Antioxidant, meat mass protein and meat production of broiler chicken due to synbiotic addition at the ration

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Abstract. This study was aimed to examine the level of antioxidant activity, meat protein mass, and meat production of broiler chicken due to synbiotic addition at the ration. This study used unsexed broiler chicken was 144 birds with an initial body weight of 45.68 ±1.52g. The birds placed into 4 treatments and 6 replications with 6 birds per cage and reared for 42 d. The treatment consist of P₀ (basal diet without of synbiotic), P₁ (basal diet with synbiotic of 1 ml/100g of ration), P₂ (basal diet with synbiotic of 2 ml/100g of ration) and P₃ (basal diet with synbiotic of 3 ml/100g of ration). The basal diet contains crude protein 22 % and metabolizable energy 3,000 kcal/kg. The completely randomized design (CRD) with 4 treatments and 6 replications was used in this research. The result of this study showed that the increase of synbiotic levels significantly (p<0.05) increase antioxidant, meat protein mass, and meat production of broiler chicken. In conclusion, the level of synbiotic 3 ml/100g of ration (P₃) increased antioxidant status, meat protein mass, and meat production.

1. Introduction
Oxidation is a natural reaction which in excess can cause damage to lipids, proteins, and DNA [1,2]. Climate change, stocking density, transportation, and nutritional deficiencies are several factors that can cause stress in broiler chickens thereby increases free radicals which can cause oxidative stress [3]. This increased oxidative stress affects the decrease in livestock productivity and in the unsaturated fatty acid content in poultry meat products [4] and this greatly affects consumer preferences. Increased oxidation stability in livestock can be improved by the supplementation of antioxidants. Antioxidants are compounds that can inhibit oxidation reactions [5]. Antioxidants added by farmers are synthetic antioxidants. Due to the increased public awareness on the importance of health and food safety, the application of synthetic antioxidants needs to be replaced with natural antioxidants such as synbiotic. Synbiotic was the combination between prebiotics and probiotics that have synergy a beneficial effect [6]. This research used synbiotic from combination of prebiotic (inulin extracted from Gembili tuber) and probiotic (Lactobacillus plantarum). Several studies have shown that the supplementation of probiotic Lactobacillus plantarum, prebiotic MOS and synbiotic improved the antioxidant status in broiler chickens [7–9].

Synbiotics were also reported to improve intestinal health, growth performance and carcass [10, 11]. Based on Setyaningrum et.al (2019) study, the synbiotic (inulin extracted from gembili tuber combined with Lactobacillus plantarum) had a positive effect on broiler chickens [12]. Increased growth
performance supported the meat production in broiler chicken. Symbiotic supplementation can increase broiler chicken carcass production. Therefore, this study was aimed to examine the level of antioxidant activity, meat protein mass, and meat production of broiler chicken due to symbiotic addition at the ration [13].

2. Method

2.1. Inulin extraction from gembili tuber and symbiotic preparation
Inulin extraction was conducted based on modified [14] method. Nine months of age of Gembili tuber prepared into powder. Process of extraction was conducted by adding 90°C hot water with the comparison of 1:15; w/v. The next process was heating for 1 h in an 80°C water bath. After that conducted of was cooling and filtering. The filtrate was then precipitated with ethanol (concentration 40%) as much as 40% from the filtrate, frozen (6 h), was thawing and centrifuge for 5 min (5000 rpm), dried, and ground to obtain the powder.

Inulin powder obtained used for symbiotic preparation. Symbiotic preparation was conducted by mixing of inulin extracted from gembili tuber 7 ml/100 ml distilled water and Lactobacillus plantarum 10 ml with bacterial concentration 10^9 cfu/ml. Then the culture was incubated for 24 h at temperature 37°C.

