



Effects of Processed Tomato (*Lycopersicon esculentum*) Wastes in the Diet on the Serum Lipid Profile of Laying Hens

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Abstract | Processed tomatoes (*Lycopersicon esculentum*) waste are rejected tomatoes that have been processed by steaming at 98° C, then adding 0.5% coconut oil. This processing aims to increase the release of lycopene from the matrix tomato, change its structure from trans to cis lycopene, and increase the absorption of lycopene in the digestive tract of laying hens. Lycopene is a compound in tomatoes and is known to inhibit cholesterol synthesis. Cholesterol in the body is transported in the blood by lipoproteins. Therefore, that is important to know the effect of giving processed tomatoes wastes (PTW) on laying hens to lipid profile of blood serum consist of cholesterol total, triglycerides, LDL (Low Density Lipoprotein), and HDL (High Density Lipoprotein). Two hundred laying hens of the Lohman Brown were healthy, had no physical defects, weighed 1600–1800 g, and produced 82% at the age of 32 weeks at the beginning of the treatment. They were randomly housed in cages (one bird per cage, ten cages per replicate) and subjected to one of five experimental diets. This experiment used a completely randomized design (CRD) with 5 different ration treatments, consist of Ration A = 0% PTW, Ration B = 0% PTW + 0.03% simvastatin, Ration C = 6% PTW, Ration D = 12% PTW, and Ration E = 18% PTW. The administration of PTW in laying hens affected cholesterol total, LDL, and triglycerides very significant ($P < 0.01$). The highest cholesterol is in administration 0% PTW (119.20 Mg/dL), and the lowest is in administration 0.03% Simvastatin (101,55 Mg/dL). The triglyceride content of 0% PTW (693.08 Mg/dL) and 6% PTW (691.08 Mg/dL) was higher ($P < 0.05$) than administration 12 PTW (615.98 Mg/dL) and 18% PTW (597.95 Mg/dL), and 0.03% simvastatin supplementation (595.85 Mg/dL). The lowest LDL is in the administration of 0.03% Simvastatin (16.27 Mg/dL). While the administration of PTW or supplementation simvastatin there was no affected ($P > 0.05$) HDL content. Giving PTW up to 18% can reduce cholesterol total as much as 8.69%, triglycerides as much as 13.73%, LDL as much as 32.03%, and maintain HDL (19.09 Mg/dL).

Keywords | Kata kunci, Cholesterol, HDL, Lycopene, LDL, Triglycerides

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INTRODUCTION

Rejected tomatoes are tomatoes for consumption as a result of sorting, tomatoes that are discarded by farmers

when overproduction, and tomatoes that are not harvested at the seventh and eighth harvests. The tomatoes are still in good condition and nutrition. According to Sahin et al. (2006), Habanabashaka et al. (2014), Mahata et al. (2016),

tomatoes are known to be rich in lycopene which is useful for inhibiting cholesterol synthesis in the body of livestock. Lycopene is known to inhibit cholesterol synthesis through three mechanisms, namely: (1) inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the synthesis of intermediate compounds for cholesterol formation, namely mevalonate compounds from HMG-CoA compounds (Palozza *et al.*, 2012; Alvi *et al.*, 2017), (2) increase the activity of Low Density Lipoprotein (LDL) receptors, resulting in an increase in cholesterol endocytosis in target tissues (Palozza *et al.*, 2012; Alvi *et al.*, 2017), (3) inhibits the activity of cholesterol acyl transferase in the formation of cholesterol esters for storage in tissues (Palozza *et al.*, 2012).

