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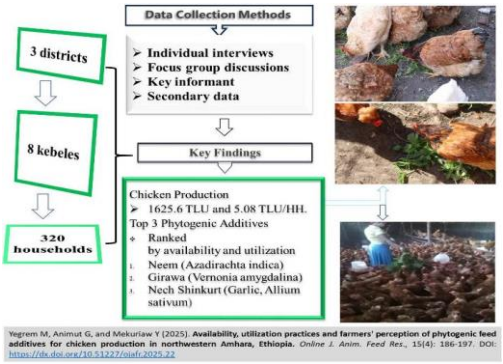
Volume 15 (4); July 2025

Research Paper

Availability, utilization practices and farmers' perception of phytogenic feed additives for chicken production in northwestern Amhara, Ethiopia

Yegrem M, Animut G, and Mekuriaw Y.

Online J. Anim. Feed Res., 15(4): 186-197, 2025; pii: S222877012500022-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.22



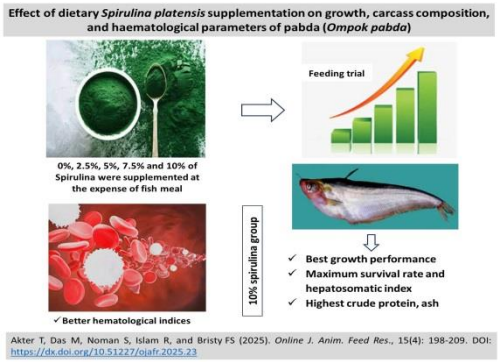
Abstract
The study was conducted to evaluate the availability, utilization practices, and farmers' perceptions of phytogenic feed additives for chicken production in Bahir Dar city and North Gojjam zone of Amhara region. The study included three areas (Bahir Dar city, North Achefer, and Bahir Dar Zuria districts), from which 320 respondents were selected from eight Kebeles. Data were collected from farm observations, individual interviews, and focus group discussions, supplemented by secondary information from agricultural offices records, and research publications. The study revealed a total of 1625.6 Tropical Livestock Units (TLU) of chickens in the study areas, and the average chicken holding per household (HH) was 5.08 TLU. Phytogenics were used as chicken feed additives by farmers in urban, peri-urban, and rural areas, with utilization rates of 58.3, 56.3, and 52.5%, respectively. Phytogenic feed additives such as Neem (*Azadirachta indica*), Girawa (*Vernonia amygdalina*), and Nech shinkurt (*Allium sativum*) ranked 1st, 2nd, and 3rd in their availability and 1st, 3rd, and 2nd in their utilization practices, respectively. The large majority of urban and peri-urban chicken producers (70.9 and 73.8%, respectively) had awareness of phytogenic feed additive utilization practices for chicken production. Phytogenic feed additives support sustainable poultry production in Ethiopia by improving food security, public health, environmental sustainability and economic resilience. Their use supports with national development goals and key sustainable development goals (SDGs), including zero hunger, good health and well-being and climate action. This finding suggests that phytogenic feed additives are readily available and utilized in the study area for chicken production at the farmer's level, albeit with no defined doses. Further research is needed to verify the effects of these phytogenic feed additives on chicken performance, and a nationwide assessment should be conducted to quantify their potential.
Keywords**:** Farmer's awareness, Girawa plant, Neem, Phytogenic feed additives, Poultry nutrition.
[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Effect of dietary *Spirulina platensis* supplementation on growth, carcass composition, and haematological parameters of pabda (*Ompok pabda*)

Akter T, Das M, Noman S, Islam R, and Bristy FS.

Online J. Anim. Feed Res., 15(4): 198-209, 2025; pii: S222877012500023-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.23



Abstract
The study was conducted to find out the effects and optimum level of dietary *Spirulina platensis* (Spirulina) supplementation on growth and haematological parameters of pabda (*Ompok pabda*). Five experimental diets were prepared containing 33% protein. Different levels of Spirulina (0% as control or S_0, $S_{2.5\%}$, $S_{5\%}$, $S_{7.5\%}$ and $S_{10\%}$) were supplemented at the expense of fish meal (FM), respectively. Up to 10% of the total dietary protein (33%) in the control diet was replaced by Spirulina protein in the experimental diets. The feeding experiment was carried out in five treatments with three replications for 10 weeks. The water quality parameters viz. ammonia, dissolve oxygen, pH, and temperature were within the suitable range for pabda culture. The best growth performance was observed in the fish fed with 10% Spirulina supplemented feed followed by 7.5%, 5% and 2.5% Spirulina supplemented diet ($P < 0.05$). Maximum survival rate (98%) and hepatosomatic index (1.37) of pabda was also found in $S_{10\%}$ Spirulina group. But the feed conversion ratio (FCR) was decreased significantly ($P < 0.05$) with the increasing level of *Spirulina* supplementation which indicated better feed utilization. In case of carcass composition of pabda, the highest percentage of crude protein and ash were observed in fish fed with 10% followed by 7.5%, 5%, 2.5% *S. platensis* supplemented diet ($P < 0.05$). Likewise, haematological condition in fish fed with 10% *Spirulina* supplemented diet resulted better in this study. Therefore, it could be concluded that

dietary supplementation of 10% *S. platensis* protein with FM protein may significantly improve the growth and the haematological parameters of *O. pabda*.
Keywords**:** Feed supplement, Fish meal, Growth, Haematology, *Ompok pabda, Spirulina platensis.*
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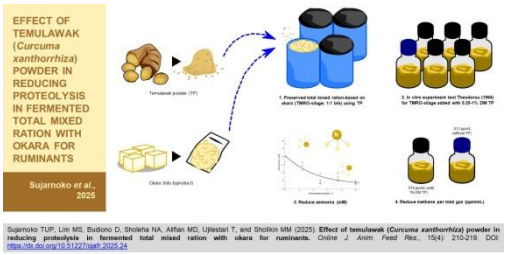
Research Paper

Effect of temulawak (*Curcuma xanthorrhiza*) powder in reducing proteolysis in fermented total mixed ration with okara for ruminants

Sujarnoko TUP, Lim MS, Budiono D, Sholeha NA, Alifian MD, Ujilestari T, and Sholikin MM.
Online J. Anim. Feed Res., 15(4): 210-219, 2025; pii: S222877012500024-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.24

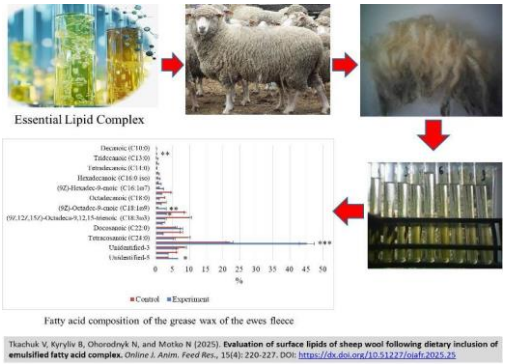


Abstract
High protein content in silage feed often triggered excessive proteolysis caused by proteases derived from both plants and spoilage bacteria, which reduced the nutritional quality for ruminants. Temulawak (*Curcuma xanthorrhiza*) is an herbal plant with antibacterial and antioxidant properties, which have been reported to reduce proteolysis in previous studies. This study aimed to evaluate the effects of temulawak powder (TP) as an anti-proteolysis additive in the fermentation of total mixed ration-based okara silage (TMRO-silage) and its impact on silage characteristics. Additionally, the study examined the effects of drying temperature on temulawak extract optimization. Temulawak was dried at 50, 65, and 80°C, followed by the measurement of phenol, flavonoid, and ferric reducing antioxidant power (FRAP) content. The TMRO-silage was composed of commercial feed and okaras (1:1 w/w), supplemented with 0-1% temulawak in increments of 0.25% (5 treatments and 4 replications). Fermentation lasted for 14 days. Proximate and *in vitro* analyses (control vs. temulawak treatments) were conducted to assess silage quality. Drying temulawak at 65°C significantly ($P < 0.01$) increased phenol (16.9 µg quercetin equivalent, QE), flavonoid (23.3 µg QE), and FRAP (30.1 µg QE) content per dry weight. Temulawak supplementation significantly reduced ammonia levels and increased the crude protein content of TMRO-silage ($P < 0.01$). Moreover, it decreased ammonia concentration in the rumen ($P < 0.01$), improved dry matter and organic matter digestibility ($P < 0.05$), and notably reduced methane production per total gas volume ($P < 0.05$). In conclusion, temulawak effectively preserves the quality of complete feed silage, enhances rumen metabolism, and mitigates methane emissions.
Keywords**:** Antibacterial activity, Antioxidant properties, Fermentation, Proteolysis, Silage quality.
[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Evaluation of surface lipids of sheep wool following dietary inclusion of emulsified fatty acid complex

Tkachuk V, Kyryliv B, Ohorodnyk N, and Motko N.
Online J. Anim. Feed Res., 15(4): 220-227, 2025; pii: S222877012500025-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.25



Abstract
Fatty acids, particularly ω-3, ω-6, and ω-9 play vital roles in sheep nutrition, but their influence on the protective properties of wool grease remained unclear. This study assessed the effects of dietary supplementation with an emulsified fatty acid complex on both the qualitative and quantitative characteristics of wool surface lipids in adult Prekos ewes and their lambs. The experimental group received a water soluble emulsion containing linoleic, oleic, palmitic, arachidonic, stearic, and α linolenic acids incorporated into the basal diet. Wax content was determined via Soxhlet extraction, and sweat salts were measured by aqueous extraction. Lipid classes were separated by thin layer chromatography, and fatty acid profiles were quantified using gas liquid chromatography. Results indicate a significant increase in wax secretion in ewes ($P < 0.01$) and lambs ($P < 0.05$), along with a decrease in sweat pH among lambs ($P < 0.05$). In ewe wax, levels of lanosterol ($P < 0.01$) and esterified cholesterol ($P < 0.05$) were elevated; lamb wax exhibited increases in lanosterol ($P < 0.05$) and dehydrocholesterol ($P < 0.05$). Both ewes and lambs showed a reduction in polar lipid content ($P < 0.05$), suggesting diminished accumulation of oxidative products. Analysis of fatty acid composition in the ewe group revealed significant increases in cerotic (C26:0; $P < 0.001$), lauric (C12:0; $P < 0.01$), and oleic (C18:1; $P < 0.01$) acids. Therefore, dietary inclusion of an emulsified fatty acid complex enhances the protective properties of wool grease by modulating wax and fatty acid composition, with potential benefits for fiber integrity and resilience.
Keywords: Emulsified fatty acids; Fleece lipids; Hexacosanoic acid; Sweat secretion; Wool wax.
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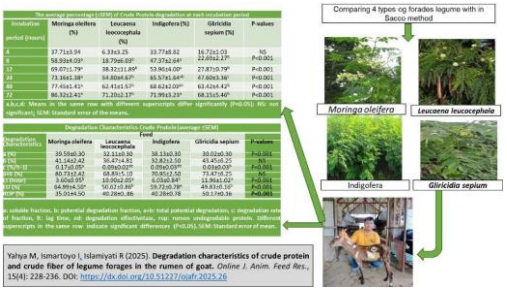
Research Paper

Degradation characteristics of crude protein and crude fiber of legume forages in the rumen of goat

Yahya M, Ismartoyo I, Islamiyati R.
Online J. Anim. Feed Res., 15(4): 228-236, 2025; pii: S222877012500026-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.26



Abstract
The nutritional value of a feedstuff depends not only on its chemical composition but also on the capacity of rumenal microbes to colonize and degrade it. This study compared the in sacco degradation kinetics of four legume forages *(Moringa oleifera, Leucaena leucocephala, Indigofera* and *Gliricidia sepium)* using three rumen fistulated goats in a 4×3 completely randomized design (CRD). Seventy-two nylon bags (10 × 5 cm, 40–50 µm pore size) containing 5 g of each forage (ground to 2 mm) were incubated for 4, 8, 12, 24, 48, or 72 hours (12 bags per time point). The study determined the soluble fraction (a), potentially degradable fraction (b), total degradable fraction (a+b), degradation rate constant of fraction b (c), lag time (Lt), degradation effectiveness (DE), and rumen undegradable protein (RUP). The results of CP degradation revealed no significant differences among forages in fractions a, b, or a + b, but fraction c, Lt, DE, and RUP differed significantly. The degradation rate (c, h^{-1}) of crude protein ranked as Moringa (0.17) > Leucaena (0.09) = *Indigofera* (0.09) > Gliricidia (0.03), while Lt was shortest for Moringa (3.60 h) and longest for Gliricidia (11.96 h). Moringa and *Indigofera* exhibited the highest DE and lowest RUP of all treatments. Similar trends were observed for crude fiber: Moringa showed the greatest DE (26.72% Lt) compared to Leucaena (18.76 h Lt). In conclusion, all four legumes were efficiently degraded in the goat rumen, through the rate and extent of degradation varied markedly among species, reflecting differences in their biochemical composition and structural carbohydrates.
Keywords: Crude Fiber, Crude Protein, Degradation Characteristics, Goat, Legumes
[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Review

Types and applications of innovative artificial intelligence in poultry farms

Abd El-Ghany WA.
Online J. Anim. Feed Res., 15(4): 237-245, 2025; pii: S222877012500027-15
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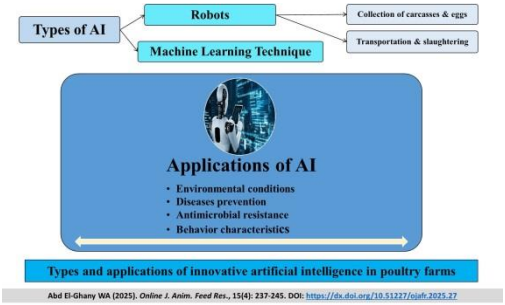

Abstract
The poultry farming world-wide face many challenges that adversely affects the production proficiency. Finding the optimal balance humans and the automation efficiency is crucial to obtain a maximum profit. Besides, improving of poultry welfare and production efficiencies necessitate some advanced modern technologies. The application of artificial intelligence (AI) and data-driven systems is regarded as an innovative solution to address many farm management problems. By the integration of AI, the industry has the opportunity to grow in terms of production quantity and poultry care quality with minimal added expense. Types of AI technology in poultry farms include machine learning techniques and robots. The machine learning technique decreases the need for big labeled data for training and helps in the transfer of knowledge, fast training, and better generalization on new tasks to enhance the performance parameters. This technique has different approaches such as Support Vector Machine, Single Shot MultiBox Detector, and Convolutional Neural Network that have a potential to reduce the labor and time and offer promising solutions for the rapid warning and accurate identification and differentiation of problems associated with poultry health. Moreover, innovative robots have been applied in poultry farms for monitoring, management, and environmental control as well as exploring of social dynamics. They are used in poultry farms for collections of eggs carcasses and eggs and transportation and slaughtering. Collectively, AI programs could be applied in poultry production for controlling environmental conditions, monitoring some behavioral conditions such as feeding, preventing some diseases, and correction of the hazardous usage of antibiotics with combating the increased incidence of antimicrobial resistance, and finally aiding in the rapid treatment. Therefore, this review highlights the types of AI models and their potential applications in poultry production.
Keywords: Antimicrobial resistance, behavior, diseases, environment, machine learning technique, poultry, robots
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AVAILABILITY, UTILIZATION PRACTICES AND FARMERS' PERCEPTION OF PHYTOGENIC FEED ADDITIVES FOR CHICKEN PRODUCTION IN NORTHWESTERN AMHARA, ETHIOPIA

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Supporting Information

RESEARCH ARTICLE
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ABSTRACT: The study was conducted to evaluate the availability, utilization practices, and farmers' perceptions of phytogenic feed additives for chicken production in Bahir Dar city and North Gojjam zone of Amhara region. The study included three areas (Bahir Dar city, North Achefer, and Bahir Dar Zuria districts), from which 320 respondents were selected from eight Kebeles. Data were collected from farm observations, individual interviews, and focus group discussions, supplemented by secondary information from agricultural offices records, and research publications. The study revealed a total of 1625.6 Tropical Livestock Units (TLU) of chickens in the study areas, and the average chicken holding per household (HH) was 5.08 TLU. Phytogenics were used as chicken feed additives by farmers in urban, peri-urban, and rural areas, with utilization rates of 58.3, 56.3, and 52.5%, respectively. Phytogenic feed additives such as Neem (*Azadirachta indica*), Girawa (*Vernonia amygdalina*), and Nech shinkurt (*Allium sativum*) ranked 1st, 2nd, and 3rd in their availability and 1st, 3rd, and 2nd in their utilization practices, respectively. The large majority of urban and peri-urban chicken producers (70.9 and 73.8%, respectively) had awareness of phytogenic feed additive utilization practices for chicken production. Phytogenic feed additives support sustainable poultry production in Ethiopia by improving food security, public health, environmental sustainability and economic resilience. Their use supports with national development goals and key sustainable development goals (SDGs), including zero hunger, good health and well-being and climate action. This finding suggests that phytogenic feed additives are readily available and utilized in the study area for chicken production at the farmer's level, albeit with no defined doses. Further research is needed to verify the effects of these phytogenic feed additives on chicken performance, and a nationwide assessment should be conducted to quantify their potential.

Keywords: Farmer's awareness, Girawa plant, Neem, Phytogenic feed additives, Poultry nutrition.

INTRODUCTION

Feed accounts for the majority of chicken production costs. The steady increase in the cost of chicken feed ingredients and compounded formulated feed is reducing profits for chicken farmers (Thirumalaisamy et al., 2016). Numerous feed additives have been widely utilized to enhance chicken production and reduce the cost of feed. Due to their therapeutic benefits of antibiotics, these components are frequently used as additives in chicken diets (Mehdi et al., 2018). The improper use of antibiotics can lead to drug-resistant microorganisms and antibiotic residues in chicken products (Mesfin et al., 2024; Ali et al., 2025). Alternative phytogenic feed additives enhance several key processes in the chicken's body (Mandey et al., 2022). Therefore, it is essential to use phytogenic feed additives for improved and unhindered chicken production (Yitbarek, 2015).

Phytogenic feed additives are gaining interest as alternatives to conventional antibiotics, probiotics, and prebiotics, due to low costs and high productive efficacy (Jachimowicz et al., 2022; Shehata et al., 2022), as consumers may accept their inclusion in chicken diets due to their natural origin (Abou-elkhair et al., 2018). Additionally, increasing the intestinal absorption surface enhances nutrient Apparent Ileal Digestibility (AID). This improved digestion promotes the development of broilers (Ravindran and Abdollahi, 2021). Overall, phytogenic feed additives could improve feed efficiency in chicken production (Aroche et al., 2018). In Ethiopia context, traditional medicinal plants continue to play a vital role in solving livestock health challenges, including those affecting chicken (Belayneh et al., 2012). Biological activity has been documented in extracts obtained from a variety of Ethiopian local plants, including antibacterial and anti-inflammatory properties (Ayalew et al., 2022). This evidence suggests that phytogenic feed additives offer a promising pathway toward sustainable poultry production in Ethiopia. Their integration into poultry systems can contribute to multiple dimensions of development enhancing food security, protecting public health, mitigating environmental impacts, and strengthening economic resilience. Accordingly, their use aligns with Ethiopia's national development priorities and global commitments under the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 13 (Climate Action).

Citation: Yegrem M, Animut G, and Mekuriaw Y (2025). Availability, utilization practices and farmers' perception of phytogenic feed additives for chicken production in northwestern Amhara, Ethiopia. *Online J. Anim. Feed Res.*, 15(4): 186-197. DOI: https://dx.doi.org/10.51227/ojafr.2025.22

Numerous studies (Shawle et al., 2016; Asrat et al., 2018; Dolle, 2020) had focused on the effect of phytogenic feed additives on chicken production, egg quality, and growth promoters. However, researchers had not given attention to farmers' knowledge of phytogenic feed additive utilization practices and the availability of these additives.

This study provides an opportunity to understand farmers' knowledge and preferences regarding phytogenic feed additives, which is crucial for developing research and strategies to improve their utilization practices and dissemination among farmers. It is hypothesized that farmers with greater knowledge of phytogenic feed additives for chicken production are more likely to prefer these additives, perceive their availability as higher, and engage in better utilization practices. The research findings will provide important baseline data for future studies with the following objective to assess farmers' knowledge of the preference, availability, and utilization practice of phytogenic feed additives for chicken production.

MATERIALS AND METHODS

Description of study areas

The study was conducted in the urban, peri-urban, and rural study areas of Bahir Dar city and North Gojjam zone of the Amhara region. These study sites were purposefully selected based on chicken production potential; the information was obtained from the Bureau of Agricultural Offices and the CSA agricultural sample survey. Bahir Dar is the capital city of Amhara National Regional State, Ethiopia. It's located about 565 km north of Addis Ababa at 11° 38′ N latitude and 37° 10′ E longitude. The elevation of the city is about 1801 meters above sea level, and it receives an average annual rainfall of 850 mm to 1250 mm, a minimum average daily temperature of 10°C, and a maximum of 32°C. Bahir Dar Zuria District (BDZD) is found in the north Gojjam Zone. The area is located about 564 km northwest of Addis Ababa. It is situated at an altitude ranging from 1700 to 2300 metres above sea level. Its extension is between 11°25′N and 11°55′N latitude and 37°04′E-37°39′E longitude. The mean annual temperature is about 20ºC, with a maximum temperature slightly above 28.3ºC and a minimum of about 10.2ºC. The annual rainfall ranges from 800-1250 mm. North Achefer is found about a distance of 101 Km and 591 Km far from Bahir Dar and Addis Ababa respectively. Its geographical coordinates are 11° 41' 0" north latitude and 36° 57' 0" east longitude. The altitude of the district ranges from 1,500 to 2,500 m above mean sea level. North Achefer is bordered on the south by the south Achefer, on the west by the Central Gondar Zone, on the north by Lake Tana, on the east by Bahir Dar Zuria, and on the southeast by the Mecha district, and one part of the Abay River defines the district's eastern boundary, as indicated by the whole study area.

Sampling method and sample size determination

A multi-stage sampling procedure was used to collect data. Stage 1: Bahir Dar and North Gojjam zone were purposively selected based on their chicken production potential; Stage 2: Within these areas, three districts were included in the study: Bahir Dar city, North Achefer, and Bahir Dar Zuria. From these districts, three urban kebeles (two from Bahir Dar and one from North Achefer), two peri-urban kebeles from Bahir Dar, and three rural kebeles (two from Bahir Dar Zuria and one from North Achefer) were randomly selected. In the urban study areas, a list of chicken-producing households was obtained from the agricultural office. In the peri-urban and rural areas, development agents assisted in identifying households with 25 or more chickens. Finally, 40 households from each selected kebele were interviewed, resulting in a total of 320 households participating in the study. All household participants agreed to provide information and images for the study, and the individuals in the pictures consented to their inclusion in the published paper, with the understanding that these materials could be used by the researcher as needed.

Data collection

Respondents' ranked major phytogenic feed additive sources for chickens based on their preferences and perceived effectiveness during utilization. A focus group discussion with community members in the study areas gathered information on utilization techniques and parts used for these additives. Additionally, semi-structured interviews were conducted with key informants including elders, long-time chicken farmers, and a veterinarian with experience in chicken farming to assess the availability and utilization practices of phytogenic feed additives. Both primary and secondary data were collected from various sources.

Conversion factor of livestock population (TLU)

Data on the livestock population in the sampled households were obtained from the interview of household heads during the survey. The number of livestock population was converted into tropical livestock unit (TLU) using the conversion factors of camel (1), cattle (0.7), sheep (0.1), goat (0.1), mules (0.7), horses (0.8), donkeys (0.5), and poultry (0.01) (Varvikko et al., 1993).

Sampling procedure for phytogenic additives

The questionnaires addressed farmers' perceptions of phytogenic feed additive utilization and availability. Following an assessment of available phytogenic feed additive resources, the top five additives were selected for chemical analysis. These major phytogenic feed additives were selected based on availability and farmer preferences. Samples of these additives were collected from the study areas to determine their chemical composition.

Proximate chemical analysis for selected phytogenic additive

The identified top 5 phytogenic feed additives were selected based on rank results. The leaf part of neem, Girawa and Tiql Gomen; the bulb part of Nech shinkurt and the rhizome of Zingible were collected in Bahir Dar and around Bahir Dar. The samples were ground into powder by using a hammer mill to pass through a 1 mm screen for chemical analysis. The samples of phytogenic feed additives were sent to the Animal nutrition laboratory of Jimma University for chemical analysis. The DM content of feed samples was determined by drying them in an oven at 105°C overnight (AOAC, 2000). Ash was determined by the complete burning of the sample at 550°C for 5 hours in a muffle furnace. Nitrogen (N) was determined using the Kjeldjhal method and then the crude protein calculates as N× 6.25 (AOAC, 1995). Crude fiber contents were analyzed using (AOAC, 1990) method. Total phosphorus contents were determined by the Vanado-Molybdate method (AOAC, 2000). The metabolizable energy (ME) was estimated according to the equation proposed by Wiseman (1987); ME (kcal/kg) = 3951 + 54.40 fat-88.70 ash. Nitrogen-free extract (NFE) was calculated by the difference between organic matter and the sum of ash, CF, EE, and CP.

Survey data management and statistical analysis

The collected data from the survey of urban, peri-urban and rural areas were entered into *the Statistical Package* for Social Sciences (SPSS version 27) software. The normality of the data was tested using the Kolmogorov-Smirnov Test. Chi-square was employed for the association of such parameters between urban, peri-urban and rural. Descriptive statistics were used to analyze the mean values of the quantitative data frequencies and percentages values. Means of quantitative data between urban, peri-urban and rural study areas were compared by employing analysis of variance (ANOVA). Means were separated using the Tukey test at P<0.05 significant level. The statistical model was used: $Y_{ij}=\mu + \alpha_i + \Sigma_{ij}$

Where: y_{ij} = is the response variable; μ = is the overall mean; α_i = the effect of i^{th} locations (urban, peri-urban and rural areas); Σ_{ij} = random error

Priority index was employed using the following formula (Kosgey, 2004).

$$Index = \frac{\Sigma\ (\text{n x No. of HHs ranked 1st}) + \ (\text{n} - 1)\text{x No. of HHs ranked 2nd}\ ) + \cdots + \ 1\ \text{x No. of HHs ranked last})\ \text{for one factor}}{\Sigma\ (\text{n x No. of HHs ranked 1st} + \ (\text{n} - 1)\text{x No. of HHs ranked 2nd} + \cdots . + 1\text{x No. of HHs ranked last})\text{for all factors}}$$

Where; n: value given for the least ranked level (example if the least rank is 5^{th} rank n-1=4, n-2=3 and.....n=1)

RESULTS AND DISCUSSIONS

Socioeconomic characteristics of the respondents

Table 1 presents the characteristics of households in the study areas. Age had a significant effect (P < 0.05) on chicken farming in the study areas. The average ages of respondents were 31.25 years in urban areas, 37.63 years in peri-urban areas, and 43.33 years in rural areas. This indicates an active working force with the potential for a positive effect on livestock development. The average family size was 3.83 people per household in urban areas, 6.25 in peri-urban areas, and 5.68 in rural areas, resulting in an overall average of 5.3 people per household. This result is comparable to Addis and Malede (2014), who reported an average family size of 5.7 in the Quara district. Significant differences (p < 0.05) in family size were observed across the study areas, which might be attributed to variations in family planning programs between urban, peri-urban, and rural communities. The majority of respondents were female household heads: 61.7% in urban areas, 67.7% in peri-urban areas, and 60% in rural areas. This suggests that women play a significant role in chicken production. There were significant differences (P < 0.05) in marital status across the study areas. 83.3% of households in rural areas, 77.5% in peri-urban areas, and 81.7% in urban areas had married household heads. Educational background also showed significant differences (P < 0.05) across the study areas. Illiterate households comprised 3.3% in urban areas, 42.5% in peri-urban areas, and 43.3% in rural areas. While 60% of urban households had a certificate or higher qualification, no households in peri-urban or rural areas held such credentials. This higher proportion of educated individuals in urban areas might contribute to the favorable acceptance of technologies like phytogenic feed additives and increased awareness about their use for improving chicken performance.

Livestock holding and composition

The average number of livestock held per household in the study area is shown in Table 2. The mean number of livestock per household in rural study areas was 4.13 heads of cattle, 0.61 heads of sheep, 0.12 heads of goats, 0.30 heads of donkey, 0.10 heads of mule, and 0.28 heads of chicken. In the peri-urban study area, the mean number of livestock per household was 3.85 heads of cattle, 0.67 heads of sheep, 0.13 heads of goats, 0.78 heads of donkey, 0.12 heads of mule, and 0.26 heads of chicken. However, livestock producers in urban study areas primarily raised cattle and chickens, with an average of 0.19 heads of cattle and 14.71 heads of chicken per household. In terms of Tropical Livestock Units (TLU), the average livestock ownership was 8.75, which differed significantly (P < 0.05) across study areas. However, urban study areas had a significantly higher average chicken keeping rate (14.71, P < 0.001) compared to peri-urban (0.26) and rural areas (0.28). Chicken accounted for the largest portion of the total livestock number in the sampled households in urban areas. This is due to population growth, rising individual consumption, and the expansion of hotels, which have led to a higher demand for chicken meat and eggs in urban areas compared to peri-urban and rural study areas.

Table 1 - Demographic and socioeconomic characteristics of the sampled households.

Parameters \ Study areas		Urban	Peri-urban	Rural	X^2	P-value
Age of HH heads (years)		31.25	37.63	43.33	148.97	***
Family size of HH heads (No.)		3.83	6.25	5.68	125.9	***
Sex of HH heads (%)	Male	38.3	32.5	45	3.23	ns
	Female	61.7	67.5	55		
Marital status (%)	Single	13.3	12.5	3.3	26.8	***
	Married	81.7	77.5	83.3		
	Widow	5	10	5		
	Divorced	0	0	8.3		
Educational level (%)	Illiterate	3.3	42.5	43.3	187.9	***
	Elementary	26.7	21.3	29.2		
	Secondary	3.3	17.5	19.2		
	Preparatory	5	6.3	2.5		
	Certificate and above	60	0	0		
	Religious	1.7	12.5	5.8		

HH= household, ns = non-significant, sig= significant value, X^2 =chi square, ***= significant at p< 0.001

Table 2 - The mean of livestock composition per household in the study areas in terms of TLU.

Livestock type \ Total livestock in TLU	Urban	Peri-urban	Rural	SEM	P-value
Cattle	0.19^b	3.85^a	4.13^a	0.042	***
Goat	-	0.13^a	0.12^a	0.004	ns
Sheep	-	0.67^a	0.61^a	0.011	ns
Donkey	-	0.78^a	0.30^b	0.010	***
Mule	-	0.12^a	0.10^a	0.004	ns
Chicken	14.71^a	0.26^b	0.28^b	0.213	***
Total	14.9	5.81	5.54	-	-

SEM= standard error of mean, ns = non-significant, ***= significant at p< 0.001

Type of additives and purpose of feeding for chickens

Table 3 presents the types of feed additives used in the study areas. In urban areas, 59.2% of respondents reported using both antibiotics and phytogenic feed additives. A smaller proportion (5.6%) used only phytogenic feed additives, while 35.2% used only antibiotics. Additionally, 4.3% of respondents in urban areas reported using both phytogenic and antibiotic feed additives. In contrast, peri-urban and rural respondents primarily relied on phytogenic feed additives, with 91.5% and 87.3% of respondents, respectively, using them. In peri-urban areas, 4.3% of respondents used only antibiotics, and 12.7% used both phytogenic and antibiotic feed additives in rural areas. The majority of respondents in urban areas (70.4%) used feed additives for improving chicken productivity, egg quality, growth, and health. In contrast, only 4.3% and 6.3% of peri-urban and rural respondents, respectively, reported using phytogenic feed additives for these purposes. Urban respondents were less likely to use feed additives for chicken treatments (9.3%) compared to peri-urban (95.7%) and rural (92.1%) respondents. A small percentage of urban respondents (7.4% and 3.7%, respectively) reported using feed additives for egg production and quality improvement. This was significantly lower than the 1.6% of rural respondents who used feed additives for this purpose. A significant proportion of respondents in all study areas reported never using phytogenic feed additives: 41.7% in urban areas, 43.8% in peri-urban areas, and 47.5% in rural areas. The primary reason cited for non-use was a lack of knowledge about phytogenic feed additives, their utilization practices, and their importance. Farmers primarily cultivate spices for human consumption and income, rather than for animal feed.

Sources of chicken feed additives in study areas

Figure 1 illustrates the sources of chicken feed additives in urban, peri-urban, and rural study areas. Backyard sources were the primary source of feed additives in peri-urban and rural areas, accounting for 22.2% and 72.2% of sources, respectively. In urban areas, both backyard and commercial sources were used, with 39.5% sourced from backyards and 36.3% from commercial suppliers. The high reliance on backyard sources in rural areas highlights the significant role of backyard cultivation of phytogenic feed additives within these communities. This reliance on backyard sources could be attributed to a decline in wild plant resources due to deforestation and lack of conservation, particularly for medicinal plants and herbs in the study area communities. This observation contradicts the findings of Regassa (2013) and Fenetahun et al. (2017), who reported that over 70% and 50% of medicinal plants, respectively, were collected from wild habitats.

Table 3 - Types and Purpose of feeding the feed additives in study areas.

Type of feed additives supplementation		Urban		Peri-urban		Rural		X^2
		N	%	N	%	N	%	
Did you provide any feed additives for your chickens?	Yes	108	90	47	58.8	63	52.5	43.17***
	No	12	10	33	41.3	57	47.5	
Did you provide phytogenic feed additives for your chicken?	Yes	70	58.3	45	56.3	63	52.5	0.84 ns
	No	50	41.7	35	43.8	57	47.5	
Antibiotics		38	35.2	2	4.3	-	-	
Phytogenic feed additives		6	5.6	43	91.5	55	87.3	154.11***
Both phytogenic and antibiotics feed additives		64	59.3	2	4.3	8	12.7	
Purpose of offering feed additives for chicken								
Production improvement		8	7.4	-	-	-	-	
Quality improvement		4	3.7	-	-	-	-	
For treatment (medicine)		10	9.3	45	95.7	58	92.1	156***
For production, quality, growth, and treatment		76	70.4	2	4.3	4	6.3	
Production and quality improvement		8	7.4	-	-	1	1.6	
Increase growth		2	1.9	-	-	-	-	

N = frequency, X^2 =chi square, ***=significant p≤ 0.001, **=p≤ 0.01

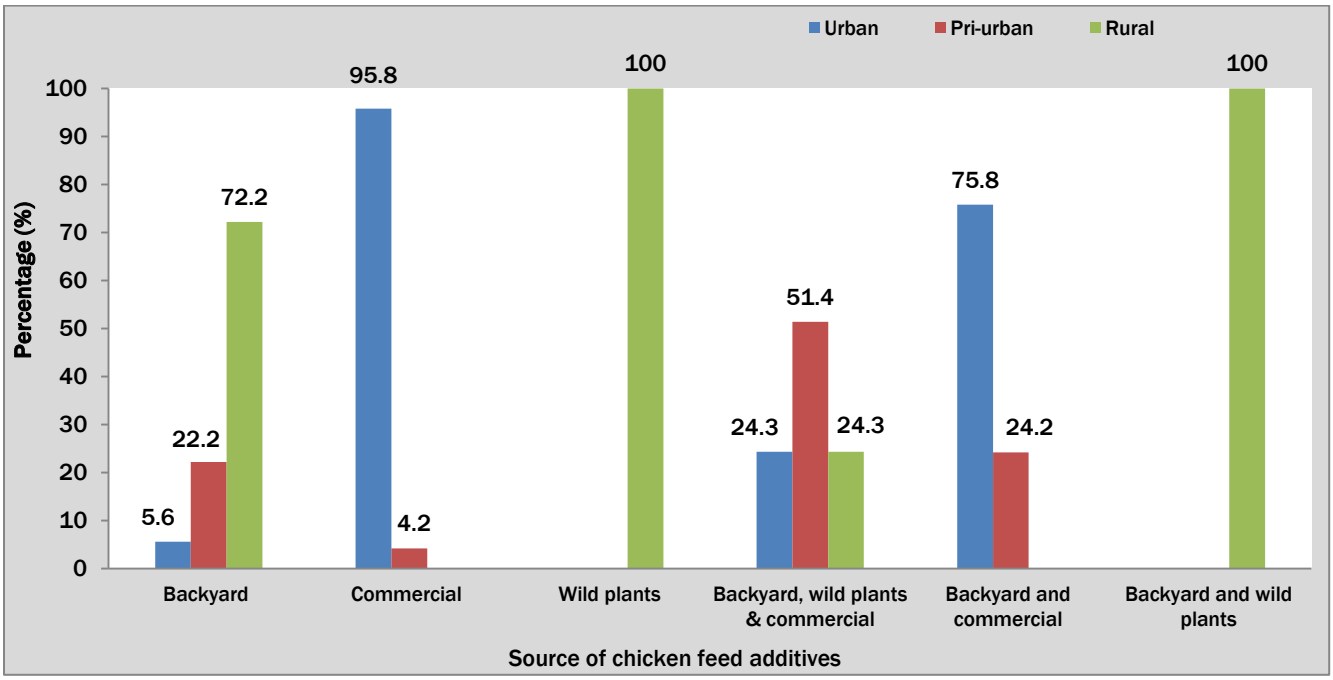

Figure 1 - Different sources of chicken feed additives in the study areas.

Availability of phytogenic feed additives in study areas

Table 4 presents the major phytogenic feed additive resources available in the study areas. The most dominant phytogenic additives, based on their availability, were Neem, Girawa, Nech Shinkurt, Tiql Gomen, Gomen, Berbere, Fenugreek, Zingible, Lomi, Tiqur Azimud, and Qariya. Other additives were available to a lesser extent. Except for Neem, Simiza, Girawa, and Damakasie, the other additives in urban study areas were purchased from the market. Their availability and affordability influenced their ranking. Neem was the top-ranked phytogenic feed additive source for chickens in urban and rural areas, and the 4th ranked source in peri-urban areas. It is available year-round in its green form. Zingible, Girawa, Nech shinkurt, and Tiql Gomen were among the top-ranked phytogenic feed additives, ranking 2nd, 3rd, 4th, and 5th in urban areas and 3rd, 1st, 2nd, and 5th in peri-urban areas, respectively. Yetibs Qtel, Tsosign, and Sinafch were not available in peri-urban and rural study areas. Respondents used spices such as Abish, Tiqur azmud, Berbere, and Feto due to their longer shelf life and availability during the dry season. Processing techniques for preserving other perishable phytogenic feed additives were not widely adopted. Feto (*Lepidium sativum*) was highly valued in peri-urban and rural areas for its medicinal properties but was scarce due to its limited occurrence and distribution. Herbicide use in other crops has also contributed to its decline.

