Buletin Peternakan 46 (2): 104-111, May 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i2.73251

Performance and Haematological Profile of Broiler Chickens Fed Diet Containing Atung (*Parinarium glaberrimum* Hassk.) Seed Powder

Sylvia Christina Hellena Hehanussa[1,3], Zuprizal[2], Chusnul Hanim[2]*, and Nanung Danar Dono[2]

[1]Departement of Animal Science, Faculty of Agriculture, Universitas Pattimura, Ambon, 97233, Indonesia
[2]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[3]Doctoral Student, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Article history
Submitted: 24 February 2022
Accepted: 16 March 2022

* Corresponding author:
Telp. +62 812 2528 7665
E-mail: c.hanim@ugm.ac.id

ABSTRACT

This study investigated the effect of feeding atung (*Parinarium glaberrimum* Hassk.) seed meal on growth performance and haematology profile in broiler chickens. A total of 140 one-day-old male New Lohmann broiler chicks were randomly assigned to five treatment groups with four replicates and seven birds in each replicate pen. The dietary treatments consisted of feeding the same corn-soybean meal as the basal diet with atung seed powder inclusions at levels of 0, 0.5, 1.0, 2.0, and 4.0%, respectively. The data were statistically analyzed using One-way with five treatments and four replications and continued with Duncan's New Multiple Range Test for data with a significant difference. Results showed that feed intake increased ($p<0.05$) in broiler chickens fed diets with 0.5 and 1% atung seed powder. At the same time, the inclusion of atung seed powder had no effect on final body weight, weight gain, and FCR. The inclusion of 1 and 2% atung seed powder significantly increased leukocyte counts, as well as the PCV value trend increased with the inclusion of atung seed powder. It can be concluded that atung seed powder additives can be supplemented in broiler chickens feed at doses of 1% by improving feed intake and some hematological profiles.

Keywords: Atung seed, Broiler chickens, Hematology profile, Performance

Introduction

In Indonesia, broiler chicken is a commodity that plays an essential role in providing animal protein requirements and supporting the community's economy. Therefore, the production and consumption numbers of broiler chickens in Indonesia are rise every year. On the other hand, broiler chickens are susceptible to disease infections that increase mortality and reduce productivity. For approximately 60 years, antibiotic growth promoters (AGP) at sub-therapeutic levels have been used in poultry feed to improve feed efficiency, growth rate, and animal health (Dibner and Richards, 2005).

The use of AGP in the poultry diet has been banned i n many countries due to the concern for the growing number of antibiotic-resistant bacteria that have adverse impacts on human health (Cheng *et al*., 2014). These antibiotic-resistant bacteria and their resistance factors can be transferred from animals to humans, which decreases the efficacy of antibiotics used for medicinal purposes (Dibner and Richards, 2005; Cheng *et al*., 2014). After the ban of antibiotics, many studies were conducted to find safer additives for organic animals production, including probiotics, prebiotics, synbiotics, organic acids, toxin binders, enzymes, and phytobiotics (Yang *et al*., 2009; Hashemi and Davoodi, 2010; Cheng *et al*., 2014; Abd El-Hack *et al*., 2022).

Phytobiotic feed additives were used in poultry feed to maintain performance and health, and it was gained increasing interest in recent decades, especially after the forbidden of AGP. Phytobiotics are plant-origin materials, such as herbs, botanicals, and essential oils, which could be improved animals' growth performance and health (Windisch *et al*., 2008; Hashemi and Davoodi, 2010). Plant-origin contains active compounds that have biological activities, especially antibacterial, anti-inflammatory, antioxidant, and immune-modulatory properties which are known to be helpful for the animal's health and enhance productivity (Windisch *et al*., 2008; Hashemi and Davoodi, 2010). Some previous studies showed that administration of phytobiotics feed additives improved the growth rate and feed efficiency (Abu-Dieyeh and Abu-Darwish, 2008; Oso *et al*., 2019; Ampode and Asimpen, 2021; Khalid *et al*., 2021) in broiler chickens. Administration of phytobiotic additives has also been reported to affect hematological parameters in broiler chickens (Ansari *et al*., 2012; Basit *et al*., 2020), which is a good indicator in evaluating the physiological and health status of animals.

