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¹ **The Evaluation of Dietary Addition of Palm and Coconut Oils in Steaming Tomato (*Lycopersicon esculentum*) Waste Powder on Digestibility of Crude Fiber and Retention of Lycopene and Nitrogen in Broiler Chickens**

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ABSTRACT

¹ Lycopene is a powerful antioxidant present in tomatoes and other vegetables and fruits. Present research was carried out to evaluate lycopene and nitrogen retention and crude fiber (CF) digestibility of steaming tomatoes waste powder which was combined with oil. Tomatoes waste in this experiment were local fresh tomato rejected from tomato field around West Sumatera province, Indonesia. The experimental factors were included the type of oil (palm and coconut oils) and dosage of oils (0.25, 0.5, 0.75, 1, and 1.25 %), and each treatment was replicated three times. The results indicated there was an interaction between the type of oil and dosage of oil on lycopene retention, and CF digestibility, while the type of oil and dosage of oil affected lycopene retention significantly. The dosage of oil also influenced lycopene retention, nitrogen retention, CF digestibility significantly. The addition of coconut oil in steaming tomato waste powder increased lycopene and nitrogen retention, and CF digestibility higher than the addition of palm oil to steaming tomato waste powder in broiler chickens. The lycopene and nitrogen retention, and CF digestibility of steaming tomato waste powder added 0.5% coconut oil was the best level for lycopene and nitrogen retention, and CF digestibility in broiler chickens.

Key words: Coconut oil, Crude fiber digestibility, Lycopene retention, Nitrogen retention, Palm oil, Tomatoes waste

INTRODUCTION

Recently, researches on poultry nutrition were conducted to find cheaper and high quality feeds. One of the famous active compound in tomato is lycopene. This compound is beneficial for human and livestock health (Bramley, 2000). Lycopene was known as a substance that has high antioxidant ability (Dewanto et al., 2002; Toor and Savage, 2005; Gupta et al., 2011; Surai, 2016). In addition, lycopene also has an important role to reduce cholesterol level in the body (Palozza et al., 2012). Lycopene can inhibit HMG-CoA reductase to synthesize mevalonate from HMG-CoA, resulting in reducing of cholesterol level in the body (Palozza et al., 2012).

Previous research showed lycopene and tomato wastes have been widely used as feed mixtures in poultry. This research shown, that semen production and viability

were affected by lycopene supplementation in broiler breeder (Mangiagalli and Taylor, 2010). It could reduce cholesterol content in meat (Mahata et al. 2016 a), and in serum of broiler chickens (Mahata et al., 2016a) and laying hen (Mahata et al., 2016b), and it also improved poultry immunity status (Olson et al., 2005; Mangiagalli and Taylor, 2010; Sun et al., 2015). Generally, lycopene structure in fresh tomato is in trans form, and it is very stable so that it is difficult to absorb by animals and humans digestive tract (Unlu et al., 2007; Knockaert et al., 2012; Meroni and Raikos 2018). Handayani et al. (2018) reported that to increase the availability of cis-lycopene and its absorption in poultry digestive tract, fresh tomato should be treated by steaming for 12 minutes at temperature 98 °C. This treating will change the structure of lycopene in fresh tomato from trans structure to cis structure.

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One of the characteristics of lycopene is lipid soluble (Clinton, 1998; Shi and Maguer, 2000; Colle et al., 2010; Colle et al., 2013; Trujillo and Mc-Clements, 2016), so that to increase lycopene absorption in the digestive tract, it must be mixed with lipid. Clark et al. (2000) found that olive oil was better than corn oil in improving lycopene absorption in the rat, because the corn oil is rich with the Polyunsaturated Fatty Acid (PUFA) like linoleic acid, while olive oil is rich with the Monounsaturated Fatty Acid (MUFA) like oleic acid. Furthermore, Clark et al. (2000) explained that lycopene is a part of carotenoid with less absorption of carotenoid in digestive tract of rat due to carotenoid oxidation which promotes by high PUFA content in corn oil and transfer of carotenoid to micelles from lipid emulsions containing large amounts of PUFA and bile salt will reduce, so that the absorption of lycopene mixed with corn oil was less than absorption of lycopene mixed with olive oil. On the other hand, Colle et al. (2012) reported the *in vitro* experiment about the addition of 5% different lipid (coconut oil, palm oil, cocoa butter, olive oil, sunflower oil, and fish oil) to row tomato pulp were significantly affected the lycopene bioaccessibility. Furthermore, in the second experiment Colle et al. (2012) added of coconut oil, olive oil, and fish oil of 0, 1, 2, 5, and 10% to row tomato pulp, and the highest lycopene bioaccessibility for olive oil and fish oil was after adding 2% and 1% of oil respectively, while the highest lycopene bioaccessibility for coconut oil was as much as 10%.