Utilization practices and preferences of phytogenic feed additives for chickens

Table 5 presents the most preferred phytogenic feed additives in the study areas, including Neem, Nech Shinkurt, Girawa, Tiql Gommen, Zingible, Simiza, Sinafich, Feto, and others. Farmers ranked these additives based on their perceived effectiveness in enhancing chicken production, product quality, and health, reflecting the criteria they prioritize

in their actual usage. Girawa ranked 1st in rural areas, 2nd in urban areas and 3rd in peri-urban areas. It was primarily used to treat chickens and improve egg quality. Neem was ranked 1st for egg production, quality improvement, and health care in urban and peri-urban areas and 2nd in rural areas. Farmers primarily used neem to treat sick chickens. Garlic (Nech shinkurt) was ranked 3rd in urban areas, 2nd in peri-urban areas, and 3rd in rural areas. Overall, it was ranked 2nd in the production system and used for improving chicken performance. The preference for phytogenic feed additives is attributed to a combination of factors: They are readily available, low Cost and practical applicability

Perception of farmers towards phytogenic feed additives

The majority of respondents reported using phytogenic feed additives for their chickens (Table 6). Awareness of their use varied across study areas: 40% of respondents in urban areas, 60% in peri-urban areas, and 33.3% in rural areas were aware of their use as treatments. Notably, 21.7% of rural farmers recognized their use for preventing several chicken diseases. Respondents consistently highlighted the significant role of phytogenic feed additives in treating sick birds. This finding suggests that many farmers in peri-urban and rural areas utilize spices and medicinal plants to maintain their chickens' health. While awareness of using phytogenic additives for improving chicken product quality and production was lower, some respondents shared insights. Only 8.8% and 5% of respondents in peri-urban areas, and 2.5% and 1.7% in rural areas were aware of this application, respectively. However, these respondents provided anecdotal evidence: they observed that older chickens' eggshells softened, but feeding them neem leaves resulted in thicker eggshells and a more yellowish yolk. This suggests a potential connection to the carotenoid compounds and calcium content in neem leaves. In urban study areas, 19.17% and 6.67% of respondents used phytogenic feed additives to enhance chicken quality and production, respectively. Furthermore, 5% of respondents in urban areas expressed a favorable view of phytogenic feed additives as an alternative to antibiotics. However, a significant portion of respondents (29.2% in urban, 26.3% in peri-urban and 40.8% in rural study areas) lacked awareness of the full benefits of phytogenic feed additives. This was particularly evident in peri-urban and rural areas, where almost all farmers lacked knowledge about using phytogenic feed additives to improve chicken product quality and production.

Table 4 - The available phytogenic feed additive resources in the study areas.

Phytogenic feed additives		Urban		Peri-Urban		Rural		Overall	
Local name	**Scientific name**	Index	Rank	Index	Rank	Index	Rank	Index	Rank
Neem	*Azadirachta indica*	0.0708	1	0.086	4	0.124	2	0.09	1
Ginger	*Zingiber officinale*	0.069	2	0.087	3	0.049	11	0.056	9
Girawa	*Vernonia amygdalina*	0.068	3	0.11	1	0.135	1	0.088	2
Nech shinkurt	*Allium sativum*	0.067	4	0.105	2	0.073	3	0.071	3
Tiql gomen	*Brassica oleracea*	0.063	5	0.065	5	0.0547	10	0.0708	4
Gomen	*Brassica carinata*	0.06	6	0.055	10	0.0657	7	0.0698	5
Qariya	*Capsicum annuum L*	0.059	7	0.044	13	0.017	16	0.051	11
Abish	*Trigenella foenum-graecum*	0.057	8	0.026	16	0.004	20	0.062	8
Berbere	*Capsicum frutescens*	0.0568	9	0.06	6	0.066	6	0.068	6
Tiqur azimud	*Nigella sativa*	0.056	10	0.049	12	0.056	8	0.0498	12
Lomi	*Citrus aurantiifolia*	0.055	11	0.05	11	0.0697	4	0.0529	10
Tena adam	*Ruta chalepensis*	0.053	12	0.044	13	0.039	13	0.0496	13
Tomato	*Lycopersicon esculentum*	0.05	13	0.0566	9	0.0678	5	0.063	7
Qey shinkurt	*Allium cepa*	0.049	14	0.034	15	0.029	14	0.038	14
Feto	*Lepidium sativum*	0.043	15	0.059	7	0.047	12	0.028	15
Simiza	*Justicia schimperiana*	0.041	16	0.012	17	0.0177	15	0.025	16
Endod	*Phytolacca dodecandra*	-	-	-	-	0.009	18	0.014	19
Tosign	*Thymus schimperi*	0.0275	17	-	-	-	-	0.024	17
Sinafich	*Sinapis alba*	0.027	18	-	-	-	-	0.015	18
Yetbs Qtel	*Rosmarinus officinalis*	0.025	19	-	-	-	-	0.0069	20
Damakase	*Ocimum urticifolium*	0.0008	20	0.057	8	0.0555	9	0.0051	21
Eret	*Aloe adigratana*	-	-	-	-	0.0045	19	0.0012	23
Serkabeba	*Senna didymobotrya*	-	-	-	-	0.0128	17	0.0017	22
Nech bahir zaf	*Eucalyptus globulus*	-	-	-	-	0.0033	21	0.0006	24

Table 5 - Utilization practice and preference of phytogenic feed additives ranked by respondents.

Phytogenic feed additives		Urban		Peri-urban		Rural		Overall	
Local name	Scientific name	Index	Rank	Index	Rank	Index	Rank	Index	Rank
Neem	*Azadirachta indica*	0.27	1	0.157	1	0.125	2	0.1	1
Girawa	*Vernonia amygdalina*	0.16	2	0.142	3	0.130	1	0.086	3
Nech shinkurt	*Allium sativum*	0.14	3	0.148	2	0.100	3	0.0865	2
Tiql Gomen	*Brassica oleracea*	0.13	4	0.117	4	0.057	8	0.08	4
Zingible	*Zingiber officinale*	0.07	5	0.116	5	0.081	4	0.075	5
Sinafich	*Sinapis alba*	0.06	6	-	-	-	-	0.062	8
Feto	*Lepidium sativum*	0.05	7	0.104	6	0.073	6	0.057	9
Tiqur azmud	*Nigella sativa*	0.045	8	0.025	11	0.043	11	0.042	11
Qariya	*Capsicum annuum L.*	0.03	9	0.036	8	0.013	17	0.0386	14
Berberie	*Capsicum frutescens*	0.016	10	0.035	9	0.019	15	0.0387	13
Qey shinkurt	*Allium cepa*	0.015	11	0.009	13	0.032	13	0.072	6
Tomato	*Lycopersicon esculentum*	0.01	12	0.01	12	0.020	14	0.041	12
Simiza	*Justicia schimperiana*	-	-	0.088	7	0.066	7	0.066	7
Damakase	*Ocimum urticifolium*	-	-	0.026	10	0.011	18	0.052	10
Nech Bahir zaf	*Eucalyptus globulus*	-	-	-	-	0.014	16	0.017	17
Endod	*Phytolacca dodecandra*	-	-	-	-	0.036	12	0.0168	18
Serkabeba	*Senna didymobotrya*	-	-	-	-	0.075	5	0.033	15
Tena adam	*Ruta chalepensis*	-	-	-	-	0.050	10	0.024	16
Eret	*Aloe adigratana*	-	-	-	-	0.055	9	0.014	19

Table 6 - Farmers' attitude and awareness of phytogenic feed additives

Parameters	Description of phytogenic feed additives importance for chicken	Study areas						X^2
		Urban (%)		Peri-urban		Rural		
		N	%	N	%	N	(%)	
Knowledge	Awareness of product quality improvement	23	19.2	7	8.8	3	2.5	85.47**
	Having an awareness of production improvement	8	6.7	4	5	2	1.7	
	Aware that used as chicken treatments	48	40	48	60	40	33.3	
	No aware of phytogenic feed additives	35	29.2	21	26.3	49	40.8	
Attitude	Used as a good alternative to antibiotics	6	5	-	-	-	-	78.56**
	Aware that phytogenic additives can be used as a prevention	-	-	-	-	26	21.7	

N=frequency, X^2= chi-square, **=significant at p<0.01

Utilization techniques and parts used of phytogenic feed additives in study areas

Farmers agreed on the utilization techniques and plant parts used for chickens during group discussions in each production system (Table 7). The most common technique involved crushing and chopping phytogenic additives into small pieces, soaking them in water, and providing this mixture as drinking water. This prevalent use of water likely relates to its ability to dissolve many active compounds. In rural areas, specific phytogenic plants are commonly used to treat Newcastle disease: Feto (*Lepidium sativum*), simiza, Eret, Nech bahir zaf, Girawa, and Nech shinkurt, often mixed with drinking water and Injera Fitfit. Crushing and chopping techniques are frequently employed to extract bioactive components from plant parts, providing an immediate response to diseases and aiding in recovery. Leaves are the most commonly used part, followed by seeds (Table 7). This aligns with previous research suggesting leaves are more widely used due to their greater availability and ease of processing, as well as their richness in secondary metabolites (Nigussie et al., 2018). However, this contrasts with Atagal (2015) study in Uganda, where roots were the most frequently used part. Similar findings were observed in other Ethiopian regions, where roots were the most commonly harvested plant part (Birhane et al., 2011; Mengistu et al., 2017).

Table 7 - List of phytogenic feed additives identified processing methods in the study areas

Local name	Plant scientific name	Part used	Method of processing	Utilization techniques of phytogenic feed additives	Role phytogenic additives for chicken in study areas
Neem	*Azadirachta indica*	Leaf	Chopped & crushed, as it is	Crushed & Chopped neem leaf soaked with water then the juice mixed with Injera & other feed or hinging neem leaf in the chicken house for direct fed	Disease treatment and prevention, egg yolk and shell improvement
Nech shinkurt	*Allium sativum*	Bulb	Crushed and chopped	Added into chicken drinking water or mixed with Injera Fitfit or other feed	Disease treatment
Zingible	*Zingiber officinale*	Rhizome	Crushed and chopped	Added into chicken drinking water	Disease treatment
Girawa	*Vernonia amygdalina*	Leaf	Crushed, rubbed & as it is	Crushed & rubbed Girawa leaf by soaking with water then the juice mixed with injera & other feed	Disease treatment
Abish	*Trigenella foenum-graecum*	Seed	Grinded and crushed	Mixed with other feed and added into drinking water	Disease treatment
Qariya		Fruit	Chopped, as it is	Direct to fed or the chopped fruit soaked with water then added into drinking water	Disease treatment
Yetbs Qtel	*Rosmarinus officinalis*	Leaf	Rubbed and as it is	The rubbed leaf of *Rosmarinus officinalis* direct fed or mixed with other feed	Disease treatment
Feto	*Lepidium sativum*	Seed	Ground and crushed	Ground and crushed Feto mixed with Injera Fitfit or added into drinking water	Disease treatment & prevention
Tena Adam	*Ruta chalepensis*	Leave and young stem	Crushed, soaked, as it is	Crushed part of Tena Adam added into drinking water	Disease treatment & prevention
Berberie	*Capsicum annuum L.*	Fruit pulp	Grinded	Berbere powder mixed with oil and then added to other feed or chicken drinking water	Disease treatment
Gomen	*Brassica carinata*	Leaf and seed	Crushed, chopped and grinded	The powder mixed with feed or water which was given to sick chicken and chopping leaf used as it is for chicken feed	Disease treatment & egg yolk improvement
Lomi	*Citrus aurantiifolia*	The liquid in the fruit	Sliced	Slicing then Squeezing it to produce juice and added it to chicken drinking water	Disease treatment
Tiql Gomen	*Brassica oleracea*	Leaf	Chopped and chopped	Chopping it and as it is to provide it for chicken	Disease treatment & egg yolk improvement
Senafch	*Sinapis alba*	Seed	Crushed and grinding	The powder mixed with feed or water which was given to sick chicken	Disease treatment
Damakase	*Ocimum urticifolium*	Leaf	Rubbed, soaked	rubbed the leaf and add water to produce leaf juice then added to drinking water and mixed with Injera	Disease treatment
Simiza	*Justicia schimperiana*	Leaf	Chopped & rubbed, soaked	Chopped leaf and rubbed with water then added into drinking water or mixed with Injera Fitfit	Disease treatment
Nech bahir zaf	*Eucalyptus globulus*	Leaf	Crushed, chopped & rubbed	Processed leaf soaked with water then added to the chicken drinking water or leaf juice mixed with Injera Fitfit	Disease treatment
Endod	*Phytolacca dodecandra*	Leaf	Crushed	The crushed leaf is soaked with water and then filter the leaf juice and added to the drinking water	Disease treatment
Serkabeba	*Senna didymobotrya*	Leaf	Chopped & rubbed, soaked	The chopped leaf rubbed with water then filter the leaf juice and added to the drinking water	Disease treatment
Eret	*Aloe adigratana*	Leaf	Chopped & crushed	The jelly juice of Eret is added into drinking water or mixed with injera	Disease treatment

Phytogenic feed additive processing techniques and feeding frequency

The respondents employed diverse processing methods for phytogenic feed additives, as shown in Table 8. A significant difference ($P < 0.05$) in processing methods was observed between study areas. Crushing, chopping, soaking, and grinding were the most common preparation methods used. Crushing was the predominant method in urban areas (41.4%), next to peri-urban areas (48.9%). In rural areas, a combination of chopping and crushing was most common (34.9%). These findings align with Fenetahun et al. (2017) who, study that identified crushing (53.70%), squeezing (25.93%), chewing (16.67%), and cooking (3.70%) as the primary processing methods for remedies. The chopping method was used by 20% of respondents in urban areas, 35.6% in peri-urban areas, and 9.6% in rural areas. Soaking was employed by 14.3% in urban areas, 13.3% in peri-urban areas, and 12% in rural areas. While 10% of urban respondents used phytogenic feed additives without processing, only 2.2% in peri-urban and 1.6% in rural areas did the same. Grinding was used by 14.3% of urban respondents, but not by any respondents in peri-urban or rural areas. The frequency of phytogenic feed additive provision to chickens also varied significantly ($P < 0.05$) between study areas. The majority of respondents (80% and 68.3%) in peri-urban and rural areas, respectively, provided phytogenic feed additives when the chickens became sick. In urban areas, 28.6% of producers offered phytogenic feed additives once daily after providing a standard diet. Another 31.7% of rural producers provided phytogenic feed additives during disease outbreaks on neighboring farms to prevent disease. Only 5.7% of urban respondents provided phytogenic feed additives twice daily after provide standard diet. A small percentage of urban respondents (11.4%) provided phytogenic feed additives three times daily and 11.4% also provided them when the chickens became sick.

Table 8 - Processing techniques and feeding frequency of phytogenic feed additives

Phytogenic feed additives processing techniques	Urban		Peri-urban		Rural		X^2
	N	%	N	%	N	%	
Chopping	14	20.0	16	35.6	6	9.5	92.58***
Soaking	10	14.3	6	13.3	8	12	
As it is	7	10.0	1	2.2	1	1.6	
Griding	10	14.3	-	-	-	-	
Crushing	29	41.4	22	48.9	16	25.4	
Chopped and rubbed	-	-	-	-	8	12.7%	
Sliced and rubbed	-	-	-	-	2	3.2%	
Chopped and crushed	-	-	-	-	22	34.9%	
Providing frequency of phytogenic feed additives							
One time per day	20	28.6	-	-	-	-	157***
Two times per day	4	5.7	-	-	-	-	
Three times per day	8	11.4	-	-	-	-	
Some times	12	17.1	-	-	-	-	
Once a week	4	5.7	-	-	-	-	
Three times a week	14	20.0	9	20	-	-	
When chickens become sick	8	11.4	36	80	43	68.3	
Disease occurrences in neighbor farm	-	-	-	-	20	31.7	

N = frequency, X^2 =chi square, ***=significant at $p \leq 0.001$

Utilization constraints of phytogenic feed additive

Table 9 presents the constraints hindering the utilization practices of phytogenic feed additives. A significant difference ($P < 0.05$) in these constraints was observed across study areas. The most frequently reported constraint was a lack of knowledge about the importance of phytogenic feed additives, mentioned by 41.7% of respondents in urban areas, 50% in peri-urban areas, and 43.3% in rural areas. While development agents and other stakeholders have conducted awareness-raising activities about using leafy greens as sources of protein and vitamins, information about the use of spices and medicinal plants to improve chicken production and product quality remains limited. In urban areas, 18.3% of respondents mentioned the high cost of certain spices as a challenge to their use as chicken feed additives.

Chemical composition of major phytogenic feed additives

Table 10 shows the chemical compositions of the top five ranked phytogenic feed additives. The current study found that *Azadirachta indica* contained 90.62% DM, 18.11% ash, 3.40% EE, 7.54% CF, 16.78% CP, 0.095 mg/g P, and 44.79% NFE. These values are lower than those reported by Bonsu et al. (2012) for CP, CF, EE, ash, moisture, and NFE, but higher for ash, CP, DM, and EE. The ash, DM, EE, and CF contents also differed from those reported by Ubua et al. (2019). These variations in chemical composition likely stem from factors such as the type of *Azadirachta indica* tree, the age of the leaves, the location, the season of harvest, the soil type, and the processing method used. The ash content of *Vernonia amygdalina* in the current study (16.29%) was higher than the value reported by Asaolu et al. (2012) for bitter leaf (9.56%). Ash content is an indicator of mineral element presence. The protein content of *Zingiber officinale* in the current proximate analysis (9.68% CP) is comparable to (Dolle, 2020) findings, but higher than the values reported by Otunola et al. (2010) (8.54% CP) and Onimawo et al. (2019) (8.91% CP).

Table 9 - The major constraint of phytogenic feed additives utilization in the study areas

Phytogenic feed additives processing techniques	Urban		Peri-urban		Rural		X^2
	N	%	N	%	N	%	
Lack of knowledge about the level of inclusion	26	21.7	28	35	36	30	44.39***
High prices of some types of spices additives	22	18.3	-	-	-	-	
Lack of extension worker advice	22	18.3	12	15	32	26.7	
Lack of knowledge about the importance	50	41.7	40	50	52	43.3	

N = frequency, X^2 =chi square, ***=significant at P ≤ 0.001

Table 10 - Chemical composition of the first five ranked phytogenic feed additives

Parameters \ Phytogenic feed additives	*Azadirachta indica*	*Allium sativum*	*Brassica oleracea*	*Vernonia amygdalina*	*Zingiber officinale*
DM, %	90.62	90.78	87.3	93.28	89.87
Ash, %	18.11	20.07	18.95	16.29	15.85
EE, %	3.4	3.46	3.53	3.6	3.66
CF, %	7.54	7.89	8.08	8.01	7.81
CP, %	16.78	11.38	12.26	22.62	11.08
P (mg/g)	0.095	0.079	0.081	0.096	0.088
OM, %	81.89	79.93	81.05	83.71	84.15
NFE, %	44.79	47.98	44.48	42.76	51.47
ME (Kcal/kg DM)	2728.27	2620.53	2653.18	2771.72	2810.68

DM: dry matter; EE: Ether extract; CF: crude fiber; CP: crude protein; P: phosphorus; OM: organic matter; NFE: nitrogen free extract; ME: Metabolizable energy.

CONCLUSIONS

The study found that chicken feed additives primarily come from backyard plants, wild plants, and commercial antibiotics. In peri-urban and rural areas, there is limited awareness of using phytogenic feed additives (plant-based additives) to improve chicken performance, rather than just for health benefits. The use of these additives is influenced by their potential to improve human food, generate income, or both. Farmers tend to select phytogenic additives based on their effectiveness in improving chicken health, production, and product quality. Based on farmers' rankings across different study areas, the top three most preferred phytogenic feed additives were *Neem*, *Girawa*, and *Nech Shinkurt*. *Girawa* emerged as the most consistently valued plant, ranking first in rural areas and among the top three in urban and peri-urban settings. These plants were primarily selected for their perceived effectiveness in improving chicken health, and supporting overall poultry productivity. This approach aligns with key Sustainable Development Goals: SDG 2 (Zero Hunger) by contributing to improved poultry productivity and food security; SDG 3 (Good Health and Well-being) by offering natural alternatives to antibiotics, thereby reducing the risks associated with antibiotic resistance; and SDG 13 (Climate Action) by helping reduce environmental impacts such as ammonia (NH_3) gas emissions from chicken farms through the use of phytogenic feed additives. Key challenges for producers include a lack of knowledge about proper usage, limited guidance from extension workers, and the high cost of some spice additives. To improve the use of locally available medicinal plants, herbs, and spices, several recommendations were made: incorporating phytogenic feed additives into packaged forms at regional and national levels, such as by the Ministry of Agriculture; providing farmers with training on proper usage and educating them through agricultural officers and development agents; raising awareness about the potential of phytogenic feed additives to replace antibiotics; and conducting further research to identify and test the effects of those additives on chicken performance.

DECLARATIONS

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Ethical consideration

This study was survey-based, and ethical approval is not required, as it was conducted using a questionnaire. Before collecting survey data, each respondent was briefed on the purpose of the survey, the confidentiality of the information, and the expected duration of the interview. The interviews were conducted with a sample of smallholder chicken producers, and their willingness to participate served as their verbal consent.

Data availability

Data are available from the corresponding author (minichle18@gmail.com) upon reasonable request.

Authors' contribution

M. Yigrem designed the study, performed data collection, analyzed the data and drafted the manuscript, while Dr. G. Animut and Prof. Y. Mekuriaw meticulously designed the study, rigorously edited the manuscript, and provided final approval of the manuscript.

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Competing interests

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

REFERENCES

Abou-elkhair R, Selim S, and Hussein E (2018). Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. Animal Nutrition, 4(4):394–400. https://doi.org/10.1016/j.aninu.2018.05.009

Addis G, and Malede B, (2014). Chicken production systems, performance and associated constraints in north Gondar zone, Ethiopia. World Journal of Agricultural Sciences, 10(1):25-33.

Ali HR, Hefny EG, Koraney NF, Ali SF, AbdAllah MI, Fadel MA, Elnomrosy SM, and. Shahein MA, (2025) Antibiotic residues correlate with antibiotic resistance of Salmonella typhimurium isolated from edible chicken meat. Scientific Reports, 15(1):15165. https://doi.org/10.1038/s41598-025-98189-4

AOAC (1990). Association of of ficial analytical chemists. Official methods of analysis, 15th Edition. Washington. DC.

AOAC (1995) Association of Official Analytical Chemists. Official Methods of Analysis, 16th Edition. Washington DC.

AOAC (2000). Association of official analytical chemists. Official methods of analysis, 17th Edition. Washington. DC.

Aroche R, Mart Y, Ruan Z, Guan G, Waititu S, Nyachoti M, Dairon M, and Lan S, (2018). Dietary inclusion of a mixed powder of medicinal plant leaves enhances the feed efficiency and immune function in broiler chickens. Journal of Chemistry, Article ID: 4073068, 6. https://doi.org/10.1155/2018/4073068

Asaolu S, Adefemi S, Oyakilome O, Ajibulu I, and Asaolu F, (2012). Proximate and mineral composition of Nigerian leafy vegetables. Journal of Food Research, 1(3):214-218. http://dx.doi.org/10.5539/jfr.v1n3p214

Asrat M, Zeryehun T, Amha N, and Urge M, (2018). Effects of supplementation of different levels of garlic (*Allium sativum*) on egg production, egg quality and hatchability of White Leghorn chicken. Livestock Research for Rural Development, 30(3):37. http:// www.lrrd.org/lrrd30/3/tesf30037.html.

Atagal G, (2015). The role of medicinal plants in treatment of cattle diseases in Serere District. Busitema University, 44. http://hdl.handle.net/20.500.12283/2151

Ayalew H, Tewelde E, Abebe B, Alebachew Y, and Tadesse S (2022). Endemic medicinal plants of Ethiopia: Ethnomedicinal uses, biological activities and chemical constituents. Journal of ethnopharmacology, 293:115307. https://doi.org/10.1016/j.jep.2022.115307

Belayneh A, Asfaw Z, Demissew S, and Bussa F, (2012). Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Wereda, Eastern Ethiopia. Journal of Ethnobiology and Ethnomedicine, 8:12–16. https://doi.org/10.1186/1746-4269-8-42

Birhane E, Aynekulu E, Mekuria W, and Endale, D (2011). Management, use and ecology of medicinal plants in the degraded drylands of Tigray, Northern Ethiopia. Journal of Medicinal Plants Research, 5(3): 309-318. https://academicjournals.org/journal/JMPR/article-full-text-pdf/39D639124118

Bonsu FK, Kagya-Agyemang JK, Kwenin WK, and Zanu HK, (2012). Medicinal response of broiler chickens to diets containing neem (Azadirachta indica) leaf meal, haematology and meat sensory analysis. World Applied Sciences Journal, 19(6):800–805 https://idosi.org/wasj/wasj19(6)12/7.pdf

Dolle A (2020). Effect of garlic, ginger and their combination as feed additives on feed intake, productive performance, hematology, serum and economics of broilers. Haramaya university, p. 65. http://ir.haramaya.edu.et/hru/handle/123456789/4384

Fenetahun Y, Eshetu G, Worku A, and Taher A, (2017). A survey on medicinal plants used by traditional healers in Harari regional State, East Ethiopia. Journal of Medicinal Plants Studies, 5(1), 85–90. https://www.plantsjournal.com/archives/2017/vol5issue1/PartB/5-1-13-432.pdf

Jachimowicz K, Winiarska-Mieczan A, and Tomaszewska E (2022). The impact of herbal additives for poultry feed on the fatty acid profile of

meat. Animals, 12(9):1054. https://doi.org/10.3390/ani12091054

Kosgey I (2004). Breeding objectives and breeding strategies for the small ruminants in the tropics (Doctoral dissertation, PhD. Thesis, Anim. Breed. Wageningen University, 272. https://edepot.wur.nl/121527

Mandey J, Sompie M, and Wolayan F (2022). Growth assessment of broiler chickens given bitter leaves (Vernonia amygdalina) as phyto-additive, potentially antimicrobial agents of lipids and amino acids. 9th International Seminar on Tropical Animal Production (ISTAP 2021) 2022 Feb 24, pp. 21-25. https://doi.org/10.2991/absr.k.220207.005

Mehdi Y, Letourneau-Montminy M, Gaucher M, Chorfi Y, Suresh G, Rouissi T, et al. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, 4(2):170–178. https://doi.org/10.1016/j.aninu.2018.03.002

Mengistu M, Kebede E, and Serda B (2017). Ethnobotanical knowledge of pastoral community for treating livestock diseases in Shinle Zone, Somali Regional State, Eastern Ethiopia. Journal of Veterinary Science and Technology, 8(5):5. https://doi.org/10.4172/2157-7579.1000474

Mesfin YM, Mitiku BA, and Tamrat Admasu H (2024). Veterinary drug residues in food products of animal origin and their public health consequences: A review. Veterinary Medicine and Science, 10(6):e7004. https://doi.org/10.1002/vms3.70049

Nigussie A, Yilkal B, Mulugeta F, Addisu A, and Gashaw A. (2018). Use and conservation of medicinal plants by indigenous people of Gozamin Wereda, East Gojjam Zone of Amhara Region, Ethiopia: an ethnobotanical approach. Evidence-Based complementary and Alternative Medicine, Article ID: 2973513. https://doi.org/10.1155/2018/2973513

Onimawo IA, Esekheigbe A, and Okoh JE (2019). Determination of proximate and mineral composition of three traditional spices. Acta Scientific Nutritional Health, 3(7):111–114. https://actascientific.com/ASNH/pdf/ASNH-03-0332.pdf

Otunola GA, Oloyede OB, Oladiji AT, and Afolayan AJ (2010). Comparative analysis of the chemical composition of three spices *Allium sativum L. Zingiber officinale* Rosc. and *Capsicum frutescens L.* commonly consumed in Nigeria. African Journal of Biotechnology, 9(41):6927–6931. https://doi.org/10.5897/AJB10.183

Ravindran V, and Abdollahi MR, (2021). Nutrition and digestive physiology of the broiler chick: state of the art and outlook. Animals, 11(10):2795. https://doi.org/10.3390/ani11102795

Regassa R (2013). Assessment of indigenous knowledge of medicinal plant practice and mode of service delivery in Hawassa city, southern Ethiopia. Journal of Medicinal Plants Research, 7(9):517–535. https://academicjournals.org/journal/JMPR/article-abstract/D374F8321001

Shawle K, Urge M, Animut G, and Zeryehun T (2016). Broiler performance, carcass characteristics, haematology and serum biochemical parameters as affected by hot red pepper (*Capsicum frutescens*) powder supplementation. Indian Journal of Animal Nutrition, 33(2):176–183. https://doi.org/10.5958/2231-6744.2016.00030.X

Shehata A, Yalçın S, Latorre J, Basiouni S, Attia Y, Abd El-Wahab A, et al (2022). Probiotics, prebiotics, and phytogenic substances for optimizing gut health in poultry. Microorganisms, 10(2):395. https://doi.org/10.3390/microorganisms10020395

Thirumalaisamy G, Muralidharan J, Senthilkumar S, Hema S, and Priyadharsini M, (2016). Cost-effective feeding of poultry. International Journal of Science, Environment and Technology, 5(6):3997-4005.

Ubua JA, Ozung PO, and Inagu PG, (2019). Dietary inclusion of neem (Azadirachta indica) Leaf meal can influence growth performance and carcass characteristics of broiler chickens. Asian Journal of Biological Sciences, 12(2):180–186. https://doi.org/10.3923/ajbs.2019.180.186

Varvikko T, Kidane G, and Gashaw G, (1993). Importance of early hay making in improving the standard of dairy cow feeding on smallholder farms in the Ethiopian Highlands. Proceedings of the VII World Conference on Animal Production, Edmonton, Alberta, https://api.semanticscholar.org/CorpusID:210617820

Wiseman J (1987). Feeding of Non-Ruminant Livestock, 1st Editon. Butterworth-Heinemann, British, Elsevier Inc. https://doi.org/10.1016/C2013-0-00782-7

Yitbarek MB, (2015). Phytogenics as feed additives in poultry production: a review. International Journal of Extensive Research, 3:49–60. https://www.academia.edu/11749929/

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EFFECT OF DIETARY *Spirulina platensis* SUPPLEMENTATION ON GROWTH, CARCASS COMPOSITION, AND HAEMATOLOGICAL PARAMETERS OF PABDA (*Ompok pabda)*

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ABSTRACT: The study was conducted to find out the effects and optimum level of dietary *Spirulina platensis* (Spirulina) supplementation on growth and haematological parameters of pabda (*Ompok pabda*). Five experimental diets were prepared containing 33% protein. Different levels of Spirulina (0% as control or S_0, $S_{2.5\%}$, $S_{5\%}$, $S_{7.5\%}$ and $S_{10\%}$) were supplemented at the expense of fish meal (FM), respectively. Up to 10% of the total dietary protein (33%) in the control diet was replaced by Spirulina protein in the experimental diets. The feeding experiment was carried out in five treatments with three replications for 10 weeks. The water quality parameters viz. ammonia, dissolve oxygen, pH, and temperature were within the suitable range for pabda culture. The best growth performance was observed in the fish fed with 10% Spirulina supplemented feed followed by 7.5%, 5% and 2.5% Spirulina supplemented diet ($P < 0.05$). Maximum survival rate (98%) and hepatosomatic index (1.37) of pabda was also found in $S_{10\%}$ Spirulina group. But the feed conversion ratio (FCR) was decreased significantly ($P < 0.05$) with the increasing level of *Spirulina* supplementation which indicated better feed utilization. In case of carcass composition of pabda, the highest percentage of crude protein and ash were observed in fish fed with 10% followed by 7.5%, 5%, 2.5% *S. platensis* supplemented diet ($P < 0.05$). Likewise, haematological condition in fish fed with 10% *Spirulina* supplemented diet resulted better in this study. Therefore, it could be concluded that dietary supplementation of 10% *S. platensis* protein with FM protein may significantly improve the growth and the haematological parameters of *O. pabda*.

Keywords: Feed supplement, Fish meal, Growth, Haematology, *Ompok pabda, Spirulina platensis.*

INTRODUCTION

Fish is an important dietary animal protein source in a healthy diet of human because of its high protein, low carbohydrate, and unsaturated fat (Mishra and Pradesh, 2020). Moreover, fisheries sector plays an important role in employment, nutrition, food security, and foreign exchange earnings in the global economy including Bangladesh which is a leading contributor in inland freshwater aquaculture. In 2022-23, the fisheries sector of Bangladesh contributed approximately 2.41% to the total gross domestic product (GDP) and around 21.47% to the agricultural GDP (DoF, 2023). However, the main constraints in this sector are increasing feed cost. Fish feed generally constitutes 60 –70% of the operational cost in intensive and semi-intensive aquaculture system of which 45% of the total feed cost is allocated to protein sources (Singh et al., 2006). Fish meal (FM) is an appropriate protein source for aquafeed because it contains a high crude protein content (65% to 72%) as well as an optimal percentage of all 10 essential amino acids (EAAs) required by all fish species (Gasco et al., 2018). One of the vital problems is the price of FM which is becoming consequently higher due to the increasing requirement of FM with the expansion and intensification of aquaculture (Hardy and Tacon, 2002).

To achieve sustainable aquaculture, novel alternative protein sources, such as cheaper plant or animal origin proteins, must be developed for sustained aqua feed production. It is proposed that increasing the use of plant protein in fish diets can minimize the cost of FM and feeds (Amer et al., 2020). But plant protein contains some factors such as cyanogenic glycosides, protease inhibitors, lectins, tannins, alkaloids, and saponins which suppress other nutrients and have negative effects in animal health (Khajali and Rafiei, 2024). Therefore, it's a crucial need to find out alternative protein sources from plant origin where anti-nutritional factors will not be a major concern like algal protein. Throughout the world, many algal species have been employed in aquaculture, mostly for nutritional purposes i.e. *Spirulina platensis, Arthrospira maxima, Chlorella vulgaris* etc. (Chen et al., 2022). Among them *Spirulina* is the most common and widely available species. It has no anti-nutritional factors, immunostimulant properties, and cheap protein source compared to FM (Amer, 2016).

Spirulina is a filamentous and multicellular blue-green microalga which belongs to two separate genera *Spirulina* and *Arthrospira*, and consists of about 15 species (El-Sheekh et al., 2014). *Spirulina* is the most common and widely

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distributed species. *Spirulina* contains high protein contents, lipids, bioactive components such as vitamins (especially vitamin A and B_{12}), minerals, polyunsaturated fatty acids, carotenes, and other pigments that have antioxidant activity (Spinola et al., 2024). Besides the nutritional properties, it also acts as a cleansing and detoxifying factor against toxic substances (Gargouri et al., 2020). *Spirulina* has no cellulose in its cell walls, being composed of soft muco-polysaccharides, thus, makes it easily digested and assimilated its 85 to 95% plant protein (Hanel et al., 2007). Muller et al. (2000) found that *Spirulina* might be used to partially or totally replace FM in formulated aqua diets. Nandeesha et al. (2001) reported that *Spirulina* may substitute up to 25% of FM, resulting in greater development in rohu (*Labeo rohita*).

Blood is a useful means of signaling and recognizing the impacts of stress, environment, and the health state of fish in a certain location (Asgah et al., 2015). The examination of haematological parameters aids in understanding the relationship between blood properties and habitat, as well as the species capacity to adapt to its surroundings. As a result, fish blood is crucial for correctly assessing species health (Celik, 2004). Including *Spirulina* in fish diets enhances haematological markers as well as immunological response, making farmed fish healthier and disease resistant.

The pabda (*Ompok pabda*) is an indigenous, freshwater small catfish belonging to the family Silurideae of the order Siluriformes. Pabda is considered as small indigenous species (SIS) and favorite food fish eagerly devoured by the consumers because of its delicious taste, palatability, high protein and minerals (Hossain, 2008). Due to overexploitation this species is declining significantly and included in the red list of IUCN (International Union for Conservation of Nature) (IUCN, 2003). Thus, natural production of pabda is decreasing day by day. In this context, pabda is a potential fish for aquaculture as it fetches very high market value which is 3-5 times higher than those of fishes having low price i.e. pangas (*Pangasius pangasius*) (Kohinoor et al., 2018). Considering its higher market price, consumer demand fish, availability of seed, cultivable in small seasonal ponds, attain marketable size with in short period, now-a-days farmers are showing considerable interest in pabda culture (Islam et al., 2021). Bangladesh has a great prospect of culture pabda in future but feed price is one of the main obstacles for its aquaculture propagation as it contains 50 to 70% of total operation cost (Hossain, 2008).

In this circumstance, the *Spirulina* can be supplemented with FM in order to enhance growth performance of pabda and make the culture more profitable for the farmers. Therefore, the efficient use of *Spirulina* should be started as a better nutrient supplement in fish culture. Though many works have been done as a replacement of FM in fishes but as far we are concerned no work has done on supplementation of *Spirulina* in pabda culture. Therefore, this study was conducted to find out the effects of dietary *Spirulina* supplementation on growth, biochemical composition, and haematological parameters of pabda.

MATERIALS AND METHODS

Experimental design

The experiment was conducted by using a completely randomized design (CRD) for a period of 10 weeks. The fingerlings of pabda were obtained from a commercial fish hatchery. Fish were acclimatized in a 300 L circular tanks with proper aeration for one week prior to the feeding trial. During their acclimatization period the fish were fed with control diet. A total 600 healthy juvenile fish (mean body weight of 0.50 ± 0.01 g) after a 1% salt solution treatment were equally divided into experimental pits; each sized 0.64 m^3 (length 1.00 m, width 0.8 m, height 0.8 m) with a water volume of 640 L. Fish in triplicate groups were provided with each experimental diet twice a day until they reached satiation. The water level in the experimental pits was maintained using water from a deep tube well. Additionally, a surface exit was installed in each pit to stop overflow in the case of rain. The experiment followed the natural photoperiod (about 10:14 light: dark) condition.

Diets preparation and fish rearing

FM, soybean meal, rice bran, wheat bran, mustard oil cake, and wheat flour were collected from the local feed market. The quality was considered during purchasing of the feed ingredients. *Spirulina* was cultured at a large scale for 3 weeks. Then it was collected, sundried, and grinded for formulating fish feed. After analyzing the proximate composition of all the feed ingredients experimental diets were formulated (Table 1).

