Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 6, 750-754

Synergistic effects of noni fruit extract, copper, and zinc encapsulated on immunity, protein digestibility, and growth performance in broiler

Savhira Aulia Rahma[1*], Mulyono[2], Vitus Dwi Yunianto[2], Lilik Krismiyanto[2]

[1]Master of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia
[2]Department of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.

ARTICLE INFO

Recieved: 07 September 2025

Accepted: 28 September 2025

*Correspondence:

Corresponding author: Savhira Aulia Rahma
E-mail address: savirarahma18@gmail.com

Keywords:

Broiler chicken, Copper, Encapsulated, Extract of noni fruit, Zinc

ABSTRACT

This research sought to explore the combined effects of noni fruit extract, copper (Cu), and zinc (Zn) encapsulation on immunity, protein digestibility, and growth performance of broiler chickens. The research used 200 Ross broilers involved, each eight days old and weighing an average of 233.69±7.47 g, which were split into four separate feeding groups, each one being repeated five times. The dietary treatments included T0 (control diet), T1 (T0 + 0.06% noni fruit extract, Cu, and Zn encapsulation), T2 (T0 + 0.12% noni fruit extract, Cu, and Zn encapsulation), and T3 (T0 + 0.18% noni fruit extract, Cu, and Zn encapsulation). ANOVA was administered for statistical analysis, followed by Duncan's test at 0.05 probability. The findings indicated that the inclusion of encapsulated noni fruit extract with Cu and Zn at the T2 level significantly ($P<0.05$) increased lactic acid bacteria (LAB) counts, protein digestibility, thymus relative weight, and daily body weight gain (DBWG), while reducing intestinal pH, E. coli counts, heterophil/lymphocyte (H/L) ratio, and feed conversion ratio (FCR) in comparison with control groups (T0). However, no significant effect ($P>0.05$) was noted on daily feed intake or the relative weights of the bursa Fabricius and spleen. In conclusion, the incorporation of 0.12% noni fruit extract, Cu, and Zn encapsulation improved intestinal bacterial balance, immunological balance, protein digestibility, and growth performance in broiler chickens.

Introduction

Indonesia's broiler chicken industry has continued to grow significantly nationwide in recent years, primarily due to the increasing demand for affordable animal protein among consumers. Broiler remains one of the most demanded animal protein sources in Indonesia. According to Indonesia's Central Statistics Agency, broiler chicken per capita average consumption reached 7.45 kg/year in 2023, or higher by 4.31% compared to 2022. Despite this growth, broiler production in tropical climates, such as Indonesia, encounters numerous environmental stressors, including elevated ambient temperatures, humidity, and pathogen exposure, which can adversely affect immune function and productivity in poultry (Ncho *et al.*, 2024). To address these challenges, the poultry industry traditionally relies on antibiotic growth promoters. However, owing to escalating concerns regarding antimicrobial resistance, the pursuit of natural and safer alternatives has emerged as a global priority (El-Hack *et al.*, 2022).

One promising alternative is the use of phytobiotics, plant-derived bioactive compounds known for their antibacterial, antioxidant, and immunomodulatory properties (Mohamed and Hasan, 2023). Tropical Indonesia offers a variety of phytobiotics, including noni fruit (*Morinda citrifolia* L.), with favorable uses as poultry feed additives. According to the Horticultural Agriculture Statistics of Indonesia (2024), noni fruits are largely cultivated in Indonesia, with the production reaching 4.16 tons in 2023. It contains active compounds such as flavonoids, anthraquinones, triterpenes, xeronines, tannins, and saponins (Zhou and Huang, 2024). The flavonoid and total phenolic contents in noni fruit extract are 5.9±0.008 mg QE/g and 39.4±0.005 mg GAE/g (Meilawati *et al.*, 2021). Flavonoid compounds help foster useful bacteria in the intestine and aid immune function, positively influencing poultry productivity (Sunder *et al.*, 2016).

However, flavonoid compounds are sensitive to environmental stress and oxidation, limiting their effectiveness when administered directly (Speisky *et al.*, 2022). A previous study by Widjastuti *et al.* (2023) reported that adding trace minerals such as copper (Cu) and zinc (Zn) enhances the stability and efficacy of noni fruit bioactive compounds by functioning as catalytic stabilizers that inhibit degradation. This mineral facilitates stabilization, not only maintains the functional integrity of the compounds but also contributes to regarding enhancements in body weight gain and feed conversion in native chickens. Cu and Zn are crucial cofactors for antioxidant enzymes, including superoxide dismutase (SOD), which help protect immune cells, including lymphocytes, from oxidative damage (Espinosa and Stein, 2021).

