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The Effects Rubber Leaf (*Hevea brasiliensis*) Flour Addition in Non-AGP Commercial Rations on Blood Cholesterol Levels of Broiler

Sri Wigati[a*], Tommy Parulian Lumban Tobing[b], Pudji Rahayu[a]

[a]Deparment of Animal Health, Animal Science Faculty, Jambi University, Jl. Raya Jambi-Muara Bulian, KM. 15 Mendalo Indah, Jambi, Indonesia 36361
[b]Under Graduated Student of Animal Science Faculty, Jambi University, Jambi, Indonesia

*Corresponding author: E-mail: sriwigati@unja.ac.id

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ABSTRACT

The research aimed to analyze the effects of adding rubber leaf *(Hevea brasiliensis)* flour in non-AGP commercial rations on the blood cholesterol levels of broiler chickens. The materials used were 200 heads of *Day Old Chick* (DOC) unsex from MB-202 strain, non-AGP commercial ration containing 22% protein, and rubber leaf flour with a tannin content of 2.5% DM basis. The experiment was designed using a completely randomized design (CRD) with four treatments and five repetitions, and each repetition unit consists of 10 DOCs. The treatment was the addition of rubber leaf flour by 0% (P0), 5% (P1), 7,5% (P2), and 10% (P3) in 100% of the commercial ration given. Broiler chickens were raised in colony battery cages for five weeks of the trial period. The observed variables were cholesterol, triglyceride, HDL, and LDL plasma levels. Data were analyzed using analysis of variance and Duncan's multiple range test. The results showed that the estimated tannin compounds in rubber leaf powder consumed were 0.11%-0.22% and that the feeding treatment significantly decreased ($P<0.05$) blood cholesterol and triglyceride levels and significantly increased ($P<0.05$) blood HDL levels. Still, it had no significant effect on the LDL levels of broiler chickens. Based on the study's results, it can be concluded that adding 7.5%-10% rubber leaf flour to commercial rations can be used as a feed additive for reducing blood cholesterol levels in broiler chickens.

Keywords: Rubber Leaf Flour, Broiler Chicken, Cholesterol, Triglycerides, HDL, LDL

INTRODUCTION

Broiler chicken is a type of broiler chicken that is often cultivated because the harvest period is short and relatively easy to rear so that it can be marketed quickly. However, the rapid growth of broiler chickens will also be accompanied by high-fat growth. This high fat causes high fat and cholesterol content in broiler chickens, which are not liked by consumers [1].

There are dilemmatic problems related to the health of chickens and consumers, especially in the chicken industry in Indonesia. Current food safety regulations prohibit synthetic antibiotics as Antibiotic Growth Promoter (AGP) due to the impact of residue accumulation in chicken products and *drug resistance,* which affects consumer health. On the other hand, the chicken industry in Indonesia must continue to produce healthy chickens, so it is necessary to

look for alternatives to these Antibiotic Growth Promoters (AGP). A safer alternative to AGP is natural antibiotics and herbs. One is the herbal antibiotic AGP, which can be used in rubber leaf powder [2]. Rubber leaves (*Hevea brasiliensis*) were reported to contain tannin compounds and have good potential as nutritional herbs [3].

The results of previous studies showed that rubber leaves (*Hevea brasiliesnsis*) had activity as an anti intestinal parasite for livestock [4, 5]. Rubber leaves in the form of flour added to non -*antibiotic growth promoter* (Non-AGP) commercial rations of 3-9% have also been reported to be able to reduce coccidia infestations in native chickens by 64% [6].

The herbal effect of rubber leaves is mediated by their tannin content, which is equal to 2.5% of the DM base [3]. Several other research results also reported that tannin compounds have antibacterial [7], antioxidant, and gastrointestinal antiparasitic activities [5, 6]. In addition, tannin compounds have also been reported as active compounds that can reduce fat in broiler chickens [8]. The mechanism of tannin compounds in lowering cholesterol levels is known in several ways, including by inhibiting cholesterol absorption or increasing cholesterol excretion through feces [9].

The administration of rubber leaf flour is expected to provide a prophylactic herbal effect and tannin effects in reducing cholesterol to improve its performance. Based on the description above, this study analyzed the impact of adding rubber leaf (Hevea brasiliensis) flour in non-AGP commercial rations on broiler chickens' plasma cholesterol, triglycerides, HDL, and LDL levels.

