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 banned 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, symbiotics, 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
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 pel 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)

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Levels of atung seed powder (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>58.50</td>
</tr>
<tr>
<td>Meat bran 1</td>
<td>6.75</td>
</tr>
<tr>
<td>Rice bran 1</td>
<td>2.00</td>
</tr>
<tr>
<td>Palm oil</td>
<td>3.00</td>
</tr>
<tr>
<td>DL-Lysine 99%</td>
<td>0.20</td>
</tr>
<tr>
<td>L-Lysine 78%</td>
<td>0.10</td>
</tr>
<tr>
<td>L-Threonine 78%</td>
<td>0.05</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.30</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.20</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.15</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.50</td>
</tr>
<tr>
<td>Atung seed powder</td>
<td>0.00</td>
</tr>
<tr>
<td>Filler (sand)</td>
<td>4.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
<tr>
<td>Nutrient composition:</td>
<td></td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>3017</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>6.54</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>3.04</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.01</td>
</tr>
<tr>
<td>Available Phosphorus (%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.15</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*Vitamin-mineral premix composed of vitamin A 1,250,000 IU, vitamin D 2,500,000 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, choline chloride 1,500 mg, copper 500 mg, iron 2,500 mg, iodine 20 mg, manganese 6,000 mg, selenium 20 mg, zinc 7,000 mg, cobalt 20 mg, Lysine 16,000 mg, DL-methionine 5,000 mg; ME = metabolizable energy.

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-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), Farhat et al. (2016), and Kickorowska 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 × 10^{12}/µl, haemoglobin 7.4-12.2 g/dl, leukocytes 9.20-28.60 × 10^{9}/µl, and PCV 24.9-40.7% (Mitruka 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), Oteforh-Okele et al. (2015), and Basit et al. (2020), who indicated that the inclusion of A. indica leaf meal, garlic water extract,
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; Adeeji 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 Olerufoh-Okeloh et al. (2015), Oloruntola (2018), Olumide et al. (2018), and Ansari et al. (2012), who reported that inclusion phytoadditive 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 phytoadditives could be due to increased white blood cells production against infection disease, reactions to drugs, and the immunomodulatory effect of phytobiological 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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