Broiler Blood Profile Feeding Contained Fish Meal with Different Levels

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ABSTRACT
The aim of the study was to analyze the blood profile of broilers fed with different levels of fish meal. The research design used a completely randomized design (CRD) with 4 treatments and 4 replications, so there were 32 experimental units. Each experiment consisted of 2 chickens and the chickens used were 100 broilers, with treatment P0 = Control (BP11 commercial feed), P1 = 43% Yellow Corn + 21% Bran + 27% CAB + 9% Fish Meal, P2 = 43% yellow corn + 23% bran + 22% CAB + 12% fish meal, P3 = 43% yellow corn + 25% bran + 17% CAB + 15% fish meal and the parameters observed were differential leukocytes. The results of the study with feeding containing fish meal with different levels had a significant effect (P<0.05) on the value of eosinophils, but had no significant effect (P>0.05) on the value of hematocrit, lymphocytes, heterophils, monocytes, basophils and H/L values. The conclusion of the study is that the provision of feed containing fish meal. The best treatment is in P3 by giving as much as 15% fish meal in the chicken ration.

Keywords: Fish Meal, Meat Race, Blood Profile

INTRODUCTION
Broiler is one of the poultry that has a relatively short maintenance time, only 4 to 6 weeks can be harvested. The existence of broilers is very important, because broilers are one of the sources of animal protein needed by the community so that they contribute to increasing the consumption of animal protein in the community. The sustainability of broiler cultivation cannot be separated from several factors, including the feed factor.

Feed is one of the important external factors in supporting the growth of broilers. Constraints faced to meet the needs of feed is the high cost of feed in the production process, it is necessary to use alternative raw materials, namely mixed fish waste, which is widely available in Southeast Sulawesi.

The feed ingredients are processed into fish meal as a mixture of broiler feed ingredients. This fish meal is produced in the Sambuli-Nambo Village of KUB Maju Jaya which is fostered directly by NSLIC/NSELRED, in accordance with standard operating procedures (SOP) in the processing process there are several fish wastes used, namely skipjack, anchovies and tuna which are used as fish meal as feed ingredients. mixed broiler. Fish meal also has a relatively high protein source composed of complex essential amino acids that can affect the growth of animal tissue cells (Purnamasari et al. 2006).

One indicator to see the health status of livestock is through a blood profile picture. Blood profile is one of the physiological and pathological parameters of the body that reflects the health condition of livestock (Satyaningtijas et al. 2010).

RESEARCH METHODS
The main material used in this study was chicken blood obtained from 32 broilers. The equipment used in this study was the Accupro tool and HB strip to determine hemoglobin.
levels. In addition, alcohol, cotton and Giemsa dye were used, a 3 ml syringe + needle and EDTA tube were needed for blood collection and a microscope to determine leukocyte differential. Meanwhile, the hematocrit pipette, creastal cell centrifugation and the hematocrit scale were used to determine the hematocrit value in percent (%).

The research design used was a completely randomized design (CRD) with four treatments and four replications. Treatment consists of:

- **P0 = Control (BP11 commercial feed)**
- **P1 = 43% Yellow Corn + 21% Bran + 27% CAB + 9% Fish Meal**
- **P2 = 43% Yellow Corn + 23% Bran + 22% CAB + 12% Fish meal**
- **P3 = 43% Yellow Corn + 25% Bran + 17% CAB + 15% Fish meal**

The mathematical model of this research is:

\[ Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \]

**RESULTS AND DISCUSSION**

The hematocrit value is the percentage of red blood cells to the entire blood volume (Soeharsono et al. 2010). Hematocrit levels will increase when there is an increase in hemoconcentration, either by an increase in blood cell levels or a decrease in blood plasma levels (Sutedjo, 2007).