Five experimental diets were prepared containing 33% protein (Table 2). Control diet contains fish meal and no Spirulina supplementation (S_0). In the experimental diets, up to 10% of the total dietary protein (i.e., 10% of 33%) was replaced by Spirulina protein at the expense of fish meal protein. Different levels (2.5%, 5%, 7.5% and 10%) of *Spirulina* were supplemented at the expense of FM protein and referred to as $S_{2.5}$, S_5, $S_{7.5}$, and S_{10} diets, respectively. Dietary feed ingredients were grinded using a laboratory mix grinder (Panasonic MX- AC300) and blended with water to prepare a dough. Then the dough was passed through a manual pellet machine to make 2 mm diameter pellets. After that the pellets were dried under the sun light for 2 days. The pellets were then stored in plastic containers and kept in refrigerator at – 18 °C for further use.

Table 1 - Proximate composition (%) of different feed ingredients used to prepare experimental diets.

Ingredient	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Mastard oil cake	35.26	10.73	9.10	11.99
Soyabean meal	44.03	5.90	6.87	15.65
Spirulina platensis	61.17	9.87	13.39	18.67
Fish meal	55.47	13.78	18.30	13.23
Wheat bran	13.55	2.11	2.04	10.64
Rice bran	12.74	13.28	7.59	11.39
Wheat flour	5.00	2.95	2.50	8.50

Table 2 - Composition of different feed ingredients and proximate composition (%) for the formulation of experimental diets.

Experimental diet composition (%) / Feed ingredients	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
Fish meal (FM)	30.10	29.40	28.70	28.00	27.40
Spirulina platensis	0.00	0.70	1.40	2.10	2.70
Soybean meal	18.00	18.10	18.10	18.15	18.10
Mustard oil cake	8.20	8.20	8.10	8.15	8.09
Rice bran	23.30	23.30	23.30	23.14	23.23
Wheat bran	17.80	17.70	17.70	17.70	17.70
Wheat flour	2.50	2.50	2.60	2.70	2.70
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Protein	32.76 ± 0.33	32.83 ± 0.12	32.95 ± 0.35	33.01 ± 0.22	33.07 ± 0.15
lipid	5.52 ± 0.17	5.74 ± 0.17	6.43 ± 0.32	5.50 ± 0.09	5.13 ± 0.21
Ash	16.35 ± 0.20	13.22 ± 0.16	15.36 ± 0.25	14.54 ± 0.23	13.7 ± 0.40
Moisture	11.81 ± 0.66	11.67 ± 0.50	10.74 ± 0.38	10.45 ± 0.36	10.11 ± 0.20
Fiber	5.95 ± 0.05	5.83 ± 0.06	6.33 ± 0.06	6.49 ± 0.10	6.87 ± 0.06

Water quality parameters

The physicochemical parameters (water temperature, dissolved oxygen: DO, pH, and total ammonia) of the experimental pit water were monitored on a regular basis to monitor the overall cultural environment. The pH, DO, water temperature (°C), and total ammonia were measured using a digital pH meter (Hach Co., Colorado, USA), a digital DO meter (Hach Co., Loveland, Colorado, USA), a Celsius thermometer (Digi-thermo WT-2), and an ammonia measurement kit (HANNA instrument Test Kit), respectively.

Determination of fish growth, feed utilization, and biological indices

At the end of the 10-week feeding trial, fish from each experimental pit were collected, counted, and group-weighed. Growth performance and feed utilization metrics werecalculated according to the following equations:

1. Weight gain (g) = Mean final weight (g) - Mean initial weight (g)

2. Percent (%) of weight gain = $\frac{\text{Mean final fish weight} - \text{Mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$

3. Specific growth rate (SGR % day^{-1}) = $\frac{\ln W_2 - \ln W_1}{\text{Time}} \times 100$

Where, W_1 = The initial live body weight (g) at time T_1 (day); W_2 = The final live body weight (g) at time T_2 (day).

4. Feed conversion ratio (FCR) = $\frac{\text{Total feed consumption (g)}}{\text{Total body weight gain of fish (g)}}$

5. Survival rate (%) = $\frac{\text{Final number of survived}}{\text{No. of actual fish stocked}} \times 100$

6. Hepatosomatic index (HSI) = Liver weight (g) ×100 / Body weight (g)

Proximate composition analysis of fish

The proximate composition of fish samples was analyzed following the methods prescribed by the Association of Official Analytical Chemists (AOAC, 2007). After oven drying to a constant dry weight at 105 °C, the moisture content was measured. Petroleum ether extraction in a Soxhlet apparatus for 16 hours was used to estimate the total lipid content, while the micro-Kjeldahl apparatus was used to evaluate the crude protein content. The weight loss following the samples' 6-hour incineration at 550°C in a muffle furnace was used to determine the amount of ash present. The crude protein of *O. pabda* was measured by measuring its total nitrogen content using the conventional Micro-kjeldahl technique. The protein, lipid, ash and moisture content were calculated using the formulas shown below.

% Protein = % Nitrogen × 5.88 (conversion factor used for plant protein)

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

$$\text{Ash content (\%)} = \frac{\text{Final weight (g)} - \text{Crucibal weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

% Protein = % Nitrogen × 6.25 (for animal protein)

$$\text{Lipid (\%)} = \frac{\text{(Final weight of beaker+ Sample weight)} - \text{Initial weight of beaker}}{\text{Initial weight of the sample}} \times 100$$

Determination of haematological parameters

Hematological parameters like white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by a fully automatic hematology analyzer (DYMIND, DH36, China).

Statistical analysis

Throughout the trial, all data were gathered, documented, and saved on a computer spreadsheet. The data were statistically analyzed using one-way ANOVA in Statistix 10 (2013), with significance assessed using the package's Least Significant Difference (LSD) option for comparing means. $P < 0.05$ was used to define statistical significance.

RESULTS

Water quality parameters

The physicochemical parameters monitored during the experiment are presented in Table 3. Throughout the trial, there were no significant variations ($P > 0.05$) in water temperature, DO, pH, or total ammonia across treatments.

Growth and feed utilization performance of fish

Table 4 displays the growth indicators of pabda catfish at the end of the feeding trial, expressed as final weight, weight gain (WG), WG%, and SGR values. In the present study, growth indicators revealed an increasing trend till the introduction of 10% *Spirulina*. The WG and WG% of pabda fed with *Spirulina* supplemented feed in S_{10} treatment was significantly higher than that of the other treatments. Significantly ($P < 0.05$) the higher SGR values were also found in treatment S_{10} (1.66 ± 0.02) where feed contained 10% *Spirulina* and lower value was found in control (1.23 ± 0.04) treatment. The mean values of FCR were observed as 2.22 ± 0.06, 2.11 ± 0.11, 1.65 ± 0.01, 1.78 ± 0.01 and 1.57 ± 0.03 in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. The means are significantly different ($P < 0.05$) among the treatments. In this study, significantly ($P < 0.05$) the lowest value of FCR (1.57 ± 0.03) was found in treatment S_{10}, where 10% *Spirulina* used. There were significant differences in survival rate ($P < 0.05$) among the treatments. The highest survivability was recorded in S_{10} where 10% *Spirulina* was used as supplement in fish feed. On the other hand, the lowest survival was found in the treatment S_0 where no *Spirulina* was supplemented. HSI values of pabda varied significantly and were 1.02, 1.35, 1.21, 1.12 and 1.37 in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$, and S_{10}, respectively (Figure 1). Hepatosomatic index of pabda fed with *Spirulina* supplemented feed in $S_{2.5}$, S_5, and S_{10} treatments were significantly ($P < 0.05$) higher compared to control and $S_{7.5}$ treatment.

Proximate composition of fish

By the end of the trial, the inclusion levels of *Spirulina* had a substantial ($P < 0.05$) impact on the proximate carcass composition of pabda catfish (Figure 2). The highest protein content (24.62 ± 0.14) % was found in S_{10}, followed by $S_{7.5}$, S_5, $S_{2.5}$, and S_0 treatments, respectively. There was an upward trend observed in the crude protein level with the increasing level of *Spirulina* supplementation (Figure 2a). In the present study, there were significant differences ($P < 0.05$) in moisture and S_0 treatment showed the highest moisture content compared to the other treatments (Figure 2b). Fish body moisture content (%) showed a downward trend in which represents that moisture content reduced with the increasing

supplementation level of *Spirulina* during the 10 weeks of experimental period. There were significant differences in lipid content ($P < 0.05$) among different treatments. The highest lipid content was found in S_0, followed by $S_{2.5}$, S_5, $S_{7.5}$, and S_{10}, treatments, respectively (Figure 2c). There was a downward trend observed in the crude lipid curve with the increasing level of *Spirulina* supplementation. The values of ash content ranged from 1.87 to 3.21% and there were significantly different ($P < 0.05$) among the treatments. The highest ash content was found in the S_{10} (3.21%) followed by S_5, $S_{7.5}$, $S_{2.5}$, respectively (Figure 2d).

Analysis of Haematological parameters of fish

Haematological parameters of fish such as Haemoglobin (Hb), White blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) are presented in Table 5. In this experiment, the RBC count of fish was 1.72×10^{12}, 1.90×10^{12}, 2.75×10^{12}, 2.35×10^{12} and 3.18×10^{12} cells L^{-1} in the treatment S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. Maximum RBC (3.18×10^{12}) was found in S_{10} where 10% *Spirulina* used as supplement. There was an upward trend of the RBC, Hb and HCTcontent observed in pabda with the increasing of *Spirulina* level in the treatments. The WBC count was significantly different ($P < 0.05$) among the treatments and the highest WBC count found in the S_{10} followed by $S_{7.5}$, S_5, $S_{2.5}$. The levels of RBC, HCT, WBC, and Hb showed a dose-dependent rise, with the largest elevations recorded in fish fed 10% *Spirulina.* The MCV of pabda in this experiment were found 89.50, 92.50, 96.50, 125.50 and 148.50 (fl) in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. Maximum MCV, MCH and MCHC values were found in S_{10} where 10% *Spirulina* was included. On the other hand, the lowest MCV, MCH, MHC values were found in the treatment S_0 where no *Spirulina* was included.

Table 3 - Ranges of water quality indicators recorded during the trial period from different treatments

Treatments	Water temperature (°C)	pH	Dissolve oxygen (mg/L)	Total ammonia (mg/L)
S_0	27.20-29.60	6.13-7.58	6.40-6.95	0.99-1.08
$S_{2.5}$	26.96-30.93	6.21-7.56	6.45-6.97	0.98-1.07
S_5	27.83-30.16	6.50-7.57	6.41-7.00	0.99-1.07
$S_{7.5}$	27.86-30.15	6.61-7.55	6.59-6.89	0.96-1.04
S_{10}	27.96-31.09	6.36-7.51	5.72-6.98	0.98-1.02

Table 4 - Growth and feed utilization performance of pabda catfish feed different levels of *Spirulina* incorporated diets for 10 weeks.

Parameters \ Treatments	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
Initial weight (g)	2.48 ± 0.07	2.50 ± 0.02	2.53 ± 0.04	2.52 ± 0.02	2.46 ± 0.05
Final weight (g)	5.88 ± 0.02^{c}	6.08 ± 0.17^{c}	7.10 ± 0.04^{b}	7.03 ± 0.41^{b}	7.91 ± 0.07^{a}
Weight gain (g)	3.40 ± 0.09^{c}	3.58 ± 0.19^{c}	4.57 ± 0.19^{b}	4.51 ± 0.38^{b}	5.43 ± 0.09^{a}
Weight gain (%)	137.25 ± 5.98^{c}	143.30 ± 6.01^{c}	457.45 ± 9.35^{b}	451.50 ± 9.97^{b}	545.30 ± 11.38^{a}
SGR (%/day)	1.23 ± 0.04^{c}	1.27 ± 0.05^{c}	1.47 ± 0.02^{b}	1.47 ± 0.07^{b}	1.66 ± 0.02^{a}
FCR	2.22 ± 0.06^{a}	2.11 ± 0.11^{a}	1.65 ± 0.01^{bc}	1.78 ± 0.01^{b}	1.57 ± 0.03^{c}
Fish Survival (%)	90.50 ± 2.11^{c}	92.25 ± 2.09^{bc}	96.25 ± 4.10^{a}	93.75 ± 3.12^{b}	97.50 ± 4.50^{a}

Values expressed as mean ± standard deviation (SD). Different lower-case letters denote a significant difference ($P < 0.05$) among the treatments. SGR: Specific growth rate, FCR: Feed conversion ratio.

Table 5 - Hematological parameters of *O. pabda* in different treatments after feeding experimental diets for 10 weeks.

Blood parameters \ Treatments	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
RBC ($\times10^{12}$ cells L^{-1})	1.72 ± 0.05d	1.90 ± 0.06^{cd}	2.75 ± 0.06^{ab}	2.35 ± 0.10^{bc}	3.18 ± 0.12^{a}
Hb (gd.L^{-1})	9.65 ± 1.10^{c}	10.35 ± 1.22^{c}	11.45 ± 1.96^{b}	11.95 ± 1.96^{ab}	12.95 ± 2.01^{a}
WBC ($\times10^{3}$cells mm^{-3})	18.34 ± 0.35^{e}	20.65 ± 0.66^{d}	23.05 ± 0.71^{c}	24.10 ± 0.71^{b}	26.67 ± 1.01^{a}
HCT(%)	24.93 ± 1.00^{e}	26.05 ± 1.01^{d}	28.43 ± 1.08^{c}	29.56 ± 1.10^{b}	32.75 ± 1.90^{a}
MCV (fl)	89.50 ± 3.79^{e}	92.50 ± 3.80^{d}	95.50 ± 4.47^{c}	125.50 ± 4.23^{b}	148.50 ± 4.77^{a}
MCH (pg)	42.45 ± 2.01^{d}	43.50 ± 2.00^{d}	45.20 ± 2.75^{c}	52.65 ± 2.80^{b}	58.05 ± 2.97^{a}
MCHC (gd.L^{-1})	46.80 ± 2.77^{d}	47.85 ± 2.75^{d}	54.50 ± 2.81^{c}	58.75 ± 2.85^{b}	69.10 ± 3.02^{a}

Values are represented by the mean ± standard deviation (SD, n = 3). Different superscripted means within the same row differ considerably ($P < 0.05$). Hb: hemoglobin, RBC: red blood cell, WBC: white blood cell, HCT: hematocrit, PCT: platelet, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

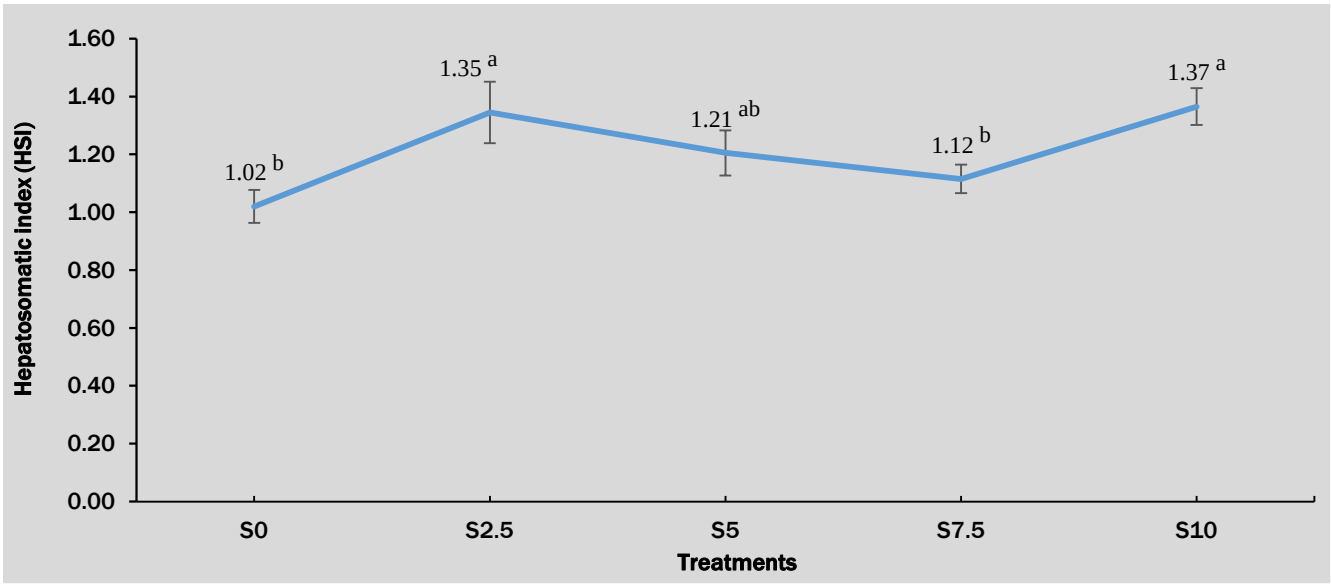

Figure 1 - Hepatosomatic index (HSI) of pabda. Different lower-case letters denote a significant difference ($P < 0.05$) among the treatments.

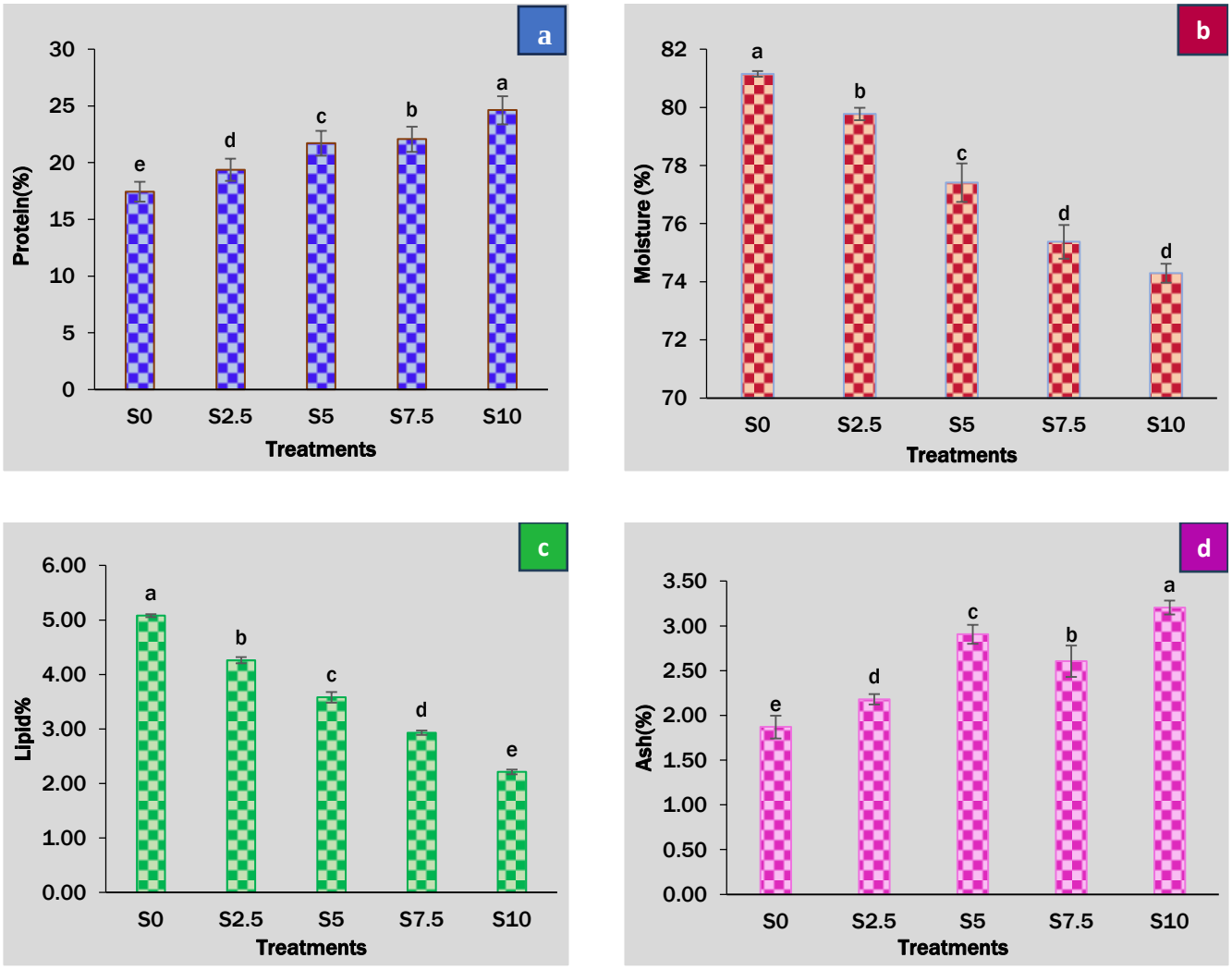

Figure 2 - Proximate carcass composition (% on fresh weight basis) of pabda (*O. pabda*) fed different level of *Spirulina* (*S. platensis*) incorporated diets for 10 weeks. Different lower-case letters denote a significant difference ($P < 0.05$) among the treatments.

DISCUSSION

Water temperature is an important physical characteristic that has a direct impact on the chemical, physical, and biological properties of a water body. Hossain (2008) reported that, the water temperature ranged from 28.9 to 32.8 °C is suitable for pabda culture. Nelson (2008) observed that fish do well in a range of 17 to 32 ^{0}C temperature depending on the species but better growth achieved at 26 °C or higher. The above statements imply that, water temperature in the present study was within the suitable range for pabda culture system. In this investigation, the pH levels varied from 6.13 to 7.58. According to Bhatnagar and Devi (2013), 6.5 to 8.5 is the ideal pH range for fish culture in ponds, while greater than 9.5 is not recommended. According to Banik et al. (2015) the optimal pH range for pabda in zooplankton-fed fish was 7.3 to 8.5. The previous studies indicate that the pH value in the current research was close to neutral, which is ideal for pabda fish rearing. Dissolve oxygenlevels in this research ranged from 6.40 to 7.0 mg.L^{-1}. According to Boyd and Tucker (2012), pond water with a Dissolve oxygenvalue of 5.0 to 7.0 mg.L^{-1} is favorable for fish production, whereas water with a DO level less than 3 mg.L^{-1} is unproductive. Malla et al. (2015) observed that the Dissolve oxygenlevels of 8.4 ± 0.28 mg.L^{-1} is suitable for pabda reared on live and artificial meals. In terms of total ammonia in the tanks' water, the primary source is fish excretion. Boyd and Tucker (2012) defined safe total ammonia levels as 1.0 mg.L^{-1}. This discussion clearly shows that the water quality parameters were within the acceptable limit for pabda catfish growth and survival. In other words, the addition of *Spirulina* to fish meals had no negative impact on water quality during fish rearing.

Spirulina includes high-quality protein as well as bioactive substances that contribute to growth acceleration. In the present study, weight gain of pabda increased with the increasing supplementation rate of *Spirulina* and all the values in different treatments are higher than control diet. Several prior researches have shown that using *Spirulina* in fish diets improves development and health of fish. Abdulrahman (2014) and Khanzadeh et al. (2016) found that common carp (*Cyprinus carpio*) and three-spot gourami (*Trichopodus trichopterus*) grew the fastest with a *Spirulina* inclusion level of 10%. The results of growth indices in our study were similar as found in previous studies. The present study showed that supplementation with *Spirulina* up to 10% did not have negative impacts on growth performance which is in line with the finding of Teimouri et al. (2013). It happened because of *Spirulina* contains a high-quality nutrient that might have an important role in growth rate compared to the algal-free diet (Spolaore et al., 2006). *Spirulina* contains various elements, including vitamins and minerals, which may aid in fish development. Nandeesha et al. (2001) found SGR values of 1.50-1.83% in experiments with Indian main carp catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets with varying levels of *Spirulina*. Promya and Chitmanat (2011) found SGR values between 2.14-2.53% on the experiment where *Spirulina* positively effect on growth, quality and immunity of catfish. This difference of SGR value could be due to the species variation as well as initial size of fish used and also may be due to the difference of culture season and different kinds of feed. The findings are consistent with those of other studies (Hirahashi et al., 2002; Hernandez et al., 2012; Palmegiano et al., 2008) who discovered that feeding *Spirulina* to fish enhanced survival and growth rates.

A low FCR value is an indication of better feed utilization efficiency of a formulated feed. Al-Deriny et al. (2020) showed that dietary *Spirulina* increased the FCR of Nile tilapia, which ascribed to the algae's involvement in improving intestinal morphometry indices and therefore increasing intestinal absorption capacity. Furthermore, adding *Spirulina* to the diet of the Oscar fish (*Astronotus ocellatus*) boosted growth performance and feed utilization efficiency (Mohammadiazarm et al., 2021). This accounts for the nutritional value of algal bioconstituents such as fatty acids, amino acids, vitamins, and minerals. Nandeesha et al. (2001) found FCR value (2.10-2.56) on their experiment with diet containing different level of *Spirulina* in Indian major carp catla and rohu. Kim et al. (2013) reported that partial substitution of FM with *Spirulina* in diets for parrot fish (*Oplegnathus fasciatus*) increased FCR from 1.98 to 2.27. The current study's findings are consistent with those of Ibrahem et al. (2013), who discovered that feeding with *Spirulina* powder increased the FCR and growth rates of striped jack (*Pseudocaranx dentex*) and *O. niloticus*.

An upward trend was observed with fluctuation in the survival rate with the use of high amount of *Spirulina* in fish feed as supplement. Dernekbasi et al. (2010) found that, in comparison to other commercial feeds, fish survival improved with an increase in the amount of *Spirulina* in the diet. *Spirulina* most likely increased growth rate and decreased death rate in the current research. According to research done by Youssef et al. (2023), *Spirulina*'s lack of cellulose makes it more readily digestible, which increases fish appetite, feed intake, and nutrient digestibility. These factors all contribute to the fish's improved health and increased resistance to infection by lowering stress levels. Hepatosomatic index (HSI) provides an indication on status of energy reserve of an animal. The present study collaborates with HIS value found by Peres et al. (2003). The present study proved that dietary supplementation of *Spirulina* enhanced fish growth. These results may possibly be due to the better digestibility and absorption of nutrient.

In present study crude protein content in fish body carcass was found better in Spirulina supplemented diet compared to non-supplemented diet. This conclusion is consistent with that of Mohammadiazarm et al. (2021) who found an increase in protease levels in fish fed with *Spirulina*, implying that it plays a role in enhancing protein utilization. According to El-Sheekh et al. (2014) crude protein content increased in tilapia with the feed increase of *Spirulina* protein percentage. Abdulrahman (2014) also found the same effect of increasing crude protein level (17.86-25.14%) with the increase of *Spirulina* protein (%) in case of common carp. Therefore, all the findings are similar to the result found in the

present study. The protein level in pabda after feeding different level of *Spirulina* protein suggested that supplementation of *Spirulina* increases the protein content in fish body. Fish protein percentages may rise as a result of *Spirulina*'s high protein content and capacity to stimulate fish somatic growth and protein synthesis (Mohammadiazarm et al., 2021). The decrease of lipid content concomitant with an increased *Spirulina* supplementation level agrees with the results found by Abdulrahman (2014) and Khanzadeh et al. (2016). The current findings demonstrated a reduction in lipid content in those administered *Spirulina*-supplemented meals. Mohammadiazarm et al. (2021) found that the polyphenol content of algae, such as β-carotene or phycocyanin, can reduce lipid levels (Kim et al., 2013; Hassaan et al., 2021). Hossain et al. (1999) conducted an experiment on nutritional value of some small indigenous fish species (SIS) of Bangladesh and found lipid content 1.87% - 9.55% fresh matter basis. In this study, lipid content ranged from 2.21% - 5.08%. *Spirulina* known to decrease lipid deposition (Nandeesha et al., 2001). Kim et al. (2013) conducted an experiment on partial replacement of FM with *Spirulina* in diets for parrot fish and found that higher the inclusion the *Spirulina* the lower crude lipid percentage which is similar to the present study. According to Nandeesha et al. (2001), the effect of dietary *Spirulina* on whole-body lipid content varies with the type of *Spirulina* utilized. The effects of *Spirulina* on whole-body protein and lipid levels are connected with their synthesis and accumulation rates in muscle, as well as the organisms' growth rate (Soivio et al., 1989). In a study of Stansby (1954) moisture content for fresh water fish was reported to be in the range of 72.1-83.6%. The finding in case of ash content was agreed with the studies of Khanzadeh et al. (2016); El-Sheekh et al. (2014), and Abdulrahman (2014). These studies found that higher inclusion levels of *Spirulina* led to increased ash content.El-Sheekh et al. (2014) found ash content in red tilapia ranged from 10.1 to 10.8 % on dry matter biases by using *Spirulina* in feed. Olvera et al. (2008) found ash content of tilapia between 3.13% and 4.17 % and the ash content was higher in control diet than the FM replacement *Spirulina* diet. Nandeesha et al. (2001) used four experimental diets for common carp by replacing FM protein through the incorporation of *Spirulina* and found ash content from 2.04 to 3.09%. In this study the ash content was measured on the dry weight basis. Though there was a fluctuation in $S_{7.5}$ treatment otherwise the ash content was increased with the raising of *Spirulina* supplementation level in the present study.

Variation in the haematological parameter is an important tool for representing the fish immunity as well as the health status (Celik, 2004). The levels of RBC, HCT, WBC, and Hb showed a dose-dependent rise, with the largest elevations recorded in fish fed 10% *Spirulina* supplemented diet. Similar to our findings, including *Spirulina* into the diet of Great Sturgeon (*Huso huso*) dramatically raised RBC and Hb (Milad et al, 2016). *Spirulina* contains a lot of iron and has considerable effects on erythropoiesis in anaemic rats by boosting the amounts of RBCs and Hb (Kapoor and Mehta, 1992). Sayed and Fawzy (2014) reported that dietary *Spirulina* significantly affected the haemoglobin content of *C. gariepinus* and varied from 7.51 to 9.83 $g.dl^{-1}$. Ibrahem et al. (2013) found that haematocrit tended to increase with increasing dietary *Spirulina* levels than the control fish. In the present study after supplementation of *Spirulina* in the experimental diets the haemoglobin counts significantly increased in the treatment, S_{10} (12.95 $g.dl^{-1}$) than the control, S_0 (9.65 $g.dl^{-1}$). Thus, the increase of RBC and Hb in our study might be ascribed to the presence of iron element in *Spirulina*. Increase in red and white blood cells of fish resulted may be due to the presence of C-phycocyanin in *Spirulina*, which can help to increase the immunity stimulating capacity of fish (Eissa et al., 2024). RBC carries glucose from blood to different cells of the whole body. Higher value of RBC represents increasing haemoglobin content in blood (Pelster, 2001). Raising the haemoglobin level of fish is an excellent way to measure their ability to transport oxygen, which allows correlations to be established between the fish's health and the oxygen concentration in their environment. It is a good immunological sign for fish because it indicates more transportation of oxygen in the blood which will prevent fish from anemia (Esmaeili, 2021). Furthermore, in the current study, nutritional supplementation with *Spirulina* resulted in greater levels of WBC compared to control, which was consistent with the findings in yellow croaker (*Pseudosciaena crocea*) (Li et al., 2014) and common carp (Samah et al., 2017). Higher WBC levels in fish given *Spirulina* supplemented diets might be due to the existence of a polypeptide-phycocyanin, which was discovered to be a significant role in boosting WBC in mice (Zhang et al., 1994). The increased WBC cells in blood usually help fish body to fight against infections and some diseases. So, the higher value of WBC indicates better immunity of fish (Kori-Siakpere et al., 2006). MCHC depends on hemoglobin (Hb) synthesis. When Hb synthesis decreases the MCHC also reduces and causes anemia (Javed et al., 2016). If hemoglobin is increased, the MCHC value is able to diminish hypochromic anemia. The results of the current study are in accordance with Ibrahem et al. (2013) who found that the MCV, MCH, MCHC in fish were increased with increasing dietary *Spirulina* levels. In the present study, the values of these parameters gradually increased with the increasing supplementation of *Spirulina* and within in suitable range which indicated a better immunity of fish than control.

CONCLUSION

The current study showed a positive dietary effect of *Spirulina* on growth performance, body composition, and haematological parameters of pabda. Among the different supplementation levels, the best growth performance of pabda was obtained in the case of 10% *Spirulina* supplementation. In addition, all the haematological parameters also improved with the increasing of *Spirulina* supplementation level during the experiment which indicates better immunity of pabda. Among the different supplementation levels, 10% *Spirulina* supplementation showed the best value of haematological

parameters. From this study, it can be concluded that, 10% *Spirulina* supplementation in pabda feed could help to get better production and health performance in the culture. However, further work is necessary to understand the effect of higher percentage of *Spirulina* supplementation, its chemical nature, mode of action and also in vivo and in vitro tests need to be conducted for better growth, quality and health of pabda.

DECLARATIONS

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Data availability

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

Ethics approval

The authors followed all applicable international, national, and institutional guidelines for the care and use of fish.

Authors' contribution

Conceptualization: T. Akter, M. Das; Data curation: S. Noman, M. Das, M.R. Islam; Formal analysis: S. Noman, F.S. Bristy; Investigation: S. Noman, M. Das, M.R. Islam, T. Akter; Methodology: S. Noman, T. Akter, M.R. Islam; Validation: S. Noman, M. Das, T. Akter; Writing – original draft: T. Akter, M. Das, S. Noman, R.R. Islam, F.S. Bristy; Writing – review & editing: T. Akter, M. Das, M.R. Islam.

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Consent to publish

All authors agree to the publication of this manuscript.

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Competing interests

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

REFERENCES

Abdulrahman NM (2014). Evaluation of *Spirulina* sp. as food supplement and its effect on growth performance of common carp fingerlings. International Journal of Fisheries and Aquatic Studies, 2: 89-92. https://www.fisheriesjournal.com/archives/2014/vol2issue2/PartB/2.pdf

Al-Deriny SH, Dawood MA, Abou Zaid AA, Wael F, Paray, BA, Van Doan H and Mohamed RA (2020). The synergistic effects of *Spirulina platensis* and *Bacillus amyloliquefaciens* on the growth performance, intestinal histomorphology, and immune response of Nile tilapia (*Oreochromis niloticus*). Aquaculture Reports, 17: 100390. https://www.sciencedirect.com/science/article/pii/S2352513420302647#:~:text=The%20application%20of%20both%20SP,feeding%20both%20SP%20and%20BA. DOI: https://doi.org/10.1016/j.aqrep.2020.100390

Amer SA (2016). Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus*). Benha Veterinary Medical Journal, 30: 1-10. https://bvmj.journals.ekb.eg/article_31332.html. DOI: https://doi.org/10.21608/bvmj.2016.31332

Amer SA, Ahmed SA, Ibrahim RE, Al-Gabri NA, Osman A and Sitohy M (2020). Impact of partial substitution of fish meal by methylated soy protein isolates on the nutritional, immunological, and health aspects of Nile tilapia, *Oreochromis niloticus* fingerlings. Aquaculture, 518: 734871. https://www.sciencedirect.com/science/article/abs/pii/S004484861932767X. DOI: https://doi.org/10.1016/j.aquaculture.2019.734871

AOAC (2007). Official methods of analysis (18th Edition). Association of Official Analytical chemists, Gaithersburg. :https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1753366

Asgah NA, Warith AW, Younis EM and Allam HY (2015). Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride. Saudi Journal of Biological Science, 22: 543-550. https://pubmed.ncbi.nlm.nih.gov/26288556/. DOI: https://doi.org/10.1016/j.sjbs.2015.01.002

Banik S and Malla S (2015). Survival and growth rate of larval *Ompok pabda* (Hamilton-Buchanan, 1822) of Tripura, India: related to efficient feed. In Proceedings of the Zoological Society. 68: 164-171. https://link.springer.com/article/10.1007/s12595-014-0111-x. DOI: https://doi.org/10.1007/s12595-014-0111-x

Bhatnagar A and Devi P (2013). Water quality guidelines for the management of pond fish culture. International Journal of Environmental Science, 3: 1980–2009. https://www.romanpub.com/resources/ijes-2019-11.pdf. DOI: https://doi.org/10.6088/ijes.2013030600019

Boyd CE and Tucker CS (2012) Pond aquaculture water quality management. Springer Science and Business Media, New York. https://www.scirp.org/reference/referencespapers?referenceid=2184634

Celik ES (2004). Blood chemistry (electrolytes, lipoproteins and enzymes) values of black scorpion fish (*Scorpaena porcus Linneaus* 1758) in the Dardanelles. Journal of Biological Science, 4: 716-719. https://scialert.net/abstract/?doi=jbs.2004.716.719 DOI: https://doi.org/10.3923/jbs.2004.716.719

Chen F, Qian J, He Y, Leng Y and Zhou W (2022). Could *Chlorella pyrenoidosa* be exploited as an alternative nutrition source in aquaculture feed? A study on the nutritional values and anti-nutritional factors. Frontiers in Nutrition, 9: 1069760. https://pubmed.ncbi.nlm.nih.gov/36570144/. DOI: https://doi.org/10.3389/fnut.2022.1069760

Dernekbasi S, Unal H, Karayucel I and Aral O (2010). Effect of dietary supplementation of different rates of *Spirulina* (*Spirulina platensis*) on growth and feed conversion in guppy (*Poecilia reticulate peters*, 1860). Journal of Animal and Veterinary Advances, 9: 1395-1399. https://acikerisim.sinop.edu.tr/items/1a3e3700-c50c-42bd-8e37-69f3ded4161f. DOI: https://doi.org/10.3923/javaa.2010.1395.1399.

DoF (2023). Yearbook of fisheries statistics of Bangladesh, 2017-18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries, 35, pp. 129. https://fisheries.portal.gov.bd/sites/default/files/files/fisheries.portal.gov.bd/download/66835738_1def_4eb0_8028_cb70be103f6f/2024-07-08-03-59-c335f1d6908e7427417e8bc610142b63.pdf.

Eissa ESH, Khattab MS, Elbahnaswy S, Elshopakey GE, Alamoudi MO, Aljàrari RM, et al. (2024). The effects of dietary *Spirulina platensis* or curcumin nanoparticles on performance, body chemical composition, blood biochemical, digestive enzyme, antioxidant and immune activities of *Oreochromis niloticus* fingerlings. BMC Veterinary Research, 20(1): 215. https://link.springer.com/article/10.1186/s12917-024-04058-z. DOI: https://doi.org/10.1186/s12917-024-04058-z.

