The effect of nano Zn fitogenik addition on broiler diet to carcass traits, relative organ weights and haematological response

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Abstract. The objective of this study was to evaluate the effects of adding nano Zn Fitogenik (NZF) on broiler diet to carcass traits, relative organ weights and haematological indices. This study used 360 heads of sexed Lohman broilers day old chick (DOC). The experimental design used was a complete design with 6 (six) treatments and 5 (five) replications, each experimental unit consisted of 12 DOC (6 males and 6 females). There were the treatment given in this study; R1 = basal diet; R2 = R1 + Zn Sulfate (90 mg Zn kg⁻¹) + 5.32 mg kg⁻¹ guava leaf flour; R3 = R1 + NZF (45 mg Zn kg⁻¹); R4 = R1 + NZF (90 mg Zn kg⁻¹); R5 = R1 + NZF (135 mg Zn kg⁻¹); R6 = R1 + NZF (180 mg Zn kg⁻¹). At 33 days old age, one male broiler from each experimental unit was slaughtered and observed for carcass weight, abdominal fat, visceral organs, and digestive tract. Haematological evaluation using 30 blood samples were taken from each experimental unit. The results showed that addition of NZF at a dose of 90 mg Zn/kg (R4) improved weight of carcass, heart, and ileum. The addition of NZF at a dose of 135 mg/kg (R5) increased the number of erythrocytes when compared to the treatment group without the addition of NZF (R1). Adding NZF tend to decrease H/L ratio. It can be concluded that adding NZF improved carcass weight and tend to suprisse heat stress in broiler.

1. Introduction

Nano Zn-Phytogenic or in Indonesian it is called Nano Zn Fitogenik (NZF) is a feed additive that containing Zn and phytogenic compound that particle size in the nanoscale. NZF was own product that produced using green synthesis process in which using inorganic Zn and guava leaf extract (Psidium guajava) as the main ingredient. Many reports stated that Zn has important function in the metabolic process of nutrients in the body [1,2]. Furthermore, Zn was reported has newly function as anti-heat stress, an agent of antibacterial, antioxidant compound [3,4]. Phytogenic compound has been reported has a positive effect on animals. Phytogenic potentially used as a growth promoter in a broiler [5]. Work mechanism of phytogenic similar to antibiotic, such as improving digestive tract healthy. Many study reported that phytogenic improved immune responce of broiler [6-9].

However, the NZF has never been tested in terms of its effect on the percentage of carcasses, carcass cuts, and hematological parameters in broilers. Therefore, the main objective of this study was to
evaluate the effects of adding NZF on broiler diet to carcass traits, relative organ weights and haematological indices.

2. Method
This experiment used 360 heads of sexed Lohman broilers day old chick (DOC) that had been vaccinated with ND IB, ND Killed, IB Transume vaccine. The average body weight of male DOC was 48.44 g/head, while the female DOC was 48.11 g/head. The cage used was the open house cage with a litter system that using rice husks on the floor. Number of cages used was 30 cages. The plot size of the cage used was 1.5 x 1 meter. Every cages were equipped with a place to eat and drinking water. The treatment diet was formulated based on Rostagno et al (2017) [10] (table 1). Nano Zn-Fitogenik (NZF) as a treatment was added at doses of 0; 45; 90; 135; and 180 mg Zn kg⁻¹. The experimental design used was a completely randomized design (CRD) with 6 (six) treatments and 5 (five) replications, each experimental unit consisted of 12 DOC (6 males and 6 females). Feed and drinking water are given ad libitum. The treatments diet used are presented in table 2.

2.1. Measurement of relative weight of carcass, carcass cuts, abdominal fat, visceral organs, and digestive tract
At 33 days old age, one male broiler from each experimental unit was slaughtered and observed for carcass weight, abdominal fat, visceral organs, and digestive tract. Previously, the chicken had fasted for 12 hours. Broilers were weighed and then slaughtered. Organs in broilers were removed to be observed and measured the percentage of the weight of visceral organs, including abdominal fat and digestive tract. Carcass weights were obtained after the broilers that have been slaughtered were removed from feathers, visceral, head and neck, and shank. The percentage of a carcass, carcass cuts, and visceral organs obtained through comparing to the body weight of broilers.

