively. However, their phosphorus levels were normal across all groups of sick goats. In conclusion, the value of an important mineral for bone healing is lower in both sick and healthy goats, which is a frightening sign for bone problems. Most goats have low levels of calcium and magnesium but average levels of phosphorous. Based on these findings, sufficient calcium and magnesium can be added to feed and given orally to further prevent bone fracture.

Keywords: Calcium, Clinically sick animals, Feeding type, Indigestion problems, Magnesium, South Asian region.

INTRODUCTION

Calcium, phosphorus, and magnesium are essential minerals in animal feed, playing crucial roles in bone healing, glucose absorption, urine formation, protein metabolism, and pH regulation (Moniem et al., 2014; Yadav et al., 2024). In Bangladesh, research on the serum minerals and trace elements in goats is considerably less than that on sheep and cattle (Rahman et al., 2018; Hossion et al., 2020; Samaddar, 2022). Rahman and Haque (2001) found hypocalcemia in 15% of cattle, 9% of goats, 7% of sheep, and 12% of cows in Bangladesh. In India, researchers found hypocalcemia in 10% of cattle, 9% of goats, 8% of sheep, and 14% of buffalo (Roche and Berry, 2006). Researchers have identified species, age, sex, breed, and stage of the production period as potential risk factors for hypocalcemia in goats (Quader et al., 2017). Researchers observe the biochemical parameters to identify metabolic, parasitic, and viral infections in ruminants (Begum et al., 2021; Saleh et al., 2022).

These parameters help to evaluate management methods, nutrition, and health conditions in a more realistic way (Tekeli et al., 2024). Metabolic problems, such as pregnant toxemia, are associated with decreased calcium levels even in both clinical and subclinical conditions (Albay et al., 2014). The required quantities of calcium and phosphorus remain within the reference values for infectious diseases such as Peste des Petits Ruminants (PPR) (Begum et al., 2018). The total concentration of biochemistry and antioxidants in healthy animals can change based on their age, race, gender, the food they eat, their environment, stress, and other things. Therefore, creating reference intervals for each animal species, based on their specific age, is vital for obtaining more precise biochemical test findings (Gül and Biçer, 2021).

There are numerous reports available to investigate serum biochemical parameters like calcium and phosphorous in healthy goats. The study makes use of the small population sizes in India, Pakistan, Nepal, and Bangladesh, which are

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approximately (<100) individuals (Gharban and Al-Shaeli, 2021; Esraa et al., 2025; Tanvir-UI-Alam et al., 2025). Research on serum biochemical factors, such as calcium, phosphorus, and magnesium, in sick goats is limited (Mbuh and Mbwaye, 2005; Begum et al., 2018). Understanding the biochemical markers that distinguish healthy from sick goats is vital for assessing their nutritional status, management methods, and overall health.

The central goal of this research is to furnish crucial information that can facilitate improved herd management practices and veterinary interventions, particularly concerning the healing of bone fractures in this vital livestock species. Furthermore, this study seeks to rectify the existing paucity of extensive data pertaining to goat biochemistry, specifically within the South Asian context.

MATERIAL AND METHODS

Study area and duration

This study was conducted in Chattogram district between July 2022 and December 2022 in the Teaching Veterinary Hospital of Chattogram Veterinary and Animal Sciences University, Bangladesh, and on three goat farms.

Animal sampling and data collection

A 5 mL sample of blood was collected from 98 clinically sick and 102 healthy goats. The sick goats were admitted to the hospital for treatment with several indications and symptoms, such as elevated body temperatures, bone fractures, urolithiasis, a pregnancy assessment, and diarrhea. The selection of healthy goats was based on well-organized data records and an adequate number of goats from different breeds. A standardized questionnaire was employed to record information about the goats, including their demographic particulars (species, age, sex, and breed), dietary patterns (grass, concentrate, or both), and farmer's income (low, middle, and high).

Sample processing and analysis

Aseptically, a standard technique was followed to collect five milliliters of blood from each goat's jugular vein. The samples for biochemical analysis were immediately processed at the Department of Medicine and Surgery (Clinical laboratory), Chattogram Veterinary and Animal Sciences University. The analysis was conducted using a biochemical analyzer (EA-200, E-LAB, Germany) following the manufacturer's instructions.

Statistical analysis

The recorded data were entered into a data recording spreadsheet of Microsoft Excel 2010. Subsequently, the data were uploaded to the statistical program JMP Pro-13 for analysis (Ye et al., 2000). Descriptive statistics analysis was conducted to summarize the data presented as mean $\pm$ SE. One-way ANOVA was used to estimate the effect of different features of serum biochemical parameters. In the present study, a p-value < 0.05 was considered statistically significant.

RESULTS

The overall mean serum calcium level of healthy goats was 8.47 ± 0.11 mg/dl and in clinically sick goats, it was 7.63 ± 0.12 mg/dl ($P=0.001$), and the value was much lower than the reference value in clinically sick goats. The two groups' serum concentrations of magnesium and phosphorus did not differ significantly from each other. The phosphorus levels were within the acceptable range, whereas the magnesium levels were near the reference values (Table 1).

In healthy goats, the serum calcium level of healthy goats varied significantly considering age ($P=0.02$), body weight ($P=0.02$), sex ($P=0.01$), feeding type ($P=0.03$), and owner's income (<0.001). However, the serum phosphorus and magnesium levels did not vary significantly across the different parameters tested, except for sex ($P=0.003$) and owner's income (<0.001) in the case of phosphorus (Table 2). In clinically sick goats, the serum calcium level was significantly lower in goats diagnosed with hypocalcemia, while the serum phosphorous level was lower in goats diagnosed with musculoskeletal defects, wounds, parasitic infestation, PPR and indigestion (Table 3).

Table 1 - Overall serum calcium, phosphorus, and magnesium levels in healthy and clinically sick goats.

Parameter	Healthy goats (N = 102) (LSM $\pm$ SEM)	Clinical sick goat (N = 98) (LSM $\pm$ SEM)	P-value	References Normal values (Pugh and Baird, 2011)
Calcium	8.47 ± 0.11	7.63 ± 0.12	0.001	8.9-11.7 mg/dl
Phosphorous	4.61 ± 0.16	4.70 ± 0.16	0.68	4.2-9.1 mg/dl
Magnesium	2.17 ± 0.04	2.15 ± 0.04	0.68	2.8-3.6 mg/dl

Table 2 - Serum calcium, phosphorus, and magnesium levels in healthy goats were determined using multiple parameters that were available in different farm goats.

Parameter	Categories	Calcium (LSM $\pm$ SEM)	Phosphorous (LSM $\pm$ SEM)	Magnesium (LSM $\pm$ SEM)
Age	<6month (n=8)	8.64 $\pm$ 0.40	4.56 $\pm$ 0.35	2.16 $\pm$ 0.12
	>6-1year (n=20)	8.34 $\pm$ 0.24	4.93 $\pm$ 0.21	2.28 $\pm$ 0.08
	>1-2year (n=22)	7.86 $\pm$ 0.23	4.14 $\pm$ 0.20	2.02 $\pm$ 0.07
	>2-3year (n=22)	8.56 $\pm$ 0.24	4.75 $\pm$ 0.21	2.19 $\pm$ 0.08
	>3year (n=30)	8.89 $\pm$ 0.20	4.65 $\pm$ 0.17	2.20 $\pm$ 0.06
	P- value	0.02	0.10	0.21
Body weight	<10kg (n=3)	8.71 $\pm$ 0.65	4.65 $\pm$ 0.58	2.25 $\pm$ 0.21
	>10-20kg (n=22)	8.28 $\pm$ 0.22	4.77 $\pm$ 0.20	2.22 $\pm$ 0.07
	>20-30kg (n=34)	8.18 $\pm$ 0.19	4.38 $\pm$ 0.17	2.17 $\pm$ 0.06
	>30-40kg (n=31)	8.55 $\pm$ 0.20	4.58 $\pm$ 0.18	2.10 $\pm$ 0.06
	>40kg (n=12)	9.45 $\pm$ 0.32	5.00 $\pm$ 0.29	2.23 $\pm$ 0.10
	P- value	0.02	0.37	0.75
Sex	Female (n=61)	8.25 $\pm$ 0.14	4.37 $\pm$ 0.12	2.13 $\pm$ 0.04
	Male (n=41)	8.80 $\pm$ 0.17	4.96 $\pm$ 0.15	2.23 $\pm$ 0.05
	P- value	0.01	0.003	0.17
Breeds	Barbari (n=3)	9.26 $\pm$ 1.14	5.25 $\pm$ 1.01	2.34 $\pm$ 0.37
	Beetal (n=10)	9.25 $\pm$ 0.34	5.13 $\pm$ 0.30	2.28 $\pm$ 0.11
	Cross (n=8)	8.16 $\pm$ 0.34	4.41 $\pm$ 0.30	2.04 $\pm$ 0.11
	Gujri (n=2)	10.32 $\pm$ 1.14	4.31 $\pm$ 1.01	2.12 $\pm$ 0.37
	JP (n=42)	8.25 $\pm$ 0.17	4.41 $\pm$ 0.15	2.11 $\pm$ 0.05
	Local (n=13)	8.37 $\pm$ 0.33	4.88 $\pm$ 0.29	2.34 $\pm$ 0.21
	Sirohi (n=3)	9.60 $\pm$ 0.66	5.29 $\pm$ 0.58	2.27 $\pm$ 0.21
	Totapuri (n=21)	8.48 $\pm$ 0.25	4.59 $\pm$ 0.21	2.20 $\pm$ 0.08
	P- value	0.06	0.35	0.45
Feed	Grass 30%+ concentrate70% (n=12)	7.87 $\pm$ 0.25	4.20 $\pm$ 0.21	2.15 $\pm$ 0.08
	Grass 70%+ concentrate30% (n=8)	8.56 $\pm$ 1.15	4.34 $\pm$ 0.99	2.12 $\pm$ 0.37
	Grass 50%+ concentrate50% (n=82)	8.62 $\pm$ 0.12	4.72 $\pm$ 0.10	2.18 $\pm$ 0.04
	P- value	0.03	0.10	0.92
Income	Low income (n=32)	7.09 $\pm$ 0.27	3.50 $\pm$ 0.24	2.04 $\pm$ 0.10
	Middle income (n=50)	8.90 $\pm$ 0.11	4.93 $\pm$ 0.10	2.20 $\pm$ 0.04
	High income (n=20)	7.87 $\pm$ 0.21	4.20 $\pm$ 0.19	2.15 $\pm$ 0.08
	P- Value	<0.001	0.00	0.34

Table 3 - Serum biochemical parameters in relation to different clinical conditions in goats presented in a teaching veterinary hospital (CVASU, Bangladesh).

Parameter	Calcium (LSM $\pm$ SEM)	Magnesium (LSM $\pm$ SEM)	Phosphorous (LSM $\pm$ SEM)
Hypocalcemia (N=3)	5.90 $\pm$ 0.50 ^b	1.93 $\pm$ 0.20 ^a	8.66 $\pm$ 0.87 ^a
Musculoskeletal (N=26)	7.87 $\pm$ 0.24 ^a	2.26 $\pm$ 0.10 ^a	4.70 $\pm$ 0.42 ^b
Wound (N=22)	7.69 $\pm$ 0.23 ^a	2.08 $\pm$ 0.09 ^a	4.24 $\pm$ 0.41 ^b
Parasites (N=18)	7.24 $\pm$ 0.26 ^{ab}	2.21 $\pm$ 0.11 ^a	4.28 $\pm$ 0.46 ^b
Urolithiasis (N=5)	7.79 $\pm$ 0.50 ^{ab}	2.41 $\pm$ 0.20 ^a	5.30 $\pm$ 0.87 ^{ab}
Anestrous (N=2)	7.06 $\pm$ 0.79 ^{ab}	1.82 $\pm$ 0.33 ^a	4.77 $\pm$ 1.39 ^{ab}
Peste des Petits Ruminants (PPR) (N=11)	8.17 $\pm$ 0.33 ^a	1.92 $\pm$ 0.14 ^a	4.57 $\pm$ 0.59 ^b
Indigestion (N=11)	7.97 $\pm$ 0.33 ^a	2.23 $\pm$ 0.14 ^a	4.37 $\pm$ 0.59 ^b
P-value	0.012	0.307	0.003

^{a, b} – values in rows with different letters differ significantly (P < 0.05)

DISCUSSION

The present investigation found that clinically sick goats had considerably lower calcium levels than reference values, supporting Karapinar et al. (2024)'s claim that adult sick cattle had lower calcium levels. Sick goats had lower calcium and magnesium levels than standard values. Their phosphorus levels were acceptable. Zhao et al. (2015) indicated reduced calcium, magnesium, and phosphorous in sick dairy cows. The present investigation revealed that goats exhibiting clinical symptoms presented with calcium concentrations that were notably diminished relative to the established normal range. This observation corroborates the findings of Karapinar et al. (2024), who documented decreased calcium levels in affected adult cattle. In addition, the sick goats exhibited reduced calcium and magnesium level when in comparison to expected values, while their phosphorus levels remained within the normal range. Zhao et al. (2015) similarly documented diminished calcium, magnesium, and phosphorus levels in afflicted dairy cows. Conversely, in healthy goats, the mean blood calcium levels were found to be lower than the reference value.

Phosphorus concentrations were deemed satisfactory; however, magnesium levels were only slightly below the reference range. [Omidi et al. \(2018\)](#) observed analogous findings, documenting a decrease in calcium and magnesium levels in captive settings, while phosphorus levels remained within the normal range. This discrepancy may have been attributable to a feed supply with inadequate calcium-to-magnesium ratios. The current investigation indicated that goats exceeding three years of age ($n = 32$) exhibited calcium, phosphorus, and magnesium levels within the reference values. Conversely, [Antunović et al. \(2019\)](#) reported that serum calcium levels in goats under one year of age were within normal limits, despite phosphorus levels being normal across all age groups. While this finding contradicted our study's results, it was consistent with the observation that phosphorus levels were standard across all ages.

[Antunović et al. \(2019\)](#) found that goats of all ages maintained normal calcium levels when given calcium-rich feed. In contrast, our study showed a decrease in calcium levels, suggesting that the calcium in the goat feed might not be enough. Also, our results showed that younger goats had higher phosphorus levels than older goats, which agrees with [Poulsen's \(1991\)](#) findings. [Radostits et al. \(2000\)](#) suggested that the decrease in blood phosphorus levels seen in adult goats is due to their reduced ability to absorb phosphorus from food. Moreover, it is important to note that growth hormone might increase phosphate levels in the blood of young animals by helping the kidneys reabsorb phosphate ([Kaneko et al., 2008](#)). This observation might explain why younger goats show higher phosphorus levels. In this study, adult and mature goats had magnesium levels below the expected range. However, [Gwaze et al. \(2012\)](#) suggest a possible link between magnesium and how the body uses this important mineral. Unlike the current study, differences in environmental conditions or the type of feed could explain this decrease.

Goats weighing more than 40 kg ($N = 12$) showed greater calcium levels than those weighing less than 40 kg, according to the present study. The present study supported by [Samardžija et al. \(2011\)](#) mentioned that goats with higher body weights have higher calcium levels. [Bayoumi et al. \(2021\)](#) confirmed the present study's finding that females are more prone to hypocalcemia than males, reporting that lower calcium levels are more common in females than in males. They explained that this was because females need more calcium during the periparturient phase. The present investigation found that females have lower phosphorus levels than males of the same age. [Kaneko et al. \(2008\)](#) found that male goats contain more phosphorus. Female may eat more and lose more phosphorus during pregnancy. The present investigation found that crossbred animals had more hypocalcemia than native breeds which support the present study by [Quader et al. \(2017\)](#) who found that crossbred goats have lower calcium levels than native breeds due to the greater loss of calcium during the milking process.

The goat raised semi intensively and fed with a diet containing 30% grass and 70% concentrate exhibited lower calcium value. However, goats fed with 70% grass and 30% concentrate, and even those fed with 50% grass and 50% concentrate, had calcium values remarkably similar to the reference value. This finding corresponds to the findings of [Adama and Moyosoluwa \(2014\)](#), who reported that goats were in semi-intensive systems had lower serum calcium values. The current study revealed that goat owners with lower and higher incomes exhibited diminished levels of calcium, magnesium, and phosphorus in comparison to middle-income goat owners. [Islam et al. \(2018\)](#) support present study, demonstrating a statistically significant reduction in the correlation between income and the levels of calcium and phosphorus among lower-income goat owners. The lower-income segment can have financial problems supplying balanced goat feed. In the present study the standard serum concentrations of calcium and phosphorus were significantly lowered in goats presenting with different clinical diseases. The present observation is consistent with the work of [Bandzaite et al. \(2005\)](#), who documented similarly reduced calcium and phosphorus levels in diseased cows with a variety of diseases. Furthermore, the present study revealed that hypocalcaemia resulted in a return of magnesium levels to their baseline values. Present findings support the conclusions of [Bayoumi et al. \(2021\)](#), who suggested that nutritional deficiencies could precipitate increased excretion during vigorous contractions. The present study showed that during musculoskeletal problem, calcium level were lower than the normal value which support present study by [Braun et al. \(2009\)](#). The present study revealed that calcium level was decreased during wound healing, which supported by [Lansdown et al. \(1999\)](#). Present study found that calcium and phosphorus levels decreased during infections like Peste des Petits Ruminants (PPR), a finding that differs from the results of [Begum et al. \(2021\)](#). As a result, a loss of appetite and diarrhoea might occur, which then leads the body to lose calcium.

The present results showed that calcium levels were lower than the standard range for goats with metabolic disorder, including digestion disorder cases. The present finding backs up by [Ahn et al.'s \(2023\)](#) hypothesis that indigestion affects the stomach's acidity, which then prevents calcium from turning into salt. If calcium is not absorbed, it is eliminated through feces. As a result, the calcium concentration decreases below the normal level.

CONCLUSION

Clinically sick goats exhibited significant variations in the concentrations of key mineral markers which help in bone healing when compared to their healthy goats. These differences, which were evident across various conditions, were modulated by factors including age, body weight, sex, dietary intake, and income in the healthy goat population. Conversely, the clinically sick goats presented a more restricted spectrum of mineral parameters.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Saroj Kumar Yadav; E-mail: shirfraaz@gmail.com; ORCID: <https://orcid.org/0000-0002-6642-1719>

Data availability

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

Authors' contribution

Saroj Kumar Yadav: Investigation, Methodology and Writing-original draft, Sunil Yadav: data collection and sample test. MdAhaduzzaman: Formal analysis, Writing- review and editing. Monor Sayeed Pallab: Supervision, Writing-review and editing. Bibek Chandra Sutradhar: Supervision, Writing-review& editing. Bhajan Chandra Das: Supervision, Writing-review and editing.

