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Effects of sugar apple (*Annona squamosa* L.) extract on cockerel's growth performance, carcass, digestive tract microbial count, and meat cholesterol

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ABSTRACT

The study aimed to determine the effect of *Annona squamosa* L. leaf extract through drinking water on the growth of cockerel`s and cholesterol content of cockerel meat. The study used a completely randomized design (CRD), with five treatments and six replications, each experimental unit consisted of 5 cockerels. The treatments consisted of chicken without added sugar apple leaf extract in drinking water (T0), added sugar apple leaf extract @1% (T1), 2% (T2), 3% (T3), 4% (T4). The addition of sugar apple leaf extract drinking water had no significant effect on the TPC variable (CFU/g) in the digestive tract, while *E. coli* and coliform were lower than the control (T0). The final weight and weight gain of cockerels that received T3 treatment were the highest compared to other treatments. The addition of sugar apple extract through drinking water had no significant effect on the variables of ration consumption and ration conversion. Cholesterol, triglycerides, HDL, and HDL in the meat of cockerels were not affected by additional treatment of sugar apple extract in drinking water. It can be concluded that the addition of *Annona squamosa* Linn leaf extract through drinking water has significant positive effects on the weight gain of cockerels.

Keywords: Cockerel, Meat cholesterol, Performance, Scum microbes

To increase the productivity of poultry, and reduce the spread of disease, farmers add potential ingredients that can be added to feed or drinking water in flocks (Seidav *et al*. 2021). Chickens are reared on feeds that are regularly supplemented with antibiotics, the aim of which is to prevent and treat disease as well as for body growth (Haque *et al*. 2020). The use of Antibiotics Growth Promoter (AGP) in animal feed has long been used as a growth promoter to improve performance and gaining economic benefits. The use of AGP aims to stimulate growth by killing bacteria to increase ration efficiency and provide benefits for farmers (Adhikari and Mukhopadhyay 2021). The use of antibiotics in animal feed in the long term causes some bacteria such as *Salmonella, Campylobacter,* and *Escherichia coli* to become resistant to antibiotics, then can transfer resistant genes to humans through the food chain or by direct contact (denBogaard *et al*. 2000). The use of antibiotics has led to antimicrobial resistance, therefore the use of antibiotics in poultry feed is prohibited.

The impact that arises from the use of antibiotics is very undesirable, so many studies have been carried out to find viable alternative sources to replace their use in the field of animal nutrition (Pashtetsky *et al*. 2020). One of the alternative natural antibiotics currently being developed is phytobiotics, namely antibiotics sourced from herbs and medicinal plants. The use of these herbal is very popular in the poultry industry after the prohibition of antibiotic growth promoters (AGP) (Lilleho *et al*. 2012). Several plants have been known to play an important role in human and animal health (Seidavi *et al*. 2021). The sugar apple (*Annona squamosa* L.) is a tropical fruit plant of the Annonaceae family. This plant has been studied for its health benefits. This is because it is associated with quite diverse phytochemical content including phenol-based compounds, alkaloids, and flavonoids. Extracts from *Annona squamosa* have been found to have potential biological activity as antimicrobial (Kumar *et al*. 2021). Sugar apple leaves contain linalool, eugenol, farnesol, and geraniol present in the extract which provides antibacterial activity against *Escherichia coli* and *coliform*, and also functions as an antioxidant (Pandey and Barve 2011).

The decrease in the population of pathogenic bacteria in the digestive tract will lead to an increase in the efficiency of nutrient absorption. The photochemical content of sugar apple leaves is a natural antibiotic with the ability to suppress pathogenic microbes, provide immunity and endurance, and also improve the appearance of production. Sugar apple is used in disorders like hyperthyroidism, and has antimicrobial, antitumor, and antioxidant properties (Narwade and Aher 2019). The flavonoid compounds contained in sugar apple leaves are a group of phenolics compounds that can inhibit the growth of pathogenic microbes. The decrease in pathogenic bacteria such as *Escherichia coli* and c*oliform* in the digestive tract will

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cause probiotic bacteria such as *Lactobacillus* sp to increase so that the efficiency of nutrient absorption increases (Nuriyasa *et al*. 2021). To develop and utilize plants as an alternative source of antibiotics, it is necessary to research to exploit the potential of sugar apple plants so that it can improve the quality of poultry products that are free from chemical drugs.

