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Blood haematology of laying hens given fermented shrimp waste extract

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Abstract

The purpose of this study was to determine the effect of using fermented shrimp waste extract in the ration on the number of erythrocytes, hemoglobin levels, and hematocrit values of laying hens. The chickens used in this study were 30 weeks old laying hens, Hyline strain, as many as 20 chickens. This study used experimental methods and the research design used a completely randomized design (CRD) with five treatments, consisting of R0 (the ration without fermented shrimp waste extract), R1 (the ration containing 0.5% fermented shrimp waste extract), R2 (the ration containing 1.0% fermented shrimp waste extract), R3 (the ration contains 1.5% fermented shrimp waste extract) and R4 (the ration contains 2.0% fermented shrimp waste extract), each treatment was repeated four times. The observed variables were the number of erythrocytes, hemoglobin levels, and blood hematocrit values. The results showed that the use of fermented shrimp waste extract had no significant effect. Giving fermented shrimp waste extract as much as 0.5-2.0% in the ration resulted in the number of erythrocytes, hemoglobin levels, and blood hematocrit values of laying hens in the normal range, and the use of 0.5% was effective in producing the number of erythrocytes (2.65×10^6/mm^3), hemoglobin levels (10.45 g/dL), and hematocrit value (28,25%) of laying hens' blood. The purpose of this study was to determine the effect of using fermented shrimp waste extract in the ration on the number of erythrocytes, hemoglobin levels, and hematocrit values of laying hens. The chickens used in this study were 30 weeks old laying hens, Hyline strain, as many as 20 chickens. This study used experimental methods and the research design used a completely randomized design (CRD) with five treatments, consisting of R0 (the ration without fermented shrimp waste extract), R1 (the ration containing 0.5% fermented shrimp waste extract), R2 (the ration containing 1.0% fermented shrimp waste extract), R3 (the ration contains 1.5% fermented shrimp waste extract) and R4 (the ration contains 2.0% fermented shrimp waste extract), each treatment was repeated four times. The observed variables were the number of erythrocytes, hemoglobin levels, and blood hematocrit values. The results showed that the use of fermented shrimp waste extract had no significant effect. Giving fermented shrimp waste extract as much as 0.5-2.0% in the ration resulted in the number of erythrocytes, hemoglobin levels, and blood hematocrit values of laying hens in the normal range, and the use of 0.5% was effective in producing the number of erythrocytes (2.65×10^6/mm^3), hemoglobin levels (10.45 g/dL), and hematocrit value (28,25%) of laying hens' blood.

Keywords: Fermented shrimp waste extract; Erythrocytes; Haemoglobin; Haematocrit; Laying hens

1. Introduction

Feeding in accordance with age needs and maintenance management can achieve increased productivity efficiently [1]. The feed given, in addition to supporting productivity in successful maintenance, is also to support the life and health of livestock. Lack of nutrients in feed can cause a decrease in productivity and interfere with health. One way to achieve nutrient balance in feed is by giving feed supplements.

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Feed supplements as additional feed serve to meet the needs of nutrients in rations which are sometimes still lacking as an effort to maintain livestock productivity and health. One of the ingredients that can be used as a feed supplement is the follow-up result of the fishing industry, such as shrimp waste because it contains protein, minerals, and active ingredients in the form of astaxanthin. The use of shrimp waste to be used as material for ration preparation has limitations due to the presence of chitin substances [2]. Chitin is a linear polysaccharide containing N-acetyl-D-glucosamine bound to a β bond, and when hydrolyzed produces 2-amino-2-deoxy-D-glucose. Chitin is found in shrimp shells in the form of complex compounds bound to proteins, inorganic salts, calcium carbonate, and lipids and pigments [3]. These bonds limit poultry digestive enzymes, causing low digestibility when consumed by livestock [4]. Therefore, processing is needed first to overcome these problems.

