The effect of zinc supplementation in different types of feed on the performance and health status of IPB-D2 chickens

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Abstract. This study aimed to evaluate the effect of zinc supplementation in various diets on the performance and health status of IPB-D2 chickens. Subsequently, about 180 one-week-old IPB-D2 chickens were used in the study and were fed the treatment diets for 12 weeks. Also, this study employed a completely randomised factorial design with six treatments and three replications of ten birds each. These treatment diets included A1, which contained nutrients based on SNI (2013) without Zn supplementation, A2 which is a combination of A1 and 60 ppm of Zinc, B1 which contained nutrients 5% higher than SNI without Zn supplementation, B2 which is a combination of B1 and 60 ppm Zinc, C1 which contains nutrients 10% higher than SNI without Zn supplementation, and lastly, C2 which is a combination of C1 and 60 ppm of Zinc. Furthermore, the data were analysed using ANOVA, and the parameters observed were the body weight, body weight gain, feed consumption, feed conversion ratio, blood profile and the villi surface area of the jejunum. Consequently, the results showed that C2 significantly (P<0.05) increased the body weight and body weight gain when fed to the finisher and grower chickens, respectively. More so, when the finisher chickens were fed the 10% SNI diet, there was an increased body weight gain and also a significantly (P<0.05) decreased feed conversion ratio. Furthermore, the addition of 60 ppm zinc increased haemoglobin significantly (P<0.05), whereas C2 decreased leukocytes significantly (P<0.05) in chicken blood. However, the treatments had no effect on feed consumption in the villi surface area of the jejunum. Therefore, the study concluded that supplementing the IPB-D2 chickens with 60 ppm zinc in a 10% SNI diet resulted in the best performance and health status.

Keywords: IPB-D2 chicken, zinc, performance, blood profile, villi surface area

1. Introduction
The IPB-D2 chicken is a crossbred species derived from the first filial generation of a male Pelung Sentul (PS) and a female Kampung x parent stock Cobb (Kampung Meat type (KM), which was bred to
the 5th generation [1]. Furthermore, it derives about 25% of its genetic content from Cobb's parent stock [1], allowing it to grow more quickly than other local chickens. Also, at the age of 12 weeks, IPB-D2 chickens have superior fast growth and can achieve slaughter weights of 1199.9 gr for males and 994.9 gr for females when fed 60 % commercial feed and 40 % rice bran [2]. Meanwhile, guidelines and standards for the manufacture of Indonesian local chicken feed are contained in SNI 7783.1-2013[3], SNI 7783.2-2013[4], and SNI 7783.3-2013[5].

To support optimal growth, a local chicken feed required several antioxidants, including zinc (Zn2+), which acts as an antioxidant, immunomodulator, and cofactor of several enzymes [6]. According to research [7], zinc supplementation in the feed up to 80 ppm can boost the immune response via humoral cell intermediaries. As a result, the right feed is required to support optimal growth, health status, and genetic conditions of local cross-bred chickens such as IPB-D2 chickens. This study aims to evaluate the effect of zinc supplementation in different types of feed on the performance and health status of IPB-D2 chickens.

2. Material and methods

2.1. Materials

In this study, about 180 one week old IPB-D2 chickens from Citra Lestari Farm Bekasi were selected and fed the treatment diets for 12 weeks. Also, they were grouped into 10 pens, each containing 18 chickens. Meanwhile, the basic tools used were an 18-piece 1x1-m2 litter pen, a feed bunk, a drink bunk, a thermo-hygrometer, and an analytic scale.

Subsequently, the chickens had been weighed previously to determine their starting body weight. In addition, the diet was given twice a day, usually morning and evening based on their needs, and water was given ad libitum. The temperature and humidity of the cage environment were measured three times, in the morning, afternoon, and evening. At the end of the week, the remaining feed and the bodyweight of the chicken are weighed, while small intestine (jejunum) samples were collected for intestinal villi surface area tests at the end of the study. Additionally, 2 ml of blood was collected for blood profile, which was carried out using a Micro-hematocrit to calculate packed cell volume (PCV), and Sahli's hemoglobinometer to calculate haemoglobin (Hb).

A Neubauer chamber was used to count erythrocytes and leucocytes. Also, a blood smear was made and stained with Giemsa for further examination under a microscope to calculate leukocyte differentiation. The blood profile test is a work procedure performed at FKH IPB's Physiology Laboratory [8].