However, the administration of tomato pulp (tomato pomace) in rations up to 10% (Nobakht and Safamehr, 2007) and 12% (Safamehr *et al.*, 2011) has not been able to reduce blood cholesterol and laying hens eggs. It was also reported that the administration of tomato pulp (tomato pomace) was 19% (Salajegheh *et al.*, 2012) and 17g/kg of tomato paste (An *et al.*, 2018) has not been able to lower blood cholesterol. Jafari *et al.* (2006) even reported that the treatment of giving tomato pulp as much as 15% could reduce egg production of laying hens, but on the contrary Habanabashaka *et al.* (2014) and Mahata *et al.* (2016) each gave tomato pulp (tomato pulp) as much as 9% and 12% of boiled tomato waste in the ration can reduce egg yolk cholesterol and increase egg yolk color. This is because the lycopene compounds in tomato cells are few or difficult to be absorbed by livestock. According to Cooperstone *et al.* (2015) lycopene compounds in tomato skin cells are generally still bound by the tomato skin matrix, and the structure is in a trans form that is difficult to absorb. In addition, lycopene is hydrophobic and lipophilic so that its absorption in the gastrointestinal tract is influenced by the presence of lipids (Colle *et al.*, 2012; Trujillo and McClements, 2016). Therefore, according to Handayani *et al.* (2018, 2019) the rejected tomatoes were first treated with heat, namely steaming at 98°C for 12 minutes and then adding 0.5% coconut oil. It aims to release lycopene from the tomato skin matrix, change its structure in the form of trans lycopene into the form of cis lycopene and increase the absorption of lycopene in the digestive tract in laying hens. So, in this research, we will evaluate the several levels of tomato waste after steaming to know their effect on cholesterol in laying hens.

The cholesterol synthesis occurs in the cytoplasm, while the main organs for cholesterol production are the liver and intestines (Kumari, 2018). Cholesterol is transported in the blood in the form of lipoproteins (Murray *et al.*, 2012). Therefore, it is important to know the lipid profile of the blood serum of laying hens given rejected tomatoes which aim to evaluate the concentration of blood lipoproteins

consisting of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides.

MATERIALS AND METHODS

This research was carried out in the February-March 2019 at the laying hens farm owned by farmers in Kampung Aro Village, Enam Lingkung sub-district, Padang Pariaman district, West Sumatera and Biotechnology Laboratory, Animal Science Faculty, Universitas Andalas, Padang.

ETHICAL APPROVAL

The animal experiments were appropriate with the guidelines 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.

BIRD, MANAGEMENT AND DIET

This research used 200 laying hens of the Lohman Brown strain (MB 402 from PT. Japfa) which were healthy, had no physical defects, weighed 1600-1800 g, and produced 82% at the age of 32 weeks at the beginning of the treatment. The feed ingredients used to prepare the experimental rations were PTW (Handayani, *et al.*, 2019), milled corn, rice bran, commercial concentrate HK 338 from PT. New Hope Indonesia, dolomite flour from CV. Usali Indonesia, sindomix-binder (premix) from PT. Otasindo Indonesia, generic simvastatin from PT. Kimia farma, Indonesia. The diet were composed of iso-protein and iso-energy as much as 16% protein and 2600 kcal/kg. The composition of the ration used can be seen in Table 1. The diet were given twice a day (06.30 and 12.00 WIB) and water was given *ad libitum*.

Chicken blood serum samples were taken at the end of the study. Prior to blood sampling, chickens were fasted for 24 hours. Blood was taken from the pectoralis vein at the bottom of the chicken wing using a 3 ml syringe. Then it was put into a vacutainer tube and brought to the laboratory to be separated between blood serum and blood solids using a centrifuge at a speed of 2500 rpm and a temperature of 4°C for 15 minutes (modification of Moriel *et al.*, 2012). Analysis of blood serum cholesterol, triglyceride, HDL, and LDL levels using a kit manufactured by DiaSys and measured using a rayto-autoanalyzer photometer (RT-5010v₅₊).

EXPERIMENTAL DESIGN AND DATA ANALYSIS

This study used a completely randomized design (CRD) with five treatments giving different levels of PTW in the ration, each treatment was repeated four times, and each replicated consist of 10 birds. The treatments were Ration A= 0% PTW; Ration B= 0% PTW + 0.03% Simvastatin; Ration C= 6% PTW; Ration D= 12% PTW;

Table 1: Feed ingredients composition of ration (%), nutrients content (%), metabolism energy (kcal/kg), and lycopene (mg/kg) of ration.