2.2. Haematological evaluation
Blood samples are taken when the broiler was 33 days old. Time of collection in the morning at 06.00 AM. Broilers were taken from each experimental unit, so 30 blood samples were obtained. Blood was taken from the brachial vein (a vein under the wing). The variables measured were erythrocytes, hemoglobin, hematocrit, leukocytes, leukocyte differentiation, (heterophil, monocytes, lymphocytes, basophils, eosinophils) and H/L ratio.

2.3. Data analysis
Data were analyzed statistically using a variety of tests (ANOVA). The mathematical model used was:

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

\[ Y_{ij} = \text{Trial response from the } i^{th} \text{ and } j^{th} \text{ tests} \]
\[ \mu = \text{general score} \]
\[ \tau_i = \text{Effect of the } i^{th} \text{ treatment} \]
\[ \varepsilon_{ij} = \text{Error treatment } i \text{ and } j \text{ replication} \]

Firstly, the data was tested for normality. Furthermore, Normal data analyzed with ANOVA, if there were significant (p<0.05) differences followed by Duncan's test. In the weight gain parameter, to determine the optimum NZF dose an orthogonal polynomial test was performed. Some parameters also performed an orthogonal contrast test to see the effect of adding and non adding NZF treatment.
Table 1. Composition and nutrient content of the basal diet for pre-starter, starter, and finisher periods.

| Feedstuff            | Prestarter (1–7 days old) | Starter (8–21 days old) | Finisher (22–33 days old) |
|----------------------|---------------------------|-------------------------|---------------------------|
| Corn (%)             | 48                        | 51.2                    | 58.5                      |
| Soybean meal (%)     | 42.68                     | 40                      | 32.71                     |
| Palm oil (%)         |                           |                         | 4.6                       |
| Crude palm oil (%)   | 4.81                      | 4.81                    |                           |
| CaCO₃ (%)            | 1.935                     | 1.72                    | 1.92                      |
| NaCl (%)             | 0.47                      | 0.45                    | 0.4                       |
| DL-methionine (%)    | 0.31                      | 0.28                    | 0.25                      |
| Lysine (%)           | 0.28                      | 0.2                     | 0.28                      |
| Tricalcium phosphate (%) | 1.35                  | 1.17                    | 1.17                      |
| Premixa) (%)         | 0.17                      | 0.17                    | 0.17                      |
| Zinc sulfate (%)     |                           |                         | 0.001                     |
| Total                | 100                       | 100                     | 100                       |

Nutrient contentsb)

| Nutrient contentsb) | Crude protein (%) | Metabolizable energy (cal kg⁻¹) | Crude fat (%) | Crude fiber (%) | Lysine (%) | Methionine (%) | Methionine + systein (%) | Calcul (%) | Available phosphorus (%) | Na (%) | Cl (%) | Zn (ppm) |
|---------------------|-------------------|---------------------------------|---------------|----------------|------------|-----------------|--------------------------|------------|----------------------------|--------|--------|---------|
|                     | 24.57             | 3,018                           | 6.82          | 2.48           | 1.53       | 0.61            | 0.92                     | 1.04       | 0.47                       | 0.23   | 0.33   | 41.29   |
|                     | 23.55             | 3,055                           | 6.92          | 2.48           | 1.39       | 0.57            | 0.88                     | 0.93       | 0.43                       | 0.22   | 0.32   | 40.06   |
|                     | 20.67             | 3,155                           | 6.96          | 2.44           | 1.26       | 0.51            | 0.88                     | 0.99       | 0.42                       | 0.20   | 0.29   | 40.02   |

a) Provide per kilogram of diet: vitamin A 15,000 IU; cholecalciferol, 3,900 IU; vitamin E 30 IU; vitamin K 3.0 mg; thiamin 2.4 mg; riboflavin, 9.0 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 0.021 mg; calcium pantothenate, 30 mg; niacin, 45 mg; folic acid 1.2 mg; biotin, 0.18 mg; choline (as choline chloride), 700 mg; Cu, 8 mg; Mn, 100 mg; Fe, 80 mg; I, 0.35 mg; Se, 0.15 mg. b) Obtained using the calculation method based on the nutrient content of the results of laboratory analysis at the Indonesian Research Institute for Animal Production, except for metabolic energy based on Rostagno et al (2017) [10]. Na: sodium. Cl: Chlorida.