MATERIALS AND METHODS

Research Materials

The research was conducted at the Fapet Farm experimental cages, Faculty of Animal Science, Jambi University. The materials used were 200 heads of *Day Old Chick* (DOC) unsex from MB-202 strain, non-AGP commercial ration containing 22% protein, and rubber leaf flour with 2.5% DM basis tannin content. The non-AGP commercial chicken feed was NOVO 511, produced by PT Charoen Pokphan, and rubber leaves were obtained from the community's rubber plantation in Pondok Meja village, Jambi, Indonesia.

The equipment for analyzing blood cholesterol levels was a Hematoanalizer, centrifuge, sample cup, automatic pipette, and yellow and blue tips.

Research Methods

Feed treatments and experimental design. A total of 200 DOC of broiler chickens were allocated in 20 colony battery cages by employing a completely randomized design (CRD). The experiment was designed with four treatments and five repetitions, and each repetition unit consists of 10 DOCs. The feed treatment given was the addition of different levels of rubber leaf flour in Non-AGP commercial broiler rations with the experimental feed composition as follows:

P0 = 100% Commercial Chicken Ration + 0% Rubber Leaf Flour
P1 = 100% Commercial Chicken Ration + 5% Rubber Leaf Flour
P2 = 100% Commercial Chicken Ration + 7.5% Rubber Leaf Flour
P3 = 100% Commercial Chicken Ration + 10% Rubber Leaf Flour

The feed treatments were given *ad libitum* starting at five days, and the chickens were kept until 35 days (5 weeks). Drinking water was also provided *ad libitum* during the trial period.

Treatment rations. The chemical composition of the feedstuffs used in treatment rations is presented in Table 1, and the feed chemical (nutrient) composition of the treatment rations is presented in Table 2.

Table 1. Feed chemical composition (nutrients) of the feedstuffs used in the treatment rations

Feed Ingredients	Composition (%)*				
	DM	OM	CP	EE	CF
Rubber Leaf Flour	94.38	94.64	21.75	4.80	27.84
Commercial Rations	89.60	92.00	22.42	4.29	3.56

*Results of proximate analysis at the Animal Feed and Nutrition Lab, Faculty of Animal Husbandry, Jambi University (November 2020). DM is dry matter, OM is organic matter, crude protein (CP), extract ether (EE), and crude fiber (CF).

Chicken weighing. The first and second weighing was to record the weights of DOCs and chickens on day 4 of age. Those weighings were also used to test the suitability of the experimental design. The subsequent weighing was carried out with an interval of 7 days.

Table 2. Feed chemical composition (nutrients) of the treatment rations.

Treatment ration	Nutrient Composition of Treatment Rations (%)**				
	DM	OM	CP	EE	CF
P0	89.60	92.00	22.42	4.29	3.56
P1	89.83	92.13	22.39	4.31	4.72
P2	89.93	92.18	22.37	4.33	5.25
P3	90.03	92.24	22.36	4.34	5.77

**Resulted by calculating the composition of treatment rations with the feed chemical composition of the feedstuffs in Table 1. P0= Control ratio (0% rubber leaf flour); P1=Ration with adding 5% rubber leaf flour; P2=Ration with adding 7.5% rubber leaf flour; P3=Ration with adding 10% rubber leaf flour.

Collection of feed and leftover feed. The feed given is weighed daily, and the remaining feed given is weighed at the end of the week and recorded to determine the amount of feed consumed and DM feed consumed.

Blood sampling. Blood samples were taken from the chickens at the age of 35 days. One chicken each from all feed treatments and replications with weight in or close to the average body weight was blood sampled from the jugular vein. The blood samples (± 3 ml/chicken) were collected in a non-anticoagulant vacutainer, tightly closed, stored in an ice box, and were then sent to the laboratory. In the laboratory, blood samples were harvested for the serum and then analyzed for cholesterol, triglyceride, HDL, and LDL levels.

