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### nut tannin

# Effects of Dietary Supplementation of *Chestnut tannin* on Growth Performance, Carcass Traits, and Meat Cholesterol in Ulu Chickens

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### **ABSTRACT**

Tannin from chestnuts has garnered interest in poultry nutrition due to its potential impact on meat quality. The current study investigated the effects of *Chestnut tannins* (CT) which were derived from natural chestnut wood, on poultry health and meat characteristics. The primary objective was to determine the effects of CT supplementation in commercial feed on performance, carcass, and meat cholesterol in Ulu chickens. A total of 48 one-day-old Ulu chickens were divided randomly based on a completely randomized design into four treatment groups, with four replications each, and raised until 63 days of age. The treatments consisted of varying doses of CT (0.1%, 0.2%, and 0.3%) supplemented with a commercial diet. The parameters measured were performance, carcass traits, and meat cholesterol. The results showed that the supplementation of different levels of CT did not significantly alter performance, carcass traits, and meat cholesterol in ulu chickens. However, correlation and trend analysis indicated that the 0.3% CT treatment yielded the best growth performance, with a body weight gain of 934.85 g and a feed conversion ratio of 2.53, respectively. Conversely, the best treatment for reducing meat cholesterol was 0.2% of CT. It can be concluded that while CT supplementation did not influence the performance and carcass characteristics, it was effective in reducing meat cholesterol levels in Ulu chickens.

Keywords: Carcass, Cholesterol, Performance, Chestnut tannins, Ulu chicken

### INTRODUCTION

Recently, the demand for indigenous chicken meat has grown alongside the increasing global population. Previous reports have highlighted that indigenous chicken meat is a valuable source of nutrients, including proteins, energy, and fat. Saidin (2010) reported differences in cholesterol content in broiler and indigenous chickens, noting that while broiler chicken meat contains approximately 100 mg of cholesterol per gram, indigenous chicken meat has a slightly higher cholesterol content of 116 mg/gram. Normally, cholesterol plays an important role in cell maintenance, but abnormal cholesterol levels in

the blood can have adverse health effects. Mozaffarian et al., (2016) reported that one of the factors that may contribute to cardiovascular disease is the high level of low-density lipoprotein (LDL).

In the past, antibiotics were widely used to enhance chicken growth performance and health. However, that condition has been stopped since 2006 when the European Union banned antibiotics in feed diets due to concerns about antibiotic residues in poultry meat and the risk of bacterial resistance (Tian et al., 2021). This shift has heightened consumer awareness of meat quality and spurred interest in products with lower fat and cholesterol

content to meet health-conscious demands. Some efforts have been conducted to achieve these targets, particularly to ensure optimal chicken performance and manage subclinical diseases (Rafiq et al., 2022). Stamler et al. (1986) previously established a clear link between high concentrations of cholesterol and the risk of cardiovascular disease. Due to such challenges, a group of potential antibiotics for promoting growth can be plant-derived compounds such as organic acids, herbs, and essential oils. Among these, tannins stand out as phytochemicals with strong antimicrobial properties. These natural compounds have shown potential not only in promoting growth but also as viable substitutes for antibiotics in poultry (Farha et al., 2020).

The use of tannins as feed supplements has gained increasing attention as an alternative to antibiotics for promoting growth in poultry. Graziani et al. (2006) stated that hydrolyzable tannins may have the potential to substitute antibiotics. In addition, numerous studies have demonstrated the efficacy of natural chestnut extracts in reducing yolk cholesterol concentrations in quail eggs (Erwan et al., 2023). For instance, Buyse et al. (2021) revealed that meat quality, intestinal growth, and increased antioxidant status in broiler chickens could be stimulated by tannin supplementation.

Tannin from chestnuts has garnered interest in poultry nutrition due to its potential impact on meat quality. Tannins can reduce ABCA1 gene expression, which may minimize the risk of cardiovascular disease (Melo et al., 2023). This gene is related to the formation of highdensity lipoprotein (HDL), thereby promoting blood pressure and reducing total cholesterol (Wang et al., 2008). Additionally, incorporating Chestnut tannins into poultry diets may enhance meat quality through their antioxidant properties, which can contribute to the improved colour, flavour, and overall nutritional value of chicken meat. Schiavone et al. (2008) reported that the supplementation of chestnut wood extract did not significantly affect apparent digestibility but exhibited quadratic or cubic effects on growth performance with increasing tannin levels in broiler chickens. Furthermore, Orzuna-Orzuna et al. (2021) reported that the inclusion of tannin in diets improved growth performance, carcass yield, and meat oxidative stability in sheep. Similarly, Chestnut tannins supplementation has been shown to reduce yolk cholesterol in Japanese quails (Erwan et al., 2023). Understanding the mechanisms through which tannins affect meat quality is important for optimizing poultry production and feed efficiency (Buyse et al., 2021). Tannins exhibit multiple beneficial functions, such as antioxidative properties, metal ion scavenging, and immune system stimulation (Fraga et al., 2010).

