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Effects of Supplementation of Saviotan Feed (Chestnut Tannin) on Blood Parameters and Yolk Cholesterol Concentration in Japanese Quails (*Coturnix japonica*)

Edi Erwan[1]*, Afriadi[2], Muhamad Rodiallah[1], Isbul Irfan[1], and Wawi Ibrah[1]

[1]*Department of Animal Science, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Riau, Indonesia*

[2]*Research and Development, Cooperation of Eurovet Indonesia, Bogor, Indonesia*

*Corresponding's author's Email: erwan_edi@yahoo.com

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ABSTRACT

Tannins are secondary metabolites and active compounds widely present in plants. Tannins have several properties, such as astringent, antiparasitic, anti-diarrheal, anti-bacterial, and antioxidant. Hence, plants containing tannins are a major study subject for a natural alternative to in-feed antibiotics or antioxidants. The functions of tannin extracted from chestnut wood, namely Saviotan Feed (SF) in poultry, especially in quails, have not yet been fully understood. The current study aimed to examine the effect of SF supplementation on some plasma metabolites, including glucose (GLU), triglyceride (TG), total cholesterol (TCHO) concentration, and yolk cholesterol in quails (*Coturnix japonica*). A total of 100 unsexed quails were divided into 4 groups, with 25 quails in each group. These quails were then placed into 20 experimental pens, with 5 quails per pen. Each treatment was replicated 5 times, and the quails were fed a commercial rations diet supplemented with different SF doses of 0% (control), 0.1%, 0.2%, and 0.3%. Quails were provided with SF supplementation from 14 to 56 days of age. A sample of 40 plasma and eggs were randomly collected and analyzed for GLU, TG, and TCHO. The results indicated no significant effects of SF on plasma GLU and TG concentration, but a significant effect was found regarding TCHO. Moreover, supplementation of SF from 0.1 to 0.3% significantly decreased TCHO concentration in the yolk. In conclusion, it has been determined that supplementation of SF may play a significant role in decreasing TCHO in yolk eggs in quails.

Keywords: Chestnut tannins, Cholesterol, Glucose, Japanese quail, Plasma metabolites, Triglycerides

INTRODUCTION

Poultry production as a monogastric animal grows continuously to produce meat and eggs (Mohammed Hassan et al., 2020). Quail farming is one of the livestock sectors that efficiently provides quality animal protein (Handarini et al., 2008). Recently, the population of quail farming has developed in Indonesia. Based on the Indonesian Directorate General Livestock and Animal Health (2020), the population of quails in Indonesia was 14,819,755 heads. Subekti and Hastuti (2013) stated that quails can quickly grow and multiply. In about 42 days, quails have been able to produce eggs and can produce three to four offspring within a year. In addition, quails can produce 250-300 eggs with relatively little feed consumption (about 20 g/head/day). Quail eggs are rich in Vitamin A, riboflavin, and thiamine which benefit vision and immunity. In addition, quail eggs could be an alternative to chicken eggs for patients who cannot consume chicken eggs due to ovomucoid (Khalifa and Noseer, 2019). It was reported by the United States Department of Agriculture (2018) that the concentration of cholesterol in quail eggs was higher than that of chicken eggs (844 and 372 mg/ 100g, respectively). Rahmat and Wiradimadja (2011) stated that cholesterol concentration in the blood may directly affect cholesterol concentration in eggs and meat. Hypercholesterolemia could be affected by consuming high cholesterol concentrations of quail eggs (Khalifa and Noseer, 2019). Hence, the effort to decrease

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cholesterol content may benefit consumers. Previous studies have reported some methods for decreasing the cholesterol content of egg yolks. For instance, Warren et al. (1988) demonstrated that blended hexane with solvent egg yolk could produce a mixture with 62.2% cholesterol. In addition, Borges et al. (1996) mixed the emulsifying of yolk and acetone with a ratio of 1:12 (weight/weight) to maintain these emulsifying properties. Tannins are found in the plant kingdom and compounds of the polyphenolic group (Huang et al., 2018). Tannins extracted from Chestnut wood (*Castanea sativa* Mill), a common plant species in the Mediterranean area, are an example of hydrolyzable polyphenols characterized by gallic acid moiety (Field et al., 2012). Tannins are valuable because they could potentially replace antibiotics in chicken feeds (Huang et al., 2018). Although the inclusion of tannins in rations of monogastric animals has been discouraged over the years due to their antinutrient contents (Huang et al., 2018), they could have positive effects on monogastric animals if tannins were supplemented at appropriate levels (Huang et al., 2018). Moreover, tannins may reduce the spread of zoonotic pathogens and some risks of diseases in animals. Recently, investigations on using tannins in the bird production sector indicated favorable results (Amirmohammadi et al., 2014; Brus et al., 2018).