Palm oil and coconut oil are rich with saturated fatty acids like palmitic acid and lauric acid respectively (Dinicolantonio and O'Keefe, 2017). Both oils (palm oil and coconut oil) have potential to dilution of lycopene for increasing its bioaccessibility by *in vitro* study (Colle et al., 2012), but the research that specifically investigates lycopene absorption by *in vivo* study in poultry is limited. Moreover, the digestibility of crude fiber and nitrogen retention from steaming tomato waste powder are important to evaluate, because maybe the steaming and oil addition to tomato waste will affect the content and structure of protein and crude fiber in tomatoes. According to Mahata et al. (2012), physical treatment (steaming) degraded crude fiber bond in juice waste mixture, and it also decreased crude fiber content. The addition of lipid in diet of poultry could reduce the rate of feed digestion in digestive tract, and it improved digestibility of feed nutrient (Baiao and Lara, 2005; Latshaw, 2008; Rizal, 2013). Therefore, this study was performed to evaluate the effects of coconut and palm oil addition in steaming tomato waste powder on lycopene and nitrogen retention, and crude fiber digestibility.

MATERIALS AND METHODS

Ethical approval

The animal experiments were carried out in accordance with the guidelines laid by institutional Ethics committee for the care of animals and were approved by Animal Ethics Committee of the Universitas Andalas, Padang, Indonesia with No:574/KEP/FK/2019.

Coconut oil and palm oil

Palm oil from one of the local brand (merk Rose Brand from Rose Brand inc, Jakarta, Indonesia) with the purity as much as 99.81%. Coconut oil was prepared by extraction of coconut milk from a mature coconut, and then it boiled until the oil from coconut milk was produced. Furthermore, the oil was separated from coconut cake by filtration. The purity of coconut oil as much as 99.85%. The fatty acid content of each coconut oil and palm oil presented in table 1.

Steaming tomato powder waste

The type of tomato used in this experiment was mature tomato (*Lycopersicon esculentum*) waste. It was collected from rejected tomatoes at an agricultural field in Alahan Panjang, West Sumatera province, Indonesia. The tomato waste was steamed in the boiled water at 98°C for 12 min (Handayani et al., 2018). Furthermore, steaming tomatoes wastes were dried in the oven at temperature 60°C for three days (Handayani et al., 2018) and then ground to be a powder. The nutrient content of tomato powder shown in table 2.

Table 1. Fatty acid composition of coconut oil and palm oil

Fatty acid	Coconut oil	Palm oil
C4 (butyric acid) (%)	0	0
C6 (caproic acid) (%)	0	0
C8 (caprylic acid) (%)	0,23	0
C10 (capric acid) (%)	4,53	0
C12 (lauric acid) (%)	55,28	0,26
C14 (myristic acid) (%)	21,8	1,26
C16 (palmitic acid) (%)	7,34	35,6
C18 (Stearic acid) (%)	3,13	5,07
C18:1 (oleic acid) (%)	4,97	39,91
C18:2 (linoleic acid) (%)	2,12	16,1

Source: analysis at the Agro-Industrial Center Central Laboratory, at Bogor city, West Java, Indonesia, 2018

Table 2. Nutrient content of tomato powder

Nutrients	Amount
Crude protein (%)	10,88
Crude lipid (%)	3,85
Crude fiber (%)	11,92
Calcium (%)	0,26
Phosphorus (%)	0,69
Metabolism Energy (KCal/kg)	1596
Methionine (%)	0,25
Lysin (%)	0,83
Lycopene (mg/100g)	52,10

Experimental animals

Totally 60 broiler chickens (strain MB 202 from Japfa comfeed inc. Indonesia) at seven weeks of age were purchased from a local broiler farm. Broiler chickens divided randomly into 10 groups combination treatment from type of oil and dosage of oil (6 bird /group) with three replicates each (2 bird/ replicate). Another birds as much as 6 birds were prepared for control treatment (tomato steaming powder without oil) and 4 other bird were used for collecting excreta from endogenous nitrogen. Then birds were kept in wire cages, and each cage was provided with water troughs and tray to collect excreta. The control treatment and endogenous nitrogen treatment were not statistically analysed. Endogenous nitrogen treatment was used for calculate retention nitrogen.

