

Addition of red tomato extract in feeds using protein sources and calcium microparticles on protein digestibility and meat production in broiler chickens

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ABSTRACT: The study aimed to assess the effect of adding red tomato extract in feeds using protein sources and calcium microparticles on protein digestibility, meat calcium mass, meat protein mass, and meat weight in broiler chickens. Two hundred day-old broiler chicks (Cobb-CP 707) unsexed aged 8 days with an average weight of 169.19 ± 5.67 g. Red tomato extract (RTE) as an antioxidant source. The study used a completely randomized design with 5 treatments and 4 replicates (each 10 birds). The treatments include T₀: control feed, T₁: T₀ + RTE 100 mg/kg, T₂: T₀ + RTE 200 mg/kg, T₃: T₀ + RTE 300 mg/kg, and T₄: T₀ + RTE 400 mg/kg. Parameters measured protein digestibility, meat calcium mass, meat protein mass and meat weight. Data were processed using analysis of variance and Duncan's test in the SPSS version 16 program. The addition of 100-400 mg/kg RTE in feeds using microparticle protein and calcium sources had a significant effect ($p < 0.05$) on protein digestibility, meat calcium mass, meat protein mass and meat weight. The conclusion is that the addition of red tomato extract at 400 mg/kg in feeds using microparticle protein and calcium sources increases protein digestibility, meat calcium mass, meat protein mass and meat weight in broiler chickens.

Keywords: Broiler, red tomato extract, protein and calcium of microparticle.

INTRODUCTION

Broiler chickens are crossbred birds that have high productivity, especially in meat production. The growth rate of broilers is fast, so the maintenance time is short with an average length of 35 days (Rini *et al.*, 2019). The success of raising broilers is influenced by 3 factors, namely genetics, nutrition and environment (Sari and Romadhon, 2017). Ration accounts for as much as 60-70% of the entire production. So, the quality of the ration is very important to maintain the stability of production efficiency in broiler chickens.

The rapid growth rate of broilers must be balanced by the provision of high ration protein levels and adequate

calcium (Ca) content. However, high protein rations are associated with high prices of protein sources such as fishmeal and soybean meal. The use of protein and Ca source materials modified into microparticles is a strategy to improve protein and Ca efficiency for optimum growth. Previous studies showed that protein digestibility and Ca retention increased in broilers fed fish meal and soybean meal treated with microparticles, even without additives (Suthama and Wibawa, 2016). Feeding using microparticle protein and calcium sources in this study is expected to be more efficient when combined with natural additives in the form of red tomato extract as a source of

antioxidants.

Red tomato (*Lycopersicon esculentum*) is a fruit that contains various vitamins, and active compounds, particularly lycopene which are very beneficial for health. Lycopene is an antioxidant that functions as an antidote to free radicals. The lycopene content in tomatoes varies because the older the colour, the higher the lycopene content. Lycopene in green tomatoes is 0.03 mg/g, in half-ripe tomatoes 0.52 mg/g and in red tomatoes as much as 48.30 mg/g (Sujana *et al.*, 2020). Lycopene in red tomatoes is acidic and can suppress the population of pathogenic bacteria by damaging cell walls and denaturing bacterial cell proteins (Dewi *et al.*, 2019).

Feeding rations using protein sources and Ca microparticles combined with red tomato extract as a source of lycopene is expected to have a synergistic effect on the healthy digestive tract. Better intestinal morphology based on villus length and crypt depth is an indication that the digestive tract is healthier in broilers fed with lycopene (Chen *et al.*, 2023). High protein digestibility followed by high Ca absorption is also due to the provision of rations using protein sources and Ca microparticles supported by the presence of lycopene from red tomato extract allows the formation of more adequate calcium binding protein (CaBP). The bound protein and Ca in the form of CaBP are transported to the target cell and deposited into the meat. Metabolism and absorption of the protein and Ca results in increased meat protein mass and meat Ca mass, respectively. Increased protein digestibility, meat protein mass, and meat Ca mass result in a better end product in the form of meat weight.

This study aims to assess the effect of red tomato extract in protein sources and Ca microparticle enriched diets on protein digestibility and broiler meat production.