Consent to publish

The publishing of this manuscript is approved by all authors.

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Competing Interests

No competing interests have been disclosed by the authors.

REFERENCES

- Adama JY, and Moyosoluwa A (2014). Calcium, phosphorus, sodium and potassium concentrations in blood serum of yearling goats raised semi-intensively in minna, north central, Nigeria. *Revue Agriculture*, 5(2): 38-41. <http://irepo.futminna.edu.ng:8080/jspui/bitstream/123456789/787/1/REVUE%20DAGRICULTURE%201.pdf>
- Ahn S, Shin W, Han Y, Bae S, Cho CU, Choi S, et al. (2023). Ruminal impaction with an indigestible foreign body in long-tailed goral (*Naemorrhodus caudatus*). *Korean Journal of Veterinary Service*, 46(2):161–165. <https://doi.org/10.7853/kjvs.2023.46.2.161>
- Albay MK, Karakurum MC, Sahinduran S, Sezer K, Yıldız R, and Buyukoglu T (2014). Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxemia. *Veterinární Medicína*, 59(7): 336-342. <https://vetmed.agriculturejournals.cz/pdfs/vet/2014/07/03.pdf>
- Antunović Z, Marić I, Klir Ž, Šerić V, Mioč B, Novoselec J (2019). Haemato-biochemical profile and acid–base status of Croatian spotted goats of different ages. *Archives Animal Breeding*, 62(2):455-463. <https://doi.org/10.5194/aab-62-455-2019>
- Bandzaite V, Klimiene I, Spakauskas V, Matusevicius A (2005). Interaction between the levels of hormones and minerals in sera of healthy and sick cows. *Polish Journal of Veterinary Sciences*, 8(4):269-274. <https://pubmed.ncbi.nlm.nih.gov/16385850/>
- Bayoumi Y H, Behairy A, Abdallah A A and Attia N E (2021). Peri-parturient hypocalcemia in goats: Clinical, hematobiochemical profiles and ultrasonographic measurements of postpartum uterine involution. *Veterinary World*, 14(3): 558–568. <https://doi.org/10.14202/VETWORLD.2021.558-568>
- Begum S, Nooruzzaman M and Mohanto N (2018). Peste des petits ruminants virus infection of Black Bengal goats showed altered haematological and serum biochemical profiles. *Onderstepoort Journal of Veterinary Research*, 85(1): 2–10. <https://doi.org/10.4102/ojvr.v85i1.1595>
- Begum S, Nooruzzaman M, Hasnat A, Islam M R and Chowdhury E H (2021). Sequential haematological and serum biochemical changes in Black Bengal goats infected with a local isolate of peste des petits ruminants virus from Bangladesh. *Veterinary Medicine and Science*, 7(2): 393–401. <https://doi.org/10.1002/vms3.373>
- Braun U, Ohlerth S, Liesegang A, Forster E, Gorber U, Tschuor A et al (2009). Osteoporosis in goats associated with phosphorus and calcium deficiency. *The Veterinary Record*, 164(7):211-213. <https://doi.org/10.1136/vr.164.7.211>
- Esraa E, Safaa N, Samia F, Mohammed K, Sabry E, Magdy E (2025). Clinical, Haematological, Biochemical Alterations, and Potential Risk Factors Associated with Ill-Thrift in Goats Raised at Smallholder Farms in Egypt. *Egyptian Journal of Veterinary Sciences*, 56(5):1031-1041. [10.21608/ejvs.2024.278076.1940](https://doi.org/10.21608/ejvs.2024.278076.1940)
- Gharban HA, and Al-Shaeli SJ (2021). Clinical and serum biochemical evaluation of goats with hypomagnesemia. *Biochemical and Cellular Archives*, 21(1):587–592. <https://connectjournals.com/03896.2021.21.587>
- Gül and Biçer O (2021). Fattening performance and carcass characteristics of yearling Damascus male goat. *Journal of Agriculture, Food, Environment and Animal Sciences*, 2(2): 116-133. <https://jafas.com/index.php/j1/article/view/18>
- Gwaze FR, Chimonyo M, Dzama K (2012). Effect of season and age on blood minerals, liver enzyme levels, and faecal egg counts in Nguni

- goats of South Africa. *Czech Journal Animal Science*, 57(10):443-53. DOI: <https://doi.org/10.17221/6345-CJAS>
- Hossain MZ, Shanta AJ, Sujana KM, Haque MI, Islam MS, and Islam MK (2020). Biochemical status of serum and corresponding milk of cows kept under open grazing system in Sirajganj, Bangladesh. *Bangladesh Journal of Veterinary Medicine (BJVM)*, 18(2):31-36. <https://doi.org/10.33109/bjvmjd2020rm1>
- Islam M A, Hossain MN, Chokrabarti SS, Rahman S, Tasnim A and Al Zabir A (2018). Socio-economic Profile of Goat Rearing Farmers and Their Management Practices in Sylhet, Bangladesh. *Journal of Agriculture and Ecology Research International*, 15(4): 1–10. <https://doi.org/10.9734/jaeri/2018/43818>.
- Kaneko JJ, Harvey JW. and Bruss ML, Eds A press (2008). Skeletal Muscle Function. In: Valberg S.G, editor. *Clinical Biochemistry of Domestic Animals*. 3rd edition. Academic press. 459-448. [Google Scholar](https://doi.org/10.1016/j.jvim.16938)
- Karapinar T, Tumer K C and Constable P D (2024). Predictors of blood ionized calcium concentration in sick adult cattle. *Journal of Veterinary Internal Medicine*, 38(2023): 520–529. <https://doi.org/10.1111/jvim.16938>
- Lansdown AB, Sampson B, Rowe A (1999). Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds. *The Journal of Anatomy*, 195(3):375-386. <https://doi.org/10.1046/j.1469-7580.1999.19530375.x>
- Mbuh J Vand Mbwaye J (2005). Serological changes in goats experimentally infected with *Fasciola gigantica* in Buea sub-division of S . W . P. Cameroon. *Veterinary Parasitology*, 131: 255–259. <https://doi.org/10.1016/j.vetpar.2005.05.013>
- Moniem A, El M A, Mohammed A A A and Mohammed F M (2014). Determine of Some Macro Minerals Potassium and Phosphors in the Blood Serum of Goats Grazing At El- Khuwei Locality , West Kordofan State , Sudan. *Global Journal of Animal Scientific Research*, 2(4): 2005–2007. <http://www.gjasr.com/index.php/GJASR/article/view/100/303>
- Omid A, Nik HA, and Nazifi S (2018). Biochemical reference values for healthy captive Persian wild goat (*Capra aegagrus*). *Comparative Clinical Pathology*, 27:483-491. DOI: [10.1007/s00580-017-2617-x](https://doi.org/10.1007/s00580-017-2617-x)
- Poulsen JSD (1991). Profiles of some clinical chemical parameters in growing dwarf and landrace kids. *Journal of Veterinary Medicine*, 38(1): 676-690. <https://doi.org/10.1111/j.1439-0442.1991.tb01064.x>
- Pugh DG. and Baird AN (2011). Reference interval and conversions: In: Pugh DG, Baird AN, editors. *Sheep and Goat Medicine*. 2nd edition. Elsevier Health Sciences, 596-600.
- Quader MNQ, Fakhru KM, Islam SJ, Kumar S, Iqbal M, Hossain A, et al (2017). Investigation of clinical hypocalcaemia in cattle and goats at the selected veterinary hospitals in bangladesh and india. *Journal of Dairy, Veterinary & Animal Research*, 5(1): 00130. <https://doi.org/10.15406/jdvar.2017.05.00130>
- Radostits OM, Gay CC, Blood DC, and Hinchcliff KW (2000). Diseases caused by *Salmonella* spp. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats, and Horses*. London, England: Harcourt Publishers, Ltd, 809-828.
- Rahman MK, Islam S, Ferdous J, Uddin MH, Hossain MB, Hassan MM, et al. (2018). Determination of hematological and serum biochemical reference values for indigenous sheep (*Ovis aries*) in Dhaka and Chittagong Districts of Bangladesh. *Veterinary world*, 11(8):1089. <https://doi.org/10.14202/vetworld.2018.1089-1093>
- Rahman MM and MN Haque (2001). Production and Reproduction performance of native cows under farm conditions. *Journal of Biological Sciences*, 1(11): 1085–1087. DOI: <https://doi.org/10.3923/jbs.2001.1085.1087>
- Roche JR and Berry DP (2006). Periparturient climatic animal and management factors influencing the incidence of milk fever in grazing systems. *Journal of Dairy Science*, 89(7): 2775–2783. [https://doi.org/10.3168/jds.S0022-0302\(06\)72354-2](https://doi.org/10.3168/jds.S0022-0302(06)72354-2)
- Saleh N, Allam T, Nayel M, Ahmed R, Elkhatam A (2022). Hematological, Serum biochemical and parasitological investigation of calf diarrhea. *Journal of Current Veterinary Research*, 4(1):58-68. DOI: [10.21608/jcvr.2022.240868](https://doi.org/10.21608/jcvr.2022.240868)
- Samaddar K (2022). Biochemical and haematological profiles of black Bengal goat in Mymensinghsadar of Bangladesh. *Research in Agriculture Livestock and Fisheries*, 8 (3): 329-337. <https://www.academia.edu/download/92887311/39970.pdf>
- Samardžija M, Dobranić T, Lipar M, Harapin I, Prvanović N, Grizelj J, et al. (2011). Comparison of blood serum macromineral concentrations in meat and dairy goats during puerperium. *Veterinarski arhiv*, 81(1):1-11. <https://hrcak.srce.hr/file/99249>
- Tanvir-Ul-Alam M, Nath C, Dhar PK, Uddin MI, Chowdhury MY, and Ahaduzzaman M (2025). Evaluation of serum biochemical parameters and acid–base balance in diarrheal goats with Peste des petits ruminants (PPR) to predict the infection and determine the appropriate intravenous fluid for rehydration using chemometrics. *Comparative Clinical Pathology*, 34(5):863-79. <https://doi.org/10.1007/s00580-025-03698-3>
- Tekeli H, Şensoy S, Aşıcı GS (2024). Determination of serum biochemical profile and oxidant-antioxidant activities in damascus goats at different ages. *Veterinary Sciences and Practices*, 19(1):1-8. <https://doi.org/10.17094/vetsci.1471422>
- Yadav SK, Shil SK, Pallab MS, Islam KN, Sutradhar BC, and Das BC (2024). Experimental long bone fracture healing in goats with cockle shell-based calcium carbonate bone paste. *Veterinary and Animal Science*, 25:100374. <https://doi.org/10.1016/j.vas.2024.100374>
- Ye C, Liu J, Ren F, and Okafo N (2000). Design of experiment and data analysis by JMP®(SAS institute) in analytical method validation. *Journal of Pharmaceutical and Biomedical Analysis*, 23: 581-589. [https://doi.org/10.1016/S0731-7085\(00\)00335-6](https://doi.org/10.1016/S0731-7085(00)00335-6)
- Zhao X, Wang X, Wang J, Wang Z, Wang L and Wang Z (2015). Oxidative stress and imbalance of mineral metabolism contribute to oxidative stress and imbalance of mineral metabolism contribute to lameness in dairy cows. *Biological Trace Element Research*, 164: 43–49. <https://doi.org/10.1007/s12011-014-0207-1>

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ANALYSIS OF DEMAND FOR WATER AND ITS IMPACT ON BROILER PRODUCTION IN IMO STATE OF NIGERIA

Kelechi Henry ANYIAM¹ , Kelechi Chima IGWE² , Taofeeq Ade AMUSA² , Cynthia Onyinyechi OBINNA-NWANDIKOM³ , Okoronkwo Christopher ENOCH¹ , Ime Godswill ISIAH¹ , Akanele Chori OBASI¹ , Frank Nna OKORO² , Doris N. IWEZOR-MAGNUS² , Henry Uchenna ANENE¹ , Emmanuel Iyke NNOROM¹ , Ujunwa Miriam OLUMBA¹ , Manfred Obinwanne IGWENAGU⁴ , Donatus Onwuha ONU² , Maurice Uchechukwu OZOR⁵ 

¹Department of Agricultural Economics, Federal University of Technology Owerri, Imo State, Nigeria

²Department of Agricultural Economics, Michael Okpara University of Agriculture Umudike, Umuahia Abia State, Nigeria

³Department of Agribusiness, Federal University of Technology Owerri, Imo State, Nigeria

⁴Department of Natural Resources and Environmental Sciences, Prairie View A&M University, Texas, United State of America

⁵Department of Agricultural Economics and Extension, Nnamdi Azikiwe University Awka, Anambra State, Nigeria

✉Email: kelechi.anyiam@futo.eu.ng

➤Supporting Information



ABSTRACT: This study analyzed the demand for water and its effect on the productivity of broiler production in Imo State, Nigeria. Specifically, the objectives were to A) estimating the determinants of water demand in broiler farms, B) examining the level of water consumption relative to recommended standards, C) evaluating the productivity of broiler farmers, and D) identifying constraints to water access. Primary data were collected from 120 broiler farmers using a structured questionnaire and analyzed with descriptive statistics, the broiler water consumption ratio (BWCR), productivity index, and multiple regression models. Results showed that the exponential functional form was the best fit, with an R^2 of 0.684, indicating that 68.4% of the variation in water demand was explained by farm and bird characteristics. Age of birds ($P < 0.05$), stock size ($P < 0.01$), and bird weight ($P < 0.10$) significantly influenced water demand. Based on the BWCR, 43.3% of broilers consumed water below optimal levels, 25.0% consumed excessively, and only 31.7% were within the recommended range. Farmers demonstrated high productivity with a ratio of 3.30, suggesting efficient input use; however, sustainability is threatened by persistent water constraints. The most pressing challenges were limited access to water distributors (18.5%), scarcity (15.0%), contamination (13.0%), high fuel costs (13.0%), and climate change (13.0%). The findings underscore the need for policy interventions to strengthen rural water infrastructure, promote solar-powered pumping systems, and improve extension services on water-use efficiency. Addressing these issues will enhance both the productivity and resilience of broiler production in water-scarce environments.

Keywords: Broiler productivity, Poultry requirements, Water consumption ratio, Water demand, Nigeria

INTRODUCTION

Water is the most critical nutrient for animal survival and productivity, yet it remains one of the least examined inputs in livestock systems. Broilers have particularly high water requirements relative to feed intake; a bird reaching 2.3 kg typically consumes about 8.2 kg of water compared to 4.6 kg of feed (Tabler, 2023). Because water underpins thermoregulation, digestion, metabolism, and waste elimination, any disruption in supply directly affects growth performance and carcass quality (Jacquie, 2020). However, climate variability, rising temperatures, and infrastructural limitations have intensified water scarcity globally, creating significant bottlenecks for poultry production (Leal Filho et al., 2022; El-Sabry et al., 2023).

In sub-Saharan Africa, water scarcity is both an environmental and livelihood concern, as many rural households depend on smallholder poultry systems for income and food security. High levels of water poverty, driven by inadequate infrastructure and reliance on seasonal rainfall, exacerbate vulnerability among rural livestock farmers (Umunakwe et al., 2021). For broiler production, insufficient or contaminated water increases mortality rates, reduces feed conversion efficiency, and lowers profitability (Ogbonna et al., 2020).

Nigeria hosts one of the largest poultry industries in Africa, contributing significantly to rural employment and national protein supply (Adeyonu et al., 2021). Nonetheless, farmers face persistent water-related challenges, including unstable electricity for pumping, high fuel costs, and rising expenses associated with water storage and distribution (Onuwa, 2022). These constraints are more severe in southeastern Nigeria, where climatic variability and uneven rainfall further widen the supply-demand gap (Emeka-Chris et al., 2022). In Imo State, most broiler farmers rely on commercial

water vendors or costly boreholes, increasing production costs and reducing margins. Disease outbreaks linked to contaminated water have also been reported, heightening production risks (Sanusi and Dries, 2025). Despite the centrality of water to poultry performance, empirical research in Nigeria has largely prioritized feed and disease management, with limited attention to water demand and its direct relationship with broiler productivity.

Although previous studies have highlighted the physiological role of water in poultry production (Barbosa et al., 2014; Jacquie, 2020; Tabler, 2023) and the influence of environmental factors on livestock water use (Leal Filho et al., 2022), empirical evidence linking water demand to broiler productivity in Nigeria remains sparse. Existing research has focused on water contamination (Ogbonna et al., 2020) and the impact of production inputs on farm performance (Onuwa, 2022), but few studies have investigated how water availability shapes growth outcomes and economic returns. This is a critical omission, as water affects feed conversion ratios, market weights, survival rates, and overall profitability, while the rising cost of accessing water through pumping, storage, and borehole systems has not been fully incorporated into productivity assessments.

This study addresses these gaps by examining the determinants of water demand and its effect on broiler productivity in Imo State, Nigeria. The specific objectives are to identify the determinants of water demand in broiler production, assess the level of water consumption relative to established standards, evaluate the productivity of broiler farmers, and identify the key constraints limiting access to adequate water supply. By integrating water demand analysis with productivity assessment, the study provides empirical evidence to support the formulation of sustainable water-use policies and strengthen the resilience of poultry production systems under conditions of increasing water scarcity.

MATERIALS AND METHODS

Study area

The study was carried out in Imo State, Nigeria. Imo State is located in the Southeastern zone of Nigeria. It is divided into three agricultural zones, viz-a-viz Orlu, Okigwe and Owerri. These divisions are for administrative and extension services and not for any agro-ecological difference. It is delineated into 27 Local Government Areas. The state lies between latitudes 4° 45'N and 7° 15'N of the equator and longitudes 6° 50'E and 7° 25'E of the Greenwich Meridian. It occupies the area between the lower River Niger and the upper and middle Imo River. It is bounded on the East by Abia State, on the West by the River Niger and Delta State; and on the North by Anambra State, while Rivers State lies to the South. Imo State covers an area of about 5,135 km², with an estimated population of 5,459,300 and a population density of about 1,063 km² (National Population Commission, 2023). The State has an average annual temperature of 24.1°C which can rise up to 32.6°C during the dry season, an average annual relative humidity of 64.2% which can rise to up to 77.9% during the rainy season, average annual rainfall of 1800mm to 2738mm and an altitude of about 100m above sea level (NBS, 2016). Agriculture is practiced by a good number of the population in the state. Crop farming is mainly regulated by the seasonal distribution of rainfall, although there are a few farmers involved in dry season farming of some food crops and vegetables. Also, livestock like cattle, sheep, goats, pigs, poultry, rabbits, and snails are reared through subsistence and commercial farming in the state (Imo Agricultural Development Programme (ADP), 2014).