One of the treatments that can be done to increase the digestibility of shrimp waste is through a biological fermentation process by microorganisms. The microorganisms used in the fermentation process of shrimp waste are *Bacillus licheniformis*, *Lactobacillus sp*, and *Saccharomyces cerevisiae*. *Bacillus licheniformis* is one of the bacteria that can help the deproteination process because it produces chitinase enzymes, the enzyme degrades glycosidic β (1,4) bonds in chitin so that it can break protein and chitin bonds in shrimp waste to decompose cellulose into glucose [5]. Followed by demineralization by *Lactobacillus sp* which can produce lactic acid from the glucose substrate, the bacteria lower the pH to acidic and then react with minerals in shrimp waste and form mineral deposits [6]. Furthermore, bioprocessing uses *Saccharomyces cerevisiae*, a yeast that produces amylase, lipase, protease, and other enzymes that can help the process of digestion of food substances in the digestive organs [7].

Feed supplement from fermented shrimp waste extract also contains active ingredients in the form of astaxanthin. Astaxanthin is a carotenoid group compound with such a structure that makes it a very active antioxidant [8]. The antioxidant activity of shrimp carotenoid extract shows its potential to be used as a natural antioxidant [9]. Antioxidants are compounds that act as protectors of cells and body tissues by binding free radicals and inhibiting other oxidative reactions. Astaxanthin with antioxidant activity can maintain health from oxidative reactions that can damage protein molecules, nucleic acids, lipids, and polysaccharides and trigger oxidative stress. Antioxidant activity can affect the physiological and biochemical conditions occurring in the body of livestock.

Physiological conditions can be known through blood profiles. Good physiological conditions can be known from the number of erythrocytes, hemoglobin levels, and hematocrit values that show normal values, so that metabolism and productivity are also normal [10]. Erythrocytes or red blood cells are indicators of body health status [11]. Erythrocytes consist of hemoglobin which has the main function as a means of transporting oxygen and nutrients to all body tissues. The amount of nutrients, especially energy and protein, has an important role in the process of erythrocyte formation so that it affects the total erythrocytes and hemoglobin levels in the blood [12]. Hemoglobin is a protein consisting of four polypeptide chains, each of which contains heme, which is a red porphyrin pigment in which iron ions are contained which function to transport oxygen and circulate it throughout the body and give red color to blood cells [13]. Hemoglobin levels in the body of cattle are influenced by oxygen levels and erythrocyte counts [14]. The rate of transport of nutrients and oxygen from hemoglobin can be seen through the hematocrit value which indicates the level of viscosity or blood viscosity. Hematocrit is the percentage of red blood cells in total blood volume. The values of such blood profile measurements can be used as health indicators.

Research related to the use of feed supplements based on fermented shrimp waste at the level of 0.5-2.0% in rations has a positive influence on the number of erythrocytes and blood hemoglobin levels of chickens [15]. Astaxanthin concentrations in the range of 10-20 μg/ml indicate optimal levels of antioxidant and antityrosinase activity [16]. Based on this description, it can be hypothesized that the administration of fermented shrimp waste extract in the ration as much as 1%, resulting in the number of erythrocytes, hemoglobin levels, and hematocrit values of laying hens is in the normal range.

2. Material and methods

2.1. Experimental Livestock

The livestock used in this study were 20 chickens laying hens *of the Hyline strain* aged 30 weeks. Chickens are divided into 5 treatments and repeated 4 times.

2.2. Trial Cage

The cages used during the study were individual cages. The size of each cage used is 40 cm long, 20 cm wide, and 20 cm high.

2.3. Research Equipment

Spuit 3 ml, vacumtube EDTA (Ethylene Diamine Tetra Acetyl Acid), tissue label, termos es, set Hemocytometer, microscope, headcounter, set Hemometer Sahli-Hellige, Micro Capillary Reader ber-EDTA, kristoseal, centrifuges, Hematocrit scale, tissue.

2.4. Feed Ingredients Constituent of Rations

Feed ingredients that make up the ration used in the study: yellow corn, soybean meal, fermented shrimp waste extract, meat bone meal, coconut oil, bone meal, stone meal, and grit. The nutrient content of feed ingredients that make up the ration can be seen in Table 1.