Table 2. NZF addition treatment given during the experiment.

| Treatment | Explanation |
|-----------|-------------|
| R1        | Basal diet (Zn content according to NRC (1994); 40 mg kg⁻¹) [11]. |
| R2        | R1 + (90 mg kg⁻¹ Zink Sulfate (conventional Zn) + 5.32 mg kg⁻¹ guava leaf flour). Phenol content in the diet was 0.63 mg kg⁻¹. |
| R3        | R1 + NZF (45 mg Zn kg⁻¹), Phenol content was 0.32 mg kg⁻¹ |
| R4        | R1 + NZF (90 mg Zn kg⁻¹), Phenol content was 0.63 mg kg⁻¹ |
| R5        | R1 + NZF (135 mg Zn kg⁻¹), Phenol content was 0.94 mg kg⁻¹ |
| R6        | R1 + NZF (180 mg Zn kg⁻¹), Phenol content was 1.26 mg kg⁻¹ |

The NZF used in the feeding trial contained 6.12% Zn and 430 mg kg⁻¹ of total phenol. Guava leaves used contained 11.85% of total phenol. NZF = Nano Zn Fytogenic.
3. Result and discussion

3.1. Carcasses, carcass cuts, and visceral organs
Effect of treatment diet on weights (g/kg body weight) and length (cm/kg body weight) of carcasses, carcass cuts, and visceral organs in broilers are presented in table 3. Addition of NZF at a dose of 90 mg Zn/kg (R4) indicated the highest weight of carcass (74.25%), meanwhile the addition of NZF at a dose of 180 mg Zn kg⁻¹ (R6) showed the lowest weight of carcasses (69.21%). When compared with the without the addition of NZF treatment (R1), it was known that the addition of NZF has varied effects. The addition of NZF showed a decrease in the weight of carcasses when given in the highest dose (180 mg Zn/kg).

The treatment diet did nothad a significant effect (P>0.05) on the weight of the abdominal fat. Ahmadi et al (2013) reported that the addition of nano ZnO up to a dose of 120 mg kg⁻¹ into a broiler diet did not changes in abdominal fat weight [12]. The treatment of feeding did not significantly influence the weight and length of the internal organs and digestive tract of broilers. Of all the internal organs and digestive tract observed, the treated feed had a significant effect (P<0.05) on the weight of the liver and ileum. This finding is consistent with previous reports that none of the animal's visceral organs were significantly affected by the dose of Zn use in feed [13]. Sharma et al (2012) reported that no significant differences were observed in the internal organ weights of mice fed control diets and supplemented with ZnO nanoparticles [14].

The treatment diet had no effect (P>0.05) on the weight of proventriculus and ventriculus. Weight of ventriculus in this study ranged from 13.7–16.6 g/kg body weight (BB) or 1.37–1.66% of body weight. Those percentages were lower than the broiler normal ventriculus weight percentage namely 1.6–2.3% [15]. A lower percentage of ventriculus indicated that the ventriculus worked lighter when digesting the feed [16]. The treatment diet had a significant effect (P<0.05) on liver weight. Liver weight in this study ranged from 22.9–26.1 g/kg or 2.29–2.61%. Those percentage was within the normal range of chicken liver percentage (1.7–2.8%) [17]. This showed that the condition of broilers in this study was in normal condition. Adding NZF made a lower heart weight than without the addition of NZF treatment (R1). Salam et al (2014) [18] stated that higher liver weight indicating the heavy work of liver cells in filtering toxins (toxic) in the blood. The treatment diet had no effect (P>0.05) on the weight of the pancreas. The weight of the pancreas in this study ranged from 2.49–3.32 g/kg or 0.24–0.33%. The pancreas weight was lower than reported by Sumiati et al (2003), namely 0.37–0.50% [19]. Pancreas weight was low showed that the pancreas worked in not heavy condition. The main pancreas function was secreting enzymes for digestion in the small intestine.