MATERIALS AND METHODS

The design used in this study was a completely randomized design (CRD) with five treatments and six replications, each experimental unit consisted of 5 cockerels, hence the total number of chickens used was 150 cockerels. The cockerels were assigned to the control diet (T0) without additional sugar apple leaf extract via drinking water. While the experimental treatments viz. T1, T2, T3, and T4, cockerels were fed which was similar to T0, but were additionally supplemented with the sugar apple leaf extract at levels 1%, 2%, 3%, and 4% in drinking. The diet's ingredients components fed and their chemical analysis are presented in Table 1.

The sugar apple leaves used were old leaves picked from 5 strands of the oldest leaf on each branch, then the leaves were washed using clean water. The sugar apple leaves obtained were weighed (1 kg), added to 1 liter of water and blended. The water extract from sugar apple leaf with a ratio of 1:1 was put into a bottle and kept until use. The liquid sample was aseptically taken from the digestive tract and placed into sterile plastics. The samples were then serially diluted in a sterile saline solution. The isolation of the bacteria was adapted from procedures described for the isolation of digestive tract bacteria (Desouky *et al*. 2021). After the incubation periods, colonies of the respective bacteria were counted.

Weight gain was obtained by subtracting the final body weight from the initial experimental weight. The ration consumption was measured once a week, namely by the difference between the amount of ration given and the rest of the ration. Feed conversion is the ratio between the amounts of feed consumed and body weight gain. Carcass weight was obtained from weighing the chicken after it was cut and carried out by removing the feathers and removing the internal organs as well as the separation between the head, neck and legs. Carcass percentage was obtained by comparing carcass weight with live weight multiplied by 100%. The physical composition of the carcass was obtained by separating the components of bone, meat and fat from the carcass. Cockerel meat cholesterol was calculated using the Boehringer method. The cockerel meat sample was blended, then put into a sterile test tube, then centrifuged at 2500 rpm for 10 min. The supernatant was taken using a microscope and then put into a microtube, and then it was ready to be analyzed for its cholesterol content using the Enzymatic Cholesterol High Performance method**.**

Data obtained were analyzed using the general linear model (GLM) and was performed with a software program IBM SPSS Statistics 25. The Least Significant Difference (LSD) test was applied to compare the differences means.

RESULTS AND DISCUSSION

The chemical content of sugar apple leaf extract was as follows: 0.10% phenol, 0.03% flavonoids, 0.23% tannins, 0.09% antioxidant capacity. In treatment without the addition of sugar apple leaf extract through drinking water (T0), the total bacteria (TPC) found in the digestive tract of cockerel was 1.03×10^5 CFU/g. For treatments T1, T2, T3 and T4, the total bacteria in the digestive tract was 27.18%, 33.98%; 45.63% and 50.49% lower than T0 but statistically not different (p>0.05). *Escherichia coli* in T0 treatment was 5.95×10^3 CFU/g. The T1 treatment caused the number of *Escherichia coli* to be 9.75% higher (p>0.05) than T0.

Table 1. Composition and nutritional content of treatment

Feed ingredient	Composition (%)				
	T0	T1	T2	T3	T4
Yellow corn	39.05	39.05	39.05	39.05	39.05
Polar	16.4	16.4	16.4	16.4	16.4
Concentrate 124	40.85	40.85	40.85	40.85	40.85
Coconut oil	3	3	3	3	3
Mineral mix	0.45	0.45	0.45	0.45	0.45
NaCl	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Sugar apple extract (%)	0	1	2	3	4
Lipid (%)	9.27	9.27	9.27	9.27	9.27
Nutrient content					
Energy metabolism (kcal/kg)	2900	2900	2900	2900	2900
Crude protein (%)	18	18	18	18	18
Lipid (%)	9.27	9.27	9.27	9.27	9.27
Crude fibre (%)	6.03	6.03	6.03	6.03	6.03
Ca (%)	1.57	1.57	1.57	1.57	1.57
P (%)	0.57	0.57	0.57	0.57	0.57

Table 2. Digestive tract microbial composition (2-8 weeks) of cockerels supplemented with sugar apple leaf extract through drinking water

Variable	Treatment					SEM[1]
	T0	T1	T2	T3	T4	
TPC (CFU/g) × 10^5	1.03^a	0.75^a	0.68^a	0.56^a	0.51^a	0.56
Total *E. coli* (CFU/g) × 10^3	5.95^a	6.53^a	4.83^b	2.63^c	1.63^c	0.36
Total *coliform* (CFU/g) × 10^5	0.91^a	0.96^a	0.46^{ab}	0.31^b	0.11^b	0.21

SEM, Standard error of treatment means; ^{abc}, Means with different superscripts within rows are significantly different (p<0.05).