Sample technique

A multistage sampling technique was used in the selection of respondents. Firstly, the 3 agricultural zones of the state were selected to enable the survey to cover the entire state. Orlu, Okigwe and Owerri zones are made up of 10, 6 and 11 Local Government Areas respectively. In the second stage, a proportionate sampling technique was used to select 4 LGAs from Orlu zone, 2 LGAs from Okigwe zone and 4 LGAs from Owerri zone bringing it to a total of 10 LGAs from the 3 Agricultural zones. This was done due to the unequal numbers of LGAs in each agricultural zone. In the third stage, four Communities each were randomly selected from the 10 LGA giving a total number of 40 communities.

In the fourth stage, 3 broiler farmers each were selected randomly from the list of registered poultry farmers in the communities selected making a total of 120 broiler farmers. These lists were obtained from Agricultural Development Programme and from the opinion leaders such as community leaders, and presidents' generals of the communities. A total sample size of 120 was used for this study. The study population consisted of registered small- and medium-scale broiler farmers. Industrial poultry operations and backyard keepers were excluded. All respondents operated commercial broiler units using deep-litter housing systems and had completed at least one production cycle within the study period. The dominant strains raised were Arbor Acres, Ross 308, and Marshall standard commercial broiler breeds in southeastern Nigeria. These strains were documented during data collection to account for potential breed-based differences in water consumption.

Methods of data collection

Primary data were obtained through a structured questionnaire administered directly to farmers. The instrument collected information on water sources, water use, production practices, flock characteristics, and productivity indicators. Data were collected on the following traits: farmer characteristics such as age, education, farming experience, flock

characteristics such as breed, flock size, age of birds, water-use traits such as daily water intake, water source, water cost, supply frequency, and productivity indicators such as mortality, feed intake, live weight, feed conversion ratio, production cost, and revenue. Water quality parameters were not assessed in this study.

The questionnaire was pre-tested with 15 farmers outside the study area to ensure clarity. A Cronbach's alpha value of 0.81 indicated strong internal consistency. Among the four functional forms tested (linear, exponential, double-log, semi-log), the exponential function was selected as the lead equation because it provided the highest explanatory power ($R^2 = 0.684$) and statistically significant coefficients for key variables such as age of birds, stock size, and weight. This implies the model better captured the nonlinear relationship between water demand and the explanatory variables.

This study relied on cross-sectional data collected within one production cycle. Seasonal variations in water availability and long-term productivity effects were not fully captured. In addition, self-reported measures of water consumption may be subject to recall bias. These limitations suggest caution in generalizing results beyond the study area.

Method of data analysis and model specification

Data collected were analyzed using descriptive statistics (mean, frequency count, and percentage), multiple regression technique, Likert scale, and demand function and productivity model. The water demand was determined and was estimated using the model as specified. The model was developed for this study considering the aim of the study.

$$D_w = b_0 + b_1AGB + b_3WB + b_4SS + b_5FI + b_6P_w + e$$

Which D_w = Quantity of water used by the broiler (Litre); AGB = Age of the broiler (weeks); WB = Weight of the broiler (kg); SS = Stock Size (Number of broilers kept); FI = Feed Intake (kg); P_w = Price of water (₦); e = error term; $b_0 - b_6$ = Parameters to be estimated.

Then, the Broiler water consumption ratio (BWC) was used to categorize the water usage level into optimal consumption level, excessive consumption level, and low consumption level. The model is specified as follows;

$$BWC = \frac{\text{Total Volume of water consumed (kg)}}{\text{Total Stock weight (Kg)}}$$

Decision rule

If $BWC = 1.50 - 3.0$, an Optimal Consumption level; If $BWC = 3.50$ - Above, an Excessive consumption level; If $BWC = 0.5 - 1.50$, a Low consumption level.

The productivity of broiler farmers in the study area was achieved using a total productivity ratio. The model as used by Anyiam et al. (2019) was adapted and remodeled to suit the study. The model is specified as:

$$P = \frac{\text{Value of Output from broiler farm (naira)}}{\text{Value of Input used in broiler farm (naira)}}$$

The output from the broiler farm consisted of the value of the broiler at maturity plus the value of the waste generated from the broiler farm. If $P = 0.5$, productive, If $P > 0.5$, highly productive, If $P < 0.5$, not productive.

RESULTS

Demand for water and level of water usage

The results for the demand for water for broiler production were achieved using the four functional forms of linear, exponential, double log and semi-log. The result is presented in Table 1.

Table 1 - The demand for water for broiler production.

Explanatory variable	Linear function	+Exponential function	Double-Log function	Semi-Log function
Constant	62.967 (1.102)	3.475 (4.854)	4.009 (2.045)	24.342 (0.813)
Age of the bird	-0.419 (-0.917)	-0.002 (-2.412)**	-0.196 (-0.518)	-20.475 (-0.966)
Weight of the bird	-43.608 (-0.564)	-0.864 (-1.891)*	-0.252 (-0.736)	-8.921 (-0.446)
Stock size	-0.059 (-1.576)*	-0.002 (-5.125)***	-0.394 (-4.445)***	-2.844 (-1.279)
Feed intake	1.378 (1.134)	0.010 (0.664)	0.168 (0.798)	10.358 (0.995)
Price of water	-0.045 (-0.075)	-0.004 (-0.550)	0.243 (0.927)	2.561 (0.125)
Functional parameters				
R^2	0.473	0.684	0.637	0.456
Adj R^2	0.411	0.636	0.585	0.408
F-Statistics	2.169	5.884	4.593	1.877

*Source: Field Survey Data Analysis, 2025. + = Lead equation *** = significant at 1%, ** = significant at 5%, * = significant at 1% values in parenthesis are the t-values

The regression results for the demand for water in broiler production are presented in Table 1. The exponential functional form was selected as the lead equation because it yielded the highest explanatory power ($R^2 = 0.684$) and statistically significant coefficients for bird age, stock size, and weight. This indicates that 68.4% of the variation in water demand was explained by the model. The negative coefficient for bird age suggests that younger birds consume less water than older birds, while the negative relationship with stock size implies that larger flocks may experience rationing behavior, whereby water per bird is reduced as stock size increases (El-Sabry et al., 2023). The relationship between bird age and water demand is illustrated in Figure 3, which shows a declining trend as birds mature.

These findings corroborate Tabler (2023), who observed that water intake in broilers changes over time and is sensitive to environmental and management conditions. Similarly, Parker and Brown (2003) reported that water demand in poultry is non-linear and influenced by both biological and management factors.

Level of water consumption

The level of water consumption for broiler production was estimated using the Broiler Water Consumption Ratio (BWCR). The results are presented in Table 2.

The Broiler Water Consumption Ratio (BWCR) revealed that 31.7% of birds consumed water at optimal levels, 25.0% consumed excessively, while 43.3% had suboptimal consumption (Table 2; Figure 1). The high proportion of suboptimal consumption suggests that many farms do not provide adequate water for their broilers, which could reduce feed intake and growth performance. This is consistent with findings by Ebrahimi et al. (2024), who demonstrated that both inadequate and excessive water supply negatively affect feed conversion efficiency and carcass quality in broilers.

Statistical analysis further supports these observations. The chi-square test ($\chi^2 = 6.20$, $p = 0.045$) shows a significant difference in water consumption levels across farms. The Standard Error of the Mean (SEM = 6.43) shows the variability in consumption frequencies, showing inconsistencies in water provision across different operations. Pairwise comparisons reveal that low water consumption differs significantly from excessive consumption ($p = 0.012$) and marginally from optimal consumption ($p = 0.038$), while no significant difference was observed between optimal and excessive consumption ($p = 0.471$). These results indicate that inadequate water supply is a more critical issue than excessive provision in the study area. The variation across farms underscores knowledge and infrastructure gaps in water management. Farmers with better access to storage and distribution facilities were more likely to achieve optimal water consumption, emphasizing the need for targeted interventions to improve water provision and management practices in broiler production.

Table 2 - Level of water consumption

Water consumption level (Litre)	Frequency	Percent	P-value
Optimal consumption	38	31.70	0.038
Excessive consumption	30	25.00	0.012
Low consumption	52	43.30	0.471
Total	120	100	
chi-square (χ^2)	6.20		
SEM	6.43		

Source: Field Survey Data, 2025, 0.5L – 1.0L (Low consumption), 1.5L – 2.0L (Optimal consumption 2.5L – above (Excessive consumption).

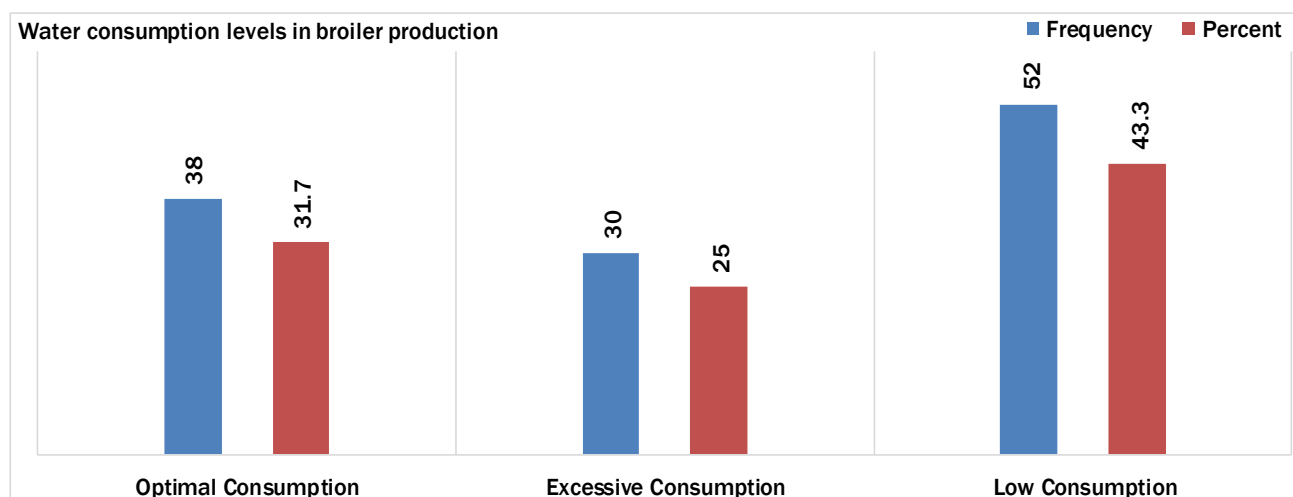


Figure 1 - Water Consumption level in Broiler Production

Productivity of broiler farmers

The productivity of the broiler farmers was determined by the productivity ratio. The result of the productivity of broiler farmers is shown in Table 3. The productivity analysis (Table 3) shows a ratio of 3.30, indicating that broiler farmers in the study area are highly productive. This suggests efficient conversion of inputs such as feed and water into outputs (mature birds and by-products). These findings align with [Onuwa \(2022\)](#), who emphasized that efficient input use strongly contributes to profitability in broiler production. However, sustaining this level of productivity may be challenged by persistent water-related constraints, as discussed below.

Table 3 - Productivity of broiler farmers.

Output (Naira)	(\$)	Input (Naira)	(\$)	Productivity	Decision
₦1306878	\$871	₦396200	\$271.34	3.299	Highly productive

*Source: Field Survey Data, 2025

Constraints faced by the farmers

The results of the constraints faced by the farmers for water is presented in Table 4. The constraints reported by farmers are summarized in Table 4 and Figure 2. Limited access to water distributors (18.5%) emerged as the most critical issue, followed by scarcity (15.0%) and contamination, high fuel costs, and climate change (13.0% each). These findings highlight systemic challenges beyond individual farm practices. Similar issues were reported by [Adeoti et al. \(2023\)](#), who noted that rural farmers in Nigeria face high costs of water procurement and limited infrastructure. Furthermore, [Ogbonna et al. \(2020\)](#) highlighted that water contamination, particularly with heavy metals and pathogens, compromises poultry health and productivity. The reported influence of climate change also reflects the findings of [Leal Filho et al. \(2022\)](#), who showed that rainfall variability exacerbates livestock water insecurity in Africa.

Table 4 - Distribution according to constraints faced by farmers in accessing water in Imo State.

Constraint ***	Frequency	Percentage
Scarcity	30	15
Contamination	26	13
Cost of borehole	15	7.5
Unstable electricity supply	6	3
High cost of fuel	26	13
Limited access to water distributors	37	18.5
Climate change	26	13
High cost of water storage tank	20	10
Water pollution	14	7

Source: Field Survey Data, 2025; *** = Multiple responses

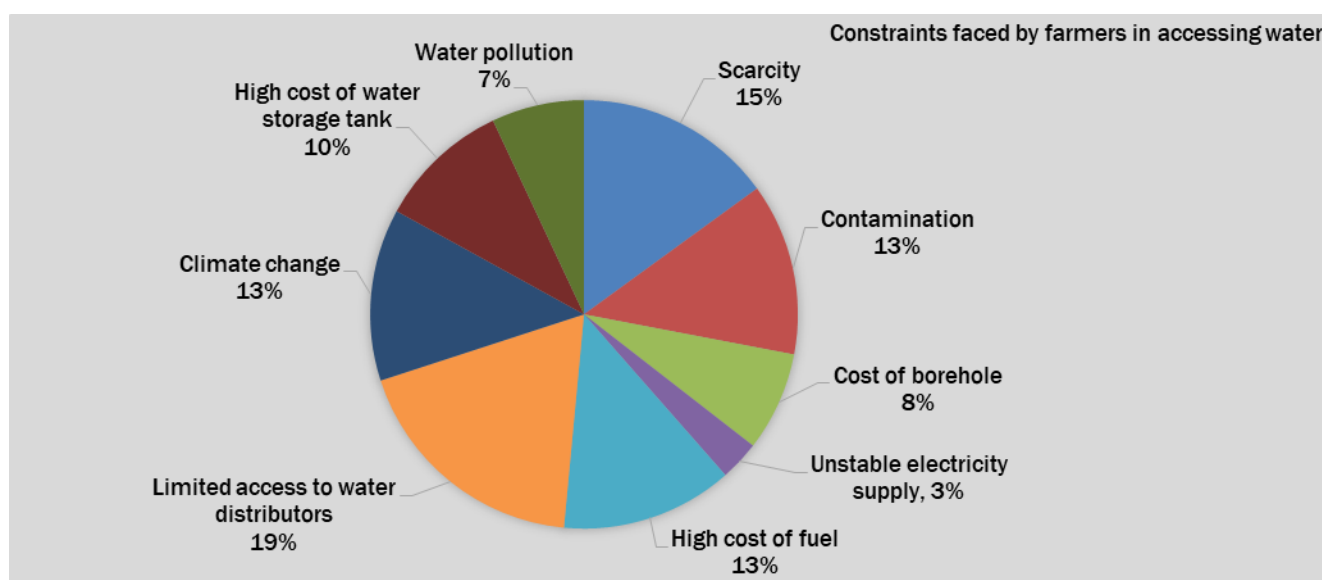


Figure 2 - Constraints faced by farmers in accessing water.

DISCUSSION

The regression analysis revealed that stock size, age, and live weight significantly influenced water demand among broiler farms. However, the negative relationship between stock size and water consumption deviates from the conventional expectation that larger flocks require proportionately more water. This counterintuitive finding suggests adaptive rationing behavior by farmers operating under conditions of water scarcity and rising production costs. Similar patterns have been documented in recent studies across water-stressed poultry systems. For instance, [El-Sabry et al. \(2023\)](#) reported that broiler farmers in semi-arid regions deliberately reduce per-bird water allocation as flock size increases in order to minimize pumping and storage costs, often at the expense of optimal bird performance. Likewise, [FAO \(2023\)](#) highlighted that water rationing has become a coping strategy among small-scale poultry producers in developing countries facing unstable water supply and high energy prices.

The observed distribution of water use levels, where 43.3% of farms operated below recommended water intake and 25% exceeded optimal levels, points to widespread inefficiencies and limited technical knowledge regarding water management. Recent empirical evidence supports this finding. [Ebrahimi et al. \(2024\)](#) demonstrated that suboptimal water provision, whether insufficient or excessive, adversely affects feed conversion ratios and growth performance in broiler production. Similarly, [Umar et al. \(2022\)](#) found that poor understanding of water quality requirements and inappropriate drinker systems contributed significantly to productivity losses among poultry farmers in sub-Saharan Africa. These findings reinforce the need for targeted extension services focusing on water-use efficiency, water quality management, and appropriate delivery systems.

The estimated productivity ratio of 3.299 suggests relatively high efficiency among broiler farmers in the study area, indicating that producers are able to generate substantial output from available inputs. This result aligns with recent studies by [Onuwa \(2022\)](#) and [Nwadiolu & Akpodiete \(2023\)](#), who reported that efficient input allocation and managerial experience significantly enhance profitability in small- and medium-scale broiler enterprises. However, despite this apparent efficiency, the long-term sustainability of productivity remains uncertain due to persistent water-related constraints. According to [World Bank \(2021\)](#), escalating costs of water storage infrastructure and energy for pumping increasingly erode profit margins in intensive livestock systems, particularly in regions with unreliable public water supply.

Furthermore, the ranking of constraints, particularly limited access to reliable water distributors and contamination risks, suggests that challenges extend beyond farm-level management to systemic supply-chain and institutional failures. Recent policy reports corroborate this observation. The International Water Management Institute ([IWMI, 2023](#)) emphasized that weak water distribution networks and inadequate quality control mechanisms disproportionately affect livestock producers, leading to inconsistent supply and heightened disease risks. This implies that improving broiler water productivity requires not only farmer-level interventions but also broader investments in water infrastructure, regulation, and private-sector participation.

CONCLUSION AND RECOMMENDATIONS

This study analyzed the demand for water and its effect on the productivity of broiler production in Imo State, Nigeria. The findings revealed that water demand is significantly influenced by bird age, stock size, and weight, with the exponential functional form providing the best fit for explaining variations. Despite high productivity levels (productivity ratio of 3.30), a large proportion of farms operate with suboptimal water consumption. Furthermore, systemic challenges such as limited access to water distributors, scarcity, contamination, high energy costs, and climate variability remain persistent constraints. These results highlight the paradox of poultry production in the study area: while farmers demonstrate efficiency in resource use, structural water-related barriers undermine sustainability. If these issues remain unresolved, the long-term viability of broiler farming, a major contributor to Nigeria's food security and rural income, could be compromised.