Ingredient	Diet (%)				
	A	B	C	D	E
Concentrate HK 338	26.30	26.30	26.30	26.30	26.30
Corn	47.40	47.40	47.40	47.40	47.40
Rice bran	21.10	21.10	15.10	9.10	3.10
Sindomix	0.50	0.50	0.50	0.50	0.50
Dolomite	4.70	4.70	4.70	4.70	4.70
PTW	0.00	0.00	6.00	12.00	18.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutrients	Nutrient content (%), Metabolism energy (kcal/kg), and Lycopene (mg/kg)				
Crude protein	16.67	16.67	16.60	16.54	16.47
Crude fat	4.88	4.88	4.47	3.78	3.65
Crude fiber	5.04	5.04	4.80	4.55	4.31
Calcium	3.32	3.32	3.32	3.31	3.31
Avail. phosphorus	0.40	0.40	0.43	0.45	0.48
Methabolizable energy	2631	2631	2629	2627	2625
Methionine	0.37	0.37	0.37	0.37	0.36
Lysine	0.60	0.60	0.61	0.63	0.64
Lycopene	0.00	0.00	31.26	62.52	93.78

Ration E = 18% PTW. The data obtained were analyzed statistically using Analysis of Variance (ANOVA). If there is a significant treatment effect, different treatment were determined with DMRT test (Duncan's Multiple Range Test) (Steel and Torrie, 1995).

PARAMETERS EVALUATED

TOTAL SERUM CHOLESTEROL (MG/DL)

Cholesterol in blood serum was tested by photometric enzymatic test method Cholesterol Oxidase Phenol Amino Phenazone (CHOD-PAP) DiaSys Diagnostiq System (Anonim, 2014). A total of 10 µl of blood serum samples were put into the tube and 1000 µl of cholesterol reagent was added. The reagents used were derived from the cholesterol assay kit from DiaSys. The reagent solution consisted of good's buffer pH 6.7 50 mmol/L, phenol 5 mmol/L, 4-Aminoantipyrine 0.3 mmol/L, cholesterol esterase (CHE) > 200 U/L, cholesterol oxidase (CHO) > 50 U/L, and Peroxidase (POD) >3 U/L. As standard, 1000 µl of cholesterol reagent was used which was added to the 10 µl cholesterol standard kit from DiaSys. The mixed solution was incubated for 10 minutes. Then standard measurements were taken and followed by samples using a rayto-autoanalyzer photometer (RT-5010_{v5+}). The cholesterol level of the blood serum sample will be read on the monitor screen of the photometer rayto autoanalyzer (RT-5010_{v5+}).

BLOOD SERUM TRIGLYCERIDES (MG/DL)

Blood serum triglycerides of laying hens were carried

out using the Glycerol Phospo Para Amino Phenazone (GPO-PAP) enzymatic photometric test method DiaSys Diagnostiq System (Anonim, 2014). A total of 10 µl of serum sample was put into a test tube and added with 500 µl of reagent solution. The reagents used were from the triglycerides assay kit, DiaSys. The reagent consists of good's buffer pH 6.7 50 mmol/L, phenol 5 mmol/L, 4-Aminoantipyrine 0.3 mmol/L, cholesterol esterase (CHE) > 200 U/L, cholesterol oxidase (CHO) > 50 U/L, and Peroxidase (POD) >3 U/L. As standard, 1000 µl of reagent was also used which was added to the 10 µl standard triglyceride kit from DiaSys. The mixed solution was incubated for 10 minutes. Then standard measurements were taken and followed by samples using a photometer rayto autoanalyzer (RT-5010_{v5+}). The triglyceride level of the blood serum sample will be read on the monitor screen of the photometer rayto autoanalyzer (RT-5010_{v5+}).