Nutrient digestibility is susceptible to increase with Cu and Zn mineral supplementation due to their role in supporting the proliferation of lactic acid bacteria (LAB) and inhibiting a proliferation of E. coli by protecting the intestinal barrier function and intestinal mucosal defense (Mirowski *et al.*, 2022; Santos *et al.*, 2020). Humidity, temperature fluctuations, and microbial contamination during storage are some reasons that can affect bioactive compounds in noni fruit extract, Cu, and Zn minerals, leading to a breakdown. This condition can be minimized with the encapsulation technology using maltodextrin as a coating material. This technology will improve the bioavailability and efficiency of bioactive compounds in the small intestine, because it is hydrophilic and easily soluble at neutral pH (Nouri, 2023; Mitsuwan *et al.*, 2023)

The use of noni fruit extract, Cu, and Zn encapsulation in broiler diets has not been reported until now. This research introduced a novel approach using encapsulation technology to improve the bioavailability and nutritional ingestion of active compounds in broiler chicken intestines. This innovation is expected to improve immune health (as indicated by the heterophil/lymphocyte (H/L) ratio and relative weight of lymphoid organs), protein digestibility, and growth performance (daily body weight gain, feed intake, and feed conversion ratio). This research aimed to find out the synergistic effects of noni fruit extract, Cu, and Zn encapsulation on immunity, protein digestibility, and growth performance of broiler chickens.

ISSN: 2090-6277/2090-6269/

Materials and methods

Research methodology, animal and diet

This research used 200 Ross broiler chickens aged 8 days. It had an average body weight of 233.69±7.47 g. The complete randomized trial with four treatments and five replicates was used in the present study. Consequently, 20 experimental units with densities of 10 birds/m2 were obtained. The treatments tested were T0 (control diet), T1 (T0 + 0.06% noni fruit extract, Cu, and Zn encapsulation), T2 (T0 + 0.12% noni fruit extract, Cu, and Zn encapsulation), and T3 (T0 + 0.18% noni fruit extract, Cu, and Zn encapsulation). Feed was provided as crumble during the research (28 days). The control diet ingredients and nutrient composition are outlined in Table 1.

Table 1. Composition and Nutrient Content of Basal Diet.

Feed Stuff	Composition (%)	
	Starter (8-21 days)	Finisher (22-35 days)
Yellow Corn	50.11	53.41
Rice bran	15.04	16.74
Soybean Meal	24	19
Fish meal	10	10
Limestone	0.3	0.3
Premix	0.25	0.25
L-Lysine	0.1	0.1
DL-Methionine	0.2	0.2
Nutrition Contents		
Metabolic Energy (kcal/kg)[1]	2993.57	3018.24
Crude Protein (%)[2]	21.22	19.33
Crude Fat (%)[2]	4.45	4.59
Crude Fiber (%)[2]	5.24	5.51
Calcium (%)[2]	1.04	1.07
Phosporus (%)[2]	0.61	0.74

[1]Based on the Bolton (1967) Formula = 40.81 [0.87(CP+2.25×CF+BETN)+k]
[2]Result of Analysis of Laboratory of Animal Nutrition, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang (2024)

Preparation of noni fruit extract, copper and zinc encapsulation

Noni fruit extract with the same level of ripeness was prepared and cleaned, which was chopped into small slices, dried in an oven at 50°C, and made into fine powder. The extraction procedure by Gouda *et al.* (2021) was used with a slight modification. Ethanol 96% mixed with noni fruit powder with a 1:10 (w/v) ratio and sonicated for 60 minutes at 37°C with a frequency of 50 Hz. The sonicated results were then filtered using filter paper and evaporated to acquire a paste shape. This extract was supplemented with Cu (5 ppm) and Zn (40 ppm) (NRC, 1994). The encapsulation procedure followed the method (Agusetyaningsih *et al.*, 2022). Maltodextrin as a coating material was dissolved in distilled water with a 1:1 (g/mL) ratio, then mixed with the noni extract enriched, Cu and Zn at a ratio of 1:5 (w/w). Freeze drying was used to obtain the encapsulated product in a powder form.