The following recommendations were made:

1. Government and development partners should expand rural water infrastructure, including community boreholes and small-scale water schemes, to reduce dependence on costly private distributors.
2. Farmers should be supported through subsidies or credit schemes to acquire solar-powered pumps, thereby reducing reliance on expensive fuel and unstable electricity supply.
3. Targeted training on water-use efficiency, quality monitoring, and proper delivery systems should be provided to ensure birds receive water at optimal levels.
4. Strengthening monitoring of water contamination and pollution will reduce risks of disease outbreaks and improve flock health.
5. Future studies should adopt longitudinal designs to capture seasonal variations in water demand, as well as explore the interactions between water quality, disease incidence, and productivity outcomes.

By implementing these measures, policymakers and stakeholders can enhance water accessibility, lower production risks, and secure the long-term sustainability of broiler farming in southeastern Nigeria.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Kelechi Henry Anyiam; E-mail: kelechi.anyiam@futo.edu.ng; ORCID: 0000-0003-3775-4714

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Author contributions

K.H. Anyiam = Conceptualization, methodology, supervision, manuscript drafting, data analysis, interpretation. K.C. Igwe = Data analysis, interpretation, manuscript review. T.A. Amusa = Field survey coordination, data curation, results validation. C.O. Obinna-Nwandikom and D.O. Onu = Statistical modelling, literature review. O.C. Enoch = Methodology development, editing. A.C. Obasi = Data collection, preparation of tables and figures. F.N. Okoro, M.U. Ozor = Review of related literature, referencing. Doris N. Iwezor-Magnus = Manuscript editing, formatting. I.G. Isaiah, E.I. Nnorom and H.U. Anene, M.O. Igwenagu = Data collection, critical revisions. Ujunwa Miriam Olumba = Policy recommendations, conclusion drafting. All authors read and approved the final manuscript.

Data availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request. Due to the sensitivity of respondents' personal and farm-level information, raw survey data cannot be publicly shared but may be accessed in anonymized form for academic and policy research purposes.

Ethics approval

The authors complied with the ARRIVE guidelines and or the Interdisciplinary Principles and Guidelines for the Use of Animal Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

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Competing Interests

The authors declare that there is no competing interest regarding the publication of this paper.

REFERENCES

- Adeyonu AG, Okunola A, Alao ME, Oyawoye EO, and Okonkwo CE (2021). An assessment of broiler value chain in Nigeria. *Open Agriculture*, 6: 296–307. <https://doi.org/10.1515/opag-2020-0168>
- Adeoti OS, Kandasamy J, and Vigneswaran, S. (2023). Water infrastructure sustainability in Nigeria: a systematic review of challenges and sustainable solutions. *Water Policy*, 25(11), 1094–1111. <https://doi.org/10.2166/wp.2023.173>
- Anyiam KH, Igwe KC, and Henry-Ukoha A (2019). Determinants of productivity of farmland in Imo State. *Journal of Agriculture and Food Sciences*, 17(2): 74–85. <https://doi.org/10.4314/jafs.v17i2.7>
- Ebrahimi NA, Nobakht A, İnci H, Palangi V, Suplata M, and Lackner M (2024). Drinking water quality management for broiler performance and carcass characteristics. *World*, 5(4): 952–961. <https://doi.org/10.3390/world5040048>
- El-Sabry MI, Romeih ZU, Stino FKR, Khosht AR, and Aggrey SE (2023). Water scarcity can be a critical limitation for the poultry industry. *Tropical Animal Health and Production*, 55(3): 215. <https://doi.org/10.1007/s11250-023-03599-z>
- Emeka-Chris CC, Ahaneku IE, Osedeke VE, Obineche CI, Onwujiariri C, and Oriaku AP (2022). Climate variability in selected locations of Southeastern Nigeria: An analysis of rainfall trends. *Global Journal of Engineering and Technology*, 1(3): 28–37. <https://gsarpublishers.com/wp-content/uploads/2023/01/GJET262022-Gelary-scriptxxxxxxxxxx.pdf>
- Food and Agriculture Organization of the United Nations. (2023). Climate-smart livestock production strategies. In *Climate-Smart Agriculture Sourcebook*. FAO. <https://www.fao.org/climate-smart-agriculture-sourcebook/production-resources/module-b2-livestock/chapter-b2-2/en/>
- Imo Agricultural Development Programme (ADP) (2014). Monthly technology review meeting. Owerri, Imo State. <http://www.citypopulation.de>
- International Water Management Institute (IWMI). 2024. IWMI Annual report 2023. Colombo, Sri Lanka: International Water Management Institute (IWMI). 44p. doi: <https://doi.org/10.5337/2024.232>.

- Jacque J (2020). Water requirements of poultry. USDA National Institute of Food and Agriculture Report. <https://poultry.extension.org/articles/feeds-and-feeding-of-poultry/basic-poultry-nutrition/water-requirements-of-poultry/>
- Leal Filho W, Totin E, Franke JA, Andrew SM, Abubakar IR, Azadi H, et al. (2022). Understanding responses to climate-related water scarcity in Africa. *Science of the Total Environment*, 806: 150420. <https://doi.org/10.1016/j.scitotenv.2021.150420>
- National Population Commission (NPC) (2023). National Population Commission Report 2023. Abuja, Nigeria: NPC.
- National Bureau of Statistics (NBS) (2016). Annual abstract of statistics report. Abuja, Nigeria: NBS.
- Nwadiolu R and Akpodiete O (2023). Technical Efficiency of Broiler Production in Delta State, Nigeria. *Journal of Agricultural Policy*, 6(1), 49–62. <https://doi.org/10.47941/jap.1586>
- Ogbonna PC, Ukpai NP, Obasi KO, and Umezuruike SO (2020). Appraising the physico-chemical characteristics and heavy metals in pond water at quarry site in Ngwogwo, Ebonyi State, Nigeria. *Nigerian Journal of Environmental Sciences and Technology*, 4(2): 237–252. https://nijest.com/wp-content/uploads/2020/08/237-252_0203_Vol.-4-No.-2_NIJEST.pdf
- Onuwa G (2022). Empirical analysis of productivity among broiler farmers. *Turkish Journal of Agriculture – Food Science and Technology*, 10(8): 1377–1381. <https://doi.org/10.24925/turjaf.v10i8.1377-1381.4821>
- Parker DB, and Brown MS (2003). Water consumption for livestock and poultry production. In *Encyclopedia of water science*. Marcel Dekker, Inc., pp. 971–974. https://www.researchgate.net/publication/269393738_Water_consumption_for_livestock_and_poultry_production
- Sanusi MM, and Dries L (2025). Smallholder rice farmers' resilience to water insecurity in Ogun State, Nigeria. *Regional Environmental Change*, 25: 30. <https://doi.org/10.1007/s10113-025-02364-2>
- Tabler T (2023). Modern broilers drink more water. *Poultry World*. Available on: <https://www.poultryworld.net/health-nutrition/modern-broilers-drink-more-water>
- Umar HS, Luka EG, Alu ES and Peter E. (2022). Analysis of profitability and technical efficiency of broiler production in North-Central Nigeria. *FUDMA Journal of Agriculture and Agricultural Technology*, 8(1), 56–63. <https://doi.org/10.33003/jaat.2022.0801.075>
- Umunakwe PC, Ukpogson MT, Aja OO, Ozor N, and Ubeh E (2021). Use of water-saving agricultural practices in Imo State, Nigeria. *World Journal of Agriculture and Soil Science*, 7(4): 1–9. <https://irispublishers.com/wjass/fulltext/Use-of-Water-Saving-Agricultural-Practices-in-Imo-State,%20Nigeria.ID.000666.php>
- UNICEF (2021). Water security for all. <https://www.unicef.org/media/95241/file/water-security-for-all.pdf>
- World Bank. (2021). Moving towards sustainability: The livestock sector and the World Bank. World Bank Group. <https://www.worldbank.org/en/topic/agriculture/brief/moving-towards-sustainability-the-livestock-sector-and-the-world-bank>

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OPTIMIZATION OF THE GROWTH AND NUTRITIONAL QUALITY OF LESSER MEALWORM (*Alphitobius diaperinus*) USING THE PROTEIN CONTENT OF THE MEDIUM

Nuraini NURAINI¹, Mirzah MIRZAH², Yuliaty Shafan NUR¹, Abdul AZIM², Dinah FATHINAH², and Rezki WAHYUDI²

¹Nutrition Science and Feed Technology Department, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia

²Animal Science Study Program, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia

✉ Email: nuraini@ansci.unand.ac.id

Supporting Information



ABSTRACT: This study aims to examine the performance and nutritional quality of lesser mealworms (*Alphitobius diaperinus*) reared with different protein levels in the culture medium. The research method used was a completely randomized design (CRD) with 4 treatments and 5 replications. The treatments involved providing different protein levels in the culture medium: A (13%), B (15%), C (17%), and D (19%). The variables observed were larval consumption (g/500 g medium), larval production (g/500 g medium), larval body weight gain (g/500 g medium), chitin content (%), crude protein (%DM), nitrogen retention (%), crude fat (%DM), crude fat digestibility (%), and metabolizable energy (kcal/kg) of lesser mealworms. The result of this study showed that differences in protein levels in the culture medium had a very significant effect ($P < 0.01$) on larval consumption, larval production, larval body weight gain, chitin content, crude protein, nitrogen retention, crude fat, crude fat digestibility, and metabolizable energy of lesser mealworms. This study concluded that 19% protein culture medium was the optimal treatment for lesser mealworm rearing, yielding larval consumption of 181.87 g/500 g medium, larval production of 122.52 g/500 g medium, larval body weight gain of 107.51 g/500 g medium, chitin content of 21.17%, crude protein of 46.77% (DM), crude fat of 25.69% (DM), nitrogen retention of 78.72%, crude fat digestibility of 79.55%, and metabolizable energy of 2994.25 kcal/kg. The findings indicate that smaller mealworms hold considerable potential as an alternative source of animal protein for poultry feed.

Keywords: Feedstuff, Lesser Mealworm, Nutritional Quality, Performance, Protein Medium.

INTRODUCTION

The increase in demand for high-quality poultry products has driven the development of the poultry industry in Indonesia. To achieve optimal results, nutritious poultry feed that is rich in protein resources is essential. Feed ingredients that are increasingly being used today are those derived from larvae. Many studies have shown that larvae are highly effective in poultry feed. For example, replacing up to 12% of soybean meal with *Hermetia illucens* larvae improves growth performance, blood hematology, intestinal morphometry, and the meat quality of broiler chickens (Sajjad et al., 2024). Kaddour et al. (2025) found the moderate supplementation with *T. molitor* can enhance feed efficiency while maintaining egg quality, positioning insect proteins as a viable pathway toward more sustainable and resilient poultry production. Another study reported that yellow mealworms (*Tenebrio molitor*) can be used in quail feed up to 12% to reduce cholesterol and egg yolk fat while maintaining eggshell thickness and egg white protein content (Nuraini et al., 2023). Larvae other than *Hermetia illucens*, and *Tenebrio molitor* that can be used as feed additives in poultry feed include lesser mealworms.

The lesser mealworm is a larval stage of the beetle *Alphitobius diaperinus*, which belongs to the family Tenebrionidae and also includes other species such as the superworm and yellow mealworm. *Alphitobius diaperinus* larvae are morphologically similar to yellow mealworms (*Tenebrio* spp.), characterized by three pairs of legs and a segmented body that tapers at the tip of the larva (Tilak et al., 2023). These larvae have a strong, yellowish-brown exoskeleton (Sammarco et al., 2023). Lesser mealworms are rich in nutrients, containing 47.8% crude protein and 23.7% crude fat in dry matter (Soetemans et al., 2020). Lesser mealworm larvae contain amino acids (mg/100 g protein), including arginine 500, histidine 380, leucine 610, lysine 620, isoleucine 400, phenylalanine 460, methionine 190, threonine 390, valine 510, alanine 700, aspartic acid 790, glycine 400, glutamate 1050, serine 310, proline 619, cysteine 210, and tyrosine 710 (Kurečka et al., 2021).

The nutritional composition of larvae depends on the media consumed by the larva (Scala et al., 2020). Lesser mealworm culture media can be derived from industrial or agricultural byproducts such as soybean meal, rice bran, and chicken manure. According to Molese et al. (2023), tofu residue can be used as a protein-rich feed ingredient with a crude protein content of 28.36%, 5.52% fat, 7.06% crude fiber, and 45.44% NFE. Energy gross of tofu residue is 4858,16

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kcal/kg. *Alphitobius diaperinus* thrives in poultry houses (poultry manure) and grain silos (Crippen et al., 2022). The nutritional content of layer chicken manure includes crude protein 13.12%, crude fiber 18.34%, crude fat 3.10%, ash 0.13%, and NFE 62.61% (Puteri et al., 2022).

The research findings of Amran et al. (2024) reported that concentrate, dried tofu residue, and rice bran are culture media for producing lesser mealworms, with the selected media being concentrate and tofu residue. To date, the protein requirements of the culture medium for producing lesser mealworms remain unknown. Generally, lesser mealworms live in chicken manure, which contains 13% crude protein (Puteri et al., 2022). Additionally, Soetemans et al. (2020) states that lesser mealworms living on a medium with 18.6% crude protein have higher larval production and nutrient content; specifically 106% larval production, 47.8% crude protein content, and 23.7% crude fat content in dry matter. Tofu residue, rice bran, and layer chicken manure complement each other as culture media. However, there has been no research on the protein requirements of culture media for lesser mealworms. Culture medium protein will influence the growth, chitin content, crude protein, crude fat, nitrogen retention, crude fat digestibility, and metabolizable energy of lesser mealworms. Based on the above explanation, research was needed to determine the influence of culture medium protein level and the optimal level to improve the nutritional quality and metabolizable energy of lesser mealworms.

MATERIALS AND METHODS

Materials

The material used in this study was culture media consisting of dried tofu residue, rice bran, and dried laying chicken manure. There were 320 parent beetles (*Alphitobius diaperinus*) per biopon. Broiler chickens of the Lohman MB 202 strain, 6 weeks old (body weight ± 1500 g), totaling 24 chickens, were used for measuring nitrogen retention, crude fat digestibility, and metabolizable energy. Chemical reagents were used for analyzing protein, fat, and chitin content. The equipment used in this study included an analytical scale, biopon (plastic breeding containers) measuring 40×30×10 cm, nets to cover the biopon, egg trays as hiding places and egg-laying sites for beetles, a blender as a media grinder, a 2-mesh sieve, a metabolizable cage measuring 60×30×20 cm for measuring nitrogen retention, crude fat digestibility, and metabolizable energy, an oven, and a set of equipment for analyzing protein, fat, and chitin content, as well as a bomb calorimeter. Lesser mealworms (*Alphitobius diaperinus*) cultivation (Figure 1).



Parent beetles *Alphitobius diaperinus*



Dried tofu residue



Rice bran



Dried laying chicken manure



Media production



Biopon set-up



Lesser Mealworm



Lesser mealworms in a single biopon



Harvesting of lesser mealworms

Figure 1 - Lesser mealworm (*Alphitobius diaperinus*) cultivation.

Method

This study used a completely randomized design (CRD) according to [Steel and Torrie \(1995\)](#), consisting of 4 treatments and 5 replicates. The treatments were the protein levels in the culture medium, consisting of: A = 13%; B = 15%; C = 17%; D = 19%.

Lesser mealworm cultivation

Lesser mealworm cultivation is carried out according to the research by [Amran et al. \(2024\)](#). The culture medium consists of tofu residue, rice bran, and laying chicken manure. Tofu pulp and laying hen feces are dried in the sun. Once dry, the media is blended until smooth. Next, each medium is filtered through a 2-mesh sieve and weighed to 500 grams for each treatment. Then, 320 adult lesser mealworms were introduced into a medium per biopon. Egg trays were placed as hiding spots and egg-laying sites for the beetles. Unripe papaya was cut into 2×2×1 cm pieces and placed at 9 points on the medium. The papaya is replaced once every three days. After 10 days, the beetles are removed from the medium by sieving. During cultivation, biopons are placed in a location that is not exposed to direct sunlight, with good air ventilation and an average room temperature and humidity of 27°C and 71%, respectively. The larvae are reared for 18 days after the beetles are removed from the medium. The larvae are harvested according to [Nuraini et al. \(2022\)](#) by sieving with a 2-mesh sieve to separate the larvae from the medium, then weighed to obtain the fresh weight. Thereafter, the larvae are steamed for 10 minutes to kill them, then drained and dried in an oven (50°C, 24 hours). The dried larvae were weighed to determine their dry weight. The variables observed were larval consumption, larval production ([Maha et al., 2021](#)), larval body weight gain ([Fitri, 2022](#)), chitin content (No, 1987), crude protein, crude fat ([AOAC, 2016](#)), nitrogen retention, crude digestibility, and metabolizable energy ([Sibbald and Wolynetz, 1985](#)).

Measurements of nitrogen retention, crude fat digestibility, and metabolizable energy were conducted using 24 broilers with a weight range of $\pm$ 1500 grams (20 broilers were used for the lesser mealworm treatment with protein levels in the culture medium, and 4 broilers were used for endogenous N), which were placed in individual metabolic cages. The broilers were first fasted for 24 hours, then force-fed 15 grams of the treatment feed. The excreta were then collected for 36 hours and sprayed with 0.3 N H₂SO₄ every 5 hours, after which the nitrogen, crude fat, and gross energy content of the excreta were analyzed.

RESULTS AND DISCUSSION

The average performance of lesser mealworms is shown in Table 1.

Table 1 - Performance of lesser mealworms.

Treatment (protein medium)	Larval consumption (g/500 g medium)**	Larval production (g/500 g medium)**	Larval body weight gain (g/500 g medium)**
A (13%)	110.30 ^d	72.54 ^b	42.47 ^d
B (15%)	124.53 ^c	80.11 ^b	57.17 ^c
C (17%)	176.14 ^b	115.94 ^a	91.21 ^b
D (19%)	181.87 ^a	122.52 ^a	107.51 ^a
SE	1.82	4.38	2.91

**Significantly different ($P < 0.01$), a,b,c,d Means within a column with different superscripts differ significantly ($P < 0.01$), SE = Standard Error.

Larval consumption

Table 1 shows that the highest consumption of larvae from lesser mealworms was found in treatment D (19% protein medium), which was 181.87 g/500 g medium, and the lowest was found in treatment A (13% protein medium), which was 110.30 g/500 g medium. The low average consumption of lesser mealworm larvae in treatment A (110.30 g/500 g medium) was due to the consumption of larvae in treatment A (13% protein medium), which originated from 100% chicken manure. This phenomenon is because the manure contains ammonia, which reduces the palatability of the lesser mealworms, leading to decreased larval consumption. Ammonia increases media moisture and pH, creating an unfavorable environment for the growth of lesser mealworms. Media with a strong odor can reduce feed palatability, making the feed unappealing to lesser mealworms ([Fernández et al., 2020](#)). [Crippen et al. \(2022\)](#) stated that high ammonia concentrations can be toxic and cause physiological stress in lesser mealworms, although lesser mealworms can utilize ammonia for metabolism; excessive levels can disrupt the nervous system and feeding behavior.

