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EFFECTIVE METHODS OF PYRIDOXINE SUPPLEMENTATION IN LAYING HENS TO ALBUMIN AND GLOBULIN LEVELS

ABSTRACT

This research investigates the effective methods of pyridoxine supplementation to enhance the protein (albumin and globulin) level of chicken egg. 12 laying hens that been ready to produce eggs were categorized into three groups based on pyridoxine supplementation methods (via drinking water, ration, and intravenous injection). Each group received supplementation pyridoxine treatment with dosage of 3 mg/kg ransoms for 68 days. The albumin and globulin levels were analyzed using Folin's Fenol technique and compared to the standard egg. We found that only albumin level could be enhanced. The intravenous injection was the most effective method to significantly increase the albumin levels compared.

Keywords: Albumin and Globulin, Egg, Laying Hens, Supplementation of Pyridoxine

INTRODUCTION

The egg is one of the familiar foods in our daily life. Egg chicken has been recognized as one of very nutritious food since it contains high-quality protein, vitamin, carbohydrate, fat, and easy to digest .[1-5]

Albumin and globulin are the main proteins of egg. Generally, albumin and globulin levels in an egg are about 4.0-5.9 and 3.0-5.0 g/dL, respectively.[6,7] Albumin has a role to control the blood osmotic pressure through spreading the body fluid, while globulin is useful to control ion circulation, hormone, and fatty acids in human's body. Moreover, globulin can bind with hemoglobin, transport iron substance, coagulation factors, and as antibodies to attack the germs[6].

Since the albumin and globulin are the vital proteins in eggs the pyridoxine as coenzymes should involve in biosynthesis of those proteins fractions. The availability of pyridoxine consumed by laying hens not only determines the rate of albumin and globulin in biosynthesis but also determines the albumin-globulin content that transferred into the chicken egg.

Eggs quality are determined by the quality and health of laying hens that produce it. If the laying hens are maintained with a right level of health and given a ration with sufficient nutritional value, the eggs quality will be high.[8,9] Researchers have made various efforts in order to produce the excellent quality of eggs which have excellent nutritional value and optimum protein content. One effort that has been done by the addition of pyridoxine (vitamin B6) in rations.[10-12] The Pyridoxine that needs for laying hens is 4.5 mg/kg of ration. However, in general pyridoxine present with a dose of 3.0 mg/kg ration has been sufficient to provide a healthy life for laying hens [13]. The gold of this study is to find the easiest and the most effective method of pyridoxine supplementation in order to produce eggs with high protein content.

EXPERIMENTAL

Materials, Experimental Design and Management

This research was conducted at Chemical Laboratory of State University of Medan. 12 adult laying hens (Isa brown kind) that had been ready to lay egg were used as the samples. The vitamin B6 (pyridoxine) with dose of 3.0 mg/kg of rations was used as coenzyme to stimulate the biosynthesis of albumin and globulin. Bovine Serum Albumin (BSA) (Sigma-Aldrich) was used as the standard protein. The commercial rations that already contained the regular dose of pyridoxine was used as the rations. All experiment was performed using a Completely Randomized Design (CRD) with three treatments and each treatment was repeated for four times. The 12 laying hens were separated into three groups based on pyridoxine supplementation treatment. The groups that threat via drinking water, via ration and via intravenous injection were denoted as S-1, S-2 and S-3, respectively. Maintenance and care of samples were carried out by following the Bugos's procedure[14].

Maintenance and Provision of Treatment on Laying Hens

Before starting the experiment, a rectangular cage with size of 45 x 30 x 45 cm was built in a row overlapping each other. It consisted of 6 cages at the front and six cages at the rear. The cage door located at the top. The cage floors made of bamboo slats that sparsely arranged to let the chicken manure directly fall down to the ground. Each laying hens was inserted into a cage and the maintenance carried out for 68 days. During the maintenance, all the laying hens was given water to drink and standard commercial rations trough ad libitum feeding. After 14 days of adaptation in the cage, the sample was given supplementation pyridoxine at a dose of 3.0 mg/kg ration. On 68th day, the eggs were collected to determine the albumin and globulin levels.

