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Effect of Tropical Alfalfa on Cholesterol (Egg and Blood) and Layer Chicken Meat Quality

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Abstract: Tropical alfalfa is a cultivar of alfalfa with high protein content and good adaptability with the local name Kacang Ratu BW. The supplementation of duck feed with tropical alfalfa or Kacang Ratu BW may reduce the cholesterol levels in duck meat with the presence of saponin. This study aimed to determine the effects of Kacang Ratu BW supplementation on egg cholesterol and meat quality in layer chickens. A total of 60 hyline brown chickens, aged 26 weeks, were reared at three different rearing periods and sacrificed at 38 weeks. The feed treatments were P_0 (Basal ration), P_1 (Basal ration +1% Kacang Ratu BW) and P_2 (Basal ration +3% Kacang Ratu BW). The measured parameters were meat quality (carcass and chemical quality), yolk quality (water, crude protein, and fat contents), and cholesterol level (yolk and blood). The results showed that Kacang Ratu BW supplementation significantly influenced egg yolk cholesterol and meat fat. However, the water and protein contents of the meat and eggs were not affected. Our findings indicated that feed supplemented with Kacang Ratu BW reduced the egg yolk cholesterol and the fat content of the layer of chicken meat.

Keywords: Alfalfa, Carcass, Cholesterol, Egg, Hens, Hyline Brown

Introduction

Alfalfa (*Medicago sativa* L) is a highly adaptable forage plant that grows in various countries (Tufarelli *et al*., 2018). Kacang Ratu BW, a tropical alfalfa cultivar, was developed at the Faculty of Animal Science Universitas Gadjah Mada Indonesia (Suwignyo *et al*., 2021a; 2022) and it has been registered as Indonesian germplasm and released by the Center of Protection Variety Plant and licensing agriculture (PPVT) ministry agriculture Republic Indonesia with number 929/PVHP/2021. Alfalfa contains high levels of protein, which is beneficial for livestock nutrition (Jiang *et al*., 2012; Tufarelli *et al*., 2018; Zheng *et al*., 2019). Depending on the variety and harvest time, the protein level of alfalfa is in the range of 17.5-30% (Laudadio *et al*., 2014; Shahsavari, 2015) The protein level of alfalfa can reach 22-32% (Suwignyo *et al*., 2020a) Alfalfa protein is a rich source of 17 different types of amino acids, which makes it a valuable component of poultry feed (Suwignyo *et al*., 2020c; 2022). It can be supplemented with fresh (chopped) or (grounded) hay (Suwignyo and Sasongko, 2019), at up to 6-10% of the diet (Suwignyo *et al*., 2020b; 2021b) without reducing the performance (Samur *et al*., 2020; Suwignyo *et al*., 2021c). However, alfalfa also contains high fiber and secondary metabolites, such as xanthophylls, β-carotene, flavonoids, polysaccharides, and saponins (Laudadio *et al*., 2014; Shi *et al*., 2014; Suwignyo *et al*., 2022).

Meat and egg are good source of protein (17-21%) but is rather high in cholesterol issues, with a standard range of 125-200 mg/dL, while the human body needs 200-300 mg cholesterol per day. Alfalfa is a legume that contains saponins, which have been shown to reduce the cholesterol content of meat. Alfalfa contains high levels of saponins (2-3% dry matter), which have hypocholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant properties (Francis *et al*., 2002; Gurfinkel, 2000). Saponins and cellulose, found in high amounts in alfalfa meal, reduced the absorption of cholesterol by binding to dietary and bile cholesterol in the intestine, thereby increasing its excretion in feces (Francis *et al*., 2002) and reducing the cholesterol level in the liver and duck meat (Suwignyo *et al*., 2022).

Therefore, the aim of this study was to determine the effects of alfalfa supplementation on cholesterol, egg chemistry, and the carcass/meat of layer chickens.

Materials and Methods

Animals and Diets

This study used Hyline Brown chickens (n = 60, aged 26 weeks). Individual cages were used to rear the chickens at three different periods and each period lasted 28 days. The chickens were fed twice daily (morning (07.00) and afternoon (14.00)) and they had access to drinking water ad libitum. This study comprised three treatment groups and each group consisted of four replicates, with five chickens in each replicate. The treatment groups included P_0 (Basal ration), P_1 (Basal ration +1% Kacang Ratu BW), and P_2 (Basal ration +3% Kacang Ratu BW) groups. Kacang Ratu BW was provided in the form of milled hay. The feed contents are listed in Table 1. This study complied with the applicable regulations with ethical clearance number 0002/EC-FKH/Ex. 2021 issued by the faculty of veterinary medicine, Universitas Gadjah Mada (UGM).

Meat and Yolk Quality

The analysis of layer chicken meat involved carcass measurements (live weight, carcass weight, and carcass percentage) and meat chemistry (water, fat, and crude protein contents). At 38 weeks, the chickens were weighed before slaughter, and one tail was cut for each repetition. Next, the non-carcass parts (feathers, head, legs, internal organs, blood, and neck) were removed to measure the carcass weight and carcass percentage (carcass weight divided by live weight multiplied by 100%). Chemical analysis of egg yolk and meat was performed using proximate analysis (AOAC, 2005). Chicken breasts were used for the chemical analysis of meat.

