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Intestinal microflora, body fat profiles and performance of male Tegal duck fed diet supplemented with red tomato extract

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ABSTRACT

The study was conducted to evaluate the effect of dietary supplementation of red tomato extract (RTE) on intestinal microflora, fat digestibility, body fat (meat fat mass, abdominal fat), and performances of male tegal duck. One hundred birds of male tegal ducks of 3 weeks old (initial body weight was 507.74±31.86 g) were randomly divided into 4 treatments with 5 replications (5 birds each) in a completely randomized design. Dietary treatments were basal diet without RTE as a control (T0), basal diet added with 0.10% RTE (T1), basal diet added with 0.15% RTE (T2) and basal diet added with 0.20% RTE (T3). Parameters measured were lactic acid bacteria (LAB), coliform, intestinal pH, fat digestibility, meat fat mass, abdominal fat, blood concentrations of malondialdehyde (MDA) and superoxide dismutase (SOD), body weight gain (BWG), carcass weight, feed consumption, and feed conversion ratio (FCR). Data were subjected to analysis of variance and continued to Duncan multiple range test when the treatment indicated significant ($p<0.05$) effect. Supplementation of RTE at higher level significanly ($p<0.05$) increased lactic acid bacteria (LAB), concentration of superoxide dismutase (SOD), carcass weight and body weight gain (BWG), while fat digestibility, meat fat mass, abdominal fat, malondialdehyde (MDA), intestinal pH, coliform counts and feed conversion ratio decreased ($p<0.05$), as compared to control. Feed consumption tended to decrease but not significant ($p>0.05$). In conclusion, feeding red tomato extract at a level of 0.20% (T3) can be categorized as effective in improving intestinal bacterial balance, body fat, increasing superoxide dismutase (SOD), an antioxidant enzyme, and performances of male tegal duck.

Keywords: Antioxidant enzyme, Body fat, Intestinal bacteria, Male duck, Tomato extract

INTRODUCTION

Tegal duck is a local Indonesian water fowl characterized with black color of beak and legs. Local female ducks are generally reared for egg production, but male ducks can be utilized as meat producers. Directorate General of Livestock and Animal Health (2022) reported the amount of duck meat consumption available only in 2017 was 0.052 kg/capita/year, but data for the following year was not listed. Consumption of duck meat is predicted to increase along with population growth. The demand of duck meat must be balanced with the increase in production. For this purpose, many efforts have been made to maintain animal health, starting from creating a

comfortable maintenance environment, vaccination and feeding additives. Additive that has been commonly used for a long time is antibiotic growth promoter (AGP) that function as growth stimulants to maintain animal health with the main purpose of inhibiting the development of pathogenic bacteria. However, dietary addition of AGP for animal has been banned by the government through the Regulation of the Minister of Agriculture Number 14/2017 starting on January 2018. The use of AGP had a negative side effect, such as causing bacterial resistance and gaining residues in poultry products that were possibly unhealthy for consumers (Teillant *et al*., 2015). The ban on the use of AGP causes the need to look for health-friendly alternative ingredients for either animal or consumers, and in hopes improving productivity.

Natural alternative ingredient as a source of active compound in this study was used red tomato. Red tomatoes are widely cultivated in Indonesia, rich in antioxidants contents that function as free radical scavengers. The active compounds of tomatoes are vitamin C, lycopene, β-carotene and flavonoids (Wala *et al*., 2022), and the lycopene content of fresh red tomatoes reaches up to 811.9 µg/g (Alenazi *et al*., 2020). The lycopene compound in red tomato is very potent antioxidant that can maintain animal health and increase productivity.

The immune system is important to be supported by the health of the digestive tract, especially the intestine, which organ is the center of nutritional absorption. Digestive tract health can be indicated by the improved balance of intestinal bacteria, namely, population of lactic acid bacteria is higher than that of pathogen. Lactic acid bacteria is gram-positive, resistant to acidic conditions, developed well under anaerobic conditions and is able to convert simple carbohydrates into lactic acid as the main fermentation product (Bintsis, 2018). This type of bacteria is beneficial because they nourish the digestive tract through the present of butyric acid one of the fermentation product called short chain fatty acid (SCFA) besides lactic acid. The diverse of intestinal microflora certainly contains harmful and pathogenic bacterial species. Ashayerizadeh *et al*. (2018) reported that high number of coliform can inhibit Lactic Acid Bacteria (LAB) activity. Lactic acid bacteria can ferment carbohydrates into short chain fatty acids (Feng *et al*. 2019), that cause the decrease in intestinal pH. The acidic atmosphere is not comfortable for the development of coliforms, but beneficial for LAB growth.