Experimental design

The experiment was performed in a 2x5 factorial (Steel and Torrie, 1980) arrangement of lipid addition to steaming tomato waste powder in completely randomized design (totally 10 treatments), and each treatment was replicated three times. The first factor was different type of oil (coconut oil and palm oil), and the second factor was different dosage of oil (0.25, 0.5, 0.72, 1, and 1.25 % in feed). The combination of treatments as follows: Addition coconut oil at different dosages as much as 0.25, 0.5, 0.75, 1, and 1.25%. Also addition of palm oil at different dosages as much as 0.25, 0.5, 0.75, 1, and 1.25% was considered.

Forced feeding

Before forced feeding, all birds were fasted for 32 hours by modified method of Sibbald (1976) to make empty digestive tracts of the birds. The water was prepared *ad libitum*. 20g of steamed tomato waste powder prepared in paste (20 ml water mixed with the tomato

powder until to be paste). Then tomato paste was added to oil as much as appropriate with each treatment (0.25, 0.5, 0.72, 1, and 1.25 % in feed), and then each bird was forced feeding. Force-feeding was accomplished by inserting a gastrointestinal tube into the esophagus. The tomato paste pushed into the crop with a syringe rod. After force-feeding, the birds were returned to their cages. A tray was placed under each cage to collect excreta. Excreta samples from each bird was collected at 48 hours after forced feeding by modified method of Sibbald (1976). Excreta on the tray was moved to another tray every three hour and contaminants of excreta, such as feathers were removed carefully before excreta were dried. The excreta were dried in the oven at temperature 60 °C for 48 hours, ground to be powder for lycopene retention, nitrogen retention, and crude fiber digestibility analysis.

Measurement of lycopene

Lycopene was analyzed by modification from Sharma and Le Maguer (1996) method. 1.25 g of excreta powder from each bird was placed in Erlenmeyer 250 ml, covered with aluminum foil, and added with 12.5 ml of mixed solution hexane: acetone: ethanol (2: 1: 1, v/v/v). That mixture solution was made with mixing 6.25 ml of hexane, 3.125 ml acetone, and 3.125 ml of ethanol. Excreta powder that has been added with a mixed solution was shaken for 30 minutes with a magnetic stirrer, then separated by funnel separate, and 10 ml of distilled water was added, and then shaken again for 15 minutes. At last, the the polar and non-polar layers were separated by separating funnel and all the top layer (non-polar) poured to a 25ml measuring flask, then added N-hexane until mark boundaries. The total lycopene content of non-polar layer with UV-Vis spectrophotometry (UV-1800 Shimadzu Kyoto, Japan) at a wavelength of 417 nm was determined. Lycopene level was calculated by standard regression.

Measurement of lycopene retention

Lycopene Retention (LR) was calculated by the modification method of Jain (1999) as follows:

$$LR (\%) = \frac{\Sigma \text{lycopene consumption} - \Sigma \text{lycopene in excreta}}{\Sigma \text{lycopene consumption}} \times 100\%$$

Measurement of nitrogen and crude fiber

Nitrogen and Crude Fiber (CF) of excreta from each bird was analyzed by proximate analysis (AOAC, 1990). Measurement of nitrogen retention and CF digestibility, nitrogen retention was calculated by the method of Sibbald (1985) and CF digestibility were calculated by the method of Mujahid et al. (2003) with few modifications as follow:

$$\text{Nitrogen Retention(\%)} = \frac{\Sigma \text{ N consumption} - (\Sigma \text{ N excreta} - \Sigma \text{ N Endogenous})}{\Sigma \text{ N consumption}} \times 100\%$$

$$\text{CF digestibility (\%)} = \frac{\text{CF consumption} - \text{CF excreta}}{\text{CF consumption}} \times 100\%$$

Analysis of data

Data were statistically analyzed by one-way analysis of variance. Differences among treatments were determined with Duncan's multiple range test (DMRT) according to Steel and Torrie (1980). The significant differences was indicated at $P < 0.05$.