In Indonesia, various types of phytobiotics to maintain broiler chickens' productivity have been widely used since the AGP post era, as Sugiharto (2021) reviewed, except atung plant. Atung (*Parinarium glaberrimum* Hassk.) is a local medicinal plant widespread in Maluku province, Indonesia. Historically, the seed of the plant has been used to treat diarrhea and bleeding in pregnant women, as well as it also used as seafood, preservatives (Moniharapon and Hashinaga, 2004). *In vitro* studies have previously shown that atung seed has antibacterial properties (Moniharapon and Hashinaga, 2004; Pacana and Galarpe, 2017b; Hehanussa *et al*., 2019) and antioxidant activities (Sarastani *et al*., 2002). Based on its biological activity, atung seed potentially be used as additives in broiler chicken feed. These antibacterial properties may likely modulate gut microflora positively, which plays an important role in enhancing birds' performance and health. On the other hand, atung seed has no economic value, with results that can be used in poultry feed without increasing feed cost significantly.

In the current study, we added atung seed powder to broiler chickens' feed as an additive to evaluate its effects on growth performance and hematological parameters. To our knowledge, no kinds of literature are available regarding the usage of atung seed as a phytobiotics feed additive, so this paper is the first report about the supplementation effect of atung seed powder in broiler chickens' feed on performance and hematological profile.

Materials and Methods

This research was conducted at the Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. A total of 140 male New Lohman MB 202 DOC (PT. Japfa Comfeed Indonesia, Indonesia) were reared in a litter-floor pen for 35 days. The DOCs were randomly assigned to five treatments with four replications. Each replication consisted of seven chickens placed in a 100 x 85 cm2 litter-floor pen. The temperature in the cage was set at 32ºC when the chickens were 1-4 days old, and then the temperature was lowered slowly according to the New Lohman MB 202 broiler maintenance standard. Temperature, humidity, ventilation, and lighting were set as similar as possible for all treatment groups.

Broiler chickens in all treatments were vaccinated against Newcastle Disease (Medivac ND La Sota, PT. Medion Farma, Bandung, Indonesia) at the age of 4 days and 21 days, respectively, through eye drops and drinking water. The vaccination against gumboro/infectious bursal disease (Medivac Gumboro A, PT. Medion Farma, Bandung, Indonesia) was administered at 18 days old through drinking water. Broiler chickens in all treatments were free to access drinking water and feed (*ad libitum*).

Atung seed powder and feed ingredients used in this study were analyzed for their nutrient content (proximate analysis, calcium, and phosphorus) (AOAC, 2005) to formulate the experimental diets. The energy metabolism of feed ingredients was following the National Research Council (NRC, 1994) database. The experimental diets were formulated following broilers' nutrient requirements according to the standard requirements recommended by the National Research Council (NRC, 1994). The control treatment group was broiler chickens that received basal feed based on a corn-soybean powder without atung seed powder supplemented. The other treatment groups were broiler chickens fed a basal diet with supplemented atung seed powder of 0.5%, 1.0%, 2.0%, and 4.0%, respectively. The composition of feed ingredients and nutrient content of the experimental diets are shown in Table 1. The experimental diet was given gradually on the first day with experimental diet and commercial feed (PT. Japfa Comfeed Indonesia, Indonesia) ratio of 0:100%, 25:75%, 50:50%, 75:25%, and 100:0%. On the seventh day, the treatment diets were given completely. Blood sampling for hematological parameters was also carried out on the 35th day for one chicken from each replication pen.

Atung seed powder preparation

Atung seeds were obtained from Soya village, Maluku Province, Indonesia. Atung seeds used in this study were collected from ripe fruit (fruit that has fallen from the tree). The seeds were removed from the pell that wrapped them, air-dried, and then crushed at a size of 40 mesh for use in mixed diet treatment.

Performance data measurement

Feed intake was measured every day from the first day until the 35th day by calculating the difference amount of the feed provided and the rest in each pen. The chickens were weighed weekly to measure their body weight gain. Weight gain was calculated by subtracting the final body weight to the initial body weight of the chickens. Feed conversion ratio (FCR) was measured weekly and presented in an average of five weeks for each replication pen.

Hematological parameter measurement

On the 35th day, one chicken in each pen was chosen for blood sampling for hematological parameters analysis. Fresh blood samples were collected from the brachial vein at the wing using a sterile syringe and put into a vacutainer tube containing Ethylene Diamine Tetra acetic Acid (EDTA) as an anticoagulant. Hematological parameters studied were red blood cells (RBC),

Figure 1. Atung (*Parinarium glaberrimum* Hassk.) tree and seed.