Previous research indicated that superoxide dismutase (SOD) is able to control pathogenic bacterial infections in the intestines of broiler chickens (Chinton-Uta *et al*. 2015). Therefore, antioxidants are needed to prevent free radicals from damaging cells and tissues. The SOD is the antioxidant enzyme that plays an important role in counteracting free radicals. Antioxidant enzyme called superoxide dismutase is the first line of defense for detoxification as well as the most powerful antioxidant in cells (Ighodaro and Akinloye, 2018). Other chemical substance known as malondialdehyde (MDA) is a sign of oxidative stress of the tissue called lipid peroxidation (Tsikas, 2017). Lipid peroxidation is the most easily observable result of free radicals. The MDA accumulation is an indicator of cell and tissue damage (Morales and Munné-Bosch, 2019). Experimental results of He *et al*. (2019) showed that ducks experiencing heat stress had high MDA levels. Therefore, high SOD and low MDA concentrations are the indications of good immune system that have positive impact on the quality of meat and productivity of ducks

In relation to meat quality, consumers mostly preferable to consume duck meat with low fat because when high fat content it will be fishy odor. The active substance lycopene can improve the growth of *Lactobacillus plantarum* bacteria (Zhang *et al*., 2022). Lactic acid bacteria in general, can produce bile salt hydrolase (BSH), an enzyme that can suppress the digestibility and absorption of fat. This enzyme can suppress fat digestibility and absorbability is due to its inhibitory effect on lipase activity (Gil-Rodríguez and Beresford, 2021), and because of its ability to deconjugate bile salts that cause the fat is unable to be emulsified and absorbed (Burhan *et al*.,

2017). The unabsorbed fat may be excreted and bring about the reduce in body fat, including low meat fat. The beneficial effect of lycopene for poultry can be known from the previous study that quail given lycopene powder at the level of 50 mg/kg result in higher carcass weight and body weight gain compared to the control group (Al-Jrrah and Abbas, 2020). Research conducted in broiler chickens showed that the provision of feed containing lycopene reduces feed conversion ratio (FCR) (Sarker *et al*., 2021; Mezbani *et al*., 2019).

Therefore, it is necessary to evaluate the feeding effect of red tomato extract as lycopene source on health status and productivity improvement of male tegal duck. Evaluation of the effect of red tomato extract supplementation was based on intestinal bacteria, body fat profiles, enzyme antioxidant activity, and performance of male tegal duck.

MATERIALS AND METHODS

Animals, Diets and Experimental Design

The reaserch was conducted for 42 days (6 weeks) using 100 birds of 3-week old male tegal ducks with an initial body weight of 507.74±31.86 g. The ducks were reared in the colony cage with 100 cm in size for either length, width or hight. There were 20 units of colony cage, and it was equipped with lightning lamp, termohygrometer, feed container and drinking water bowl. Red tomato extract (RTE) derived from fresh red tomatoes, and using the N -hexane, 95% methanol and distilled water for the preparation process. The experimental diet was composed of maize, rice bran, soybean meal, meat bone meal, $CaCO_3$, mineral and vitamin premix (Table 1). The ducks were randomly divided into 4 dietery treatments and 5 replications (5 birds each) in a completely randomized design arrangement.The RTE supplementation at the level of 0% (control), 0.10% (T1), 0.15% (T2) and 0.20% (T3) were created as treatments. The RTE, according to treatment level, was mixed with a small amount of diet, and given in the morning until it was completely consumed prior to feeding diet without supplementation for daily need.

Preparation of Red Tomato Extract

Fresh red tomatoes are thinly sliced and dried at room temperature for 7 days, after drying they were then mashed and sifted to obtain powder. Red tomato powder were soaked in N-hexane with a ratio of 1:3 (w/v). The soaked material was sonificated using a sonificator equipment for 30 minutes, then it was washed using distilled water with a ratio of 3:1 (v/v). The solution was stirred to separate precipitate from the filtrate. The precipitate was taken and then methanol was added with a ratio of 1:2 (v/v) to produce extract. The methanol solution was evaporated at the Diponegoro University Integrated Laboratory to obtain paste form, and then it was stored in the refrigerator until further use.