RESULTS

Lycopene retention from steaming tomato waste powder which was added with different types of oil (coconut and palm oils), and combined with different dosage of both of oils (0.25, 0.5, 0.75, 1, and 1.25%) was shown in table 3. There was interaction ($P < 0.05$) between type and dosage of oil on lycopene retention. The interaction between dosage of oil at 0.25% with the type of coconut oil and palm oil showed no significant ($P > 0.05$) effect on lycopene retention, as well as the interaction between dosage of oil at 0.5% with both type of oils did not significantly ($P > 0.05$) affect the lycopene retention. So there were no interactions ($P > 0.05$) between dosage of oil (0.25% and 0.5%) with the type of both different oils in lycopene retention. The interaction of both palm and coconut oil dosages at 0.75, 1, and 1.25% indicated significant ($P < 0.05$) effects on lycopene retention.

Table 3. Effect type and dosage of oil (coconut and palm oil) in steaming tomato waste on lycopene retention of broiler chicken

Type of oil	Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	47.19 ^{bc}	57.49 ^a	47.16 ^{bc}	46.21 ^{bc}	41.85 ^c	47.98 ^B
Coconut oil	49.97 ^b	61.74 ^a	57.77 ^a	56.46 ^a	58.16 ^a	56.82 ^A
Means	48.58 ^b	59.62 ^a	52.46 ^b	51.34 ^b	50.00 ^b	

^{a-c}Means values in the same row bearing different superscripts are significantly different ($P < 0.05$), ^{A-B}Means values in the same column bearing different superscripts are significantly different ($P < 0.05$), SEM=1.71

The result of nitrogen retention of steaming tomato waste powder with different oil types, and different dosage of oil are summarized in table 4. It was obtained that there was no interaction ($P > 0.05$) between types of oil (palm and coconut oils) and dosage of oil (0.25, 0.5, 0.75, 1, and 1.25%) on nitrogen retention, but nitrogen retention was affected by the type of oil (palm and coconut oils) significantly ($P < 0.05$), and also the dosages of oil (0.25, 0.5, 0.75, 1, and 1.25%) showed significant ($P < 0.05$) effects on nitrogen retention. In this study, the dosage of oil at 0.5% was the best dosage to increase nitrogen retention compared to other oil dosages (0.25, 0.75, 1, and 1.25%).

There was significantly interactions ($P < 0.05$) between the types of oil with the dosage of oil on CF digestibility (Table 5). That shows the type of oil and how much dosage of oil added affect CF digestibility in the digestive tract of broiler. CF digestibility was affected by types of oil (palm and coconut oils) significantly ($P < 0.05$), and also the dosage of oil showed significant ($P < 0.05$) effects on CF digestibility. The dosage of both of oils at 0.25% in diet showed the CF digestibility (24.50%) lesser than the dietary dosage of both of oils at 0.5% (32.16%). The highest CF digestibility was found in treatment coconut oil at the dosages of 0.25% and 0.5%. Increasing dosages of oil (0.75, 1 and 1.25) caused reduction in CF digestibility in both types of oil. It means that the dosages of oil should be added not more than 0.5% in order to obtain the best CF digestibility.

Table 4. Effect type and dosage of oil (coconut and palm oil) in steaming tomato waste on on nitrogen retention of broiler chicken

Type of oil	Dosage of Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	34.67	46.16	35.01	26.79	22.38	33.00 ^B
Coconut oil	54.43	62.39	47.69	46.79	38.93	50.05 ^A
Means	44.55 ^b	54.28 ^a	41.35 ^b	36.79 ^c	30.65 ^d	

^{a-c}Means values in the same row bearing different superscripts are significantly different (P<0.05), ^{A-B}Means values in the same column bearing different superscripts are significantly different (P<0.05), SEM= 1.37

Table 5. Type and dosage of oil (coconut and palm oil) in steaming tomato waste on on crude fiber digestibility of broiler chickens

Type of oil	Dosage of Dosage of palm oil and coconut oil (%)					Means
	0.25	0.5	0.75	1	1.25	
Palm oil	15.65 ^c	28.05 ^b	11.60 ^{cd}	11.35 ^{dc}	9.29 ^c	15.19 ^B
Coconut oil	33.36 ^a	36.26 ^a	15.34 ^{cd}	14.82 ^{cd}	13.03 ^{cd}	22.56 ^A
Means	24.50 ^b	32.16 ^a	13.47 ^c	13.09 ^c	11.16 ^c	