Escherichia coli treatment of T2, T3 and T4 respectively caused *Escherichia coli* to be 18.82%; 55.80% and 72.61% lower (p<0.05) than the control treatment. In control treatment (T0), the total *Coliform* bacteria was 0.91 CFU/g. T1 treatment caused *Coliform* bacteria 5.49% higher (p>0.05) than T0. Treatments T2, T3 and T4 each caused *Coliform* bacteria to be 49.45%; 65.93% and 87.91% lower (p<0.05) than the control treatment (T0), as in Table 2.

Sandeep and Mittal (2017) stated that sugar apple leaves have been an important source of medicine since the beginning of human civilization. *Annona squamosa* Linn has been widely used as an antioxidant, antidiabetic, hepatic protective, and anti-lice agent. Sugar apple leaves contain alkaloids, carbohydrates, tannins, and phenolics which function as antibacterial pathogens. Sugar apple leaves have various pharmacological activities such as antidiabetic, analgesic, anti-inflammatory, wound healing, antimalarial, cyst toxic, anti-oxidant, and anti-microbial. Pandey and Barve (2011) stated that the volatile compounds of this plant has strong anti-bacterial activity. The results of the analysis at the Udayana University Laboratory found that sugar apple leaf extract contained 0.10% phenol; 0.03% flavonoids; 0.23% tannin; 0.09% antioxidant and IC 50% by 0.87%. The active ingredients in sugar apple leaf extract can reduce the population of pathogenic bacteria such as *Escherichia coli* and *coliform* in the digestive tract of the cockerel that received T2, T3, and T4 treatment compared to the control (T0) treatment. Treatments T1, T2, T3, and T4 had no significant effect (p>0.05) on the total bacterial count (TPC) compared to the control treatment (T0). This indicates that the beneficial bacteria, namely bacillus bacteria in the digestive tract of laying hens in treatment T1, T2, T3, and T4 were more than those without additional sugar apple leaf extract through drinking water compared to the control treatment (T0).

The data in Table 2 shows that the weight of cockerels at the beginning of the study did not show a significant difference (p>0.05). This indicates that the cockerels used at the beginning of the study had a homogeneous body weight. The body weight at the end of the cockerel that received T0 treatment was 842.48 g. The final body weight of cockerel which received additional treatment of 1% sugar apple leaf extract through drinking water (T1), 2% (T2), and 4% (T4) respectively were 3.53%, 6.05%, and 1.22% higher than cockerel who received control treatment (T0) but not significantly different (p>0.05). The addition of sugar apple leaf extract @3% through drinking water (T3) caused the cockerel's body weight to be significantly higher than the control (T0). The addition of sugar apple leaf extract through drinking water did not cause any difference in the feed consumption of cockerel. Cockerel that received T3 treatment resulted in higher body weight gain (p<0.05) than the control treatment (T0). There was no significant difference in feed conversion between treatments. The consumption of cockerel rations given T0 treatment was 39.66 g/day (Table 2). Treatment with sugar apple leaf extract added through drinking water, namely T1, T2, T3, and T4 3.78% respectively; had 4.97%; 4.51%; higher ration consumption (p>0.05 than the control treatment but treatment T4 was 4.49% lower (p>0.05) than T0.

Treatment without the addition of sugar apple leaf extract through drinking water (T0) increased the body weight by 16.59 g/day. Treatments T1, T2, and T4 had 3.80%, 6.75%, and 6.69% higher body weight gain (p>0.05) than the control treatment (T0). Cockerels that received T3 treatment had 14.59% higher body weight gain (p<0.05) than the control treatment (T0). The feed conversion of cockerels that did not receive additional sugar apple leaf extract through drinking water (T0) was 2.39. Treatment T1, T2, T3 and T4 had feed conversion of 0% respectively; 1.67%; 8.79% and 10.46% lower but not statistically different (p>0.05) compared to the control treatment (T0).