Table 1. Ingredients and nutrient composition of dietary treatments for broiler chickens (0-35 d of age)

Ingredients (%)	Levels of atung seed powder (%)				
	0	0.5	1	2	4
Yellow corn	58.50	58.50	58.50	58.50	58.50
Soybean meal	24.25	24.25	24.25	24.25	24.25
Meat bone meal	6.75	6.75	6.75	6.75	6.75
Rice bran	2.00	2.00	2.00	2.00	3.00
Palm oil	3.00	3.00	3.00	3.00	2.00
DL-Methionine 99%	0.20	0.20	0.20	0.20	0.20
L-Lysine 78%	0.10	0.10	0.10	0.10	0.10
L-Threonine 78%	0.05	0.05	0.05	0.05	0.05
NaCl	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix[1]	0.20	0.20	0.20	0.20	0.20
Dicalcium phosphate	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.50	0.50	0.50	0.50	0.50
Atung seed powder	0.00	0.50	1.00	2.00	4.00
Filler (sand)	4.00	3.50	3.00	2.00	0.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient composition:					
ME[2] (kcal/kg)	3017	3035	3053	3088	3101
Crude protein (%)	21.01	21.04	21.07	21.13	21.35
Crude fat (%)	6.54	6.55	6.56	6.58	5.70
Crude fiber (%)	3.04	3.05	3.07	3.10	3.42
Calcium (%)	1.01	1.02	1.03	1.05	1.09
Available Phosphorus (%)	0.47	0.47	0.47	0.47	0.48
Methionine (%)	0.52	0.52	0.52	0.52	0.52
Lysine (%)	1.15	1.15	1.15	1.15	1.16
Threonine (%)	0.84	0.84	0.84	0.84	0.84

[1]Vitamin-mineral premix composed of vitamin A 1.250.000 IU, vitamin D 250.000 IU, vitamin E 750 IU, vitamin K3 200 mg, vitamin C 5.000 mg, vitamin B1 250 mg, vitamin B2 400 mg, vitamin B6 100 mg, vitamin B12 1.2 mg, biotin 20 mg, folic acid 50 mg, nicotinic acid 3.000 mg, Ca-d pantothenate 400 mg, cholin chloride 1.500 mg, copper 500 mg, iron 2.500 mg, iodine 20 mg, manganese 6.000mg, selenium 20 mg, zinc 7.000 mg, cobalt 20 mg, Lysine 16.000 mg, DL-methionine 5.000 mg; [2]ME = metabolizable energy.

white blood cells (WBC), heterophils, lymphocytes, haemoglobin, and packed cell volume (PCV), which were analyzed according to the method described by Jain (1986). Determination of total plasma protein was carried out using a microcapillary tube. The microcapillary tube containing blood samples was centrifuged using a microcentrifuge for 15 minutes, the section of plasma layer was broken, and plasma was dropped to a refractometer, and then the total plasma protein was measured.

Statistical analysis

Data were statistically analyzed using Oneway with five treatments and four replications and continued subsequently with Duncan's New Multiple Range Test for the data with significant differences. Data were statistically analyzed using SPSS for Windows tools (SPSS, version 25, IBM, New York, USA). The statistical significance was assumed when probability value $p<0.05$ and the tendency to be a significant difference when $p>0.05$ and $p<0.10$.

Results and Discussion

Growth performance

Table 2 showed that dietary supplementation of 0.5% and 1% atung seed powder increased ($p<0.05$) feed intake but subsequently decreased at 2 and 4% of the supplementation. The inclusion of phytobiotic additives in animal feed improved the flavor and palatability, the digestive tract secretions, and total feed intake (Windisch *et al*., 2008), even though the flavor effect on feed consumption in poultry was less compared to ruminants. The specific compound in atung seed that can increase feed intake is unknown, but atung seed contains aromatic compounds of phenol and its' derivates. Tipu *et al.* (2006) reported that aromatic compounds of herbs and spices stimulated appetite and digestive tract secretion.