Determination of Albumin and Globulin Levels

Determination albumin protein and globulin levels in egg sample were done by Folin's Fenol procedure[15]. In detail, 0.5 mL of egg sample was poured into a tube reaction followed by adding 9.5 mL Na_2SO_4 (22.5%) solution. After that the sample was soaked into another beaker glass containing 37°C water for 2 hours. Sample was then filtered by using filter paper to obtain albumin filtrate and globulin sediment. Afterward, the obtained sediment was moved on the top of measure flask 50 mL. The filter paper was carefully perforated and then washed with NaOH 0.01 M solution followed by distilled water. 20 µL of protein standard Bovine Serum Albumin (BSA) from Sigma-Aldrich was poured out into standard tube. Three flask measures were prepared with flask 1 for albumin protein, flask 2 for globulin protein and flask 3 for standard protein (see Table-1).

Finally, its absorbance was measured at a wavelength of 570 nm to determine the albumin and globulin level according to below formula:

$$Albumin\ levels = \frac{Sample\ absorbance}{Standard\ absorbance}\ x\ Standard\ concentration\ (5\ g\ /\ 100\ mL) \qquad (1)$$

$$Globulin\ levels = \frac{Sample\ absorbance}{Standard\ absorbance}\ x\ Standard\ concentration\ (5\ g\ /\ 100\ mL) \qquad (2)$$

Table-1: The Reaction Analysis of Albumin and Globulin Levels using Folin's Phenol Method

Reaction	Flask 1	Flask 2	Flask 3
Filtrate albumin or globulin	5 mL	5 mL	-
BSA Standards	-	-	4 mL
Aquades	25 mL	25 mL	25 mL
NaOH	2 mL	2 mL	2 mL
Folin's Fenol	3 mL	3 mL	2 mL

Data Analysis

The albumin and globulin levels that obtained from measurements were further statistically analyzed using analysis of variance (ANOVA). Hypothesis test was done at the significant level of $\alpha = 0.05$. If there was significant effect then least statistics different (LSD) test was then further conducted. Fig.- 1 schematically shows the flow chart of this research.