El-Sheekh M, El-Shourbagy I, Shalaby S and Hosny S (2014). Effect of feeding *Arthrospira platensis* (Spirulina) on growth and carcass composition of hybrid red Tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*). Turkish Journal of Fisheries and Aquatic Sciences, 14: 471–78. https://www.trjfas.org/abstract.php?id=87. DOI: https://doi.org/10.4194/1303-2712-v14_2_18

Esmaeili N (2021). Blood performance: a new formula for fish growth and health. Biology, 10(12): 1236. https://www.mdpi.com/2079-7737/10/12/1236. DOI: https://doi.org/10.3390/biology10121236

Gargouri M, Akrouti A, Magne C, El Feki A and Soussi A (2020). Protective effects of spirulina against hemato-biochemical alterations, nephrotoxicity, and DNA damage upon lead exposition. Human & experimental toxicology, 39: 855-869. https://pubmed.ncbi.nlm.nih.gov/32003233/. DOI: https://doi.org/10.1177/0960327120903490

Gasco L, Gai F, Maricchiolo G, Genovese L, Ragonese S, Bottari T and Caruso G (2018). Fishmeal alternative protein sources for aquaculture feeds. Feeds for the aquaculture sector: current situation and alternative sources, 1-28. https://link.springer.com/chapter/10.1007/978-3-319-77941-6_1. DOI: https://doi.org/10.1007/978-3-319-77941-6_1

Hanel R, Broekman D, Graaf S and Schnack D (2007). Partial replacement of fishmeal by lyophilized powder of the microalgae *Spirulina platensis* in pacific white shrimp diets. The Open Marine Biology Journal, 1: 1-5. https://benthamopenarchives.com/abstract.php?ArticleCode=TOMBJ-1-1. DOI: http://dx.doi.org/10.2174/1874450800701010001

Hardy RW and Tacon AGJ (2002). Fishmeal: Historical uses, production trends and future outlook for sustainable supplies. Responsible Marine Culture, 3: 311- 325. https://www.researchgate.net/publication/284849886_Fish_meal_Historical_uses_production_trends_and_future_outlook_for_sustainable_supplies. DOI: http://dx.doi.org/10.1079/9780851996042.0311

Hassaan MS, Mohammady EY, Soaudy MR, Sabae SA, Mahmoud AM and El-Haroun ER (2021). Comparative study on the effect of dietary β-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in Nile tilapia, *Oreochromis niloticus*. Fish & Shellfish Immunology, 108: 63-72. https://www.sciencedirect.com/science/article/abs/pii/S1050464820307166. DOI: https://doi.org/10.1016/j.fsi.2020.11.012

Hernandez LHH, Flores GH, Araiza MAF and López OA (2012). Effects of total replacement of fishmeal with Spirulina powder and soybean meal on juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum). The Israeli Journal of Aquaculture, 12: 24-46. https://www.researchgate.net/publication/286186894_Effects_of_Total_Replacement_of_Fishmeal_with_Spirulina_Powder_and_Soybean_Meal_on_Juvenile_Rainbow_Trout_Oncorhynchus_mykiss_Walbaum. DOI: http://dx.doi.org/10.46989/001c.20612

Hirahashi T, Matsumoto M, Hazeki K, Saeki Y, Ui M and Seya T (2002). Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. International Immunopharmacology, 2: 423-434. https://pubmed.ncbi.nlm.nih.gov/11962722/. DOI: https://doi.org/10.1016/s1567-5769(01)00166-7.

Hossain MA (2008). Development of a suitable diet for culture of pabda (*Ompok pabda*) in cage using locally available feed ingredients. Annals of Bangladesh Agriculture, l2(2): 55-62. https://www.researchgate.net/publication/309010874_Development_of_a_suitable_diet_for_culture_of_pabdaOmpok_pabda_in_cage_using_locally_available_feed_ingredients. DOI: https://www.cabidigitallibrary.org/doi/full/10.5555/20113107939

Hossain MA, Shah A and Afsana K (1999). Nutritional value of some small indigenous fish species (SIS) of Bangladesh. Bangladesh Journal of Fisheries Resources, 3: 77-85. Article link: https://core.ac.uk/download/pdf/33720152.pdf

Ibrahem MD, Mohamed MF and Ibrahim MA (2013). The role of *Spirulina platensis* (*Arthrospira platensis*) in growth and immunity of Nile tilapia (*Oreochromis niloticus*) and its resistance to bacterial infection. Journal of Agricultural Science, 5: 109-117. https://www.researchgate.net/publication/270505988_The_Role_of_Spirulina_platensis_Arthrospira_platensis_in_Growth_and_Immunity_of_Nile_Tilapia_Oreochromis_niloticus_and_Its_Resistance_to_Bacterial_Infection. DOI: https://doi.org/10.5539/jas.v5n6p109

Islam MA, Samad MA, Paul D, Al Asif A and Hossain A (2021). Feeding frequency on the growth and production of endemic near-threatened *Ompok pabda* (Hamilton 1822) in pond setup. Asian-Australasian Journal of Bioscience and Biotechnology, 6(2): 89-102. https://www.banglajol.info/index.php/AAJBB/article/view/56144. DOI: https://doi.org/ 10.3329/aajbb.v6i2.56144.

IUCN (2003). Bangladesher Bipanna Prani. IUCN, The world Conservation Union, p 294. https://portals.iucn.org/library/node/8338

Javed M, Ahmad I, Ahmad A, Usmani N and Ahmad M (2016). Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. SpringerPlus, 5: 1-9. https://pubmed.ncbi.nlm.nih.gov/27386247/. DOI: https://doi.org/10.1186/s40064-016-2478-9

Kapoor R and Mehta U (1992). Iron bioavailability from *Spirulina platensis*, whole egg and whole wheat. Indian Journal of Experimental Biology, 30 :904–907. https://pubmed.ncbi.nlm.nih.gov/1293017

Khajali F and Rafiei F (2024). A review of plant anti-nutritional factors in animal health and production: the classification, biological properties, and the passivation strategy. Journal of Agriculture and Food Research, 101290. DOI: https://doi.org/10.1016/j.jafr.2024.101290

Khanzadeh M, Fereidouni AE and Berenjestanaki SS (2016). Effects of partial replacement of fish meal with *Spirulina platensis* meal in practical diets on growth, survival, body composition, and reproductive performance of three-spot gourami (*Trichopodus trichopterus*) (Pallas, 1770). Aquaculture international, 24: 69-84. https://link.springer.com/article/10.1007/s10499-015-9909-4. DOI: https://doi.org/10.1007/s10499-015-9909-4

Kim SS, Rahimnejad S, Kim KW and Lee KJ (2013). Partial replacement of fish meal with *Spirulina pacifica* in diets for parrot fish (*Oplegnathus fasciatus*). Turkish Journal of Fisheries and Aquatic Sciences, 13: 197-204. https://www.trjfas.org/abstract.php?id=330. DOI: http://dx.doi.org/10.4194/1303-2712-v13_2_01

Kohinoor AHM, Rahman MM, Rashid J, Chowdhury P and Islam MS (2018). Production potentials of pabda (*Ompok pabda*, Hamilton) in semi-intensive management under different stocking densities. Bangladesh Journal of Fisheries, 30: 37–45. https://fsb.bau.edu.bd/bjf/index.php/home/article/download/16/48

Kori-Siakpere O, Ake JEG and Avworo UM (2006). Sub-lethal effects of cadmium on some selected hematological parameters of *Hetero clarias* (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). Internmational Journal of Zoological Research, 2 (1): 77–83. https://scialert.net/abstract/?doi=ijzr.2006.77.83#:~:text=Exposure%20to%20cadmium%20caused%20a,decreased%20apart%20from%20one%20case. DOI: https://doi.org/10.3923/ijzr.2006.77.83

Li M, Wu W, Zhou P, Xie F, Zhou Q and Mai K (2014). Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. Aquaculture, 434: 227–232. https://www.sciencedirect.com/science/article/abs/pii/S0044848614004141. DOI: https://doi.org/10.1016/j.aquaculture.2014.08.022

Malla S and Banik S (2015). Larval rearing of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) with live and artificial diets. International Journal of Fauna and Biological Studies, 2: 16-21. https://www.faunajournal.com/vol2Issue5/pdf/2-4-19.1.pdf

Milad A, Yeganeh S, Dadar M, Sakai M and Dawood MAO (2016). Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso Linnaeus*, 1754). Fish Shellfish Immunology, 56: 436–444. https://www.sciencedirect.com/science/article/abs/pii/S1050464816304788. DOI: https://doi.org/10.1016/j.fsi.2016.08.003

Mishra SP and Pradesh U (2020). Significance of fish nutrients for human health. International Journal of Fisheries and Aquatic Research, 5: 47-49. https://www.fishjournals.com/assets/archives/2020/vol5issue3/5-3-19-141.pdf. DOI: http://dx.doi.org/10.35841/AAJPHN.6.4.156

Mohammadiazarm H, Maniat M, Ghorbanijezeh K and Ghotbeddin N (2021). Effects of spirulina powder (*Spirulina platensis*) as a dietary additive on Oscar fish, *Astronotus ocellatus*: Assessing growth performance, body composition, digestive enzyme activity, immune-biochemical parameters, blood indices and total pigmentation. Aquaculture nutrition, 27: 252-260. https://onlinelibrary.wiley.com/doi/abs/10.1111/anu.13182. DOI: https://doi.org/10.1111/anu.13182

Nandeesha MC, Gangadhara B, Manissery JK and Venkataraman LV (2001). Growth performance of two Indian major carp catla (*Catla catla*) and rohu (*Labeo rohita*) fed diet containing different level of *Spirulina*. Bioresource Technology, 80: 117-20. https://www.sciencedirect.com/science/article/abs/pii/S0960852401000852. DOI: https://doi.org/10.1016/S0960-8524(01)00085-2

Nelson RL (2008). Aquaponic Food Production - Raising fish and plants for food and profit. Montello, WI: Nelson and Pade, 2:22p. https://archive.org/details/aquaponicfoodpro0000nels

Olvera-Novoa MA, Domínguez-Cen LJ, Olivera-Castillo L and Martínez-Palacios CA (2008). Effect of the use of the microalga Spirulina maxima as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquaculture Research, 29: 709–15. https://www.researchgate.net/publication/230241662. DOI: https://doi.org/10.1046/j.1365-2109.1998.29100709.x

Palmegiano GB, Gai F, Dapra F, Gasco L, Pazzaglia M and Peiretti PG (2008). Effects of *Spirulina* and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. Aquaculture Research, 39: 587–595. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2109.2008.01914.x. DOI: https://doi.org/10.1111/j.1365-2109.2008.01914.x

Pelster B (2001). The generation of hyperbaric oxygen tensions in fish. Physiology, 16: 287-291. https://journals.physiology.org/doi/full/10.1152/physiologyonline.2001.16.6.287. DOI: https://doi.org/10.1152/physiologyonline.2001.16.6.287

Peres H, Lim C and Klesius PH (2003). Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*). Aquaculture, 225: 67-82. DOI: https://doi.org/10.1016/S0044-8486(03)00289-8

Promya J and Chitmanat C (2011). The effects of *Spirulina platensis* and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the African sharp-tooth catfish (*Clarias gariepinus*). International Journal of Agriculture Biology, 13: 77-82. https://agris.fao.org/search/en/providers/122650/records/64725353e17b74d2224fd54f

Samah R, Rasha M and Ashraf A (2017). Efficacy of *Spirulina platensis* diet supplements on disease resistance and immune-related gene expression in *Cyprinus carpio* exposed to herbicide atrazine. Fish Shellfish Immunology, 67: 119–128. https://www.sciencedirect.com/science/article/abs/pii/S1050464817303170. DOI: https://doi.org/10.1016/j.fsi.2017.05.065

Sayed AE and Fawzy MA (2014). Effect of dietary supplementation of *Spirulina platensis* on the growth and haematology of the Catfish *Clarias gariepinus*. Journal of Advances in Biology, 5: 32-38. https://rajpub.com/index.php/jab/article/view/3769. DOI: https://doi.org/10.1016/j.fsi.2017.05.065

Singh PK, Gaur SR and Chari MS (2006). Growth performance of *Labeo rohita* fed on diet containing different levels of slaughter house waste. Journal of Fisheries and Aquatic Science, 1: 10-16. https://scialert.net/fulltext/fulltextpdf.php?pdf=academicjournals/jfas/2006/10-16.pdf. DOI: https://doi.org/10.3923/jfas.2006.10.16

Soivio A, Niemisto M and Bacstrom M (1989). Fatty acid composition of Coregonus muksun Pallas: Changes during incubation, hatching, feeding and starvation. Aquaculture, 79: 163-168. https://www.sciencedirect.com/science/article/abs/pii/0044848689904572. DOI: https://doi.org/10.1016/0044-8486(89)90457-2

Spinola MP, Mendes AR and Prates JA (2024). Chemical composition, bioactivities, and applications of Spirulina (*Limnospira platensis*) in food, feed, and medicine. Foods, 13: 3656. https://www.mdpi.com/2304-8158/13/22/3656. DOI: https://doi.org/10.3390/foods13223656

Spolaore P, Joannis-Cassan J, Duran E and Isambert A (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101: 87–96. https://www.sciencedirect.com/science/article/abs/pii/S1389172306705497. DOI: https://doi.org/10.1263/jbb.101.87

Stansby ME (1954). Composition of certain species of fresh-water fish. 1. Introduction: the determination of the variation of composition of fish. Food Research, 19: 231-234. https://ift.onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1954.tb17444.x. DOI: http://dx.doi.org/10.1111/j.1365-2621.1954.tb17444.x

Teimouri M, Amirkolaie AK and Yeganeh S (2013). The effects of dietary supplement of *Spirulina platensis* on blood carotenoid concentration and fillet color stability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 414: 224-228. https://www.researchgate.net/publication/273425860. DOI: https://doi.org/10.1016/j.aquaculture.2013.08.015

Youssef IM, Saleh ES, Tawfeek SS, Abdel-Fadeel AA, Abdel-Razik ARH and Abdel-Daim AS (2023). Effect of *Spirulina platensis* on growth, hematological, biochemical, and immunological parameters of Nile tilapia (*Oreochromis niloticus*). Tropical Animal Health and Production, 55(4): 275. https://link.springer.com/article/10.1007/s11250-023-03690-5. DOI: https://doi.org/10.1007/s11250-023-03690-5

Zhang C (1994). The effects of polysaccharide and phycocyanin from *Spirulina platensis* variety on peripheral blood and hematopoietic system of bone marrow in mice. Second Asia-Pacific Conference on Alga Biotechnology, 58pp. https://www.scirp.org/reference/referencespapers?referenceid=1960300

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EFFECT OF TEMULAWAK (*Curcuma xanthorrhiza*) POWDER IN REDUCING PROTEOLYSIS IN FERMENTED TOTAL MIXED RATION WITH OKARA FOR RUMINANTS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: High protein content in silage feed often triggered excessive proteolysis caused by proteases derived from both plants and spoilage bacteria, which reduced the nutritional quality for ruminants. Temulawak (*Curcuma xanthorrhiza*) is an herbal plant with antibacterial and antioxidant properties, which have been reported to reduce proteolysis in previous studies. This study aimed to evaluate the effects of temulawak powder (TP) as an anti-proteolysis additive in the fermentation of total mixed ration-based okara silage (TMRO-silage) and its impact on silage characteristics. Additionally, the study examined the effects of drying temperature on temulawak extract optimization. Temulawak was dried at 50, 65, and 80 °C, followed by the measurement of phenol, flavonoid, and ferric reducing antioxidant power (FRAP) content. The TMRO-silage was composed of commercial feed and okaras (1:1 w/w), supplemented with 0-1% temulawak in increments of 0.25% (5 treatments and 4 replications). Fermentation lasted for 14 days. Proximate and *in vitro* analyses (control vs. temulawak treatments) were conducted to assess silage quality. Drying temulawak at 65 °C significantly ($P < 0.01$) increased phenol (16.9 µg quercetin equivalent, QE), flavonoid (23.3 µg QE), and FRAP (30.1 µg QE) content per dry weight. Temulawak supplementation significantly reduced ammonia levels and increased the crude protein content of TMRO-silage ($P < 0.01$). Moreover, it decreased ammonia concentration in the rumen ($P < 0.01$), improved dry matter and organic matter digestibility ($P < 0.05$), and notably reduced methane production per total gas volume ($P < 0.05$). In conclusion, temulawak effectively preserves the quality of complete feed silage, enhances rumen metabolism, and mitigates methane emissions.

Keywords: Antibacterial activity, Antioxidant properties, Fermentation, Proteolysis, Silage quality.

INTRODUCTION

Ensuring high-quality feed for ruminants, particularly beef cattle, requires a high-protein intake, commonly achieved through high-protein silage. This need becomes even more critical when the silage has a high digestibility rate (Li et al., 2021). High-protein silage, such as alfalfa or leguminous plants, serves as a key commodity. Similarly, tofu byproducts still contain a significant amount of protein. Tofu byproduct, or okara, is a soybean-processing residue rich in protein (9.9-32.8%), fat (6.2-22%), crude fiber (4.1-23.4%), and essential minerals such as calcium, iron, and copper (Kamble and Rani, 2020; Ginting et al., 2024). However, its high moisture content (74-80.3%) makes it highly susceptible to microbial spoilage, which can degrade its nutritional quality and cause an unpleasant odor (Kamble and Rani, 2020). Fermenting okara with microorganisms such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* enhances its nutritional profile. This process increases the concentration of small peptides (from 7.35% to 39.58%), enriches its amino acid and organic acid content, and improves digestibility (Suwarsito and Purbomartono, 2018; Kamble and Rani, 2020; Heng et al., 2022). As a result, fermented okara becomes a more stable and nutrient-dense alternative protein source for animal feed, and potentially for functional food applications. However, the absence of a controlled ensiling system presents several challenges, particularly the risk of excessive proteolysis (Hadidi et al., 2023). Therefore, supplementation with temulawak, an herbal additive containing bioactive compounds, became necessary to inhibit proteolysis. Reports also indicated its potential to improve ruminant production parameters (Adli et al., 2024; Sujarnoko et al., 2020).

Proteolysis occurs when proteins in the silage break down into non-protein nitrogen compounds, reducing the silage's nutritional value (Jayanegara et al., 2019; Okoye et al., 2023). This process is often exacerbated by plant protease activity

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and spoilage microorganisms, including *Clostridium* sp., *Enterobacteria*, *Pseudomonas*, molds (*Aspergillus* sp. and *Fusarium* sp.), and yeasts (*Candida* sp. and *Saccharomyces* sp.) (Ahangari et al., 2021; He et al., 2024; Snyder et al., 2024). These microorganisms not only degrade high-protein silage quality but also produce mycotoxins, such as those from *Aspergillus* sp. and *Fusarium* sp., which are toxic (Ekwomadu et al., 2021; Navale et al., 2021). Therefore, incorporating additives, such as *Curcuma xanthorrhiza* (temulawak), has become a primary strategy to mitigate these issues.

Curcuma xanthorrhiza has the potential to reduce protein degradation by inhibiting the growth of spoilage microorganisms (Yogiara et al., 2020; Septama et al., 2022; Nurcholis et al., 2024). It acts as an antimicrobial agent that suppresses pathogenic bacteria like *Clostridium* sp., *Enterobacteria*, and *Pseudomonas*, as well as mycotoxin-producing fungi such as *Aspergillus* sp. and *Fusarium* sp. Consequently, the conversion of protein into non-protein nitrogen and ammonia decreases. Additionally, temulawak contains curcuminoids and xanthorrhizol, which can reduce protease activity from both plants and spoilage microbes, thereby minimizing protein degradation (Seseogullari-Dİrihan et al., 2018; Al-Amin et al., 2024). Furthermore, yeast and *C. xanthorrhiza* supplementation in goats fed a high-PUFA diet reduced methane production and rumen protozoa populations while improving nutrient metabolism efficiency *in vitro* (Sulistyowati, 2014).

This study hypothesizes that the secondary metabolites of temulawak can inhibit proteolysis during the fermentation of total mixed ration-based okara silage (TMRO-silage) containing tofu by-products by reducing the protease activity of plants and spoilage microorganisms. This study introduces a novel application of temulawak as a natural additive in complete feed silage, which has not been widely explored in previous research. It not only acts as an antimicrobial agent to suppress the growth of spoilage bacteria and mycotoxin-producing molds but also serves as an anti-proteolytic agent that helps preserve protein content in the silage. This study aims to evaluate temulawak's effectiveness in reducing proteolysis during the fermentation of tofu by-product-based TMRO-silage, optimize its drying method to enhance bioactive metabolite content, and analyze its impact on silage quality, feed digestibility, and methane gas production in the rumen.

MATERIALS AND METHODS

Preparation temulawak powder

Fresh temulawak (*C. xanthorrhiza*) was harvested from Dramaga District, Bogor (6.5829°S, 106.7338°E) following a three-month cultivation period, consistent with local agronomic practices. The rhizomes were sliced into 3 mm-thick pieces and dried in an oven at 50°C, 65°C, and 80°C until they reached a stable weight. The dried temulawak was then ground and passed through a 40-mesh sieve. The resulting powder was extracted using an ultrasonic Branson 1510 with an oscillation frequency of 40 kHz in an aqueous-acetone solution (70:30 v/v). The extract was then analyzed for total phenolic content, flavonoid content, and antioxidant activity using the ferric reducing antioxidant power (FRAP) method.

Determination of total phenolic, total flavonoid, and FRAP

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, following Makkar's protocol (Makkar, 2003). First, 10 µL of 10% Folin-Ciocalteu reagent was added to the sample and incubated for 5 minutes. Then, 20 µL of 7.5% Na_2CO_3 was introduced into the mixture, which was subsequently incubated in a dark room for 30 minutes. The absorbance of the resulting solution was measured at a wavelength of 750 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH). Gallic acid was used to construct a standard curve for determining the TPC of temulawak powder, expressed as mg gallic acid equivalent (GAE) per gram of dry weight (DW). The standard curve was prepared using seven concentration levels: 0, 50, 100, 150, 200, 250, and 300 ppm. The TPC was calculated using the equation $y = 0.006x + 0.198$, where y represents the absorbance value and x denotes the gallic acid concentration.

The total flavonoid content (TFC) was determined using $AlCl_3$ colorimetric method, following the protocol described by Chang (Chang et al. 2002). First, 250 µL of the sample extract was mixed with 75 µL of 5% $NaNO_2$ and incubated for 5 minutes. Then, 150 µL of 10% $AlCl_3$ was added, followed by additional 5-minute incubation. Afterward, 500 µL of 1 M NaOH was introduced into the mixture, and the final volume was adjusted to 2 mL with distilled water. The absorbance of the solution was measured at 510 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH). Quercetin was used to generate the standard curve, and TFC was expressed as mg quercetin equivalent (QE) per gram of dry weight (DW). The standard curve was prepared with quercetin concentrations of 0, 20, 40, 60, 80, and 100 ppm. The TFC was calculated using the equation $y = 0.005x + 0.102$, where y represents the absorbance value, and x denotes the quercetin concentration. The antioxidant capacity of temulawak powder was determined using the Ferric Reducing Antioxidant Power (FRAP) assay, following Benzie and Strain (1996). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. Then, 100 µL of the sample extract was mixed with 900 µL of the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance of the resulting solution was measured at 593 nm using a nano-spectrophotometer

(SPECTROstar Nano, BMG LABTECH). A standard curve was prepared using $FeSO_4 \cdot 7H_2O$ at concentrations of 0, 200, 400, 600, 800, and 1000 µM. The FRAP value was expressed as mg Trolox equivalent (TE) per gram of DW and calculated using the equation y = 0.004x + 0.123, where y represents the absorbance value, and x denotes the Fe^{2+} concentration.

Preparation of fermented okaras for complete feed preservation

This study examined the effect of temulawak powder dosage (0%, 0.25%, 0.5%, 0.75%, and 1% DM) on complete feed supplemented with fermented okaras, using a fresh okara-to-complete feed ratio of 1:1. The fermentation process lasted 14 days. Fresh okaras were sourced from a home-based tofu industry in Dramaga District, Bogor. The complete feed was a commercial product from Agro Apis Palacio Company in Bubulak. The feed ingredients were formulated and analyzed for proximate composition at the Feed Science and Technology Laboratory, Faculty of Animal Science, IPB University. Proximate components were analyzed following AOAC (2023) methods, including dry matter (934.01), crude protein (990.03), crude fiber (962.09), crude fat (920.39), and ash (942.05); nitrogen-free extract was calculated by difference. Table 1 presents the composition and nutritional content.

Table 1 - Feed composition and nutritional content

Ingredient	Level (% DM)
Okara	12
Palm kernel meal	25
Casava dreg	17
Coffee husk	22
Copra meal	10
Pollard	8
Molasses	5
Urea	1
Nutrition content (%)	
Dry matter	56.5
Crude protein	16.9
Crude fiber	18.5
Non-nitrogen extract	52.3
Crude fat	5.28
Ash	7.04

***In vitro* model of Theodorou (1994) and gas production assessment**

The fermented okara and complete feed included a control group without temulawak powder (TMRO-0) and with treatment groups supplemented with 0.25% to 1% temulawak powder (TMRO-0.25, TMRO-0.5, TMRO-0.75, TMRO-1), were oven-dried at 60°C for 24 hours. The samples were then incubated *in vitro* with a rumen fluid and buffer mixture following the Theodorou method (Theodorou et al. 1994). Rumen inoculum from beef cattle was collected at a government-operated slaughterhouse in Bogor, West Java. The facility complied with animal welfare standards established by the National Research and Innovation Agency (BRIN). A 500 mg sample was placed into a 125 ml serum bottle, followed by the addition of 15 ml of rumen fluid and 60 ml of buffer mixture. The treatment allocations followed a randomized complete design. Incubation was conducted in four replicates, with four bottles per replicate.

Immediately after sample preparation, the serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals. The incubation process lasted for 24 hours at 39°C, with gas production recorded throughout. After incubation, the supernatant was collected to measure total volatile fatty acids (VFA) and ammonia concentration, following the Jayanegara method (Jayanegara et al., 2016). The remaining residue was weighed to determine *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD).

Data analysis

The assessment of drying temperature on the chemical characteristics of temulawak extract followed a completely randomized design (CRD) with different treatments. In the drying experiment, the effect on flavonoid content, phenol levels, and ferric reducing antioxidant power (FRAP) was evaluated. Temulawak was dried at three different temperatures: 50°C (T50), 65°C (T65), and 80°C (T80), with each treatment replicated four times. The average drying time was then presented graphically. In the silage experiment, the addition of temulawak powder followed a CRD. The treatments consisted of temulawak powder at 0% (TP0), 0.25% (TP0.25), 0.5% (TP0.5), 0.75% (TP0.75), and 1% (TP1) of the total TMRO-silage weight. If the ANOVA results were significant ($p < 0.05$), further analysis was conducted using Duncan's multiple range test. A graphical illustration was employed to observe the dynamic changes in ammonia production in TMRO-silage following temulawak powder addition. In the *in vitro* experiment, the treatments included a control diet consisting of complete feed and okaras (TP0) and a diet supplemented with 1% DM temulawak (TP1). Each treatment was replicated four times. Data analysis was conducted using an independent t-test in SPSS 16 to compare the means between the two groups. The t-test statistic (t) was calculated using the formula where X_1 and X_2 represent the mean values of groups TP0 and TP1, respectively; s_1^2 and s_2^2 denote their variances; n_1 and n_2 indicate the sample sizes of each group. A significance level (α) of 0.05 was used to determine statistical differences between the treatments. Gas production was fitted to the following non-linear regression model: $V_t = (a + b) \times (1 - e^{-ct})$,

where V_t represents the volume of gas production (ml) at time t, t is the incubation period (h), a + b denotes the gas production potential, e is the base of the natural logarithm, and c is the gas production rate constant. The model's fit was assessed using the coefficient of determination (R^2) and mean square error (MSE), both of which were reported to validate the regression accuracy.

RESULTS AND DISCUSSION

Impact of thermal drying conditions on bioactive compound stability in temulawak

Drying is one of the most common methods for preserving herbal materials (Calín-Sánchez et al., 2020). Using an oven at a specific temperature influences the drying rate (Torki-Harchegani et al., 2016). Additionally, oven temperature affects the quality of the active compounds produced (Abd Ghani et al., 2023). Optimizing drying temperature is essential to maintaining the quality of bioactive compounds in temulawak. Temulawak is an herbal plant with antibacterial and antioxidant properties and can influence rumen metabolism (Rosidi et al., 2016; Sujarnoko et al., 2023). The effect of temperature on drying speed is shown in Figure 1. Drying at 50°C takes approximately 25 hours to reach a stable dry weight. At 65°C, the material reaches a stable dry weight in 9 hours, while at 80°C, it achieves equilibrium after 7 hours of drying (Figure 1).

Oven temperature significantly influenced ($P<0.01$) total flavonoid content, with the highest levels observed at 65°C, followed by 50 and 80°C (Table 2). The lower flavonoid content at 80℃ resulted from excessive heat exposure, which degraded a significant portion of the flavonoids. High temperatures caused structural damage to the functional groups of flavonoids (Zhang et al., 2023). For instance, rutin, naringin, and luteolin degrade at 130°C after two hours, while high temperatures also inhibit anthocyanin biosynthesis (Chaaban et al., 2017; Yang et al., 2024). Drying at 50°C preserved more flavonoid content than at 80°C because lower temperatures minimized functional group degradation (Sharma et al., 2015; Červenka et al., 2018). However, drying at 65°C resulted in significantly higher flavonoid content than at 50℃, likely due to prolonged heat exposure enhancing flavonoid release.

Phenols are among the compounds in temulawak that exhibit antibacterial and antioxidant properties (Abd Rashid et al., 2022). Drying temulawak at 80°C significantly reduces the total phenol content compared to drying at 50 and 65°C (Table 2). This reduction occurs because phenolic groups degrade and oxidize when exposed to high temperatures, leading to a significant decline in total phenol content. Heat exposure causes structural damage to these compounds (Cheng et al., 2014; Wang et al., 2021). In contrast, drying at 50 and 65°C does not result in a significant difference in total phenol content. This stability likely occurs because the temperatures used do not exceed the degradation threshold of phenols (Levén and Schnürer, 2005). Temulawak is an herbal plant with antioxidant properties that enhance its ability to absorb free radicals (Rosidi et al., 2016). Drying at 65°C increases its antioxidant activity compared to drying at 80°C (Table 2). This occurs because high temperatures accelerate the thermal degradation of antioxidant compounds, reducing temulawak's ability to neutralize free radicals (Levén and Schnürer, 2005; Peron et al., 2017). Meanwhile, drying at 50°C results in lower antioxidant activity than at 65°C. This may be due to prolonged exposure to heat and oxygen, which promotes oxidation (Poljšak and Fink, 2014; Bahrololoumi et al., 2022).

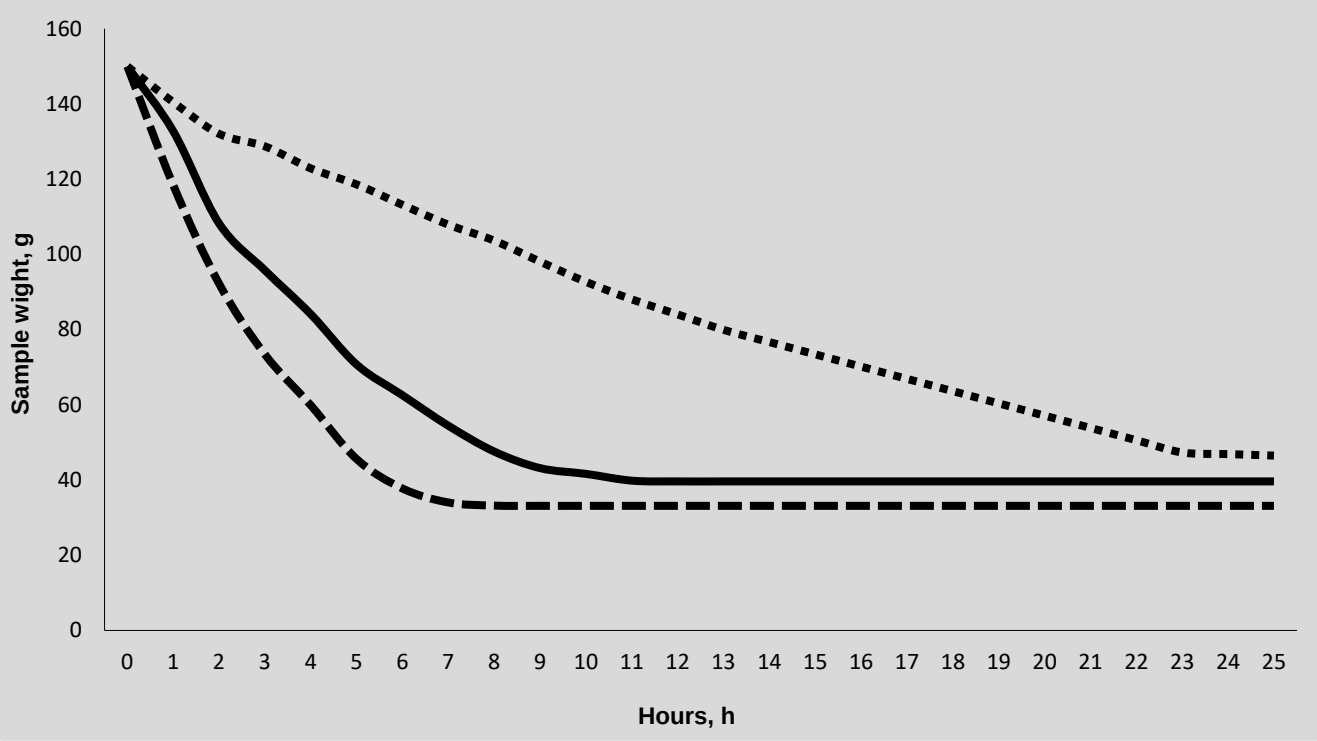

Figure 1 - Oven temperature influenced the sample weight (g) of temulawak powder over time (h), where 50°C (solid), 65°C (small dashed), 80℃ (long dashed).

Table 2 - Flavonoid content, phenol levels, and FRAP under drying treatments at 50, 65, and 80 °C in temulawak powder.

Drying temperature (°C) / Flavonoid content	50 °C	65 °C	80 °C	P value
Total flavonoid (µg QE/g DW)	18.1 ± 1.45[b]	23.3 ± 1.72[a]	14.5 ± 1.71[c]	<0.01
Total phenol (µg GAE/g DW)	17 ± 1.36[a]	16.9 ± 1.73[a]	12.4 ± 1.63[b]	0.01
FRAP (mg TE/g DW)	19.7 ± 2.25[b]	30.1 ± 2.42[a]	9.18 ± 2.91[c]	<0.01

Different superscripts within the same row are significantly different at P<0.05. FRAP=ferric reducing antioxidant power, GAE=gallic acid equivalent, QE=quercetin equivalent.

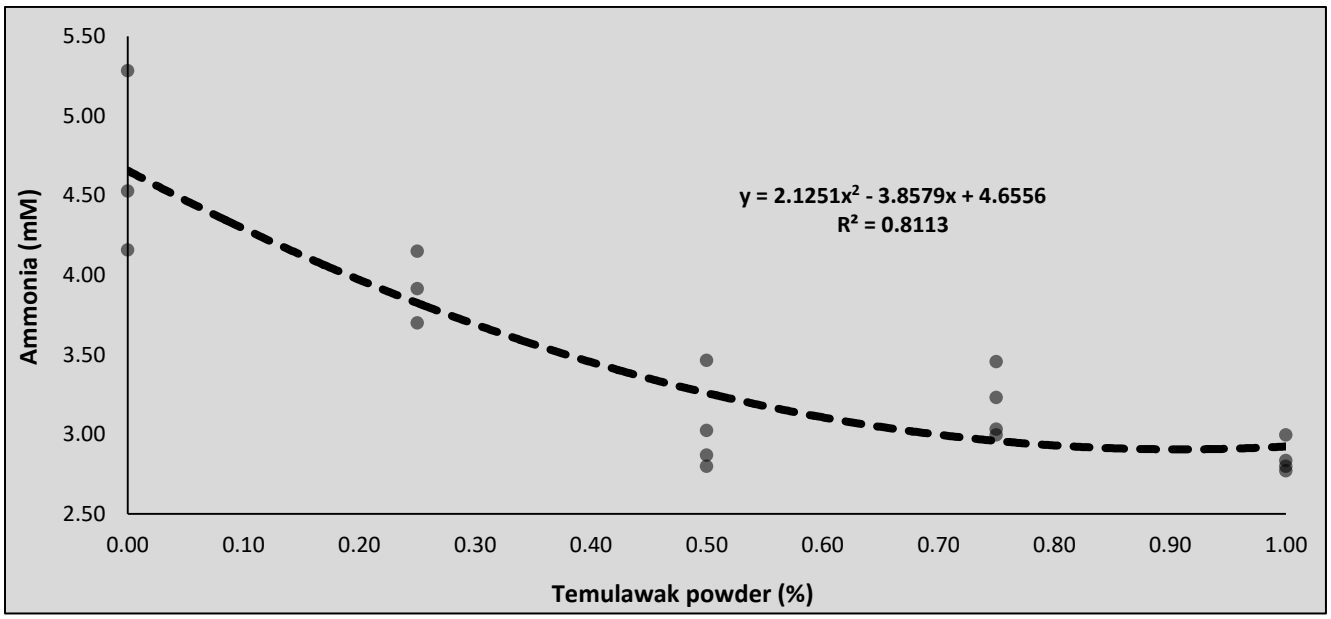

Figure 2 - The impact of temulawak powder addition (%, x-axis) to TMRO-silage on ammonia production (mM, y-axis).

Effect of temulawak powder on TMRO-silage

Preserving high-protein feed ingredients during the rainy season presents a significant challenge (Wang et al., 2019). In the silage process, spoilage bacteria such as *Clostridium* sp. and *Escherichia coli* deaminate protein chains, converting them into ammonia. This reaction makes it more difficult to lower the silage pH (Jayanegara et al., 2019). Temulawak, a rhizome with antibacterial properties, can help prevent protein degradation during silage production (Rahmat et al., 2021). Adding 0.5% temulawak powder to TMRO-silage effectively inhibited deamination, as indicated by a significant decrease in ammonia levels ($P < 0.01$; Table 3). Lower ammonia levels help stabilize silage pH during storage (Jayanegara et al., 2019). Temulawak reduces ammonia content by using its phenolic compounds to inhibit spoilage bacteria (Sadarman et al., 2019; Figure 2). Additionally, tannins in temulawak bind to proteins, further limiting deamination (Kondo et al., 2006). Adding 1% temulawak powder effectively prevented crude protein loss, significantly increasing the feed protein percentage on TMRO-silage ($P < 0.05$; Table 4). Soluble carbohydrates in the feed are converted into lactate, carbon dioxide, and water, which leads to a relative increase in protein content within the silage. Phenols can inhibit microbial activity, while tannins help protect proteins from degradation (Jayanegara et al., 2019; Sujarnoko et al., 2020). However, temulawak powder did not significantly lower the pH, which remained within the acceptable range for silage (pH 4-4.5). The addition of temulawak powder effectively preserved the dry matter content of TMRO-silage, as indicated by the significantly higher dry matter values in the treatment group compared to the control ($P < 0.01$; Table 4). The preservation process for complete feed and tofu by-products improved because the nutrients were protected from spoilage. Additionally, nutrient degradation was prevented (Rahmat et al., 2021; Abd Rashid et al., 2022).