The increasing levels of atung seed powder at 2 and 4% decreased feed intake of the birds compared to the bird in groups fed of 0.5 and 1% atung seed powder, which was most likely related to the anti-nutritional factors (tannins)

Table 2. The effect of atung seed powder supplementation on performance of 35 d old broiler chickens

Items	Levels of atung seed powder (%)					SEM[1]	*p*
	0	0.5	1	2	4		
Feed intake (g/bird)	3185[a]	3434[b]	3433[b]	3177[a]	3168[a]	40.9	0.03
Final body weight (g/bird)	1896	1992	1867	1862	1819	30.1	0.49
Weight gain (g/bird)	1848	1944	1820	1815	1772	30.1	0.49
FCR	1.72	1.77	1.89	1.76	1.80	0.02	0.19

[a,b] Mean in the same row without common letter are different at $p<0.05$; [1]SEM=standard error of mean.

content in atung seed. Some studies have previously shown that atung seed contains phenols, tannins, flavonoids, alkaloids, and saponins (Pacana and Galarpe, 2017a; Hehanussa *et al*., 2019). Tannins are considered anti-nutritional for animals, and non-ruminant is more sensitive to tannin when compared to ruminant (Mueller and Harvey, 2006). The presence of tannins in high amounts in the non-ruminants feed could be decreased feed intake, which was in agreement with the studies by Biswas and Wakita (2001) and Sarker *et al.* (2010). In addition, several studies by Khalaji *et al*. (2018), Olumide *et al*. (2018), and Oloruntola (2018) have found that high doses in the inclusion of phytobiotic additives of Camellia L extract, *Ocimum gratissimum*, and glyciridia leaf meal, respectively, reduced feed intake and weight gain of broiler chickens. Oloruntola (2018) suggested that phytobiotic inclusion at high doses could be impaired the palatability of feed caused by anti-nutritional factors was contained in the plant origin.

The result showed that supplementation of atung seed powder to a level of 4% had no effect on final body weight, weight gain, and feed conversion. Inclusion of atung seed powder at 0.5 and 1% increased feed intake compared to other groups but at the same time did not increase weight gain significantly, which therefore could not improve feed conversion. It might be related to anti-nutritional content (tannins) in atung seed, which affects nutrient digestibility and broiler chicken's performance. Tannins can bind to the protein of feed and enzymes, and complex protein-tannins form causes a decrease in protein digestibility and digestive enzyme activity (Mueller and Harvey, 2006). Several studies showed that inclusion of polyphenol-rich phytobiotic feed additives at higher doses in poultry feed leads to a fall in feed intake (Sarker *et al*., 2010; Jelveh *et al*., 2018), final body weight and weight gain (Biswas and Wakita, 2001; Sarker *et al.,* 2010; Chamorro *et al*., 2013; Jelveh *et al*., 2018) and feed efficiency (Chamorro *et al*., 2013; Jelveh *et al*., 2018).

In concordance with our findings, Khalaji *et al*. (2018) reported that supplementation of black cumin and *Artemisia sieberi* phytobiotic additives did not affect weight gain and feed conversion in broiler chickens. Similar results have been obtained by Aditya *et al*. (2016), Farahat *et al*. (2016), and Kiczorowska *et al*. (2016). Yang *et al*. (2009) suggested that supplementation of herbal plants may result in varying effects on the growth performance of broiler chickens due to influenced by several factors, such as plant parts, physical properties, the doses of administration, and compatibility with other ingredients. In addition, Alzawqari *et al*. (2016) also stated that the effectiveness of phytobiotics was influenced by several factors such as the content of active compounds, physiological conditions of animals, feed, and environment (sanitary condition). During the study, the chickens were reared in good sanitation, which may explain why supplementation of atung seed powder has not affected growth performance significantly. On the other hand, some phytobiotics may contain harmful substances even though they belong to the natural additives group (Yang *et al*., 2009). The no significant difference in the final weight, weight gain, and FCR of broiler chickens in the current study could be attributed to the presence of antinutritional factors contained in atung seed.

Haematological parameters

Basit *et al*. (2020) suggested that blood haematology testing in the animal experiment is significant for evaluating the toxic effects of feed or feed additives, such as phytobiotics or plant-origin supplemented. In addition, blood haematology values also could be used to determine animals' physiological and pathological status.