2.2. Method

This study used a completely randomised factorial design with six treatments and three replications of ten birds from each pen. Based on the recommendations of Leeson and Summers [9] and SNI 7783 [3,4,5], the diets in this study were modified to meet 5% and 10% higher nutrient requirements, respectively. Table 1 shows the nutrient content of the ration. The treatment diets included:

A1= diet contained nutrients according to SNI without Zn supplementation
A2= A1 + 60 ppm Zn
B1= diet contained nutrients 5% higher than SNI without Zn supplementation
B2= B1 + 60 ppm Zn
C1= diet contained nutrients 10% higher than SNI without Zn supplementation
C2= C1 + 60 ppm Zn.
Table 1. Nutrient content of the diet on starter, grower and finisher period*.

|       | Nutrient       | A  | B  | C  |
|-------|----------------|----|----|----|
| **Starter** |                |    |    |    |
| ME (kkal/kg) | 2900 | 3000 | 3100 |
| Crude Protein (%) | 19  | 20  | 21  |
| Lipid (%) | 3   | 3.1 | 3.2 |
| Crude Fibre (%) | 2.4 | 1.7 | 1.4 |
| Ca (%) | 0.92 | 0.93 | 0.99 |
| Pavailable (%) | 0.57 | 0.55 | 0.57 |
| Lysine (%) | 1.04 | 1.13 | 1.19 |
| Methionine (%) | 0.34 | 0.36 | 0.38 |
| Cystine (%) | 0.25 | 0.27 | 0.28 |
| Meth+Cys (%) | 0.59 | 0.63 | 0.66 |

|       | Nutrient       | A  | B  | C  |
|-------|----------------|----|----|----|
| **Grower** |                |    |    |    |
| ME (kkal/kg) | 2950 | 3050 | 3150 |
| Crude Protein (%) | 17.00 | 18.10 | 19.00 |
| Lipid (%) | 4.95 | 5.16 | 5.44 |
| Crude Fibre (%) | 2.70 | 1.98 | 1.24 |
| Ca (%) | 0.88 | 0.90 | 0.93 |
| Pavailable (%) | 0.62 | 0.59 | 0.56 |
| Lysine (%) | 0.90 | 0.99 | 1.04 |
| Methionine (%) | 0.32 | 0.34 | 0.35 |
| Cystine (%) | 0.21 | 0.24 | 0.25 |
| Meth+Cys (%) | 0.53 | 0.58 | 0.61 |

|       | Nutrient       | A  | B  | C  |
|-------|----------------|----|----|----|
| **Finisher** |                |    |    |    |
| ME (kkal/kg) | 3005 | 3100 | 3201 |
| Crude Protein (%) | 15.00 | 16.00 | 17.00 |
| Lipid (%) | 5.00 | 5.62 | 5.99 |
| Crude Fibre (%) | 2.62 | 1.90 | 1.11 |
| Ca (%) | 0.85 | 0.88 | 0.90 |
| Pavailable (%) | 0.61 | 0.57 | 0.46 |
| Lysine (%) | 0.75 | 0.83 | 0.86 |
| Methionine (%) | 0.29 | 0.31 | 0.31 |
| Cystine (%) | 0.18 | 0.21 | 0.22 |
| Meth+Cys (%) | 0.48 | 0.52 | 0.53 |

*calculation result.

2.3. Parameters

The parameters observed were body weight, body weight gain, feed consumption, feed conversion ratio, blood profile and villi surface area of the jejunum.

2.4. Data analysis

Analyses of variance (ANOVA) were used to analyse the data, and significant differences were analysed using Tukey’s test [10].
3. Result and discussion

3.1. Performance of 2 - 12 weeks IPB-D2 chicken.

The parameters observed were body weight, body weight gain, feed consumption, feed conversion ratio from the age of 2 to 12 weeks. The result showed in Table 2, 3, and 4.