Sugar apple leaf extract (*Annona squamosa* L.) functions as an antioxidant, antidiabetic, antitumor activity, and anti-

Table 3. Performance of cockerel (2 – 8 weeks) added sugar apple extract through drinking water

Variable	Treatment				
	T0	T1	T2	T3	T4
Initial cockerel weight (g)	133.98±2.85^a	128.87±2.71^a	129.86±2.73^a	129.5±52.72^a	131.14±2.84^a
Final cockerel weight (g)	842.48±23.65^b	872.2±23.97^b	893.47±25.17^{ab}	951.93±25.21^a	852.77±23.91^b
Feed consumption (g/day)	39.66±2.78^a	41.16±2.83^a	41.63±2.86^a	41.45±2.85^a	37.88±2.71^a
Weight gain (g/day)	16.59±1.81^b	17.22±1.80^{ab}	17,71±1.89^{ab}	19,01±1.92^a	17.70±1.83^{ab}
Feed conversion	2.39±0.07^a	2.39±0.08^a	2.35±0.06^a	2.18±0.05^a	2.14±0.05^a

SEM, Standard error of treatment means; ^{abc}Means with different superscripts within rows are significantly different (p<0.05).

Table 4. Carcass characteristics of cockerel (2 – 8 weeks) fed sugar apple leaf extract through drinking water

Variable	Treatment				
	T0	T1	T2	T3	T4
Live weight (g)	819.65±20.45^{b2}	864.8±21.73^{ab}	896.63±22.96^{ab}	914.4±25.42^{a}	852.57±25.19^{ab}
Carcass weight (g)	440.89±15.72^{b}	458.17±16.51^{ab}	476.92±17.37^{ab}	508.86±19.28^{a}	456,3±16.74^{ab}
Carcass percentage (%)	53.79±1.03^{a}	52.98±1.08^{a}	53.19±1.11^{a}	55.65±1.17^{a}	53.52±1.04^{a}
Bone percentage (%)	63.46±2.28^{a}	63.92±2.29^{a}	63.25±2.30^{a}	64.24±2.39^{a}	62.39±2.21^{a}
Meat percentage (%)	28.50±1.70^{a}	29.04±1.84^{a}	29.7±1.83^{a}	28.90±1.73^{a}	30.45±1.82^{a}
Fat percentage (%)	8.04±1.05^{a}	7.04±0.98^{a}	7.05±0.99^{a}	6.86±0.81^{a}	7.16±0.75^{a}

SEM, Standard Error of Treatment Means; ^{abc}Means with different superscripts within rows are significant different (p<0.05).

lice agent. The chemical content of plants in sugar apple leaves such as alkaloids, carbohydrates, fixed oils, tannins, and phenolics as antibacterial pathogens. It has various pharmacological activities such as antidiabetic, analgesic, anti-inflammatory, wound healing, anti-oxidant, and anti-microbial (Sandeep and Mittal 2017). Volatile compounds from sugar apple leaves were also studied for antibacterial activity (Pandey and Barve 2011). The addition of sugar apple leaf extract through drinking water causes the total TPC bacteria (CFU/g) to increase and decrease the pathogenic bacteria *Escherichia coli* (CFU/g) and *Coliform* bacteria causing physically healthier chickens, better feed digestion so that the resulting weight gain higher than the control feed. The addition of sugar apple leaf extract to a level of 3% had an impact on the feed becoming more palatable so that the consumption of rations is quantitatively higher although statistically, the difference is not significant. The addition of 4% sugar apple leaf extract caused a decrease in feed consumption, this was due to the high tannin content at the level of 4% additional sugar apple leaf extract, the bitter taste of drinking water was at a disturbing level resulting in a decrease in feed consumption. Higher feed consumption in cockerel that received T3 treatment also led to higher consumption of energy, protein, minerals, and vitamins, as nutrients needed for growth. In poultry nutrition, feeding higher protein, can assist early gut development and digestive physiology and improve growth performance and immunity (Beski *et al*. 2015). The more nutrient content absorbed by the cockerel's body, the higher the final body weight because it can increase the development of tissues in the animal's body so that the growth of chickens increases. Natural antibiotic with their unique properties and positive impact on poultry production commonly used to improve bird production performance (El-Hack *et al*. 2022).