In the three parts of the small intestine (duodenum, jejunum and ileum), the treatment diet had a significant effect (P<0.05) on ileal weight. When compared with the treatment group without the addition of NZF (R1), it was seen that the addition of NZF at doses of 135 and 180 mg Zn/kg significantly (p<0.05) decreased ileal weight. Weight and length of intestinal showed the number of villi and the ability to secret digestive enzymes [20]. The treatment diet did not have a significant effect (p>0.05) on the relative weight and length of the caecum. It showed that adding NZF had no influenced the role of the caecum. The weight of caecum in this study ranged from 5.5–6.5 g/kg or 0.55–0.65%. Pond et al (1995) explained that caecum functions in helping the absorption of water, digestion of carbohydrates, and protein with the help of the bacteria that live in it. Caecum also functions to degrade cellulose with the help of microbes, cholesterol metabolism and enhance the immune response of young chickens by enlarging lymphoid tissue [21].
Table 3. The effect of adding NZF on relative weight (g/kg body weight) and relative length (cm/kg body weight) of carcasses, carcass cuts, and visceral organs of broilers.

| Organ                  | Treatment | SEM | p-value |
|------------------------|-----------|-----|---------|
| Carcass (g kg\(^{-1}\))| R1        |     |         |
|                        | 717.5bc   |     |         |
| Abdominal fat (g kg\(^{-1}\)) | R2        | 7.0 | 0.46    |
|                        | 6.2       |     | 0.025   |
| Proventriculus (g kg\(^{-1}\)) | R3        | 4.60| 0.13    |
|                        | 4.13      |     | 0.111   |
| Ventriculus (g kg\(^{-1}\)) | R4        | 13.78| 0.40    |
|                        | 16.08     |     | 0.308   |
| Heart (g kg\(^{-1}\)) | R5        | 26.15| 0.44    |
|                        | 24.86ab   |     | 0.023   |
| Pancreas (g kg\(^{-1}\)) | R6        | 2.84| 0.10    |
|                        | 3.32      |     | 0.262   |
| Duodenum (g kg\(^{-1}\)) | R1        | 9.93| 0.38    |
|                        | 10.28     |     | 0.498   |
| Duodenum (cm kg\(^{-1}\)) | R2        | 21.04| 0.72    |
|                        | 21.74     |     | 0.390   |
| Jejunum (g kg\(^{-1}\)) | R3        | 20.72| 0.52    |
|                        | 19.26     |     | 0.258   |
| Jejunum (cm kg\(^{-1}\)) | R4        | 20.00| 1.29    |
|                        | 47.70     |     | 0.490   |
| Ileum (g kg\(^{-1}\)) | R5        | 16.64a| 0.50    |
|                        | 14.64ab   |     | 0.050   |
| Ileum (cm kg\(^{-1}\)) | R6        | 53.78| 1.32    |
|                        | 51.27     |     | 0.176   |
| Caecum (g kg\(^{-1}\)) | R1        | 6.05 | 0.28    |
|                        | 5.58      |     | 0.975   |
| Caecum (cm kg\(^{-1}\)) | R2        | 24.24| 0.73    |
|                        | 22.63     |     | 0.553   |

Different superscripts in the similar row showed significantly different (P<0.05). R1 = basal diet; R2 = R1 + Zn Sulphate (90 mg Zn kg\(^{-1}\)) + 5.32 mg kg\(^{-1}\) guava leaf flour; R3 = R1 + NZF (45 mg Zn kg\(^{-1}\)); R4 = R1 + NZF (90 mg Zn kg\(^{-1}\)); R5 = R1 + NZF (135 mg Zn kg\(^{-1}\)); R6 = R1 + NZF (180 mg Zn kg\(^{-1}\)).
3.2. Hematological evaluation