BLOOD SERUM LDL (MG/DL)

Blood serum LDL of laying hens was measured by Cholesterol Oxidase Phenol Amino Phenazone (CHOD-PAP) enzymatic photometric test method DiaSys Diagnostiq System (Anonim, 2014). The LDL cholesterol test kit used was from DiaSys Ltd. Laying hens blood serum supernatant was obtained by mixing 100 µl of blood serum sample with 1,000 µl of LDL reagent solution placed in a centrifuge tube. After that, it was centrifuged for 10 minutes at a speed of 2500 rpm. The reagent contains heparin 100,000 U/L and Sodium citrate 64 mmol/L. Next, 100 µl of supernatant was added with 1,000 µl of

cholesterol reagent. Each sample was mixed properly and then incubated for 10 minutes. Then, it was measured with a photometer rayto autoanalyzer (RT-5010_{v5+}). The cholesterol level of the supernatant blood serum sample will be read on the monitor screen of the photometer rayto autoanalyzer (RT-5010_{v5+}). After that the blood serum LDL is calculated based on the following formula:

$$LDL = \text{Total cholesterol (Mg/dL)} - \text{cholesterol supernatant (Mg/dL)}$$

BLOOD SERUM HDL (MG/DL)

Blood serum HDL of laying hens was measured by Cholesterol Oxidase Phenol Amino Phenazone (CHOD-PAP) enzymatic photometric test method DiaSys Diagnostiq System (Anonim, 2014). The HDL cholesterol test kit used was from DiaSys Ltd. A total of 250 µl of blood serum samples were put into the tube and added 500 µl of HDL reagent solution and then centrifuged for 10 minutes at a speed of 2,500 rpm. The reagent contains phosphoric acid 1.4 mmol/L and magnesium chloride 8.6 mmol/L. The results of the centrifuge (supernatant) were taken 10 µl, then added 1,000 µl of cholesterol reagent then homogenized, and incubated for 10 minutes. Furthermore, it was measured with a photometer rayto autoanalyzer (RT-5010_{v5+}). The HDL level of the blood serum sample will be read on the monitor screen of the photometer rayto autoanalyzer(RT-5010_{v5+}).

RESULTS AND DISCUSSION

TOTAL BLOOD SERUM CHOLESTEROL

Based on analysis of variance, treatment with PTW and simvastatin in laying hens rations had a very significant effect ($P < 0.01$) on blood serum cholesterol content. The results of the follow-up test with DMRT showed that the cholesterol content of the 0% PTW treatment (control) was not significantly different ($P > 0.05$) with the treatment with 6% PTW and 12% PTW, but the treatment with 6% PTW was not significantly different ($P > 0.05$) with 12% PTW treatment, while the cholesterol content of 0% PTW (control) and 6% PTW was significantly higher ($P < 0.05$) with 0.03% simvastatin supplementation and 18% PTW,

while the 18% PTW treatment was not significantly different ($P > 0.05$) treated with 12% PTW and 0.03% simvastatin supplementation (Table 2).

In this study, there was a decrease in blood serum cholesterol from laying hens from 119.20 Mg/dL to 108.83 Mg/dL along with the increase in PTW levels in the ration which was repeated 4 times in different chickens, and to 101.55 Mg/dL with simvastatin 0.03%. Increasing the level of PTW in the ration, the lycopene content also increases, and blood serum cholesterol in laying hens decreases. Lycopene is a carotenoid compound that has the ability to lower cholesterol (Rao et al., 2006; Palozza et al., 2012). The mechanism of cholesterol-lowering by lycopene through three pathways, namely the first pathway inhibits the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzym A reductase (HMG-CoA) in the synthesis of mevalonate compounds from HMG-CoA compounds (Palozza et al., 2012; Alvi et al., 2017). Alvi et al. (2016) explained that lycopene inhibits the HMG-CoA reductase enzyme in a reversible competitive manner, namely by occupying almost half of the active site of the enzyme so that the number of enzyme-substrate complexes is reduced. The second pathway is to increase the activity of the Low Density Lipoprotein (LDL) receptor at the cellular level, resulting in an increase in the breakdown/disposal of LDL in the blood serum (Palozza et al., 2012; Alvi et al., 2017). The inhibited activity of the HMG-CoA reductase enzyme will increase LDL receptors on the surface of liver cells thereby increasing LDL degradation to meet cell needs in the form of cholesterol (Fuhramn et al., 1997). The third pathway, inhibits the activity of the enzyme acyl-coenzyme A cholesterol acyltransferase (ACAT) in the formation of cholesterol esters in the liver and other tissues (Elkin et al., 1999; Palozza et al., 2012). Cholesterol is absorbed in the small intestine in the form of cholesterol which has been esterified into cholesterol esters by ACAT (Feingold and Grunfeld, 2018). In addition, lycopene is also known to increase the work of the alpha-7 Hydroxaz enzyme which can convert cholesterol into bile salts, therefore cholesterol in the blood will decrease (Murray et al., 2003).