Cholesterol

Cholesterol analysis involves the determination of cholesterol levels in the blood and yolk. Blood cholesterol was measured by placing 2 mL of chicken blood into a blood tube containing ethylenediaminetetraacetic acid to prevent blood clots, followed by storage in an ice flask for laboratory analysis. The blood cholesterol levels of the chickens were determined with a photometer using the cholesterol oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) method at a wavelength of 546 nm and a temperature of 37°C.

Table 1: Nutrient composition of Hyline Brown's diet

Nutrient content (%)	P_0	P_1	P_2
Metabolizable energy (kcal/kg)	2.798,73	2.778,19	2.778,27
Crude protein	16,92	16,65	16,54
Crude fiber	2,56	2,85	3,38
Crude fat	4,50	4,49	4,96
Ca	4,52	4,5	4,53
Available P	0,47	0,58	0,59
Lysine	1,32	1,20	1,17
Methionine	0,58	0,57	0,56

P_0 = Basal ration; P_1 = Basal ration +1% Kacang Ratu BW; P_2 = Basal ration +3% Kacang Ratu BW

Yolk cholesterol was measured by collecting one chicken egg for each repetition and the analysis was carried out according to the method of (Hammad *et al*., 1996), with slight modifications by Eren and Uyanik, (2007). The eggs were hard-boiled for 15 min; the yolks were separated and 0.1 g samples of yolks were weighed. Yolk lipids were extracted with isopropanol (4 mL/0.1 of yolk), vortex-mixed, and centrifuged at 3000 rpm for 5 min. The yolk lipids in the samples were determined using a spectrophotometer with a commercial kit (HUMAN Cholesterol Liquicolor for cholesterol).

Statistical Analysis

All obtained data were subjected to variance analysis and where the results were significant, Duncan's multiple range test was used for further analysis. Statistical analysis was performed using Statistical Product and Service Solutions software (SPSS) version 16.0 software (SPSS Inc., Chicago, IL, USA).

Results

Meat Quality

The average of the meat quality values is presented in Table 2. The result show that ration supplemented with Kacang Ratu BW, up to 3%, resulted in no significant difference ($p>0.05$) in live weight, carcass weight, and carcass percentage.

Table 2, the moisture content and crude protein of the meat of layer chickens that received Kacang Ratu BW supplementation of up to 3% was not significantly different from the meat of those that did not receive the supplemented feed. In contrast, the fat content of the meat was significantly different ($p<0.05$) across the treatment groups and 3% Kacang Ratu BW supplementation was able to reduce the fat content in the meat of layered chicken for 38 weeks.

Yolk Quality

The yolk quality values of the water, crude protein, and fat contents of the laying hens can be seen in Table 3. The results show that the Kacang Ratu BW supplementation at different levels (3%, 1%, and 0%) had no significant effect ($p<0.05$) on yolk quality.

Table 2: Production and meat quality of hyline brown supplemented by Kacang Ratu BW

Treatment	P_0	P_1	P_2
Live weight	1920±30.00	1876±53.64	1856±38.44
Carcass weight	1105±2.880	1096±13.64	1085±12.58
Carcass percentage	57.57±0.760	58.50±1.360	58.47±0.930
The water content of meat	73.33±0.320	73.37±0.330	72.88±1.170
Crude protein of meat	21.10±0.030	21.10±0.020	21.05±0.820
Fat content of meat	1.16±0.02[a]	0.95±0.01[b]	0.98±0.02[b]

P_0 = Basal ration; P_1 = Basal ration +1% Kacang Ratu BW; P_2 = Basal ration +3% Kacang Ratu BW

Table 3: Blood and yolk cholesterol of hyline brown that supplemented with Kacang Ratu BW

Treatment	P_0	P_1	P_2
Yolk cholesterol (mg/dl)	739.01±4.16[a]	632.85±9.56[b]	627.43±11.76[b]
Blood cholesterol	118.56±3.88	106.85±7.30	104.46±6.520
The water content of yolk	49.25±2.07	49.42±1.06	49.52±2.670
Crude protein of yolk	15.69±0.47	14.10±0.57	14.86±2.670
The fat content of yolk	25.53±0.29	26.14±1.21	25.57±0.500

P_0 = Basal ration; P_1 = Basal ration +1% Kacang Ratu BW; aP_2 = Basal ration +3% Kacang Ratu BW

Cholesterol

Analysis of the blood and yolk cholesterol of the laying hens is shown in Table 3. Supplementation of layer chicken rations with Kacang Ratu BW had a significant effect ($p<0.05$) on yolk cholesterol. On the other hand, supplementation has no effect on blood cholesterol.

Discussion

The results of the first experiment show that Kacang Ratu BW supplementation, up to 3%, did not have a negative effect on the carcass quality of laying hens. This finding was attributed to the provision of a balanced diet with adequate nutritional practices (Leeson and Summers, 2001). Daud *et al*., (2017) reported that the increase in carcass weight and carcass percentage would be directly proportional to the increase in age and body weight of chickens. Nearly the same final body weight will result in a proportional carcass weight, such that the carcass percentage achieved is relatively the same.