Data collection

The procedure for the total count of small intestinal bacteria (lactic acid bacteria and E. coli) refers to previous research by Sugiharto *et al.* (2017). The digesta specimens from each small intestine were analyzed using total plate count methods to obtain total bacteria (LAB and E. coli). MacConkey agar was used for culturing E. coli and incubated at 38°C for 24 hours. The colonies of E. coli bacteria were counted as red. Lactic acid bacteria were grown on de Man, Rogosa, and Sharpe (MRS) agar and incubated for 48 hours at 38°C under anaerobic conditions. A digital pH meter (Ecotest; pH 1) was used for determining the pH value of the small intestine.

Protein digestibility was determined using the total collection procedure, combined with 0.5% Fe2O3 indicator. Every 2 hours, the excreta were sprayed with 0.2 N HCl, and the collection trays were replaced daily. The collection began when the first red excreta was released on the second day, and it changed in color. On the second day, chickens received a treatment diet without an indicator. The indicator-containing diet was reintroduced on the third day, followed by a treatment diet without the indicator on the fourth day (Krismiyanto *et al.*, 2023).

The heterophil/lymphocyte (H/L) ratio was calculated according to the differences in the count percentage of leukocytes (basophils, eosinophils, and heterophils) as granular cells and non-granular cells such as monocytes and lymphocytes (Sunu *et al.*, 2021). The relative weight of lymphoid organs (Bursa Fabricius, thymus, and spleen) as immune organs was measured using an analytical balance of 0.01 g precision. The weight of each organ was divided by the weight of the living body and multiplied by 100 to get the relative percentage (Agusetyaningsih *et al.*, 2023)

Growth performance data included total daily feed intake (DFI), total daily body weight gain (DBWG), and feed conversion ratio (FCR). The daily feed intake was calculated by subtracting the amount delivered from the amount remaining. Subtracting the final body weight (35 days old) from the initial body weight (8 days old) and subsequently dividing the result by the 28-day rearing period was used for calculating daily body weight gain. Dividing the total daily feed intake by the daily body weight gain was used to determine FCR.

Statistical analysis

Analysis of variance (ANOVA) with IBM SPSS Statistics 25 utilized for statistical analysis, with a significance threshold of 5% (P < 0.05). Duncan's multiple range (DMRT) test, administered to determine significant variations among the treatments (Steel and Torrie, 1995).

Results

Total bacterial, intestinal pH, and protein digestibility

The addition of noni fruit extract and Cu and Zn encapsulation in the diets significantly influenced (P<0.05) the total LAB, E. coli bacteria, small intestinal pH, and protein digestibility (Table 2). Furthermore, the highest total LAB count was observed in T3 and T2, which was significantly different from that in T0 and T1. The lowest total number of E. coli bacteria was observed in T3, which differed significantly from T0 and T1 but was not significantly different with T2. Treatments T2 and T3 also resulted in significantly lower small intestinal pH values than those at T0 and T1. The highest protein digestibility was observed in T3, which differed considerably from T0 and T1 but showed no significant difference from T2.

Immune indicators in broiler chickens

The inclusion of noni fruit extract, along with Cu and Zn encapsulation in the diets, significantly influenced (P<0.05) the decrease in H/L ratio and increase in relative weight of the thymus. In contrast, no significant differences (P>0.05) were found in the relative weights of the bursa Fabricius and the spleen (Table 3). The lowest H/L ratios were recorded at T3 and T2, which were notably different from those at T0 and T1. The relative weight of the thymus in T3 was significantly greater than in the other treatments.

Growth performance of broiler chickens

The inclusion of noni fruit extract, along with Cu and Zn encapsula-

Table 2. Total bacterial, small intestinal pH and protein digestibility.