The level of hemoglobin in the blood is an indication of the adequacy of oxygen transported in the body. Low oxygen content in the blood causes an increase in hemoglobin production and erythrocyte count (Ali et al. 2013). The average hematocrit and hemoglobin values of broiler blood obtained in this study are presented in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0U1/SD</td>
</tr>
<tr>
<td>Hematokrit (%)</td>
<td>26.88±2.36</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>16,23±1,75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The results of the analysis of variance showed that the addition of fish meal with different levels in the feed had no significant effect (P>0.05) on the blood hematocrit value of broilers. The highest hematocrit value in this study was P3 (27.75%) not much different from P2 (27.63%) and P0 (26.88), while the lowest hematocrit level was P1 (24.75%) this value was still in the range normal.

The general average hematocrit value obtained is 26.75% lower than the report by Jola et al (2018) that the normal hematocrit value in broilers ranges from 27-30%. An increase or decrease in the value of the hematocrit in the blood will have an impact on the viscosity (thickness) of the blood. The greater the percentage of hematocrit, the viscosity will increase. Yanti (2015) states that sufficient protein requirements will increase the formation of erythrocytes and a high number of erythrocytes will increase the formation of the hematocrit value. Vice versa, the decrease in hematocrit value can be caused by erythrocyte damage, decreased erythrocyte production or can also be influenced by the number and size of erythrocytes (Dawson and Whittow, 2000). According to Rini et al. (2013) the low hematocrit is influenced by feed consumption and low nutrient content.

The hematocrit values in the control treatment (without fish meal) and the administration of fish meal were both in the normal range. This shows that fish meal does not contain toxic substances that can disrupt the process of blood cell formation. According to Alfian et al. (2017), there are several factors that affect the blood picture (erythrocyte count, hemoglobin level and hematocrit value).
including age, gender, work activity, race, nutritional status, lactation, altitude, and environmental temperature.

The results of the analysis of variance showed that the addition of fish meal with different levels in the feed had a significant effect (P<0.05) on the blood hemoglobin level of broilers. The results of the DMRT test showed that the blood hemoglobin level of chickens in the control treatment (16.23 g/dL) was significantly higher than that of the chicken blood hemoglobin levels in the P1 treatment (13.77 g/dL), but not significantly different from the P2 treatment (14.60 g/dL) and P3 (14.91 g/dL). Although statistically there was a difference in blood hemoglobin levels between the control and P1, P2 and P3 treatments, physiologically, the chicken blood hemoglobin levels obtained in this study were still in the normal range.

The high value of hemoglobin in the P3 treatment was due to the glycine content found in fish meal, so the higher the percentage of fish meal given, the hemoglobin also increased. Zuprizal et al. (2001) stated that fish meal has a glycine content of 7.40%. Glycine is a heme-forming protein in hemoglobin biosynthesis, so it is able to form hemoglobin when there is a decrease in hemoglobin concentration in the blood (Broto et al. 2017). The low level of hemoglobin in erythrocytes causes the metabolic rate to decrease and the energy produced is low.

The general mean obtained in this study (14.88 g/dL) resulted in hemoglobin levels similar to the recommendations of Nwani et al. (2017) stated that normal blood hemoglobin levels in chickens ranged from (14.67 g/dL) higher than the report Darmawan (2002) stated that the normal range of hemoglobin levels in chicken blood was in the range of 7.0-13.0 g/dL. Rosita et al. (2015) stated that the high protein content in feed causes an increase in nutrient absorption in facilitating metabolic processes so that the increase in hemoglobin remains within the normal range. In addition, hemoglobin levels are higher if the nutritional content and the amount of ration consumption is high (Rini et al. 2013). While the report of Aeni et al. (2016) suggested that the low level of hemoglobin in the blood of chickens is due to the low number of erythrocytes, the hemoglobin level is positively correlated with the number of erythrocytes and the low protein content in the ration causes a decrease in hemoglobin levels.

