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Supplementation of Zinc on Antioxidant Activity, Blood Profile, Mineral Availability, Abdominal Fat, Digestive and Accessory Organs of Sikumbang Janti Duck

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ABSTRACT

The present research aimed to evaluate the addition of zinc (Zn) on antioxidant activity, blood profile, mineral availability, and abdominal fat of Sikumbang Janti duck. A total of 96 female Sikumbang Janti ducks aged 8 weeks were used in this research. This study used a completely random design with four treatments and four replications (6 duck/replications). The treatments were as follows: control diet (Z0), the addition of 30 mg Zn/kg (Z1), 60 mg Zn/kg (Z2), and 90 mg Zn/kg(Z3). Variables observed were antioxidant activity, blood profile, mineral content in the tibia, and abdominal fat. The results showed that Zn addition on feed significantly increased antioxidant activity (DPPH), Zn concentration in thigh, leukocytes, mineral availability (Ca, P, and Zn) (P<0.01), and decreased weight of abdominal fat in Sikumbang Janti duck (P<0.01). Blood profiles (except leukocytes) were not affected by the addition of Zn in the diet (P>0.05). It is concluded that the Z2 (60 mg Zn/kg) addition improves antioxidant activity, blood leukocytes, zinc content in thigh meat, mineral availability, and decreases abdominal fat weight of Sikumbang Janti duck.

Keywords: Abdominal fat, Antioxidant, Local duck, Meat, Zinc

1. Introduction

Zinc (Zn) is a mineral (micro) that is important for the growth and development of poultry, especially in the process of enzymes catalysis, structural, and regulatory (1,2). In recent years, research related to Zn in poultry has been expanding. Zinc addition in poultry feed can improve performance (3), egg quality (4), antioxidant activity (3,5), immune system (5–7) and reduce stress caused by increasing environmental temperature (8).

Zinc minerals are necessary for tropical nations such as Indonesia, where the average daily temperature exceeds the comfort zone of poultry, including laying ducks. The Sikumbang Janti duck is one of the local egg-laying ducks that has the potential to be developed in the Indonesian state of West Sumatra. The Sikumbang Janti duck is a laying duck from Payakumbuh City with a dominant white coat color, except for green primary wing feathers and a black beak (9–11). However, Sikumbang Janti duck egg production is still relatively low, with an average production of 190-210 eggs/year (10). Therefore, supplementation of the mineral Zn in feed is expected to increase production. Apart from functioning as an antioxidant, the mineral Zn plays an essential role in regulating the hormone system, cell growth, immunity, and reproduction (12).

Previous research has demonstrated that zinc increases the egg production of Magelang ducks (13). It was also mentioned that the Zn mineral could overcome the effects of heat stress by reducing oxidative damage to cell membranes caused by free radicals and reducing the heterophil/lymphocyte ratio and the mortality rate of laying duck embryos (14). Nevertheless, there is no data on zinc addition to the diet of Sikumbang Janti ducks, especially antioxidant activity, blood profile, mineral availability, abdominal fat, etc. Therefore, the present study aimed to evaluate the effect of zinc addition on antioxidant activity, blood profile, mineral availability, and abdominal fat of Sikumbang Janti duck.

2. Materıals and Methods

2.1. Animal, design, and diet

This study used 96 female Sikumbang Janti ducks aged eight weeks and weighing an average of 752.41 ± 81.99 g/bird. The Sikumbang Janti duck was purchased from a farmer from Payakumbuh City, West Sumatra. The current research lasted eight weeks using a completely randomized design with four treatments and four replicates Z0 (control diet); Z1 (Z0 + 30 mg Zn/kg), Z2 (Z0 + 60 mg Zn/kg), and Z3 (Z0 + 90 mg Zn/kg) (6 birds per replicate). The cages used in this study were 1.5m x 1.5m. The cage provided a place to eat and drink. The Zn used was 75% ZnO (Zn-O-India). The ration (Table 1) was formulated with an energy metabolism of 2,700 kcal/kg and 17% crude protein (15).