Effect of adding temulawak powder on *in vitro* rumen fermentation

The addition of 1% temulawak powder to TMRO-silage significantly reduced rumen ammonia levels compared to the other treatment ($P < 0.01$; Table 5). This reduction is likely due to temulawak's phenolic and flavonoid compounds, which can inhibit protease activity and microbial proliferation (Landis-Piwowar et al., 2008; Hernández-Rodríguez et al., 2019). The decrease in ammonia production tends to lower pH levels since ammonia, which has a basic nature due to its ($-NH_2$) group, is reduced (Calsamiglia et al., 2008). Additionally, a strong correlation exists between phenolic content and lactic acid bacteria populations (Pianpumepong and Noomhorm, 2010). Temulawak powder also increases total gas production

and digestibility (IVDMD and IVOMD). However, an important finding is the reduction in methane production per total gas ($P < 0.05$; Table 5). Previous *in vitro* studies also reported methane reduction with other phenolic-containing compounds (Sujarnoko et al., 2020; 2023). The positive correlation between temulawak powder and rumen fermentation characteristics is primarily attributed to its phenolic and flavonoid content. However, drying methods should be optimized to ensure the best residual compound retention. Future research should explore not only the application of temulawak powder in high-protein silage preservation, such as TMR-silage with okaras, but also in other high-protein silage applications, including fermented food products.

Table 3 - Influence of the temulawak powder on the ammonia production (mM) of TMRO-silage

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
Ammonia (mM)	4.52 ± 0.66^{c}	3.91 ± 0.23^{b}	3.1 ± 0.37^{a}	2.9 ± 0.35^{a}	2.84 ± 0.13^{a}	0.01

Different superscripts within the same row are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP.

Table 4 - Influence of the temulawak powder on pH and proximate composition of TMRO-silage

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
pH	4.32 ± 0.02	4.3 ± 0.07	4.3 ± 0.03	4.33 ± 0.03	4.3 ± 0.05	0.34
Dry matter (%)	54.1 ± 0.64^{a}	56 ± 0.7^{b}	57.4 ± 0.63^{c}	57.2 ± 0.75^{c}	55.3 ± 0.76^{b}	<0.01
Crude protein (%)	15.2 ± 0.1^{a}	15.5 ± 0.65^{ab}	15.7 ± 0.32^{ab}	15.8 ± 0.52^{b}	17.1 ± 0.41^{c}	<0.01
Ether extract (%)	6.72 ± 0.25^{b}	5.56 ± 0.22^{a}	7.8 ± 0.19^{c}	7.07 ± 0.16^{b}	7.67 ± 0.46^{c}	<0.01
Crude fiber (%)	22.3 ± 0.49^{b}	22.6 ± 0.92^{b}	21.9 ± 0.84^{b}	21.7 ± 1.16^{ab}	20.4 ± 0.82^{a}	0.03
Ash (%)	8.74 ± 0.26^{a}	8.77 ± 0.45^{a}	9.03 ± 0.65^{a}	9.17 ± 0.67^{a}	10.1 ± 0.83^{b}	0.03
NFE (%)	47 ± 46^{c}d	47.6 ± 0.5d	45.5 ± 0.43^{ab}	46.3 ± 0.86^{bc}	44.7 ± 0.53^{a}	<0.01

Different superscripts within the same row are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; NFE=nitrogen-free extract. TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP.

Table 5 - *In vitro* characteristics of TMRO-silage added with temulawak powder

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
Ammonia (mM)	12.3 ± 1.14^{b}	11.4 ± 0.87^{b}	10.3 ± 0.79^{ab}	9.7 ± 0.53^{a}	9.03 ± 0.62^{a}	<0.01
pH	6.99 ± 0.17^{b}	6.97 ± 0.14^{b}	6.91 ± 0.21^{b}	6.87 ± 0.07^{b}	6.81 ± 0.83^{a}	0.01
VFA (mM)	125 ± 5.63	123 ± 6.01	120 ± 5.99	118 ± 6.3	115 ± 6.46	0.07
IVDMD (%)	49.6 ± 0.85^{a}	51.0 ± 0.56^{ab}	50.5 ± 0.48^{b}	50.8 ± 0.38^{b}	51.1 ± 0.6^{b}	0.03
IVOMD (%)	47.2 ± 0.89^{a}	47.8 ± 0.75^{ab}	48.2 ± 0.86^{b}	48.3 ± 0.71^{b}	48.9 ± 0.78^{b}	0.03
Total gas (mL)	113 ± 2.95^{a}	115 ± 2.53^{ab}	118 ± 2.16^{b}	117 ± 2.01^{b}	119 ± 1.97^{b}	0.03
Methane (ppm)	72,166 ± 6,135	70,135 ± 5,345	69,140 ± 4,866	68,450 ± 3,852	68,114 ± 3,313	0.26
MTG (ppm/mL)	631 ± 54.7^{b}	611 ± 47.2^{b}	595 ± 41.6^{ab}	582 ± 35.7^{ab}	576 ± 30.6^{a}	0.05

Different superscripts within the same column are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; NFE=nitrogen-free extract. TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP. VFA=volatile fatty acid; IVDMD=*in vitro* dry matter digestibility; IVOMD=*in vitro* organic matter digestibility; MTG=methane per total gas.

CONCLUSION

Adding 1% dry matter temulawak powder effectively reduces the risk of protein degradation during the optimal storage of total mixed ration-based okara silage (TMRO-silage). It also improves the nutritional characteristics of TMRO-silage. Additionally, incorporating 1% temulawak powder enhances *in vitro* digestibility and protects feed protein from deamination. This dosage optimally reduces methane relative to total gas production while increasing total gas production. This benefit is likely related to the higher levels of total phenols, flavonoids, and ferric reducing antioxidant power (FRAP) in temulawak processed at 65 °C.

DECLARATIONS

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Data availability

The data are available upon request from the corresponding author via the provided contact information.

Ethical considerations

Rumen donors were obtained from slaughtered beef cattle, based on approval from the relevant institutional ethics committee (Approval No. 078/KE.02/SK/10/2022).

Authors' contribution

The authors contributed equally to data analysis and manuscript writing. T.U.P.Sujarnoko led the conceptualization. M.S.Lim conducted data curation. M.S.Lim and D.Budiono carried out formal analysis. T.U.P.Sujarnoko secured the funding and performed validation. N.A.Sholeha conducted the investigation and visualization. N.A.Sholeha and M.D.Alifian developed the methodology. D.Budiono and M.D.Alifian administered the project. N.A.Sholeha provided the resources. M.S.Lim, T.Ujilestari, and M.M. Sholikin developed the software. T.U.P. Sujarnoko and M.M. Sholikin provided supervision. M.S.Lim, T.Ujilestari, and M.M.SHOLIKIN drafted the original manuscript, while T.Ujilestari and M.M.SHOLIKIN reviewed and edited the manuscript.

Consent to publish

Each author reviewed and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

REFERENCES

Abd Ghani A, Abd Rashid NY, Abd Razak DL, Jamaluddin A, Abdul Manan M, and Mansor A (2023). The effect of heat treatments on the bioactive compounds, antioxidant activity, and cosmeceutical properties (anti-pigmentation and anti-ageing) of fermented broken rice. Food Research, 6: 155–162. DOI: https://doi.org/10.26656/fr.2017.6(S2).009

Abd Rashid SNA, Hasham R, Abd. Rashid ZI, Cheng KK, Aziz AA, Shafin N et al. (2022). Formulation and characterization of the physicochemical, antioxidant activity and sensory attributes of curcuma-based herbal drink. Materials Today: Proceedings, 57: 1061–1066. DOI: https://doi.org/10.1016/j.matpr.2021.09.272

Adli DN, Sholikin MM, Ujilestari T, Ahmed B, Sadiqqua A, Harahap M A, et al. (2024). Effect of fermentation of herbal products on growth performance, breast meat quality, and intestinal morphology of broiler chickens: A meta-analysis. Italian Journal of Animal Science, 23(1): 734–750. DOI: https://doi.org/10.1080/1828051X.2024.2351441

Ahangari H, Yazdani P, Ebrahimi V, Soofiyani S R, Azargun R, Tarhriz V, et al. (2021). An Updated review on production of food derived bioactive peptides: Focus on the psychrotrophic bacterial proteases. Biocatalysis and Agricultural Biotechnology, 35: 102051. DOI: https://doi.org/10.1016/j.bcab.2021.102051

Al-Amin M, Fazalul Rahiman S S, Khairuddean M, and Muhamad Salhimi S (2024). (R)-(−)-Xanthorrhizol inhibits the migration and invasion of triple-negative breast cancer cells by suppressing matrix metalloproteinases via the nf-κb signaling pathway. Planta Medica, 90(10): 785–791. DOI: https://doi.org/10.1055/a-2339-2633

AOAC (2023). Association of Official Analytical Chemists. Official Methods of Analysis, 21th Edition. New York DOI: https://doi.org/10.1093/9780197610145.002.001

Bahrololoumi A, Shaafaey M, and Dargazany R (2022). Constitutive modeling of the cross-linked polymers during the trio-aging mechanism: Effects of water, oxygen, UV. ASME 2022 International Mechanical Engineering Congress and Exposition, 2022: IMECE2022-95558. DOI: https://doi.org/10.1115/IMECE2022-95558

Benzie IFF, Strain JJ. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239(1): 70–76. DOI: https://doi.org/10.1006/abio.1996.0292

Calín-Sánchez Á, Lipan L, Cano-Lamadrid M, Kharaghani A, Masztalerz K, Carbonell-Barrachina A A, et al. (2020). Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. Foods, 9(9): 1261. DOI: https://doi.org/10.3390/foods9091261

Calsamiglia S, Cardozo P W, Ferret A, and Bach A (2008). Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. Journal of Animal Science, 86(3): 702–711. DOI: https://doi.org/10.2527/jas.2007-0146

Červenka L, Červenková Z, and Velichová H (2018). Is air-drying of plant-based food at low temperature really favorable? A meta-analytical approach to ascorbic acid, total phenolic, and total flavonoid contents. Food Reviews International, 34(5): 434–446. DOI: https://doi.org/10.1080/87559129.2017.1307389

Chaaban H, Ioannou I, Chebil L, Slimane M, Gérardin C, Paris C, et al. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. Journal of Food Processing and Preservation, 41(5): e13203. DOI: https://doi.org/10.1111/jfpp.13203

Cheng Y, Xu Q, Liu J, Zhao C, Xue F, and Zhao Y (2014). Decomposition of five phenolic compounds in high temperature water. Journal of the Brazilian Chemical Society, 25(11): 2102-2107. DOI: https://doi.org/10.5935/0103-5053.20140201

Ekwomadu T I, Akinola S A, and Mwanza M (2021). Fusarium mycotoxins, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. International Journal of Environmental Research and Public Health, 18(22): 11741. DOI: https://doi.org/10.3390/ijerph182211741

Ginting E, Elisabeth DAA, Khamidah A, Rinaldi J, Ambarsari I, and Antarlina SS (2024). The nutritional and economic potential of tofu dreg (okara) and its utilization for high protein food products in Indonesia. Journal of Agriculture and Food Research, 16: 101175. DOI: https://doi.org/10.1016/j.jafr.2024.101175

Hadidi M, Orellana Palacios JC, McClements DJ, Mahfouzi M, and Moreno A (2023). Alfalfa as a sustainable source of plant-based food proteins. Trends in Food Science & Technology, 135: 202–214. DOI: https://doi.org/10.1016/j.tifs.2023.03.023

He Y, Degraeve P, and Oulahal N (2024). Bioprotective yeasts: Potential to limit postharvest spoilage and to extend shelf life or improve microbial safety of processed foods. Heliyon, 10(3): e24929. DOI: https://doi.org/10.1016/j.heliyon.2024.e24929

Heng X, Chen H, Lu C, Feng T, Li K, and Gao E (2022). Study on synergistic fermentation of bean dregs and soybean meal by multiple strains and proteases. LWT, 154: 112626. DOI: https://doi.org/10.1016/j.lwt.2021.112626

Hernández-Rodríguez P, Baquero L P, and Larrota H R (2019). Flavonoids: Potential Therapeutic Agents by Their Antioxidant Capacity. In: M. R. S. Campos (Editor), Bioactive Compounds, p. 265–288 (Bg). DOI: https://doi.org/10.1016/B978-0-12-814774-0.00014-1

Jayanegara A, Sujarnoko T U P, Ridla M, Kondo M, and Kreuzer M (2019). Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis. Journal of Animal Physiology and Animal Nutrition, 103(2): 456–465. DOI: https://doi.org/10.1111/jpn.13050

Jayanegara A, Dewi S, Laylli N, Laconi E, Nahrowi, and Ridla M (2016). Determination of cell wall protein from selected feedstuffs and its relationship with ruminal protein digestibility *in vitro*. Media Peternakan, 39: 134–140. DOI: https://doi.org/10.5398/medpet.2016.39.2.134

Kamble DB and Rani S (2020). Bioactive components, *in vitro* digestibility, microstructure and application of soybean residue (Okara): A review. Legume Science, 2(1): 1–9. DOI: https://doi.org/10.1002/leg3.32

Kondo M, Kita K, and Yokota H (2006). Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. Asian-Australasian Journal of Animal Sciences, 19(4): 533–540. DOI: https://doi.org/10.5713/ajas.2006.533

Landis-Piwowar KR, Milacic V, and Dou QP (2008). Relationship between the methylation status of dietary flavonoids and their growth-inhibitory and apoptosis-inducing activities in human cancer cells. Journal of Cellular Biochemistry, 105(2): 514–523. DOI: https://doi.org/10.1002/jcb.21853

Levén L and Schnürer A (2005). Effects of temperature on biological degradation of phenols, benzoates and phthalates under methanogenic conditions. International Biodeterioration & Biodegradation, 55(2): 153–160. DOI: https://doi.org/10.1016/j.ibiod.2004.09.004

Li R, Zheng M, Jiang D, Tian P, Zheng M, and Xu C (2021). Replacing alfalfa with paper mulberry in total mixed ration silages: Effects on ensiling characteristics, protein degradation, and *in vitro* digestibility. Animals, 11(5): 1273. DOI: https://doi.org/10.3390/ani11051273

Makkar H P S (2003). Quantification of Tannins in Tree and Shrub Foliage. Springer Netherlands, Dordrecht, pp. 49-51 DOI: https://doi.org/10.1007/978-94-017-0273-7

Navale V, Vamkudoth K R, Ajmera S, and Dhuri V (2021). *Aspergillus* derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. Toxicology Reports, 8: 1008–1030. DOI: https://doi.org/10.1016/j.toxrep.2021.04.013

Nurcholis W, Rahmadansah R, Astuti P, Priosoeryanto B P, Arianti R, and Kristóf E (2024). Comparative analysis of volatile compounds and biochemical activity of *Curcuma xanthorrhiza* Roxb. essential oil extracted from distinct shaded plants. Plants, 13(19): 2682. DOI: https://doi.org/10.3390/plants13192682

Okoye C O, Wang Y, Gao L, Wu Y, Li X, Sun J, et al. (2023). The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. Microbiological Research, 266: 127212. DOI: https://doi.org/10.1016/j.micres.2022.127212

Peron D V, Fraga S, and Antelo F (2017). Thermal degradation kinetics of anthocyanins extracted from Juçara (*Euterpe edulis* Martius) and "Italia" grapes (*Vitis vinifera* L.), and the effect of heating on the antioxidant capacity. Food Chemistry, 232: 836–840. DOI: https://doi.org/10.1016/j.foodchem.2017.04.088

Pianpumepong P and Noomhorm A (2010). Isolation of probiotic bacteria from turmeric (*Curcuma longa* Linn.) and its application in enriched beverages. International Journal of Food Science & Technology, 45(12): 2456–2462. DOI: https://doi.org/10.1111/j.1365-2621.2010.02337.x

Poljšak B and Fink R (2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. Oxidative Medicine and Cellular Longevity, 2014(1): 1–22. DOI: https://doi.org/10.1155/2014/671539

Rahmat E, Lee J, and Kang Y (2021). Javanese Turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evidence-Based Complementary and Alternative Medicine, 2021: 1–15. DOI: https://doi.org/10.1155/2021/9960813

Rosidi A, Khomsan A, Setiawan B, Riyadi H, and Briawan D (2016). Antioxidant potential of Temulawak (*Curcuma xanthorrhiza* Roxb.). Pakistan Journal of Nutrition, 15(6): 556–560. DOI: https://doi.org/10.3923/pjn.2016.556.560

Sadarman, Ridla M, Nahrowi, Sujarnoko T U P, Ridwan R, and Jayanegara A. (2019). Evaluation of ration based on soy sauce by-product on addition of acacia and chestnut tannin: An *in vitro* study. IOP Conference Series: Materials Science and Engineering, 546(2): 022020. DOI: https://doi.org/10.1088/1757-899X/546/2/022020

Septama AW, Tasfiyati AN, Kristiana R, and Jaisi A (2022). Chemical profiles of essential oil from Javanese turmeric (*Curcuma xanthorrhiza* Roxb.), evaluation of its antibacterial and antibiofilm activities against selected clinical isolates. South African Journal of Botany, 146: 728–734. DOI: https://doi.org/10.1016/j.sajb.2021.12.017

Seseogullari-Dİrihan R, Tekbas Atay M, Pashley D H, and Tezvergil-Mutluay A (2018). Inhibitory effect of curcuminoid pretreatments on endogenous dentin proteases. Dental Materials Journal, 37(3): 445–452. DOI: https://doi.org/10.4012/dmj.2017-116

Sharma K, Ko E Y, Assefa AD, Ha S, Nile SH, Lee ET, et al. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23(2): 243–252. DOI: https://doi.org/10.1016/j.jfda.2014.10.005

Snyder A B, Martin N, and Wiedmann M (2024). Microbial food spoilage: Impact, causative agents and control strategies. Nature Reviews Microbiology, 22(9): 528–542. DOI: https://doi.org/10.1038/s41579-024-01037-x

Sulistyowati E, Sudarman A, Wiryawan K G, and Toharmat T (2014). *In vitro* goat fermentation of PUFA-diet supplemented with yeast and *Curcuma xanthorrhiza* Roxb. Media Peternakan, 37(3): 175–181. DOI: https://doi.org/10.5398/medpet.2014.37.3.175

Sujarnoko TUP, Ridwan R, Nahrowi N, and Jayanegara A (2020). Extraction of tannin from Acacia (*Acacia mangium*) bark and its use as a feed additive for protecting *in vitro* ruminal degradation of tofu dregs. Advances in Animal and Veterinary Sciences, 8(7): 761-765. DOI: https://doi.org/10.17582/journal.aavs/2020/8.7.761.765

Sujarnoko TUP, Saputra Lim M, Budiono D, Amalia Sholeha N, Firmansyah D, Zahera R, et al. (2023). The effect of Temulawak (*Curcuma xhantorrhiza*) dose on tofu dreg plus complete feed silage with methane relative mitigation: *In vitro*. E3S Web of Conferences, 454: 02010. DOI: https://doi.org/10.1051/e3sconf/202345402010

Suwarsito S and Purbomartono C (2018). The effect of use of tofu dregs fermented on feed efficiency and growth of African cat fish (*Clarias gariepinus*). IOP Conference Series: Materials Science and Engineering, 434(1): 012128. DOI: https://doi.org/10.1088/1757-899X/434/1/012128

Theodorou MK, Williams BA, Dhanoa M S, McAllan AB, and France J (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology, 48(3–4): 185–197. DOI: https://doi.org/10.1016/0377-8401(94)90171-6

Torki-Harchegani M, Ghasemi-Varnamkhasti M, Ghanbarian D, Sadeghi M, and Tohidi M (2016). Dehydration characteristics and mathematical modelling of lemon slices drying undergoing oven treatment. Heat and Mass Transfer, 52(2): 281–289. DOI: https://doi.org/10.1007/s00231-015-1546-y

Wang D, Ding J, Wang B, Zhuang Y, and Huang Z (2021). Synthesis and thermal degradation study of polyhedral oligomeric silsesquioxane (POSS) modified phenolic resin. Polymers, 13(8): 1182. DOI: https://doi.org/10.3390/polym13081182

Wang H, Wang C, Li C, Xu X, and Zhou G (2019). Effects of phenolic acid marinades on the formation of polycyclic aromatic hydrocarbons in charcoal-grilled chicken wings. Journal of Food Protection, 82(4): 684–690. DOI: https://doi.org/10.4315/0362-028X.JFP-18-420

Yang J, Guo C, Chen F, Lv B, Song J, Ning G, et al. (2024). Heat-induced modulation of flavonoid biosynthesis via a LhMYBC2-Mediated regulatory network in oriental hybrid lily. Plant Physiology and Biochemistry, 214: 108966. DOI: https://doi.org/10.1016/j.plaphy.2024.108966

Yogiara, Mordukhova EA, Kim D, Kim W-G, Hwang J-K, and Pan J-G (2020). The food-grade antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in *Escherichia coli*. Bioorganic & Medicinal Chemistry Letters, 30(24): 127651. DOI: https://doi.org/10.1016/j.bmcl.2020.127651

Zhang X, Li L, He Y, Lang Z, Zhao Y, Tao H, et al. (2023). The CsHSFA-CsJAZ6 module-mediated high temperature regulates flavonoid metabolism in *Camellia sinensis*. Plant, Cell & Environment, 46(8): 2401–2418. DOI: https://doi.org/10.1111/pce.14610

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EVALUATION OF SURFACE LIPIDS OF SHEEP WOOL FOLLOWING DIETARY INCLUSION OF EMULSIFIED FATTY ACID COMPLEX

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ABSTRACT: Fatty acids, particularly ω-3, ω-6, and ω-9 play vital roles in sheep nutrition, but their influence on the protective properties of wool grease remained unclear. This study assessed the effects of dietary supplementation with an emulsified fatty acid complex on both the qualitative and quantitative characteristics of wool surface lipids in adult Prekos ewes and their lambs. The experimental group received a water-soluble emulsion containing linoleic, oleic, palmitic, arachidonic, stearic, and α-linolenic acids incorporated into the basal diet. Wax content was determined via Soxhlet extraction, and sweat salts were measured by aqueous extraction. Lipid classes were separated by thin-layer chromatography, and fatty acid profiles were quantified using gas-liquid chromatography. Results indicate a significant increase in wax secretion in ewes ($P < 0.01$) and lambs ($P < 0.05$), along with a decrease in sweat pH among lambs ($P < 0.05$). In ewe wax, levels of lanosterol ($P < 0.01$) and esterified cholesterol ($P < 0.05$) were elevated; lamb wax exhibited increases in lanosterol ($P < 0.05$) and dehydrocholesterol ($P < 0.05$). Both ewes and lambs showed a reduction in polar lipid content ($P < 0.05$), suggesting diminished accumulation of oxidative products. Analysis of fatty acid composition in the ewe group revealed significant increases in cerotic (C26:0; $P < 0.001$), lauric (C12:0; $P < 0.01$), and oleic (C18:1; $P < 0.01$) acids. Therefore, dietary inclusion of an emulsified fatty acid complex enhances the protective properties of wool grease by modulating wax and fatty acid composition, with potential benefits for fiber integrity and resilience.

Keywords: Emulsified fatty acids; Fleece lipids; Hexacosanoic acid; Sweat secretion; Wool wax.

INTRODUCTION

Sheep nutrition is one of the most important factors affecting their productivity (Burezq and Khalil, 2022). Diet is the most accessible means by which meat (Costa et al., 2023), milk (Vargas-Bello-Pérez et al., 2021), and wool yield (Chishti et al., 2021) can be systematically enhanced, as well as the physicochemical and consequently technological properties of wool (Kitaeva et al., 2023). As Li et al. (2024) report, lipids play a crucial role in sheep nutrition; a deficiency leads to growth retardation, impaired reproductive performance, reduced productivity, and deterioration of product quality. Alba et al. (2021) emphasize that fat digestibility in sheep is high and depends on fat's physicochemical properties, fatty acid composition, and dietary balance. Furthermore, Gelaye et al. (2021) report a significant influence of dietary factors on both the quantitative and qualitative parameters of sheep fleece grease.

Wool grease is the product of the secretory activity in the sebaceous and sweat glands (Aissani et al., 2022). Wool grease is the product of secretory activity in the sebaceous and sweat glands (Aissani et al., 2022). Although the precise function of sebaceous secretion remains under investigation, it is generally believed to prevent skin dryness, impart softness and elasticity to the epidermis, and provide water repellency. The presence of active hydrolytic enzymes particularly arylsulfatase in sebaceous secretion suggests additional roles in detoxifying endogenous and exogenous compounds, disinfecting the hair-follicle cavity, and potentially participating in the desquamation of cells within the follicular sheath (Raghav et al., 2022).

Wool grease or its purified form, lanolin is widely used in pharmacology and cosmetology (Abou Taleb and El-Sayed, 2021). Owing to its content of unsaturated fatty acids such as oleic, linoleic, and α-linolenic acids (Cholewinska and Michalak, 2018), lanolin exerts a beneficial effect on the skin by forming a protective film on epidermal surface, maintaining moisture levels by reducing transepidermal water loss by 20–30% (Souto et al., 2021), thereby preventing cracking (Kang et al., 2022).

Albanell et al. (2018) and Duzelbayeva et al. (2023) describe sheep sebaceous secretions as a complex mixture of esters of primary and secondary alcohols alongside free long- and medium-chain fatty acids. The principal constituents are esters of cholesterol, lanosterol, and three additional C_{30} alcohols analogous to lanosterol; minor components include cerebrosterol and 25-oxycholesterol, the latter arising via autoxidation (Ruttler et al., 2022). Sweat predominantly contains alkali metal salts—chiefly potassium, with lesser sodium—and, under high humidity, the presence of potassium

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soaps and organic acids renders wool grease a natural detergent (Molik et al., 2023). Wool wax is the sole grease component that positively influences wool's physicochemical properties (Dominguez et al., 2003). By coating fibers with a thin layer, wax promotes inter-fiber adhesion forming staples and braids that contribute to a compact fleece. This protective coating shields wool from mechanical and botanical contaminants, as well as environmental stressors (e.g., solar radiation, precipitation) during growth, storage, and initial processing. Lanolin's protective efficacy stems primarily from its specific lipid composition and the optimal balance among lipid classes (Jenkins and Belsito, 2023).

Wool quality depends heavily on both the quantity and quality of wax, which vary with breed, individual characteristics, husbandry practices, seasonal and climatic conditions, and diet (El-Sayed et al., 2018). Given these influences, the present study aimed to investigate the effects of dietary supplementation with an emulsified fatty acid complex on the qualitative and quantitative characteristics of sheep wool grease.

MATERIALS AND METHODS

Experimental animals and design

The study was conducted during the winter stall-housing period on adult Prekos ewes maintained at the Educational and Scientific Production Center "Komarnivske," Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine. Using paired-analogue matching for breed, age, and live weight, two groups of ten ewes each were formed: a control group and an experimental group. At the start of the trial, all ewes were in the late gestation period; from mid-trial onward, they entered early lactation.

Following a 10-day adaptation period during which all animals received a basal diet balanced according to established feeding standards, a 95-day experimental period commenced. Control ewes continued on the basal diet, whereas experimental ewes received, in addition, 3 % (w/w) of a water-soluble fatty acid complex ("Essential Lipid Complex," LLC EcoProFeed, Ukraine; 880 kcal per 100 g). This emulsion comprised linoleic acid (C18:2 ω-6; 54.5 %), oleic acid (C18:1 ω-9; 24 %), palmitic acid (C16:0; 10 %), arachidonic acid (C20:4 ω-6; 6 %), stearic acid (C18:0; 4 %), and α-linolenic acid (C18:3 ω-3; 1.5 %). It was produced by enzymatic treatment of oils using a lipolytic enzyme complex from *Bacillus pseudomonas* and *Bacillus subtilis* in the presence of glycolipids and polysaccharides. This supplementation increased dietary crude-fat intake by 12 g (from 46.41 g to 58.41 g) in pregnant ewes and by 18 g (from 62.81 g to 80.81 g) in lactating ewes.

All animals were group-fed, with daily weighing of the provided feed, and had *ad libitum* access to water. Feed and water intakes were recorded throughout the experimental period.

Sampling

At the end of the experiment, wool samples including surface grease were collected from the experimental ewes and their lambs in the region posterior to the scapula for subsequent biochemical analyses.

Determination of the total amount of wax, sweat, and pH of sweat

Wool fat (wax) was extracted from fleece samples with tetrachloromethane (Sigma-Aldrich, USA) using a Soxhlet apparatus for 5 h (Daly and Carter, 1954). After cooling and phase separation, the extract was evaporated to dryness. The residue was dissolved in 10 mL of chloroform–methanol (2:1; Chemico Group, UK/SRP Ltd, Ukraine), and 3 mL of 7.5 % potassium chloride solution (Luxion Co., China) was added. Samples were shaken and allowed to separate for 24 h; the upper aqueous–methanol layer was removed by suction, and the lower chloroform layer containing lipids was retained for analysis. The defatted wool was washed, dried to constant mass, cleared of debris, and weighed. Wax content was determined gravimetrically and expressed as a percentage of clean, dry fiber. Sweat salts were extracted aqueously, and the pH of the extract was measured using a universal ion-selective meter.

Lipid-class separation by thin-layer chromatography (TLC)

The lipid extract was re-dissolved in chloroform–methanol (2:1) and applied to 100 × 100 mm silica-gel TLC plates (Sorbfil; particle size 90–120 µm). Lipid classes were separated using a mobile phase of petroleum ether–diethyl ether (4:1, v/v; Carlo Erba, Italy). After development and drying, plates were sprayed with 50 % sulfuric acid (Alhim, Ukraine) and charred at 105 °C. Individual lipid classes were identified by comparison with reference standards (cholesterol, stearic acid, lanosterol; Sigma-Aldrich, USA) and published R_f values.

Quantitative determination of individual lipid classes

Lipid bands were scraped from the silica, transferred to centrifuge tubes, and treated with 5 mL concentrated sulfuric acid. Tubes were mixed thoroughly, heated in a boiling water bath for 20 min, then cooled and centrifuged at 3,000 rpm for 20 min. The absorbance of the supernatant was measured at 400 nm in a 10 mm path-length cuvette. Concentrations of each lipid class were calculated against calibration curves and expressed as percentages of total wax.

Determination of the fatty acid composition of wax

Surface lipids were converted to fatty acid methyl esters via direct transesterification (Stoffel et al., 1959). Separation was performed on a "Chrom-4" GLC (Czech Republic) equipped with a 2,400 mm × 3 mm metal column packed with Chromosorb (60–80 mesh) coated with 15 % polyethylene glycol succinate. Operational conditions were: column thermostat, 190 °C; injector, 240 °C; airflow, 400 mL/min; carrier gas (N_2), 25 mL/min. Fatty acids were identified by comparison to Supelco standard mixtures, and retention times (t_R) were recorded. Individual fatty acid percentages were calculated using standard quantitative formulas.

Statistical analysis

All data were processed using Statistica 12.0 (StatSoft Inc., USA). Results are presented as mean ± SD. Group comparisons were made by one-way ANOVA, followed by post-hoc tests for pairwise significance. Differences were considered significant at $P < 0.05$.

RESULTS

The conducted studies (Table 1) first revealed that feeding ewes, a water-soluble complex of fatty acids, leads to a significant increase in wax secretion in the experimental animals from 12.21 to 14.89% ($P < 0.01$). Consequently, the wax-to-sweat ratio in grease significantly improved from 1:1.15 in controls to 1:0.96 in treated ewes. No significant differences were observed in total sweat content or sweat pH between control and experimental ewes under the conditions of this study.

Table 1 - Indicators of grease content of ewe's fleece of ewes, (x ± SD, n = 4)

Indicator	Control	Experiment
Amount of wax, %	12.21 ± 1.08	14.89 ± 0.37**
Amount of sweat, %	14.04 ± 0.91	14.26 ± 1.22
pH of sweat	8.61 ± 0.21	8.43 ± 0.23
Wax: sweat ratio	1: 1.15	1: 0.96

SD= Standard deviation; **=P < 0.01.

The lipid composition of the wax in ewes of the experimental group was altered (Figure 1). Specifically, the nutritional factors applied led to a significant increase in esterified cholesterol, from 37.08% to 39.91% ($P < 0.05$), and lanosterol, from 7.36% to 10.65% ($P < 0.01$), as well as a decrease in polar lipids, from 21.97% to 18.39% ($P < 0.05$). In contrast, the fractions of esterified cholesterol, non-esterified fatty acids, dehydrocholesterol, and squalene did not exhibit significant changes. Similar changes observed in the grease of ewes were also found in the grease of their lambs (Table 2). Specifically, in the experimental group, the wax content significantly increased from 13.50% to 14.99% ($P < 0.05$). This increase, in turn, led to alterations in the wax-to-sweat ratio. In the control group, the ratio was 1:1, while in the experimental group, it was 1:0.81. Unlike ewes, in the lambs from the control group, the pH of sweat significantly decreased from 7.78 to 7.04 ($P < 0.05$), while the amount of sweat also showed a tendency to decrease. In the lipid composition of the wax in lambs (Figure 2), as observed in ewes, the experimental group exhibited an increase in lanosterol, from 7.60% to 9.47% ($P < 0.05$), and a decrease in polar lipids, from 9.35% to 7.64% ($P < 0.05$). However, unlike ewes, the fraction of dehydrocholesterol in the wax of lambs significantly increased from 11.43% to 13.48% ($P < 0.05$). No significant changes were observed in esterified and non-esterified cholesterol, non-esterified fatty acids, or squalene in the lambs of the experimental group.

The fatty acid composition of ewe wax consists of 23 acids, including both saturated and unsaturated, as well as iso-acids (Figure 3). It is noteworthy that five of these acids have not yet been identified. The fatty acid composition of the wax in ewes from the experimental group differs from that of the control group in terms of the content of individual acids. Specifically, the experimental group exhibits a significantly higher content of lauric acid (dodecanoic C12:0) ($P < 0.01$), oleic acid (9Z)-octadec-9-enoic C18:1ω9) ($P < 0.01$), cerotic acid (hexacosanoic C26:0) ($P < 0.001$), and one unidentified acid ($P < 0.05$), as well as a lower content of linoleic acid (9E,12E)-octadeca-9,12-dienoic C18:2ω6) ($P < 0.05$). The total amount of unsaturated fatty acids in the wax of the control group is 27.92%, while in the experimental group, it is 13.21%.

These findings demonstrate that inclusion of a water-soluble fatty-acid complex in the diet of ewes modifies both the lipid-class composition and fatty-acid profile of wool grease, with implications for its protective functions and, by extension, the physicochemical properties of wool fibers.

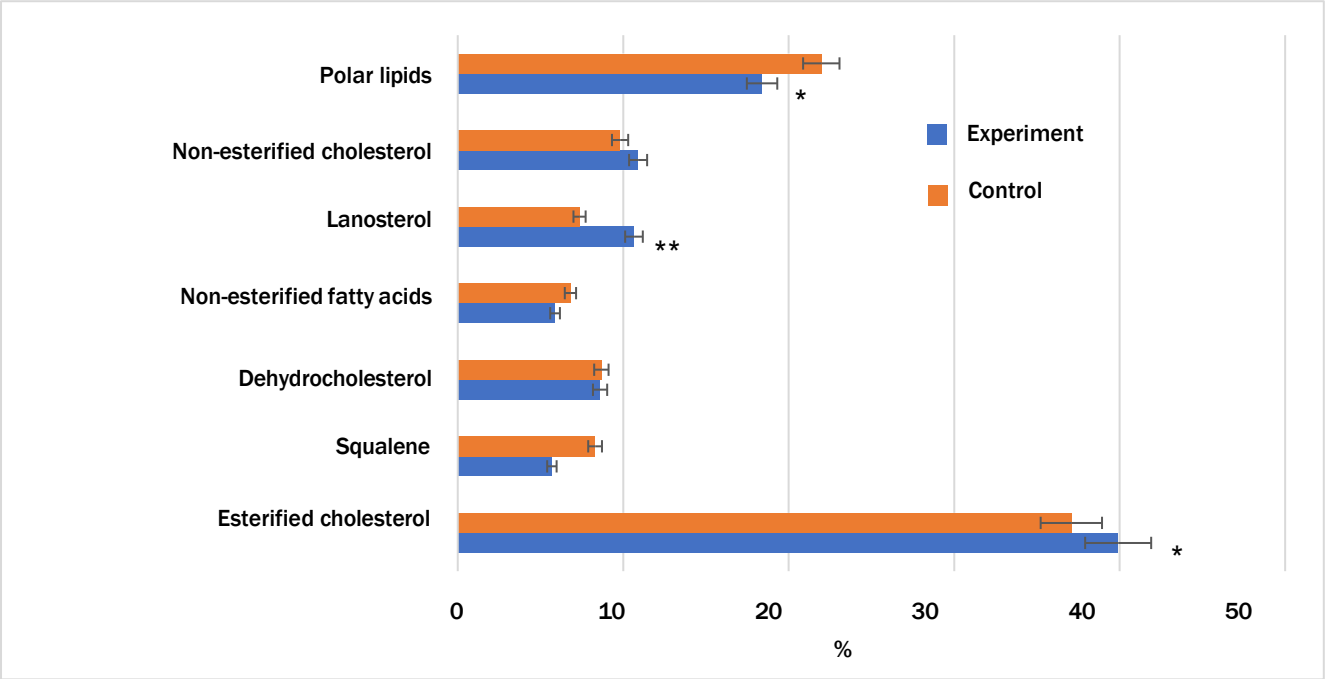

Figure 1 - Lipid composition of the grease of the ewe's fleece (M ± SD, n = 4). SD= Standard deviation; *=P < 0.05, **=P < 0.01

Table 2 - Indicators of fleece grease of lambs, (x ± SD, n = 4)

Indicator	Control	Experiment
Amount of wax, %	13.50 ± 0.77	14.99 ± 0.91*
Amount of sweat, %	13.48 ± 0.96	12.10 ± 1.49
pH of sweat	7.78 ± 0.23	7.04 ± 0.50*
Wax : sweat ratio	1: 1	1: 0.81

SD= Standard deviation; *=P < 0.05

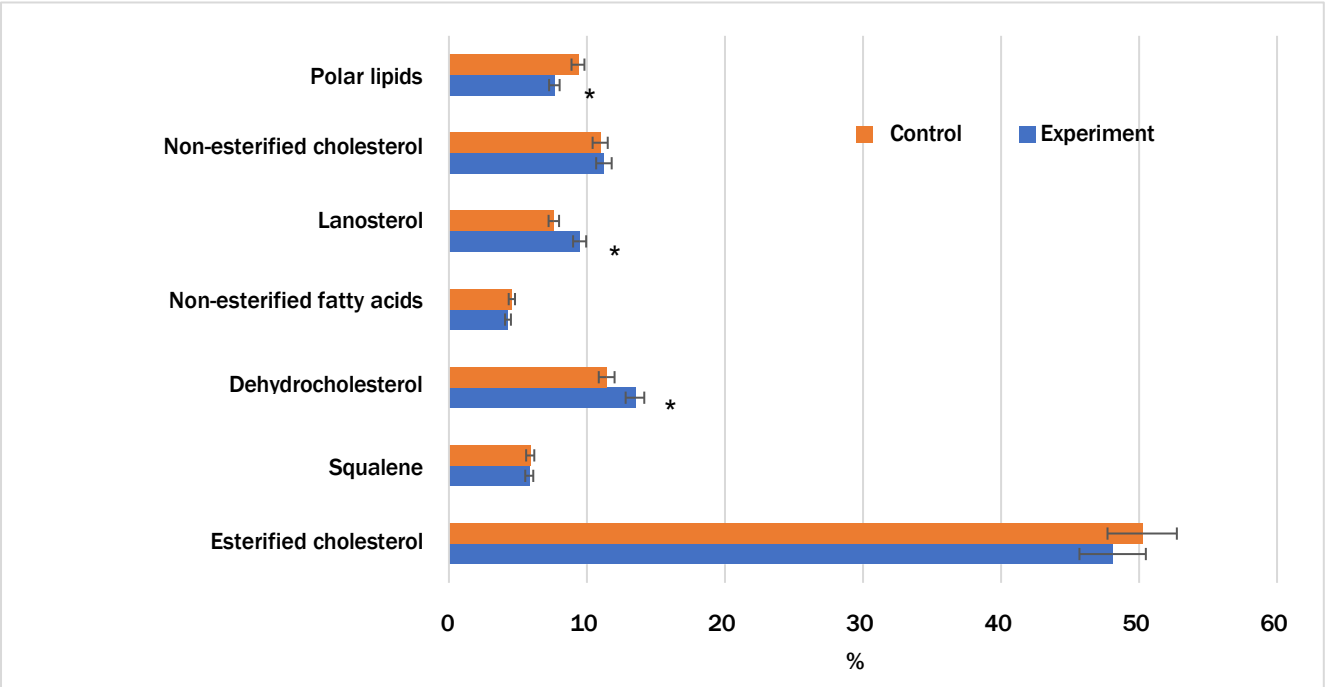

Figure 2 - Lipid composition of the wool grease wax of lambs, (M ± SD, n = 4). SD= Standard deviation; *=P < 0.05.