Measurement of blood cholesterol, triglyceride, **HDL, and LDL.** The cholesterol, triglyceride, HDL, and LDL levels were measured using a photometer with the method of the cholesterol oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) with the use of a wavelength of 564 and a temperature of 37℃. The procedure for measuring complete

cholesterol levels refers to the SOP (*Standard Operational Procedure*) of the Jambi City Health Laboratory.

Observed Variables and Data Analysis

The variables observed in this study were estimated tannin consumption, blood (serum) cholesterol, triglyceride, HDL, and LDL levels of boiler chickens.

The terminations used in determining the observed variables were:

1. Estimated consumption of rubber leaf flour tannin is calculated based on consumption from DM of rations, DM of rubber leaf flour, and total tannin content in DM of rubber leaf flour by using the following formula:

$$\text{Consumption of DM rations} = \frac{\text{DM rations given (g)} - \text{DM rations remaining (g)}}{\text{(number of chickens)}}$$

2. Total Cholesterol (TC) = LDL + HDL + 20% Triglicerides
3. LDL = TC - HDL - 20% Triglicerides
4. HDL = TC – LDL - 20% Triglicerides

Data obtained were analyzed using analysis of variance (ANOVA). If the treatments affected the variable observed, it was continued with a follow-up test, i.e., Duncan's multiple range test [10].

RESULTS AND DISCUSSIONS

Estimated Consumption of Tannins of Broiler Chickens

Dry matter consumption was measured to be used as an estimate of the amount of tannin consumed by broiler chickens in each treatment. Rubber leaf flour has a total tannin content of 2.5% and condensed tannin of 2.3% on a dry matter basis [3]. The average dry matter (DM) consumption of the experimental rations and estimation of tannin consumption of broiler chickens fed Non-AGP commercial rations with the addition of rubber leaf flour were presented in Table 3.

Table 3. Average dry matter consumption of the experimental rations and broiler chickens' estimated total tannin consumption.

Treatment	DM consumption		Estimated total tannin consumption	
	Ration (g/head)	Rubber leaf flour (g/head)	Amount (g/head)	Percentage (%/head)
P0	3018 ± 75.35	0.00	0.00	0.00
P1	3242 ± 162,10	162.10	4.10	0.11
P2	2853 ± 341.44	213.90	5.30	0.17
P3	2856 ± 365.60	285.60	7.10	0.22

Note: The ANOVA results on DM consumption were non-significant (P>0.05).
DM of Rubber leaf flour = 94.38%; the total tannin content in rubber leaf flour = 2.5% DM Basis (Wigati et al., 2014). P0 = addition of 0% rubber leaf flour, P1 = addition of 5% rubber leaf flour, P2 = addition of 7.5% rubber leaf flour, P3 = addition of 10% rubber leaf flour

The result of the analysis of variance showed that there was no significant difference (P>0.05) in DM consumption between treatments. The DM consumptions of the rations were used to determine the DM consumption of rubber leaf flour. Hence, an estimate of the total tannin consumption can then be determined. Table 3 showed that estimated total tannin consumption increased with increasing rubber leaf flour addition levels. Thus, the increase in the rubber leaf flour addition level was still in line with the rise in tannin consumption and was still by the tannin doses designed to be given in the experiment.

Given the addition of rubber leaf powder, the estimations of total tannins consumed by broiler chickens were between 0.12 – 0.25% of the DM ratio. These tannin concentrations were still within tolerable limits for chickens. According to Akmal and Mairizal [11], the tannin content of 0.25 – 0.42% in the ration containing sengon leaves (*Albizzia falcataria*) was not yet anti-nutritive. It was still within the tolerance limits for livestock. Hadiyanto et al. [2] also stated that the consumption of tannins between 0.08 – 0.23% of the DM ration containing rubber leaf flour in native chickens was still within tolerance limits.

Blood Cholesterol Levels of Broiler Chickens

Cholesterol is a fatty substance from metabolism, mainly found in blood and bile fluid. Cholesterol is a precursor to form bile acids, which are synthesized in the liver and absorb triglycerides and soluble vitamins in food. According to Swenson [12], normal blood cholesterol levels for broiler chickens are 125-200 mg/dl. The results of measuring cholesterol, triglycerides, HDL, and LDL levels in the blood of broiler chickens given Non-AGP commercial rations with the addition of rubber leaf flour (*Hevea brasiliensis*) were presented in Table 4.