### Table 2. Average body weight (gr) at starter, grower and finisher period.

| Period         | Zn (ppm) | Diets          | Average |  |
|---------------|----------|----------------|---------|---|
|               |          | SNI            | 5% SNI  | 10% SNI |  |
| Starter       | 0        | 233.79 ± 27.80 | 215.15 ± 10.98 | 251.45 ± 28.24 | 233.46 ± 22.34 |
| (2-4 weeks)   | 60       | 228.00 ± 18.34 | 224.41 ± 15.45 | 222.53 ± 15.26 | 224.98 ± 16.35 |
| Average       |          | 230.89 ± 23.07 | 219.78 ± 13.22 | 236.99 ± 21.75 |  |
| Grower        | 0        | 445.24 ± 33.36 | 491.61 ± 26.35 | 445.01 ± 16.44 | 460.62 ± 32.55 |
| (4-8 weeks)   | 60       | 461.67 ± 28.88 | 470.24 ± 17.84 | 499.24 ± 18.05 | 477.05 ± 25.70 |
| Average       |          | 453.45 ± 29.32 | 480.93 ± 23.28 | 472.13 ± 23.48 |  |
| Finisher      | 0        | 747.07 ± 33.46 | 853.19 ± 2.39 | 753.52 ± 27.92 | 784.59 ± 61.77 |
| (8-12 weeks)  | 60       | 800.95 ± 31.73 | 822.28 ± 4.42 | 889.82 ± 47.15 | 837.69 ± 55.92 |
| Average       |          | 774.01 ± 53.32 | 837.73 ± 37.31 | 821.67 ± 52.31 |  |

*Different letters in the same row and column indicate significant differences (P <0.05).*

### Table 3. Average body weight gain (gr/week/bird) at starter, grower and finisher period.

| Period         | Zn (ppm) | Diets          | Average |  |
|---------------|----------|----------------|---------|---|
|               |          | SNI            | 5% SNI  | 10% SNI |  |
| Starter       | 0        | 138.43 ±36.45  | 118.80 ±3.33  | 190.42 ±17.08 | 149.21 ±18.96 |
| (2-4 weeks)   | 60       | 129.18 ±17.62  | 139.72 ±13.34 | 104.17 ±5.00  | 124.36 ±11.98 |
| Average       |          | 133.81 ±27.04  | 129.26 ±8.33  | 147.29 ±11.04 |  |
| Grower        | 0        | 349.96 ±8.82   | 352.44 ±17.14 | 339.37 ±14.45 | 346.93 ±13.40 |
| (4-8 weeks)   | 60       | 382.33 ±17.39  | 361.33 ±12.11 | 391.64 ±11.58 | 378.44 ±18.07 |
| Average       |          | 365.65 ±22.05  | 356.89 ±14.14 | 365.51 ±30.93 |  |
| Finisher      | 0        | 313.22 ±19.16  | 355.15 ±16.08 | 361.72 ±28.04 | 325.30 ±52.54 |
| (8-12 weeks)  | 60       | 281.33 ±20.55  | 386.98 ±19.94 | 389.40 ±25.56 | 340.04 ±65.21 |
| Average       |          | 287.56 ±46.48  | 336.06 ±54.56 | 374.40 ±41.64 |  |

*Different letters in the same row and column indicate significant differences (P <0.05).*
Table 4. Average feed consumption (gr/week/bird) at starter, grower and finisher period.

| Period           | Zn (ppm) | Diets                  | Average   |
|------------------|----------|------------------------|-----------|
|                  |          | SNI 5% SNI 10% SNI     |           |
| Starter (2-4 weeks) | 0        | 846.94 ± 82.40         | 833.43 ± 95.00 |
|                  | 60       | 906.02±56.61           | 888.27 ± 46.14 |
| Average          |          | 876.48±69.51           | 840.65±28.85 |
| Grower (4-8 weeks) | 0        | 1535.78±35.67          | 1437.46 ± 27.43 |
|                  | 60       | 1487.33±36.90          | 1446.15 ± 24.16 |
| Average          |          | 1511.56±37.86          | 1367.09±31.65 |
| Finisher (8-12 weeks) | 0       | 2326.59±23.21          | 2235.19 ± 24.23 |
|                  | 60       | 1897.63±26.90          | 2167.73 ± 26.71 |
| Average          |          | 2112.11±24.53          | 2111.80±34.16 |

*Different letters in the same row and column indicate significant differences (P <0.05).