Table 2. Differential leukocytes of broilers fed a diet containing fish meal with different levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P0U1/SD</th>
<th>P1U2/SD</th>
<th>P2U3/SD</th>
<th>P3U4/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limfosit</td>
<td>49.00±2.74</td>
<td>51.19±3.28</td>
<td>51.88±2.97</td>
<td>50.38±1.11</td>
</tr>
<tr>
<td>Heterofil</td>
<td>37.31±2.63</td>
<td>37.44±1.71</td>
<td>37.13±2.60</td>
<td>38.13±2.42</td>
</tr>
<tr>
<td>Monosit</td>
<td>7.19±0.83</td>
<td>5.94±0.72</td>
<td>6.25±1.49</td>
<td>6.06±1.72</td>
</tr>
<tr>
<td>Eusonofil</td>
<td>6.50±0.20</td>
<td>5.44±1.43</td>
<td>4.75±0.29</td>
<td>5.44±0.85</td>
</tr>
<tr>
<td>Basofil</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Rasio H/L</td>
<td>0.76±0.09</td>
<td>0.73±0.08</td>
<td>0.72±0.09</td>
<td>0.76±0.06</td>
</tr>
</tbody>
</table>

Limfosit berperan dalam merespon antigen (benda-benda asing) dengan membentuk antibodi yang bersirkulasi dalam darah atau dalam pengembangan imunitas (Arjuna dkk. 2019). Kadar limfosit darah yang diperoleh pada penelitian ini berkisar antara 49.00-52.19%. Nilai ini masih berada pada kisaran normal. Hasil ini sesuai dengan pendapat Harahap (2014) bahwa persentase limfosit pada darah unggas berkisar 42-66%. Hasil analisis ragam menunjukkan bahwa penambahan tepung ikan dengan level berbeda pada pakan tidak berpengaruh nyata (P>0,05) terhadap kadar limfosit darah ayam ras pedaging. Persentase limfosit ayam ras pedaging yang diberi pakan mengandung tepung ikan cenderung meningkat dengan persentase tertinggi pada P3 (51,88%) dan terendah pada P0 (49,00%).

The average percentage of lymphocytes obtained in this study ranged between (49.00-51.88%) with the general average (50.61%) lower than the Harahap report (2014) that the
percentage of lymphocytes in chicken blood ranged (42-66%), while Kusnadi (2009) reported that the administration of heat stress would reduce the percentage of lymphocytes. Low lymphocyte count (lymphocytopenia) occurs when the leukocyte count is 30% lower than normal (Latimer and Bientzle, 2010). From the observations of feeding without treatment (control) showed a lower lymphocyte percentage when compared to other treatments with feeding containing fish meal.

The value of normal lymphocyte levels indicates that feeding fish meal does not interfere with the immune system and livestock health. This shows that feeding fish meal can form antibodies that play an important role in fighting microorganisms. Almatsir (2010) stated that protein-rich fish meal can form antibodies, transport nutrients, and as an energy source.

The condition of chickens that are stressed or attacked by disease have a lymphocyte content that tends to increase, this is in response to attacks by disease agents. According to Moenek et al. (2019) that the increase in the lymphocyte value and above the normal value can be caused by the response of the chicken body to the incoming antigen (endoparasites, bacteria, or viruses). Almatsir (2010) stated that protein-rich fish meal can form antibodies, transport nutrients, and as an energy source.

Heterophiles work by phagocytosis by confining microorganisms in the cytoplasm (Sismanto, 2007). Heterophiles are phagocytic cells that function to phagocytose germs and viruses. Heterophil levels of chicken blood in this study ranged from 27.31-38.13%. The heterophile values in this study were classified as normal. Hendro et al. (2013) stated that the normal percentage of heterophile in broiler blood was in the range of 20-40%. The percentage of broiler heterophile values based on the results of this study were 37.13±2.63% (P0), 37.44±1.71 (P1), 37.13±2.60 (P2), 38.13±2.42 (P3).