2.2. Parameters Observed

2.2.1. Antioxidant Activity

At the end of the study, one duck per repetition was slaughtered, and thigh meat was taken to analyse antioxidant activity. Antioxidant activity was performed with the 1.1-diphenyl-2picrylhydrazil (DPPH) method (16).). The specimen was dissolved (1 mg ml-1) in methanol. The mixture was vortexed after adding 500 µl of DPPH (125 µM in methanol) to 500 µl of the sample in a test tube. After 30 min at room temperature, the absorbance of the solution was measured at a wavelength of 517 nm using a spectrophotometer (Shimadzu UV VIS -1800, Japan). The IC_{50} value was determined using the linear regression equation, which describes the relationship between sample concentration (test compound) with Symbol X and average radical scavenging activity (measurement replication series, symbol Y). The higher the antioxidant activity, the lower the IC_{50} value (17).

$$\text{DPPH radical scavenging activity } (\%) = \frac{\text{A Control - A Sample}}{\text{A Control}} \times 100$$

2.2.2. Blood Profile

Blood sampling was carried out at the end of rearing (1 duck per replication) in the brachial vein using a

Table 1. Feed formulation and nutrient content of the diet (As fed)

Ingredients	%
Corn	55.00
Rice bran	15.40
Soybean meal	18.00
Fish meal	8.50
$CaCO_3$	2.50
Top mix[a]	0.50
Dl-Methionine[b]	0.10
Total	**100.00**
Metabolizable Energy (Kcal/kg)	2744.55
Crude Protein (%)	17.36
Crude Fibre (%)	3.63
Crude Fat (%)	1.58
Avaiable Phosphorus (%)	0.59
Methionine (%)	0.54
Lysine (%)	1.22
Zn (mg/kg)	29.59

Note: [a]top mix provided (in mg/kg) = Vitamin A 12.000 IU; Vitamin D3 2.000.000 IU; Vitamin E 8.000 IU; Vitamin K_3 2.000 mg; Vitamin B_1 2000; Vitamin B_2 5.000 mg; Vitamin B_6 500 mg; Vitamin $B1_2$ 1.200 µg; Vitamin C 25.000 mg; Ca-D-Pathotenate 6.000 mg; Niacin 40.000; Cholin Chloride 10.000 mg; Lysine 30.000 mg; Methionine 30.000 mg; Manganese 120.000 mg; Iron 20.000 mg.
[b]Dl-Methionine (Shandong Nhu Amino Acid Co. LTD)

sterile syringe. Blood samples were put into tubes containing ethylene diamine tetra acetic acid (EDTA). Blood analysis consisted of erythrocytes, leukocytes, heterophiles, lymphocytes, and monocytes using a hemocytometer and microscope (Olympus CX 23, China), hemoglobin using a complete reagent kit (Merckotest®) and hematocrit using Micro-Capillary reader (USA).

2.2.3. Mineral Availability of Tibia

The tibia of each duck was cleaned, and muscle and attached cartilage were removed, washed with water, and oven-dried (Memmert, Germany) at 60ºC for 24 h. The procedure for analysing the minerals Calcium (Ca), phosphor (P), and Zn followed the analysis method of association of official analytical chemist (18).

2.2.4. Abdominal Fat

Abdominal fat following the method outlined by Reski et al. (19). Sikumbang Janti ducks were slaughtered (one duck per repetition) at the end of the experiment (16 weeks). A digital balance (Osaka-HWH®, Japan) was used to test parameters.

2.3. Data analysis

An analysis of variance was performed on the data based on a 4 x 4 complete randomized design, and Duncan's multiple range test was performed on the means. A $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Antioxidant Activity

The average antioxidant activity and Zn concentration in Sikumbang Janti duck meat are presented in Table 2. Zn addition on feed significantly increased antioxidant activity (DPPH) compared to the treatment without Zn ($P<0.01$). Zinc concentration in thigh meat was significantly affected by Zn feed ($P<0.01$). Adding up to 90 mg Zn/kg of feed enhanced the Zn concentration in thigh meat compared to treatment without Zn. However, the Zn addition of 90 mg Zn/kg did not affect Zn concentration in thigh meat compared to the Zn addition of 30 mg Zn/kg feed.

Zinc addition in feed has a positive effect on antioxidant status. In this study, the measurement of antioxidant activity using the DPPH method showed that Zn has antioxidant properties with a mechanism as a radical scavenger. The higher the Zn addition in the Sikumbang Janti duck ration, the higher the Zn deposited in the meat, which has an impact on the higher antioxidant activity. The lower the value of the antioxidant activity (IC_{50}) reflects, the higher the antioxidant activity and vice versa (17).