Table 5. Average feed conversion ratio of IPB-D2 chicken at starter, grower and finisher period.

| Period           | Zn (ppm) | Diets                  | Average   |
|------------------|----------|------------------------|-----------|
|                  |          | SNI 5% SNI 10% SNI     |           |
| Starter (2-4 weeks) | 0        | 6.32 ± 1.31            | 6.19±1.39 |
|                  | 60       | 7.09±0.91              | 7.13±1.36 |
| Average          |          | 6.71±1.11              | 6.14±1.80 |
| Grower (4-8 weeks) | 0        | 5.62±1.07              | 5.28±0.76 |
|                  | 60       | 5.02±0.19              | 4.79±0.63 |
| Average          |          | 5.32±0.76              | 4.89±0.94 |
| Finisher (8-12 weeks) | 0       | 8.10±1.27              | 7.02±1.34 |
|                  | 60       | 6.83±1.15              | 6.52±1.22 |
| Average          |          | 7.46±1.29              | 5.72±1.11 |

*Different letters in the same row and column indicate significant differences (P <0.05).

Analysis of variance results showed that a 10% SNI diet with 60 ppm zinc (C2) supplementation significantly (P <0.05) increased the bodyweight of IPB-D2 chickens by 889.82 ± 47.15 gr at finisher period, which is usually between 4 - 8 weeks. Also, the body weight gain of the IPB-D2 chicken in the starter period significantly increased (P <0.05) when given the C1 diet, which comprises 10% SNI without zinc supplementation. Similarly, the body weight gain of chickens when given 10% SNI feed with 60 ppm zinc supplementation (C2) at the grower period increased significantly (P <0.05) by 391.64 ± 11.58 gr. Lastly, the body weight gain significantly (P <0.05) increased by 374.40 ± 41.64 gr in chickens at the finisher period treated with a 10% SNI (C) diet.

In this treatment, it is clear that both the zinc-supplemented diet and the diet with a nutrient composition greater than 10% SNI affect chicken bodyweight performance and body weight gain. This is due to zinc’s antioxidant role, which can reduce the free radicals (ROS) that cause tissue damage [11]. More so, zinc contains metalloenzyme components, which include DNA polymerase, carboxy A and B peptidases, and alkaline phosphatase, all of which play a role in DNA proliferation. As a result, it will affect protein synthesis, protein digestion, and absorption of amino acids, as well as energy metabolism.
[12] leading to increased muscle mass. Meanwhile, when compared to other feeds, 10% SNI feed has a dense nutrient composition that can increase the body weight and body weight gain of IPB-D2 chickens. However, feed consumption was unaffected by diet treatment in this study, but feed conversion was significantly (P<0.05) lower in the 10% SNI diet (C) by 5.72±1.11. Therefore, these results indicate that zinc supplementation and a 10% SNI diet can accelerate IPB-D2 chicken growth without affecting feed consumption, resulting in better feed conversion.

3.2. Blood profile
Blood profile parameters can be used to determine the health status of livestock, since it has an important role in measuring the physiological, pathological and nutritional status of livestock. Also, animal blood profile will change along with physiological changes, both internal changes by age, nutritional status, health, body temperature and stress, or externally by disease, microorganisms and environmental temperature [13]. The blood profile result of IPB-D2 chickens at the age of 12 weeks are presented in Table 6.

Table 6. Blood profile IPB-D2 chicken aged 12 weeks.