The average consumption rate of lesser mealworm larvae in treatment D (19% protein medium) was 181.87 g/500 g medium. This was due to the medium mixture consisting of 47% tofu residue, 38% rice bran, and 15% chicken manure. The preferred medium texture for lesser mealworm habitats is dry, loose, not too dense, and not wet. Media such as dry tofu residue and concentrates like rice bran have proven optimal for lower mealworm growth and production, as their

textures are easy to dig into, do not clump, and maintain sufficient moisture without becoming soft or watery. The fine, loose, and non-clumping texture of dried tofu residue makes it easier for lesser mealworms to consume and digest the culture medium, thereby enhancing palatability and increasing larval consumption. This assertion is supported by [Amran et al. \(2024\)](#), who stated that the use of dried tofu residue and concentrates can promote high larval consumption in lesser mealworm. Larval consumption of the selected lesser mealworm larvae was observed in treatment D (19% protein medium), amounting to 181.87 g/500 g medium. The research findings of [Safitri and Widiyaningrum \(2024\)](#) indicated that the average larval consumption of lesser mealworm larvae for 50 individuals was 1.138 grams. Metabolizable energy factors also influence the consumption rate of lesser mealworm larvae. Lower metabolizable energy in the culture medium leads to greater consumption of mealworm larvae, as fewer mealworms require it.

Larval production

Table 1 shows that the highest larval production from lesser mealworms was found in treatment D (19% protein medium), which was 122.52 g/500 g medium, and the lowest was found in treatment A (13% protein medium), which was 72.54 g/500 g medium. The low larval production from lesser mealworms in treatment A, which was 72.54 g/500 g medium, was due to the low ratio energy-protein of the culture medium, which was 182.42:1 in treatment A. One of the important components in the culture medium for lesser mealworms is energy and protein, which play a crucial role in cell synthesis and tissue formation. Low protein content in the culture medium can reduce mealworm growth, resulting in lower larval production. This aligns with the research by [Amran et al. \(2024\)](#), which found that the low live weight of lesser mealworms is influenced by the low protein content of their culture medium. [Scott et al. \(1992\)](#) also emphasized that the balance of nutrients, particularly protein and energy, significantly influences the rate of body weight gain in larvae. The protein-to-energy ratio is also an important factor in lesser mealworm production. According to [Schøn et al. \(2025\)](#), a higher protein-to-carbohydrate ratio accelerates larval growth and development by increasing protein deposition.

Treatment C achieved the highest larval production from lesser mealworms, with 115.94 g/500 g medium, followed by treatment D with 122.52 g/500 g medium. This was due to the high energy-protein ratio of the culture medium in treatments C and D, which were 233.15 : 1 and 227.81 : 1, respectively. The high protein content of the lesser mealworm culture medium can influence larval production because energy and protein play a crucial role in the growth and development of the lesser mealworm's body. Protein is the primary source of amino acids used by lesser mealworms to build and repair their body tissues, as well as to support various important metabolizable functions. Culture media with high protein content provide lesser mealworms with sufficient nutrients to support their optimal growth. This conclusion is supported by [Kotsou et al. \(2021\)](#), who showed that high protein content in lesser mealworm feed can increase growth and survival rates as well as shorten development time.

Larval body weight gain

Table 1 shows that the highest body weight gain of lesser mealworm larvae was found in treatment D (19% protein medium), which was 107.51 g/500 g medium, while the lowest body weight gain of lesser mealworm larvae was found in treatment A (13% protein medium), which was 42.47 g/500 g medium. The lower average body weight gain of lesser mealworm larvae in treatment A (42.47 g/500 g medium) was due to the medium's lower protein content (13%). The low protein content of the culture medium fails to meet the protein requirements for lesser mealworm growth, resulting in low body weight gain of lesser mealworm larvae. Low protein content in the medium can disrupt metabolizable processes, leading to suboptimal conversion of nutrients into energy and building materials for the body, thereby reducing growth and body weight gain of lesser mealworm larvae. Research shows that culture media with low nutrient content reduce metabolism in lesser mealworms, resulting in lower fresh weights and lengths than media with high nutrient content, such as dried tofu residue or concentrate. This aligns with the findings of [Amran et al. \(2024\)](#), where the low live weight of lesser mealworm was influenced by the low protein content of the culture medium.

The highest average body weight gain of lesser mealworm larvae in treatment D (19% protein content) was 107.51 g/500 g medium. This is due to the high crude protein content of the medium in this treatment, with crude protein content in treatment D at 19%. Crude protein content influences the magnitude of body weight gain in lesser mealworm larvae, as they are selective in the feed they consume. Protein plays a crucial role in metabolic processes, the growth of new tissues, and the repair of damaged tissues. The higher the protein content in the medium, the more nutrients are available to support the growth and development of lesser mealworm, resulting in a significant increase in their body weight. [Scott et al. \(1992\)](#) also emphasized that the balance of nutrients, particularly protein and energy, significantly influences the rate of body weight gain in larvae. The high body weight gain in larvae is also due to their high consumption. High larval consumption provides sufficient nutrient intake, particularly protein and energy, which are the primary factors in accelerating the growth and body weight gain of lesser mealworm larvae.

Digestibility analysis

The average nutritional content and nutritional digestibility of lesser mealworms are shown in Table 2.

Table 2 - Nutritional content and nutritional digestibility of lesser mealworms.

Treatment (protein medium)	Chitin (%)**	Crude Protein (%DM)**	Nitrogen Retention (%)**	Crude Fat (%DM)**	Crude Fat Digestibility (%)**	Metabolizable Energy (kcal/kg)**
A (13%)	9.35 ^d	23.35 ^c	30.33 ^c	17.70 ^c	71.62 ^d	2378.85 ^d
B (15%)	13.58 ^c	25.63 ^c	52.08 ^b	20.98 ^b	74.12 ^c	2561.18 ^c
C (17%)	18.22 ^b	37.26 ^b	62.75 ^b	22.59 ^b	76.78 ^b	2762.94 ^b
D (19%)	21.17 ^a	46.77 ^a	78.72 ^a	25.69 ^a	79.55 ^a	2994.25 ^a
SE	0.60	2.66	5.01	0.49	0.71	7.78

Note: **: Significantly different (P<0.01); a,b,c,d: Means within a column with different superscripts differ significantly (P<0.01); DM = Dry Matter; SE =Standard Error.

Chitin content

Table 2 shows that the highest chitin content in lesser mealworm was observed in treatment D (19% protein medium; 21.17%) and the lowest in treatment A (13% protein medium; 9.35%). The low chitin content of lesser mealworm in treatment A (9.35%) is related to the low larval production of lesser mealworm in treatment A. Lesser mealworms with low larval production have low live weight, which leads to reduced skin production. This is because chitin is located in the outer skin of the lesser mealworm. [Iber et al. \(2022\)](#) stated that chitin is the main component of the exoskeleton or shell of crustaceans and insects, as well as the cell walls of yeast, algae, and fungi. The chitin content of the lesser mealworm in treatment D was higher than in other treatments, at 21.17%. This is related to the high larval production in treatment D. Lesser mealworm living on a high-protein medium can meet their protein needs, resulting in fewer mealworm with higher live weight and larger body size, thereby increasing chitin content. This aligns with the findings of [Alhidayat et al. \(2025\)](#), who reported that lesser mealworm culture media with high crude protein content produce lesser mealworms with larger body weights, thereby increasing chitin content. The chitin content of lesser mealworm is high in treatment D, but its administration in poultry feed certain limits (not in high concentrations because of chitin), will not interfere with poultry productivity, as can be seen from the research of [Wandira et al. \(2016\)](#), which found no difference in body weight gain between ducks not given chitin and those given 1%, 2%, and 3% chitin in the ration. Another opinion by [Sedgh-Gooya et al. \(2022\)](#) states that 5% chitin in dry matter does not harm broilers. Regarding the effect of chitin on nitrogen retention, [Khempaka et al. \(2011\)](#) reported that feeding shrimp meal at 20% with a chitin content of 3.8% resulted in nitrogen retention of 50.89%.

Crude protein

Table 2 shows that the highest crude protein content of lesser mealworms was found in treatment D (medium protein 19%) at 46.77% and the lowest was found in treatment A (medium protein 13%) at 23.35%. The crude protein content of lesser mealworms in treatments A and B is low, at 23.35% and 25.63%, respectively, due to the low crude protein content in the culture media, which are 13% and 15%, respectively. If lesser mealworms lack protein in the culture media, their bodies will experience limitations in metabolism. The enzymes formed from protein will also decrease in number, so that the mealworm's metabolism cannot function optimally. Digestive enzymes function to break down food into smaller nutrients that can be absorbed. Protein deficiency disrupts enzyme synthesis, which ultimately affects nutrient absorption and crude protein production in the mealworm's body. This finding is supported by [Nuraini et al. \(2022\)](#), who found that mealworm protein content is influenced by the nutrient composition of the culture medium.

The high crude protein content of lesser mealworms in treatment D (46.77%) is related to the high crude protein content of the culture medium in treatment D (19%). The crude protein content in the culture medium directly affects the crude protein content of the mealworms. If the culture medium contains a high amount of crude protein, then the mealworms raised in that medium will also exhibit a high crude protein content, and vice versa. Protein functions in tissue formation through protein synthesis within the lesser mealworm's body. According to [Fuso et al. \(2021\)](#), the protein level achieved by larvae is greatly influenced by the amount of protein and fiber available in their food substrate.

The crude protein content of the selected lesser mealworms was found in treatment D (culture medium protein 19%) at 46.77%. Other studies indicate that lesser mealworms have a protein content of 48% when fed a 18.6% crude protein culture medium ([Soetemans et al., 2020](#)). The difference in crude protein content is due to the difference in the culture medium used, which consisted of wheat flour mixed with 15% rapeseed flour and beer waste.

Nitrogen retention

Table 2 shows that the highest nitrogen retention in lesser mealworms was found in treatment D (19% protein medium) at 78.72%, while the lowest was found in treatment A (13% protein medium) at 30.33%. The low protein intake in treatment A (3.11 g/head) influences the lower nitrogen retention in lesser mealworms (30.33%). The low protein consumption is due to the low crude protein content of lesser mealworms in treatment A, at 23.35%. The culture

medium's low protein content of 13% contributes to the low crude protein content of lesser mealworms. If the culture medium has low protein content, the lesser mealworm's body will also have a limited amount of available amino acids. As a result, the lesser mealworm's body has difficulty maintaining or storing nitrogen for metabolic needs, leading to low nitrogen retention. This phenomenon can be seen from the research by [Alhidayat et al. \(2025\)](#), which found that yellow mealworms living in a culture medium with crude protein of 22% had lower nitrogen retention of 72.27% compared to a culture medium with crude protein of 26%, which had nitrogen retention of 80.89%.

Lesser mealworms in treatment D exhibited a high nitrogen retention of 78.72%. This was influenced by the high protein intake in treatment D, which was 6.25 g/head. The high protein intake was due to the high crude protein content of lesser mealworms in treatment D, which was 46.77%. The high crude protein content of lesser mealworms in this treatment was due to the high protein content of the culture medium, which was 19%. Lesser mealworms with adequate protein intake have better nitrogen balance between intake and excretion. This aligns with the opinion of [Ren et al. \(2025\)](#) that to achieve nitrogen balance in insects, there needs to be a balance between food intake, metabolic conversion, and excretion. Digested protein provides nitrogen used for tissue synthesis. High nitrogen retention indicates that lesser mealworms can utilize most of the nitrogen intake.

The high nitrogen retention in treatment D reflects the satisfactory protein quality of the lesser mealworms. This aligns with the research by [Alhidayat et al. \(2025\)](#), which found that lesser mealworms with high nitrogen retention also have high protein quality. The high protein quality in treatment D is also reflected in the amino acid composition of the lesser mealworms. The results of amino acid content of lesser mealworms in treatment D contain several amino acids, namely lysine 0.39%, methionine 0.56%, threonine 1.31%, tryptophan 0.47%, arginine 1.11%, glutamic acid 4.07%, alanine 3.32%, aspartic acid 2.45%, glycine 2.31%, histidine 1.08%, isoleucine 1.73%, cysteine 1.20%, leucine 2.70%, valine 2.26%, phenylalanine 1.93%, proline 2.32%, serine 1.44%, and tyrosine 2.07%.

The highest nitrogen retention in lesser mealworms in this study was observed in treatment D (19% protein medium), at 78.72%. This result is lower than the nitrogen retention of yellow mealworms (*Tenebrio molitor*), which was 80.89% ([Alhidayat et al., 2025](#)). The nitrogen retention of lesser mealworms in treatment D was lower than that of superworms (*Zophobas morio*), which was 80.36% ([Nuraini et al., 2025](#)).

Crude fat

Table 2 shows that the highest crude fat content was found in treatment D (culture medium protein 19%) at 25.69%, and the lowest crude fat content was found in treatment A (culture medium protein 13%) at 17.70%. The low crude fat content of the lesser mealworms in treatment A (17.70%) is related to the low crude fat content of the culture medium in treatment A (3.1%), which affects fat metabolism as an energy reserve for the lesser mealworms. In treatment A, the culture medium used was 100% chicken feces. Chicken feces can also have toxic properties due to their ammonia content, which inhibits mealworm growth, resulting in low fat storage in the mealworm's bodies. According to [Rumpold and Schlüter \(2013\)](#), although chicken manure can be used as a physical medium for mealworm growth, its nutrient content is low, which increases physiological stress on the mealworms, causing available energy to be primarily used for survival rather than fat storage.

The high crude fat content of larvae in treatment D (crude protein 19%) at 25.69% is due to the high protein content of the medium. The high protein content of the culture medium is not entirely used for body tissue synthesis; the larvae undergo deamination, where the amino groups of amino acids are released, and the remaining carbon is utilized in energy metabolism pathways. If the energy produced from this metabolism is not immediately utilized, the excess energy is converted into energy reserves in the form of fat through the process of lipogenesis. This process leads to an increase in crude fat content in the mealworm's body. Additionally, the high protein content promotes faster growth, causing the mealworms to require more energy reserves for the subsequent metamorphosis phase, which are stored in the form of body fat. Research conducted by [Kim et al. \(2019\)](#) shows that media with high protein content produce mealworms with higher crude fat content compared to media with low protein content. Thus, it can be concluded that the higher the protein content in the culture medium, the greater the potential for increased crude fat content in the mealworm's body.

The crude fat content of lesser mealworms is greatly influenced by the feed medium; lesser mealworms grown on high-quality medium tend to have high crude fat content. The fat content of the culture medium greatly influences the growth of lesser mealworms. According to [Suwannayod et al. \(2025\)](#), fat-rich culture media significantly increase the fat content in larval bodies.

The crude fat content of the selected mealworms was 25.69% in treatment D, which used a mixture of tofu pulp, feces, and rice bran (crude protein 19%) as the medium. Another study stated that media using industrial waste produced lesser mealworms with a crude fat content of 26% ([Soetemans et al., 2020](#)).

Crude fat digestibility

Table 2 shows that the highest crude fat digestibility was found in treatment D (culture medium protein 19%) at 79.55%, and the lowest crude fat digestibility was found in treatment A (culture medium protein 13%) at 71.62%. The low

crude fat digestibility of lesser mealworms in treatment A (71.62%) is attributed to the low crude fat content of lesser mealworms in treatment A (17.70%). The low crude fat content of lesser mealworms (*Alphitobius diaperinus*) may be one of the causes of low fat digestibility when they are used as feed for poultry. This is because the amount of fat substrate available in the feed significantly determines the activity and efficiency of lipid-digesting enzymes in poultry, such as pancreatic lipase. When the fat content in the ration is very low, lipase activity in the digestive tract tends to decrease because the stimulus for enzyme secretion is reduced. This condition prevents some of the available fat fractions from being optimally digested and absorbed. The crude fat had the highest digestibility in treatment D, at 79.55%. This was due to the high fat content of the culture medium (25.69%). The high fat content led to increased fat digestibility because more fat substrate was absorbed. In addition to the high fat content of the mealworms, the medium's protein content also affected their fat digestibility. According to Miguéns-Gómez et al. (2020), when the protein requirements of the larvae are met, energy and fat metabolism occur more efficiently, resulting in larvae with tissue structure and fat composition that are easier to digest by the poultry digestive system. It is further explained that lesser mealworms reared on a protein-rich medium tend to produce softer body tissue with a fat composition that is more easily digested by the poultry digestive system.

Metabolizable Energy

Table 4 shows that the highest metabolizable energy (ME) was found in treatment D (crude protein 19%) at 2994.25 kcal/kg, while the lowest metabolizable energy (ME) was found in treatment A (crude protein 13%) at 2378.85 kcal/kg. The low metabolizable energy (ME) of the lesser mealworms in treatment A, which is 2378.85 kcal/kg, is due to the low protein content (CP 13.12%) and fat content (Fat 3.1%) of the culture medium in treatment A. The nutritional content of the culture medium also affects the growth and energy metabolism of the lesser mealworms. Culture media with low fat content will inhibit caterpillar growth, resulting in low metabolizable energy content in caged caterpillars because fat is the highest source of energy in insects, so a decrease in fat is directly proportional to a decrease in metabolizable energy (Schiavone et al., 2017). The highest metabolizable energy content in lesser mealworms was observed in treatment D (crude protein 19%), with 2994.25 kcal/kg. This indicates that the culture medium has high nutritional quality. High-quality culture media enhance the feed conversion efficiency of lesser mealworms. This means that lesser mealworms can convert feed into body tissue with higher energy content, such as fat and protein. High-quality media rich in protein, fat, and energy increases the metabolizable energy content of lesser mealworm bodies, due to higher growth and nutrient absorption rates. This aligns with the research by Fowles and Nansen (2020), which found that protein-rich media also promote optimal growth and metabolism in larvae. Adequate protein supports enzyme synthesis, the formation of body tissues, and the conversion of nutrients into energy reserves.