The present study aims to determine whether CT supplementation can reduce meat cholesterol levels in Ulu chickens. To advance experiments on CT, the effects of its supplementation on growth performance and carcass characteristics were also examined.

### MATERIAL AND METHODS

### **Ethical approval**

This study was approved by the Ethical Clearance Committee of the Faculty of Animal Sciences, Jambi University, Indonesia (Approval Number: 06UN21.7/ECC/2023).

### **Experimental design**

A total of 48 one-day-old (DOC) Ulu chicks were randomly selected based on a completely randomized design with four treatments. Each treatment group contained three chickens per pen, and each treatment was replicated four times. These cages were maintained at a constant temperature of  $30 \pm 1$  °C and continuous lighting. All Ulu chickens were raised in groups from doc until 63 days of age at the Poultry Division Field Laboratory, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia. Throughout the experiment, feed (Charoen Pokphand Ltd, Indonesia) and water were provided with ad libitum access. The treatments consisted dietary supplementation with varying levels of CT, derived from chestnut wood extract, mixed into a commercial feed. The treatment groups were as follows 0% CT (control), 0.1% CT (low), 0.2% CT (medium), and 0.3% CT (high). The CT was a commercial feed supplement obtained from chestnut wood (Erwan et al., 2023). The chemical analysis of the commercial feed is presented in Table 1.

**Table 1.** The chemical analysis of commercial feed provided for Ulu chicken

| Nutrient          | Starter period <sup>1</sup> | Finisher period <sup>2</sup> |
|-------------------|-----------------------------|------------------------------|
| Crude protein (%) | 23.50                       | 19.00                        |
| Crude fiber (%)   | 1.88                        | 6.00                         |
| Crude fat (%)     | 5.87                        | 5.00                         |
| Ca (%)            | 0.29                        | 0.80                         |
| P (%)             | 0.15                        | 0.45                         |
| ME (Kcal/kg)      | 3,050                       | 2,910                        |

Ca: Calcium, P: Phosphor, ME: Metabolizable Energy, <sup>1</sup>CP511 and <sup>2</sup>CP512 Produce by PT. Charoen Pokphand, Indonesia.

### Performance parameters measurement

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were recorded weekly throughout the experimental period. The FCR was calculated by dividing the total feed intake by the BWG of chickens during the experiment.

#### Meat cholesterol measurement

Meat cholesterol concentrations were analyzed using an enzymatic color method with the following steps 1) One milliliter of cholesterol reagent kit was pipetted into a test tube, and 0.01 ml of the extracted sample was added, 2) The mixture was incubated for 20 minutes until the solution turned red, 3) A blank was prepared by pipetting 1 ml of the cholesterol reagent kit into a separate test tube. Blanks were prepared for each series of analyses, 4) The blank was placed into the spectrophotometer cell (Clinicon Autoanalyzer), and the reading was adjusted to zero at a wavelength of 500 nm before inserting the sample for measurement. Cholesterol levels were calculated by the numbers displayed on the spectrophotometer monitor. Carcass weight, breasts, whole thighs, wings, and abdominal fat were measured following the methodology described by Erwan et al. (2009).

### Statistical analysis

All data were analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test as a post-hoc test. Regression equations were fitted for data relating to the level of CT and performance, carcass characteristics, and meat cholesterol concentration. Significant differences were implied by p < 0.05. Results were presented as means  $\pm$  standard error mean (S.E.M). All statistical analyses were performed using the commercially available package StatView (Version 5, SAS package, 1998). Prior to analysis, the Thompson rejection test was applied to all data to remove outliers (p < 0.05), with the remaining data used for analysis.

### **RESULTS**

## Effects of supplementing different levels of *Chestnut tannins* in commercial feed on performance in Ulu chickens

Table 2 presented the changes in growth performance after CT supplementation. There were no significant effects of CT supplementation on growth performance including FI, BWG, and FCR in Ulu chickens. However, FI and BWG exhibited a tendency to increase as the levels of CT in the diets increased. The correlation between CT and body weight is shown in Figure 1.

At a supplementation level of 0.3%, as shown in Figure 2, there was a significant negative correlation between CT levels and FCR (p < 0.05).