MATERIALS AND METHODS

Livestock, materials and equipment

The livestock used in the study were 200 broiler chickens strain Cobb-CP 707 (unsexed) aged 8 days with an average body weight of 169.19 ± 5.67 g. The experimental diet consisted of a mixture of ground corn, rice bran, microparticle soybean meal, microparticle fish meal, microparticle eggshell meal, premix, lysine and methionine with an energy content of 3,034.62 kcal/kg and 18.62% protein (Table 1). Red tomato extract is the test ingredient as a source of additives in the form of lycopene. Materials for the preparation of microparticles include distilled water, fish meal, soybean meal, eggshell meal, virgin coconut oil (VCO) and filter paper. Materials used for the production of tomato extract include red tomatoes, N-Hexan, distilled water, methanol, aluminium foil and Whatman 41 filter paper. Equipment used for the preparation of microparticle feeds includes a Power Sonic 45 brand sonicator, 2 litre

Table 1. Composition and feed nutrient content.

Feed stuff	Composition
Yellow Corn	57.25
Rice Bran	12.52
Soybean Meal of Microparticle	20.38
Fish Meal of Microparticle	9.00
Eggshell Meal of Microparticle	0.30
Premix	0.25
Lysine	0.10
Methionine	0.20
Total	100.00
Nutrient content	
Metabolizable Energi (kcal/kg)**	3,013.88
Crude Protein (%)*	18.26
Crude Fat (%)*	4.27
Crude Fiber (%)*	5.50
Calcium (%)*	1.09
Phosphor (%)*	0.84

*Feed was analyzed for proximate and minerals in the laboratory of animal nutrition, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (2023). ** Based on the Bolton calculation formula (1967).

glass beaker, 500 ml measuring cup, stirring rod, 0.3 mm sieve and grinder with a sieve size of 100 mesh. The tools used to extract red tomatoes consist of a blender, aluminium tray, analytical balance with 1 g accuracy and 2-litre glass beaker.

Methods

One kilogram of red tomato fruit was weighed and washed, and insert pulverized using a blender without water, and oven dried at the provided temperature and time with unit and mashed. The dried tomato sample from the blender was weighed as much as 100 g and soaked in hexane solvent (N-Hexan) as much as 300 ml (the ratio of sample and hexane 1:3) for 72 hours while stirring twice, then the sample was filtered using filter paper. The preparation of tomato extract was carried out repeatedly with respect to the ratio of 1: 3 (w/v). The sample was filtered using Whatman 41 filter paper to take the filtrate. The filtrate obtained was then sonicated for 30 minutes. The filtrate was then washed using distilled water in a ratio of 3:1 v/v. The solution was homogenized using a stirrer for 10 minutes until two layers were formed, namely orange filtrate and distilled water. The collected filtrate was collected in a glass beaker and antisolvent (methanol) was added in a ratio of 1:2 v/v. In the next stage, the solution was evaporated. The evaporated red tomato extract was ready to be added to the ration according to the treatment.

The procedure used for the synthesis of protein microparticles refers to the method of Suthama and Wibawa (2018). Protein source feed ingredients, namely fish meal and soybean meal, were first pulverized using a grinder and then sieved with a size of 100 mesh. The fish meal was sieved using a 0.3 mm sieve. Fish meal and soybean meal were dissolved in distilled water in a ratio of 1:4 (b/v) and 2 ml of virgin coconut oil (VCO) was added for every 500 ml. The solution was sonified for 60 min using an ultrasound transducer at 40Hz and then dried in the sun. The procedure of making microparticle calcium source refers to the method of Ain *et al.* (2020), namely eggshells cleaned with water, then dried in the sun, then ground using a grinder and then sieved with a size of 100 mesh.

The study was arranged in a completely randomized design (CRD) with 5 treatments and 4 replicates, each consisting of 10 birds. The treatments were as follows: T₀: control feed, T₁: T₀ + RTE 100 mg/kg, T₂: T₀ + RTE 200 mg/kg, T₃: T₀ + RTE 300 mg/kg, and T₄: T₀ + RTE 400 mg/kg. The treatment feeds were given from 8 to 35 days of age.

The parameters measured were protein digestibility, meat Ca mass, meat protein mass and meat weight. Protein digestibility was carried out on 4 days at the end of rearing using the total collection method combined with indicators. On day 32, one chicken was taken as sampling for the digestibility test. Chicken is given treatment feed mixed with Fe₂O₃ indicator as much as 0.5% of feed consumption. The collection of excreta began when it appeared red which is the colour of the indicator. The collected excreta were sprayed using 0.1 N HCl every 2 hours to prevent nitrogen evaporation. Excreta were dried under the sun until dry (1-2 day), then mashed and protein content was analyzed to determine protein digestibility. Protein digestibility is calculated using the Wahju formula (1997) as follows:

$$PD (\%) = \frac{PI - (AExP - AEnP)}{PI} \times 100\%$$

Where: PD = Protein Digestibility, PI = Protein Intake, AExP = Amount of Excreta Protein, and AEnP = Amount of Endogenous Protein