The results of the analysis of variance showed that feeding fish meal at different levels had no significant effect (P>0.05) on the heterophile value of broilers. However, numerically the percentage of broiler heterophile values tended to increase in each treatment from the lowest to the highest average of 37.13±2.63% (P0), 37.13±2.60 (P2), 37.44±1, 71 (P1), 38.13±2.42 (P3) with a general average (37.50%). Even though there was an increase, the heterophil level was still in the normal range.

Hendro et al. (2013) stated that the normal percentage of heterophile in broiler blood was in the range (20-40%). This shows the average percentage of heterophils in this study is in the normal range (37.13-38.13%). Isroli et al (2009) stated that a high percentage of heterophils in the blood results in an increase in heterophil production or the condition of chickens experiencing stress, while Addas et al. (2012) reported that low or decreased heterophile values mean that the body is unable to fight bacteria or viruses optimally, making it more susceptible to infection. Heterophiles are the main line of defense against infection (Latimer and Bienzle, 2010).

Normal heterophile values in this study indicate that feeding containing fish meal contains antimicrobial substances to inhibit the growth of microorganism’s activity against disease agents so that the condition of chickens in feeding fish meal is able to normalize the number of leukocytes and leukocyte differentiation associated with disease resistance in the body and influenced by the genetic control of the livestock (Lokhande et al. 2009). The condition of chickens when stressed and inflammation or inflammation occurs, the number of heterophils will increase rapidly (Maxwell and Robertson, 1998 in Rosmalawati, 2008). The increase in the number of heterophils is thought to be due to the presence of complex compounds contained in fish meal, this is in accordance with the opinion of Rengganis and Baratawidjaja (2012) adding that the heterophile
working system is to destroy pathogens through independent exogenous pathways (lysosomes, proteolytic enzymes and cationic proteins) and oxygen dependent.

Monocytes are the second line of defense after heterophils which have phagocytic activity, when inflammation occurs monocytes migrate to tissues and turn into macrophages (Mitchell and Johns 2008). Monocytes are immature macrophages. When they migrate to tissues and turn into active macrophages, they act as phagocytic cells (Wibawan and Soejoedono 2013). The value of monocytes in this study ranged from 5.94-7.19%. The monocyte value in this study was classified as normal, this was in accordance with the opinion of Eroschenko (2008) which stated that the normal limit for the monocyte value in broiler blood was 3-10%. The percentage of broiler monocytes in each treatment were 7.19±0.83 (P0), 5.94±0.72 (P1) 6.25±1.49 (P2), and 6.06±1.72 (P3). The results of the analysis of variance showed that feeding fish meal at different levels had no significant effect (P>0.05) on the monocyte value of broilers. The monocyte value of the research results was in the range (5.94-7.19). The P0 treatment showed the monocyte value that was equal to (7.19%) and the P1 treatment showed the lowest monocyte value (5.94%).

The average percentage of monocytes obtained (6.36%) is lower than the report by Eroschenko (2008) which states that the normal limit for monocyte blood in broiler is 10%. The results of this study are in line with the research of Atlan et al. (2003) with a range of monocytes (5-7%), normal monocyte values, indicating that feeding containing fish meal at different levels had no significant effect (P>0.05) on the monocyte value of broilers. The monocyte value of the research results was in the range (5.94-7.19). The P0 treatment showed the monocyte value that was equal to (7.19%) and the P1 treatment showed the lowest monocyte value (5.94%).