### Effects of supplementing different levels of Chestnut tannins in commercial feed on carcass traits of Ulu chickens

Table 3 shows the effects of different levels of CT supplementation on carcass and carcass cutting. The findings indicated no significant effect on this parameter among all treatments (p > 0.05). The weight of the carcass, breasts, whole thighs, and wings were not altered by CT supplementation. However, as shown in Figures 3, and 4 a significant positive correlation was found between the levels of CT and carcass weight, breasts, and whole thighs (p < 0.05).

### Effects of supplementing different levels of *Chestnut tannins* in commercial feed on abdominal fat and meat cholesterol of Ulu chicken

Figure 5 shows the effect of the supplementation of CT in commercial feed on the abdominal fat of Ulu chicken. While no statistically significant differences in abdominal fat were detected among the treatment groups, a decreasing trend was observed with increased CT supplementation. As seen in Figure 6, CT supplementation tended to reduce the cholesterol levels in Ulu chicken meat to low and medium levels compared to other groups (P > 0.3) though this reduction was not statistically significant (P > 0.3).

**Table 2.** The effect of supplementation of *Chestnut tannins* at different levels on the performance of Ulu chicken

| Parameters            | Treatments   |            |              |            | D l       |
|-----------------------|--------------|------------|--------------|------------|-----------|
|                       | 0            | 0.1        | 0.2          | 0.3        | - P-value |
| Final body weight (g) | 1,059±56.6   | 1,113±49.6 | 1,060±17.9   | 1,171±12.7 | 0.2       |
| Average gain (g)      | 821±53.2     | 874±45.8   | 830±19.9     | 935±7.1    | 0.2       |
| Feed intake (g)       | 2,305±76.8   | 2,233±9.4  | 2,234±51.9   | 2,362±29.4 | 0.2       |
| FCR                   | $2.84\pm0.2$ | 2.58±0.1   | $2.73\pm0.1$ | 2.53±0.0   | 0.3       |

FCR: Feed conversion ratio

**Table 3.** The effect of different supplementation levels of *Chestnut tannins* in commercial feed on carcass characteristics in Ulu chicken

| Parameters       | Treatments |             |          | D value       |           |
|------------------|------------|-------------|----------|---------------|-----------|
|                  | 0          | 0.1         | 0.2      | 0.3           | - P-value |
| Carcass (g)      | 570±22.9   | 625±55.9    | 618±24.8 | 692±30.6      | 0.2       |
| Breasts (g)      | 160±9.4    | 169±10.4    | 174±4.4  | $185 \pm 7.0$ | 0.2       |
| Whole thighs (g) | 212±14.9   | $222\pm9.6$ | 209±6.9  | $245\pm2.1$   | 0.1       |
| Wings (g)        | 90±5.1     | 96±3.4      | 93±1.8   | 101±3.5       | 0.2       |

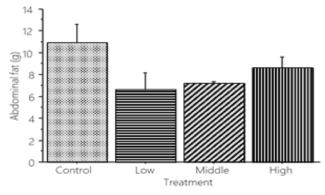


Figure 1. The correlation between *Chestnut tannins* and body weight

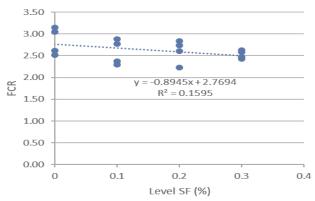


Figure 2 The correlation between *Chestnut tannins* and feed conversion ratio

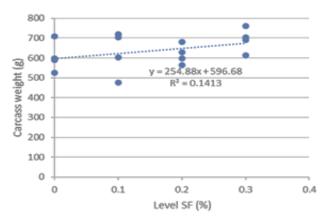
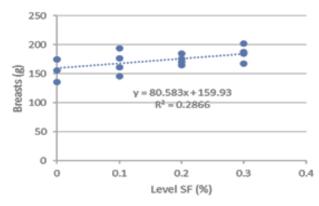