Parameter	Treatment				P Value
	T0	T1	T2	T3	
Small Intestine					
LAB (log cfu/g)	6.75±0.11[b]	6.96±0.22[b]	7.50±0.20[a]	7.67±0.15[a]	0
E. coli (log cfu/g)	4.59±0.14[a]	4.43±0.12[a]	3.99±0.16[b]	3.91±0.31[b]	0
pH	6.64±0.19[a]	6.41±0.06[b]	6.23±0.10[c]	6.19±0.08[c]	0
Protein Digestibility (%)	76.82±3.64[c]	80.77±2.68[b]	84.36±1.91[ab]	86.17±2.41[a]	0

[a,b,c] Means marked with superscript letter in the same row are significantly different (P<0.05). T0 (control diet), T1 (T0 + 0.06% noni fruit extract, Cu, and Zn encapsulation), T2 (T0 + 0.12% noni fruit extract, Cu, and Zn encapsulation), and T3 (T0 + 0.18% noni fruit extract, Cu, and Zn encapsulation). LAB: Lactic Acid Bacteria. pH: Potential Hydrogen.

Table 3. H/L ratio and the relative weight of lymphoid organs (% body weight).

Parameter	Treatment				P Value
	T0	T1	T2	T3	
H/L Ratio	0.81±0.05[a]	0.70±0.06[b]	0.55±0.06[c]	0.51±0.04[c]	0
Bursa of Fabricius	0.068±0.009	0.068±0.004	0.068±0.001	0.071±0.005	0.85
Spleen	0.095±0.004	0.095±0.004	0.097±0.018	0.099±0.010	0.89
Thymus	0.236±0.031[b]	0.261±0.014[ab]	0.289±0.033[ab]	0.316±0.071[a]	0.05

[a,b,c]Means marked with superscript letter in the same row are significantly different (P<0.05). T0 (control diet), T1 (T0 + 0.06% noni fruit extract, Cu, and Zn encapsulation), T2 (T0 + 0.12% noni fruit extract, Cu, and Zn encapsulation), and T3 (T0 + 0.18% noni fruit extract, Cu, and Zn encapsulation). H/L Ratio: Heterophils/lymphocytes ratio.

Table 4. Growth performance of broiler chickens (Days 8 to 35).

Parameter	Treatment				P Value
	T0	T1	T2	T3	
DFI (g)	112.83±7.31	112.18±3.32	107.07±9.89	103.66±6.32	0.17
DBWG (g)	59.83±1.31[b]	60.78±1.78[b]	63.45±1.47[a]	63.80±2.48[a]	0.00
FCR	1.89±0.13[a]	1.85±0.06[a]	1.69±0.15[b]	1.63±0.10[b]	0.01

[a,b] Means marked with superscript letter in the same row are significantly different (P<0.05). T0 (basal diet); T1 (T0 + 0.06% encapsulated noni fruit extract with Cu Zn); T2 (T0+ 0.12% encapsulated noni fruit extract with Cu Zn); T3 (T0 + 0.18% encapsulated noni fruit extract with Cu Zn). DFI : Daily Feed Intake. DBWG : Daily Body Weight Gain. FCR: Feed Conversion Ratio.

tion in diets, significantly influenced (P<0.05) the increase in daily body weight gain (DBWG) and decrease in feed conversion ratio (FCR). But had no significant impact (P>0.05) on daily feed intake (DFI) (Table 4). Additionally, the daily weight gain observed in the T3 and T2 groups was notably greater than T0 and T1 groups. The FCR values were significantly reduced in T2 and T3 compared with those in T0 and T1.

Discussion

The increase in lactic acid bacteria (LAB) and the simultaneous decrease in Escherichia coli populations observed in the T2 and T3 groups, in comparison to the control group (T0), indicate that these treatments were effective in maintaining bacterial balance in the small intestine. Noni fruit has several bioactive compounds that can act as antibacterial agents, such as flavonoids, anthraquinones, and alkaloids (Liu *et al.*, 2025; Su *et al.*, 2024). These compounds can provide a positive effect in suppressing the multiplication of pathogenic bacteria and supporting the stability of intestinal bacteria. Flavonoids act as antibacterial agents, one of the mechanisms being through interaction with extracellular proteins of the pathogenic bacteria's membrane, thereby disrupting the integrity of the bacterial membrane (Donadio *et al.*, 2021). These disruptions will affect protein denaturation, suppress vital cellular functions, and eventually result in the pathogenic bacterial cell breaking down.

The potential hydrogen (pH) was observed to have a significant reduction in the treatment groups (T1, T2, and T3) compared to the control group (T0). The acidification of the intestinal environment, due to an increase in LAB population and inhibition of E. coli population, is suspected to have contributed to the lower pH in the small intestine. The acidic environment in the small intestine is maintained through a mechanism that decreases pH from LAB, which can produce organic acids through carbohydrate fermentation (Tang *et al.*, 2023). This situation is not etiological for the life of pathogenic bacteria, but beneficial for lactic acid bacteria (Liu *et al.*, 2021).

