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Blood fat profile of laying hens consuming rations fortified with citric acid and beta-carotene

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Abstract. Extreme climate change greatly affects the metabolism of laying hens due to their intolerance to high environmental temperatures. Hence, to improve the metabolism, the feed quality also needs to be improved. This study aims to determine the blood lipid profile of laying hens consuming a diet rich in citric acid and beta-carotene (β-carotene). During the study, 200 laying hens of the Lohmann strain aged 35 weeks and a ration with a crude protein content of 18.39% and metabolic energy of 2960 Kcal/kg were used. The treatment was fortification with various levels of β-carotene sources in the diet and 0.2% citric acid, namely T0 (without fortification of β-carotene sources and citric acid), T1 (0.1% fortification of β-carotene sources + 0.2% citric acid), T2 (0.15% fortification of β-carotene + 0.2% of citric acid), and T3 (0.2% fortification of β-carotene + 0.2% of citric acid). The data were analyzed by analysis of variance and further testing by Duncan's Multiple Range Test. Furthermore, the observed variables were blood cholesterol content and HDL, as well as triglycerides. The results showed that the fortification of citric acid and β-carotene significantly affected blood cholesterol levels but had no significant effect on the concentration of HDL and triglycerides. Considering these results, it was concluded that fortification of citric acid and β-carotene sources in the ratio of laying hens decreased the amount of fat in the blood.

1. Introduction

Laying hens are animals that provide a wellspring of creature protein, such as eggs, which have a high health benefit and moderately low purchasing costs. It experiences various phases in their growth, beginning from the starter, grower, and layer phase. It also experience physiological stress at production, specifically during peak production. However, this worsens due to high environmental temperature, which causes heat stress for laying hens. It is also sensitive to hot weather and noise, with an average body temperature between 39 – 41°C [1].

The efforts made in dealing with heat stress in laying hens include providing a source of antioxidants such as *Indigofera* leaves in their rations. A report showed that *Indigofera sp* leaves contain β-carotene of 507.6 mg/kg [2]. Therefore, adding an acidifier in the form of citric acid is necessary to improve the digestive performance of feed substances in the ration. The effectiveness of using *Indigofera zollingeriana* leaves as an antioxidant in the diet and the addition of citric affects the blood fat profile of laying hens. This study aims to evaluate the blood lipid profile of laying hens with rations fortified

as antioxidants from *Indigofera* leaves and citric acid under physiological conditions and tropical environments to support productivity.

2. Materials and methods

2.1. Research materials

The sample used was 200 laying hens, placed in an experimental cage plot in the form of a battery size of 40 x 30 x 40 cm. Furthermore, it was placed randomly according to treatment and replication, consisting of 10 laying hens.

2.2. Rations

The feed ingredients used in preparing the ration consist of 50% milled corn, 35% concentrate, 15% rice bran, and β-carotene source for treatment. The rations are prepared and stirred once a week with a mixer. Then it was given three times a day, with 50% dose in the morning, 30% in the afternoon, and 20% in the evening. The nutritional contents of the rations used are listed in Table 1.

Table 1. Nutrient content of study rations.

Nutrient composition	Nutrient content
Crude protein (%)	18.39
Crude fat (%)	2.50
Crude fiber (%)	5.14
Calcium (%)	3.16
Phosphor (%)	1.81
Metabolism energy (Kcal)	2960

2.3. Drinking water

There is an unlimited provision of drinking water, and ration mixing is performed every week. Therefore, to determine the consumption rate of the ration, the remaining quantity is weighed alongside the dose administered

2.4. Experimental design

The method used was a completely randomized design consisting of 4 treatments and 5 replicates comprising 10 laying hens. Furthermore, the treatment includes the fortification of β-carotene from *Indigofera* leaves and citric acid in the ration consisting of the followings:
T0: Without fortification of β-carotene and citric acid in the ration.
T1: Fortification of 0.10% β-carotene *Indigofera* leaves and 0.10% citric acid in the ration.
T2: Fortification of 0.15% β-carotene *Indigofera* leaves and 0.10% citric acid in the ration.
T3: Fortification of 0.20% β-carotene *Indigofera* leaves and 0.10% citric acid in the ration.