Microbial Population

Microbial counts in the intestine were determined with slight modifications of the method described by Shang *et al*. (2016). One duck from each replication (5 ducks from each treatment group) was selected for slaughter at 63 days of age to measure the intestinal bacteria. The contents of the small intestine were collected by gentle pressing and placed in sterile plastic bottles, which were immediately refrigerated until further analysis. Coliform counts were determined as red colonies in MacConkey agar (Merck KGaA) media after 24 hours of aerobic incubation at 38°C. The LAB were counted in media of de Man Rogosa and Sharpe (MRS; Merck KGaA) agar after 48 hours of anaerobic incubation at 38°C. The counts of microbial populations were expressed in colony-forming units (log cfu/g).

Body Fat

Fat digestibility was measured according to total collection method combined with indicator of Fe_2O_3 in 10-week-old ducks (4 days before the end of the study) using 20 samples with one bird was a representative of each replication. The feed treatment was mixed with Fe_2O_3 indicator at a concentration of 0.5%, and given during 4 days.

Table 1. Composition and nutrition content of basal ration

Ingredient	Composition (%)
Maize	55,11
Brand	19,04
Soybean meal	16,85
Meat Bone Meal	8,00
$CaCO_3$	0,30
Mineral and vitamin premix*	0,40
Lysine	0,10
Methionine	0,20
Total	100
Nutrient content**	
Metabolizable Energy (kcal/kg)***	3047,45
Crude Protein (%)	19,45
Crude Fiber (%)	6,54
Ether Extract (%)	5,24
Calcium (%)	1,18
Phosphor (%)	0,66

*Content per kg: calcium 32.5%, phosphor 1%, iron 6 g, mangan 4 g, iodine 0.075 g, copper 0.3 g, zinc 3.75 g, vitamin B12 0.5 mg, vitamin D3 50,000 IU
**Based on result analyse.
*** Based on the calculation formula Bolton (1967).

When the first red-colored excreta appeared it was collected using a special tray placed at the bottom of the cage. Excreta were collected every day and periodically sprayed with 0.2N HCL with an interval of approximately 2 hours. The collected excreta were then dried, ground, and taken to the laboratory for fat analysis using the Soxhlet method. The crude fat digestibility was calculated based on Krismiyanto *et al*. (2020) formula as follows:

Fat digestibility (%) = (fat intake – amount of fat in excreta)/fat intake X 100%

The meat fat mass was determined from the breast, back, and leg meat samples (both upper and lower parts). The meat was separated from the bones, mixed, ground, and then sampled for fat content analysis. The fat content was analyzed using the Soxhlet method, and then the meat fat mass was calculated same as to meat protein mass measurement according to formula of Suthama (2003) as follows:

Meat fat mass (g) = meat fat content (%) X meat weight (g)

The ducks were slaughtered and cleaned from feathers, and guts were removed, head and claws were separated, then weighed to determine the carcass weight. Abdominal fat was taken from the abdominal and visceral areas and weighed using a digital scale with an accurancy of 0.01 g. The percentage of abdominal fat was calculated using the formula of Krismiyanto *et al*. (2020) as follows:

Percentage of abdominal fat (%) = fat weight (g)/body weight (g) X 100%

Blood Serum

Three milliliters of blood were drawn from the jugular vein using a syringe before slaughtering. The collected blood samples were then stored in a vacum tube containing anticoagulant of ethylene diamine tetraacetic acid tripotassium (EDTA K3). The EDTA K3-containing tube was temporarily stored in an ice box, and the blood samples were then centrifuged at a speed of 3000 rpm for 5 minutes. The blood serum was used to

measure the concentrations of MDA and SOD, according to Yuanita *et al*. (2019). The MDA level was determined using thiobarbituric acid (TBA) reagent, and the measurement of SOD concentration was performed using a UV-Vis spectrophotometer at 505 nm with Ransod Superoxide Dismutase Manual Rx Monza reagent.