Anti-bacterial substances can lyse toxins that stick to the intestinal wall, so that the absorption of nutrients becomes better, as is the mechanism of action of antibiotics as growth promoters. The decrease in pathogenic bacteria in the digestive tract will lead to an increase in the efficiency of nutrient absorption. The addition of natural antibiotic to drinking water supports digestion and metabolism in the body, stimulates the growth and development of a useful microbiome and limits the multiplication and adhesion of pathogens (Krauze 2021). The pharmacologic properties of of sugar apple leaves as a natural antimicrobial to inhibit bacterial growth (Kumar *et al*. 2021). The ability to inhibit pathogenic microbes, provide immunity and endurance, and also improve the appearance of production.

The content of secondary metabolites such as essential oils, phenols, alkaloids, terpenoids, and flavonoids in sugar apple leaves can inhibit the growth of microbes or bacteria (Simon *et al*. 2016). The decrease in pathogenic bacteria such as *Escherichia coli* and *coliform* in the digestive tract will cause lactic acid bacteria, such as *Lactobacillus* sp to increase so that the efficiency of nutrient absorption increases (Nuriyasa *et al*. 2021). The addition of sugar apple leaf extract had no effect on the feed conversion ratio of cockerel. Higher weight gain is obtained by increasing feed consumption, not efficiency in feed use.

Cockerels that received control treatment (T0) produced a slaughter weight of 819.65 g while treatments T1, T2 and T4 produced a slaughter weight of 5.51%; 9.39% and 4.02% higher but not statistically significantly different (p>0.05). The cutting weight in the T3 treatment was 11.56% higher which was statistically significantly (p<0.05) than the control treatment (T0). The T0 treatment resulted in the lowest carcass weight, which was 440.89 g. Treatments T1, T2 and T4 resulted in carcass weight of 10.05%; 8.17% and 3.5% respectively higher (p>0.05) than T0. Cockerel that received T3 treatment produced 15.42% higher carcass weight which was statistically significant (p<0.05) than T0 treatment. Additional treatment of sugar apple leaf extract

Table 5. Meat cholesterol in cockerel (2 – 8 weeks) given sugar apple leaf extract via drinking water

Variable	Treatment				
	T0	T1	T2	T3	T4
Total cholesterol (mg/dL)	134.25±7.01^{a2}	134.07±7.02^{a}	130.13±6.98^{a}	125.43±6.94^{a}	125.04±6.93^{a}
Triglycerides (mg/dL)	120.23±13.71^{a}	115.76±13.52^{a}	113.98±13.51^{a}	112.34±13,48^{a}	112 .67± 13.49^{a}
HDL (mg/dL)	98.12±4.86^{a}	87.44±4.21^{a}	88.14±4.25^{a}	97.08±4.87^{a}	92.57±4.35^{a}
LDL (mg/dL)	43.63±3.15^{a}	48.26±3.98^{a}	47.45±3.92^{a}	47.21±3.81^{a}	49.54±3.99^{a}

SEM, Standard Error of Treatment Means; ^{abc}Means with different superscripts within rows are significant different (p<0.05).

through drinking water had no significant effect on the variable percentage of cockerel carcass. The lowest bone percentage occurred in treatment T4 which was 62.39%. Treatments T3, T2, T1 and T0 produced bone percentages of 2.96%; 1.38%; 2.45% and 1.72% higher but not statistically different (p>0.05). The lowest percentage of carcass meat occurred in T0 treatment, which was 28.50%, while T1, T2, T3 and T4 treatments were respectively 1.89%; 4.21%; 1.40% and 6.84% higher than treatment T0 but not statistically different (p>0.05). The T3 treatment resulted in the lowest percentage of fat, namely 6.86%. Treatments T4, T2, T1 and T0 were 10.34%; 2.78%; 2.62% and 17.20% higher but not significantly different (p>0.05).

Cockerels that received additional treatment of sugar apple leaf extract through drinking water at a level of 3% (T3) produced the highest cutting weight compared to other treatments. Chemical content such as phenols, flavonoids, tannins, and antioxidants can kill some pathogenic bacteria (*Escherichia coli* and *Coliform*), so the composition of *Bacillus* bacteria in the digestive tract is beneficial to be higher. This condition can accelerate the absorption of nutrients in the intestine so that it will have an impact on higher feed consumption. Nuriyasa *et al*. (2022) stated that higher rations in feeds with the same energy and protein content resulted in higher consumption of nutrients, especially energy, and protein. Energy and protein are nutrients as constituents of body organs so that the cutting weight becomes higher.