Table 1 Metabolizable Energy Content and Nutrients of Research Feed Ingredients

Feed Ingredients	**ME**	**CP**	**EE**	**CF**	**Ca**	**P**	**Lys**	**Meth**
	(Kcal/kg)	**……………………… (%)……………………..**						
Yellow corn	3350	8.60	3.80	2.20	0.02	0.08	0.26	0.18
Soybean meal	2230	44.00	0.80	7.00	0.29	0.27	2.69	0.62
Fermented shrimp waste extract	3033	25.15	0.96	0.00	6.81	2.83	0.85	0.28
Meat bone meal	2375	38.84	10.93	2.46	9.80	4.50	2.08	0.54
Coconut oil	8600	-	-	-	-	-	-	-
Bone meal	-	-	-	-	24.00	12.00	-	-
Stone flour	-	-	-	-	40.00	-	-	-
Grit	-	-	-	-	30.87	1.11	-	-

Source: Analysis results of the Ruminant Animal Nutrition and Fodder Chemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University (2021).

2.5. Research Ration Arrangement

The rations used in the study were prepared based on control rations (R0) with metabolic protein and energy requirements of 17.5% and 2750 kcal/kg respectively. Feeding is carried out 2 times a day in the morning with a time span of around 06:00 – 06:30 and during the day around 12:00 – 12:30 with the amount of each feeding 60 g / head or 120 g / head / day. The arrangement of rations is presented in Table 2.

Table 2 Trial Ration Formulation

No	**Feed Ingredients**	**R0**	**R1**	**R2**	**R3**	**R4**
		……………………… (%)……………..				**………**
1	Yellow corn	58.10	57.70	57.50	57.30	57.10
2	Soybean meal	21.00	20.90	20.60	20.30	20.00
3	Fermented shrimp waste extract	0.00	0.50	1.00	1.50	2.00
4	Meat bone meal	8.50	8.50	8.50	8.50	8.50
5	Coconut oil	1.00	1.00	1.00	1.00	1.00
6	Bone meal	3.15	3.15	3.15	3.15	3.15
7	Stone flour	4.50	4.50	4.50	4.50	4.50
8	Grit	3.75	3.75	3.75	3.75	3.75

Information: R0 = Ration does not contain fermented shrimp waste extract; R1 = Ration contains 0.5% fermented shrimp waste extract; R2 = Ration contains 1% fermented shrimp waste extract; R3 = Ration contains fermented shrimp waste extract1.5%; R4 = Ration contains fermented shrimp waste extract2 %.

Table 3 Metabolizable Energy Content and Nutrients of Experimental Rations

Nutrient Content	R0	R1	R2	R3	R4	Necessity
ME (kcal/kg)	2702,53	2702.06	2703,84	2705,61	2707,30	2700-2750
Crude protein (%)	17.54	17.59	17.56	17.54	17.52	17.5
Ca (%)	4.62	4.65	4.69	4.72	4.75	≥4.3
P (%)	0.91	0.92	0.93	0.95	0.96	≥0.80
Lysine (%)	0.89	0.89	0.89	0.88	0.88	≥ 0.88
Methionine (%)	0.52	0.52	0.52	0.52	0.51	≥ 0.48

Source: Needs based on Hyline International (2016).

2.6. Data Collection and Sample Analysis

Blood sampling was carried out in the last week of the study, blood samples were taken from one chicken in each treatment unit and repeated so that it became 20 samples. Blood is taken through the pectoralis vein of the humerus part of the chicken wing as much as 3 cc. Blood samples are taken using a syringe and then accommodated in EDTA vacuum tubes that have been labeled according to treatment and put in a cooler box so that the sample does not clot.

2.7. Counting the Number of Erythrocytes (millions/mm3)

The number of erythrocytes is calculated using a hemocytometer. Blood thinning is done with Hayem solution which is isotonic which has been diluted in a hemocytometer pipette, then calculated under a microscope with a magnification of 40 times. The number of squares calculated to establish the number of erythrocytes is 40 small squares.