2.5. Observed variables

The variables observed were the blood fat profile of laying hens, including cholesterol, HDL, and triglyceride. Furthermore, the process was performed by collecting blood samples in each treatment at the end of the first and second months through the jugular vein and accommodated in a tube of approximately 6 ml. Afterward, they were separated from the serum, prepared using a blood collection tube without EDTA/heparin as a 9 ml anti-coagulant. The tube is angled at around 450 degrees to broaden the blood surface and obtain a significant volume of serum. As a result, a yellowish transparent liquid appears at the top of the blood, the liquid is accommodated into a 4 ml cuvette. In addition, the cholesterol, HDL, and triglyceride levels were analyzed using spectrophotometric techniques.

2.6. Data analysis

The data collected were analyzed using a completely randomized design (CRD). Then, Duncan's multiple range test was performed with a significant difference less than 0.05 [3].

3. Results and discussion

Climate change affects the hens' metabolism, specifically when the temperature is high. Furthermore, the health of laying hens is harmed due to the long-time persistence of this condition. The fortification of β-carotene and citric acid in the diet of laying hens affects ($P<0.05$) their blood characteristics. The average blood profile of laying hens in the form of cholesterol, HDL, and triglycerides, which have rations were fortified with β-carotene sources from *Indigofera* leaves and citric acid is shown in Table 2.

Table 2. Average blood fat profile of laying hens during the study.

Treatments	Blood cholesterol(mg/100ml)	HDL (mg/100ml)	Triglyceride (mg/100ml)
T0	$168.56^{b} \pm 2.45$	42.43 ± 0.56	$169.41^{b} \pm 5.23$
T1	$147.31^{a} \pm 4.11$	39.76 ± 1.19	$153.83^{a} \pm 2.77$
T2	$153.24^{a} \pm 3.72$	44.02 ± 1.83	$155.89^{a} \pm 3.14$
T3	$149.98^{a} \pm 2.33$	41.89 ± 3.47	$159.17^{a} \pm 3.26$

Note: Different characters in the same column indicate significant differences ($P < 0.05$).

The analysis of variance showed that the fortification treatment with β-carotene and citric acid had a significant effect ($P<0.05$) on blood cholesterol levels in laying hens in the first production phase. The higher the concentration of β-carotene in the diet, the lower the blood cholesterol levels, which shows the presence of lower fat catabolism in laying hens. Furthermore, the increased cholesterol in the blood negatively affects health [4]. β-carotene fortification acts as an antioxidant in the body of laying hens, thereby reducing the formation of free radicals, which are a by-product of various metabolisms. A report stated that an increase in the use of *Indigofera* leaves in the diet increases the level of antioxidants in the blood, which positively affects the health status of livestock [1].

The analysis of variance on the HDL concentration of laying hens' blood showed that the fortification treatment with β-carotene and citric acid had no significant effect ($P > 0.05$) on blood HDL. Furthermore, the average HDL value of laying hens' blood ranged from 39.76 - 44.02 mg/100ml, implying that it was in the normal range. The average value of HDL cholesterol in broiler blood is above 22 mg/100 ml. This value implies that fortification of β-carotene from *Indigofera* leaves and citric acid did not affect normal HDL levels in blood [5].

The analysis of variance on triglyceride values showed that the fortification of β-carotene and citric acid had a significant effect ($P < 0.05$) on laying hens. The average triglyceride values were 153.83 - 169.41 mg/100 mL. The antioxidant content in *Indigofera* leaves reduces the synthesis of fatty acids in the liver caused by the Acetyl-CoA carboxylase enzyme. Also, the decrease in triglyceride levels in the blood is caused by a reduction in fatty acid synthesis by the acetyl CoA carboxylase enzyme activity and the possibility of large fat degradation in the liver. [6]

4. Conclusions

Feed fortification with β-carotene from *Indigofera zollingeriana* up to 0.20% and 0.10% citric acid in the ration improved the metabolism of laying hens. Also, the fortification of β-carotene and citric acid in the diet reduces blood cholesterol levels and does not negatively affect HDL and triglycerides in laying hens.

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