Since the EU banned antibiotic growth promoters (AGP) in animal feed in 2006, many strategies have been proposed for replacing AGPs and maximizing growth performance (Schiavone et al., 2008; Mirzaei et al., 2022). Previous reports revealed that supplementation of 3% grape extract tannins in the ration of broiler chicken decreased growth performance, but 1% of grape extract tannins inhibited the growth of pathogenic bacteria (Hughes et al., 2005). In addition, Schiavone et al. (2008) revealed that supplementation of 0.2% chestnut wood tannins accelerated growth and reduced mortality in broilers. In terms of its effect on physiology, it was revealed that supplementation of tannin extract at 75 mg/kg body weight/day significantly reduced total cholesterol (TCHO) and low-density lipoprotein (LDL) levels but did not affect TG and high-density lipoprotein (HDL) levels in hypercholesterolemia in white rats (Umarudin et al., 2012). Budiarto et al. (2016) revealed that tannin compounds can precipitate proteins on the small intestine's surface to reduce fat absorption.

The metabolic performance of the body could be evaluated by plasma metabolite measurements (Weikard et al., 2010). Some factors may contribute to metabolic changes in plasma metabolites, such as natural factors, genetic type, farm conditions, age, physiological state, and nutrients (Erwan et al., 2014; 2018; 2021). Several studies have been carried out on the influence of tannins on several types of livestock, both ruminants and non-ruminants, but a few studies have been carried out on quails. Therefore, this study was conducted to determine the effect of Saviotan Feed (SF) supplementation in the commercial ration on plasma metabolites and egg yolk cholesterol in quails.

MATERIALS AND METHODS

Ethical approval

This research was conducted under strict regulations in accordance with the recommendations in the Guide for the Care and Use of Animal, at the Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia.

Study animals

One-day-old Japanese quails were obtained from a commercial farm located at Garuda Sakti, Indonesia. The study was carried out from May to July 2023 at the Livestock Production Technology, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia. The current study investigated 100 laying quails from hatching to 56 days of age. The quails were kept in 20 cages, and each cage was 50×50×20 cm. Before being treated, quails were weighed to obtain a homogeneous initial weight of 100 heads and transferred to cage units of 5 heads each. The addition of SF to the commercial ration started when the quails were 14 days old and continued until they reached 56 days old. The study lasted for 42 days, during which ration and water were provided *ad libitum*.

Each pen unit consisted of 5 quails. A commercial diet (Metabolizable energy of 3050.34 kcal/kg, crude protein of 23.5%) was supplemented with different SF doses (0%, 0.1%, 0.2%, and 0.3% of diet). The SF was provided from Gruppo Mauro Saviola, Mantova, Italy. Table 1 shows the nutritional contents of the commercial feed (Indonesia).

Table 1. Nutrient contents of commercial feed of quails

Nutrients	Content values
Crude protein (%)	23.50
Crude fiber (%)	1.88
Crude fat (%)	5.87
Calcium (%)	0.29
Phosphor (%)	0.15
Metabolizable Energy (Kcal/kg)	3050.34

Research procedure

Preparation of Saviotan Feed

The commercial feed was mixed with SF powder every week for each treatment group.

Preparation of cage

The cages and equipment were cleaned and sanctified using disinfectants. Treatment groups were selected on the basis of their body weight to ensure uniformity among the groups. The location of each cage was determined through randomization and each cage was then coded, making it easier to record data.

Measured parameters

The parameters measured in this study were plasma metabolites, including glucose (Glu), triglyceride (TG), and TCHO, using the method by Erwan et al. (2018). The cholesterol concentration of egg yolk was also measured. Blood sampling was carried out at 56 days of age. Two heads of quails from each cage were used as samples, resulting in 40 quails. Blood sampling was carried out after the quail was fasted for 10 hours. This involved slaughtering the quails by cutting the jugular vein and then collecting approximately 3 ml of blood samples using an Eppendorf tube containing ethylenediaminetetraacetic acid (EDTA) to prevent clotting. The blood samples were stored in an ice flask. The quails were decapitated without anesthesia. Then, the samples were taken to the livestock production technology laboratory of the faculty of agriculture and animal science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia. The cholesterol concentration of egg yolks was analyzed from 40 eggs using the Liebermann-Burchard method. The analysis was performed using a UV-visible spectrophotometer at Andalas University Biotechnology Laboratory, Padang City, West Sumatera, Indonesia.