Table 4. The average values of blood cholesterol, blood triglycerides, blood HDL, and HDL of broiler chickens given a Non-AGP commercial ration added with rubber leaf flour at day 35 of the age.

Treatments	Variables			
	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
P0	148.40^{a}±20.50	41.20^{a}±7.20	73.00^{a}±7.90	36.40^{a}±9.20
P1	124.60^{ab}±4.40	30.60^{b}±2.50	73.00^{a}±7.90	26.00^{a}±6.60
P2	119.80^{b}±13.30	30.00^{b}±3.50	82.60^{ab}±7.40	25.00^{a}±2.90
P3	118.80^{b}±14.70	28.60^{b}±6.80	86.40^{b}±6.50	24.60^{a}±6.50

Note: Superscripts with different lowercase letters in the same column show significant differences (P<0.05). P0 = addition of 0% rubber leaf flour, P1 = addition of 5% rubber leaf flour, P2 = addition of 7.5% rubber leaf flour, P3 = addition of 10% rubber leaf flour

The analysis of variance showed that the addition of rubber leaf flour in the commercial ration had a significant effect (P<0.05) on the blood cholesterol levels of broiler chickens. Duncan's further test showed that blood cholesterol levels in the P1 treatment were not significantly different (P>0.05) from those of the control (P0). Still, blood cholesterol levels in P2 and P3 treatments were significantly different (P<0.05) from those of the control (P0).

Meanwhile, between treatments, blood cholesterol levels were not significantly different (P>0.05).

The results showed that adding rubber leaf flour with 7.5-10% levels in commercial rations can reduce broiler blood cholesterol levels slightly below their normal level. The ability of rubber leaf flour to lower blood cholesterol was thought to be due to the tannin content in rubber leaf flour and to be due to an increase in the amount of crude fiber (CF)) in the treatment rations in line with the rise in the levels of rubber leaf flour addition (Table 2). According to Wahyudi [13], tannins can reduce cholesterol and fat absorption through mucosal protein deposition on the surface of the small intestine. In line with Wahyudi [13], Lajuck [14] also stated that tannins can react with mucosal proteins and intestinal epithelial cells to inhibit fat absorption in the intestine. Furthermore, Dorisandi et al. [15] reported that feeding broiler rations composed of Seduduk leaf meal containing tannin compounds significantly reduced blood cholesterol levels in broiler chickens.

Concerning the role of crude fiber in decreasing blood cholesterol, Sutardi [16] stated that fiber can reduce fat absorption so that fat deposition into the chicken's body can be suppressed. Sitepoe [17] also noted that the addition of crude fiber (CF)) to the ratio can reduce broiler chicken blood plasma cholesterol and LDL. The mechanisms involved in suppressing cholesterol synthesis in the presence of crude fiber in feed are increasing intestinal peristalsis so that feed is not absorbed optimally, reducing precursor of essential compounds for cholesterol-forming materials in the blood circulation, and increasing the loss of bile salts in the duodenum so that the liver requires more cholesterol to produce bile salts by taking cholesterol reserves in the tissues [18].

Blood Triglyceride Levels of Broiler Chickens

Triglycerides are the central lipid storage in adipose tissue; this form of lipid will be released after hydrolysis by the hormone-sensitive lipase enzyme into free fatty acids and glycerol [19]. The liver is an organ that plays a vital role in the formation of triglycerides because the liver can convert carbohydrates into free fatty acids and transform them back into triglycerides [20]. According to Melluzi et al. [21], normal triglyceride levels in broiler chickens range between 43.3 and 168 mg/dl. Genes and consumption of foods such as carbohydrates, fats, and alcohol can influence increases in blood triglycerides. The measurement results of blood triglyceride levels in broiler chickens fed Non-AGP commercial rations with the addition of rubber leaf (*Hevea brasiliensis*) flour were presented in Table 4.