2.2. Birds, diet and management
This study used unsexed broiler chicken was 144 birds with an initial body weight of 45.68±1.52 g. The birds placed into 4 treatments and 6 replications with 6 birds per cage and reared for 42 d. The treatment consist of P0 (basal diet without of symbiotic), P1 (basal diet with symbiotic of 1 ml/100g of ration), P2 (basal diet with symbiotic of 2 ml/100g of ration) and P3 (basal diet with symbiotic of 3 ml/100g of ration). Composition of the basal diet (table 1) according to National Research Council (1994) [15].

| Items                | Composition (%) |
|----------------------|-----------------|
| Rice bran            | 12.90           |
| Corn                 | 48.00           |
| Meat bone meal       | 10.80           |
| Soy bean meal        | 28.00           |
| Mineral mix¹         | 0.30            |
| Total                | 100             |

Proximate analysis

| Crude fiber (%)      | 5.19            |
| Crude fat (%)        | 3.53            |
| Crude protein (%)    | 22.08           |
| Metabolizable Energi (kcal/kg) | 3,038.33 |
| Methionine(%)        | 0.37            |
| Arginine(%)          | 1.54            |
| Lysine(%)            | 1.22            |
| Phosphorus(%)        | 0.68            |
| Calcium (%)          | 1.07            |

¹per kilogram contains of P 1.00%; Ca 32.5%; Mn 4.00 g; Fe 6.00 g; Zn 3.75 g; Cu 0.30 g; Vitamin D₃ 50,000 IU; Vitamin B₁₂ 0.15 g

2.3. Sample collection
Blood sample for measuring the superoxide dismutase (SOD) activity and malondialdehyde (MDA) was conducted on 42-day of age, one broiler for each replicate. Blood sample was obtained from the wing vein and punctured into EDTA tubes; centrifuged at 2,500 rpm for 10 minutes to separate the plasma and serum. SOD activity and MDA are measured based on [7].
Measurement of meat protein mass and meat production were conducted at 42 days of the age of broiler chicken. One bird is selected from each replicate. The birds underwent fasting for 8 h, slaughtering, scalding, evisceration; the head and feet were separated from the carcass obtained. Meat production was measured by separating the meat from the bone of the whole carcass, then weighed. The meat was homogenized to be analyses for protein [16]. Meat protein mass was measured based on Sutama (2003) [17].

2.4. Statistical analysis
The completely randomized design (CRD) with 4 treatments and 6 replications was used in this research. Data was analyzed by one-way analysis of variance (ANOVA). The Duncan’s multiple range tests were used when there are significant differences between treatments.

3. Results and discussion

3.1. Antioxidant status
Superoxide dismutase (SOD) was the enzymes of antioxidant that has a function as the first-line against damage of oxidative [18]. Malondialdehyde (MDA) was the lipid peroxidation end product, where MDA accumulation was expressed as lipid peroxidation index, so the greater amount of MDA produced will cause more tissue damage [8,19]. Table 2 showed the average of the SOD and MDA. The results showed that the addition of synbiotic at ration significantly (p<0.05) increased the SOD and decreased (p<0.05) the MDA of broiler chicken. The highest SOD status value was observed in P3 (addition synbiotic of 3 ml/100 g of ration) and was significantly different (p<0.05) compared to the others. The value of the lowest SOD was achieved in P0 (addition synbiotic of 0 ml/100 g of ration). The results showed that MDA values in P1 (addition synbiotic of 1 ml/100 g of ration) was able to decrease the MDA values when compared to controls. This was in line with Popović et.al (2015), synbiotic supplementation increases antioxidant activity and reduces the damage caused by free radicals in broiler chickens [20]. Synbiotics stimulate scavenging ROS, inhibit lipid peroxidase and increase antioxidant capability by activating various antioxidant enzymes [21]. The synbiotic also play a role in detoxification at free radical production to prevent the stress of oxidative [22]. Probiotics can stimulate antioxidant enzymes activities so that enabling natural antioxidants that protects the animals from oxidative stress induced by ROS [23,24].