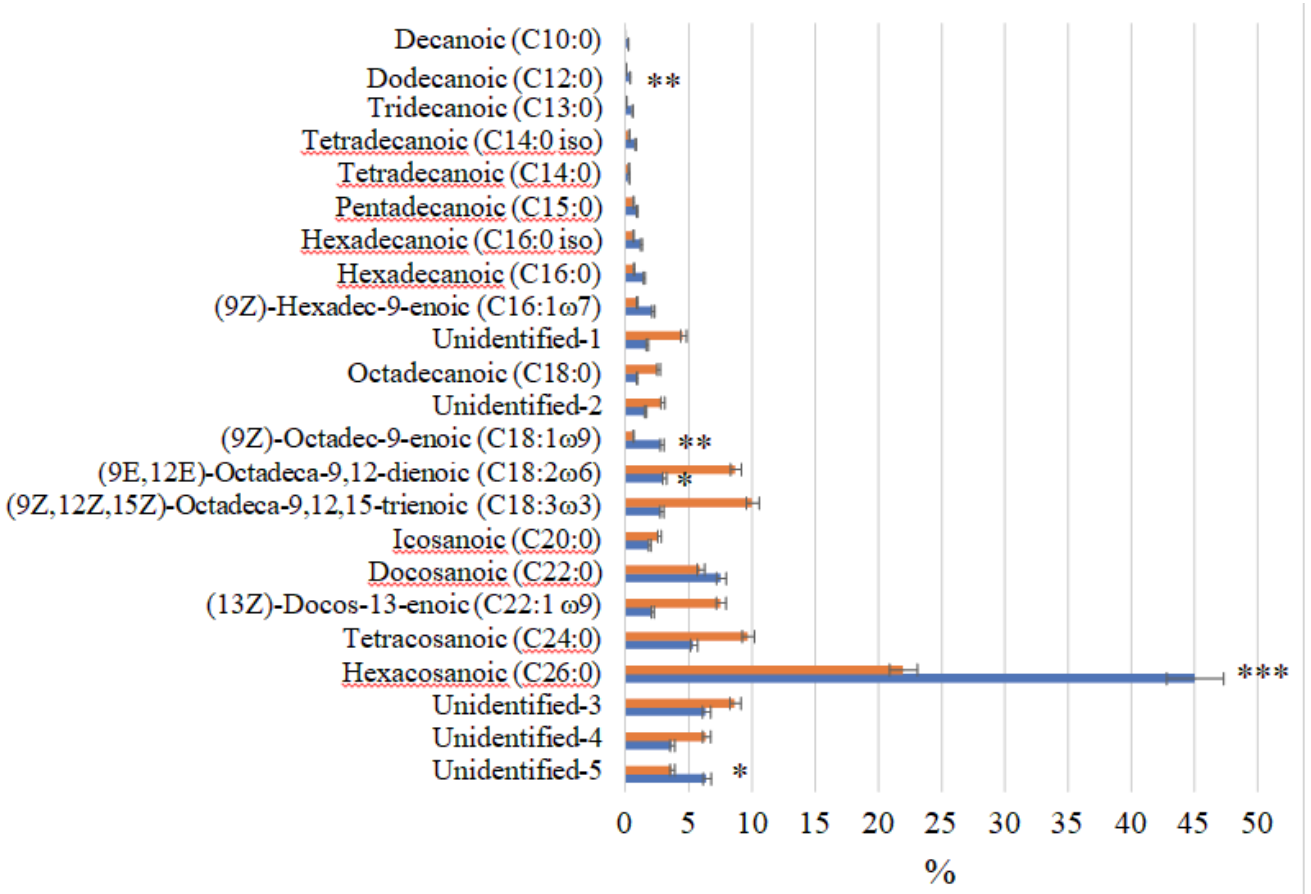

Figure 3 - Fatty acid composition of the grease wax of the ewe's fleece, (M ± SD, n = 3). SD= Standard deviation; *=P < 0.05, **=P < 0.01, ***=P < 0.001.

DISCUSSION

Enhancing the productivity and quality of sheep products depends largely on balanced nutrition, particularly the provision of optimal energy levels, which profoundly influence metabolic processes (Hervás et al., 2021; Oualyou et al., 2024). In recent years, researchers in small-ruminant nutrition have increasingly focused on improving feeding efficiency (Dijkstra et al., 2025). Alba et al. (2021) highlight that, given the diverse demands of sheep for meat, milk, and wool production, high-energy feeds are critical. Tajonar et al. (2023) emphasize the essential role of unsaturated fatty acids, noting that their deficiency disrupts phospholipid metabolism, impairs membrane binding capacity and fluidity, and compromises lipoprotein formation and lipid transport.

Despite extensive research on energy-dense supplements, data remain scarce regarding the effects of ω-3, ω-6, and ω-9 fatty acids on the protective properties of sheep fleece grease. To address this gap, we supplemented the basal diet of ewes with a water-soluble fatty acid complex containing both saturated (palmitic C16:0; stearic C18:0) and unsaturated acids linoleic (C18:2 ω-6), oleic (C18:1 ω-9), arachidonic (C20:4 ω-6), and α-linolenic (C18:3 ω-3). Rumen fermentation hydrogenates these unsaturated acids to saturated forms, reducing methane production and mitigating the risk of acute tympany (Yang et al., 2022).

Wool grease secreted by sebaceous glands as lanolin plays a vital role in maintaining fiber integrity by preventing moisture ingress (Molik and Potocka, 2019; Lis, 2024; Meng et al., 2025). Present findings demonstrate that dietary inclusion of the lipid complex significantly increased wax content in both ewes ($P < 0.01$) and their lambs ($P < 0.05$), improving the wax-to-sweat ratio. A wax-to-sweat ratio of less than 1: 1 corresponds to superior protective properties. Additionally, lambs in the experimental group exhibited a trend toward reduced sweat volume accompanied by a significant decrease in sweat pH ($P < 0.05$). As we reported previously, elevated sweat concentrations, particularly under alkaline conditions accelerate wax degradation (Tkachuk et al., 2024).

The findings of Johnson et al. (2023) indicate that lanolin's qualitative characteristics depend primarily on its specific lipid composition. Present results corroborate this assertion: experimental ewes exhibited significant increases in lanosterol ($P < 0.01$) and esterified cholesterol ($P < 0.05$), while their lambs showed elevated levels of lanosterol ($P < 0.05$) and dehydrocholesterol ($P < 0.05$), accompanied by a decrease in polar lipids ($P < 0.05$). The reduction in polar lipids may reflect diminished accumulation of oxidative products.

Regarding the fatty acid profile of the surface lipids of wool, the amount of oleic acid (9Z)-octadec-9-enoic C18:1ω9) in the ewes of the experimental group significantly increased ($P < 0.01$). This is consistent with the fact that the water-soluble lipid complex contains 24% of this acid. However, the observed decrease in linoleic acid (9E,12E)-octadeca-9,12-dienoic C18:2ω6) ($P < 0.05$), despite its 54.5% presence in the complex, remains somewhat unclear. In the wax of the animals in the experimental group, an increase in lauric acid (dodecanoic C12:0) ($P < 0.01$) and, particularly, cerotic acid (hexacosanoic C26:0) ($P < 0.001$) was observed, with cerotic acid being the most abundant of all fatty acids. Notably cerotic acid plays a crucial role due to its antimicrobial properties (Singh and Singh, 2003; Rehan et al., 2020), functioning as a natural disinfectant within the fleece. Although five of the twenty-three identified fatty acids remain uncharacterized, one of these unknowns increased significantly under dietary treatment ($P < 0.05$). Changes in the fatty-acid composition reduced the proportion of unsaturated acids to 13.21 % in the experimental group versus 27.92 % in controls. This shift likely enhances oxidative stability, as higher unsaturated-acid content correlates with increased susceptibility to peroxide oxidation (Cao et al., 2024).

Therefore, dietary inclusion of a fatty acid emulsion enhances the protective properties of sheep wool grease by increasing total wax content and optimizing its lipid and fatty acid composition.

CONCLUSION

Dietary supplementation of ewes with a water-soluble fatty acid complex increased the secretion of wool surface lipids, resulting in an improved wax-to-sweat ratio; in lambs, sweat pH also decreased. The lipid composition of wool wax shifted, with a reduction in polar lipids in both supplemented ewes and their offspring—likely reflecting lower accumulation of oxidation products. Enhanced protective properties of wool grease in the experimental group were evidenced by elevated levels of lanosterol, esterified cholesterol, and dehydrocholesterol. The fatty acid profile of experimental ewes' wax was characterized by higher concentrations of oleic acid (C18:1 ω-9), lauric acid (C12:0), and notably cerotic acid (C26:0); the latter, owing to its antimicrobial activity, may serve as a natural disinfectant within the fleece. Overall, these findings demonstrate that dietary inclusion of a fatty acid emulsion enhances the protective functions of wool grease, with positive implications for fleece quality. However, the current study does not fully address the impact of these nutritional interventions on the physicochemical and technological properties of wool fibers, highlighting avenues for future research.

DECLARATIONS

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Data availability

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

Ethical approval

All manipulations with animals were carried out by the international principles of the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» and the resolutions of the National Congress of Ukraine on Bioethics (2010), which comply with the current legislation of Ukraine, in particular the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty» as amended on 15.11.2024. According to protocol No 93 (03.06.2021) from the bioethical commission of the Institute of Animal Biology NAAS have obtained ethical approval for the study.

Authors' contribution

T.Vitalii: Writing – original draft, Validation, Supervision, Methodology, Conceptualization. K.Bogdan: Writing – original draft, Resources, Funding acquisition, Conceptualization, O.Nataliia: Writing – original draft, Project administration, Methodology. M.Nataliia: Writing – original draft, Methodology.

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Competing interests

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

REFERENCES

Abou taleb M, and El-Sayed H (2021). Preparation and characterization of lanolin-based condensate and its utilization as a nonionic softener for wool fabric surface. Journal of Applied Research and Technology, 19(5): 508–520.

https://doi.org/10.22201/icat.24486736e.2021.19.5.1443

Aissani N, Ghidaoui M, and Sebai H (2022). Wool wax extraction from washing effluent and effect on olea europea germination and growth. Dose-Respose, 20(3): 15593258221121202. https://doi.org/10.1177/15593258221121202

Alba HDR, Freitas Junior de JE, Leite LC, Azevedo JAG, Santos SA, Pina DS, et al. (2021). Protected or unprotected fat addition for feedlot lambs: feeding behavior, carcass traits, and meat quality. Animals, 11(2): 328. https://doi.org/10.3390/ani11020328

Albanell CB, Carrer V, Marti M, Iglesias J, Iglesias J, and Coderch L (2018). Solvent- extracted wool wax: thermotropic properties and skin efficacy. Skin Pharmacology and Physiology, 31(4): 198–205. https://doi.org/10.1159/000488247

Burezq H, and Khalil F (2022). Multifarious feed additives on lamb performance on Kuwait farms. Veterinary World, 15(12): 2785-2794. https://doi.org/10.14202/vetworld.2022.2785-2794

Cao H, Xiong SF, Dong LL, and Dai ZT (2024). Study on the mechanism of lipid peroxidation induced by carbonate radicals. Molecules, 29(5): 1125. https://doi.org/10.3390/molecules29051125

Chishti AA, Khan HM, Pattoo RA, and Sofi AH (2021). Effect of housing and nutritional interventions during winter on wool attributes of Corriedale sheep. Tropical Animal Health and Production, 53(3): 348. https://doi.org/10.1007/s11250-021-02792-2

Cholewinska P, and Michalak M (2018). Fatty acid profile of wool grease from Olkuska lambs. Wiadomosci Zootechniczne, 1: 29-33. https://wz.iz.edu.pl/files/WZ_2018_1_art04_en.pdf

Costa da TSA, Silva da JAR, Faturi C, Silva AGME, Rego do AC, Monteiro EMM, et al. (2023). Evaluation of the quality of meat and carcasses from sheep fed diets containing three types of oils. Frontiers in Veterinary Science, 10: 1103516. https://doi.org/10.3389/fvets.2023.1103516

Daly RA, and Carter HB (1954). A method of fractionating raw fleece samples and some errors encountered in its use in experimental studies of fleece growth. Australian Journal of Agricultural Research, 5(2): 327-344. https://doi.org/10.1071/AR9540327

Dijkstra J, Bannink A, Congio GFS, Ellis JL, Eugene M, Garcia F, et al. (2025). Feed additives for methane mitigation: modeling the impact of feed additives on enteric methane emission of ruminants — approaches and recommendations. Journal of Dairy Science, 108(1): 356-374. https://doi.org/10.3168/jds.2024-25049

Dominguez C, Jover E, Bayona J.M, and Erra P (2003). Effect of the carbon dioxide modifier on the lipid composition of wool wax extracted from raw wool. Analytica Chimica Acta, 477(2): 233-242. https://doi.org/10.1016/S0003-2670(02)01418-6

Duzelbayeva S, Kassenova B, Akhatova Z, and Konuspayev S (2023). Analysis of fatty acids included in wool fat and their discussion. News of National Academy of Science Republic of Kazakhstan - Chemical sciences series, 3: 61–70. https://doi.org/10.32014/2023.2518-1491.177

El-Sayed H, Mowafi S, El-Kheir A, and El-Khatib EM (2018). A comprehensive critique on wool grease extraction, properties and applications. Egyptian Journal of Chemistry, 61(6): 1151- 1159. https://doi.org/10.21608/ejchem.2018.4214.1372

Gelaye G, Sandip B, and Mestawet T (2021). A review on some factors affecting wool quality parameters of sheep. African Journal of Food, Agriculture, Nutrition and Development, 21(10): 18980-18999. https://doi.org/10.18697/ajfand.105.19330

Hervás G, Toral PG, Fernandez-Diez C, Badia AD, and Frutos P (2021). Effect of dietary supplementation with lipids of different unsaturation degree on feed efficiency and milk fatty acid profile in dairy sheep. Animals (Basel), 11(8): 2476. https://doi.org/10.3390/ani11082476

Jenkins BA, and Belsito DV (2023). Lanolin. Dermatitis, 34(1): 4-12. https://doi.org/10.1089/derm.2022.0002

Johnson H, Norman T, Adler BL, and Yu JD (2023). Lanolin: the 2023 American contact dermatitis society allergen of the year. Cutis, 112(2): 78-81. https://doi.org/10.12788/cutis.0825

Kang ST, Um JY, Chung BY, Lee SY, Park JS, Kim JC, et al. (2022). Moisturizer in patients with inflammatory skin diseases. Medicina (Kaunas), 58(7): 888. https://doi.org/10.3390/medicina58070888

Kitaeva A, Mamedova V, Bezaltychna O, Slyusarenko I, and Novichkova A (2023). Productivity of the Tsigai sheep breed under different feeding regimens. Online Journal of Animal and Feed Research, 13(6): 451-459. https://dx.doi.org/10.51227/ojafr.2023.62

Li Q, Xu G, Yang D, Tu Y, Zhang J, Ma T, and Diao Q (2024). Effects of feed ingredients with different protein-to-fat ratios on growth, slaughter performance and fat deposition of small-tail han lambs. Animals, 14(6): 859. https://doi.org/10.3390/ani14060859

Lis K (2024). Hypersensitivity to lanolin: an old–new problem. Life, 14(12): 1553. https://doi.org/10.3390/life14121553

Meng X, Nan Q, Zeng Q, Zhang J, Liu S, Tan L, et al. (2025). Antibacterial and mothproofing wool fabrics modified by M-Arg/PHMG and ZnO. Applied Surface Science, 680: 161341. https://doi.org/10.1016/j.apsusc.2024.161341

Molik E, and Potocka A (2019). Wybrane zagadnienia związane z możliwością wykorzystania wełny owczej [Selected issues associated with the potential uses of sheep wool]. Przeglad Hodowlany, 3: 31–33. https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-4edfa34a-4997-464e-88e5-c825918dc759

Molik E, Zapletal P, Pustkowiak H, and Kaminska D (2023). Effectof day length on fatty acid content of sheep wool fat. Roczniki Naukowe Zootechniki, 50(1): 105-113. https://doi.org/10.58146/qtcw-9a30

Oualyou O, Gnanda IB, Zampaligre N, Kere M, Traore K, Yoda GLM, et al. (2024). Estimating net energy requirements of male Djallonke sheep. Animal Production Science, 64: AN23294. https://doi.org/10.1071/AN23294

Raghav S, Uppal V, and Gupta A (2022). Comparative study on distribution of sebaceous and sweat glands in skin of different domestic animals. Indian Journal of Animal Research, 56(11): 1356- 1360. https://doi.org/10.18805/IJAR.B-4228

Rehan M, Shafiullah, Ansari FA, and Singh O (2020). Isolation, identification, antibacterial activity and docking of fatty acid and fatty alcohol from Rumex dentatus leaf extract. International Journal of Pharmaceutical Sciences Review and Research, 64(1): 7-11. https://doi.org/10.47583/ijpsrr.2020.v64i01.002

Ruttler F, Hammerschick T, Schlag S, and Vetter W (2022). Isolation of lanosterol and dihydrolanosterol from the unsaponifiable matter of lanolin by urea complexation and countercurrent chromatography in heart-cut recycling mode. Journal of Chromatography B Analytical Technology in the Biomedical and Life Science, 1210: 123470. https://doi.org/10.1016/j.jchromb.2022.123470

Singh B, and Singh S (2003). Antimicrobial activity of terpenoids from Trichodesma amplexicaule Roth. Phytotherapy Research, 17(7): 814-816. https://doi.org/10.1002/ptr.1202

Souto EB, Yoshida CMP, Leonardi GR, Cano A, Sanchez-Lopez E, Zielinska A, et al. (2021). Lipid-polymeric films: composition, production and applications in wound healing and skin repair. Pharmaceutics, 13: 1199. https://doi.org/10.3390/pharmaceutics13081199

Stoffel W, Chu F, and Ahrens EH (1959). Analysis of long chain fatty acids by acids by gas- iquid chromatography. Journal of Analytical Chemistry, 31(2): 307-308. https://doi.org/10.1021/ac60146a047

Tajonar K, Gonzalez-Ronquillo M, Relling A, Nordquist RE, Nawroth C, and Vargas-Bello- Perez E (2023). Toward assessing the role of dietary fatty acids in lamb's neurological and cognitive development. Frontiers in Veterinary Science, 10: 1081141. https://doi.org/10.3389/fvets.2023.1081141

Tkachuk VM, Stapay PV, Ohorodnyk NZ, and Motko NR (2024). Internal lipids and their fatty acids composition in a sheep wool fiber under biodestruction with fleece microorganisms. The Ukrainian Biochemical Journal, 96(3): 98–108. https://doi.org/10.15407/ubj96.03.097

Vargas-Bello-Perez E, Darabighane B, Miccoli FE, Gomez-Cortes P, Gonzalez-Ronquillo M, and Mele M (2021). Effect of dietary vegetable sources rich in unsaturated fatty acids on milk production, composition, and cheese fatty acid profile in sheep: a meta-analysis. Frontiers in Veterinary Science, 8: 641364. https://doi.org/10.3389/fvets.2021.641364

Yang Z, Liu S, Xie T, Wang Q, Wang Z, Yang H, et al. (2022). Effect of unsaturated fatty acid ratio in vitro on rumen fermentation, methane concentration, and microbial profile. Fermentation, 8(10): 540. https://doi.org/10.3390/fermentation8100540

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DEGRADATION CHARACTERISTICS OF CRUDE PROTEIN AND CRUDE FIBER OF LEGUME FORAGES IN THE RUMEN OF GOAT

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ABSTRACT: The nutritional value of a feedstuff depends not only on its chemical composition but also on the capacity of ruminal microbes to colonize and degrade it. This study compared the in sacco degradation kinetics of four legume forages *(Moringa oleifera, Leucaena leucocephala, Indigofera* and *Gliricidia sepium)* using three rumen fistulated goats in a 4×3 completely randomized design (CRD). Seventy-two nylon bags (10 × 5 cm, 40–50 µm pore size) containing 5 g of each forage (ground to 2 mm) were incubated for 4, 8, 12, 24, 48, or 72 hours (12 bags per time point). The study determined the soluble fraction (a), potentially degradable fraction (b), total degradable fraction (a+b), degradation rate constant of fraction b (c), lag time (Lt), degradation effectiveness (DE), and rumen undegradable protein (RUP). The results of CP degradation revealed no significant differences among forages in fractions a, b, or a + b, but fraction c, Lt, DE, and RUP differed significantly. The degradation rate (c, h^{-1}) of crude protein ranked as Moringa (0.17) > Leucaena (0.09) = *Indigofera* (0.09) > *Gliricidia* (0.03), while Lt was shortest for Moringa (3.60 h) and longest for *Gliricidia* (11.96 h). Moringa and *Indigofera* exhibited the highest DE and lowest RUP of all treatments. Similar trends were observed for crude fiber: Moringa showed the greatest DE (26.72% Lt) compared to Leucaena (18.76 h Lt). In conclusion, all four legumes were efficiently degraded in the goat rumen, through the rate and extent of degradation varied markedly among species, reflecting differences in their biochemical composition and structural carbohydrates.

Keywords: Crude Fiber, Crude Protein, Degradation Characteristics, Goat, Legumes

INTRODUCTION

Feed is one of the main factors influencing livestock production and its economic efficiency. Forage is often provided as a combination of grasses and legumes to complement the nutritional requirements of ruminants (Phelan et al., 2015; Richards et al., 2021). Primary forages comprise grasses and legumes, which supply essential nutrients such as crude protein and crude fiber (Katoch et al. 2022).

A quality feed ingredient, depends not only on its nutritional composition but also on the capacity of rumen microbes to adapt and degrade it; degradation efficiency—particularly of lignin—strongly influences overall digestibility (Suhartanto et al, 2000; Humer and Zebeli, 2017). Feed degradation refers to the fraction of feed that is solubilized and fermented by rumen microbes, thereby supplying nutrients to the host animal (Orskov and McDonald, 2009).

Evaluation of feed ingredient degradation in ruminants can be done by the in sacco method, where ground feed is enclosed in nylon bags and incubated in the rumen for defined intervals to assess degradation kinetics (Reis et al., 2017). Feed degradation value can be predicted from the in sacco feed degradation characteristic value (Akhirany et al. 2013; Babangida et al., 2021). The in sacco method allows precise determination of the time-dependent degradation rate of the feedstuffs (Wati et al., 2012) and enables direct measurement of ruminal degradability under physiological conditions (Harfiah, 2009). Thus, the quality of a feed ingredient can be determined from the nutritional content it has, so it is very important to know the quality of protein and crude fiber content in *Moringa oleifera, Leucaena leucocephala, Indigofera* and *Gliricidia sepium*. This study therefore aimed to characterize the in sacco degradation kinetics—rate, extent, and lag time—of these four legume forages.

The selected forages including *Moringa oleifera, Leucaena leucocephala, Indigofera* and *Gliricidia sepium* offer advantages over grasses, notably higher crude protein content and improved nutritional value, which can enhance growth, production, and reproductive performance in ruminants.

MATERIALS AND METHODS

Ethical approval

Experimental procedures on live animals were conducted in compliance with animal welfare principles and were approved by the Health Research Ethics Committee of Hasanuddin University Makassar: Approval No. 150/UN4.6.4.5.31/PP36/2024 prior to the commencement of the study.

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Experimental animals and diets

The study was conducted from April to May 2024 at Hasanuddin University in Makassar, Indonesia. The method used was a 4×3 Completely Randomized Design (CRD) involving three rumen-fistulated goats (n=3) aged 1.5-2.0 years, with an average body weight of 21-25 kg. The animals received the same diet, *Moringa oleifera, Leucaena leucocephala, Indigofera* and *Gliricidia sepium*, and bran, each offered to satisfy 3% of the initial body weight (BW) on a dry-matter basis, provided twice daily (morning and evening), with water available ad libitum throughout the study.

Experimental design

This study evaluated four leguminous forages in the rumen of goats over incubation periods of 4, 8, 12, 24, 48, and 72 hours, to determine their degradation kinetics and nutritional quality. The experiment employed a 4 × 3 completely randomized design (CRD) with four treatments and three replicates. Each goat received four nylon bags—one per forage species (*Moringa oleifera, Leucaena leucocephala, Indigofera*, and *Gliricidia sepium*) resulting in 12 nylon bags per incubation time. A total of 72 nylon bags were used across six incubation periods (4, 8, 12, 24, 48, and 72 hours). Each bag measured 10×5 cm and had a porosity of 40-50 µm. For each incubation time, four bags were placed in the rumen of each fistulated goat. The feed ingredients tested were as follows: L1, Moringa (*Moringa oleifera*); L2, Leucaena (*Leucaena leucocephala*); L3, *Indigofera*; and L4, Gliricidia (*Gliricidia sepium*), all harvested 70 days after the previous cutting. The legume foliage was oven-dried at 60 °C for five days, ground to a particle size of approximately 2 mm, and then subjected to proximate analysis (AOAC, 1995). Five grams of each ground feed sample were placed into individual nylon bags and incubated in the rumen of goats for the designated times. After incubation, the bags were removed, drained, and dried in an oven at 60 °C for 48 hours. The overall trial duration was approximately 14 days, during which each animal received a diet consisting of 70% elephant grass, 20% legume, and 10% rice bran.

The crude protein content of the feed samples, both before and after incubation, was determined by proximate analysis using the Kjeldahl method (AOAC, 2001) at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The loss of crude protein recovered from each nylon bag after incubation reflects ruminal degradation and is used to calculate feed protein degradation by forage type and incubation time. Degraded crude protein (CP) in each sample is calculated by comparing its initial and final CP contents. The percentage losses of crude protein and crude fiber (CF) are calculated as follows: % CP Loss = (% CP Initial x Initial Sample Weight) - (% CP Final × Final Sample Weight), while the formula for calculating the percentage of crude fiber (CF) is % CF Loss = (% CF Initial × Initial Sample Weight) - (% CF Final × Final Sample Weight).

Furthermore, the crude protein and crude fiber lost during the incubation period were used to measure the value of Y by calculating the values of a, b, c and a+b which were entered into the exponential equation according to Ørskov and McDonald (1979) as follows:

$$Y = a + b\,(1\text{-}e^{-ct})$$

The characteristic values of CP, CF, and ED degradation in feed can be calculated by the following formula:

$$DE = a+[(bxc)]/[(c+k)]$$

which Y = Feed degradation by rumen microbes at time t (incubation time); DE = degradation effectiveness, a = soluble feed fraction; b = feed fraction with potential for degradation; c = Degradation rate of fraction (b); a + b = Total degradation potential, including material that escapes the bag without being degraded; K = constant 0.05/hour, (Srakaew et al., 2021). Proteins that is degraded in the rumen perfectly is called rumen degradable protein (RDP), and proteins that cannot be degraded are called Rumen Undegradable Protein (RUP) of each sample calculated by the following equation: RUP = 100% - RDP (Terefe et al., 2022). Degradation curves and patterns of feed degradation in the goat rumen, determined by the in sacco method, were analyzed using the Neway program (Ismartoyo, 2011).

Statistical analysis

The data were analyzed as a 4 × 3 completely randomized design (CRD) with four treatments and three replicates. Each incubation period (4, 8, 12, 24, 48, and 72 h) employed 12 nylon bags, for a total of 72 bags. Degradation characteristics of the legumes were evaluated by generating degradation curves using SPSS Version 16.0 and Microsoft Excel 2010. Significant differences among treatments were determined by Duncan's multiple range test (Gaspersz, 1991).

RESULTS AND DISCUSSION

Feed nutrient content

The feed provided to the animals was analyzed for nutrient content at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. Table 1 presents the nutrient composition of the diets used in this study. High-quality feed contains a complete spectrum of nutrients to satisfy goats' requirements for maintenance, growth, and production (Roy and Rana, 2024). Feed quality is largely determined by its protein and energy contents (Ullah-Khan et al., 2019; Rouillé et al., 2023), and a balanced nutrient profile promotes optimal livestock performance.

Table 1 - Feed nutrient content

Nutrient content	*Moringa oleifera*	*Leucaena leucocephala*	*Indigofera*	*Gliricidia sepium*
Dry matter (%)	16.14	21.4	24.67	23.54
Organic matter (%)	83.28	85.23	86.65	87.29
Crude protein (%)	32.70	25.63	29.50	22.79
Crude fiber (%)	13.95	20.52	20.06	23.43
Crude fat (%)	3.03	2.25	2.22	2.12
Ash (%)	16.72	14.77	13.35	12.71
NFE (%)	33.60	36.83	34.86	38.94
NDF (%)	23.38	39.00	30.99	43.48
ADF (%)	15.13	33.75	23.74	35.42
Cellulose (%)	10.93	14.61	16.52	15.72
Hemicellulose (%)	8.25	5.25	7.25	8.06
Lignin (%)	3.88	18.98	7.11	19.52

Feed Chemistry Laboratory Analysis Results, Faculty of Animal Husbandry, Hasanuddin University 2024; NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NFE: Nitrogen free Extract.

Table 2 - The average percentage (±SEM) of Crude Protein degradation at each incubation period

Incubation period (Hours)	*Moringa oleifera (%)*	*Leucaena leococephala (%)*	*Indigofera (%)*	*Gliricidia sepium (%)*	P-values
4	37.71±3.94	6.33±3.25	33.77±8.82	16.72±1.03	NS
8	58.93±4.03^{a}	18.79±6.03^{b}	47.37±2.64^{a}	22.69±2.27^{b}	P < 0.001
12	69.07±1.79^{a}	38.32±11.89^{b}	53.96±4.00^{a}	27.87±0.79^{b}	P < 0.001
24	73.16±1.38^{a}	54.80±4.67^{b}	65.57±1.64^{ab}	47.60±3.36^{c}	P < 0.001
48	77.45±1.41^{a}	62.41±1.57^{b}	68.62±2.03^{ab}	63.42±4.42^{b}	P < 0.001
72	86.32±2.41^{a}	71.20±2.17^{b}	71.99±3.23^{a}	68.15±5.46^{b}	P < 0.001

a,b,c,d: Means in the same row with different superscripts differ significantly (P<0.05); NS: not significant; SEM: Standard error of the mean.

Crude protein degradation

The quality of a feed ingredient is reflected in its nutritional composition, particularly its crude protein content, which supports livestock productivity. In ruminants, the evaluation of feed ingredients extends beyond protein concentration to include fermentability and resistance to degradation in the rumen (Iommelli et al., 2022).

Protein degradation kinetics are therefore critical for assessing the nutritional value of dietary proteins. Using the in sacco method, crude protein degradation in the rumen is quantified by placing feed samples in nylon bags and incubating them for 4, 8, 12, 24, 48, and 72 hours. The resulting percentages of crude protein degradation are presented in Table 2.

Table 2 shows that the four legume types differenced (P < 0.05) at each incubation time, likely due to variations in their structural characteristics, protein content, and fiber composition. After 4 h, Moringa exhibited the highest crude protein degradation (37.71 %), followed by *Indigofera* (33.77 %), *Gliricidia* (16.72 %), and *Leucaena* (6.33 %). Suhartanto et al. (2000) reported that feed degradation by rumen microbes is influenced by the nutrient composition of the substrate—particularly lignin content—which affects overall digestibility. At 8, 12, 24, 48, and 72 h, degradation values also differed significantly (P < 0.05) among the feeds. Duncan's multiple range test revealed that, after 72 h, Moringa again showed the highest degradation (86.32 %), followed by *Indigofera* (71.99 %), *Leucaena* (71.20 %), and *Gliricidia* (68.15 %). These findings are consistent with Akhirany et al. (2013), who observed peak degradation of fibrous forages at 72 h. Throughout the 8–48 h incubations, Moringa and *Indigofera* consistently degraded more rapidly than Leucaena and *Gliricidia*. As shown in Table 1, *Leucaena* and *Gliricidia* have lower crude protein contents (25.63 % and 22.79 %, respectively) compared to Moringa (32.70 %) and *Indigofera* (29.50 %), which likely contributed to their reduced degradation values. Hartadi et al. (2008) noted that differences in the potentially soluble fraction and the degradation rate of the potentially degradable fraction are affected by feed nutrient composition, rumen residence time, and substrate availability for microbial activity.

Crude protein degradation curve

Figure 1 presents the in sacco crude protein degradation curves for the four legumes at incubation times of 4, 8, 12, 24, 48, and 72 hours. The degradation kinetics demonstrates a progressive increase in crude protein loss with longer

incubation. The most pronounced increase occurs between 4 and 12 hours, after which degradation rates begin to plateau between 24 and 72 hours as the available substrate in the rumen diminishes (Jiang et al., 2020). Low measured crude protein can result from microbial breakdown: rumen microbes hydrolyze feed proteins into amino acids, which are further deaminated into ammonia and other small compounds, thus reducing the recoverable protein fraction (Pranoto et al., 2013). Microbial proteolytic activity therefore lowers the crude protein content over time. Our results indicate that longer rumen incubation times correspond to higher crude protein degradation, reflecting progressive substrate utilization by rumen microbes. Among the four legumes tested, Moringa exhibited the highest degradation curve, while Gliricidia showed the lowest, consistent with its comparatively lower initial protein content. This observation aligns with Puastuti et al. (2015), who reported that feeds with higher protein concentrations are degraded more rapidly by rumen microbes.

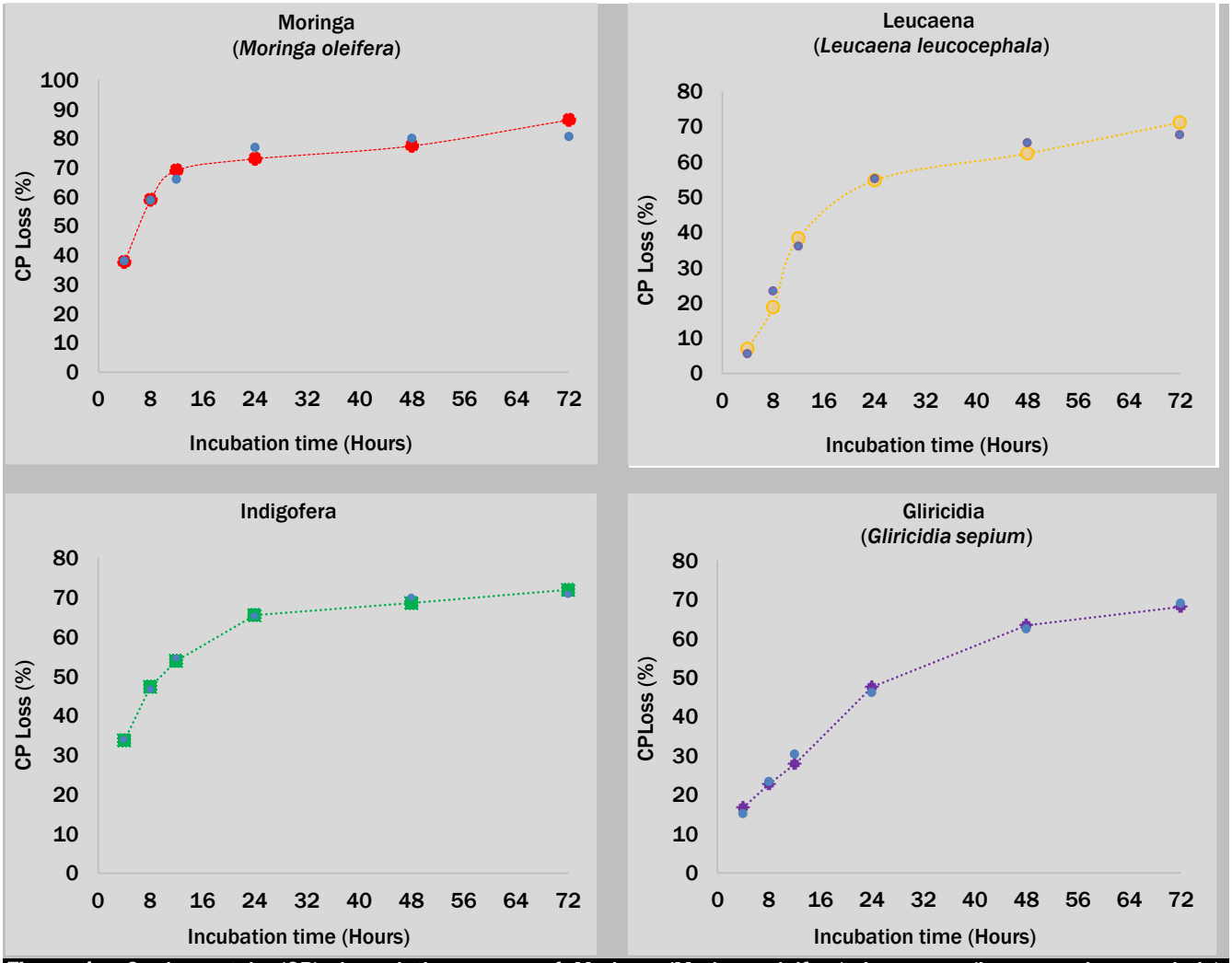

Figure 1 - Crude protein (CP) degradation curves of Moringa *(Moringa oleifera)*, Leucaena *(Leucaena leucocephala)*, *Indigofera*, Gliricidia *(Gliricidia sepium)*.