Table 2. Antioxidant activity (1.1-diphenyl-2picrylhydrazil) and zinc content in thigh meat of Sikumbang Janti Duck fed by a zinc-contained diet

Variables	Treatments				SEM	*P-value*
	Z0	Z1	Z2	Z3		
Antioxidant activity (%)	25.78^{a}	17.10^{b}	14.52^{c}	9.22^{d}	1.55	0.001
Zn content (ppm)	156.65^{a}	169.38^{b}	182.93^{c}	166.28^{b}	2.55	0.001

Note: Z0 (control diet); Z1 (Z0 + 30 mg Zn/kg), Z2 (Z0 + 60 mg Zn/kg), and Z3 (Z0 + 90 mg Zn/kg). Means in the same row with diffrent superscripts differ significantly ($P<0.01$)

Table 3. Blood profile of Sikumbang Janti Duck fed by a zinc-contained diet

Variables	Treatments				SEM	*P-value*
	Z0	Z1	Z2	Z3		
Erythrocytes ($10^6/mm^3$)	2.35	2.47	2.34	2.62	0.08	0.56
Hemoglobin (%)	12.25	12.26	11.75	11.60	0.15	0.28
Hematocrit (%)	26.33	27.00	25.33	27.66	0.47	0.37
Leukocytes ($10^6/mm^3$)	22.07^{b}	23.69^{ab}	24.17^{a}	22.69^{ab}	0.32	0.06
Heterophils (H) (%)	33.33	31.79	31.95	33.20	033	0.73
Lymphocytes (L) (%)	63.51	64.14	64.61	64.15	0.31	0.16
Monocytes (%)	2.43	2.37	2.33	2.35	0.06	0.95
H:L ratio	0.52	0.49	0.49	0.51	0.01	0.34

Note: Z0 (control diet); Z1 (Z0 + 30 mg Zn/kg), Z2 (Z0 + 60 mg Zn/kg), and Z3 (Z0 + 90 mg Zn/kg). Means in the same row with diffrent superscripts differ significantly ($P<0.05$)

Some research results also showed that Zn addition in duck feed (2,20), broiler (3), laying hens (5,21), and quail (22) can improve or enhance antioxidant status. Zinc prevents oxidation of the sulfhydryl group and reduces the generation of reactive oxygen species. Zinc is a cofactor for the enzyme Cu Zn-superoxide dismutase, which inhibits free radicals by facilitating the conversion of superoxide anions to hydrogen peroxide (21). In addition, Zn increases the formation of metallothionein, which serves as a hydroxyl radical scavenger (23).

3.2. Blood Profile

Table 3 presents the average blood profile of Sikumbang Janti ducks. Leukocytes increased ($P<0.05$) when Zn feed addition (60 mg Zn/kg) was compared to without Zn. Zinc addition did not affect erythrocytes, hemoglobin, hematocrit, heterophils, lymphocytes, monocytes, or the H:L ratio ($P>0.05$).

Animal blood profile describes the nutritional and physiological status according to the internal and external environment. In general, the blood profile of poultry is influenced by physiological (age, sex, reproductive status and season) and pathological (disease) factors (24). In this study, Zn addition up to 90 mg Zn/kg in the diet did not affect blood profile (except leukocytes) with average range values. This shows that the Sikumbang Janti ducks were in normal physiological conditions during the study. In this study, Z2 treatment resulted in the highest number of leukocytes compared to Z0 treatment. Zinc has a relationship with the formation of leukocytes, and this is because Zn has a role in Thymulin hormone activity. Thymulin is secreted by thymic epithelial cells, which plays a role in the maturation of T lymphocytes, where Zn deficiency can cause apoptosis in lymphoid cells, which results in a reduction in total leukocytes and lymphocytes (25).

3.3. Mineral Availability of Tibia

Table 4 shows the average availability of the minerals Ca, P, and Zn in the tibia. Zinc addition significantly affected Ca, P, and Zn content in the tibia (*P<0.01*). The addition of 60 mg Zn/kg resulted in higher Ca, P, and Zn content compared to the 30 mg Zn/kg treatment and the treatment without Zn. However, addition at the highest level (90 mg Zn/kg) showed a decrease in the P and Zn content of the tibia (*P<0.01*).

Table 4. Calcium, phosphorus, and zinc in the tibia of Sikumbang Janti Duck fed by a zinc-contained diet

Variables	Treatments				SEM	*P-value*
	Z0	**Z1**	**Z2**	**Z3**		
Calcium (%)	38.82[a]	42.03[b]	44.06[c]	43.65[c]	0.54	0.001
Phosphorus (%)	3.72[a]	4.09[b]	4.28[c]	4.14[b]	0.05	0.001
Zinc (ppm)	127.95[a]	133.67[b]	141.72[c]	128.46[a]	1.57	0.001

Note: Z0 (control diet); Z1 (Z0 + 30 mg Zn/kg), Z2 (Z0 + 60 mg Zn/kg), and Z3 (Z0 + 90 mg Zn/kg). Means in the same row with diffrent superscripts differ significantly (*P<0.01*)