The number of erythrocytes, hematocrit, and hemoglobin levels in this study is presented in Table 4. Generally, the average erythrocytes, hematocrit, and hemoglobin in each treatment were normal as reported by Samour (2015) [22]. This indicated that the nutrients from the diet able to meet the nutrient requirements for the formation of red blood cells such as proteins and vitamins. The addition of NZF at a dose of 135 mg/kg (R5) increased the number of erythrocytes when compared to the treatment group without the addition of NZF (R1) and the addition of conventional Zn at a dose of 90 mg Zn/kg (R2). R2 (90 mg Zn kg\(^{-1}\) from conventional Zn) and R4 (90 mg Zn kg\(^{-1}\) from NZF) had sub-standard levels of erythrocytes than reported by Samour (2015), namely 2.5–3.9 x106 mm\(^{-3}\) [22]. In contrast, the number of erythrocytes for R5 (135 mg Zn kg\(^{-1}\) from NZF) showed the number of erythrocytes above the standard number of erythrocytes reported by Samour (2015) [22]. Smith et al (2000) stated that erythrocytes formed in the bone marrow have the function of carrying hemoglobin into the blood circulation [23]. The high value of erythrocytes for NZF addition treatment showed that NZF was able to increase the formation and maturation of red blood cells thereby increasing the supply of oxygen (O\(_2\)) to be flowed to the body's cells and carrying carbon dioxide (CO\(_2\)) from cells back to the lungs. Tuerk and Fazel (2009) stated that Zn plays a special catalytic role in the activity of alpha-aminolevulinic acid dehydratase which is responsible for hem synthesis [24]. Donmez et al (2002) found a significant change in the number of chicken erythrocytes given Zn addition to their diet [25].

Table 4. The effect of adding NZF on number of erythrocytes, hematocrit, and hemoglobin.

| Treatment | Erythrocytes (10\(^6\) mm\(^{-3}\)) | Hematocrit (%) | Hemoglobin (g 100ml\(^{-1}\)) |
|-----------|----------------------------------|----------------|-----------------------------|
| R1        | 2.57\(^{BC}\)                   | 29.00          | 12.52                       |
| R2        | 2.07\(^{BC}\)                   | 26.40          | 11.80                       |
| R3        | 2.67\(^{BC}\)                   | 29.60          | 12.20                       |
| R4        | 2.15\(^{C}\)                    | 27.80          | 11.36                       |
| R5        | 4.26\(^{A}\)                    | 31.20          | 12.16                       |
| R6        | 3.29\(^{AB}\)                   | 25.60          | 12.00                       |
| SEM       | 0.18                             | 0.94           | 0.14                        |
| p-value   | 0.0032                           | 0.555          | 0.248                       |

Different letter in the similar column showed significantly different (P<0.05). R1 = basal diet; R2 = R1 + Zn Sulfate (90 mg Zn kg\(^{-1}\)) + 5.32 mg kg\(^{-1}\) guava leaf flour; R3 = R1 + NZF (45 mg Zn kg\(^{-1}\)); R4 = R1 + NZF (90 mg Zn kg\(^{-1}\)); R5 = R1 + NZF (135 mg Zn kg\(^{-1}\)); R6 = R1 + NZF (180 mg Zn kg\(^{-1}\)).

Mangkoewidjojo and Smith (1988) stated that hematocrit or Packed Cell Volume (PCV) was a description of the percentage of the volume of blood solids consisting of red blood cells [26]. The hematocrit concentration in this study was still in the normal interval as reported by Samour (2015) who stated that the normal range of hematocrit in chickens was 24–43% [22]. It showed that the addition of NZF to a dose of 180 mg Zn/kg does not interfere with blood circulation in the broiler body. Hemoglobin (Hb) functions to carry O\(_2\) in red blood cells to be transferred throughout the body [27]. The hemoglobin concentration in this study was in normal condition as reported by Samour (2015), namely 10.2–15.1 g/100ml [22]. This showed that hemoglobin was functioning properly, or there was no disruption in the broiler’s metabolic processes and was able to carry and bind O\(_2\) to the tissue and secrete CO\(_2\) from the tissue. Rifkind and Heim (1997) stated that the bond of Zn with hemoglobin increased oxygen affinity, therefore Zn can reduce stress [28]. In general, the condition of broilers in this study was not affected by anemia, which was a condition where there was a low number of erythrocytes and hemoglobin levels or hematocrit concentration [29]. White blood cells and their differentiation are indicators that are generally used to indicate the health status of livestock, including broilers. Examination of leucocyte...
concentration, leucocyte differentiation, and H/L ratio was one way to determine the presence or absence of stress and disease in the broiler. The effect of the treatment diet on leucocyte concentration, differentiation (lymphocytes, heterophils, basophils, eosinophils, monocytes), and H/L ratio are presented in table 5. Leucocyte concentrations and leucocyte differentiation of broilers fed by treatment appear to be within the normal concentration range of broilers. In this study, the number of leucocytes from each treatment ranged from 23.56–27.70 thousand mm$^{-3}$. This number was still in the standards reported by Mangkoewidjojo and Smith (1988), which ranges from 16–40 thousand points mm$^{-3}$ [26]. This showed that broilers in this study were not infected with the disease during maintenance.