Degradation characteristics of crude protein

The in sacco method evaluates protein degradation by incubating feed samples directly in the rumen. This approach also characterizes degradation parameters, including the soluble fraction (a), the potentially degradable but non-soluble fraction (b), and the degradation rate of fraction b (c). The crude protein degradation characteristics for the four diets are presented in Table 3. Table 3 shows that fraction (a) did not differ significantly among the four legumes ($P > 0.05$). Fraction (a) reflects the truly soluble portion of the feed that dissolves readily in the rumen and during initial washing (Wati et al., 2012). Duncan's multiple range test indicated that Moringa had the highest fraction a (39.59 %), followed by *Indigofera*, Leucaena, and Gliricidia, confirming Moringa's superior solubility and Gliricidia's relative resistance. Katongole et al. (2021) noted that a high soluble CP fraction in forages is often associated with low acid detergent fiber (ADF) content. Moreover, lignin, which cannot be degraded by rumen microbes, substantially reduces cell-wall degradability (Hatfield and Kalscheur, 2020; Wang et al., 2022). Fraction b represents the potentially degradable but insoluble protein fraction. Statistical analysis indicated no significant differences in fraction b among the four legumes ($P > 0.05$). This fraction is expected to consist of amino acid–rich proteins that escape ruminal degradation and are absorbed in the

intestine. Duncan's multiple range test ranked fraction b as follows: Gliricidia (43.45 %) > Moringa (41.14 %) > Leucaena (36.47 %) > *Indigofera* (32.82 %). La Goffe (1991, cited in Widyobroto et al., 1995) noted that such proteins are often bound to fibrous cell-wall components, rendering them resistant to enzymatic attack. This resistance may also reflect tannin–protein complex formation, which reduces ruminal protein degradation (Min et al., 2003; Patra & Saxena, 2010). The degradation rate constant (c) quantifies the rate at which fraction b is degraded. Here, c differed significantly among legumes ($P < 0.05$). Moringa exhibited the highest rate (0.17 h^{-1}), followed by Leucaena (0.09 h^{-1}), *Indigofera* (0.09 h^{-1}), and Gliricidia (0.03 h^{-1}). Higher c values indicate greater microbial accessibility and faster degradation, influenced by cell-wall composition and substrate availability (Van Soest, 1994; Wati et al., 2012). Noviandi et al. (2021) further emphasized that nutrient composition, incubation time, and cell-wall content modulate both the soluble fraction and the degradation rate of the potentially degradable fraction. The low c value in Gliricidia may result from its specific cell-wall constituents (Aye and Adegun, 2013). Lag time—the interval before the onset of measurable degradation—also differed significantly ($P < 0.05$). Duncan's test identified Gliricidia as having the longest lag time (11.96 h), indicating that its proteins require more time to become accessible to rumen microbes.

Effectiveness of crude protein degradation

Feed degradation effectiveness (DE) —which integrates the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c)—varied significantly among the four legume species ($P < 0.05$). Moringa exhibited the highest DE (64.99 % ± 7.80), closely matching the 64.29 % reported by Sumadi et al. (2017). Leucaena's DE was 50.62 % ± 1.49, similar to values of 50.74 % (Sumadi et al., 2017) and 55.61 % (Yustisiana and Kustantinah, 2011). *Indigofera* showed a DE of 59.72 % ± 1.76, higher than the 48.00 % observed by Syamsi et al. (2022). Gliricidia's DE was 49.83 % ± 0.28, differing from previously reported values of 66.14 % (Hadi et al., 2011) and 47.00 % (Syamsi et al., 2022). These discrepancies likely reflect differences in cell-wall composition, plant maturity, and cutting age, all of which influence forage nutrient profiles. Suhartanto et al. (2000) emphasized that feed degradability is strongly affected by nutrient composition—particularly lignin content—which constrains microbial access and digestibility in the rumen.

Crude fiber degradation

The in sacco method was used to assess crude fiber degradation in the rumen by incubating feed samples in nylon bags for 4, 8, 12, 24, 48, and 72 hours. Table 4 presents the percentages of crude fiber degradation obtained by this method. Analysis of variance indicated that, at 4 hours of incubation, crude fiber degradation did not differ significantly among the four legumes ($P > 0.05$); the ranked order was Gliricidia > Indigofera > Moringa > Leucaena. This ranking likely reflects inherent differences in fiber content and composition. At 8, 12, 24, 48, and 72 hours, degradation values differed significantly among the legumes ($P < 0.05$). After 72 hours, Gliricidia showed the highest degradation (54.92 %), followed by Leucaena (54.13 %), Moringa (53.41 %), and *Indigofera* (52.23 %). These losses during ruminal incubation are assumed to represent the proportion of crude fiber digestible by rumen microbes.

Crude fiber degradation curves

The crude fiber loss of the tested feed during incubation periods of 4, 8, 12, 24, 48, and 72 hours is presented in the corresponding table, while the degradation curves or patterns— distinguished by smooth and jagged lines—are illustrated in Figure 2. The peak of fiber degradation occurred at 72 hours of incubation, as shown in Figure 2. According to Orksov et al. (1980), the optimal rumen incubation time for fibrous feed ranges from 48 to 72 hours. This is supported by Suparjo (2010), who stated that incubation intervals of 12, 24, 48, and 72 hours are most appropriate for fibrous feeds. These findings suggest that the 24–72 hour period provides optimal conditions for rumen microbes to interact with and degrade the incubated feed substrate (Ambar and Djajanegara, 1982).

Degradation characteristics of crude fiber

The in sacco method characteristics feed degradation by estimating the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c). These parameters for the forage feeds are presented in Table 5. Fraction a represents the truly soluble cell contents. Analysis of variance indicated no significant differences in fraction (a) among the feeds ($P > 0.05$), reflecting similar water-solubility profiles. Feedstuffs with low water solubility dissolve and degrade less readily in rumen fluid, corresponding to lower quality. According to Duncan's test, Gliricidia had the lowest mean fraction a (30.02 %), while Moringa had the highest (39.59 %). Regression analysis showed that fraction (a) was not significantly correlated with fraction b or c ($P > 0.05$), suggesting that the soluble cell components measured in fraction a do not predict the quantity or rate of the potentially degradable fraction. Factors influencing fraction a are therefore limited to the water-soluble constituents of the plant cells (Van Soest, 1982; Lestari et al., 2012) Differences in nutrient composition among feeds also affect degradability. The high fraction (a) of streptokinase (SK) in legumes reflects their neutral detergent fiber (NDF) and hemicellulose contents. A greater hemicellulose-to-crude-fiber ratio enhances forage quality (Parakkasi, 1998). This concurs with Tillman et al. (1991), who reported that SK degradation is strongly influenced by crude fiber content and by fiber constituents such as cellulose, hemicellulose, and lignin.

Table 3 - Degradation characteristics crude protein (average ±SEM)

Degradation Characteristics \ Feed	*Moringa oleifera*	*Leucaena leococephala*	*Indigofera*	*Gliricidia Sepium*	P-values
a (%)	39.59±0.30	32.11±0.30	38.13±0.30	30.02±0.30	P<0.001
b (%)	41.14±2.42	36.47±4.81	32.82±2.50	43.45±6.25	NS
c (%/h^{-1})	0.17±0.05^{a}	0.09±0.02^{ab}	0.09±0.03^{ab}	0.03±0.03^{b}	P<0.001
a+b (%)	80.73±2.42	68.89±5.10	70.95±2.50	73.47±6.25	NS
Lt (hour)	3.60±0.95^{b}	10.90±2.05^{a}	6.03±0.84^{b}	11.96±1.02^{a}	P<0.001
DE (%)	64.99±4.50^{a}	50.62±0.86^{b}	59.72±0.78^{a}	49.83±0.16^{b}	P<0.001
RUP (%)	35.01±4.50	40.28±0..86	40.28±0.78	50.17±0.16	P<0.001

a: soluble fraction, b: potential degradation fraction, a+b: total potential degradation, c: degradation rate of fraction, lt: lag time, de: degradation effectiveness, rup: rumen undegradable protein. Different superscripts in the same row indicate significant differences (P<0.05). SEM: Standard error of mean.

Table 4 - The average percentage (±SEM) of Crude fiber degradation of legume forages at each incubation period

Incubation period (Hours)	*Moringa oleifera (%)*	*Leucaena leococephala (%)*	*Indigofera (%)*	*Gliricidia sepium (%)*	P-values
4	11.97±4.517	10.19±4.48	13.16±3.54	17.01±1.33	NS
8	22.41±0.91^{a}	17.27±4.15^{ab}	20.78±1.87^{a}	20.76±1.76^{ab}	P<0.001
12	26.94±0.75^{b}	27.53±4.47^{ab}	31.00±4.50^{a}	24.92±1.44^{b}	P<0.001
24	41.02±3.57^{a}	38.34±4.83^{c}	39.86±2.05^{b}	36.79±1.84^{d}	P<0.001
48	44.51±2.43^{b}	46.24±3.60^{ab}	43.44±1.69^{b}	46.48±4.20^{a}	P<0.001
72	53.41±4.66^{b}	54.13±0.77^{a}	52.23±1.52^{b}	54.92±5.20^{a}	P<0.001

a,b,c,d, : Means in the same row with different superscripts differ significantly (P<0.05); NS: not significant; SEM: Standard error of the mean.

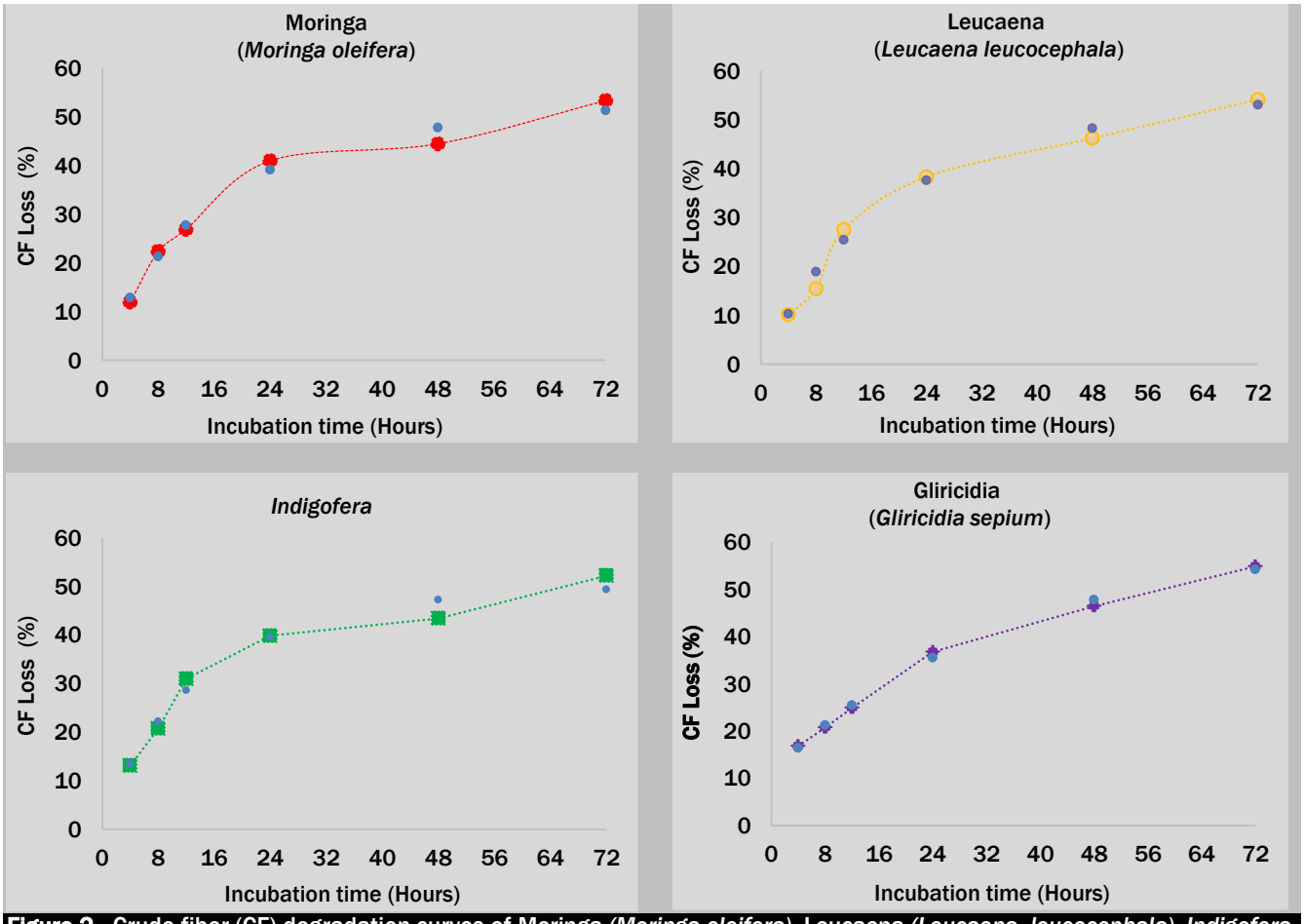

Figure 2 - Crude fiber (CF) degradation curves of Moringa *(Moringa oleifera)*, Leucaena *(Leucaena leucocephala)*, *Indigofera*, Gliricidia *(Gliricidia sepium)*.

Fraction (b) represents the slowly degradable feed fraction. Statistical analysis revealed no significant differences in fraction (b) among the four forages ($P > 0.05$), likely reflecting similar structural carbohydrate contents (NDF and ADF). Duncan's test ranked fraction b as follows: Gliricidia (32.11 %) > Leucaena (26.71 %) > Moringa (18.60 %) > Indigofera (12.38 %). The variability in this fraction correlates with the fiber composition of each forage (Wati et al., 2012). Chemical analysis showed the following ADF and NDF contents: Moringa, 15.13 % ADF and 23.38 % NDF; Leucaena, 33.75 % ADF and 39.00 % NDF; Indigofera, 23.74 % ADF and 30.99 % NDF; Gliricidia, 35.52 % ADF and 43.48 % NDF. As noted by Lopez et al. (2000) and Van Soest (1994), ADF and NDF levels can strongly influence forage digestibility. Moreover, fiber fractions bound by lignin resist microbial attack (Harfiah et al., 2009), and high lignin content further impedes ruminal degradation (Zhong et al., 2021).

The constant c describes the degradation rate of fraction b in the feed. Statistical analysis indicated no significant differences in c among the four legumes ($P > 0.05$). The highest fiber degradation rates were observed in Moringa and Indigofera (0.05 % h^{-1}), followed by Leucaena (0.04 % h^{-1}), with Gliricidia exhibiting the lowest rate (0.02 % h^{-1}). A lower degradation rate constant can correspond to higher overall in sacco digestibility, as reported by Rasjid and Ismartoyo (2014). Degradation speed is influenced by the degradability of cell-wall constituents (Van Soest et al., 1982). Variations in the parameters a, b, c, and effective degradability (ED) among the legume forages likely reflect differences in their nutrient and cell-wall compositions (Hadi et al., 2011). Gharechahi et al. (2023) noted that both inter- and intra-species differences in plants result in varying proportions of cellulose, hemicellulose, and lignin. Lag time (t) represents the period required for rumen microbes to adapt to the feed substrate. Analysis of variance indicated no significant differences in lag time among the feeds tested ($P > 0.05$). However, Duncan's multiple range test showed that Moringa exhibited the longest lag time, whereas Gliricidia had the shortest.

Effectiveness of crude fiber degradation

Feed degradation (DE) effectiveness integrates the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c) to estimate the proportion of feed that is digested. Crude fiber DE did not differ significantly among the four legumes ($P > 0.05$). Moringa exhibited the highest DE (43.98 % ± 2.87), followed by Indigofera (42.39 % ± 1.24), Leucaena (40.52 % ± 1.80), and Gliricidia (39.37 % ± 3.48), although these differences were not statistically significant. Feed DE is influenced by factors such as species, plant maturity, lignification level, and rumen incubation time. Moringa's superior DE likely reflects its lower crude fiber content, since cellulose and hemicellulose bound to lignin are resistant to microbial and enzymatic attack, reducing digestibility (Komar, 1984; Tillman et al., 1998). Pangestu (2005) similarly noted that fiber composition and lignin associations vary among forages, leading to differential degradation in the digestive tract. According to Mehrez and Orskov (1977), DE depends on fractions (a) and (b), the degradation rate c, and the feed passage rate. Liyama and Lam (2001) further emphasized that degradation characteristics vary with plant part, age, and lignification, reflecting intrinsic feedstuff properties.

CONCLUSION

The degradation kinetics of crude protein in the four legumes over 4, 8, 12, 24, 48, and 72 hours of ruminal incubation are summarized as follows: Moringa (c = 0.17 h^{-1}; lag time = 3.60 h; DE = 64.99 %), Leucaena (c = 0.09 h^{-1}; lag time = 10.90 h; DE = 50.62 %), Indigofera (c = 0.09 h^{-1}; lag time = 6.03 h; DE = 59.72 %), and Gliricidia (c = 0.03 h^{-1}; lag time = 11.96 h; DE = 49.83 %). All four forages are readily degraded by rumen microbes, although they require varying adaptation periods before reaching maximal degradation. Overall, longer incubation times correspond to higher degradation extents.

DECLARATIONS

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Author's contribution

M.Yahya contributed to data collection and data analysis, as well as drafting and writing the manuscript. I.Ismartoyo, and R.Islamiyati contributed to the experiments, ideas, and research design.

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Consent to publish

All authors agree to the publication of this manuscript.

Data availability

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

Competing interests

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

REFERENCES

Akhirany N, Ismartoyo I, Jusoff K, Natsir A, and Ako A (2013). The digestibility, degradation and index value of four local feeds goat. World Applied Sciences Journal, 26: 60–66. https://www.idosi.org/wasj/wasj26(nrrdsi)13/11.pdf

Ambar AR, and Djajanegara A (1982). Effect of rice straw storage process with urea on solubility of crude fiber components of rice straw. Proceeding of Seminar on Animal Science. Cisarua, Bogor.

AOAC (1995). Association of Official Analytical Chemists. Official Method of Analysis. 16th Edition. Washington, DC.

AOAC (2001). Protein (crude) in animal feed, forage (plant tissue), grain, and oilseed. AOAC Official Method. http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=672

Aye PA, and Adegun MK (2013). Chemical composition and some functional properties of moringa, leucaena and gliricidia leaf meals. Agriculture and Biology Journal of North America, 4(1):71-7. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=3aa8e5a92bf42def2a4bd5943aa95bf4451bef47

Babangida L, Nasir A, Muhammad IR, Mansur SJ, Audu R, Bature N, et al. (2021). In sacco rumen degradation of ensiled maize cobs treated with non protein nitrogen (urea). Nigerian Journal of Animal Science and Technology (NJAST): 4(4):65-73. https://www.njast.com.ng/index.php/home/article/view/177

Gharechahi J, Vahidi MF, Sharifi G, Ariaeenejad S, Ding XZ, Han JL, et al. (2023). Lignocellulose degradation by rumen bacterial communities: New insights from metagenome analyses. Environmental Research, 229:115925. https://doi.org/10.1016/j.envres.2023.115925

Gaspersz V (1991). Experimental design method. CV. ARMICO, Bandung.

Hadi RF, Kustantinah, and Hartadi H (2011). Kecernaan in sacco hijauan leguminosa dan hijauan non- leguminosa dalam rumen sapi peranakan ongole. [In Sacco digestibility of leguminous forage and non-leguminous forage in the rumen of ongole peranakan cattle] Buletin Peternakan, 35(2): 79-85. https://doi.org/10.21059/buletinpeternak.v35i2.594

Harfiah, Mide MZ, and Rasyid S (2009). Potensi mikroba selulolitik dan lignolitik dalam mendegradasi selulosa dan lignin limbah pertanian. [Potential of cellulolytic and lignocellulolytic microbes in degrading cellulose, hemicellulose, and lignin of agricultural waste]. Competing grant report of the research institute of Hasanuddin University, Makassar. https://core.ac.uk/works/17370011/

Hartadi H, Kustantinah, Zuprizal, Indarto E, and Dono ND (2008). Animal Feed Nutrition. Faculty of Animal Science, Gadjah Mada University, Yogyakarta.

Hatfield RD, and Kalscheur KF (2020). Carbohydrate and protein nutritional chemistry of forages. Forages, John Wiley & Sons Ltd. pp. 595–607. https://doi.org/10.1002/9781119436669.ch33

Humer E, and Zebeli Q (2017). Grains in ruminant feeding and potentials to enhance their nutritive and health value by chemical processing. Animal feed science and technology, 226:133-151. https://doi.org/10.1016/j.anifeedsci.2017.02.005

Iommelli P, Zicarelli F, Musco N, Sarubbi F, Grossi M, Lotito D, et al. (2022). Effect of cereals and legumes processing on in situ rumen protein degradability: A review. Fermentation, 8(8): 363. https://doi.org/10.3390/fermentation8080363

Ismartoyo (2011). Introduction to research techniques for ruminant feed degradation; Introduction to ruminant feed degradation research techniques. Brilian Internasional, Sidoarjo, Indonesia.

Jiang B, Yuxiang Z, Tian W, and Fei L (2020). Nutritive value and ruminal degradation of seven Chinese herbs as forage for Tan sheep. Bioengineered, 11(1), 1159–1169. https://doi.org/10.1080/21655979.2020.1834740

Katoch R (2022). Nutritional quality of important forages. Techniques in forage quality analysis, Singapore: Springer Nature, Singapore. pp. 173-185. https://doi.org/10.1007/978-981-19-6020-8_14

Katongole CB, Lumu R, and Lindberg JE (2021). Comparative chemical composition and rumen degradation of common reed and elephant grass in urban/peri-urban dairying systems in Uganda. Agroecology and Sustainable Food Systems, 45(6): 892–906. https://doi.org/10.1080/21683565.2021.1896618

Komar (1984). Management technology: Straw rice as food for livestock. Dian Grahita Foundation, Indonesia. https://scholar.google.com/scholar?cluster=14668443931230164541&hl=en&oi=scholarr

Lestari T, Nuswantara LK, and Surono (2012). Degradasi bahan kering dan bahan organik dengan berbagai level jerami padi secara in sacco pada kambing jawarandu [Degradation of dry matter and organic matter with various levels of rice straw in sacco in goat]. Animal Agriculture Journal, 1(1): 875-888. https://ejournal3.undip.ac.id/index.php/aaj/article/view/938

Liyama K, and Lam TB. Structural characteristics of cell walls of forage grasses-their nutritional evaluation for ruminants–Review. Asian-Australasian Journal of Animal Sciences, 14(6):862-879. https://www.animbiosci.org/journal/view.php?doi=10.5713/ajas.2001.862

Lopez S, Dijkstra J, and France J (2000). Predicción del suministro de energía en rumiantes con énfasis en forrajes [Prediction of energy supply in ruminants with emphasis on forages]. CAB International, Wallingford, UK, pp: 63-94 https://doi.org/10.1079/9780851993447.0063

Mehrez AZ and Ørskov ER (1977). A study of the artificial fibre bag technique for determining the digestibility of feed in the rumen. Journal of Agricultural Science, 88: 645 - 650. https://doi.org/10.1017/S0021859600037321

Min BR, and Hart SP (2003). Tannins for suppression of internal parasites. Journal of Animal Science. 81 (14): E102 E109. https://doi.org/10.2527/2003.8114_suppl_2E102x

Noviandi CT, Kustaantinah K, Irawan A, Widyobroto BP, and Astuti A. (2021) Determination of in vitro Gas Production Kinetics by Adding Leucaena leucecophala and Corn Oil to the Ration in Different Ratios. Iranian Journal of Applied Animal Science, 11(1): 23-31. https://sanad.iau.ir/journal/ijas/Article/680281?jid=680281

Orskov ER (1992). Protein Nutritional in Ruminant. Academic Press, London. Hal. 41-93

Orskov ER, and McDonald I (2009). The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. Journal of Agricultural Science, 92(2), 499-503 https://doi.org/10.1017/S0021859600063048

Pangestu E (2005). Evaluasi serat dan suplementasi zink dalam ransum berbahan hasil samping industry pertanian pada ternak ruminansia [Evaluation of fiber level and zinc supplementation in agroindustrial byproduct-based diet on ruminants]. Dissertation. Bogor Agricultural Institute https://repository.ipb.ac.id/handle/123456789/41292

Parakkasi A (1998). The science of nutrition and feeding of ruminant livestock. Jakarta: Universitas Indonesia (UI-Press), pp.811-852. ISSN: 979-456-185-1. https://digilib.unsri.ac.id/opac/index.php?p=show_detail&id=70990

Patra AK, and Saxena J (2010). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture, 91(1): 24–37. https://doi.org/10.1002/jsfa.4152

Phelan P, Moloney AP, McGeough EJ, Humphreys J, Bertilsson J, O'riordan EG, et al. (2015). Forage legumes for grazing and conserving in ruminant production systems. Critical Reviews in Plant Sciences, 34(1-3):281-326. https://doi.org/10.1080/07352689.2014.898455

Pranoto Y, Anggrahini S, and Efendi Z (2013). Effect of natural and Lactobacillus plantarum fermentation on invitro protein and starch digestibilities of sorghum flours. Food Bioscience, 2: 46-52. http://dx.doi.org/10.1016/j.fbio.2013.04.001

Puastuti W, Widiawati Y, and Wina E (2015). Digestion and ruminal fermentation of cocoa pod silage based ration enriched by gliricidia and calliandra leaves on goat. Jurnal Ilmu Ternak dan Veteriner, 20(1): 31-40. http://dx.doi.org/10.14334/jitv.v20i1.1114

Rasyid S, and Ismartoyo (2014). Goat feed index value based on in sacco and in vivo studies. Buletin Nutrisi dan Makanan Terna, 10(1):1-11. https://journal.unhas.ac.id/index.php/bnmt/article/download/908/579

Reis MJ, Santos SA, Prates LL, Detmann E, Carvalho GG, Santos AC, et al. (2017). Comparing sheep and cattle to quantify internal markers in tropical feeds using in situ ruminal incubation. Animal Feed Science and Technology, 232: 139-147. https://doi.org/10.1016.j.anifeedsci.2017.08.013

Richards N, Nielsen BD, and Finno CJ (2021). Nutritional and non-nutritional aspects of forage. Veterinary Clinics: Equine Practice, 37(1):43-61. https://doi.org/10.1016/j.cveq.2020.12.002

Rouillé B, Jost J, Fança B, Bluet B, Jacqueroud MP, Seegers J, et al. (2023). Evaluating net energy and protein feed conversion efficiency for dairy ruminant systems in France. Livestock Science, 269:105170. https://doi.org/10.1016/j.livsci.2023.105170

Roy A, and Rana T (2024). Feeds, feeding equipment and feeding habits of goats. Trends in Clinical Diseases, Production and Management of Goats. Elsevier Inc. Pp.113-133. https://doi.org/10.1016/B978-0-443-23696-9.00002-X

Srakaew W, Wachirapakorn C, Cherdthong A, and Wongnen C (2021). Ruminal degradability and bypass nutrients of alkaline or steam-treated cassava chip and corn grain. Tropical Animal Science Journal, 44: 451-461. https://doi.org/10.5398/tasj.2021.44.4.451

Suhartanto B, Kustantina, and Padmowijoto S (2000). In sacco degradation organic matter and crude protein four different feedstuffs was measured using bag. Rowett research institute. Buletin Peternakan, 24(2): 82-93. https://doi.org/10.21059/buletinpeternak.v24i2.1410

Sumadi S, Subrata A, and Sutrisno S (2017). Total protein production and protein digestibility Moringa leaves in vitro. Jurnal Sain Peternakan Indonesia, 12(4): 419-423. https://ejournal.unib.ac.id/jspi/article/view/3571

Suparjo (2010). In sacco feed evaluation. Laboratory Faculty of Animal Feed University of Jambi. Available online: https://123dok.com/document/qv9mglgy-evaluasi-pakan-secara-in-sacco.html

Syamsi, AN, Ifani M, and Subagyo Y (2022). Protein energy synchronization index of tropical legumes for ruminant livestock. Jurnal Peternakan, 19(1): 29-37. https://ejournal.uin-suska.ac.id/index.php/peternakan/article/view/15415

Terefe G, Faji M, and Mengistu G (2022). Nutritional value and in situ degradability of selected forages, browse trees, and agro-industrial by-products. Online Journal of Animal and Feed Research, 12(2): 97-102. https://dx.doi.org/10.51227/ojafr.2022.13

Tillman AD, Hartadi H, Reksohadiprojo S, Prawirokusumo S, and Lebdosoekojo S (1998). Basic animal feed science. Gadjah Mada University Press. Yogyakarta.

Ullah-Khan K, Tellechea-Rodrigues A, Menegasso-Mansano CF, Queiroz DM, Kazue-Sakomura N, Romaneli RD, et al. (2019). Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Latin American journal of aquatic research, 47(2):232-239. https://www.scielo.cl/scielo.php?pid=S0718-560X2019000200232&script=sci_arttext&tlng=en

Van Soest, PJ (1982). Nutritional ecology of the ruminant. commstock pulishing assciates. A devision of cornell University Press. Ithaca and London. Pages 488/ISBN 9781501732355, 1501732358

Van Soest, PJ (1994). Nutritional ecology of the ruminant. Comstock Publishing Associates a Division of Cornell University Press. (2nd Eds.). Ithaca and London. Pages 476/ISBN 080142772X, 9780801427725

Wang YL, Wang WK, Wu QC, and Yang HJ (2022). The release and catabolism of ferulic acid in plant cell wall by rumen microbes: A review. Animal Nutrition, 9: 335–344. https://doi.org/10.1016/j.aninu.2022.02.003

Wati, NE, Achmadi J, and Pangestu E (2012). Nutrient degradation of agricultural waste feed ingredients in the goat rumen in sacco. Animal Agriculture Journal, Vol. 1. (1): 485-498. http://ejournal-s1.undip.ac.id/index.php/aaj

Widyobroto BP, Padmowiyoto S, and Utomo R (1995). In Sacco degradation of organic matter and protein of five tropical grasses. Buletin Peternakan (Bulletin of Animal Science), 19: 43-53. https://doi.org/10.21059/buletinpeternak.v19i1.1711

Yustisiana Z, Kustantinah DEA. (2011). In Sacco evaluation of laucaena, Hibiscus tiliaceus, Tamarindus indica L, and Manihot esculenta Crantz in the Rumen. Faculty of Animal Science, Gadjah Mada University, Yogyakarta. https://etd.repository.ugm.ac.id/penelitian/detail/172533

Zhong H, Zhou J, Abdelrahman M, Xu H, Wu Z, Cui L, et al (2021). The effect of lignin composition on ruminal fiber fractions degradation from different roughage sources in water buffalo (Bubalus bubalis). Agriculture, 11(10):1015. https://doi.org/10.3390/agriculture11101015

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TYPES AND APPLICATIONS OF INNOVATIVE ARTIFICIAL INTELLIGENCE IN POULTRY FARMS

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REVIEW

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ABSTRACT: The poultry farming world-wide face many challenges that adversely affects the production proficiency. Finding the optimal balance humans and the automation efficiency is crucial to obtain a maximum profit. Besides, improving of poultry welfare and production efficiencies necessitate some advanced modern technologies. The application of artificial intelligence (AI) and data-driven systems is regarded as an innovative solution to address many farm management problems. By the integration of AI, the industry has the opportunity to grow in terms of production quantity and poultry care quality with minimal added expense. Types of AI technology in poultry farms include machine learning techniques and robots. The machine learning technique decreases the need for big labeled data for training and helps in the transfer of knowledge, fast training, and better generalization on new tasks to enhance the performance parameters. This technique has different approaches such as Support Vector Machine, Single Shot MultiBox Detector, and Convolutional Neural Network that have a potential to reduce the labor and time and offer promising solutions for the rapid warning and accurate identification and differentiation of problems associated with poultry health. Moreover, innovative robots have been applied in poultry farms for monitoring, management, and environmental control as well as exploring of social dynamics. They are used in poultry farms for collections of eggs carcasses and eggs and transportation and slaughtering. Collectively, AI programs could be applied in poultry production for controlling environmental conditions, monitoring some behavioral conditions such as feeding, preventing some diseases, and correction of the hazardous usage of antibiotics with combating the increased incidence of antimicrobial resistance, and finally aiding in the rapid treatment. Therefore, this review highlights the types of AI models and their potential applications in poultry production.

Keywords: Antimicrobial resistance, behavior, diseases, environment, machine learning technique, poultry

INTRODUCTION

Recently, the poultry production systems have faced significant obstacles resulted from the different infectious and non-infectious causes. These challenges have induced adverse economic losses in the production and posed important threats to the health of humans. Thus, through monitoring and controlling of such conditions have become a critical issue for the poultry producers. Conventional methods that have been used for handling of these problems are often unreliable, labor-intensive, and time-consuming. Therefore, there is a crucial necessity for more efficient and accurate methods to monitor and control of such conditions.

The developed automated farm management strategies can monitor the environment as well as the physiological and behavioral characteristics, and provide essential benefits to maximize bird's welfare and minimize production losses. The application of smart technology tools in poultry farming is promising and helps in evaluation of huge data, monitoring flocks, optimizing environmental conditions, detecting diseases, and enabling the farmers to make data-driven decisions to achieve sustainable growth in their businesses (Sharma and Patil, 2018). The previous studies showed the importance of AI technology applications in the poultry farming using advanced sensors, automation technology, internet of things, big data analysis, robotics, and transportation (Ren et al., 2020; Abbas et al., 2022; Park et al., 2022; Wu et al., 2022). The AI facilitates the analysis and integration of information, enabling data-driven decision-making, and enhancing business efficiency (Dwivedi et al., 2021). A combination of internet of things and AI spotlight on the field of the real-time monitoring of poultry and advance analytics and automation (Debauchea et al., 2020). Smart poultry farming system using AI could contribute to achieve the Sustainable Development Goals (SDGs) particularly when considering some key goals including no poverty, zero hunger, good health and well-being, and responsible consumption and production.

Overall, this article highlights the types of AI models and their potential applications in poultry production.

The recent advancement in monitoring systems in poultry flocks is illustrated in table 1.

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Table 1 - The recent advancement in monitoring systems in poultry flocks

Findings	Reference
Utilized a CNN pattern recognition structure to identify and classify healthy and diseased infected layers with *Clostridium perfringens* type A. They detected classification accuracies of 66.6% and 100% of the tested samples.	Sadeghi et al. (2015)
Used a CNN pattern (YOLO-V3 and Faster R-CNN) as an automated detector to differentiate between healthy and diseased broilers according to their droppings' shape, color, and water content.	Wang et al. (2019)
Improved the Feature Fusion Single Shot MultiBox Detector (IFSSD) to enhance the performance of the SSD model on Inception V3. They reached detection precision of 99.7% using a local dataset of an industrial base.	Zhuang and Zhang (2019)
Applied video surveillance, a depth camera, and an automatic health status classifier with an accuracy ranged from 0.975-0.978% to observe broiler chickens.	Okinda et al. (2020)
Achieved a recognition accuracy of 95.6% after using a machine learning classification algorithm method which was attached to a foot ring of each chicken to identifying the state of chickens.	Bao et al. (2021)
Used a combination of DenseFCN and the point supervision methodology and achieved an accuracy of 93.84% and a frame rate of 9.27 frames/second to count the number of chickens on a farm.	Cao et al. (2021)
Applied transfer learning on pre-trained image classification and CNN techniques to identify diseases from the images of chicken droppings. The validation accuracy of these approaches was 94%.	Mbelwa et al. (2021)
Achieved a high accuracy for the detection of chicken's behavior using YOLOv4 and Mask R CNN models.	Joo et al. (2022)
Developed a deep learning model based on YOLOv5x-hens and a CNN to track the number of hens in a real-time.	Yang et al. (2022)

SOME TYPES OF AI TECHNOLOGY THAT ARE APPLIED IN POULTRY FARMS MANAGEMENT

Machine learning technique

The transfer learning has been used in several domains such as computer vision, natural language processing, and speech recognition. The machine learning is a transfer learning developing computer program which can use the input information to produce new knowledge or improve the existing ones. Besides, it uses labelled data to develop accurate predictions as well as non-pre-assigned labels to identify datasets (Milosevic et al., 2019).

Machine learning technique has been employed beneficially in case of a limited dataset or when the manual annotating data are costly and time-consuming. Machine learning reduces the need for a large amount of labeled data for training. Moreover, it helps in knowledge transfer, fast training, and better generalization on new tasks with limited data to improve the performance parameters. The fine-tuning and feature extractors are the approaches of the transfer learning technique that convert raw data to feature vectors (Park et al., 2022). The deep learning approach is derived from the conventional machine learning technique and it can identify features from raw data without the need for notable engineering knowledge on feature extraction (LeCun et al., 2015).