2.7.1. Calculating Hemoglobin Level (g/dL)

- The hemometer tube is filled with 0.1 HCL to the limit line.
- The blood sample is sucked with a Sahli-Hellige Hemometer pipette to the 20mm line mark.
- Then the blood is inserted into the Sahli-Hellige Hemometer tube which already contains 0.1N HCL.
- Mixed or stirred with the available stirrer (blood will look brown). This indicates the presence of hemolysis.
- Then aquadest is added drop by drop until the color corresponds to the standard color of Sahli.
- Then the height of the meniscus (surface) of the fluid on the Sahli Hemometer tube is read, the number that is read indicates the hemoglobin level of the blood sample (units Hb = g / dL)

2.7.2. Calculating the hematocrit value (%)

- Blood is introduced into the hematocrit capillaries that already contain anticoagulants by sucking to 1 cm from the upper end.
- One of the capillaries is closed with a cristoseal.
- Then the capillaries that already contain blood are centrifuged using a centrifugation device at a speed of 3000 rpm for 15 minutes.
- After centrifuging, the blood will separate between the blood cells and their plasma.
- Then the volume of blood cells that have separated in the capillaries is read using a microcapillary reader (micro capillary reader or hematocrit scale).
- The calculation of hematocrit value is as follows:
- Hematocrit value (%) = $\frac{Blod\ cell\ volume}{Blod\ volume} \times 100\ \%$

2.8. Experiment Design and Statistical Analysis

The type of research carried out is experimental research. The experimental design used was a Complete Randomized Design with 5 treatments. Each treatment was repeated 4 times, bringing the total to 20 experimental units. The treatment is:

- R0 = Ration without fermented shrimp waste extract (Basal ration)
- R1 = Ration contains 0.5% fermented shrimp waste extract
- R2 = Ration contains 1.0% fermented shrimp waste extract

- R3 = Ration contains 1.5% fermented shrimp waste extract
- R4 = Ration contains 2.0% fermented shrimp waste extract

After collection, the data is further analyzed using variety analysis. Differences between treatments using the Duncan Multiple Distance Test.

3. Results and discussion

3.1. Effect of Treatment on the Number of Erythrocytes of Laying Breed Chickens

Erythrocytes or red blood cells are components in the blood that function to carry oxygen from the lungs to all body tissues. The number of erythrocytes is an indicator of health status and adequacy of nutrients in the body [11]. The average number of erythrocytes in each treatment can be seen in Table 4.

Table 4 Average Number of Erythrocytes of Laying Hens Results of Experiments

Deuteronomy	Treatment				
	R0	R1	R2	R3	R4
	…..………………. ($x10^6/mm^3$) ………………………				
1	2.68	2.75	2.81	2.59	3.02
2	2.88	2.54	2.70	2.71	2.30
3	2.87	2.56	2.06	2.38	2.95
4	3.09	2.77	2.51	2.72	2.59
Total	11.52	10.62	10.08	10.40	10.86
Average	2.88	2.65	2.52	2.60	2.71

Based on Table 4. The number of erythrocytes in the results ranged from $2.52\times10^6/mm^3$ - $2.88\times10^6/mm^3$. The lowest erythrocyte value was produced in the R2 treatment with the mean value of the number of erythrocytes ($2.52x10^6/mm^3$) and the highest average value of erythrocytes was produced in the R0 treatment with the number ($2.81x10^6/mm^3$). The results of fingerprint analysis (ANOVA) showed that the administration of fermented shrimp waste extract in the ration had no real effect ($P>0.05$) on the number of erythrocytes of laying hens. The number of erythrocytes that have no noticeable effect, is still within the normal range.