The addition of sugar apple leaf extract through drinking water at a level of 4% (T4) caused growth to decrease although not significantly, due to sugar apple leaves containing tannin. Poultry is sensitive to tannins. Tannins have several negative effects on poultry (Hidayat *et al*. 2021). High amounts of tannins cause decreased broiler growth (Buyse *et al*. 2022). The impaired growth is because tannins can reduce appetite, reduce feed intake, and poor nutrient digestibility and absorption (Choi and Kim 2020). In addition, tannin compounds can also cause injury to the digestive tract of animals which can cause digestive disorders such as decreased protein digestion.

Carcass weight on cockerel that received T3 treatment was the highest compared to other treatments. Carcass weight is related to slaughter weight, the higher the cut weight, the higher the carcass weight produced, following the opinion of Nuriyasa *et al*. (2021). Giving sugar apple leaf extract through drinking water did not affect the percentage of carcasses produced. This was due to the higher carcass weight produced by higher slaughter weight, so the percentage of carcass produced was not significantly different. The physical composition of the carcass which included the percentage of bone, meat, and fat was not affected by additional treatment of sugar apple leaf extract through drinking water. This was due to the higher weight of the carcass's physical composition (bone, meat, fat) produced by a higher carcass weight as well.

The total cholesterol in cockerel meat that was treated without additional sugar apple leaf extract through drinking water (T0) was 134.25 mg/dl. Meat cholesterol in treatment T1, T2, T3 and T4 were 0.13%; 3.07%; 6.57% and 6.86% lower (p>0.05) compared to treatment T0. The triglyceride content of meat in cockerel was 120.23 mg/dl. Treatments T1, T2, T3, and T4 had 3.72%; 5.20%; 6.56%, and 6.29% lower (p>0.05) triglyceride content T0. HDL of cockerel meat that received T0 treatment was 98.12 mg/dl. Treatments T1, T2, T3 and T4 had 10.88%; 10.17%; 1.06% and 5.66% lower HDL (p>0.05) than T0. The LDL content of meat in cockerel that received T0 treatment was 48.26 mg/dl, while T1, T2, T3, and T4 treatments had 10.61%; 8.76%, 8.21% and 13.55% higher (p>0.05) LDL compared to treatment T0.

Additional sugar apple leaf extract through drinking water can reduce pathogenic bacteria *Escherichia coli* and *Coliform*. Total bacteria in the digestive tract (TPC) were not affected by additional treatment of sugar apple leaf extract. This situation indicates that the number of *Bacillus* bacteria increases in the digestive tract. Lactobacillus with high bile salt hydrolyses activity can reduce cholesterol levels. *Lactobacillus acidophilus* is a type of lactic acid bacteria that is able to bind cholesterol in its cells. Bacterial cell membranes play a role in binding cholesterol. Cholesterol binding and bile acid deconjugation are carried out by lactic acid bacterial cells. This causes the cholesterol content of the meat in laying hens given sugar apple leaf extract in drinking water to produce meat with quantitatively lower cholesterol and triglyceride content compared to the control treatment. Adding sugar apple leaf extract through drinking water, can quantitatively reduce LDL levels of meat in cockerel. The tannin content in apple sugar can cause a significant reduction in monounsaturated fatty acids (Ebrahim *et al*. 2015). The HDL content of meat in cockerel increased slightly, which was due to the process of releasing cholesterol esters into the liver by hepatic lipase which hydrolyzes HDL and triglycerides to provide cholesterol for bile acid production. The increase of HDL indicated that endogenous cholesterol metabolism was probably disturbed.

Treatment of sugar apple leaf extract through drinking water did not affect total bacteria (TPC) but could reduce the number of *Escherichia coli* and *coliform* bacteria. The addition of sugar apple leaf extract through drinking water at a level of 3% resulted in higher performance and carcass in cockerel than in other treatments. The addition of sugar apple leaf extract through drinking water in cockerel did not affect the cholesterol content of meat, triglycerides, HDL, and LDL.

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