^{a-c}Means values in the same row bearing different superscripts are significantly different (P<0.05), ^{A-B}Means values in the same column bearing different superscripts are significantly different (P<0.05), SEM=1.24

DISCUSSION

Based on the result in this experiment, there was interaction between type and dosage of oil on lycopene retention. The lycopene retention at the dosage of 0.25% and 0.5% for both types of oils was not different. While, lycopene retention in tomato waste powder of steaming tomato with coconut oil at dosages of 0.75, 1, and 1.25% was as much as waste powder of steaming tomato with coconut oil at dosages of 0.5%. Lycopene retention in waste powder of steaming tomato with palm oil at dosages of 0.75, 1, and 1.25% decreased lycopene retention in comparing with 0.5% dosage. This condition showed when the dosage of oil is low (less than 0.5%), fatty acid from coconut oil and palm oil (Table 1) are able to solve the lycopene in micelle (an aggregate of molecules in a colloidal solution) of poultry digestive tract. Therefore lycopene absorption can be easily in digestive tract. When the oil dosage is high (more than 0.5%) in waste powder of steaming tomato, the medium chain saturated fatty acid in coconut oil appear better than palm oil which contains long chain saturated fatty acid to solve lycopene in micelle. According to Li et al. (2011) when in digestive tract the lipid load is high, triglycerides with long chain fatty acids will hydrolysis by enzyme (lipase), although this hydrolysis was lesser for triglycerides with medium chain fatty acids. According to Agarwal and Rao (1998) Lycopene is a part of carotenoid. An experiment on simulation of gastric-duodenal fluid that showed the

dilution of carotenoid in oil which not combined with micelles is higher than carotenoid in oil which combined with micelle (Malaki et al., 2010). That is the reason why the lycopene from steaming tomato waste powder which added with palm oil which contains long-chain fatty acid less absorption when high lipid load in digestive tract in this experiment. According to Huo et al. (2007) the addition of 0.25, 0.5, 1, 2.5% of coconut oil to salads which consist of 20% spinach, 35% tomatoes, 25% carrots, 10% lettuce, 10% yellow peppers, compared to salad which obtained 0.25% to 1% of coconut oil did not affect the micellarization of lycopene, but the micellarization increased after adding of coconut oil at dosage 2.5%. Colle et al. (2012) in an *in vitro* experiment, reported that lycopene bioaccessibility increased after addition of 0, 1, 2, 5, to 10% coconut oil. Moreover, Colle et al. (2012) expressed the addition of 2% olive oil with high long-chain fatty acid to tomato pulp, caused the highest lycopene bioaccessibility but this bioaccessibility was decreased in higher levels of olive oil. Beside that, this experiment describes that lycopene retention in tomato waste by emulsification with coconut oil better than emulsification with palm oil. Because coconut oil contain less unsaturated fatty acids than palm oil (table 1). The oils with high unsaturated fatty acid highly susceptible to oxidation, forming highly reactive radicals, resulting lycopene depletion. Because lycopene prevent unsaturated fatty acids from being oxidised and resulting in less

absorption of available lycopene in intestinal tract (Clark *et al.*, 2000).

Lycopene retention in this study was obtained 40.19% (data not displayed) at the control treatment (no oil addition), and 41.85% to 61.74% for the treatment combination the type and dosage of oil in steaming tomato waste powder. Lycopene retention in this study showed a good influence to increase the absorption of lycopene in poultry digestive tract, because of both types of oil (palm and coconut oils) were supporting factor for lycopene absorption in poultry digestive tract. The increasing of lycopene absorption in the digestive tract of poultry due to lycopene is a part of carotenoid. These carotenoid compounds are soluble in oil (Reboul, 2019). Both of oils and lycopene in the digestive tract of poultry would be absorbed in the micelles form. Micelles consist of carotenoid compounds, monoglycerides, and free fatty acids produced by hydrolysis of lipids in digestive tract (Yonekura and Nagoa, 2007). Monoglycerides, and free fatty acids produced by hydrolysis of oil would increase the formation of micelles, consequently lycopene will be more entered into the micelle and absorbed by the body through enterocytes. So that presence lipid would increase lycopene absorption (Hof *et al.*, 2000; Yonekura and Nagoa, 2007; Colle *et al.*, 2012; Trujillo and Mc-Clements, 2016).