The number of erythrocytes of laying hens given fermented shrimp waste extract (R1, R2, R3, R4) had a lower yield value than the chicken group that was not given fermented shrimp waste extract (R0). The number of erythrocytes in the treatment of R1, R2, R3, R4 although lower than R0 the average range of the number of erythrocytes is still within the normal range of the number of erythrocytes in chickens. This is in accordance with research by [17] which states that the normal range of erythrocytes in chickens is in the range of 2.5-3.5 ($10^6/mm^3$). Furthermore [18] stated that the normal number in chickens ranged from 2.3-3.5 ($10^6/mm^3$), and according to [19] that the number of normal erythrocytes in purebred chickens ranged from 2.17-2.86 ($10^6/mm^3$). Lower erythrocyte levels ($P>0.05$) in laying hens given fermented shrimp waste extract (R1, R2, R3, R4) is a physiological impact on astaxanthin's ability to reduce heat stress.

The lower number of erythrocytes in laying hens given the addition of fermented shrimp waste extract in the ration is a physiological impact on the antioxidant ability of astaxanthin which can prevent oxidative stress from free radicals, one of which is caused by heat stress. Free radicals are compounds that contain one or more unpaired electrons in their outermost orbitals, so they are highly reactive and able to oxidize surrounding molecules [20]. Astaxanthin as an antioxidant can dampen reactive oxygen species thereby reducing oxidative in lipids (including membranes and lipoproteins), proteins and DNA. Astaxanthin in shrimp waste shows strong antioxidant activity [9], then [21] state that astaxanthin shows antioxidant activity that can treat and prevent diseases caused by oxidative tests by reducing oxidative stress in target tissues in antioxidant therapy.

Erythrocyte levels in the group of chickens given fermented shrimp waste extract showed that the chickens did not experience oxidative stress due to hot environmental temperatures. This is because, in addition to astaxanthin as an antioxidant, there are also amino acids methionine and lysine in fermented shrimp waste extract. Methionine according to [22] and [23] acts as a neurotransmitter. The ability of methionine causes heat exposure to be received by the nervous system and responded very slowly by the central nervous system so that the physiological response of cells to heat becomes slow [24]. The physiological response to slow heat makes chickens not experience heat stress so that the number of erythrocytes is in normal numbers, in accordance with [25] that the number of erythrocytes is influenced by various environmental factors including environmental temperature.

3.2. Effects of Treatment on Hemoglobin Levels of Laying Hens

Hemoglobin is the main component of red blood cells in the form of iron-rich proteins that give red color to the blood. Hemoglobin functions in gas exchange and oxygen distribution into cells [26]. The average hemoglobin level in the study of laying hens given fermented shrimp waste extract in rations with each different treatment can be seen in Table 5.

Table 5 Average hemoglobin levels of laying hens' experimental results

Deuteronomy	Treatment				
	R0	R1	R2	R3	R4
	.... (g/dL)				
1	8.60	10.00	8.00	12.80	9.00
2	8.60	6.80	7.40	9.00	7.00
3	10.80	12.80	5.80	6.80	8.40
4	10.40	12.20	7.00	8.20	8.00
Total	38.40	41.80	28.20	36.80	32.40
Average	9.0	10.45	7.05	9.20	8.,10

Based on Table 5. hemoglobin levels in the results ranged from 7.05 g / dL - 10.45 g / dL. The lowest hemoglobin content was produced in the R2 treatment with an average value of hemoglobin content (7.05 g / dL) and the highest average value of hemoglobin content was produced in the R1 treatment with a total (10.45 g / dL). The results of fingerprint analysis (ANOVA) showed that the administration of fermented shrimp waste extract in the ration had no real effect (P>0.05) on hemoglobin levels of laying hens. Hemoglobin levels that have no real effect, are still in the normal range in accordance with the research [18] and [17] which states that normal hemoglobin levels in chickens range from 7.0-13.0 g / dL.

Fermented shrimp waste extract contains bioactive astaxanthin which acts as an antioxidant. Antioxidant activity can protect hemoglobin from oxidation [27]. Antioxidants protect membrane lipids and hemoglobin from oxidation attack by H2O2, thus preventing hemolysis caused by peroxide attack [28 in 29]. Many studies have reported that astaxanthin has much stronger antioxidant activity than other carotenoids. Astaxanthin has antioxidant activity 10 times stronger than zeaxanthin, lutein, and canthaxanthin in splitting singlet oxygen [8]. Furthermore [30] said astaxanthin has beneficial effects in reducing oxidative stress, enhancing immune response, with its main mode of action being a scavenger of reactive oxygen species (ROS).