The carcass percentages in the present study were within the normal range. Meanwhile, the carcass percentage of male layer chickens ranged from 58.15-58.39% (Daud *et al*., 2017) and another study reported that the carcass percentages of broilers ranged from 58.04-60.08% (Fenita *et al*., 2011).

The water content in the meat remained at normal levels. According to (Forrest *et al*., 1975), the water content of normal chicken meat is in the range of 70-75%. The moisture content of meat is affected by the age of the livestock (Soeparno, 1994), environment, and feed composition (Wahyu, 2004).

Meat protein levels are still within normal range. According to Soeparno (1994); Forrest *et al*. (1975), the protein content of broiler chickens is in the range of 16-22 %. Age, species, stress, feed, and sex cause variations in the chemical composition of meat (Lawrie, 1995).

Supplementation 3% Kacang Ratu BW was able to reduce the fat content in the meat of layered chicken for 38 weeks. This finding was attributed to saponins in Kacang Ratu BW that can bind endogenous bile salts (endogenous bile cholesterol) (Nurhayati *et al*., 2020). Fat has a positive correlation with cholesterol, as evidenced by the decrease in cholesterol content in both the blood and yolk layers in this study. Cholesterol is a component of fat (Djaelani and Tana, 2015); hence, the higher the fat content in poultry meat is, the higher the cholesterol content in meat, and vice versa (Ismoyowati and Widyastuti, 2003).

Yolk Quality

An important component that influences the quality of the interior quality of the eggs is water content. When stored, the water content of the egg whites decreases not only due to water evaporation but also due to the diffusion of water from the egg whites to the yolks. The moisture content of eggs is affected by the rate of evaporation, temperature, and humidity at which they are stored (Sudaryani, 2000). The water content of egg yolks in this study was almost the same as that reported by Hutapea *et al*. (2016) (49.4 %).

Supplementation had no effect on yolk crude protein, which could be due to the ability of poultry to maintain egg protein content during the deposition process (Sari *et al*., 2017). Quantity and quality of feed given to livestock very important for production and quality eggs both physical or external and chemical or internal (Tugiyanti and Eriyanti, 2012).

In this study, the fat content of egg yolk was not significantly influenced by Kacang Ratu BW supplementation. The fat content (25.57-26.14%) was consistent with the report of Lai *et al*. (2010), which stated that the fat content in chicken egg yolks ranges from 31.8-35.5%. The fat content of the yolk is influenced by the nutritional content of the laying chicken ration (Zeweil *et al*., 2006).

Cholesterol

Supplementation with 3% Kacang Ratu BW resulted in the lowest yolk and blood cholesterol levels (Table 3). Similar data were reported by (Suwignyo *et al*., 2022), where the cholesterol levels in the liver and meat of ducks were significantly decreased (3-6%) by alfalfa-supplemented feed. This result could be due to the fiber and saponin contents in Kacang Ratu BW. According to (Sudarmono, 2003), the limit of fiber consumption in laying hens during the egg-laying period is between 3-4 %.

The presence of fiber and saponins in the feed reduces cholesterol levels because the cholesterol present in the body is reduced as bile acid is excreted through feces. Increased excretion of bile acids through feces will spur the body, especially the liver, to synthesize new bile acids derived from cholesterol, resulting in a decrease in the cholesterol level (Francis *et al*., 2002; Parwiastuti, 2001). Bile acids, which function to emulsify fat, return into the gallbladder through the liver and blood vessels, then into the growing follicles that occur with receptor media processes, and finally into the egg yolk (Wahyuni and Jacob, 2007).

These results are similar to those of (McNaughton, 1978), who reported that alfalfa meal lowered egg yolk cholesterol. Zheng *et al*. (2019) reported that the addition of 5.8 and 10% alfalfa meal can reduce yolk cholesterol in laying hens. Similarly, (Kocaoğlu Güçlü *et al*., 2004) stated that feeding 9% alfalfa in a laying quails diet can reduce the levels of egg yolk cholesterol and blood cholesterol. The blood cholesterol levels in this study were normal. Normal blood cholesterol levels in laying hens are 52-148 mg/dL (Basmacioğlu and Ergül, 2005).

Conclusion

The results of this study indicated that supplementation of laying hens' diet with up to 3 % Kacang Ratu BW can reduce blood cholesterol, yolk cholesterol, and meat fat. Kacang Ratu BW did not have any significant effects on other meat quality parameters.

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Author's Contributions

Bambang Suwignyo: Designed the research plan, organized the study, covered all budget for research, coordinated the data analysis and contributed to the written of the manuscript.

Eprilia Aristia Rini: Participated in experiments in the field (data gathering) and processing research data.

Heru Sasongko: Advised during constructing the research plan, data collection, and written manuscript.

Bambang Ariyadi: Advised during data analyses and written manuscript.

Siti Helmyati: Advised during the written manuscript, and evaluated the final draft before submission.

Ethics

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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