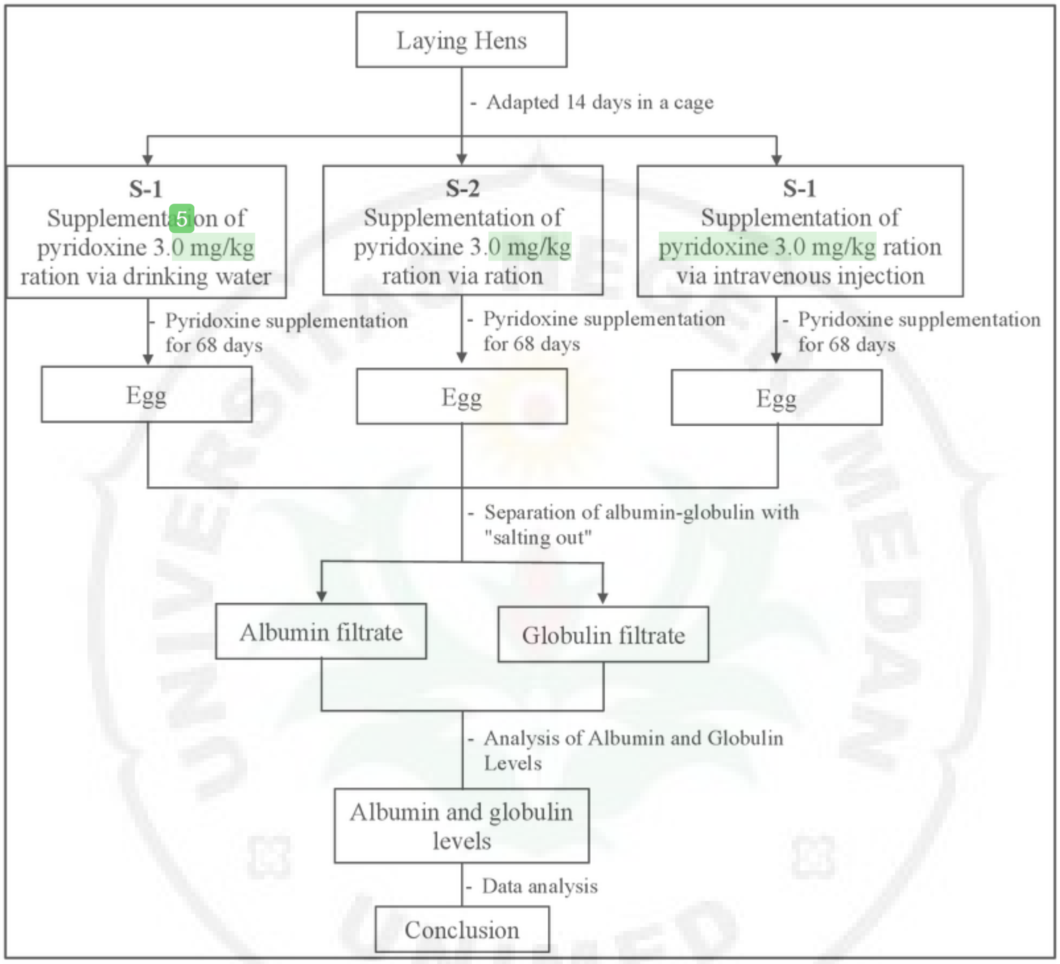

Fig. -1: Research stages of effective methods of pyridoxine supplementation in laying hens.

RESULTS AND DISCUSSION

Analysis of Albumin Levels

Table 2 lists the albumin levels from pyridoxine supplementation with dosage of 3.0 mg/kg rations by different methods. The average of albumin levels from pyridoxine supplementation via drinking water, ration and intravenous injection are 6.16, 7.29 and 9.76 g/dL, respectively. It is found that the intravenous injection was the most effective method to give the highest albumin level. The albumin levels of pyridoxine supplementation by those three methods were significantly higher compared to the standard egg with albumin level of about 4.0-5.9 g/dL

Table-2: Average Albumin Level by Different Methods with Pyridoxine Dosage of 3.0 mg/kg Ration

Variables	Variations of pyridoxine supplementation methods		
	Via drinking water	Via ration	Via intravenous injection
Average albumin level (g/dL)	6.16 ±0.87	7.29 ±0.76	9.76 ±1.18

Table-3 shows the result of analysis of variance for albumin level of chicken egg after pyridoxine supplementation with different methods. Since the $F_{calculate}$ (13.01) is greater than F_{table} (10.92) then the hypothesis (Ho) is rejected which means there is a significant effect of pyridoxine supplementation on albumin level. Furthermore, the Least Significance Different (LSD) test also proved that albumin level via intravenous injection was significantly higher compared to via drinking water and via ration, as shown in Fig.-2.

Table-3: Analysis of Variance Albumin Levels of Chicken Egg Via Drinking Water, Ration, and Intravenous Injection

Sources of Diversity	DF	SS	MS	$F_{cal.}$	F_{table} (0.01 DF)
Treatment	2	20.35	10.17	13.01	10.92
Error	6	4.69	0.78		
Total	8	25.04	10.95		

