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

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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 bypassed in 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 EI-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.
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 glaberimum 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 fish also used as seafood, preservatives (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 32°C 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 Gumboro A, 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 experimental diets were formulated following broilers' nutrient requirements according to the standard requirements recommended by the National Research Council (NRC, 1994). The experimental diets were formulated following broilers' nutrient requirements according to the standard requirements recommended by the National Research Council (NRC, 1994). The experimental diets were formulated following broilers' nutrient requirements according to the standard requirements recommended by the National Research Council (NRC, 1994). The experimental diets were formulated following broilers' nutrient requirements according to the standard requirements recommended by the National Research Council (NRC, 1994).

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),
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 One-way 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).
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-ruminant 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 et al. (2018) have found that high doses in the inclusion of phytobiotic additives of Camellia L extract, Ocimum gratissimum, and glycinidia 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% (Mitraka and Rawsley,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-Okeleh et al. (2015), and Basit et al. (2020), who indicated that the inclusion of A. indica leaf meal, garlic water extract, garlic water extract,
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-Okokh 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.

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