Meat protein mass and calcium mass were measured using the same chicken to measure protein digestibility. One chicken was taken from each experimental unit to be slaughtered, and carcasses were separated between meat and bones. The meat was weighed using the indicated weight balance model and specification. Meat samples from each part of the carcass with the same weight were ground until homogeneous. The protein and calcium content of the homogenized meat mixture was analyzed. The mass of meat protein and calcium is known through calculations based on Suthama (2003) as follows:

Meat protein mass (g) = meat protein content (%) x meat weight (g)

Meat calcium mass (g) = meat calcium content (%) x meat weight (g)

Meat weight is obtained by weighing the meat on the carcass of broiler chickens after being separated from the bones.

Data were analyzed using analysis of variance (ANOVA) applying SPSS 16.0 statistical software. Duncan's multiple range tests examined the differences between the treatments' means. The significance level was p<0.05.

RESULTS AND DISCUSSION

Protein digestibility

Feeds with microparticle protein sources supplemented with red tomato extract (RTE) had a significant effect (p<0.05) on protein digestibility in broiler chickens (Table 2). The highest protein digestibility was observed on the treatment supplemented with 400 mg/kg ETM (T₄) and 300 mg/kg ETM (T₃). The increase in protein digestibility in T₃ and T₄ is related to the provision of rations using microparticle protein sources that can facilitate protein digestion and absorption. The results of this study are supported by previous reports that protein digestibility and some essential amino acids increased in broilers fed fish meal and microparticle soybean meal (Suthama and Wibawa, 2018). Similarly, protein digestibility and Ca retention increased in broilers fed complete diets using microparticle protein sources plus acidifier additives (Suthama *et al.*, 2021). Lycopene from ETM as an antioxidant has a positive effect on the health of the digestive tract, resulting in improved nutrient digestibility, especially protein (Table 2). Protein digestibility is related to improved digestive tract health due to better bacterial balance caused by the influence of lycopene from RTE. Comparative research on weaning pigs fed lycopene showed that not only did the balance between *Lactobacillus* sp. and *Escherichia coli* improve, but other bacteria also became better so that villi height increased and mucosal defense function strengthened (Meng *et al.*, 2022). The effect of lycopene to improve the condition of the digestive tract also occurs in poultry, especially broilers, associated with increased protein digestibility. Tomato extract as a source of natural antioxidants in the form of lycopene can increase the immune system humorally, and the small intestine systemically related to the development of beneficial bacteria. Previous research reported by Alagawany *et al.* (2022) showed that there was a link between a decrease in bacterial population and an increase in nutrient digestibility in quail fed pomace (tomato waste). Healthy small intestine conditions, as

Table 2. Protein digestibility, meat calcium mass, meat protein mass and meat weight in broiler.

Treatment	T0	T1	T2	T3	T4
Protein Digestibility (%)	78.77 ^c	83.34 ^b	84.09 ^b	86.33 ^{ab}	89.20 ^a
Meat Calcium Mass (mg)	26.35 ^d	30.49 ^c	36.01 ^b	39.27 ^b	44.46 ^a
Meat Protein Mass (g)	115.84 ^b	154.25 ^a	167.53 ^a	167.75 ^a	173.56 ^a
Meat Weight (g)	585.00 ^b	644.25 ^{ab}	689.25 ^a	692.75 ^a	712.75 ^a

Different superscripts on mean values in the same row indicate significant differences ($p < 0.05$).

discussed earlier, have a positive impact on the digestive process, resulting in increased nutrient digestibility, especially protein (Table 2). Jalalinasab *et al.* (2014) stated that the use of dried tomato waste "dried tomato pomace" can reduce the percentage of heterophils and increase lymphocytes, which indicates an increase in body immunity in laying hens. Lycopene has the potential to increase humoral body resistance characterized by higher activity of antioxidant enzymes such as SOD and GSH-Px in broilers (Mezban *et al.*, 2019; Wang *et al.*, 2022). Tomato extract with lycopene content, besides being assumed to be humoral in increasing endurance, is also systemic for the small intestine because it can modulate the development of better beneficial bacteria. Thus, the addition of RTE as a source of lycopene antioxidants strongly supports the effect of feeding feeds using microparticle protein sources in relation to increasing protein digestibility.