The hemoglobin content of laying hens given fermented shrimp waste extract (R1, R2, R3, R4) when averaged overall had a lower yield than the group of chickens who were not given fermented shrimp waste extract (R0). This can be due to the role of astaxanthin as a potential antioxidant that can prevent oxidative stress. The results of [31] research that giving fermented shrimp waste extract can increase the digestibility value of protein higher than basal rations without giving fermented shrimp waste extract, meaning that nutrient absorption becomes higher. Higher nutrient absorption positively affects the formation of red blood cells, where hemoglobin levels are directly proportional to the number of erythrocytes [32]. Normal hemoglobin levels in this study were directly proportional to the normal number of erythrocytes.

3.3. Effect of Treatment on Hematocrit Value of Laying Breed Chickens

Hematocrit is the percentage of the number of red blood cells in the overall blood volume. The hematocrit value is highly dependent on the number of erythrocytes, since erythrocytes are the largest mass of cells in the blood [33]. The average hematocrit value in the study of laying hens given fermented shrimp waste extract in rations with each different treatment can be seen in Table 6.

Table 6 Average hematocrit value of laying hens' experimental results

Deuteronomy	**Treatment**				
	R0	**R1**	**R2**	**R3**	**R4**
	…………………………. (%)……………………………				
1	28	28	27	25	27
2	28	27	23	31	29
3	23	30	23	23	29
4	29	28	27	25	26
Total	108	113	100	104	111
Average	27.00	28.25	25.00	26.00	27.75

Based on Table 6, the hematocrit value of the study results ranged from 25% - 28.25%. The lowest hematocrit value was produced in R2 treatment with an average hematocrit value (25%) and the highest average hematocrit value was produced in R1 treatment with a total (28.25%). The results of fingerprint analysis (ANOVA) showed that the administration of fermented shrimp waste extract in the ration had no real effect ($P>0.05$) on the hematocrit value of laying hens. The hematocrit value that has no real effect is still within the normal range, in accordance with the research of [17;12] which states that normal hematocrit values range from 22%-35%.

The use of astaxanthin derived from fermented shrimp waste as much as 10-20 μg / ml or equivalent to 10-20 ppm can show strong antioxidant activity [16]. Giving fermented shrimp waste extract containing active ingredients in the form of astaxanthin has a positive impact on digestion. This is in accordance with what was said by [34] that astaxanthin has benefits for digestion ungags. Furthermore [31] suggests that giving fermented shrimp waste extract can increase the digestibility of feed ingredients. Increased nutrient absorption has the potential to increase the formation of red blood cells (erythropoiesis). Especially protein as one of the precursors forming red blood cells. Precursors of sufficient red blood cells are very likely to form red blood cells with a larger size [35].

Apart from the influence of the nutrient absorption process, protein and Zn deficiency can also affect hematocrit values. Protein and Zn deficiency will affect the low hematocrit value, according to [36] protein and iron minerals are nutrients for the formation of hemoglobin. Lack of protein and iron will interfere with the formation of erythrocytes (erythropoiesis). Iron in the erythropoiesis process is only needed in small amounts, but it must remain in feed because iron is not converted from other nutrients [37]. Zn has a role in the conformation of membrane proteins and protein interactions in the cell membrane directly [38]. When viewed from the results of the study above, the percentage of hematocrit shows the possibility of no protein and Zn deficiency, this indicates that the health status of chickens is in good condition.

4. Conclusion

The use of fermented shrimp waste extract at the level of 0.5% in the ration was effective in producing erythrocyte count, hemoglobin level, and blood hematocrit value of laying hens with values of $2.65\times10^6/mm^3$, 10.45 g/dL, and 28.25%, respectively. Fermented shrimp waste extract can be used in the range of 0.5-2% in the ration to maintain the hematological value of the blood of laying hens (erythrocyte count, hemoglobin level, and hematocrit value).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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