The statistical analysis showed that adding rubber leaf flour into the commercial ratio significantly affected broiler blood triglycerides (P<0.05). Further result tests showed that the blood triglyceride levels in the P1, P2, and P3 treatments were significantly lower (P<0.05) than that of the control (P0). However, blood triglyceride levels were not significantly different (P>0.05) between treatments. The results showed that adding 5-10% rubber leaf flour in commercial rations can reduce broiler blood triglyceride levels below their average. It was suggested that the decrease in blood triglyceride levels is due to tannin effects and crude fiber effects in inhibiting the absorption of cholesterol, which lead to a reduction in the synthesis of bile acids, which are used for the absorption of nutrients, including triglycerides from the feed.

Blood HDL Levels in Broiler Chickens

High-density lipoprotein (HDL) is the smallest lipoprotein particle synthesized by the liver. HDL is called good cholesterol, so its level in the blood is expected to be high [22]. HDL transforms excessive free cholesterol found in peripheral tissue endothelium, including blood vessels, to HDL receptors in the liver to be used as a precursor for bile. Then, it is excreted through bile [23]. The results of measuring blood HDL levels in broiler chickens fed a Non-AGP commercial ration with the addition of rubber leaf flour (*Hevea brasiliensis*) were presented in Table 4.

The results of the analysis of variance showed that the addition of rubber leaf flour in commercial rations had a significant effect ($P<0.05$) on the level of blood HDL (*High-Density Lipoprotein*) in broiler chickens. Duncan's follow-up test showed that the levels of blood HDL in P1 and P2 treatments were not significantly different ($P>0.05$) from that of the control (P0) and were not also significantly different ($P>0.05$) between those of P2 and P3 treatments. However, the levels of blood HDL in P3 treatment were significantly higher ($P<0.05$) than that of the control (P0). These results indicated that adding rubber leaf flour with levels of 10% in commercial rations can increase blood HDL levels of broiler chickens.

Increased HDL levels in the experiment indicated that there was a response to these treatments. Increasing HDL levels in broiler blood will increase its role in transporting excess cholesterol from blood vessels to the liver, which is then degraded or converted into bile acids. Hence, increasing HDL levels in the experiment will reduce blood cholesterol levels in broiler chickens.

Blood LDL Levels in Broiler Chickens

Low-density lipoprotein (LDL) is a metabolite of VLDL (Very Low-Density Lipoprotein), also called bad cholesterol because it is atherogenic. It means that it easily sticks to the walls of blood vessels and causes a buildup of fat, which can cause narrowing of the blood vessels. Basmacioglu and Ergul [24] show purebred chickens' average blood LDL level is <130 mg/dl. The results of measuring blood LDL levels in broiler chickens fed non-AGP commercial rations with the addition of rubber leaf flour (Hevea brasiliensis) are presented in Table 4.

The results of the statistical analysis showed that adding rubber leaf flour into the commercial ration had no significant effect ($P>0.05$) on the levels of blood LDL in broiler chickens. The level of the blood LDL in broiler chicken in all treatments (P0, P1, P2, and P3) was still at normal levels, even though they tended to be at low levels.

According to Murray et al. [25], LDL contains half to two-thirds of cholesterol; a decrease in blood cholesterol levels will cause a reduction in LDL levels because 65% of cholesterol is in the form of LDL. In the experiment, adding rubber leaf flour into non-AGP commercial rations reduced blood cholesterol and triglyceride levels but did not significantly reduce LDL levels in broiler chickens. According to Deviana et al. [26], decreasing cholesterol levels in the liver will stimulate an increase in LDL receptors (up*regulation*) on the surface of the liver. LDL receptor functions as a clearance for LDL cholesterol; thus, when blood cholesterol levels decrease, such as in the experiment, the cholesterol levels in the liver will also decrease and then downregulate to LDL receptor level, resulting in a decrease of blood LDL levels in broiler chickens. However, the levels of blood LDL in the experiment were nonsignificatly reduced. It was suggested that the

effect of tannins contained in rubber leaf flour in lowering blood cholesterol should also be accomplished by its impact on HDL levels since HDL functions to transport excessive free cholesterol such as LDL found in peripheral tissue endothelium, including blood vessels [23, 27, 28]

CONCLUSIONS

Based on the study's results, it can be concluded that adding 7.5%-10% rubber leaf flour to commercial rations can be used as a feed additive for reducing blood cholesterol, triglyceride, and LDL levels and increasing blood HDL levels in broiler chickens.

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