Results in Table 3 showed that inclusion of atung seed powder up to 4% did not affect RBC, haemoglobin, plasma protein, and H/L ratio in broiler chickens. The RBC value ranged from 1.58-3.82 x 10^6/µl, haemoglobin 7.4-12.2 g/dl, leukocytes 9.20-28.60 x 10^3/µl, and PCV 24.9-40.7% (Mitruka and Rawnsley,1981) for a healthy bird. It was shown that haematological parameters of RBC, PCV, and haemoglobin were still within the normal range in birds fed diets with atung seed powder supplemented and birds in the control group. It could be indicated that broiler chickens fed diets with atung seed powder supplemented were not disturbed in their physiological status and did not lack oxygen-carrying for their body's metabolic needs.

The PCV values in birds fed diets with atung seed powder supplemented trend to increased (p=0.085) compared to the birds in the control group. The hematocrit is generally an indicator to determine the ability of the blood to carry oxygen (O2) and nutrient (Olumide *et al*., 2018), which indicates that the inclusion of atung seed powder has a positive effect on oxygen and nutrient transport. The current study finding is in agreement with several studies of Ansari *et al.* (2012), Oleforuh-Okoleh *et al.* (2015), and Basit *et al*. (2020), who indicated that the inclusion of *A. indica* leaf meal, garlic water extract,

Table 3. The effect of atung seed powder supplementation on the selected haematological parameters of 35 d old broiler chickens

Item	Levels of atung seed powder (%)					SEM[1]	*p*
	0	0.5	1	2	4		
RBC ($x10^6$/µl)	3.04	3.00	3.66	2.94	2.87	0.140	0.518
Haemoglobin (g/dl)	8.93	7.40	7.83	7.95	7.25	0.290	0.549
PCV (%)	27.50	31.75	32.67	33.25	32.00	0.750	0.085
Plasma protein (g/dl)	3.63	3.78	3.47	3.58	3.27	0.090	0.555
WBC ($x10^3$/ul)	5.08[a]	7.67[a]	13.71[bc]	15.48[c]	9.42[ab]	1.182	0.005
H/L rasio	0.58	0.52	0.57	0.63	0.72	0.039	0.652

[a,b,c] Mean in the same row without common letter are different at p<0.05; [1]SEM=standard error of mean.

Persicaria odorata leaf meal, respectively, increased PCV values in broiler chickens.

The findings in the current study also showed that inclusion of atung seed powder at 1 and 2% significantly increased (p<0.05) WBC counts compared to that of in the control group. Odoh and Bratte (2015) suggested that an increase or decrease in leucocyte counts from the normal ranges indicates inflammation, autoimmune disorders, leukemia, allergies, bone marrow depression, or stress. On the other hand, the increase in WBC counts (still within the normal ranges) indicates a better ability to against infections disease (Olumide *et al*., 2018; Adedeji *et al*., 2019), which is seen in the birds fed diets with 1 and 2% atung seed powder. This result is comparable with some previous studies of Oleforuh-Okoleh *et al.* (2015), Oloruntola (2018), Olumide *et al*. (2018), and Ansari *et al*. (2012), who reported that inclusion phytobiotic additives of garlic water extract, Glisiridia leaf meal, *Ocimum gratissimum* leaf meal, and *A. indica* leaf meal, respectively, increased WBC numbers significantly in broiler chickens. The WBC counts of birds in the control group were lowest, which could be assumed that these birds have less ability to defend against infection disease when compared to the other groups. Oloruntola (2018) suggested that the high WBC numbers in animals fed phytobiotic additives could be due to increased white blood cells production against infection disease, reactions to drugs, and the immunomodulatory effect of phytobiotic compounds.

The increase in WBC counts in birds supplemented with atung seed powder was likely due to the beneficial biological properties of flavonoids contained in atung seed. Flavonoids are claimed as health-promoting and disease-preventing substances (Middleton, 1998) for both animals and humans, which has known that they possess antibacterial, antiviral, anti-inflammatory, antioxidant (Cushnie and Lamb, 2005), and immunomodulatory (Nagarathna *et al*., 2014; Kamboh *et al*., 2016) properties. These properties of flavonoids may likely work synergistically to improve the immune system in animals. Nagarathna *et al*. (2014) stated that the immune system is a complex organization that involves WBC, antibodies, and other blood factors that protect the body from a foreign substance. Middleton (1998) suggested that flavonoids stimulated various cell types, including basophils, neutrophils, eosinophils, T and B lymphocytes, macrophages, platelets, and others involved in immune function. Kamboh *et al.* (2016) and Nagarathna *et al.* (2014) reported that flavonoids increased humoral immunity. The supplementation of flavonoids was also reported to increase the responsiveness of T and B lymphocytes for antibody synthesis (Kamboh *et al*., 2016) and WBC counts (Kamboh *et al*., 2018) in broiler chickens.