Performances

Performance data that measured including body weight gain, feed consumption, feed conversion ratio, and carcass weight. Body weight was weighed weekly using a digital scale. Carcass weight was weighed at the end of the study using a digital scale after the ducks were slaughtered and cleaned from non-carcass parts. Feed consumption was measured every day by subtracting the previous day's remaining feed from the given feed. Feed conversion was calculated by dividing the feed consumption by the body weight gain.

Statistical Analysis

Data were statistically analyzed using SPSS version 25 in a one-way arrangement of completely randomized design by considering a significance level of $p<0.05$. Difference values among treatments were determined by Duncan test at a probability level of 5%, when the treatment indicated significant effect.

RESULTS AND DISCUSSION

Intestinal Microflora

Dietary supplementation of RTE indicated significant effect ($p<0.05$) on the intestinal bacteria and potential hydrogen (pH) (Table 2). Diet added with RTE at a level of 0.20% (T3) significantly increased population of LAB and decreased coliform counts. Intestinal bacteria in T3 treatment (0.20% ETM) did not differ from that in T2 (0.15% ETM) treatment, but it showed better balance as compared to either T0 (without RTE) or T1 (0.10% RTE). The lowest intestinal pH was due to feeding effect of 0.20% RTE (T3) as compared to other treatments.

Dietary supplementation of RTE, either at the level of 0.15% (T2) or 0.20% (T3), increased intestinal population of LAB and a reduction in the number of coliform bacteria in tegal duck (Table 2). Lycopene derived from RTE serve as an antioxidant that can promote the growth of beneficial bacteria as evidenced by the increased total LAB observed in both treatments, T2 and T3. Antioxidant function of lycopene is known to be potentially have a positive impact on LAB viability during its growing phase (Jain *et al.,* 2020; Li *et al.,* 2016). The LAB was higher and, conversely, the number of coliforms was lower in T2 and T3 compared to either none of supplementation (T0) or low addition level of RTE (T1). The fermentation ability of LAB can produce short-chain fatty acids (Feng *et al.,* 2018), and thus, resulted in a decrease in gut pH. The higher production of short-chain fatty acids was observed in T2 and T3, although it was not measured in the present study, but lower gut pH bring about the higher LAB population was the proof (Table 2). Short-chain fatty acids can be connected with the decrease in pH, creating an unfavorable environment for pathogen (coliform) but conducive for the growth of beneficial bacteria in the avian gut (Nabi *et al.*, 2020). The phenomenon of low intestinal pH conditions causes pathogen (coliform) is unable to grow, while LAB population is stimulated to increase.

Body Fat

Fat digestibility, abdominal fat, and meat fat mass were significantly decreased ($p<0.05$) by feeding dietary supplementation of RTE (Table 3). Fat digestibility and meat fat mass in T3 treatment were the lowest and different from those in other treatments (Table 3). Abdominal fat in T3 treatment numerically tended to indicate the lowest value but it did not differ from that in T2 and T3. However, that in the three treatments (T1, T2, and T3) was significantly lower compared to T0 (control).

The treatment with the addition of 0.2% RTE (T3) significantly reduced crude fat digestibility, fat meat mass and abdominal fat (Table 3). The higher level of lycopene brought about the lower fat digestibility and meat fat mass, alt-

hough abdominal fat was the same. These results were the indication of regulatory effect of lycopene function as antioxidant derived from RTE that can cause the improvement of intestinal bacterial balance, higher LAB and lower pathogen (coliform) populations. The reduced fat digestibility can be correlated with high population of LAB (Table 2), because this LAB is able to produce bile salt hydrolase (BSH) enzyme, although this enzyme did not measuared in the present study. Thus, it can be assumed that BSH enzyme via the separation of glycine and taurine from steroids resulting in deconjugated bile salts. The present result is supported by the report of Burhan *et al*. (2017) who stated that *Lactobasillus plantarum* IIA-1A5 is a lactic acid bacteria species that exhibits BSH activity by separating glycine from steroids, and resulting deconjugated bile salts. Similarly, a report of Hernández-Gómez *et al.* (2021) indicated that *Lactobacillus plantarum* DGIA1 is a potentially probiotic strain with its BSH enzyme exhibit an excellent deconjugation activity. The deconyugated bile salts bring about the inhibition of detary fat emulsification and reduce hydrolysis activity of lipase, so that dietary lipid can not be reabsorbed by the intestine.