Table 2: Effect of treatments on total blood serum cholesterol (Mg/dL), triglycerides (Mg/dL), LDL (Mg/dL), and HDL (Mg/dL) layer hens.

Treatments (%)	Blood serum cholesterol (Mg/dL)	Blood serum triglycerides (Mg/dL)	Blood serum LDL (Mg/dL)	Blood serum HDL (Mg/dL)
0 PTW	119.20 ^a	693.08 ^a	40.28 ^a	20.33
0.03 Simvastatin	101.55 ^c	595.85 ^b	16.27 ^c	18.53
6 PTW	118.60 ^a	691.08 ^a	38.00 ^a	19.98
12 PTW	111.58 ^{ab}	615.98 ^b	31.05 ^b	18.80
18 PTW	108.83 ^{bc}	597.95 ^b	27.38 ^b	19.09
SE	2.99	24.36	1.72	0.44

^{a,b,c} Different superscripts in the same column showed significantly different ($P < 0.05$). SE, Standard Error; PTW, processed tomatoes.

In this study, it was also known that 0.03% simvastatin supplementation in the diet significantly reduced blood serum cholesterol compared to the control treatment and the administration of PTW (6 and 12%) in the ration, but its activity was still equivalent to the administration of 18% PTW. This is because simvastatin is a type of statin drug, namely 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor which works as an inhibitor of the hydrolysis activity of the HMG-COA reductase enzyme whose mechanism of action is the same as lycopene (Kumari, 2018). A decrease in total cholesterol in the blood of laying hens after being given simvastatin at a dose of 0.03% in the ration has also been reported by Kim et al. (2004). In the previous year Elkin et al. (1999) reported that administration of simvastatin at a dose of 0.03% significantly reduced the cholesterol ester content in the blood plasma of laying hens.

In this study it was found that the dose of lycopene PTW as much as 62.52 mg/kg or the administration of PTW as much as 12% in the ration had shown its activity in lowering blood serum cholesterol of laying hens, while the dose of 31.26 mg/kg had not been able to reduce blood serum cholesterol of laying hens. This result differs with Sun et al. (2014a) that lycopene supplementation was 10.45; 26.56; and 35.96 mg/kg in the ration that given for 35 days, the three doses were able to reduce blood serum cholesterol of laying hens. However, An et al. (2018) reported that commercial lycopene supplementation up to 20 mg/kg in laying hens rations has not been able to reduce blood serum cholesterol. This is presumably due to differences in the digestibility of commercial lycopene and lycopene derived from PTW. Handayani et al. (2019) it was found that the retention of lycopene in tomato waste with adding coconut oil was only around 61.74%. This study showed the same results with the report of Mahata et al. (2016) that the administration of 12% tomato flour which had been processed by boiling for 8 minutes could reduce blood serum cholesterol of laying hens. The level of administration of tomato products in its effect on lowering blood serum cholesterol in this study was lower than the report of Rahmatnejad et al. (2009), that blood cholesterol of laying hens decreased after administration of tomato pulp (tomato pomace) as much as 24% in the ration. Nobakht and Safamehr (2007) reported that giving 10% tomato pulp (tomato pomace) to laying hens did not reduce blood serum cholesterol. The same was reported by Safamehr et al. (2011) and Salajegheh et al. (2012) 12 and 19% tomato pulp (tomato pomace) added with multi-enzymes in laying hens rations also had no effect on decreasing blood serum cholesterol.