Sugiharto *et al.* (2016) suggested that the H/L ratio correlated with stress in animals. Adrenal stress response in poultry consistently increases the H/L ratio (Grasman, 2002). The H/L ratio amongst all treatments was no different, which indicates that birds fed diets containing atung seed powder up to 4% were in the same stress response condition as birds in the control group. However, the inclusion of 4% atung seed powder elicited the highest H/L ratio. It could be related to the lowest weight gain of birds in that treatment, even though there were no differences in statistical analysis.

Conclusions

The inclusion of atung seed powder had no improved growth rate and FCR, but it can improve the haematological value of PCV and leucocyte counts. Atung seed powder additives can be supplemented in broiler chickens feed at 1% by improving feed intake and some haematological profiles.

Acknowledgement

The authors are grateful to the Ministry of Research, Technology, and Higher Education for funding support through the Domestic Education Scholarship (BPDN) and the Research Directorate of Universitas Gadjah Mada for funding support through the Final Assignment Recognition Program (RTA) 2019.

References

Abd El-Hack, M. E., M T. El-Saadony, H. M. Salem, A. M. El-Tahan, M. M. Soliman, G. B. A. Youssef, A. E. Taha, S. M. Soliman, A. E. Ahmed, A. F. El-kott, K. M. Al Syaad and A. A. Swelum. 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult. Sci. 101: 101-696. https://doi.org/10.1016/j.psj.2022.101696

Abu-Dieyeh, Z. H. M. and M. S. Abu-Darwish. 2008. Effect of feeding powdered black cumin seeds (*Nigella sativa* L.) on growth performance of 4-8 week-old broilers. J. Anim. Vet. Adv. 7: 292-296.

Adedeji, O. S., S. R. Amao, and K. S. Olugbemiga. 2019. The effect of dietary inclusion of *Sesame indicum* leaves on haematology and serum parameters of broiler chickens. Pan Afri. J. Life Sci. 2: 73-78. http://dx.doi.org/10.36108/pajols/9102/20(0130)

Aditya, S., M. Ahammed, S. H. Jang, and S. J. Ohh. 2016. Effects of dietary onion (*Allium cepa*) extract supplementation on performance, apparent total tract retention of nutrients, blood profile and meat quality of broiler chicks. Asian-australas. J. Anim. Sci. 30: 229-235. https://doi.org/10.5713/ajas.16.0553

Alzawqari, M. H., A. A. Al-Baddany, H. H. Al-Baadani, I. A. Alhidary, R. U. Khan, G. M. Aqil, and A. Abdurab. 2016. Effect of feeding dried sweet orange (*Citrus sinensis*) peel and lemon grass (*Cymbopogon citratus*) leaves on growth performance, carcass traits, serum metabolites and antioxidant status in broiler during the finisher phase. Environ. Sci. Pollut. Res. 23: 17077 - 17082. https://doi.org/10.1007/s11356-016-6879-7

Ampode, K. M. B. and S. M. Asimpen. 2021. Neem (A*zadirachta indica*) leaf powder as phytogenic feed additives improves the production performance, and immune organ indices of broiler chickens. Journal of Animal Health and Production 9: 362-370. http://dx.doi.org/10.17582/journal.jahp/2021/9.4.362.370

Ansari, J., S. H. Khan, A. ul Haq, and M. Yousaf. 2012. Effect of the levels of Azadirachta indica dried leaf meal as phytogenic feed additive on the growth performance and haemato-biochemical parameters in broiler chicks. J. Appl. Anim. Res. 40: 336 - 345. https://doi.org/10.1080/09712119.2012.692329

AOAC. 2005. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Basit, M. A., A. A. Kadir, T. C. Loh, S. A. Aziz, A. Salleh, U. Kaka, and S. B. Idris. 2020. Effects of inclusion of different doses of *Persicaria odorata* leaf meal (POLM) in broiler chicken fed on biochemical and haematological blood indicators and liver histomorphological changes. Animals. 10: 1-18. https://doi.org/10.3390/ani10071209

Biswas, A. H. M. and M. Wakita. 2001. Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profiles in broilers. J. Poult. Sci. 38: 50-57. http://dx.doi.org/10.2141/jpsa.38.50