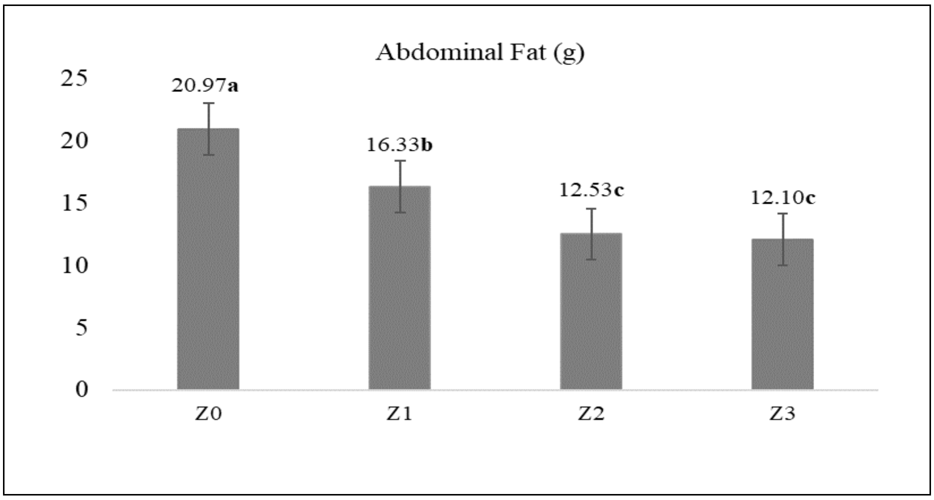

Figure 1. Abdominal fat of Sikumbang Janti Duck fed by a zinc-contained diet. Means in the same bar chart with different superscripts differ significantly (*P<0.01)*

Bone is a complex tissue that supports muscle, body growth and development and is extremely important as a reserve of calcium and phosphorus in the formation of eggshells (26). This study shows the content of minerals (Ca, P, and Zn) in the tibia bone is affected by Zn addition in the diet. Zinc has an essential role in bone calcification (27). Zinc addition up to 60 mg Zn/kg (Z2 treatment) in the ration increases the content of Ca, P, and Zn in the tibia. Some research indicates that supplementing broiler diets with 80 ppm of zinc leads to a calcium content of up to 51.79% (29). A high dose (80 ppm) of zinc addition resulted in a more significant zinc content in the tibia than a low dose (20 ppm) (30). Zinc in bone is a functional Zn reserve that can be easily mobilized for tissue needs. Zinc addition up to 48 mg Zn/kg laying hens had higher tibial bone strength and calcium deposition than other treatments (5).

In addition, Zn influences the average physiological effect of vitamin D on calcium metabolism, influencing calcium deposition in bone tissue (31). In this study, addition at high levels (90 mg Zn/kg) showed a decrease in tibial bone phosphorus concentrations, indicating that excessive Zn levels harm the bioavailability of other minerals. The antagonistic nature of Zn and phosphorus minerals may explain this phenomenon (23).

3.4. Abdominal Fat

Figure 1 shows the average abdominal fat of the Sikumbang Janti duck. Zinc addition at 60 mg/kg significantly (*P<0.05*) reduced abdominal fat compared to Z0 and Z1 treatment. However, it was no different from the Z3 group (90 mg/kg).

The weight of abdominal fat in this study decreased as Zn addition of the diet was raised (Figure 1). According to Mohammadi (32), supplementing the diet with zinc (Zn-SO_4 and Zn-Met) lowered the proportion of abdominal fat compared to the control treatment. In different studies, the lack of an increase in abdominal fat could be accounted for by differences in species and Zn content in the diet (33).

These findings are consistent with previous research indicating that organic and inorganic zinc addition in the feed had no negative impact on the visceral and digestive organs of Pekin ducks (34).

Addition of dietary Zn at 60 mg Zn/kg in diet increases antioxidant activity, leukocytes, zinc concentration in thigh meat, mineral availability in the tibia, and decreases abdominal fat weight of Sikumbang Janti duck.

Authors' Contribution

Study concept and design: R.K.R, Acquisition of data: R.K.R; R. A, Analysis and interpretation of data: R.K.R, Drafting of the manuscript: R.K.R; A.D; Z, Critical revision of the manuscript for important intellectual content: K.S; K, Statistical analysis: R. A, Administrative, technical, and material support: S. R

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All research methods were approved by The Research Ethics Committee Faculty of Medicine Universitas Andalas, number: 31/UN.16.2/KEP-FK/2023.

Conflict of Interest

The authors declares that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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