A higher amount of BSH enzyme can deconjugate more bile salts, and causes dietary fat can not be emulsified and absorbed, and thus, allowing it to be excreted via excreta. This mechanism bring about the decrease in fat digestibility, and provide a positive impact on body fat content. The increased excretion of fat suggests a decrease in fat absorption, and lead to a decrease in the fat content of target tissue (meat and abdominal fat). However, fat digestibility and meat fat mass in T2 were higher than those in T3 can be connected with lower LAB population with weaker effect to deconjugate bile salts, and this gives a meaning that the mechanism was not as effective as in T3. Lycopene is a potential antioxidant in affecting fat metabolism, also in modulating immune response as indicated by the high SOD and low MDA concentrations (Table 2). The improvement of humoral immunity through the increased SOD and GSH-Px levels due to addition of lycopene was beneficial for broiler (Mezbani *et al*., 2019; Wang *et al.,* 2022), and for weaning pig (Meng *et al*., 2022). Therefore, RTE with lycopene content would be also effectively beneficial for ducks because it function as humoral also systemic immunity related to the regulation of fat metabolism.

High crude fat digestibility lead to a high deposition of fat in T0 and T1, but abdominal fat was the same in the three treatments (T1, T2, and T3) added with RTE (Table 3). This finding suggested that the low level of RTE (T1) is not been able to suppress fat metabolism as effectively as T3. The extreme condition was occurred in T0 due to none RTE supplementation, which was indicated by highest coliform and lowest LAB populations (Table 2), and resulted high body fat (Table 3). Research on broiler chickens has shown that dietary supplementation of 100 mg/kg of lycopene reduced the percentage of ab-

Table 2. Effect of Red Tomato Extract on Intestinal Microflora, Malondialdehyde and Superoxide Dismutase of Male Tegal Duck

Variables	Treatment			
	T0	T1	T2	T3
LAB (log cfu/g)	7,39±0,18^{c}	8,59±1,14^{b}	10,88±0,65^{a}	10,91±0,37^{a}
Coliform (log cfu/g)	3,43±0,40^{a}	2,76±0,48^{b}	2,29±0,03^{c}	2,12±0,16^{c}
pH value	6,26±0,05^{a}	6,14±0,06^{b}	6,08±0,07^{bc}	6,01±0,03^{c}
MDA (nmol/mL)	4,21±0,20^{a}	3,86±0,31^{ab}	3,70±0,38^{bc}	3,38±0,16^{c}
SOD (Unit/mL)	24,49±2,49^{b}	26,94±1,70^{ab}	28,57±2,04^{a}	30,20±3,06^{a}

Means in the same row with different superscripts differ significanly ($p<0,05$). T0=control; T1=basal diet added with 0,1% red tomato extract; T2= basal diet added with 0,15% red tomato extract; T3=basal diet added with 0,2% red tomato extract.

dominal fat (Man *et al*., 2021). The amount of fat deposited in the meat and abdomen was not significantly different. The physiological and nutritional metabolism phenomenon, particularly fat, did not occur in the same way in either T0 or T1 as those in T3, as previously described.

The low fat digestibility was closely related to the reduce fat absorption because of the limited ability of LAB to produce BSH enzyme, as it has been discussed previously. In addition to the factor described above, it was also due to the effect of lycopene in reducing *de novo* cholesterol formation. Lycopene inhibited cholesterol synthesis through the action of the enzyme called as 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase (Periago *et al*., 2016). The HMG-CoA reductase enzyme converts HMG-CoA to mevalonic acid, which is the initial precursor of cholesterol. Lycopene in this process function as catalysts that inhibit the substrate from reaching the active site. This mechanism is a process in preventing mevalonic acid formation, thereby reducing cholesterol synthesis.

Bile salts consist of cholic acid and chenodeoxycholic acid that are synthesized in the liver using cholesterol (Ridlon *et al*., 2016). The decrease in cholesterol synthesis can be interpreted as low bile salt formation, which affects the process of fat emulsification in the digestive process. This condition can reduce fat digestibility and further lowering fat deposition in the form of meat fat mass, especially when high level of RTE addition as a source of lycopene (Table 3). On the other hand, meat fat mass in the three treatments, T0, T1, and T2, was lower than that in T3 seem to be closely related to fat digestibility due to the weaker influence of lycopene or nothing, such as in T0 treatment. However, previous study reported that addition of tomato juice fermented with LAB of either *Lactobacillus plantarum* or *Lactobacillus casei* markedly delayed cholesterol oxidation (Liu *et al*., 2018). Thus, the effect of lycopene either in a state of intact or fermented product on fat metabolism in general, and cholesterol in particular, depend on the level of availability.