Chamorro, S., A. Viveros, C. Centeno, C. Romero, I. Arija, and A. Brenes. 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. Animal. 7: 555-561. https://doi.org/10.1017/S1751731112001851

Cheng, G., H. Hao, S. Xie, X Wang, M. Dai, L. Huang, and Z. Yuan. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front. Microbiol. 5: 1-15. https://dx.doi.org/10.3389%2Ffmicb.2014.00217

Cushnie, T. P. T. and A. J. Lamb, 2005. Antimicrobial activity of flavonoid. Int. J. Antimicrob. Agents. 26: 343 - 356. https://dx.doi.org/10.1016%2Fj.ijantimicag.2005.09.002

Dibner, J. J. and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84: 634 – 643. https://doi.org/10.1093/ps/84.4.634

Farahat, M., F. Abdallah, T. Abdel-Hamid, and A. Hernandez-Santana. 2016. Effect of supplementing broiler chicken diets with green tea extract on the growth performance, lipid profile, antioxidant status and immune response. Br. Poult. Sci. 57: 714-722. https://doi.org/10.1080/00071668.2016.1196339

Grasman, K. A. 2002. Assessing immunological function in toxicological studies of avian wildlife. Integr. Comp. Biol. 42: 34 - 42. https://doi.org/10.1093/icb/42.1.34

Hashemi, S. R. and H. Davoodi. 2010. Phytogenics as new class of feed additive in poultry industry. J. Anim. Vet. Adv. 9: 2295-2304. http://dx.doi.org/10.3923/javaa.2010.2295.2304

Hehanussa, S. C. H., Zuprizal, N. D. Dono, and C. Hanim. 2019. Potential of atung seeds (*Parinarium glaberrimum* Hassk.) as a phytobiotic candidate in poultry ration based on nutrient composition, phytochemical and antibacterial properties. IOP Conf Series: Earth and Environmental Science 387(2019) 012072.

Jain, N. C. 1986. Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia.

Jelveh, K., B. Rasouli, A. Seidavi, and S. S. Diarra. 2018. Comparative effects of Chinese green tea (*Camellia sinensis*) extract and powder as feed supplements for broiler chickens. J. Appl. Anim. Res. 46: 1114–1117. https://doi.org/10.1080/09712119.2018.1466707

Kamboh, A. A., M. A. Khan, U. Kaka, E. A. Awad, A. M. Memon, M. Saeed, N. A. Korejo, M. Bakhetgul, and C. Kuma. 2018. Effect of dietary supplementation of phytochemicals on immunity and haematology of growing broiler chickens. Ital. J. Anim. Sci. 17: 1038–1043. https://doi.org/10.1080/1828051X.2018.1438854

Kamboh, A. A., S. Q. Hang, M. A. Khan, and W. Y. Zhu. 2016. *In vivo* immunomodulatory effects of plant flavonoids in lipopolysaccharide-challenged broilers. Animal. 10: 1619–1625. https://doi.org/10.1017/s1751731116000562

Khalaji, S., M. Zaghari, K. H. Hatami, S. Hedari-Dastjerdi, L. Lotfi, and H. Nazarian. 2018. Black cumin seeds, Artemisia leaves (*Artemisia sieberi*), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poult. Sci. 90: 2500–2510. http://dx.doi.org/10.3382/ps.2011-01393

Khalid, N., S. F. Sakhawat, H. Mehmood, M. M. Ali, R. Ali, Y. Amin, and M. Ayaz. 2021. Effects of *Moringa oleifera* leaf powder as feed additives on growth performance and blood parameters of broiler. J. Anim. Health Production 9: 271-276. http://dx.doi.org/10.17582/journal.jahp/2021/9.3.271.276

Kiczorowska, B., A. R. M. Al-Yasiry, W. M. Samolińska, A. Marek, and E. Pyzik. 2016. The effect of dietary supplementation of the broiler chicken diet with *Boswellia serrata* resin on growth performance, digestibility, and gastrointestinal characteristics, morphology, and microbiota. Livest. Sci. 191: 117-124. https://doi.org/10.1016/j.livsci.2016.07.019

Middleton, E. Jr. 1998. Effect of plant flavonoids on immune and inflammatory cell function. In: Flavonoids in the Living System. Plenum Press, New York.