BLOOD SERUM TRIGLYCERIDES

The results of the analysis of variance, the administration PTW and 0.03% simvastatin supplementation had

a very significant effect ($P < 0.01$) on the blood serum triglyceride content of laying hens (Table 2). After testing of DMRT, it was found that the 0% treatment (control) was not significantly different ($P > 0.05$) with the 6% PTW treatment. The triglyceride content of 0% (control) and 6% PTW treatment was significantly higher ($P < 0.05$) with 12 and 18% PTW treatment, and 0.03% simvastatin supplementation treatment. In the 12 and 18% PTW treatments were not significantly different ($P > 0.05$) with 0.03% simvastatin supplementation. The higher level of PTW administration will increase the lycopene content in the ration, and result in a decrease in the triglyceride content in the blood serum of laying hens.

The blood serum triglyceride content decreased significantly after administration of 12% and 18% PTW containing lycopene as much as 62.52 mg/kg and 93.76 mg/kg respectively in the ration, but not at the lower dose of 6% PTW (contains lycopene 31.26 mg/kg in the ration). Sahin et al. (2006) that the provision of lycopene from 50 to 200 ppm in the ration can reduce triglycerides of quail egg yolks. In contrast to that reported by Sun et al. (2014b) the administration of lycopene in the ration from 10.45 to 35.96 mg/kg had no effect on the blood serum triglyceride content of laying hens. In addition, simvastatin supplementation of 0.03% also significantly reduced blood serum triglycerides in laying hens in this study. Similar results were also reported by Kim et al. (2004) that blood plasma triglyceride levels in laying hens decreased after being given 0.03% simvastatin supplementation in their rations.

The decrease in serum triglycerides is due to the ability of lycopene or simvastatin to inhibit the process of triglyceride synthesis in the liver. This is explained by Agarwal and Rao (2000), Ševčíková et al. (2008), Hsu et al. (2015) that lycopene works by inhibiting the synthesis of triglycerides and cholesterol in the liver tissue, and causes a significant decrease in triglycerides in the blood.

BLOOD SERUM LDL

The results of the analysis of variance showed that the administration of PTW had a very significant effect ($P < 0.01$) on the LDL content of laying hens blood serum. After testing DMRT showed that the LDL content of laying hens blood serum at 0% treatment (control) was not significantly different ($P > 0.05$) with 6% PTW treatment. The LDL content of blood serum treatment with 0% (control) and 6% PTW was significantly higher ($P < 0.05$) with 12 and 18% PTW treatment, and 0.03% simvastatin supplementation treatment. The 12% PTW treatment was not significantly different ($P > 0.05$) with the 18% PTW treatment, but the LDL content in the 0.03% simvastatin supplementation treatment was significantly lower ($P < 0.05$) with the 12 and 18% PTW treatment.

This condition shows that increasing levels of PTW use in the ration will reduce LDL content. However, simvastatin 0.03% showed a much greater ability to reduce the LDL content of the blood serum of laying hens compared to all levels of PTW in the ration.