| Blood profile       | Zn (ppm) | Diet       | Average | Normal value |
|---------------------|----------|------------|---------|--------------|
|                     |          | SNI        | 5% SNI  | 10% SNI      |              |
| Hematocrit (%)      | 0        | 28.00±3.00 | 27.67±5.51 | 27.67±2.08  | 27.78±3.31  | 24.00-43.00 |
|                     | 60       | 29.00±1.73 | 26.33±2.08 | 30.00±5.00  | 28.44±3.28  | [14]        |
|                     | Average  | 28.50±2.26 | 27.00±3.79 | 28.83±3.66  |              |             |
| Hemoglobin (g/dl)   | 0        | 10.07±1.01 | 10.00±0.40 | 9.80±0.53   | 9.96±0.61b  | 10.20-15.10 |
|                     | 60       | 10.73±0.46 | 10.60±1.40 | 11.33±1.03  | 10.89±0.96a | 15.10       |
|                     | Average  | 10.40±0.79 | 10.30±0.98 | 10.57±1.11  |              | [14]        |
| Erythrocyte (10⁶ mm⁻³) | 0        | 1.71±0.43  | 2.80±1.35  | 3.29±1.41   | 1.93±1.22   | 2.50-3.90   |
|                     | 60       | 1.59±0.14  | 2.15±0.40  | 2.06±0.30   | 2.59±0.37a  | [14]        |
|                     | Average  | 1.64±0.29  | 2.47±0.96  | 2.67±1.13   |              |             |
| Leukocyte (10³ mm⁻³) | 0        | 20.37±2.79ab| 21.82±2.60a| 17.05±2.44ab| 19.74±3.10b| 7.00-32.00  |
|                     | 60       | 18.72±3.51ab| 22.12±5.34a| 11.12±3.08b| 17.31±6.03b| [15]        |
|                     | Average  | 19.54±2.97 | 21.96±3.76 | 14.08±4.09  |              |             |
| Heterophile (%)     | 0        | 36.53±6.43 | 24.09±3.26 | 36.58±8.16  | 32.40±8.27  | 20.00-65.00 |
|                     | 60       | 38.38±5.78 | 32.43±3.86 | 27.41±4.77  | 32.74±6.81  | [15]        |
|                     | Average  | 37.45±6.37a| 28.265±5.57b| 31.99±7.81b|              |             |
| Lymphocyte (%)      | 0        | 58.31±7.18 | 56.38±6.88 | 53.80±3.51  | 56.16±5.62  | 20.00-75.00 |
|                     | 60       | 56.20±5.92 | 48.45±4.64 | 61.83±7.05  | 55.49±7.77  | [15]        |
|                     | Average  | 57.26±6.06 | 52.41±6.81 | 57.81±6.64  |              |             |
| Eosinophil (%)      | 0        | 2.67±1.35  | 2.93±1.91  | 6.62±4.77   | 4.07±3.27   | 0.00-7.00   |
|                     | 60       | 1.96±1.63  | 4.40±1.66  | 3.36±2.37   | 3.23±1.97   | [15]        |
|                     | Average  | 2.31±1.39  | 3.66±1.79  | 4.99±3.81   |              |             |
| Monocyte (%)        | 0        | 0.74±0.05  | 0.89±0.09  | 1.02±0.52   | 0.88±0.29   | 2.00-5.00   |
|                     | 60       | 1.21±0.43  | 0.68±0.06  | 0.79±0.09   | 0.89±0.33   | [15]        |
|                     | Average  | 0.97±0.37  | 0.78±0.13  | 0.90±0.36   |              |             |
| H/L                 | 0        | 0.64±0.18  | 0.43±0.06  | 0.69±0.19   | 0.59±0.18   | 0.20-0.80   |
|                     | 60       | 0.70±0.21  | 0.68±0.14  | 0.45±0.13   | 0.61±0.19   | [16]        |
|                     | Average  | 0.66±0.18  | 0.55±0.17  | 0.57±0.19   |              |             |

*Different letters in the same row and column indicate significant differences (P <0.05).
The hematocrit value indicates the percentage of erythrocyte volume in 100 ml of blood, where the normal hematocrit value in chicken blood ranges from 24.00 to 43.00 % [14]. In this study, the hematocrit ranged from 27.00 to 28.83 %, which is still within the normal range. Also, the hematocrit value of livestock correlates positively with erythrocyte size and negatively with body fluid concentration.

Haemoglobin contains a blood respiration pigment (heme) that has an oxygen affinity by forming oxyhemoglobin [17], implying that it plays a role in oxygen and carbon dioxide transport [18]. In this study, the diet treatments resulted in a haemoglobin value ranging between 9.96 to 11.33 g / dl, which is still within the normal range of 10.20 to 15.10 g / dl chicken haemoglobin [14]. Meanwhile, the analysis of variance from blood profile tests showed that diet with 60 ppm zinc supplementation (2) significantly (P <0.05) increased the haemoglobin value in IPB-D2 chicken blood by 10.89 g / dl. In addition, this increased haemoglobin value was caused by zinc which plays a role in increasing the affinity of oxygen in the blood through the interaction of haemoglobin with 2,3-diphosphoglyceric acid (2,3-DPG) [19].