Statistical analysis

One-way ANOVA was used for analyzing all the data and the Tukey-Kramer test was performed as a post-doc test. Significant differences were indicated by $p < 0.05$. Values were presented as means ± Standard Error Mean. Statistical analysis was performed using the commercially available package (SAS, 1998). Thompson rejection test as ($p < 0.05$) to eliminate outliers was applied for all data before being used for data analysis.

RESULTS AND DISCUSSION

Plasma glucose concentration

Table 2 shows the effect of dietary supplementation of SF on Glu levels. No Significant effect was observed between treatments regarding Glu ($p > 0.05$). This outcome was predictable since tannin content in chestnut wood was relatively small (0.1-0.3%/kg), so it did not affect blood glucose levels. Feed consumption also may influence glucose levels. According to Purnamasari (2009), the influential factors in blood glucose are metabolic food intake and glucostatic activity of the liver. Although there were no significant effects among treatments, the trend of Glu concentrations decreased in SF-supplemented treatments, compared to control.

Tannins can also have the function of stimulating glucose and fat metabolism. Hence, the deposits of both sources of calories in the blood can be avoided, resulting in cholesterol and blood sugar decrease (Kurnia et al., 2010).

Table 2. Effects of supplementation of different Saviotan doses on plasma glucose of quails aged 14 to 56 days

Treatment	Glucose (mg/dL)
Control (0%)	424.80 ± 57.80
SF (0.1%)	332.20 ± 39.07
SF (0.2%)	395.60 ± 33.96
SF (0.3%)	339.00 ± 38.20

Values are mean ± SEM, SF: Saviotan Feed

Total plasma triglycerides

The TG concentration of plasma in quails treated with commercial rations supplemented with SF is shown in Table 3. As can be seen in Table 3, supplementation of commercial ration of quails with 0.3% of SF up to 56 days of age did not have a significant effect on TG levels ($p > 0.05$). Generally, TG serves as energy reserves. This result is consistent with that of Jannah et al. (2018), who reported that high TG levels can cause a rise in triglycerides leading to disturbances in blood circulation.

The SF supplementation level in the current study was probably not optimal to affect TG level. Schiavone et al. (2008) reported that hydrolyzed tannins (HT) from chestnut wood, such as castalagin, have antimicrobial effects against several types of microbes, like *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritica*, *Clostridium perfringens*, and *Staphylococcus aureus*.

Based on Table 3, the TG content with the supplementation of 0.1-0.3% SF was 198.9-328.30 mg/dL.

The results of this study were relatively higher than the research by Widowati et al. (2012), indicating that turmeric flour (*Curcuma longa L.*) influences TG levels of quails ranging from 86.8-115.8 mg / dL.

Table 3. Effects of supplementation of different Saviotan doses on plasma triglycerides of quails aged 14 to 56 days

Treatments	Triglycerides (mg/dL)
Control (0%)	242.80 ± 47.32
SF (0.1%)	328.30 ± 34.69
SF (0.2%)	198.90 ± 59.55
SF (0.3%)	247.60 ± 86.92

Values are mean ± SEM, SF: Saviotan Feed.

Plasma total cholesterol

Table 4 shows the plasma concentration of TCHO levels at the end of treatment. As can be seen, there was a significant difference among the treatments ($p < 0.05$).