CONCLUSION

This study concludes that crude protein at 19% is the optimal protein content for the selected lesser mealworms culture medium, resulting in lesser mealworm with a larval consumption rate of 181.87 g/500 g medium, larval production of 122.52 g/500 g medium, larval body weight gain of 107.51 g/500 g medium, chitin content of 21.17%, crude protein content of 46.77% (dry matter), nitrogen retention of 78.72%, crude fat content of 25.69% (dry matter), crude fat digestibility of 79.55%, and metabolizable energy of 2994.25 kcal/kg. This result is due to the availability of adequate protein media at 19%, which efficiently support the synthesis of body tissue protein and lipogenesis, producing larvae with greater body mass and a rich nutritional profile, making them a highly valuable alternative feed source for poultry.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Nuraini Nuraini; E-mail: nuraini@ansci.unand.ac.id; ORCID: <https://orcid.org/0000-0001-9963-1328>

Availability of data and materials

The raw data, study data, and materials are accessible upon request.

Ethical consideration

Before publication in this journal, all authors have reviewed ethical concerns, including data fabrication, duplicate publication and submission, redundancy, plagiarism, consent to publish, and misconduct.

Authors' contribution

Nuraini designed the research, coordinated the research and data analysis, drafted and edited the publication manuscript, and provided final approval. Mirzah and Yuliaty Shafan Nur assist with laboratory and data analysis, and with editing publication manuscripts. Abdul Azim, Dinah Fathinah, and Rezki Wahyudi conducted the cultivation of lesser mealworms, analyzed the data, and drafted the manuscript.

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Competing interests

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

REFERENCES

- Alhidayat R, Nuraini N, Rias MI and Akbar M (2025). Effect of culture media composition on chitin content, nitrogen retention, crude fat digestibility, and growth of mealworms (*Tenebrio molitor*) [Pengaruh komposisi media biakan terhadap kandungan kitin, retensi nitrogen, pencernaan lemak kasar, dan pertumbuhan dari ulat hongkong (*Tenebrio molitor*)]. Jurnal Ilmu Peternakan Terapan, 8(1): 100-109. <https://publikasi.polije.ac.id/jipt/article/view/5400>
- Amran M, Haryadi H and Trisna A (2024). The effect of different media on lesser mealworm production (*Alphitobius diaperinus*) as a source of protein feed for poultry [Pengaruh media berbeda terhadap produksi ulat kandang (*alphitobius diaperinus*) sebagai pakan sumber protein ternak unggas]. Jurnal Peternakan Lokal, 6(1): 44-52. <https://doi.org/10.46918/peternakan.v6i1.2143>
- AOAC (2016). Official Methods of Analysis of the Association of Analytical Chemist. Association of Official Analytical Chemist, Virginia. <https://www.aoac.org/?s=aoac+2016>
- Crippen TL, Singh B, Anderson RC and Sheffield CL (2022). Management practices affecting lesser mealworm larvae (*Alphitobius diaperinus*) associated microbial community in a broiler house and after relocating with the litter into pastureland. Frontiers in microbiology, 13, 875930. <https://doi.org/10.3389/fmicb.2022.875930>
- Fernández NF, Defagó MT, Palacios SM and Arena JS (2020). Antifeedant effect of plant extracts on the poultry pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae): an exploratory study. Revista De La Sociedad Entomológica Argentina, 79(4). <https://www.biotaxa.org/RSEA/article/view/66244>
- Fitri WN (2022). The effect of different culture media compositions on crude protein, crude fat, crude fiber, and fresh weight yellow mealworms (*Tenebrio molitor*) [Pengaruh komposisi media biakan berbeda terhadap protein kasar, lemak kasar, serat kasar dan berat segar ulat hongkong (*Tenebrio molitor*)]. Thesis, Animal Science Faculty, Andalas University. <http://scholar.unand.ac.id/id/eprint/201057>
- Fowles TM and Nanse C (2020). Insect-based bioconversion: value from food waste using black soldier fly larvae. Waste Management, 109: 505–517. <https://doi.org/10.1016/j.wasman.2020.05.038>
- Fuso A, Barbi S, Macavei LI, Luparelli AV, Maistrello L, Montorsi M, et al. (2021). Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. Foods, 10 (8). <https://doi.org/10.3390/foods10081773>
- Iber BT, Kasan NA, Torsabo D and Omuwa JW (2022). A review of various sources of chitin and chitosan in nature. Journal of Renewable Materials, 10(4): 1097-1123. <https://doi.org/10.32604/jrm.2022.018142>
- Kaddour A, Diaw MT, Saidj D, Yahi K, and Moula N (2025). Effects of a mealworm larvae-based diet on laying performance and egg quality in laying hens. Journal of the Indonesian Tropical Animal Agriculture, 50 (3): 235-244, <https://doi.org/10.14710/jitaa.50.3.235-244>
- Khempaka S, Chitsatchapong C and Molee W (2011). Effect of chitin and protein constituents in shrimp head meal on growth performance, nutrient digestibility, intestinal microbial populations, volatile fatty acids, and ammonia production in broilers. Journal of Applied Poultry Research, 20(1): 1-11. <https://doi.org/10.3382/japr.2010-00162>
- Kim HW, Bae S, Park YS, Kim MJ and Hwangbo J (2019). Growth performance and body composition of mealworms (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) fed diets with varying protein levels. Livestock Science, 226: 31–36. <https://doi.org/10.1016/j.livsci.2019.04.001>
- Kotsou K, Rumbos CI, Baliota GV, Gourgouta M and Athanassiou CG (2021). Influence of temperature, relative humidity, and protein content on the growth and development of larvae of the lesser mealworm, *Alphitobius diaperinus* (Panzer). Sustainability, 13(19). <https://doi.org/10.3390/su131911087>
- Kurečka M, Kulma M, Petříčková D, Plachý V and Kourímská L (2021). Larvae and pupae of *Alphitobius diaperinus* as promising protein alternatives. European Food Research and Technology, 247: 2527-2532. <https://doi.org/10.1007/s00217-021-03807-w>
- Maha IV, Elfrida E and Sarjani TM (2021). Utilization of organic waste as *Tenebrio molitor* Feed [Pemanfaatan limbah organik sebagai media pakan *Tenebrio molitor*]. Jurnal Jeumpa, 8 (1): 516-524. <https://doi.org/10.33059/jj.v8i1.3987>
- Molese LT, Mulis M and Suherman SP (2023). The effect of fermented tofu pulp on silkworm biomass (*Tubifex sp*) [Pengaruh pemberian fermentasi ampas tahu terhadap biomassa cacing sutra (*Tubifex sp*)]. Journal of Fisheries Agribusiness, 1(2): 77-84. <http://dx.doi.org/10.56190/jfa.v1i2.17>
- Miguéns-Gómez A, Rumpold BA, Nørgaard JV, Smetana S, Dalle ZA and Jansson A (2020). Insect larvae as a sustainable source of proteins and fats for feed and food – A review. Journal of Insects as Food and Feed, 6(3): 221–231. <https://doi.org/10.3920/JIFF2019.0044>
- No HK (1987). Application of Crawfish Chitosan as a Coagulant in Recovery of Organic Compounds from Seafood Processing Wastes. LSU Historical Dissertations and Theses, Louisiana State University, 4468. https://repository.lsu.edu/gradschool_disstheses/4468
- Nuraini N, Nur YS, Djulardi A, Amizar R and Sari YC (2022). Medium for cultivation of *Tenebrio molitor* larvae and its effects as alternative animal protein source in the diet on the performance of broiler. Advances in Animal and Veterinary Sciences Journal, 10(11): 2335-2346. <http://dx.doi.org/10.17582/journal.aavs/2022/10.11.2335.2346>
- Nuraini N, Nur YS, Djulardi A and Sari YS (2023). The effect of dietary supplementation of Hong Kong caterpillar (*Tenebrio molitor*) on quail egg quality. Journal of World's Poultry Research, 13 (2): 253-260. <https://dx.doi.org/10.36380/jwpr.2023.28>

- Nuraini N, Mirzah M, Nur YS, Syabil KM, Ismaliza and Hijr F (2025). Performance, carcass quality, and physiological organ assesment of broilers fed *Zophobas morio* caterpillar in the diet. International Journal of Veterinary Science, 14(4): 668-675. <https://doi.org/10.47278/journal.ijvs/2025.023>
- Puteri RE, Sa'adah R and Laras RG (2022). Evaluation of nutritional value and amino acid content in poultry manure for catfish feed (*Clarias gariepinus*) [Evaluasi nilai gizi dan kandungan asam amino pada kotoran unggas untuk pakan ikan lele (*Clarias gariepinus*)]. Jurnal Perikanan, 12(4): 691-698. <https://doi.org/10.29303/jp.v12i4.397>
- Ren H, Pu Q, Yang X, Kashyap S and Liu S (2025). Regulatory mechanisms of nitrogen homeostasis in insect growth and development. Insect Science. <https://doi.org/10.1111/1744-7917.70059>
- Rumpold BA and Schlüter OK (2013). Nutritional composition and safety aspects of edible insects. Comprehensive Reviews in Food Science and Food Safety, 12(2): 191–210. <https://doi.org/10.1111/1541-4337.12014>
- Safitri N and Widiyaningrum P (2024). Analysis of feeding ability and mortality of lesser mealworms (*Alphitobius diaperinus*) exposed to lantana leaf extract (*Lantana camara*) [Analisis kemampuan makan dan mortalitas ulat kandang (*Alphitobius diaperinus*) yang terpapar ekstrak daun lantana (*Lantana camara*)]. Prosiding Seminar Nasional Sains dan Teknologi Seri 02, Science and Technology Faculty, Terbuka University. 1(2), 792–801. <https://conference.ut.ac.id/index.php/saintek/article/view/2718>
- Sajjad M, Sajjad A, Chisti GA, Khan EU, Mozuraitis R and Binyameen M. (2024). Insect larvae as an alternate protein source in poultry feed improve the performance and meat quality of broilers. Animals, 14 (14). <https://doi.org/10.3390/ani14142053>
- Sammarco BC, Hinkle NC and Crossley MS (2023). Biology and management of lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in broiler houses. Journal of Integrated Pest Management, 14 (1): 1-8. <https://doi.org/10.1093/jipm/pmad003>
- Scala A, Cammack JA, Sallia R, Scieuzo C, Franco A, Bufo SA, Tomberlin JK and Falabella P. (2020). Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale. Scientific Report, 119448. <https://doi.org/10.1038/s41598-020-76571-8>
- Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Biasibetti ED, et al. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: Apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology, 8(1): 51. <https://doi.org/10.1186/s40104-017-0181-5>
- Schøn ML, Jensen, K, Schow-Madsen M, Gold M, Mathys A, Schou TM, et al. (2025). Using gas exchange measurements to monitor growth, energy expenditure, and body composition of black soldier fly larvae (*Hermetia illucens*) on diets varying in protein-to-carbohydrate ratio. bioRxiv. <https://doi.org/10.1101/2025.09.08.674872>
- Scott ML, Nesheim and Yaoung (1992). Nutrition of The Chicken. M.L. Scott and associates. Ithaca., New York. https://books.google.com/books/about/Nutrition_of_the_Chicken.html?id=Zly8QgAACAAJ&utm_source=chatgpt.com
- Sedgh-Gooya S, Torki M, Darbemamieh M, Khamisabadi H and Abdolmohamadi A (2022). Growth performance and intestinal morphometric features of broiler chickens fed on dietary inclusion of yellow mealworm (*Tenebrio molitor*) larvae powder. Veterinary Medicine and Science, 8(5): 2050-2058. <https://doi.org/10.1002/vms3.881>
- Sibbald IR and Wolynetz MS (1985). Estimates of retained nitrogen are used to correct estimates of bioavailable energy. Poultry Science, 64: 1506-1513. <https://doi.org/10.3382/ps.0641506>
- Soetemans L, Gianotten N and Bastiaens L (2020). Agri-Food Side-Stream Inclusion in the Diet of *Alphitobius diaperinus*. Part 2: Impact on Larvae Composition. Insects, 11(3): 190. DOI: <https://doi.org/10.3390/insects11030190>
- Steel RGD and Torrie JH (1995). Statistical Principles and Procedures, PT Gramedia, Jakarta. <https://lib.ui.ac.id/detail.jsp?id=20129245>
- Suwannayod S, Setthaya P, Limsopatham K and Harnpornchai N. (2025). The effect of growth and nutrition in black soldier fly larvae fed by hemp seed oil mixed diets. Insects, 16 (11): 1081. <https://doi.org/10.3390/insects16111081>
- Tilak R, Sharma A, Kapoor S and Bajaj S (2023). A rare case of gastrointestinal canthariasis caused by *Alphitobius diaperinus*. Journal of Communicable Diseases, 5(1): 74-77. <https://doi.org/10.24321/0019.5138.202311>
- Wandira IA, Ichsan M and Wiryawan KG (2016). Weight gain and plasma cholesterol concentration in Mojo sari ducks fed different levels of chitin extracted from crab wastes. Proceedings of International Seminar on Livestock Production and Veterinary Technology, pp. 390-394. https://www.researchgate.net/publication/326555475_Weight_Gain_and_Plasma_Cholesterol_Concentration_in_Mojosari_Ducks_Fed_Different_Levels_of_Chitin_Extracted_from_Crab_Wastes

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BENEFICIAL EFFECTS OF *Canavalia gladiata* SEED MEAL FERMENTATION ON PROXIMATE COMPOSITION, BIOACTIVE COMPOUNDS, FATTY ACID PROFILE AND AMINO ACID PROFILE

Gningnini Alain KONE¹ , N'Da Amani Sylvère Bertrand KOKO¹ , Faustin Parfait KOUTOUAN¹ ,
Konan Raphaël N'GUESSAN¹ , and Maryline KOUBA² 

¹Joint Research and Innovation Unit for Agricultural Sciences and Transformation Processes, National Polytechnic Institute Felix Houphouët Boigny, Yamoussoukro P.o Box: 1313, Côte d'Ivoire

²Joint research unit For Physiology, Environment, and Genetics for the Animal and Livestock Systems, Institut Agro, National Research Institute for Agriculture, Food and Environment, Saint Gilles 35590, France

✉ Email: alain.kone@inphb.ci

➤ Supporting Information



ABSTRACT: Soybean meal is the dominant protein source in animal feed, but rising costs have encouraged the search for alternatives. *Canavalia gladiata*, a tropical legume with good yield potential and resistance to pests, contains about 26% protein. However, its use in non-ruminant diets is constrained by anti-nutritional factors. Fermentation with *Saccharomyces cerevisiae* has been shown to enhance protein content and reduce anti-nutrients in various feed ingredients, making it a promising strategy for improving *C. gladiata* seed. The aim of this study was to evaluate the effect of yeast fermentation on *C. gladiata* seed meal proximate composition, bioactive compounds, fatty acid profile and amino acid profile. In this study, raw seed meal (RCSM) was prepared from *C. gladiata* seed grown in Côte d'Ivoire, while fermented seed meal (FCSM) was obtained through a two-step aerobic and anaerobic fermentation with yeast. Proximate composition, bioactive compounds, antioxidant activity, fatty acid and amino acid profiles were determined using standard analytical methods. Fermentation increased dry matter by 4% and crude protein by 23%, while reducing carbohydrates and ash. It also enhanced total polyphenols by 11.11% and antioxidant activity by 32.14%, though flavonoids decreased slightly and tannin levels increased. Fatty acid profiles showed stability of polyunsaturated fatty acid (PUFA) and n-6/n-3 ratios within optimal ranges. Amino acids increased overall, with notable rises in several essential amino acids, though lysine, cystine, and glutamic acid declined. Overall, yeast fermentation significantly improved the nutritional and bioactive properties of *C. gladiata* seed meal, supporting its potential as an affordable alternative protein source for monogastric animal diets.

Keywords: Bioactive compounds, *Canavalia gladiata*, Fermentation, Nutritional value, Yeast.

INTRODUCTION

Soybean meal (SBM) remains the primary protein ingredient in animal diets due to its rich protein concentration and favorable amino acid composition (Cherdthong et al., 2014; Gunun et al., 2022). However, the recent increase in its market price has limited its use in feed formulation, prompting nutritionists to explore more affordable alternative protein supplements (Gunun et al., 2022).

Canavalia gladiata is a leguminous plant that originated in the Asian continent and spread throughout the tropics (Sasipriya and Siddhuraju, 2012). Eaten as a green vegetable in Asia, this plant has particular agronomic traits, including a high cultivation temperature (Nishizawa and Aii, 2018). They are cultivated on a limited scale throughout Asia, the West Indies, Africa and South America (Ekanayake et al., 2006). Moreover, its average yield is comparable to that of the soybean, and it is relatively resistant to pests and diseases (Nishizawa and Aii, 2018). Concerning nutritional properties, the *C. gladiata* seed contains approximately 26% protein, 3% fat, and 62% carbohydrate (Sridhar and Seena, 2006; Nishizawa and Aii, 2018). But, presence of anti-nutrients limits its use as a feed ingredient for livestock particularly non-ruminant animals (Sasipriya and Siddhuraju, 2013).

Yeast (*Saccharomyces cerevisiae*) has been applied to enhance the nutritional quality of feed ingredients (Polyorach et al., 2016; Promkot et al., 2017; Gunun et al., 2022) and to mitigate anti-nutritional compounds present in some animal diets (Boonnop et al., 2009). Indeed, Cherdthong and Supapong (2019) reported that cassava waste from bioethanol production fermented with yeast had a crude protein content of 25.1% of dry matter, twice that of unfermented cassava waste. Also, Gunun et al. (2022) reported that rubber seed kernel contained crude protein at 21.2%

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of dry matter, while the yeast-fermented rubber seed kernel product contained crude protein at 33.6% of dry matter, an increase in crude protein of 12.4 % of dry matter. Moreover, as a food-safe strain (Son et al., 2023; Wang et al., 2024), *S. cerevisiae* plays a pivotal role as a chassis organism for the efficient production of fatty acids and their derivatives (Wang et al., 2024). The aim of this study was to evaluate the effect of yeast fermentation on *C. gladiata* seed meal proximate composition, bioactive compounds, fatty acid profile and amino acid profile.

MATERIALS AND METHODS

Production of *C. gladiata* seeds

The raw seeds of *C. gladiata* were produced at the graduate school of agriculture experimental station, at the National Polytechnic Institute Felix Houphouët Boigny (INP-HB) in Yamoussoukro, located in central Côte d'Ivoire, from April to July 2024. Moreover, the *C. gladiata* seeds were sun-dried for 5 days after harvest, sorted, and stored in a dry environment until use.