Treatments T₁ and T₂ revealed similar protein digestibility to T₃. The protein digestibility values in both treatments can be stated to be at a medium level because the amount of lycopene from ETM is low, so it is not as effective as T₄. Previous studies have shown that lycopene can improve the development and performance of the digestive tract in quail (Al-Jrrah *et al.*, 2020). This implies that when the digestive tract is unhealthy with low performance, nutrient digestion, including protein, is not maximized. The use of feeds with microparticle protein sources in T₁ and T₂ should be able to increase protein digestibility to a higher level, but due to low lycopene levels, it is not supportive and causes the resulting protein digestibility to be lower than T₃ and T₄ (Table 2).

However, this is contrary to the T₀ treatment which produced significantly the lowest protein digestibility (Table 2) because it was without RTE so the condition of the digestive tract is contrary to T₄. Lycopene as an antioxidant plays a role in improving the histopathology of the digestive tract and suppressing the negative effects of *Escherichia coli* (Fararh *et al.*, 2019). Antioxidant deficiency in poultry can cause a decrease in cell work affecting productivity due to the stress experienced (Candra, 2014). Unhealthy digestive tract conditions have an impact on the effectiveness of protein utilization from microparticle protein sources at T₀ which is not as effective as T₄. Thus, due to the absence of antioxidant supply from

outside, there was no decrease in pathogenic bacteria and the condition of the digestive tract did not support the process of nutrient digestion, especially protein. T₀ treatment because without additional RTE as a source of lycopene, the improvement of digestive tract conditions did not occur, resulting in low protein digestibility (Table 2).

Meat calcium mass

Feeds with protein sources and calcium microparticles supplemented with RTE had a significant effect ($p < 0.05$) over the control diet on meat calcium mass in broiler chickens (Table 2). Rations with protein sources and calcium microparticles plus ETM at 400 mg/kg (T₄) produced significantly the highest meat calcium mass. The feed uses protein and Ca sources made into microparticles to facilitate the process of digestion and absorption. The increased meat Ca mass in T₄ can be attributed to the lycopene antioxidant effect of RTE in improving the health of the digestive tract, so as to increase protein digestibility (Table 2) and Ca retention. The availability of both nutrients, in the form of protein and high Ca retention, allows more calcium binding protein (CaBP) to be formed. Increased calcium availability that binds protein in the form of CaBP can increase meat calcium mass (Syafitri *et al.*, 2015). High amounts of CaBP make transportable Ca more available to target tissues, including meat. Calcium binding protein is transported to target tissues such as bone and meat (Maharani *et al.*, 2013). Calcium enters the blood circulation in the form of CaBP through transecural absorption in the duodenum (Pu *et al.*, 2016). Meat Ca mass is also influenced by the age of livestock that are not in the growth period because Ca requirements for bones are reduced so that Ca deposition in meat increases. The use of feeds with protein sources and Ca microparticles can be maximized by the positive effects of lycopene additives from RTE.

Treatments T₂ and T₃ statistically produced the same meat Ca mass, and significantly lower than T₄ (Table 2). This result can be assumed that the action of lycopene was not as effective as in T₄. The rations used were similar in protein and Ca microparticle sources, the contribution of lycopene from ETM can be concluded to be lower than that of T₄, making it less supportive of the digestive tract, which

was characterized by low protein digestibility (Table 2). Intestinal morphology, including villus length and crypt depth, was better in broilers fed with adequate levels of lycopene, but otherwise less favourable with lower lycopene (Chen *et al.*, 2023). The condition of intestinal morphology that is not optimal in T₂ and T₃ can be assumed to have not supported the formation of CaBP, so that Ca transport into the meat is that of T₄, which finally resulted in medium levels of meat Ca mass (Table 2).

The mechanism in the T₁ treatment supplemented with lycopene from ETM 100 mg/kg is almost the same as T₂ and T₃ but the effectiveness of lycopene in helping the Ca retention process is the lowest. The results of research by Chen *et al.* (2023) showed that the addition of low levels of lycopene is less able to contribute to the health of the digestive tract or improve intestinal morphology. The T₀ treatment produced the lowest meat Ca mass since it was not supplemented with RTE. Red tomato extract as a source of natural antioxidants added to the diet, as discussed earlier, has an effect on improving the condition of the digestive tract and enhancing nutrient absorption. Alagawany *et al.* (2022) proved that feeding tomato waste can reduce the growth of pathogenic bacteria and improve the digestive tract.