Erythrocytes are the largest blood components consisting of haemoglobin, which plays an important role in the transportation of oxygen and nutrients. In this study, it ranged between 1.64 to 3.29 x 106 mm-3, which was still within the normal range of 2.50 to 3.90 x 106 mm-3 of chicken erythrocytes [14]. Furthermore, the erythrocytes of chicken fed the SNI (A) diet and without zinc supplementation (1) diet were significantly lower than those of the other diet treatments, although not statistically significant. This lower erythrocyte count indicates a lack of ability of chickens to use oxygen for nutrient metabolism, in contrast to chickens treated with zinc supplementation, which had a higher count of erythrocytes due to zinc's role in the synthesis of the enzyme carbonic anhydrase, which transports carbon dioxide into the lungs [20].

Leukocytes are the body's defence system [21], and the normal range of chicken leukocytes is 7.00 to 32.00 x 103 mm-3 [15]. In this study, they ranged from 11.12 to 22.12 x 103 mm-3, which is still within the range of normal chicken leukocytes, implying that the chickens in this study were not infected with the disease. Meanwhile, chickens fed 10% SNI with 60 ppm zinc (C2) supplementation had a lower leukocyte count by 11.12±3.08 x 103 mm-3 (P<0.05). This result corresponds with previous research that zinc supplementation in feed up to 80 ppm can boost immune response through humoral cell intermediaries [22].

Similarly, lymphocytes are specific immune system, which consists of humoral specific (B) cells and cellular specific (T) cells. Subsequently, high environmental temperature triggers the secretion of corticosteroid hormones which can inhibit lymphocyte formation [23]. In this study, the percentage of lymphocyte count ranged between 20.00 to 65.00%, which is still within the normal range of chicken lymphocytes [15].

Heterophils are phagocytic cells with a broad spectrum of microbial activity, and there was a significant decrease (P <0.05) in the number of heterophils in the group of hens treated with 5% SNI (B) feed, whereas there was a significant increase (P <0.05) in heterophils by 37.45 ± 6.37% observed in the chickens fed with SNI (A). Therefore, this high heterophile value indicates that chickens have a high potential to fight bacterial or viral infections through a non-specific immune response [24].

In this study, the percentage of eosinophils and monocytes were in the normal range [15], indicating that the chicken did not infect the disease. Eosinophils and monocytes participate in parasite, bacterial, and allergen phagocytosis [25]. Also, if there are antigens that cause the formation of a specific defence system, blood monocytes will be elevated.

The value of the heterophile / lymphocyte (H / L) percentage ratio [26] can be used to identify stress indicators. Also, the H / L ratio can be divided into three levels, which include low at 0.2, medium at 0.5, and high at 0.8 or more [16]. In this study, the H / L ratio ranged from 0.43 to 0.70, which is in the medium to the high range [16], indicating that the chicken is stressed. In addition, the stress in chickens can be caused by high environmental temperatures, due to the secretion of the hormone corticosterone, which leads to an increase in the number of heterophile in the blood and their release from the bone marrow [27].
3.3. **Villi Surface area of the jejunum**

The small intestine is made up of three parts including the duodenum, jejunum, and ileum, which have nearly identical histological structures. The surface area of the villi can indicate nutrient absorption efficiency. Table 7 shows the villi surface area of the jejunum in IPB-D2 chickens aged 12 weeks.

| Histologi | Zn Diet | Avg. Surface Area (µm²) |
|-----------|---------|------------------------|
| Jejunum’s surface area | SNI 0% | 1554.73±577.56 |
| | 5%SNI 10% | 1547.47±590.88 |
| | 10%SNI | 1345.71±464.69 |

From the results obtained in this study, the diet treatment did not affect the villi surface area of the jejunum in IPB-D2 chickens, but it can be seen from the data that the largest villi surface area was in chickens treated with SNI feed without zinc supplementation (A1). Therefore, the greater the number of villi, the greater the surface area of the villi, which can increase nutrient absorption in the bloodstream [28].

4. **Conclusion**

Therefore, it can be concluded that the diet containing 10% SNI supplemented with 60 ppm zinc (C2) yielded the best effect on the performance and health status of IPB-D2 chickens by increasing body weight and body weight gain in the finisher and grower period, respectively. Also, it reduces feed conversion, increases hemoglobin levels in the blood, which serves as an oxygen transporter and lastly, reduces the number of leukocytes in the blood. As a result, it can be said that the chicken is not infected with certain diseases.

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