This study showed that the content of lycopene derived from PTW can affect the LDL blood serum of laying hens. Lycopene PTW as much as 62.52 mg/kg in the ration in the treatment of giving 12% PTW was able to significantly reduce the LDL blood serum of laying hens. The ability of lycopene in lowering LDL has also been reported by several previous researchers such as giving lycopene as much as 12 mg/kg (Hu et al., 2008), 42.6 to 127.8 mg/kg (Verghese et al., 2008), 5 mg/kg (Lorenz et al., 2012), and 10 mg/kg (Mulikalwar et al., 2012) in rabbits significantly reduced their serum LDL content. Basuny et al. (2009) also reported that administration of 100 to 800 ppm tomato lycopene or 200 commercial lycopene in rat diets can significantly reduce LDL in rat blood serum. In contrast to the report of Hsu et al. (2015) that the administration of commercial lycopene up to 18 mg/kg in the ration has not been able to reduce blood serum LDL in laying quail. Salajegheh et al. (2012) also reported that the addition of tomato pulp up to 19% did not affect the LDL level of the blood serum of laying hens. Furthermore, Mahata et al. (2016) reported that the administration of boiled tomato powder up to 12% did not affect the LDL level of the blood serum of laying hens.

The decrease in LDL in the blood serum of laying hens in this study was an indicator of a decrease in blood serum cholesterol levels. This is because LDL is the main cholesterol carrier, which is as much as two-thirds of the cholesterol (Karney et al., 2017) or about 67% of the cholesterol (Venugopal and Jialal, 2019) circulating in blood serum is in LDL. The decrease in the LDL content of laying hens blood serum after being given PTW and simvastatin in this study was caused by the inhibition of cholesterol synthesis which resulted in increased LDL receptor activity on the liver cells, thereby increasing the degradation of LDL in blood serum (Palozza et al., 2012; Alvi et al., 2017).

BLOOD SERUM HDL

Analysis of variance showed that treatment with PTW and 0.03% simvastatin supplementation had no significant effect ($P > 0.05$) on blood serum HDL content (Table 2). This shows that the administration of PTW maintains the HDL of the blood serum of laying hens equivalent to the ration that is not given PTW (0%). The same result can also be seen from the effect of simvastatin 0.03% which can maintain HDL blood serum of laying hens. This study showed that the levels of HDL in the blood of laying hens were maintained even though the levels of cholesterol,

LDL, and triglycerides in the blood serum decreased. Similar results were also reported by Jiang et al. (2015) that lycopene supplementation in lambs up to 200 mg/kg ration can reduce blood plasma cholesterol, triglycerides, and LDL but has no effect on blood plasma HDL content.

Table 2 shows the mean HDL of blood serum of laying hens given PTW in the ration ranging from 18.53 to 20.33 Mg/dL. The HDL level in this study was lower than the report from Mahata et al. (2016), namely the HDL content in laying hens fed with boiled tomato powder to a level of 12% ranging from 37.75-48.67 Mg/dL. Blood HDL levels of laying hens supplemented with vitamin B12 and bile salts in the ration were 47.17-51.63 Mg/dL (El-Katcha et al., 2019). Basmacioglu and Ergul (2005) also stated that normal HDL levels in laying hens were >22 Mg/dL. However, the blood serum HDL of laying hens in this study was greater than the results of An et al. (2018) study, ranging from 5 Mg/dL to 7.1 Mg/dL in laying hens treated with lycopene or tomato pulp. The same is true for the report of An et al. (2018) that HDL levels of laying hens fed pure lycopene ranged from 5.6 to 7.1 Mg/dL.

CONCLUSION

Adding PTW up to a level of 18% in the ration of laying hens can reducing total cholesterol in blood serum with a percentage decrease of 8.69% (119.20 to 108.83 Mg/dL), triglycerides with a percentage decrease of 13, 73% (693.08 to 597.95 Mg/dL), LDL with a decreased percentage of 32.03% (40.28 to 27.38 Mg/dL), and maintaining HDL (19.09 Mg/dL).

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NOVELTY STATEMENT

The novelty of this case report is to provide new information about the appropriate percentage of tomato waste in the ration for farmers or researchers interested in using tomato waste powder to lower laying hen cholesterol.

AUTHOR'S CONTRIBUTION

All authors conceived and designed the study. UFH, W, YR, IS, and MEM conducted the experiments, analyzed the data and wrote the paper. All Author contribute to

manuscript revisions. All authors approved the final version of the manuscript and agree to be held accountable for content there in.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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