Table 2. Average of SOD and MDA of broiler chicken due to the addition of synbiotic.

| Items                | Treatments | SEM  | p        |
|----------------------|------------|------|----------|
|                      | P0         | P1   | P2       | P3       |
| SOD (U/mL)           | 21.09c     | 24.29b | 25.84a   | 22.50c   |
| MDA (ng/mL)          | 482.4a     | 397.81b | 403.19b  | 425.22b  |

abc Different superscript in the same row showed significantly different p<0.05.

The results show that synbiotic supplementation was able to increase endurance in broilers, characterized by the increased SOD values and low MDA values. This is in line with the research of [25], the mixture of inulin from the tuber of Dahlia with Lactobacillus sp increased the antioxidant activity on crossbred kampong chicken. Synbiotic supplementation increased the intestinal health of broiler chicken and it will affect the increase of antioxidant activities in body tissues [10,12,25,26]. According to Sohail et al (2010) intestinal microbial has an important function in broilers with regard to immune modification, metabolic regulation, and antioxidant activity [27].

The average of meat protein mass and meat production showed at table 3. This research showed that the addition of synbiotic at ration significantly (p<0.05) increases meat protein mass and meat production.
Table 3. Average of meat protein mass and meat production of broiler chicken due to the addition of synbiotic.

| Items                     | Treatments | SEM | p       |
|---------------------------|------------|-----|---------|
| Meat protein mass (%)     | P0         | 52.52<sup>c</sup> | 59.78<sup>c</sup> | 77.2<sup>b</sup> | 91.40<sup>a</sup> | 3.9   | <0.0001 |
| Meat production (g)       | P0         | 207.55<sup>c</sup> | 226.00<sup>c</sup> | 255.95<sup>b</sup> | 287.55<sup>a</sup> | 11.4  | <0.0001 |

abc Different superscript in the same row showed significantly different p<0.05.

Table 3 showed that supplementation of synbiotic at 3 ml/100 g of ration increased (p<0.05) the meat protein mass compared to control. The highest meat protein mass was achieved by the T3 (synbiotic 3 ml / 100 g of ration) and the lowest meat protein mass was achieved by the control treatment. The result of this study similar to Abdurrahman et al (2016) report that the mixture of Dahlia tuber powder and Lactobacillus sp increases meat protein mass of kampung chicken crossbreed [25]. Study of Rofle (2000) showed that combination of prebiotics and probiotics has a synergistically beneficial effect for the host, by way of prebiotics providing substrates to be utilized by beneficial microbial in the fermentation process to produce lactic acid [28]. The increase of lactic acid concentration decreases the pH of the digestive tract, where this condition increases the growth of beneficial bacteria to compete with pathogenic bacteria, so that the growth of pathogenic bacteria was suppressed [29]. The decrease of pH in the digestive tract also increases the production of digestive enzymes thereby increasing the digestibility of nutrients, especially protein [30]. This result was supported by data in previous studies on protein digestibility, increasing with synbiotic supplementation [12]. The increased protein digestibility would increase the absorption of protein and therefore the meat protein mass also increases.

Data on meat production of broiler chicken showed in table 3. The result showed that the synbiotic supplementation increased (p<0.05) meat production. Supplementation of synbiotic at P3 significantly increased meat production compared to P0, P1 dan P2. Increased meat production in this study was supported by data of net protein utility values which increases with synbiotic supplementation [12]. Synbiotic supplementation lead to healthy intestinal environmental conditions for growth of beneficial bacteria, so as to create a balance of intestinal microbes, as the result, the intestine will be protected from damage created by pathogenic bacteria, and resulting in better intestinal structure and function [31, 32]. This is indicated by the intestinal morphology consisting of the height of villi and ratio of the height of villi to crypt depth, which increases with synbiotic supplementation [12]. The increased height of villi also indicates that intestinal health positively influences the process of protein digestion and absorption, allowing the protein to be deposited throughout all the muscle tissues and ultimately increasing meat production.

4. Conclusion
The study concludes that the level of synbiotic 3 ml/100g of ration (P3) increased antioxidant status, meat protein mass, and meat production of broilers.

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