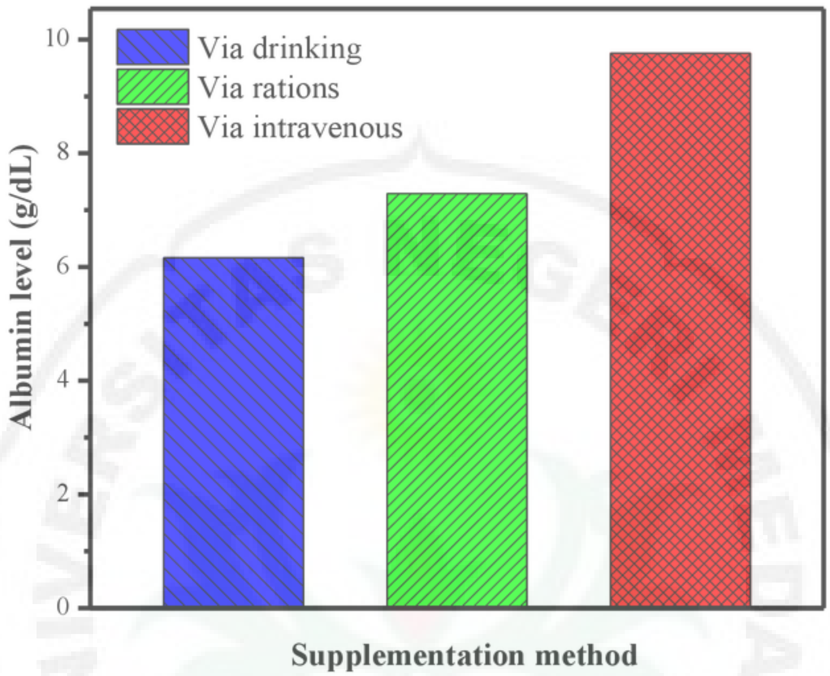

Fig.-2: Albumin Levels of Chicken Egg by Different Methods with Pyridoxine Dosage of 3.0 mg/kg Ration

Analysis of Chicken Egg Globulin Protein Levels

Table-4 shows the globulin levels of egg that supplemented with pyridoxine (3.0 mg/kg ration) for various treatments. The globulin levels via drinking water, ration and intravenous injection are 4.44 g/dL, 3.20 g/dL and 3.94 g/dL, respectively. The average globulin levels for those treatments are similar to the standard globulin level of 3-5 g/dL. Furthermore, analysis of variance indicates that globulin level of chicken egg after pyridoxine supplementation via drinking water, through ration, and intravenous injection was not significantly different, as shown in Table-5.

Table 4. Average Globulin Levels of Chicken Egg Supplemented with Pyridoxine Dose 3.0 mg/kg Ration

Variables	Variations of Pyridoxine Supplementation Methods		
	Via Drinking Water	Via Ration	Via Intravenous Injection
Mean globulin egg level (g/dL)	4.44 ± 0.88	3.204 ±1.01	3.941 ±0.30

Table-5: Analysis of Variance Globulin Level of Chicken Egg Via Drinking Water, Ration, and Intravenous Injection

Sources of Diversity	DF	SS	MS	$F_{cal.}$	F_{table} (0.01 DF)
Treatment	2	2.33	1.16	1.84	10.92
Error	6	3.80	0.63		
Total	8	6.14	1.80		

Pyridoxine is a water-soluble compound and acts as a coenzyme to help facilitate the metabolism of carbohydrates, fats, and proteins.[16] Vitamin supplementations in layer diet remained indispensable due to their participation in all biochemical processes and chicken gut flora provides minimum vitamin synthesis but competes with the host for dietary vitamins.[4,17,18] Chicken eggs contain high-quality proteins, carbohydrates, easily digestible fats, and minerals, as well as valuable vitamins.[19,20] Poultry is susceptible to vitamin deficiency because the microorganisms can cause it in the digestive tool of poultry. Poultry needs large amounts of vitamins since they cannot synthesize vitamins for the ongoing reactions of metabolism in the body.[21-23]

Normal albumin level in chicken eggs are about 4.0 to 5.9 g / dL. It is concluded that the albumin levels after pyridoxine supplementation by each method is increased compared to the normal levels. It causes pyridoxal phosphate (PLP) is a versatile coenzyme. It can play a role to catalyse the important reactions in the metabolism of amino acids and proteins.[24-28] As it is known that there are about 60 types of amino acid reactions involving pyridoxal phosphate.[29] Therefore, the pyridoxine plays the important role in the formation of protein in chicken eggs, especially in albumin level.

CONCLUSION

Three different methods of pyridoxine supplementation via drinking water, ration, and intravenous injection could only significantly enhance the albumin protein levels of chicken egg. Supplementation of pyridoxine via intravenous injection was the most effective method for increasing levels of egg albumin protein. The globulin level after pyridoxine supplementation was similar to the standard chicken egg.

ACKNOWLEDGMENT

This work was supported by Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan-Indonesia.

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