Processing methods of RSCM and FCSM

The raw *C. gladiata* seed meal (RSCM) was prepared from *C. gladiata* seed ground using a universal grinder. The fermented *C. gladiata* seed meal (FCSM) was prepared by fermenting the RSCM with commercial dry yeast (*Saccharomyces cerevisiae*). The fermentation process was carried out according to the method reported by Shi et al. (2017), with some minor modifications. Each kilogram of seed meal as fermentation substrate was mixed and inoculated with one liter of distilled water containing 2% (w/w) dry yeast (*Saccharomyces cerevisiae*). Aerobic fermentation was carried out at 37 °C for 24 hours. After the first step, the aerobically fermented mixture was transferred to a vacuum oven and then fermented under anaerobic conditions at 37 °C for 24 hours. After fermentation, the mixture was oven-dried at 60 °C for 72 hours to obtain FCSM.

Chemical analyses

Proximate composition of RSCM and FCSM was analyzed using the methods of AOAC (2000). Lipids were extracted from samples using the method of Folch et al. (1957). Bioactive compounds were extracted from samples according to the hydroalcoholic method described by Sinan et al. (2023). The total polyphenols were quantified by the method described by Lezoul et al. (2020) and Tiho et al. (2017). The total flavonoids were quantified by aluminium chloride colorimetric method described by Tiho et al. (2017). The total tannins were quantified by the Folin-Ciocalteu method described by Hossain et al. (2020). The condensed tannins were quantified by the vanillin acid method described by Price et al. (1978). The antioxidant activity of samples was measured by the 2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS^{•+}) radical cation method described by Tiho et al. (2017). Fatty acid profile of RSCM and FCSM was analyzed according to the methods described by Koné et al. (2020). Amino acids from RSCM and FCSM were analyzed by acid hydrolysis method (AFNOR, 2005).

Statistical analysis

Raw and fermented *C. gladiata* seed meals parameters data were subjected to the independent samples t-test at 5% significance, using R version 4.5.1 software.

RESULTS AND DISCUSSION

Proximate composition of RSCM and FCSM

The results of this study showed that fermentation improved the nutritional profile of *C. gladiata* seed meal, particularly in terms of dry matter and crude protein content by 4% and 23%, respectively (Table 1). The increase in dry matter content and the decrease in carbohydrate and ash contents observed in the fermented seed meal compared to the raw meal is likely due to microbial degradation of soluble carbohydrates and moisture reduction during the processing, which has been similarly reported in legume-based substrates (Ojokoh et al., 2013; Arise et al., 2022). Moreover, Yang et al. (2018) reported that the crude protein content significantly increased by 12% in fermented soybean meal produced by a combination of *B. subtilis*, *L. casei*, and yeast, as compared with those in raw soybean meal.

The increase in crude protein content following fermentation indicates a concentration effect often attributed to the microbial breakdown of non-protein components, such as fiber and anti-nutritional factors, as well as the biosynthesis of microbial proteins (Hidalgo-Fuentes et al., 2024). This aligns with findings from previous studies on other legumes, where solid-state fermentation enhanced the crude protein content of cowpea (Ojokoh et al., 2013) and jack bean (Arise et al., 2022).

Table 1 - Proximate composition of RSCM and FCSM in % of dry matter (DM)

Proximate composition ¹	RSCM	FCSM	SEM	p-value
Dry matter (in % of crude matter)	91.23 ^b	94.89 ^a	0.82	6.67 x 10 ⁻⁶
Crude Protein	25.62 ^b	30.38 ^a	1.14	6.87 x 10 ⁻³
Ether Extract	4.95	5.09	0.04	1.15 x 10 ⁻¹
Crude Fiber	8.28	7.76	0.25	3.51 x 10 ⁻¹
Ash	4.24 ^a	3.95 ^b	0.07	6.84 x 10 ⁻⁴
Total Carbohydrate ²	65.19 ^a	60.58 ^b	1.13	4.02 x 10 ⁻²
ME (kcal/kg of dry matter) ³	3366.73	3330.01	9.99	4.24 x 10 ⁻¹

RSCM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; ME: Metabolizable energy; SEM: Standard error of the mean. ¹ Values are the means of three analyses per sample. ² Total carbohydrate (Carb_Tot) = 100 - [Protein (% DM) + Fat (% DM) + Ash (% DM)] (Tiho et al., 2024); ³ ME (kcal/kg of dry matter) = [2.44 × Protein (% DM) + 8.37 × Fat (% DM) + 3.57 × Carb_Tot (% DM)] × 10 (Tiho et al., 2024). ^{a,b} Parameter means within rows with no common superscript differ (p < 0.05).

Bioactive compounds of RSCM and FCSM

The fermentation of *C. gladiata* flour induced an increase in total polyphenol content by 11.11% (Table 2). This increase can probably be explained by the release of phenolic compounds bound or their biotransformation into more active molecules by microbial enzymes such as tannases and glycosidases (Yang et al., 2023). As polyphenols are major antioxidants, this increase could strengthen the antioxidant potential of fermented seed, corroborating several studies on fermented vegetables and legumes (Asensio-Grau et al., 2020; Emkani et al., 2022; Knez et al., 2023). The enhancement of total polyphenols is consistent with previous findings in fermented legumes such as soybeans and pigeon peas, where microbial fermentation has been shown to release bound phenolics from the cell wall matrix or synthesize new bioactive compounds (Duenas et al., 2005; Hur et al., 2014).

Interestingly, a slight decrease in flavonoid content by 7.17% was observed after fermentation. This reduction may be attributed to the partial degradation, transformation, or use of certain flavonoids by fermenting microorganisms (Maria John et al., 2014; Zhao et al., 2021; Emkani et al., 2022).

Contrary to the commonly anticipated effects of fermentation (Adebo et al., 2022; Yang et al., 2023; Hidalgo-Fuentes et al., 2024), an increase in tannin content was observed in the present study. Similarly, a rise in total tannin levels has also been reported during the fermentation of pea protein concentrate (Çabuk et al., 2018). This may be explained by either the liberation of bound tannins from the seed matrix (Çabuk et al., 2018) or the biosynthesis of tannin-like phenolics by certain microbial strains during the fermentation process (Emkani et al., 2022). In contrast, condensed tannin content remained unchanged, suggesting that these compounds are more chemically stable and less affected by fermentation conditions (Makkar, 2003). This finding agrees with previous studies reporting the resilience of condensed tannins during biotransformation processes (Francis et al., 2002).

Finally, the significant enhancement in antioxidant activity by 32.14% following fermentation could be directly linked to the observed increases in total polyphenols and tannins. According to Zhao et al. (2021) and Kim et al. (2024), the antioxidant activity is positively correlated with yeast fermentation. Both classes of compounds are known to contribute to antioxidant capacity through mechanisms such as hydrogen atom donation, metal chelation, and inhibition of lipid peroxidation (Shahidi and Ambigaipalan, 2015). This link is well established: these families of compounds contribute strongly to free radical scavenging capacities and oxidative stabilization (reduction of oxidative stress) in various fermented systems (Zhao et al., 2021; Emkani et al., 2022). This result supports the hypothesis that fermentation could be an effective tool to enhance the biofunctional potential of *C. gladiata*, particularly its antioxidant properties.

Table 2 - Bioactive compounds of RSCM and FCSM

Bioactive compounds ¹	RSCM	FCSM	SEM	P-value
Total Polyphenols (mg GAE/g of dry matter)	82.21 ^b	91.34 ^a	2.30	1.82 x 10 ⁻²
Total Flavonoids (mg QE/g of dry matter)	52.98 ^a	49.18 ^b	0.90	5.33 x 10 ⁻³
Total tannins (mg TAE/g of dry matter)	0.34 ^b	0.45 ^a	0.02	4.91 x 10 ⁻¹¹
Condensed Tannins (mg CatE/g of dry matter)	0.08	0.07	0.01	4.60 x 10 ⁻¹
Antioxidant Activity (µmol TE/g of dry matter)	0.56 ^b	0.74 ^a	0.04	1.35 x 10 ⁻⁸

RSCM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAE: Tannic acid equivalent; CatE: Catechin equivalent; TE: Trolox equivalent; SEM: Standard error of the mean. ¹ Values are the means of three analyses per sample. ^{a,b} Parameter means within rows with no common superscript differ (p < 0.05).

Fatty acid (FA) profile of RSCM and FCSM

The unsaturated fatty acids (74.81% and 71.88%, respectively for raw and fermented seed meal) were more abundant in *C. gladiata* seed oil than saturated fatty acids (25.17% and 28.53%, respectively for raw and fermented seed meal) (Table 3). These results confirm those of [Qian et al. \(2025\)](#), who reported that *C. gladiata* seed oil contained 69.1 to 81.8% unsaturated fatty acids and 18.2 to 30.9% saturated fatty acids. The fermentation of *C. gladiata* resulted in a decrease in unsaturated fatty acids by 3.92%, and an increase in saturated fatty acids (SFA) by (13.35%), notably palmitic acid (8.31%) and stearic acid (53.58%). This development is consistent with several recent studies that report that fermentation of food breaks down nutrients, including proteins associated with allergic reactions, making them easily digestible, and it also creates beneficial nutrients, including fatty acids ([Borresen et al., 2012](#); [Song et al., 2013](#); [Kim et al., 2015](#)). *Saccharomyces cerevisiae* plays a role in food production and also exhibits fatty acid composition changes depending on its environment and growth conditions ([Son et al., 2023](#)). The yeast can synthesize fatty acids, particularly saturated fatty acids like palmitic acid (16:0), and unsaturated fatty acids like oleic acid (18:1), with the latter's synthesis influenced by oxygen availability ([Wang et al., 2024](#)).

In contrast, the balance between polyunsaturated fatty acid (PUFA) contents, notably C18: 2n-6 and C18: 3n-3, were little affected by fermentation, as indicated by the relative stability of the C18: 2n-6/C18: 3n-3 and n-6/n-3 ratios, increasing respectively from 1.89 to 2.01 and from 1.89 to 1.98. The C18:2n-6/C18:3n-3 ratio is considered balanced, particularly in animal feed, when it is less than 4 as recommended by [ANSES \(2011\)](#) and [Simopoulos \(2016\)](#) to optimize the immune response. [Simopoulos and Di-Nicolantonio \(2016\)](#) concluded that n-3 fatty acids participate to a decrease adipose tissue development and lead to weight loss, while n-6 fatty acids increase adipose tissue synthesis and lead to obesity. After examining various effects of n-6/n-3 ratios on experimental animals, [Simopoulos and Di-Nicolantonio \(2016\)](#) indicated that the preferred ratio for an optimal health could be 1:1 or 2:1. This n-6/n-3 ratio interval 1:1 to 2:1 was increased, and set to be optimal between 1:1 and 5:1 according to [Gonzalez-Becerra et al. \(2023\)](#). Anyhow, high n-6/n-3 ratios between 10:1 and 20:1 should be stickily avoided, because they increase the risk of inflammatory diseases and obesity ([Simopoulos and Di-Nicolantonio, 2016](#); [Gonzalez-Becerra et al., 2023](#)).

Table 3 - Fatty acid profile of RSCM and FCSM in % of total fatty acid.

Fatty acid ¹	RSCM	FCSM	SEM	P-value
Myristic acid, C14:0	0.51 ^a	0.45 ^b	0.02	2.13 × 10 ⁻²
Pentadecanoic acid, C15:0	0.60	0.62	0.02	6.50 × 10 ⁻¹
Palmitic acid, C16:0	17.70 ^b	19.17 ^a	0.36	1.08 × 10 ⁻²
Stearic acid, C18:0	2.93 ^b	4.95 ^a	0.45	6.52 × 10 ⁻⁶
Arachidic acid, C20:0	0.87	0.83	0.03	5.23 × 10 ⁻¹
Behenic acid, C22:0	0.43	0.38	0.02	1.11 × 10 ⁻¹
Tricosanoic acid, C23:0	0.21	0.20	0.00	7.05 × 10 ⁻²
Lignoceric acid, C24:0	1.50	1.47	0.02	4.10 × 10 ⁻¹
Hyenic acid, C25:0	0.42 ^b	0.46 ^a	0.01	2.19 × 10 ⁻³
Total of saturated fatty acids (SFA)	25.17 ^b	28.53 ^a	0.79	4.45 × 10 ⁻³
9-hexadecenoic acid, C16:1 n-9	0.18 ^b	0.22 ^a	0.01	6.08 × 10 ⁻⁴
Palmitoleic acid, C16:1 n-7	1.62 ^b	3.68 ^a	0.46	3.03 × 10 ⁻⁵
Oleic acid, C18:1 n-9	43.81 ^a	39.97 ^b	0.90	2.56 × 10 ⁻³
Cis-vaccenic acid, C18:1 n-7	2.95	2.84	0.03	5.44 × 10 ⁻²
Gondoic acid, C20:1 n-9	0.75	0.69	0.02	2.16 × 10 ⁻¹
Total of monounsaturated fatty acids (MUFA)	49.92	48.01	0.56	7.74 × 10 ⁻²
Linoleic acid, C18:2 n-6	16.23 ^a	15.40 ^b	0.22	2.76 × 10 ⁻²
Arachidonic acid, C20:4 n-6	0 ^b	0.23 ^a	0.05	6.30 × 10 ⁻⁴
Total of n-6 FA	16.23	15.63	0.17	7.37 × 10 ⁻²
Linolenic acid, C18:3 n-3	8.61 ^a	7.77 ^b	0.20	6.77 × 10 ⁻³
Total of polyunsaturated fatty acids (PUFA)	24.89 ^a	23.47 ^b	0.37	2.57 × 10 ⁻²
Total of unsaturated fatty acids (UFA)	74.81 ^a	71.48 ^b	0.72	4.99 × 10 ⁻²
PUFA/MUFA	0.50	0.49	0.01	6.17 × 10 ⁻²
UFA/SFA	2.9 ^a	2.5 ^b	0.10	6.75 × 10 ⁻⁴
C18:2 n-6/C18:3 n-3	1.89 ^b	2.01 ^a	0.02	4.87 × 10 ⁻³
n-6/n-3	1.89 ^b	1.98 ^a	0.03	5.30 × 10 ⁻³

RSCM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; FA: Fatty acid; SFA: Sum of saturated fatty acids; MUFA: Sum of monounsaturated fatty acids; PUFA: Sum of polyunsaturated fatty acids; UFA: Sum of unsaturated fatty acids; n-6/n-3: Sum of n-6 fatty acids/sum of n-3 fatty acids ratio; SEM: Standard error of the mean. ¹ Values are the means of three analyses per sample. ^{a,b} Parameter means within rows with no common superscript differ (p < 0.05).

Amino acid (AA) profile of RCSM and FCSM

The quantity of AAs was much higher in fermented *C. gladiata* seed meal with *S. cerevisiae* than in raw *C. gladiata* seed meal (93.36 vs 87.52 g for 100 g of protein, respectively) (Table 4). Fermentation, one of the earliest techniques for preserving perishable foods, also enhances their nutritional quality by increasing amino acid levels (Elhalis et al., 2023; Yang et al., 2024). Additionally, the fermentation of food breaks down nutrients, including proteins associated with allergic reactions, making them easily digestible, and it also creates beneficial nutrients, including amino acids (Borresen et al., 2012; Song et al., 2013; Kim et al., 2015). The present study confirmed increases in several essential AAs and non-essential AAs in the FCSM, when compared with the RCSM. In fact, all AAs increased by 4.36% to 15.04% in fermented *C. gladiata* seed meal, excepted, the contents of lysine, cystine and glutamic acid that decreased respectively by 8.74%, 19.33% and 2.84%. Moreover, Kim et al. (2015) showed that the amounts of essential amino acids and non-essential amino acids were little increased respectively by 1.98% and 3.98% in fermented *C. gladiata* with 3% *A. oryzae* at 25 ± 2 °C and $55 \pm 5\%$ at ambient humidity for 36 h and dried. Also, Yang et al. (2018) indicated that total amino acid contents significantly increased by 5% in fermented soybean meal produced by a combination of *B. subtilis*, *Lactobacillus casei*, and yeast, as compared with those in soybean meal. In addition, the contents of phenylalanine, valine, and methionine were increased by 9-42% after fermentation. The increase in the content of sweet amino acids such as glycine and alanine after fermentation could increase the fermented *C. gladiata* seed meal in sweetness (Yang et al., 2024).

Table 4 - Amino acid profile of RCSM and FCSM in g/100 g protein

Amino acid	RCSM	FCSM	SEM	p-value
Arginine	5.50 ^b	6.04 ^a	0.12	2.51×10^{-5}
Histidine	3.06 ^b	3.44 ^a	0.09	1.49×10^{-3}
Isoleucine	4.02 ^b	4.57 ^a	0.12	1.76×10^{-4}
Leucine	7.65 ^b	8.41 ^a	0.17	7.41×10^{-4}
Lysine	6.18 ^a	5.64 ^b	0.14	2.13×10^{-2}
Methionine	0.96 ^b	1.13 ^a	0.04	2.56×10^{-2}
Phenylalanine	4.71 ^b	5.08 ^a	0.09	1.46×10^{-2}
Serine	5.39 ^b	5.98 ^a	0.14	3.83×10^{-3}
Threonine	4.53 ^b	4.97 ^a	0.10	1.53×10^{-3}
Tryptophan	1.08	1.13	0.03	4.87×10^{-1}
Valine	4.65 ^b	5.31 ^a	0.15	8.56×10^{-5}
Total of essential AAs	47.73 ^b	51.70 ^a	0.95	5.91×10^{-3}
Alanine	4.65 ^b	5.08 ^a	0.10	4.60×10^{-4}
Aspartic acid	11.17 ^b	11.91 ^a	0.17	1.12×10^{-4}
Cystine	1.19 ^a	0.96 ^b	0.05	2.14×10^{-3}
Glutamic acid	11.62 ^a	11.29 ^b	0.07	1.76×10^{-4}
Glycine	3.97 ^b	4.57 ^a	0.14	4.66×10^{-4}
Proline	3.68 ^b	4.18 ^a	0.12	1.57×10^{-3}
Tyrosine	3.51	3.67	0.06	1.88×10^{-1}
Total of non-essential AAs	39.79 ^b	41.66 ^a	0.45	7.09×10^{-3}

RCSM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; AAs: Amino acids; SEM: Standard error of the mean. ¹ Values are the means of three analyses per sample. ^{a,b} Parameter means within rows with no common superscript differ ($p < 0.05$).