Carotenoid absorption such as β -carotene was reported by Williams *et al.* (1998) ranged from 2 to 50%. The absorption is possible from food ingredients and independently from outside of foods. Jain (1999) stated that from 143 μg of lycopene given daily to mice, just 105 μg (73%) could absorb. According to Hof *et al.* (2000) and Trujillo and Mc-Clements (2016) absorption in foods containing carotenoids can be improved by processing. The heat processing will disrupt cell wall structure in plant thus can be released carotenoids from chromoplasts and increase their bioaccessibility (Trujillo and Mc-Clements, 2016) as well as it can change trans form to cis form (Basaran *et al.*, 2017). In present study, lycopene retention have high value compared to previous studies, which was because of tomato waste powder that was a product from steam processing. Steaming of tomato wastes for 12 minutes at 98°C supported releasing of lycopene from tomato matrix and undergo isomerization of lycopene from trans form to cis form. The release of lycopene from tomato matrix will increase the availability of lycopene, while the cis form of lycopene will be absorbed more easily than to trans lycopene form. According to Boileau *et al.* (1999) and Knockaert *et al.* (2012), the cis isomer of

lycopene was more easily absorbed than the trans lycopene isomer in the digestive tract.

Nitrogen retention and CF digestibility of steaming tomato waste powder increased after adding both of palm and coconut oils compared with nitrogen retention and CF digestibility at tomato waste powder without oil addition (data not displayed). It means, the addition of both type of oils to waste powder of steaming tomato affected the digestion rate in the digestive tract of poultry. The reason can be related to the actions of protease enzyme for hydrolyze protein and lipase enzyme to hydrolyze lipid from waste powder of steaming tomato in intestinal lumen of animals. The addition fat to animal feed reduced the passage rate of the digesta in the gastrointestinal tract, and allowed better absorption of all nutrients presented in the feed (Baiao and Lara, 2005; Latshaw, 2008; Rizal, 2013). The addition of 0.25% of oil to steaming tomato waste powder resulted in nitrogen retention 44.55%, and increased to 54.28% when dosage of oil increased to 0.5%, however in the dosage of oil at 0.75, 1, and 1.25 %, nitrogen retention decreased to 41.28%, 36.79% and 30.65% respectively. The decreasing of nitrogen retention caused by addition of higher dosages of oil (more than 0.5%), can be explained by accumulation of lipids in gastrointestinal, which causes low hydrolyze of triglycerides contained in the oil via lipase enzymes, and it mixed with CF contained in the steaming tomatoes waste, so triglycerides would be taken out of the digestive tract and nitrogen retention will be reduced.

The CF content in tomato waste powder was high (12%), which increase the viscosity of the digestive tract, and accelerate the rate of feed in the digestive tract. Therefore the digestibility of CF decreased when the dosage of both oils (palm and coconut oils) increased from 0.5% to 0.75, 1, and 1.25%. Paudel (2013) reported the effect of different types of oil like soybean oil (4%), and the combination of 2% rapeseed oil with 2% flaxseed oil on fat digestibility in broiler chickens and reported that the volume and viscosity of feed in the small intestine increased and the excreta from jejunum had much more liquid and so this might be a reason of low digestion. The range of CF digestibility in present study was from 9.29% to 36.26%. This finding was lower than the report of Mahata *et al.* (2018) that indicated CF digestibility of unboiled and boiled tomatoes added to broiler diet was from 37.61% to 51.28% respectively.

CONCLUSION

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The addition of coconut oil in steaming tomato waste powder increased lycopene and nitrogen retention, and crude fiber digestibility higher than the addition of palm oil to steaming tomato waste powder in the broiler. The best level for lycopene and nitrogen retention, and crude fiber digestibility in broiler was for group that added 0.5% coconut oil to the diet.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Author's contribution

Handayani were involved in the data collecting, statistical analysis and drafting of the manuscript. Wizna, Suliansyah, Rizal, and Mahata read and approved the final manuscript.

Consent to publish

All authors gave their informed consent prior to their inclusion in the study.

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