3.3. Differentiation of leucocytes

The addition of 45 mg Zn/kg gave the highest number of leucocytes (29.91x10$^3$ mm$^{-3}$), compared to other treatments, including the treatment of adding 90 mg Zn/kg from conventional Zn. This showed that Zn from NZF was stable in the digestive tract protected from the formation of complexes with other nutrients so that it allowed a good absorption so the impact on the number of leucocytes. Akbari et al (2008) reported that the addition of 60 mg Zn kg/ZnO to the basal diet significantly increased the leucocytes of a broiler. Guyton and Hall (1997) and Akbari et al (2008) explained that leucocytes (white blood cells) play a role in the immune system, and the formation of antibodies, so they have an important role in defending the body from foreign body infections [30, 31]. Table 5 showed that the addition of NZF at a dose of 135 mg Zn/kg increased lymphocyte concentrations compared to the group of without adding NZF (R1) and the addition of conventional Zn (R2). R5 (135 mg Zn kg$^{-1}$) produced the highest lymphocyte concentration (59.20%) compared to other treatments, including the treatment of adding 90 mg Zn/kg from conventional Zn (R2). This showed that the supply of Zn from NZF was better than Zn from conventional Zn. Lymphocytes were formed by lymphoid cells, which Zn has an important role in the hormone thymulin, which has an important function in the maturation of T lymphocytes [32].

Lymphocytes are the most abundant type of leucocytes in chicken blood. The average value of lymphocytes for all treatments in this study was in the range of 55.20–59.20% or was still within the normal interval reported by Campbell and Ellis (2007) which was around 29–84% [33]. This showed that the number of lymphocytes in this study was considered to be able to maintain the immunity of broilers when attacked by a disease. Lymphocytes function to form antibodies and cellular immunity that function to respond to the presence of antigens and stress by increasing the circulation of antibodies in the immune system [34, 35]. Previous reports showed that the addition of 60 mg Zn/kg ZnO to basal feed significantly increased the number of lymphocytes in broilers [30]. The average percentage of heterophile in this study for all treatments ranged from 30.40% to 35%. Latimer and Bienzle (2010) stated that heterophile was the main line of defense against infection [37]. The addition of NZF tended to reduce heterophile concentrations compared to the treatment group without NZF addition (R1) and the addition of 90 mg Zn/kg from conventional Zn (R2). Broilers treated with NZF at a dose of 90 mg Zn/kg showed the lowest percentage of heterophil (30.40%). This showed that the addition of NZF at a dose of 90 mg Zn/kg reduced stress levels in broilers. Heterophile was mature cells that can destroy bacteria and harmful agents that attack the body, so heterophile in poultry become the first defense system in the body. Tizard (1982) revealed that heterophile defense processes were nonspecific that work phagocytosis by confining foreign organisms in the cytoplasm that contains proteolytic enzymes [38]. The high number of heterophils showed high potential in suppressing the infection of disease agents through the process of phagocytosis.

The average number of basophils for all treatments in this study ranged from 1.00–1.4% or still included in the normal interval reported by Nambol et al (2016), namely 0–2% [39]. This showed that broilers in this study were not exposed to parasitic infections. Tizard (1982) stated that basophils increase when poultry was infected with parasites. Basophils play a role in various allergic reactions and can prevent blood coagulation by releasing heparin [38]. Melvin and William (1993) reported that Basophils can fight antigens in inflammatory regions by producing heparin, histamine, bradykinin, and
lysozyme enzymes [40]. Campbell (1995) suggested that eosinophils in poultry have a role in the inflammatory response, phagocytosis, and killing bacteria or parasites [41]. Eosinophils declined in stressful conditions [42]. Eosinophils have two main functions, first, being able to attack and destroy pathogenic bacteria, second being able to produce enzymes that can neutralize inflammatory factors [43]. The average number of eosinophils for all treatments in this study ranged from 6.20–7.20% or still included in the normal interval reported by Jain (1986), namely 2–8% [44]. This confirmed that the condition of broilers in this study was in a healthy condition. The average number of monocytes for all treatments in this study ranged from 1.80–3.20% or still within the normal range reported by Campbell and Ellis (2007), namely 0–7% [33]. This showed that the condition of broilers in this study was in good physiological condition. Ganong (2008) stated that monocytes were leucocytes that function as a second defense response after heterophils that act as macrophages (swallowing and destroying cells of pathogenic microorganisms) [27].