The stabilization of pH and bacterial balance of the small intestine is also influenced by supplementation of copper (Cu) and zinc (Zn) minerals. The improvement of LAB growth is inhibited by zinc through the mechanism of an antioxidant to minimize free radicals in the small intestine (Chang *et al.*, 2022). This condition supports an acidic intestinal environment, which is influenced by the role of copper minerals in supporting LAB to produce antibacterial metabolites such as short-chain fatty acids (SCFAs), lactic acid, and acetic acid, which can inhibit the colonization of E. coli as a pathogenic bacterium (Wu *et al.*, 2025).

Protein digestibility, as shown in Table 2, increased significantly in the treatment groups (T1, T2, and T3) compared to the control group (T0). The maintained bacterial balance and reduction in intestinal pH are the factors that improve the ability of nutritional digestion (Han *et al.*, 2023). The protease enzyme contributed to increasing the protein digestibility in the treatment groups (T1, T2, and T3). The increase of digestive enzyme activity, especially protease, contributes to improving protein digestibility in the treatment groups. Carneiro *et al.* (2022) explained that LAB can produce more compact extracellular protein, thereby supporting nutrient digestibility by degrading protein complexes into peptides and amino acids.

The heterophil/lymphocyte (H/L) ratio was observed to decrease, and the relative weight of thymus increased significantly in the treatment groups (T1, T2, and T3) compared to the control group (T0). This effect is likely due to the bioactive compounds in noni fruits that can stimulate the immune system of broilers. According to Ribeiro *et al.* (2024) and

Hirakawa *et al*. (2020), a marker of reduced hematological stress in poultry is by lower H/L ratio and an increase in the thymus relative weight. Flavonoid components in noni fruit are considered to protect the body, especially the thymic structure, from lipid peroxidation by acting as donors of hydroxyl groups (–OH) to neutralize free radicals (Gari, 2024). The modulating immune responses in poultry are due to anti-inflammatory mechanisms by inhibiting proinflammatory cytokine production and antioxidant effects via restricting T cell activation (Hasted *et al*., 2021; Zaragozá *et al*., 2020).

The decreased H/L ratio and increased thymus weight offer advantages in the responsive immune function due to copper (Cu) and zinc (Zn) mineral supplementation. This is likely contributed by copper mineral that enhances the phagocytic activity to protect from pathogenic cells and inhibits the production of reactive oxygen species (ROS) that positively impacts immunity function (Ruiz *et al*., 2021; Stafford *et al*., 2013). Superoxide dismutase (SOD), as an antioxidant enzyme, and the differentiation of lymphocytes (T and B cells) are vital in protecting the immune system from free radicals (Lu *et al*., 2024; Cheng *et al*., 2022; Hojyo and Fukada, 2016).

The growth performance results showed that T2 and T3 enhanced daily body weight gain (DBWG) while reducing the feed conversion ratio (FCR) compared with the other treatments (Table 4). This outcome was likely related to improvements in intestinal health, as indicated by the improvement in the total LAB population in the small intestine (Table 2), thereby indicating an improved feed utilization efficiency. According to Hassan *et al*. (2024), a lower FCR indicates that broilers can achieve greater body weight gain with the same or even lower feed intake, resulting in more optimal feed conversion. The insignificant difference in daily feed intake ($P>0.05$) further supports the assumption that the treatment enhanced nutrient utilization efficiency rather than increased consumption. Similarly, Ayalew *et al*. (2022) confirmed that incorporation of feed additives, including phytogenic compounds, can improve nutrient utilization and growth performance in broilers, even when feed intake does not significantly increase.

Encapsulation of Cu and Zn is essential for improving the stability and bioactivity of functional compounds in noni fruit extract to maximize their physiological efficacy, especially the bioactive state during the digestion process in the gastrointestinal tract. According to López *et al*. (2024), the bioactive compounds will be protected by encapsulation as a coating process from degradation during feed processing or storage and passage through the digestive tract. The increased DBWG and decreased FCR values as production performances are attributed to improved gastrointestinal function, which leads to an impact on nutrient utilization efficiency. Furthermore, Dlamini *et al*. (2023) explained that encapsulation technology is designed to protect bioactive compounds from exposure to low pH (acidic conditions) in the proventriculus and mechanical pressure in the ventriculus, thereby optimizing their availability in the small intestine (neutral to slightly alkaline).