Table 4. Effects of supplementation of different Saviotan doses on plasma total cholesterol of quails aged 14 to 56 days

Treatment	TCHO (mg/dL)
Control (0%)	211.00 ± 26.14^{a}
SF (0.1%)	230.10 ± 27.07^{ab}
SF (0.2%)	319.20 ± 49.84^{b}
SF 0.3%)	161.20 ± 18.28^{a}

^{ab}Means different superscript letters were significantly different at $p < 0.05$). Values are mean ± SEM, SF: Saviotan Feed, TCHO: Total cholesterol

Some factors, such as feed, influence the concentration of TCHO in the blood. Cholesterol is a hydrophobic molecule that can be transferred in the blood through spherical macromolecules in the plasma lipoproteins, such as chylomicrons, HDL, LDL, and VLDL. The SF supplementation affected quail blood plasma TCHO levels in the current study. Based on the results, the concentration of TCHO was the lowest in the group supplemented with 0.3% SF, compared to other treatments. It was speculated that SF could lower the TCHO content in the direction of the flow of free fatty acids, which can reduce the lipoprotein formation that carries cholesterol. Previous studies have demonstrated that tea tannins inhibit increased serum cholesterol concentration when administered to rats fed a peroxidized corn oil diet (Okuda et al., 1984).

In addition, Gato et al. (2013) reported that plasma total cholesterol levels significantly decreased when humans consumed tannin-rich fiber. The TCHO concentrations in this study ranged from 161.20 to 319.20 mg/dl, which was higher than a study by Blaszczyk et al. (2006), who reported that quail blood cholesterol content ranges from 180 to 220 mg /dl.

Egg yolk cholesterol of quail

Changes in egg yolk TCHO levels following supplementation of different doses of SF are shown in Table 5. The tannin content of chestnut wood may decrease the cholesterol concentration of yolk eggs in quails. The concentration of active compounds in the form of tannins decreases cholesterol concentration in egg yolk by inhibiting cholesterol absorption in the digestive tract. Previous research has reported that tannin compounds may inhibit fat absorption by binding fat to intestinal mucosal epithelial cells and increasing cholesterol binding in fiber. As a result, cholesterol can be excreted through feces and not absorbed into the body (Josten et al., 2006). Furthermore, Kurnia et al. (2010) stated that tannins can stimulate glucose and fat metabolism, so the accumulation of both sources of calories in the blood can be avoided, resulting in decreased cholesterol and blood sugar.

Results of the current study indicated a decrease in cholesterol concentration of egg yolk in quails fed SF supplemented ration. Minieri et al. (2016) revealed that a diet with chestnut tannin extract decreased cholesterol by about 17% in Mugellese and 9% of yolk in the Leghorn chicken breed. Mello and Santos (2004) reported that the content of tannin compounds in feed can inhibit the performance of several digestive enzymes, including trypsin, amylase, and lipase, then decrease the availability of amino acids that support the egg formation process, including cholesterol content. Tugiyanti et al. (2016) stated that tannins could inhibit the absorption of food substances, including fat and cholesterol, in the digestive tract by the mucus layer. Winarno (1989) stated that the cholesterol concentration could decrease in the body through two pathways, firstly by being converted into bile acids, and secondly by excreted from the body in the form of neutral sterols in feces. The dietary supplementation of SF in a practical dose may decrease the cholesterol concentration. For human consumption, eggs with a lower cholesterol concentration could be recommended as an alternative for controlling heart disease (Minieri et al., 2016). Further studies are needed to evaluate the relationships between SF and other factors to regulate cholesterol in other poultry species.

Table 5. Effects of supplementation of different Saviotan doses on yolk total cholesterol of quails aged 14 to 56 days

Treatment	Egg yolk cholesterol (mg/dL)
Control (0%)	1,031.14 ±56.95[a]
SF (0.1%)	869.30 ± 53.85[b]
SF (0.2%)	826.26 ± 27.66[b]
SF (0.3%)	869.20 ± 41.32[b]

SF: Saviotan Feed, [ab]Means different superscript letters were significantly different at $p < 0.05$, Values are Mean ± SEM.

CONCLUSION

Supplementation of SF in the commercial ration at a 0.1-0.3% level could decrease cholesterol in the egg yolk of quails but did not affect plasma Glu and TG. It is concluded that the optimal level of dietary supplementation of SF for decreasing the cholesterol concentration of yolk eggs in quails is 0.2%.

DECLARATIONS

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Availability of data and materials

Data from the current study are available at the editors' request.

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Not Applicable.

Authors' contributions

Edi Erwan, Isbul Irfan, and Wawi Ibrah conducted the research, prepared data, performed the statistical analysis, and wrote the first draft. Afriadi and Muhammad Rodiallah edited the manuscript. All authors have checked and approved the final version of the manuscript.

Competing interests

The authors declare no conflicts of interest.

Ethical consideration

The authors have checked the ethical issues, including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy.

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