Table 5. Effect of adding NZF to broiler diet on leucocyte concentrations, leucocyte differentiation (lymphocytes, heterophils, basophils, eosinophils, monocytes), and H/L ratio.

| Parameters                | Treatment | R1     | R2     | R3     | R4     | R5     | R6     | SEM  | p-value |
|---------------------------|-----------|--------|--------|--------|--------|--------|--------|------|---------|
| Leucocite (10³ mm⁻³)      |           | 23.66b | 23.56b | 29.91a | 23.70b | 23.67ab| 25.79b | 0.61 | 0.0112  |
| Leucocyte differentiation |           |        |        |        |        |        |        |      |         |
| Lymphocyte (%)            |           | 55.80  | 55.20  | 56.40  | 58.00  | 59.20  | 56.25  | 0.48 | 0.131   |
| Heterophil (%)            |           | 32.50ab| 35.00a | 32.00ab| 30.40b | 31.60b | 32.20ab| 0.44 | 0.092   |
| Basophil (%)              |           | 1.00   | 1.40   | 1.20   | 1.20   | 1.20   | 1.20   | 0.074| 0.818   |
| Eosinophil (%)            |           | 6.60   | 7.20   | 7.20   | 7.80   | 6.20   | 6.80   | 0.21 | 0.374   |
| Monosit (%)               |           | 2.60   | 2.60   | 3.20   | 2.60   | 1.80   | 2.20   | 0.18 | 0.386   |
| H/L Ratio                 |           | 0.57   | 0.61   | 0.57   | 0.52   | 0.53   | 0.56   | 0.012| 0.345   |

Different superscripts in the similar row showed significantly different (P<0.05). R1 = basal diet; R2 = R1 + Zn Sulfate (90 mg Zn kg⁻¹) + 5.32 mg kg⁻¹ guava leaf flour; R3 = R1 + NZF (45 mg Zn kg⁻¹); R4 = R1 + NZF (90 mg Zn kg⁻¹); R5 = R1 + NZF (135 mg Zn kg⁻¹); R6 = R1 + NZF (180 mg Zn kg⁻¹).

Treatment diet did not have a significant effect (P>0.05) on the heterophils lymphocytes (HL) ratio. These results as shown by El-Katcha et al (2017) who reported no influence for the addition of nano ZnO to 45 mg/kg to H/L ratio [45]. H/L ratio was a real index as an indicator of stress in poultry [46]. Fawzy et al (2016) stated that Zn was needed for normal lymphocyte development [47]. The Zn content in the basal diet was thought to be sufficient for the development of lymphocytes so that the treatment diet does not influence the H/L ratio. The average H/L ratio for all treatments in this study ranged from 0.52–0.61 or above from the normal interval reported by Jain (1993), namely 0.45–0.5 [48]. This showed that the condition of the broiler in this study was under conditions of heat stress. Temperature conditions in this study were higher from the ideal temperature for the broiler. In the day time, the temperature can reach up to 35°C, causing the chicken had heat stress. Mujahid et al (2007) stated that increasing 5°C that exceeds the comfort zone caused oxidative stress in broilers. In this study, there was a decrease in the H/L ratio for the experimental chicken group which was given the addition of NZF (R3; R4; R5; and R6). This showed that the addition of NZF into diet had a tendency to reduce heat stress [49]. Siegel (1995) divides the value of the H/L ratio into three parts, i.e., 0.2 for low-stress levels, 0.5 for moderate stress levels, and 0.8 for high-stress levels. In this study, the broiler appeared to experience moderate stress [50].

4. Conclusion
Addition of NZF at a dose of 90 mg Zn/kg (R4) improved weight of carcass, heart, and ileum. The addition of NZF at a dose of 135 mg/kg (R5) increased the number of erythrocytes when compared to the treatment group without the addition of NZF (R1). Adding NZF tent to decrease H/L ratio. It can be concluded that adding NZF imporoved carcass weight and tend to suprise heat stress in broiler.
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