Different innovative models have been applied to observe the behavior of birds and predict the infection early as possible. These proposed approaches include Support Vector Machine (RBF-SVM), Single Shot MultiBox Detector (SSD), and Convolutional Neural Network (CNN) such as You Only Look Once (YOLOv4), YOLOv5x, Visual Geometry Group (VGG16), Residual Networks (ResNet50), Extreme Inception (XceptionNet), and MobileNet (Okinda et al., 2020). These models can reduce the labor and time and they are promising for the rapid warning and accurate identification of problems associated with poultry health and welfare. The transfer learning using VGG16, ResNet50, and MobileNet approaches have been used for the precise and thorough processing of images. Moreover, the VGG16 approach achieved the highest accuracy when compared to the ResNet-50 and MobileNet models for the differentiation between heathy and diseased birds based on the bird's posture and feathers texture. The CNN is a type of the deep learning algorithms which has been emerged in the field of digital image processing. It is able to detect and classify objects in computer vision tasks based on the observation of the bird's physical features. Furthermore, YOLOv4 algorithm can recognize dead birds in the farms (Bochkovskiy et al., 2020).

Robots

Robots are applied in poultry farms for monitoring, management, and environmental control (Sahoo et al., 2022). This innovative approach permits exploring of social dynamics in different species (Romano et al., 2019). Standardized, controlled, replicable, and reproducible robots can interact with animals for investigation of a social behavior (Gribovskiy et al., 2018; Dennis et al., 2020; Parajuli et al., 2020). They can collect eggs and dead birds, thus save labor and facilitate

production (Astill et al., 2020; Zhao, 2021; Wu et al., 2022). Researchers have focused on developing equipping robots with sensors to identify diseased birds, allow monitoring of disease birds, and dispose dead birds from the flock (Abbas et al., 2022; Park et al., 2022).

Most of robotic studies have been conducted under controlled experimental environmental conditions or in small-scale poultry houses (Vroegindeweij et al., 2018; Wu et al., 2022). Additionally, robotic systems have experienced experimental investigations to demonstrate their capabilities to monitor poultry houses and broader their future applications.

Robot's uses in poultry farms

Collection of carcasses

Nanny robot can monitor the movement and body temperature of birds in conventional 3-layer cage systems using thermal cameras. This robot can sense morbid and dead birds by identifying the inactivity and the abnormal temperature values of them. Moreover, the robot consists of two modes; one mode works as a remote control and the other is autonomous (Liu et al., 2021). The autonomous robot scout employs an infrared and visible light camera to identify morbid chickens. Both mods can screen the bird's temperature and movements to detect diseased and dead birds in cages and cage-free systems. Despite this robot shows high reliability (97.5%), accuracy (95.24%), precision (95.24%), and recall rates (100%), detecting dead chickens is somewhat difficult because their shapes are similar to healthy birds either in the sitting or lying position. An effective vision system is important for robots to accurately detect deceased birds. Also, drinkers and feeders in the deep litter closed systems may act as obstacles to identifying and collect dead carcasses. Therefore, the movement flexibility of dead bird's collection robots in around equipment's or the integration of flexible robotic arms and grippers should be improved to increase the mobility and accessibility (Althoefer, 2018). A robot equipped with two grippers and a camera at the end of a robot's arm was designed to remove dead chickens (Li et al., 2022a).

Collection of eggs

Automatic egg collection robots have been developed to reduce the human-induced problems and the need for human labor in egg collection, besides they could be used in the dense environments (Vroegindeweij et al., 2018). An autonomous robot (PoultryBot), equipped with a spiral spring on the front, has been successfully developed for collecting over 95% of the floor eggs in layers farms. This robot drives separately for more than 3000 m in the house and collects 46% of 300 eggs with a collection failure in approximately 37% of eggs (Vroegindeweij et al., 2018). Also, GohBot is an autonomous egg-collecting robot that uses a mechanical arm with a vacuum mirror to collect eggs with succeeding rate of 91.6% (Joffe and Usher, 2017). An egg-collecting robot consisting of a deep learning-based egg detector, arm, gripper, and camera has been developed (Li et al., 2021). Moreover, this robot can collect white and brown eggs in a rate of 94% using arms and grippers after eggs detection by image processing algorithms. Another type of robots has been designed to recognize white and brown eggs in free-range layers (Chang et al., 2020). This type used a computer vision based platform to move toward the eggs, collects 60% and 88% of the eggs on flat and surrounded floors, and then stores them in its chamber. Moreover, it could efficiently collect 8 eggs in 25 m^2 area within 10 minutes based on its contents of egg shaped stones within its operational area (Chang et al., 2020). However, there are some obstacles facing egg collection robotic techniques in the cage systems including the mobility within the poultry house, detection of eggs without breaking them, storage, and the possibly to classify them according to their weight and shape.

Transportation and slaughtering

Recently, the stunning and slaughtering of birds can be applied in the farm instead of the processing plants using robots which are able to transfer birds to the stunning sites for shackling (Park et al. 2022). Farm Processing and Transport (FPaT) is a system that has been used to reduce the birds stressors during handling during transportation from the flock to the slaughter houses, decrease the amount of water for scalding, and thus improves the carcass yield (Park et al., 2022). This system is composed of two mobile units; processing and transport trailers which are designed on standard 53-ft trailers (Park et al., 2022). Moreover, FPaT system also allows the non-significant differences in the major food quality matrix, visual properties, myopathy scores, water holding capacity, yield, and texture properties when compared with the traditional techniques (Park et al., 2022).

THE DIFFERENT AI APPLICATIONS USED IN POULTRY PRODUCTION

The different applications of AI in poultry farms are summarized in table 2.

Environment conditions

The AI approaches monitor environmental parameters around birds in a real-time and adjust them to create an optimal and stress-free environment. Debauchea et al. (2020) implemented an AI algorithm (Gated Recurrent Unit) to validate and predicate some environmental parameters. For instance, sensors such as camera and data acquisition

systems could detect relative humidity, temperature, ventilation, and lighting systems (Fernandez et al., 2018; Lahlouh et al., 2020; Lorencena et al., 2020). Moreover, Monte Carlo simulation system could be used to monitor the litter moisture (Rico-Contreras et al., 2017). The edge computing solution system was applied to screen the temperature, humidity, and light intensity and transmit their levels by the end nodes ZigBee to the gateway network (Yang et al., 2019). One hand type of sensors also controlled the fan and light intensity, while the other hand uploaded data to the cloud. Additionally, the MQ137 gas sensor was implemented to measure ammonia (Raj and Jayanthi, 2018) and hydrogen sulfide (Handigolkar et al., 2016) levels in air. In comparison to the traditional chemical sensors, the multifunction electro-thermal system showed a faster response and a lower power consumption for the detection of ammonia level (Lotfi et al., 2019). An adsorbing material has been used to extract ammonia from the chicken's litter (Xu et al., 2017). An automated anti-epidemic or disinfection sprayer robot, comprising of transport vehicle, sensors, spraying unit, and controller, has been also designed and applied in poultry farms (Feng and Wang, 2020).

Table 2 - The different applications of AI in poultry farms

Applications	Reference
Detection of avian influenza virus using an interferometric biosensor	Xu et al. (2007)
Using of infrared spectroscopy and artificial neural networks for detection of uropathogenic *Escherichia coli* strains' susceptibility to cephalothin	Lechowicz et al. (2013)
Detecting jumping and landing force in laying hens using wireless wearable sensors	Banerjee et al. (2014)
Application of wireless activity sensor network to avian influenza monitoring system in poultry farms	Okada et al. (2014)
Using of intelligent procedure for the detection and classification of chickens infected by *Clostridium perfringens* based on their vocalization	Sadeghi et al. (2015)
Estimating broiler weights based on machine vision and artificial neural network	Amraei et al. (2017)
Prediction of moisture content in poultry litter using artificial intelligence techniques and Monte Carlo simulation to determine the economic yield from energy use	Rico-Contreras et al. (2017)
Removal of phosphate using aluminum-doped magnetic nanoparticles	Xu et al. (2017)
Management of broiler breeder feed intake and flock uniformity	Zuidhof et al. (2017)
Real-time monitoring of broiler flock's welfare status using camera-based technology	Fernandez et al. (2018)
Monitoring and health status identification using Iot-based real-time poultry	Raj and Jayanthi (2018)
Detection of sick broilers using an early warning algorithm	Zhuang et al. (2018)
Diagnosis of infectious bursal disease with RNA microarray and machine Learning	Fang (2019)
Comparing between random forest and gradient boosting machine methods for predicting *Listeria* spp. prevalence in the environment of pastured poultry farms	Golden et al. (2019)
Detecting ammonia sensing using a platinum cantilever-based thermal conductivity and 3-omega technique	Lotfi et al. (2019)
Environmental monitoring of chicken house based on edge computing in internet of things	Yang et al. (2019)
Detection of automated sneeze using sound-based poultry health monitoring tool	Carpentier et al. (2019)
Detection of avian influenza-infected chickens based on a chicken sound convolutional neural network	Cuan et al. (2020)
Monitoring poultry behavior using edge computing and artificial intelligence	Debauchea et al. (2020)
Designing disinfection robot for livestock breeding	Feng and Wang (2020)
Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics	Khaledi et al. (2020)
Implementation of multi input multi output fuzzy-PID behavior controller in poultry house systems	Lahlouh et al. (2020)
Assessing layer pullet drinking behaviors under selectable light colors using convolutional neural network	Li et al. (2020a)
Analysis of feeding and drinking behaviors of group-reared broilers via image processing	Li et al. (2020b)
Designing a framework for modelling, control, and supervision of poultry farming	Lorencena et al. (2020)
Microbial identification and antimicrobial susceptibility testing using Machine learning for matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) spectra	Weis et al. (2020)
Detecting poultry eating behavior based on vocalization signals	Huang et al. (2021)
Application of artificial intelligence for antimicrobial resistance	Lv et al. (2021)
Acceleration of antibiotic discovery through artificial intelligence	Melo et al. (2021)
Application of artificial intelligence in combating high antimicrobial resistance rates	Rabaan et al. (2022)

Behavior characteristics

Some behavior conditions of birds including resting, running, and feeding could be remotely and automatically monitored using internet of things technologies such as sensors, microphones, mobile phones, and cameras that are connected to a server or cloud for prompt processing and visualization (Ojo et al., 2022). The feeding systems schedules have been improved according to the environment, health, and behavior of individual birds (Zuidhof et al., 2017). The AI systems precisely control the timing and quantity of feed and tailor it according to the specific needs of the birds. These results in reduced feed wastage, healthier birds, and ultimately enhanced production. The changes in feed and water intake, feed conversion ratio, and body weight could be detected based on vocalization signals, machine learning technique, digital image analysis, and CNN approaches (Amraei et al., 2017; Li et al., 2020a, b; Huang et al., 2021). The piezoelectric crystals technique may evaluate the broilers locomotion deficiency based on analyzing the peak vertical force exerted on both feet at the weakness conditions. Moreover, it enables the identification of asymmetry between each foot force, which explained the uneven gait of layers (Banerjee et al., 2014). Under natural conditions, chicks reared with mother hens have learned some behavior conditions include resting times, pecking habits, and food preferences (Edgar et al., 2016). These chicks exhibit synchronization in resting activity and behavior. They spend more times feeding and less time standing and perches when compared with chicks raised without a mother hens (Roden and Wechsler, 1998). However, robotic researches allow robots to interact with chicks and support their social learning and development (de Mesquita Souza Saraiva et al., 2011).

Diseases prevention

The traditional manual methods for the diagnosis of poultry diseases are mostly laborious, time-consuming, unreliable, and sometimes fail to detect the specific infections accurately. Therefore, developing of a more rapid, accurate, and effective methods to overcome such challenge is an urgent need. The AI technologies can detect the possible diseases occurrence and trace their vectors or modes of transmission via analyzing some historical data. This line can help in the early warning capabilities to prevent the future outbreaks (Ojo et al., 2022; Park et al., 2022). The machine learning algorithms excel, deep regression network, digital image processing, and ResNet residual network have received increased attention for the rapid prediction and diagnosis of diseased flocks. The previous AI techniques are based on analyzing some behavioral and biological conditions such as sound, movement, and eating, or tracking production performance (Zhuang et al., 2018; Carpentier et al., 2019; Fang et al., 2020). Poultry diseases conditions including avian influenza (Cuan et al., 2020), infectious bursal disease (Fang, 2019), salmonellosis (Hwang et al., 2020), closteridiosis (Sadeghi et al., 2015), and listeriosis (Golden et al., 2019) could be diagnosed using CNN, random forest, and gradient boosting machines. Some wireless devices depend on the body temperature sensors and accelerometers have been applied for the chicks with a highly pathogenic avian influenza up to six hours before death (Okada et al., 2014). Moreover, Xu et al. (2007) developed a new, portable, and inexpensive biosensor to identify several strains of pathogenic avian influenza infected birds within few minutes. Infectious bronchitis and infectious laryngotracheitis of chickens could be also monitored using auditory sensing systems including machine learning techniques and digital signal processing (Carroll, 2018). These techniques of AI could provide more information to manage the flocks in a more rounded manner.

Antimicrobial resistance

The continuous development of antimicrobial resistance is an important challenge facing poultry industry (Lv et al., 2021). The AI programs may help in the correction of the hazardous usage of antibiotics, combating the increased incidence of antimicrobial resistance, and aiding in the rapid treatment without waiting the bacterial culture results (Rabaan et al., 2022). Consequently, the AI approaches reduce time to discover new drugs, ensure accuracy of the diagnosis and treatment, and lower the treatment costs (Lv et al., 2021; Rabaan et al., 2022). The infrared spectroscopy with artificial neural network can short the time for the detection of antimicrobial resistance from days to hours or even 30 minutes (Lechowicz et al., 2013), beside they can find new mutants of resistance (Melo et al., 2021). The different technologies of AI can control the antimicrobial resistance by gathering of data to construct decision support systems, designing new antimicrobials, and investigating drug combinations synergism (Boolchandani et al., 2019; Rodríguez-González et al., 2019; Khaledi et al., 2020). For the early and effective prediction of the antimicrobial resistance, the construction of comprehensive antimicrobial resistance databases for the integration of more cutting edge algorithms is important (Rabaan et al., 2022). Lv et al. (2021) used artificial resistance algorithms methods including nave Bayes, decision trees, random forests, RBF-SVM, and artificial neural networks for the monitoring of antimicrobial resistance problems. Similarly, flow-cytometer antimicrobial susceptibility testing, infrared spectrometer, and k-mer-based machine learning were applied for combating antibiotic resistance (Mulroney et al., 2017; Mahé and Tournoud, 2018; Inglis et al., 2020). By increasing the amount of the whole-genome sequence data and understanding the structural basis of resistance and rational design principle (Klevens et al., 2007), the AI models are better able to induce a high accuracy in surveillance programs (Deng et al., 2016, Argimón et al., 2020) and develop new, effective, and broad-spectrum antimicrobials (Weis et al., 2020). However, there are some challenges face the application of AI models to combat the antimicrobial resistance; 1) most programs do not consider the intermediate category of resistance that overlaps the susceptible and resistant categories and, 2) the only resistant or susceptible resistance can result in a false diagnosis.

CONCLUSION

There is no doubt on the significant effects of AI in the poultry farming and production. In the near future, AI is expected to alter the poultry sector and contribute positively by improving efficiency and accuracy at all aspects of the industry. Despite the AI technology models have been already implemented in the chain of poultry industry with immense potentials, they address several challenges and obstacles that cannot be overcome without the integration of all aspects. AI technology will lead to the understanding of sustainable industry which suppresses the generation of greenhouse gases and helps in gaining some SDGs including the decrease in wasted feed and breeding tasks. It is expected the continuous development of the broiler industry to meet the hunger and support the world's food. From a global standpoint of view, adoption of AI technology information systems is important to ensure that humans are taking environmentally friendly and use the feedback information to produce safe poultry production. Therefore, AI acts as a potential method for the next generation of poultry farming system. Encouraging further researches is needed for widening the scale of AI technology applications in large-capacity poultry houses in order to gain some of SDGs.

DECLARATIONS

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Author's contribution
Abd El-Ghany WA has collected and drafted the manuscript, formatted it, and approved the final manuscript.

Competing of interests
The author has not declared any competing interests.

REFERENCES

Abbas G, Jaffery S, Hashmi AH, Tanveer AJ, Arshad M, Amin QA, et al. (2022). Prospects and challenges of adopting and implementing smart technologies in poultry production. Pakistan Journal of Science, 74(2): 108-127. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20220489512

Althoefer K. (2018). Antagonistic actuation and stiffness control in soft inflatable robots. Nature Reviews Materials, 3(6): 76-77. DOI: https://doi.org/10.1038/s41578-018-0004-0

Amraei S, Abdanan MS, and Salari S. (2017). Broiler weight estimation based on machine vision and artificial neural network. British Poultry Science, 58(2): 200-205. DOI: https://doi.org/10.1080/00071668.2016.1259530

Argimón S, Masim MAL, Gayeta JM, Lagrada ML, Macaranas PKV, Cohen V, et al. (2020). Integrating whole-genome sequencing with in the National Antimicrobial Resistance Surveillance Program in the Philip pines. Nature Communications, 11(1): 2719. DOI: https://doi.org/10.1038/s41467-020-16322-5

Astill J, Dara RA, Fraser ED, Roberts B, and Sharif S. (2020). Smart poultry management: Smart sensors, big data, and the internet of things. Computers and Electronics in Agriculture, 170: 105291. DOI: https://doi.org/10.1016/j.compag.2020.105291

Banerjee D, Daigle CL, Dong B, Wurtz K, Newberry RC, Siegford JM, et al. (2014). Detection of jumping and landing force in laying hens using wireless wearable sensors. Poultry Science, 93(11): 2724-2733. DOI: https://doi.org/10.3382/ps.2014-04006

Bao Y, Lu H, Zhao Q, Yang Z, and Xu W. (2021). Detection system of dead and sick chickens in large scale farms based on artificial intelligence. Mathematical Biosciences and Engineering, 18 (5): 6117-635. DOI: https://doi.org/10.3934/mbe.2021306

Bochkovskiy A, Wang CY, and Liao HYM. (2020). Yolov4: Optimal speed and accuracy of object Detection, ArXiv Prepr. ArXiv2004.10934. DOI: https://doi.org/10.48550/arXiv.2004.10934

Boolchandani M, D'Souza AW, and Dantas G. (2019). Sequencing-based methods and resources to study antimicrobial resistance. Nature Reviews Genetics, 20(6): 356-370. DOI: https://doi.org/10.1038/s41576-019-0108-4

Cao L, Xiao Z, Liao X, Yao Y, Wu K, Mu J, et al. (2021). Automated chicken counting in surveillance camera environments based on the point supervision algorithm: LC DenseFCN. Agriculture, 11(6): 493. DOI: https://doi.org/10.3390/agriculture11060493

Carroll BT. (2018). Characterizing acoustic environments with OLAF and ELSA: PhD Thesis, Georgia Institute of Technology, pp. 1-108. Carpentier L, Vranken E, Berckmans D, Paeshuyse J, and Norton T. (2019). Development of sound-based poultry health monitoring tool for automated sneeze detection. Computers and Electronics in Agriculture, 162: 573-581. DOI: https://doi.org/10.1016/j.compag.2019.05.013

Chang CL, Xie BX, and Wang CH (2020). Visual guidance and egg collection scheme for a smart poultry robot for free-range farms. Sensors (Basel), 20(22): 6624. DOI: https://doi.org/10.3390/s20226624

Cuan K, Zhang T, Huang J, Fang C, and Guan Y (2020). Detection of avian influenza-infected chickens based on a chicken sound convolutional neural network. Computers Electronics in Agriculture, (178): 105688. DOI: https://doi.org/10.1016/j.compag.2020.105688

de Mesquita Souza Saraiva M, Lim K, do Monte DFM, Givisiez PEN, Alves LBR, de Freitas Neto OC, et al. (2022). Antimicrobial resistance in the globalized food chain: A One Health perspective applied to the poultry industry. Brazilian Journal of Microbiology, 53(1): 465-486. DOI: https://doi.org/10.1007/s42770-021-00635-8

Debauchea O, Mahmoudia S, Mahmoudia SA, Mannebacka P, Bindelle J, and Lebeau F. (2020). Edge Computing and Artificial Intelligence for Real-time Poultry Monitoring. Procedia Computer Science, 175 (2020):534-541. DOI: http://dx.doi.org/10.1016/j.procs.2020.07.076

Deng X, den Bakker HC, and Hendriksen RS. (2016). Genomic Epidemiology: Whole genome-sequencing–powered surveillance and outbreak investigation of foodborne bacterial pathogens. Annual Review of Food Science and Technology, 7: 353-374. DOI: https://doi.org/10.1146/annurev-food-041715-033259

Dennis IC, Abeyesinghe SM, and Demmers TGM. (2020). The behaviour of commercial broilers in response to a mobile robot. British Poultry Science, 61(5): 483-492. DOI: https://doi.org/10.1080/00071668.2020.1759785

Dwivedi YK, Hughes L, Ismagilova E, Aarts G, Coombs C, Crick T, et al. (2021). Artificial Intelligence (AI): Multidisciplinary perspectives on emerging challenges, opportunities, and agenda for research, practice and policy. International Journal of Information Management, 57: 101994. DOI: https://doi.org/10.1016/j.ijinfomgt.2019.08.002

Edgar J, Held S, Jones C, and Troisi C. (2016). Influences of maternal care on chicken welfare. Animals, 6(1): 2. DOI: https://doi.org/10.3390/ani6010002

Fang C, Huang J, Cuan K, Zhuang X, and Zhang T. (2020). Comparative study on poultry target tracking algorithms based on a deep regression network. Biosystems Engineering, 190: 176-183. DOI: https://doi.org/10.1016/j.biosystemseng.2019.12.002

Fang T. (2019). Rapid diagnosis of infectious bursal disease with RNA microarray and machine learning. In Proceedings of the International Conference on Bioinformatics & Computational Biology (BIOCOMP). 2019; pp. 67-73: The Steering Committee of the World Congress in Computer Science. Available at: https://www.proquest.com/docview/2278745749?pqorigsite=gscholar&fromopenview=true&sourcetype=Conference%20Papers%20&%20Proceedings

Feng QC and Wang X. (2020). Design of disinfection robot for livestock breeding. Procedia Computer Science, 166: 310-314. DOI: https://doi.org/10.1016/j.procs.2020.02.093

Fernandez AP, Norton T, Tullo E, van Hertem T, Youssef A, Exadaktylos V, et al. (2018). Real-time monitoring of broiler flock's welfare status using camera-based technology. Biosystems Engineering, 173: 103-114. DOI: https://doi.org/10.1016/j.biosystemseng.2018.05.008

Golden CE, Rothrock JR, MJ, and Mishra A. (2019). Comparison between random forest and gradient boosting machine methods for predicting *Listeria* spp. prevalence in the environment of pastured poultry farms. Food Research International, 22: 47-55. DOI: https://doi.org/10.1016/j.foodres.2019.03.062

Gribovskiy A, Halloy J, Deneubourg JL, and Mondada F. (2018). Designing a socially integrated mobile robot for ethological research. Robotics and Autonomous Systems, 103: 42-55. DOI: https://doi.org/10.1016/j.robot.2018.02.003

Handigolkar LS, Kavya M, and Veena P. (2016). Iot based smart poultry farming using commodity hardware and software. Bonfring International Journal of Software Engineering and Soft Computing, 6: 171-175. DOI: http://dx.doi.org/10.9756/BIJSESC.8269

Huang J, Zhang T, Cuan K, and Fang C. (2021). An intelligent method for detecting poultry eating behaviour based on vocalization signals. Computers and Electronics in Agriculture, 180: 105884. DOI: https://doi.org/10.1016/j.compag.2020.105884

Hwang D, Rothrock Jr, MJ, Pang H, Guo M, and Mishra A. (2020). Predicting *Salmonella* prevalence associated with meteorological factors in pastured poultry farms in southeastern United States. Science of the Total Environment, 713: 136359. DOI: https://doi.org/10.1016/j.scitotenv.2019.136359

Inglis TJ, Paton TF, Kopczyk MK, Mulroney KT, and Carson CF. (2020). Same-day antimicrobial susceptibility test using acoustic enhanced flow cytometry visualized with supervised machine learning. Journal of Medical Microbiology, 69(5): 657-669. DOI: https://doi.org/10.1099/jmm.0.001092

Joffe BP, and Usher CT. (2017). In: Autonomous robotic system for picking up floor eggs in poultry houses. ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers, p. 1.

Joo KH, Duan S, Weimer SL, and Teli MN. (2022). Birds' eye view: Measuring behavior and posture of chickens as a metric for their well-being. arxiv.org/abs/2205.00069v1. DOI: https://doi.org/10.48550/arXiv.2205.00069

Khaledi A, Weimann A, Schniederjans M, Asgari E, Kuo TH, Oliver A, et al. (2020). Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics. EMBO Molecular Medicine, 12(3): e10264. DOI: https://doi.org/10.15252/emmm.201910264

Klevens RM, Edwards JR, Richards CL Jr, Horan TC, Gaynes RP, Pollock DA, et al. (2007). Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Reports, 122(2): 160-166. DOI: http://org/doi:10.1177/003335490712200205

Lahlouh I, Rerhrhaye F, Elakkary A, and Sefiani N. (2020). Experimental implementation of a new multi input multi output fuzzy-PID controller in a poultry house system. Heliyon, 6 (8): e04645. DOI: https://doi.org/10.1016/j.heliyon.2020.e04645

Lechowicz L, Urbaniak M, Adamus-BiałekW, and Kaca W. (2013). The use of infrared spectroscopy and artificial neural networks for detection of uropathogenic *Escherichia coli* strains' susceptibility to cephalothin. Acta Biochimica Polonica, 60(4): 713-718. Available at: https://pubmed.ncbi.nlm.nih.gov/24432322/

LeCun Y, Haffner P, and Bottou LGH. (2015). Deep learning. Nature, 521: 436-444. DOI: https://doi.org/10.1038/nature14539

Li G, Chesser GD, Huang Y, Zhao Y, and Purswell JL. (2021). Development and optimization of a deep-learning-based egg-collecting robot. Trans. ASABE, 64: 1659-1669. Available at: https://elibrary.asabe.org/abstract.asp?aid=52807

Li G, Ji B, Li B, Shi Z, Zhao Y, Dou Y, et al. (2020a). Assessment of layer pullet drinking behaviors under selectable light colors using convolutional neural network. Computers Electronics in Agriculture, 172: 105333. DOI: https://doi.org/10.1016/j.compag.2020.105333

Li G, Zhao Y, Purswell JL, Du Q, Chesser Jr, GD, and Lowe JW JC. (2020b). Agriculture E I. Analysis of feeding and drinking behaviors of group-reared broilers via image processing. Computers and Electronics in Agriculture, 175: 105596. DOI: https://doi.org/10.1016/j.compag.2020.105596

Liu HW, Chen CH, Tsai YC, Hsieh KW, and Lin HT. (2021). Identifying images of dead chickens with a chicken removal system integrated with a deep learning algorithm. Sensors, 21(11): 3579. DOI: https://doi.org/10.3390/s21113579

Lorencena MC, Southier LFP, Casanova D, Ribeiro R, and Teixeira M. (2020). A framework for modelling, control and supervision of poultry farming. International Journal of Production Research, 58(10): 3164-3179. DOI: https://doi.org/10.1080/00207543.2019.1630768

Lotfi A, Navaei Mm, and Hesketh PJ. (2019). A platinum cantilever-based thermal conductivity detector for ammonia sensing using the 3-omega technique. ECS Journal of Solid State Science Technology, 8: Q126. DOI: https://doi.org/10.1149/2.0231906jss

Lv J, Deng S, and Zhang L. (2021). A review of artificial intelligence applications for antimicrobial resistance. Biosafety and Health, 3(1): 22-31. DOI: https://dx.doi.org/10.1016/j.bsheal.2020.08.003

Mahé P, and Tournoud M. (2018). Predicting bacterial resistance from whole-genome sequences using k-mers and stability selection BMC Bioinformatics, 19(1): 383. DOI: https://doi.org/10.1186/s12859-018-2403-z

Mbelwa H, Mbelwa J, and Machuve D. (2021). Deep convolutional neural network for chicken diseases detection. International Journal of Advanced Computer Science and Applications, 12(2): 759-765. DOI: https://dx.doi.org/10.14569/IJACSA.2021.0120295

Melo MCR, Maasch JRMA, and de la Fuente-Nunez C. (2021). Accelerating antibiotic discovery through artificial intelligence. Communications Biology, 4: 1050. DOI: https://doi.org/10.1038/s42003-021-02586-0

Milosevic B, Ciric S, Lalic N, Milanovic V, Savic Z, Omerovic I, et al. (2019). Machine learning application in growth and health prediction of broiler chickens. World's Poultry Science Journal, 75(3): 401-410. DOI: https://doi.org/10.1017/S0043933919000254

Mulroney KT, Hall JM, Huang X, Turnbull E, Bzdyl NM, Chakera A, et al. (2017). Rapid susceptibility profiling of car bapenem-resistant *Klebsiella pneumoniae*. Scientific Reports, 7(1): 1903. DOI: https://doi.org/10.1038/s41598-017-02009-3

Ojo RO, Ajayi AO, Owolabi HA, Oyedele LO, and Akanbi LA. (2022). Internet of things and machine learning techniques in poultry health and welfare management: a systematic literature review. Computers and Electronics in Agriculture, 200: 107266. DOI: https://doi.org/10.1016/j.compag.2022.107266

Okada H, Suzuki K, Kenji T, and Itoh T. (2014). Applicability of wireless activity sensor network to avian influenza monitoring system in poultry farms. Journal of Sensor Technology, 4(1): 18-23. DOI: http://dx.doi.org/10.4236/jst.2014.41003

Okinda C, Nyalala I, Korohou T, Okinda C., Wang J, Achieng T, et al.. (2020). A review on computer vision systems in monitoring of poultry: A welfare perspective. Artificial Intelligence in Agriculture, 4: 184-208. DOI: https://doi.org/10.1016/j.aiia.2020.09.002

Parajuli P, Huang Y, Tabler T, Purswell JL, DuBien JL, and Zhao Y. (2020). Comparative evaluation of poultry-human and poultry-robot avoidance distances. Transactions of the ASABE, 63 (2):477-484. DOI: http://dx.doi.org/10.13031/iles.18-142

Park M, Britton D, Daley W, McMurray G, Navaei M, Samoylov A, et al. (2022). Artificial intelligence, sensors, robots, and transportation systems drive an innovative future for poultry broiler and breeder management. Animal Frontiers, 12(2): 40-48. DOI: https://doi.org/10.1093/af/vfac001

Rabaan AA, Alhumaid S, Mutair AA, Garout M, Abulhamayel Y, Halwani MA, et al. (2022). Application of artificial intelligence in combating high antimicrobial resistance rates. Antibiotics (Basal), 11(6): 784. DOI: https://doi.org/10.3390/antibiotics11060784

Raj AAG, and Jayanthi JG. (2018). Iot-based real-time poultry monitoring and health status identification, in: 2018 11th International Symposium on Mechatronics and its Applications (ISMA), 2018; pp. 1-7. DOI: https://doi.org/doi:10.1109/ISMA.2018.8330139.

Ren G, Lin T, Ying Y, Chowdhary G, and Ting KC. (2020). Agricultural robotics research applicable to poultry production: A review. Computers and Electronics in Agriculture, 169:105216. DOI: https://doi.org/10.1016/j.compag.2020.105216

Rico-Contreras JO, Aguilar-Lasserre AA, Méndez-Contreras JM, López-Andrés JJ, and Cid-Chama G. (2017). Moisture content prediction in poultry litter using artificial intelligence techniques and Monte Carlo simulation to determine the economic yield from energy use. Journal of Environmental Management, 202 (1): 254-267. DOI: https://doi.org/10.1016/j.jenvman.2017.07.034

Roden C, and Wechsler B. (1998). A comparison of the behaviour of domestic chicks reared with or without a hen in enriched pens. Applied Animal Behaviour Science, 55(3-4): 317-326. DOI: https://doi.org/10.1016/S0168-1591(97)00073-7

Rodríguez-González A, Zanin M, and Menasalvas Ruiz E. (2019). Public health and epidemiology informatics: Can artificial intelligence help future global challenges? An overview of antimicrobial resistance and impact of climate change in disease epidemiology. Yearbook of Medical Informatics, 28(1): 224-231. DOI: https://doi.org/10.1055/s-0039-1677910

Romano D, Donati E, Benelli, G, and Stefanini C. (2019). A review on animal–robot interaction: from bio-hybrid organisms to mixed societies. Biological Cybernetics, 113(3): 201-225. DOI: https://doi.org/10.1007/s00422-018-0787-5

Sadeghi M, Banakar A, Khazaee M, and Soleimani MR. (2015). An intelligent procedure for the detection and classification of chickens infected by *Clostridium perfringens* based on their vocalization. Brazilian Journal of Poultry Science, 17(4): 537-544. DOI: https://doi.org/10.1590/1516-635X1704537-544

Sahoo PK, Kushwaha DK, Pradhan NC, Makwana Y, Kumar M, Jatoliya M, et al. (2022). Robotics Application in Agriculture. In: 55 Annual Convention of Indian Society of Agricultural Engineers and International Symposium, pp. 60-76. Google Scholar

Sharma M, and Patil C. (2018). Recent trends and advancements in agricultural research: An overview. Journal of Pharmacognosy and Phytochemistry, 7(2): 1906-1910. Google Scholar

Vroegindeweij BA, Blaauw SK, IJsselmuiden JM, and van Henten EJ. (2018). Evaluation of the performance of PoultryBot, an autonomous mobile robotic platform for poultry houses. Biosystems Engineering, 174, 295-315. DOI: https://doi.org/10.1016/j.biosystemseng.2018.07.015

Wang J, Shen M, Liu L, Xu Y, and Okinda C. (2019). Recognition and classification of broiler droppings based on deep convolutional neural network. Journal of Sensors, 2019; Article ID 3823515. DOI: https://doi.org/10.1155/2019/3823515

Weis CV, Jutzeler CR, and Borgwardt K. (2020). Machine learning for microbial identification and antimicrobial susceptibility testing on MALDI-TOF mass spectra: a systematic review. Clinical Microbiology and Infection, 26 (10): 1310-1317. DOI: https://doi:10.1016/j.cmi.2020.03.014.

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

Xu J, Luu L, and Tang Y. (2017). Phosphate removal using aluminum-doped magnetic nanoparticles. Desalination Water Treatment. 58: 239-248. DOI: https://doi.org/10.5004/dwt.2017.0356

Xu J, Suarez D, and Gottfried DS. (2007). Detection of avian influenza virus using an interferometric biosensor. Analytical and Bioanalytical Chemistry, 389(4): 1193-1199. DOI: https://doi.org/10.1007/s00216-007-1525-3

Yang X, Chai L, Bist RB, Subedi S, and Wu Z. (2022). A deep learning model for detecting cage-free hens on the litter floor. Animals, 12(15): 1983. DOI: https://doi.org/10.3390/ani12151983

Yang X, Zhang F, Jiang T, and Yang D. (2019). Environmental monitoring of chicken house based on edge computing in internet of things, in: 2019 IEEE 8th Joint International Information Technology and Artificial Intelligence Conference (ITAIC), IEEE, pp. 617-620. DOI: http://dx.doi.org/10.1109/ITAIC.2019.8785634

Zhao Y. (2021). In: Current Status and Industrialization Development of Industrial Robot Technology. Springer, Cham, 2021; pp. 804-808. DOI: https://doi.org/10.1007/978-3-030- 79197 1_117

Zhuang X, and Zhang T. (2019). Detection of sick broilers by digital image processing and deep learning. Biosystems Engineering, 179: 106-116. DOI: https://doi.org/10.1016/j.biosystemseng.2019.01.003

Zhuang X, Bi M, Guo J, Wu S, and Zhang T. (2018). Development of an early warning algorithm to detect sick broilers. Computers and Electronics in Agriculture, 144: 102-113. DOI: https://doi.org/10.1016/j.compag.2017.11.032

Zuidhof M, Fedorak M, Ouellette C, and Wenger I. (2017). Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poultry Science, 96(7): 2254-2263. DOI: https://doi.org/10.3382/ps/pex013

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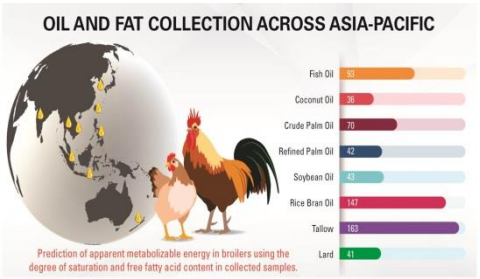

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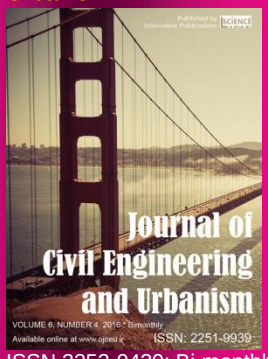

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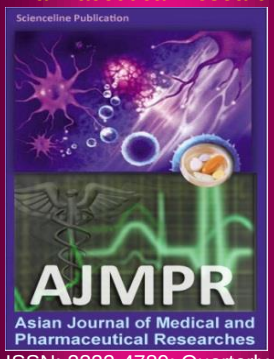

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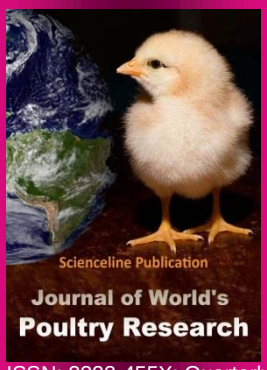

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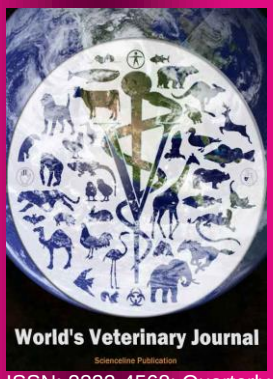

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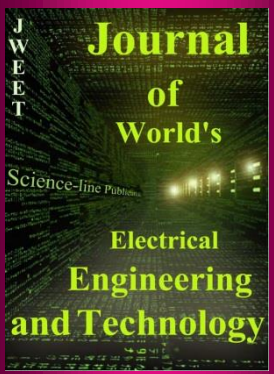

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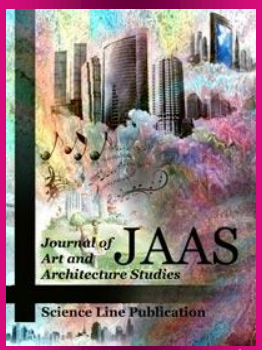

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