Malondialdehyde and Superoxide Dismutase

Dietary supplementation of RTE significantly ($p<0.05$) affect blood concentrations of malondialdehyde (MDA) and superoxide dismutase (SOD) (Table 2). Supplementation of RTE at a level of 0.20% (T3) significanly increased SOD blood concentration, and decreased MDA level. However, MDA level in T3 treatment did not differ from that in T2 (0.15% RTE) treatment. Blood SOD concentration due to suplementation of 0.30% RTE (T3) was not different when compared to that in T1 (0.10% RTE) and T2 (0.15%) treatments. Concentration of blood SOD in both treatments (T2 and T3) significantly higher than that in T0 group, however, there was no differences between T1 and T0 treatments.

Diet containing RTE at both level 0.15% (T2) and 0.20% (T3) increased SOD and decreased MDA blood concentrations of ducks. Antioxidant enzymes such as SOD act as defencing agent against free radicals, while MDA is an indicator of the level of tissue damage. Red tomatoes contain lycopene that can function as an antioxidant (Alenazi *et al*., 2020). Lycopene indicates a favorable function in maintaining or improving antioxidant systems, and can exert a potentially beneficial effect on poultry industry system. The increased antioxidant enzyme activities of SOD, and on the contrary, the decreased blood serum MDA concentration found in present study were consistence with the previous report (Surai, 2025). The SOD was reported to have a protective effect on heat stress or on other oxidative-stress related conditions in poultry production. Similar report indicated that lycopene maintains oxidative balance in birds through various ways including free radical scavenger, activating host antioxidant enzymes, such as SOD and GSH -Px (Arain *et al*., 2018). Research conducted by Wang *et al*. (2022) showed that lycopene addition at 30 mg/kg increase SOD and decrease MDA concentrations in the blood serum of 21-day-old broiler chickens. Similarly, peking ducks given 100 mg/kg of lycopene for 10 days reduce MDA and increase antioxidant enzyme catalase (El-Sheshtawy *et al*., 2021).

Table 3. Effect of Red Tomato Extract on Body Fat and Performances of Male Tegal Duck

Variables	Treatments			
	T0	T1	T2	T3
Fat Digestibility (%)	84,12±0,40[a]	83,94±0,40[a]	82,10±1,60[b]	79,83±1,32[c]
Meat Fat Mass (g)	25,64±3,18[a]	22,73±2,19[ab]	20,32±3,27[b]	15,19±3,51[c]
Abdominal Fat (%)	0,57±0,09[a]	0,48±0,05[b]	0,43±0,04[b]	0,39±0,03[b]
Body Weight Gain (g/bird)	968,20±31,30[b]	982,69±16,30[b]	1005,23±27,81[b]	1049,17±47,48[a]
Carcass Weight (g)	816,50±47,35[c]	906±22,77[b]	927±36,29[b]	1011,25±25,22[a]
Feed Convertion Ratio	4,99±0,23[a]	4,95±0,28[a]	4,76±0,310[ab]	4,51±0,23[b]
Feed consumption (g/d)	115,10±3,66	115,96±7,66	113,97±3,59	112,62±3,48

Means in the same row with different superscripts differ significanly (p<0,05). T0=control; T1=basal diet added with 0,1% red tomato extract; T2= basal diet added with 0,15% red tomato extract; T3=basal diet added with 0,2% red tomato extract.

The LAB population in T2 and T3 treatments (Table 2) can also be associated with the enhancing antioxidant enzymes activity. Lactic acid bacteria group, especially *Lactobacillus plantarum* and *Lactobacillus casei*, could ferment tomato juice to produce effective compound that have an important effect in disrupting the *E. coli* cell membrane, and, on the contrary, stimulate their growth its self (Liu *et al*., 2018). Similar previous report indicated that the LAB could prevent the *E. coli*-induced passage to across the intestinal wall (Mangeli *et al*., 2002). This mechanism causes more healthy gut due to intestinal barrier improvement, and suggests that it can be correlated with the increased blood SOD concentration as an indication of humoral immunity response. Therefore, the higher LAB population in T2 and T3 treatments could be interpreted as an effect on the increasing amount of lycopene supplementation. The higher level of RTE was given, the higher amount of lycopene intake, that bring about more free radicals can be captured, and leads to high SOD and low MDA levels (Table 2).