Eosinophils are part of leukocytes that play an active role in helping to regulate the severity of allergies or killing a number of parasites that infect the body. An increase in the number of eosinophils can be affected by helminthic or parasitic infections (Arjuna et al. 2019). The eosinophil values of broilers in this study were 6.50±0.20% (P0), 5.44±1.43% (P1), 4.75±0.29% (P2), and 5.44±0.85% (P3). The value of eosinophils in this study ranged from 4.75 to 6.50%. This value is still in the normal range. Rosamalawati (2008) stated that the normal range of chicken eosinophil levels is 3-8%. The results of the analysis of variance showed that feeding fish meal at different levels had a significant effect (P<0.05) on the eosinophil value of broilers. The DMRT test results showed that the eosinophil value of broilers in the control treatment (6.50%) was significantly higher than the eosinophil value of chicken blood in the P1 treatment (5.44%), but not significantly different from the P2 (4.75) and P3 treatments. (5.44%) with a general average value of 5.53%. Although statistically there was a difference in the eosinophil value between the control treatment and the fish meal treatment, physiologically, the eosinophil value of broilers obtained in this study was still in the normal range. Rosamalawati (2008) stated that the normal range of chicken eosinophils was 3-8% higher than Arfah's (2015) report that the normal range of chicken eosinophils was (2-5%) while Suriansyah et al. (2016) reported that factors that can affect the level of eosinophils are excessive.
reactions in the body of chickens or hypersensitivity of the immune response to allergies and parasites and the level of inflammation. This shows that fish meal which contains high protein is able to facilitate metabolism in the blood, this is in accordance with the opinion of Adriani and Wirjatma (2012) fish meal has a relatively high protein which can form body tissues, repair and replace damaged or dead tissue.

The value of eosinophils as a good toxicant to foreign proteins that enter the body through the lungs or digestive tract, as well as toxins produced by bacteria and parasites (Kayadoe et al. 2008), this shows that feeding fish meal contains proteins that form immunity, in the fight against parasites, especially in allergies. the body responds by increasing eosinophils, on giving fish meal the percentage of eosinophils becomes normal. Basophils are a type of white blood cell that contain and can release histamine and serotonin during the body's immune response. Basophils are granulocytes that are rarely found in mammalian blood circulation, but are probably more common in poultry blood (Schalm, 2010).

The results of the analysis of variance showed that feeding fish meal at different levels had no significant effect (P>0.05) on the basophil value of broilers. Blood basophil levels in this study were not detected, it does not mean that in the blood of broilers there are no basophil cells, but this is rarely found. Although the concentration of basophils is very small, their presence is very important because basophil cells contain heparin which can inhibit the blood clotting process (Akramullah, 2020). Basophils play an important role in the immune response, which begins with contact with allergy-causing substances by producing chemical mediators such as histamine which then attracts other immune cells (Moreira et al. 2013). The results of the analysis of variance showed that the feeding of fish meal at different levels had no significant effect (P>0.05) on the basophil value of broiler blood. The ratio of the highest heterophil-lymphocyte values was P0 (0.76) and P3 (0.76) and the lowest was P2 (0.72) but not much different from P1 (0.73) Ernadi and Khermshahi (2007) that the ratio constant value heterophil-lymphocyte was in the range of 0.2-0.8%.

The value of the heterophil-lymphocyte ratio showed that the feeding containing fish meal was able to reduce the effect on heat stress due to heat stimulators as indicated by the heterophil-lymphocyte ratio in broilers being normal. These results indicate that the chickens in each treatment were resistant to a hot environment. The condition of chickens when stressed and inflammation or inflammation occurs, the number of heterophil-lymphocyte ratios increases. The higher the heterophil-lymphocyte ratio, the higher the heat stress level. Kusnadi (2008) states that the higher the ratio, the higher the stress level as a form of adaptation to the environment. This also means that the resistance of the chicken body to a hot environment is getting lower, while according to Mahmoud and Yaseen (2005) it is stated that the condition of the chicken that is always in a comfortable state triggers the low value of heterophile-lymphocytes with a decrease in heterophils and an increase in lymphocytes.

CONCLUSION

Based on the results of this study, it can be concluded that feeding containing fish meal at different levels had a significant effect (P<0.05) on the value of hemoglobin and eosinophils, but had no significant effect (P>0.05) on the value of hematocrit, lymphocyte, heterophile, monocytes, basophils and H/L values. The best treatment is in P3 by giving as much as 15% fish meal in the chicken ration.
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