Data on physiological parameters and production performance exhibited the most advantageous when adding T2 treatment, comprising 0.12% encapsulated noni fruit extract with Cu and Zn minerals. The T2 treatment significantly supported intestinal health, as indicated by an improvement in LAB population, a decrease in E. coli count, and a decrease in pH value in the small intestine. In addition, T2 also enhances immune function, as indicated by the optimal H/L ratio and thymus weight, thereby contributing to growth performance characterized by improved protein digestibility and DBWG, and a decrease in FCR. The recommended optimal dose is therefore 0.12% encapsulated noni extract with Cu and Zn in broiler feed formulations.

Conclusion

The addition of noni fruit extract, copper, and zinc encapsulation at 0,12% level (T2) improved the total bacterial intestines, protein digestibility, immune health, and growth performance in broiler chickens.

Acknowledgments

The authors thank Mulyono, Vitus Dwi Yunianto and Lilik Krismiyanto for their guidance and the Noni Fruit Team for their technical support.

Conflict of interest

The authors state that there are no conflicts of interest, such as budgetary, personal, or institutional, that might influence the content, interpretation, or presentation of the data and findings of this manuscript.

References

Agusetyaningsih, I., Widiastuti, E., Wahyuni, H.I., Yudiarti, T., Murwani, R., Sartono, T.A., Sugiharto, S., 2022. Effect of encapsulated *Cosmos caudatus* leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Ann. Anim. Sci. 22, 653 – 662.

Agusetyaningsih, I., Marifah, B., Widiastuti, E., Wahyuni, H.I., Yudiarti, T., Sartono, T.A., Sugiharto, S., 2023. Physiological condition, gut morphology and immune responses of broilers supplemented with *Moringa oleifera* leaf extract, whey protein or their combination. Journal of the Indonesian Tropical Animal Agriculture 48, 126 – 142.

Ayalew, H., Zhang, H., Wang, J., Wu, S., Qiu, K., Qi, G., Tekeste, A., Wassie, T., Chanie, D., 2022. Potential feed additives as antibiotic alternatives in broiler production. Front. Vet. Sci. 9, 1 – 15.

Carneiro, A.L.M., Café, M.B., Carvalho, F.B., Oliveira, H.F., Carvalho, D.P., Carvalho, G.B., Gomides, L.P. da S., Stringhini, J.H., 2022. Effects of a monocomponent protease from Bacillus licheniformis on broiler performance, digestibility, and carcass yield. Acta Scientiarum. Animal Sciences 45, 1 – 11.

Chang, Y., Mei, J., Yang, T., Zhang, Z., Liu, G., Zhao, H., Jia, G., 2022. Effect of dietary zinc methionine supplementation on growth performance, immune function, and intestinal health of cherry valley ducks challenged with avian pathogenic Escherichia coli. Front. Microbiol. 13, 1 - 19.

Cheng, F., Peng, G., Lu, Y., Wang, K., Ju, Q., Ju, Y., Ouyang, M., 2022. Relationship between copper and immunity: The potential role of copper in tumor immunity. Front. Oncol. 12, 75 – 82.

Dlamini, S.B., Gigante, A.M., Hooton, S.P., Atterbury, R.J., 2023. Efficacy of different encapsulation techniques on the viability and stability of diverse phage under simulated gastric conditions. Microorganisms J. 11, 1 – 15.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Piaz, D.F., 2021. Interactions with microbial proteins driving the antibacterial activity of flavonoids. Pharmaceutics 13, 66 – 74.

El-Hack, A.M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B., Swelum, A.A., 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult. Sci. 101, 1 – 20.

Espinosa, C. D., Stein, H.H., 2021. Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: a review. Animal Science and Biotechnology J. 12, 1-12.

Gari, J. 2024. Dietary plant flavonoid supplementation for poultry as a potent scavenger of hydroxyl radicals and antioxidants: a review. Peer J. Organic Chem. 6, 1 – 17.