Mitruka, B. M. and H. M. Rawnsley.1981. Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals. Masson Pub., New York.

Moniharapon, E. and F. Hashinaga. 2004. Antimicrobial activity of atung (*Parinarium glaberimum* Hassk.) fruit extract. Pak. J. Biol. Sci. 7: 1057-1061. http://dx.doi.org/10.3923/pjbs.2004.1057.1061

Mueller and I. Harvey. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86: 2010-2037. http://dx.doi.org/10.1002/jsfa.2577

Nagarathna, P. K. M., K. Reena, S. Reddy, and J. Wesley. 2014. Evaluation of immunomodulatory activity of the flavanoid of *Kigelia Africana*. Int. J. Pharm. Sci. Res. 5: 4359-4365. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4359-65

NRC. 1994. National Research Council. Nutrient Requirements of Poultry Ninth Revised Edition, National Academy Press, Washington DC.

Odoh, L. I. and L. Bratte. 2015. Effects of varying levels of neem (*Azadirachta indica*) leaf meal in layer diets on the haematological and serological indices, and faecal bacterial ocunts of layers. J. Nat. Sci. Res. 5: 37-45.

Oso, A. O., R. U. Suganthi, G. B. M. Reddy, P. K. Malik, G. Thirumalaisamy, V. B. Awachat, S. Selvaraju, A. Arangasamy, and R. Bhatta. 2019. Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poult. Sci. 98: 4755-4766. https://doi.org/10.3382/ps/pez191

Oleforuh-Okoleh, V. U., H. M. Ndofor-Foleng, S. O. Olorunleke, and J. O. Uguru. 2015. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. J. Agric. Sci. 7: 167-173. http://doi.org/dx.doi.org/10.5539/jas.v7n4p167

Oloruntola, O. D. 2018. Gliricidia leaf meal in broiler chickens diet: effects on performance, carcass, and haemato-biochemical parameters. J. Appl. Life Sci. Int. 18: 1-9.

Olumide, M. D., G. O. Chioma, O. A. Ajayi and O. E. Akinboye. 2018. Performance, haematological and serum biochemical profile of broilers chicken fed diets supplemented with *Ocimum gratissimum* meal. Int. J. Mod. Biol. Res. 6: 27-34.

Pacana, J. M. and Van R. K. R. Galarpe. 2017a. Phytochemical screening and toxicity of *Atuna racemosa* Rafin. chrysobalanaceae shell and seed extracts. IJAAS. 4: 110-115. https://doi.org/10.21833/ijaas.2017.011.017

Pacana, J. M. and Van R. K. R. Galarpe. 2017b. Antibacterial property *of Atuna racemosa* Rafin. chrysobalanaceae shell and kernel extracts (aqueous, methanol, ethyl acetate, and decoction). Int. J. Biosci. 11: 443-448. http://dx.doi.org/10.12692/ijb/11.1.443-448

Sarastani D., S. T. Soekarto, T. Muchtadi, D. Fardiaz, and A. Apriyantono. 2002. Aktivitas antioksidan ekstrak dan fraksi ekstrak biji atung. Jurnal Teknologi dan Industri Pangan 8: 149-156.

Sarker, M. S. K., G. M. Kim, and C. J. Yang, 2010. Effect of green tea and biotite on performance, meat quality and organ development in ross broiler. Egyp. Poult. Sci. J. 30: 77-88.

Sugiharto, S., T. Yudiarti and I. Isroli. 2016. Haematological and biochemical parameters of broilers fed cassava pulp fermented with filamentous fungi isolated from the Indonesian fermented dried cassava. Livest. Res. Rural. Dev. 28: 2016. http://www.lrrd.org/lrrd28/4/sugi28053.htm

Sugiharto, S. 2021. Herbal supplements for sustainable broiler production during post antibiotic era in Indonesia - an overview. Livest. Res. Rural. Dev. 33: 2021. http://www.lrrd.cipav.org.co/lrrd33/8/33103sgh_u.html

Tipu, M. A., M. S. Akhtar, M. I. Anjum, and M. L. Raja. 2006. New dimension of medicinal plants as animal feed. Pakistan Vet. J. 26: 144-148.

Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86: E140-E148. https://doi.org/10.2527/jas.2007-0459

Yang, Y., P. A. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. Worlds Poult. Sci. J. 65: 97-114. https://doi.org/10.1017/S0043933909000087