CONCLUSION

To our knowledge, the fermentation of *C. gladiata* seed meal with yeast (*Saccharomyces cerevisiae*) has not been previously reported. The results of this study indicate that yeast fermentation could be an effective biotransformation approach for enhancing the protein quality and amino acid bioavailability of *C. gladiata* seed meal, thereby making its nutritional profile more competitive. Yeast-based fermentation appears to be a promising method for improving the bioactive potential of *C. gladiata* seeds. Consequently, the rise in bioactive compounds may positively influence the functional properties of the fermented seed meal, particularly with regard to shelf-life extension and gut health in animal feeding applications. This shows its potential as a value-added alternative protein source for monogastric animal diets.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Gningnini Alain Koné; E-mail: alain.kone@inphb.ci; ORCID: <https://orcid.org/0000-0002-5143-3311>

Data availability

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

Authors' contribution

Gningnini Alain Koné designed the study, contributed to data analysis and manuscript writing, and coordinated the contributions of all other authors; N'Da Amani Sylvère Bertrand Koko and Konan Raphaël N'Guessan contributed to study design, data collection, and manuscript writing; Faustin Parfait Koutouan designed the study and manuscript writing; Maryline Kouba contributed to the study design and critical revision of the manuscript, as well as to the analysis of the samples.

Ethical regulations

Not applicable.

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Competing Interests

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

Abbreviations

SBM: Soy Bean Meal; INP-HB: National Polytechnic Institute Felix Houphouët Boigny; RSCM: Raw *C. gladiata* Seed Meal; FCSM: Fermented *C. gladiata* Seed Meal; AAs: Amino Acids.; CP: Crude Protein; AOAC: Association of Official Analytical Chemists; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; TAE: Tannic Acid Equivalent; CatE: Catechin Equivalent; TE: Trolox Equivalent; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); Abs: Absorbance; DF: Dilution Factor; AFNOR: French Association for Standardization; ISO: International Organization for Standardization; ANSES: French Agency for Food, Environmental and Occupational Health & Safety; ME: Metabolizable Energy; FAO: Food and Agricultural Organization of the United Nations; DM: Dry Matter; FA: Fatty acid; SFA: Sum of Saturated Fatty Acids; MUFA: Sum of Monounsaturated Fatty Acids; PUFA: Sum of Polyunsaturated Fatty Acids; UFA: Sum of Unsaturated Fatty Acids; SEM: Standard error of the mean.

REFERENCES

- Adebo JA, Njobeh PB, Gbashi S, Oyedele OM, Oyeyinka SA, et al. (2022). Fermentation of cereals and legumes: impact on nutritional constituents and nutrient bioavailability. *Fermentation*, 8(2): 63. <https://doi.org/10.3390/fermentation8020063>
- ANSES (2011). Actualisation des apports nutritionnels conseillés pour les acides gras. ANSES, Maisons-Alfort, France, 323 p. <https://www.anses.fr/en/system/files/NUT2006sa0359Ra.pdf>
- AOAC (2000). Association of official analytical chemists. official methods of analysis, 17th Edition. Arlington. VA. USA. <https://groupdocs.vip/reviews/E08035/312322/4983933-aoac-2000-official-methods-of-analysis-17th-ed>
- Arise AK, Malomo SA, Cynthia CI, Aliyu NA and Arise RO (2022). Influence of processing methods on the antinutrients, morphology and in-vitro protein digestibility of jack bean. *Food Chemistry Advances*, 1: 100078. <https://doi.org/10.1016/j.focha.2022.100078>
- Asensio-Grau A, Calvo-Lerma J, Heredia A, Andrés A (2020). Enhancing the nutritional profile and digestibility of lentil flour by solid state fermentation with *Pleurotus ostreatus*. *Food & Function*, 11(9): 7905-7912. <https://doi.org/10.1039/D0FO01527J>
- Boonnop K, Wanapat M, Nontaso N and Wanapat S (2009). Enriching nutritive value of cassava root by yeast fermentation. *Scientia Agricola*, 66(5): 629-633. <https://doi.org/10.1590/S0103-90162009000500007>
- Borresen EC, Henderson AJ, Kumar A, Weir TL and Ryan EP (2012). Fermented foods: patented approaches and formulations for nutritional supplementation and health promotion. *Recent Patents on Food, Nutrition & Agriculture*, 4(2): 134-140. <https://doi.org/10.2174/2212798411204020134>
- Çabuk B, Nosworthy MG, Stone AK, Korber DR, Tanaka T, House JD, et al. (2018). Effect of fermentation on the protein digestibility and levels of non-nutritive compounds of pea protein concentrate. *Food Technology & Biotechnology*, 56: 257-264. <https://doi.org/10.17113/ftb.56.02.18.5450>
- Cherdthong A and Supapong C (2019). Improving the nutritive value of cassava bioethanol waste using fermented yeast as a partial replacement of protein source in dairy calf ration. *Tropical Animal Health and Production*, 51: 2139-2144. <https://doi.org/10.1007/s11250-019-01911-4>
- Cherdthong A, Wanapat M, Saenkamsorn A, Waraphila N, Khota W, Rakwongrit D, et al. (2014). Effects of replacing soybean meal with dried rumen digesta on feed intake, digestibility of nutrients, rumen fermentation and nitrogen use efficiency in Thai cattle fed on rice straw. *Livestock Science*, 169: 71-77. <https://doi.org/10.1016/j.livsci.2014.09.008>
- Duenas M, Fernandez D, Hernandez T, Estrella I and Munoz R (2005). Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L.). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum*. *Journal of the Science of Food and Agriculture*, 85(2): 297-304. <https://doi.org/10.1002/jsfa.1924>
- Ekanayake S, Nair BM, Asp NG and Jansz ER (2006). Effect of processing of sword beans (*Canavalia gladiata*) on physicochemical properties of starch. *Starch-Stärke*, 58 (5): 215-222. <https://doi.org/10.1002/star.200500449>
- Elhalis H, See XY, Osen R, Chin XH and Chow Y (2023). Significance of fermentation in plant-based meat analogs: a critical review of nutrition, and safety-related aspects. *Foods*, 12(17): 3222. <https://doi.org/10.3390/foods12173222>
- Emkani M, Oliete B and Saurel R (2022). Effect of lactic acid fermentation on legume protein properties, a review. *Fermentation*, 8(6): 244. <https://doi.org/10.3390/fermentation8060244>

- Folch J, Lees M and Sloane Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1): 497-509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5)
- Francis G, Kerem Z, Makkar HPS and Becker K (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, 88(6): 587-605. <https://doi.org/10.1079/BJN2002725>
- French Association for Standardization (AFNOR) (2005). Aliments des animaux - détermination de la teneur en acides aminés. NF EN ISO 13903. 25p. Retrieved on 23 August 2012 from <http://www.afnor.org/en>
- Gonzalez-Becerra K, Barron-Cabrera E, Muñoz-Valle JF, Torres-Castillo N, Rivera-Valdes JJ, Rodriguez-Echevarria R, et al. (2023). A balanced dietary ratio of n-6:n-3 polyunsaturated fatty acids exerts an effect on total fatty acid profile in RBCs and inflammatory markers in subjects with obesity. *Healthcare*, 11(16): 2333. <https://doi.org/10.3390/healthcare11162333>
- Gunun N, Oupppamong T, Khejornsart P, Cherdthong A, Wanapat M, Polyorach S, et al. (2022). Effects of rubber seed kernel fermented with yeast on feed utilization, rumen fermentation and microbial protein synthesis in dairy heifers. *Fermentation*, 8(6): 288. <https://doi.org/10.3390/fermentation8060288>
- Hidalgo-Fuentes B, de Jesús-José E, Cabrera-Hidalgo AdJ, Sandoval-Castilla O, Espinosa-Solares T, González-Reza RM, et al. (2024). Plant-based fermented beverages: nutritional composition, sensory properties, and health benefits. *Foods*, 13(6): 844. <https://doi.org/10.3390/foods13060844>
- Hossain MA, Disha NK, Shourave JH and Dey P (2020). Determination of antioxidant activity and total tannin from drumstick (*Moringa oleifera* Lam.) leaves using different solvent extraction methods. *Turkish Journal of Agriculture-Food Science and Technology*, 8(12): 2749-2755. <https://doi.org/10.24925/turjaf.v8i12.2749-2755.4038>
- Hur SJ, Lee SY, Kim YC, Choi I and Kim GB (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, 160: 346-356. <https://doi.org/10.1016/j.foodchem.2014.03.112>
- Kim A, Balanov P and Smotraeva I (2024). Study of changes of antioxidant activity during fermentation of various types of legumes as functional food product. *Functional Foods in Health and Disease*, 14(11): 751-765. <https://doi.org/10.31989/ffhd.v14i11.1452>
- Kim OK, Chang JY, Nam DE, Park YK, Jun W and Lee J (2015). Effect of *Canavalia gladiata* extract fermented with *Aspergillus oryzae* on the development of atopic dermatitis in NC/Nga mice. *International Archives of Allergy and Immunology*, 168(2): 79-89. <https://doi.org/10.1159/000441654>
- Knez E, Kadac-Czapska K and Grembecka M (2023). Effect of fermentation on the nutritional quality of the selected vegetables and legumes and their health effects. *Life*, 13(3): 655. <https://doi.org/10.3390/life13030655>
- Koné GA, Good M and Kouba M (2020). Performance of guinea fowl fed hevea seed meal or cashew nut meal as a partial substitute for soya bean meal. *Animal*, 14(1): 206-214. <https://doi.org/10.1017/S175173111900185X>
- Lezoul NEH, Belkadi M, Habibi F and Guillén F (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules*, 25(20): 4672. <https://doi.org/10.3390/molecules25204672>
- Makkar HPS (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, 49(3): 241-256. [https://doi.org/10.1016/S0921-4488\(03\)00142-1](https://doi.org/10.1016/S0921-4488(03)00142-1)
- Maria John KM, Thiruvengadam M, Enkhtaivan G and Kim DH (2014). Variation in major phenolic compounds and quality potential of CTC black tea elicited by *Saccharomyces cerevisiae* and its correlation with antioxidant potential. *Industrial Crops and Products*, 55: 289-294. <https://doi.org/10.1016/j.indcrop.2014.02.006>
- Nishizawa K and Arai Y (2018). A crude sword bean (*Canavalia gladiata*) extract is gelled by cooling. *Bioscience, Biotechnology, and Biochemistry*, 82(1): 120-126. <https://doi.org/10.1080/09168451.2017.1403884>
- Ojokoh AO, Daramola MK and Oluoti OJ (2013). Effect of fermentation on nutrient and anti-nutrient composition of breadfruit (*Treculia africana*) and cowpea (*Vigna unguiculata*) blend flours. *African Journal of Agricultural Research*, 8(27): 3566-3570. <https://doi.org/10.5897/AJAR12.1944>
- Polyorach S, Pongchompu O, Wanapat M and Cherdthong A (2016). Optimal cultivation time for yeast and lactic acid bacteria in fermented milk and effects of fermented soybean meal on rumen degradability using nylon bac technique. *Asian-Australasian journal of animal sciences*, 29(9): 1273-1279. <https://doi.org/10.5713/ajas.15.0798>
- Price ML, Scoyoc SV and Butler LG (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26(5): 1214-1218. <https://doi.org/10.1021/jf60219a03>
- Promkot C, Nitipot P, Piamphon N, Abdullah N and Promkot A (2017). Cassava root fermented with yeast improved feed digestibility in brahman beef cattle. *Animal Production Science*, 57(8): 1613-1617. <https://doi.org/10.1071/AN15685>
- Qian H, Meng D, Lu Yue L, Xu H, Feng K and Wang J (2025). Ethnobotanical use, phytochemistry, pharmacology, and toxicity of *Canavalia gladiata*. *Drug Design, Development and Therapy*, 19: 3779-3803. <https://doi.org/10.2147/DDDT.S519102>
- Sasipriya G and Siddhuraju P (2012). Effect of different processing methods on antioxidant activity of underutilized legumes, *Entada scandens* seed kernel and *Canavalia gladiata* seeds. *Food and Chemical Toxicology*, 50(8): 2864-2872. <https://doi.org/10.1016/j.fct.2012.05.048>
- Sasipriya G and Siddhuraju P (2013). Evaluation of growth performance, serum biochemistry and haematological parameters on broiler birds fed with raw and processed samples of *Entada scandens*, *Canavalia gladiata* and *Canavalia ensiformis* seed meal as an alternative protein source. *Tropical animal health and production*, 45(3): 811-820. <https://doi.org/10.1007/s11250-012-0293-z>
- Shahidi F and Ambigaipalan (2015). Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - a review. *Journal of Functional Foods*, 18(B): 820-897. <https://doi.org/10.1016/j.jff.2015.06.018>
- Shi C, Zhang Y, Yin Y, Wang C, Lu Z, Wang F, et al. (2017). Amino acid and phosphorus digestibility of fermented corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* fed to pigs. *Journal of Animal Science*, 95(9): 3996-4004. <https://doi.org/10.2527/jas2017.1516>
- Simopoulos AP (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, 8(3): 128. <https://doi.org/10.3390/nu8030128>
- Simopoulos AP and Di-Nicolantonio JJ (2016). The importance of a balanced ω -6 to ω -3 ratio in the prevention and management of obesity. *Open Heart*, 3(2): e000385. <https://doi.org/10.1136/openhrt-2015-000385>

- Sinan KI, Yagi S, Llorent-Martínez EJ, Ruiz-Medina A, Gordo-Moreno AI, Stefanucci A, et al. (2023). Understanding the chemical composition and biological activities of different extracts of *Secamone afzelli* leaves: a potential source of bioactive compounds for the food industry. *Molecules*, 28(9): 3678. <https://doi.org/10.3390/molecules28093678>
- Son YJ, Jeon M-S, Moon HY, Kang J, Jeong DM, Lee DW, et al. (2023). Integrated genomics and phenotype microarray analysis of *Saccharomyces cerevisiae* industrial strains for rice wine fermentation and recombinant protein production. *Microbial Biotechnology*, 16(11): 2161-2180. <https://doi.org/10.1111/1751-7915.14354>
- Song SH, Lee C, Lee S, Park JM, Lee HJ, Bai DH, et al. (2013). Analysis of microflora profile in Korean traditional nuruk. *Journal of Microbiology Biotechnology*, 23(1): 40-46. <http://dx.doi.org/10.4014/jmb.1210.10001>
- Sridhar KR and Seena S (2006). Nutritional and antinutritional significance of four unconventional legumes of the genus canavalia - a comparative study. *Food Chemistry*, 99: 267-288. <https://doi.org/10.1016/j.foodchem.2005.07.049>
- Tiho T, Koné GA, Silué N, Djako MJ and Djaah G (2024). *Acacia auriculiformis* leaves' powder as an alternative for antibiotics uses in broilers organic farming. *World Journal of Advanced Research and Reviews*, 21(01): 2675–2685. <https://doi.org/10.30574/wjarr.2024.21.1.0357>
- Tiho T, Yao NJC, Brou YC and Adima AA (2017). Drying temperature effect on total phenols and flavonoids content, and antioxidant activity of *Borassus aethiopum* mart ripe fruits' pulp. *Journal of Food Research*, 6(2): 50-64. <http://dx.doi.org/10.5539/jfr.v6n2p50>
- Wang Z, Su C, Zhang Y, Shangguan S, Wang R and Su J (2024). Key enzymes involved in the utilization of fatty acids by *Saccharomyces cerevisiae*: a review. *Frontiers in Microbiology*. 14: 1294182. <https://doi.org/10.3389/fmicb.2023.1294182>
- Yang A, Zuo L, Cheng Y, Wu Z, Li X, Tong P, et al. (2018). Degradation of major allergens and allergenicity reduction of soybean meal through solid-state fermentation with microorganisms. *Food Function*, 9(3): 1899-1909. <https://doi.org/10.1039/c7fo01824j>
- Yang F, Chen C, Ni D, Yang Y, Tian J, Li Y, et al. (2023). Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: a review. *Foods*, 12(17): 3315. <https://doi.org/10.3390/foods12173315>
- Yang X, Hong J, Wang L, Cai C, Mo H, Wang J, et al. (2024). Effect of lactic acid bacteria fermentation on plant-based products. *Fermentation*, 10(1): 48. <https://doi.org/10.3390/fermentation10010048>
- Zhao Y-S, Eweys AS, Zhang J-Y, Zhu Y, Bai J, Darwesh OM, et al. (2021). Fermentation affects the antioxidant activity of plant-based food material through the release and production of bioactive components. *Antioxidants*, 10(12): 2004. <https://doi.org/10.3390/antiox10122004>

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Miller BA and Lu CD (2019). Current status of global dairy goat production: An overview. *Asian-Australasian Journal of Animal Sciences*, 32(8): 1219. [Link](#), [DOI](#)

Xu P, Zhang Z, Peng P, Yang J, Li X, Yuan T, et al. (2022). Study on vacuum drying kinetics and processing of the *Lonicera japonica* Thunb. aqueous extracts. *LWT - Food Science and Technology*. 167: 1-9. [Link](#), [DOI](#)

b) For symposia reports and abstracts

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (*Pampus argentens euphrasens*) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. *Asian Fisheries Society Manila, Philippine*, 13: 191-199. [Link](#), [DOI](#)

c) For edited symposia, special issues, etc., published in a journal

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), *Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science*, 31: 17-27. [Link](#), [DOI](#)

d) For books

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. [Link](#), [DOI](#)

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603. [Link](#), [DOI](#)

e) Books, containing sections written by different authors

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg). [Link](#), [DOI](#)

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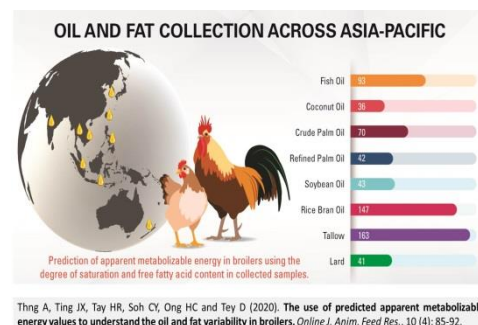
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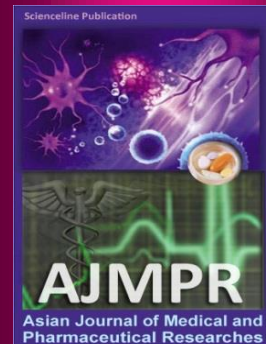
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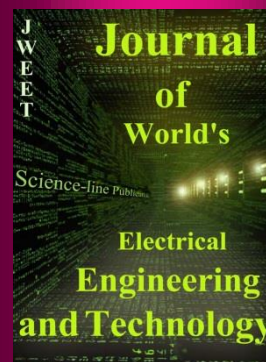
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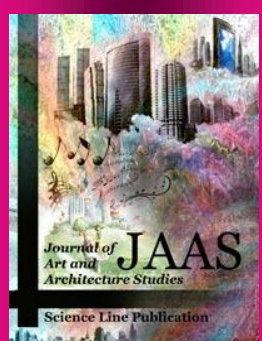
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