Performance

There was a significant effect of RTE supplementation on performance of male Tegal ducks, especially for body weight gain (BWG) and feed conversion ratio (FCR) (Table 3). Ducks fed diets supplemented with 0,20% RTE (T3) showed significantly highest (p<0.05) body weight gain (BWG) and carcass weight (CW) compared to other three treatments (T0, T1, and T2). Body weight gain was the same in T0, T1 and T2 treatments, but carcass weight between T1 and T2 did not different, and that in T0 was significantly (p<0.05) the lowest.

Supplementation of 0.2% RTE was able to produce better performances, indicated by higher body weight gain and carcass weight, and lower meat fat mass and feed conversion ratio with unchanged feed consumption (Table 3). The improvement of performance was attributed to the contribution of digestive tract health conditions due to better gut bacterial balance and immune status with high SOD and low MDA (Table 2). The increased LAB population and the decreased total coliform (Table 2) lead to a healthier digestive tract caused the increased nutrient digestibility, particularly protein, and this was supported by higher body resistance (high SOD and low MDA) that have an positive impact on weight gain. *In vivo* and *in vitro* studies indicated that lycopene is a powerful antioxidant among other carotenoid components. Supplementation of lycopene derived from tomato (*Solanum lycopersicum*) revealed numerous health promoting activities in poultry that related to antioxidant effect and immunomodulator (Arain *et al*., 2018). Therefore, supplementation of lycopene derived from RTE in the present study was beneficial for improvement in performances with better meat yield. Research conducted by Alagawani *et al*. (2022) showed that the addition of 6% dried tomato suppresses coli-

form growth and produces higher protein digestion compared to the control treatment in quails. The same experimental result with addition of 100 mg/kg of lycopene for 10 days increased total serum protein in Peking ducks (El-Sheshtawy *et al*., 2021). The more protein is absorbed, it should be the more protein can be deposited, which in turn improves weight gain in ducks.

The RTE feeding treatment affected FCR but not for feed consumption (Table 3). This can be assumed that feed or nutrient utilization for weight gain is better with the addition of RTE up to 0.2%, compared to the control group. Previous study on broiler chickens fed diet containing lycopene indicated the reduce in FCR (Sarker *et al*., 2021; Mezbani *et al*., 2019). The present study was supported by the previous result that Mojosari ducks fed diet with inclusion of tomato powder combined with sardine oil did not affect feed consumption (Andri *et al*., 2018), but it can increase the final body weight of Mallard ducks (Omar *et al*., 2019).

The addition of RTE either in T1 or T2 treatments was found to be less effective as compared to T3 in relation to carcass weight. The lower amount of lycopene derived from RTE in both treatments (T1 and T2) was assumed less substrate that can be fermented by endogenous LAB, in turn resulting limited availability of lycopene, which condition was unfavorable effect for final production. Carcass production in term of weight is a part of BWG, and since BWG statistically indicated similar pattern in T1 and T2 treatments, so it would be logic that carcass weight was also the same (Table 3). The availability of lycopene beside related to the real amount of addition also due to the result of LAB fermentation activity. The present results were supported by the report of Bartkiene *et al*. (2013) who stated that selected lactic acid bacteria (LAB) is able to ferment tomato juice produce greater amount of lycopene. However, red tomato extract (RTE) in both treatments (T1 and T2) did not undergone such process, so that the availability of lycopene did not support muscle protein deposition to produce higher carcass weight, unlike as in T3. As it was reported previously that tomato can be fermented by selected LAB to produce greater lycopene bioavailability (Bartkiene *et al*., 2013), which is potentially provided a beneficial impact on production performance.

CONCLUSION

Feeding red tomato extract at a level of 0.20% (T3) can be categorized as effective in improving intestinal bacterial balance, body fat, increasing superoxide dismutase (SOD), an antioxidant enzyme, and performances of male tegal duck.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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