Gouda, M., Bekhit, A.E., Tang, Y., Huang, Y., Huang, L., He, Y., Li, X., 2021. Recent innovations of ultrasound green technology in herbal phytochemistry: A review. Ultrasonics Sonochemistry J. 73, 1-15.

Han, Y., Xu, X., Wang, J., Cai, H., Li, D., Zhang, H., Yang, P.Y., Meng, K., 2023. Dietary Bacillus licheniformis shapes the foregut microbiota, improving nutrient digestibility and intestinal health in broiler chickens. Front. Microbiol. 14, 1 – 14.

Hassan, W.H., Mustafa, M.M., Isa, R.H., 2024. Effect of herbal extracts as alternatives to antibiotics in the first week of age on broiler performance, serum biochemistry, and intestinal morphology under commercial farm conditions. South African Journal of Animal Science 3, 455–465.

Hasted, T.L., Boerlin, P., Sharif, S., Diarra, M.S., 2021. Immunostimulatory potential of fruits and their extracts in poultry. Front. Immunol. 12, 1 – 11.

Hirakawa, R., Nurjana, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T., Toyomizu, M., 2020. Heat stress causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Front. Vet. Sci. 7, 1 – 13.

Hojyo, S., Fukada, T., 2016. Roles of zinc signaling in the immune system. Journal of Immunology Research 2, 1 – 21.

Krismiyanto, L., Suthama, N., Yunianto, V.D., Mulyono, Mangisah, I., Hijriyanti, I., 2023. Protein deposition and carcass weight in broiler given noni fruit extract (*Morinda citrifolia* L.) compared to zinc bacitracin. Journal of Animal Science and Veterinary Medicine 8, 59 – 64.

Liu, L., Li, Q., Yang, Y., and Guo, A., 2021. Biological function of short-chain fatty acids and its regulation on intestinal health of poultry. Front. Vet. Sci. 8, 1 – 21

Liu, Z., Su, C., Yang, M., Wang, F., Zhang, L., Liu, L., Kang, J., 2025. Antibacterial mechanism of noni fruit (*Morinda citrifolia* L.) essential oil against Staphylococcus aureus and its application in beef. Food Control 176, 1 – 11.

López, C.G., Carrillo, L.L.M., Vargas, B.P.E., García, G.I.A., 2024. Microencapsulation of feed additives with potential in livestock and poultry production: a systematic review. Chilean J. of Agric. Anim. Sci. 40, 229 – 249.

Lu, J., Liu, X., Li, X., Li, H., Shi, L., Xia, X., Yang, X., 2024. Copper regulates the host innate immune response against bacterial infection via activation of ALPK1 kinase. In: Proceedings of the National Academy of Sciences, Illinois, USA, pp. 1 – 10.

Meilawati, L., Ernawati, T., Dewi, R.T., Megawati, M., Sukirno, S., 2021. Study of total phenolic, total flavonoid, scopoletin contents and antioxidant activity of extract of ripened noni juice. Indonesian Journal of Applied Chemistry 23, 55-62.

Mirowski, K. S., Biesiada-Drzazga, B., Andraszek, K., Saleh, M.A., Banaszewska, D., 2022. The effect of feed supplementation with Cu and Zn chelates on the content of these elements in the blood of broiler chickens and their body weight and feed conversion. Acta Scientiarum Polonorum seria Zootechnica J. 21, 3 – 10.

Mitsuwan, W., Saengsawang, P., Jeenkeawpieam, J., Nissapatorn, V., Pereira, M.L., Kitpipit, W., Vimon, S., 2023. Development of a microencapsulated probiotic containing Pediococcus acidilactici WU222001 against avian pathogenic Escherichia coli. Veterinary World 16, 1131 – 1140.

Mohamed, M.A., Hassan, H.M.A., 2023. Phytogenic substances as safe growth promoters in poultry nutrition. Inter. J. Vet. Sci. 12, 89-100.

Ncho, C. M., Berdos, J.I., Gupta, V., Rahman, A., Mekonnen, K.T., Bakhsh, A., 2024. Abiotic stressors in poultry production: A comprehensive review. Animal Physiology and Animal Nutrition J. 1, 1 – 12.

Nouri, A. 2023. Encapsulated organic acids as a safe alternative or synergistic compound with anticoccidials for more effective control of coccidiosis in Eimeria-challenged broilers. Livestock Science J. 278, 1 – 14.