Figure 3 The correlation between *Chestnut tannins* and carcass weight



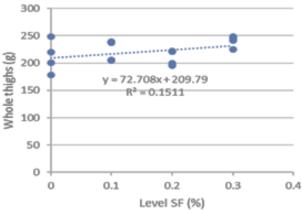


Figure 4 The correlation between *Chestnut tannins* and whole thighs

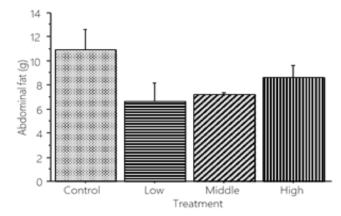


Figure 5. The effect of Chestnut tannins on abdominal fat

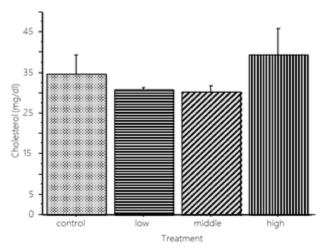


Figure 6. The effect between Chestnut tannins on meat cholesterol

### DISCUSSION

The present study confirmed that supplementation of CT in commercial feed did not significantly impact the performance of Ulu chickens. However, a trend was observed where body weight at 63 days of age increased with higher levels of CT supplementation. These findings suggest that CT can support the growth of Ulu chickens. The findings were inconsistent with a previous report (Cengiz et al., 2017) which indicated that tannin supplementation from barley (2g/kg or 0.2% feed) decreased body weight, feed intake, and FCR while it can be considered as an alternative strategy to address foot dermatitis in broiler chickens.

Tannins in feed can impart a bitter, astringent taste, which may alter the poultry's perception of the feed. Chickens tend to avoid feed with a bitter or astringent taste, which can reduce their feed intake. The mechanism involved tannins binding to proteins in the chicken's saliva, which contains proline and hydrophobic groups, forming complex bonds that cause the feed to become astringent. Houshmand et al. (2015) noted that the primary adverse effects of tannins in monogastric animals were related to their protein-binding capacity, which reduces protein, starch, and overall digestibility. This ultimately leads to reduced palatability and decreased feed consumption in chickens. However, when administered in appropriate amounts, tannins have been reported to enhance feed intake in some studies.

In another study, hydrolyzed tannins were used as feed additives for broiler chickens. Tonda et al. (2018) evaluated the effects of hydrolyzed tannins at 0.5 g/kg of

feed, both alone and in combination with *Bacillus coagulans*. Their study assessed the impact of hydrolyzed tannins on the performance and intestinal health of broiler chickens. Ethydrolyzed tannins could reduce intestinal lesion scores, the number of oocysts in feces, and the feed conversion ratio in broiler chickens challenged with *Eimeria* spp. Tannins from proanthocyanidin extract of grape seeds significantly reduced mortality and increase weight gain in broiler chickens after infection with *E. tenella* (Wang et al., 2008).

Investigation into the effects of tannins on carcass and meat quality in chickens revealed crucial findings. Perić et al. (2022) reported that the supplementation of Chestnut tannin extract in linseed oil-enriched diets improved intestinal morphology in broiler chickens. Additionally, the inclusion of sorghum with varying tannin levels in broiler diets resulted in inconsistent trends in performance and carcass characteristics, indicating that tannin levels may not be the sole factor affecting nutritional quality (Milton et al., 2023). Furthermore, substituting maize with low tannin sorghum in broiler diets was found to enhance the nutritional value of chicken meat, leading to lower cholesterol content and improved vitamin E levels in the thigh meat of chickens (Ochieng et al., 2020). However, a meta-analysis by Hidayat et al. (2021) cautioned that high dietary tannin levels might negatively affect amino acid digestibility, broiler performance, and lymphoid organs.

Chestnut tannins supplementation showed potential in reducing the cholesterol levels in Ulu chicken meat to a medium level. This result was in line with the findings of Starčević et al. (2015). Tannins, such as those from Galla chinensis and quebracho extracts, were significantly

effective in reducing total cholesterol concentrations in both the serum and liver of broiler chickens (Perin et al., 2019; Ren et al., 2023). Additionally, the inclusion of low-tannin sorghum in broiler diets has shown a hypocholesterolemic effect, leading to decreased cholesterol levels in the liver (Starčević et al., 2015). These findings suggested that dietary supplementation of tannins can effectively modulate cholesterol content in chicken meat, highlighting the potential of tannins as a natural approach to improve the nutritional quality of poultry products.

#### CONCLUSION

The optimal performance of Ulu chickens aged 63 days was observed with supplementation of 0.3% chestnut CT, resulting in a body weight gain of 934.85 g and an FCR of 2.53. The CT supplementation at 0.3% in commercial feed did not cause differences in the carcass characteristics, whereas supplementation levels of 0.1 and 0.2% tended to reduce cholesterol content in Ulu chicken meat. Further studies were recommended to determine the effect of CT supplementation on growth performance and plasma metabolites in other poultry species.

### **DECLARATIONS**

### **Authors' contributions**

Edi Erwan, Deni Fitra, Evi Irawati, and Afriadi conducted the experiment, performed statistical analyses, and prepared the draft manuscript. Vebera Maslami, Mozhdeh Emadi, and Edi Erwan further revised the manuscript. All authors have checked and approved the final version of the paper before submission.

### **Competing interests**

The authors declare no conflicts of interest.

### Availability of data and materials

All the data and materials are available upon request from the corresponding author.

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#### **Ethical considerations**

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

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