Meat protein mass

Feeding diets using protein sources and Ca microparticles supplemented with RTE had a significant effect ($p < 0.05$) on meat protein mass in broiler chickens (Table 2). The addition of higher levels of RTE from 100 to 400 mg/kg resulted in statistically not different meat protein mass but tended to be higher even though the trend was not tested. The effect of lycopene from RTE is humoral for the immune system, and systemic for gastrointestinal health. Increased activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) is evidence of humoral effects in broilers fed lycopene (Mezban *et al.*, 2019; Wang *et al.*, 2022). The effect of lycopene depended on the level of RTE. Lycopene from RTE increases the efficient use of microparticle protein sources, resulting in meat protein mass that depends on the level of RTE, although statistically not different. Previous reports on broilers fed diets supplemented with lycopene showed improvements in duodenal morphology and crypt (Chen *et al.*, 2023). This mechanism might imply that protein digestibility as a substrate intake for the protein deposition process in the form of meat protein mass, although the effectiveness of lycopene differs depending on the RTE level, but is overcome by feeds with microparticle protein sources. Maharani *et al.* (2013) stated that if more protein can be retained, it contributes positively to protein deposition, resulting in high protein mass.

The control treatment (T₀) without added RTE as a

source of lycopene produced the lowest meat protein mass. Lycopene, as discussed earlier, can have a humoral effect on increasing endurance and improving the health of the digestive tract systemically. Previous research showed that there was no improvement in duodenal morphology and crypt in broilers fed diets without lycopene (Chen *et al.*, 2023). Suthama *et al.* (1991) stated that protein deposition determined by protein degradation is related to high protein intake. Low protein digestibility (Table 2) in this study had an impact on meat protein deposition not being maximized, causing low meat protein mass at T₀.

Meat weight in broiler

Feeding diets using microparticle protein and Ca sources supplemented with RTE had a significant effect ($p < 0.05$) on broiler meat weight (Table 2). Meat weight in the four treatments T₁ to T₄ was not statistically different but the higher the RTE level (100 to 400 mg/kg), the higher the meat weight tended to be. This condition is an indication that a healthy digestive tract, increases protein digestibility and meat protein mass, resulting in higher meat weight. Previous research showed that broilers fed lycopene led to better gut morphology, including villus length and crypt depth (Chen *et al.*, 2023). The improvement of bacterial balance and intestinal morphology is a dual effect of lycopene from RTE that can help increase protein availability from the feed using microparticle protein sources. The results of this study are in agreement with previous reports that increased protein digestibility supports improved productivity in broiler chickens fed rations with microparticle protein plus a natural acidifier (Suthama *et al.*, 2021).

Increased protein digestibility means more substrate is available for meat protein synthesis which can ultimately increase meat weight. Red tomato extract containing lycopene helps to increase the protein utilization efficiency of feeds with microparticle protein sources and has a positive effect on meat protein mass, resulting in high meat weights. The results of this study demonstrated that protein availability is directly proportional to meat protein deposition due to the bioactivity of RTE in meat weight with high values, except for treatment T₁ which is statistically similar to T₀. Lycopene feeding is reported to increase meat production in broiler chickens (Arain *et al.*, 2018), but reduce blood fat through regulating lipogenesis (Wan *et al.*, 2021). The mechanism can be assumed that the effect of lycopene administration from RTE is more dominant in protein regulation, resulting in an increase in meat weight.

Meat weight in T₀ was significantly lower than those of T₁₋₄ but statistically similar to T₁. Protein digestibility was inadequate and resulted in low protein mass, which did not support productivity in the form of meat weight. The process of protein deposition that has a positive impact on

meat weight starts from the condition of the digestive tract in relation to the availability of protein substrates. Even though the ration uses microparticle protein sources, without the addition of RTE, it does not support the health of the digestive tract, causing low protein intake. Lycopene as an antioxidant is stated to play a role in improving digestive tract conditions by suppressing the effects of *Escherichia coli* (Fararh *et al.*, 2019). Protein intake in the form of insufficient protein digestibility has an impact on low meat protein deposition, resulting in low meat weight. Cholis *et al.* (2018) reported that meat protein mass is directly proportional to meat weight, the higher the mass of meat protein, the higher the weight of the resulting meat, and vice versa.

Conclusion

The addition of red tomato extract at the level of 400 mg/kg to the ration using protein sources and calcium microparticles (T₄) increased protein digestibility, meat calcium mass, meat protein mass and meat weight.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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