Ribeiro, L.R.R., Sans, E.C.O., Santos, R.M., Taconelli, C.A., Farias, R., Molento, C.F.M., 2024. Will the white blood cells tell? a potential novel tool to assess broiler chicken welfare. Front. Vet. Sci. 11, 1 – 16.

Ruiz, L.M., Elorza, A.A., Libedinsky, A., 2021. Role of copper on mitochondrial function and metabolism. Front. Mol. Biosci. 8, 1 – 17.

Santos, T.S.D., Teng, P.Y., Yadav, S., Castro, F.L.D.S., Gould, R.L., Craig, S.W., Kim, W.K., 2020. Effects of inorganic Zn and Cu supplementation on gut health in broiler chickens challenged with Eimeria spp. J. Front. Vet. Sci. 7, 1 - 8.

Speisky, H., Shahidi, F., Camargo, A.C., Fuentes, J., 2022. Revisiting the oxidation of flavonoids: loss, conservation or enhancement of their antioxidant properties. Antioxidants 11, 1 – 28.

Stafford, S. L., Bokil, N.J., Achard, M.E., Kapetanovic, R., Schembri, M.A., McEwan, A.G., Sweet, M.J. 2013. Metal ions in macrophage antimicrobial pathways: emerging roles for zinc and copper. Bioscience Reports 33, 1 – 14.

Statistics Indonesia. 2024. Biopharmaceutical Crop Production by Province and Crop Type. Directorate of Statistics, Jakarta. https://www.bps.go.id/id/statistics-table/3/ (accessed 4 December 2024)

Statistics Indonesia. 2024. Broiler Meat Consumption by Province. Directorate of Statistics, Jakarta. https://www.bps.go.id/id/statistics-table/ (accessed 15 November 2024)

Steel, R. G. D., Torrie, J. H., 1995. Principles and procedures of statistics. McGraw-Hill Book Company, New York, USA.

Su, C., Yang, M., Chen, S., Fu, C., Zhang, L., Liu, S., Li, C., 2024. Multiple metabolite profiles uncover remarkable bioactive compounds and metabolic characteristics of noni fruit (*Morinda citrifolia* L.) at various stages of ripeness. Food Chemistry 450, 1 – 11.

Sugiharto, S., Yudiarti, T., Isroli. I., Widiastuti, E., Putra, F.D., 2017. Effect of dietary supplementation with Rhizopus oryzae or *Chrysonilia crassa* on growth performance, blood profile, intestinal microbial population, and carcass traits in broilers exposed to heat stress. Arch. Anim. Breed 60, 347-356.

Sunder, J., Sujatha, T., Kundu, A., 2016. Effect of *Morinda citrifolia* in growth, production and immunomodulatory properties in livestock and poultry: a review. Journal of Experimental Biology and Agricultural Sciences 4, 249-265.

Sunu, P., Sunarti, D., Mahfudz, L.D., Yunianto, V.D., 2021. Effect of synbiotic from Allium sativum and Lactobacillus acidophilus on hematological indices, antioxidative status and intestinal ecology of broiler chicken. Journal of the Saudi Society of Agric. Sci. 20, 103-110.

Tang, H., Huang, W., Yao, Y.F., 2023. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. Microbial Cell 10, 49–62.

Widjastuti, T., Komala, I., Tanwiriah, W., Nurlaeni, L., 2023. Application of noni fruit (*Morinda citrifolia* L.) extract with Cu and Zn supplemented in the ration on performance chicken sentul of phase developer. International Journal on Advanced Science, Engineering and Information Technology 13, 1 – 6.

Wu, X., Zhou, Y., Lu, Z., Zhang, Y., Zhang, T., Jiang, Q., 2024. Cupric citrate supplementation improves growth performance, nutrient utilization, antioxidant capacity, and intestinal microbiota of broilers. Animal Bioscience 38, 530 – 538.

Zaragozá, C., Villaescusa, L., Monserrat, J., Zaragozá, F., Álvarez-Mon, M., 2020. Potential therapeutic anti-inflammatory and immunomodulatory effects of dihydroflavones, flavones, and flavonols. Molecules 25, 1 – 13.

Zhou, S., Huang, G., 2024. The chemical composition and pharmacological activities of *Morinda citrifolia*. Applied Biological Chemistry 67, 1 – 18.