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Antioxidant Enzymes and Growth of Broiler Fed Microparticle Protein Diet with Inulin or *Lactobacillus acidophillus* Supplementation

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ABSTRACT

The effect of dahlia tuber extract or *Lactobacillus acidophilus* inclusion on intestinal physiology, antioxidant enzymes, and growth performance of broiler chicken given a microparticle protein-composed diet was evaluated in the present study. Three dietary treatments were applied, Control: 21% intact protein diet without additive, MP-DTE: 21% microparticle protein diet + 1.2% dahlia tuber extract as inulin source, and MP-La: 21% microparticle protein diet + 1.2 mL *L. acidophilus* (1 mL *L. acidophilus*/La equal to 10^8 cfu). Microparticle proteins were obtained from common protein source ingredients for poultry, fish meal, and soybean meal. A completely randomized design was assigned with 3 treatments and replicated 8 times, 10 broilers in each replication. Experimental animals were 240 broilers for treatment and 10 birds for endogenous correction. Digestibility of protein and essential amino acids, villi height, intestinal bacterial counts (LAB and *Eschericia coli*), short chain fatty acids/SCFA (acetate, propionate, and butyrate), antioxidant enzymes (GSH-Px and SOD), and growth performances (meat protein/MPM and fat mass/MFM, feed consumption, body weight gain/BWG, and feed conversion ratio/FCR) were variables measured. Data were statistically processed based on analysis of variance and continued to the Duncan test ($p<0.05$). Supplementation of dahlia inulin extract or *L. acidophilus* to the micropaticle protein diet significantly ($p<0.05$) increased N retention, villi height, LAB population, SCFA, antioxidant enzymes, and improved MPM and BWG, but decreased *E. coli* count, MFM, and FCR. However, feed consumption was not affected by any treatment. It can be concluded that *L. acidophilus* supplementation to the microparticle protein diet (MP-La) improves antioxidant enzymes, and growth performance with high meat protein and low fat mass.

Keywords: antioxidant enzyme; broiler; dahlia inulin; Lactobacillus; microparticle protein

INTRODUCTION

Broiler chickens are modern poultry experiencing genetic engineering to have a high growth rate for meat production at a relatively short rearing period. Fast-growth broilers should be supported by feeding a high-quality diet using enough protein ingredients. Protein sources, fish meals, and soybean meals are commonly used in poultry feed formulation; however, the price of those two ingredients fluctuates and is very often higher than low. Processing both ingredients to become micro in size is a possible method to increase nutrient utilization efficiency, especially protein, and thus, reduce production cost. The smaller particle size of the feed brought about the more intensive accessibility of digestive enzymes to feed substrate (Jambrak *et al*., 2014; Huang & Stein, 2016), thus increasing digestibility.

Microparticle protein can be basic information in establishing the use of processed protein source ingredients for poultry that gives impact in maximizing nutrient utilization and minimizing gut diseases due to the pathogenic bacteria, and finally, improved growth (Suthama *et al*., 2021b). Lactic acid bacteria (LAB) population and villus height increased, and growth performance improved in broiler given graviola (*Annona muricata* Linn.) leaf extract with a normal protein diet (Maesaroh *et al*., 2022). Feeding a microprotein diet with the inclusion of prebiotics or probiotic would be an ideal combination ensuring the host animal's health and productive efficiency in the absence of using antibiotic growth promoters (AGPs) (Suthama *et al*., 2021a, b). However, since the use of AGPs is prohibited, encouraging researchers to obtain and investigate beneficial natural feed additives with improving effects on poultry production without harming either host animal or consumer health. The previous result indicated that dietary herbal additives could be an alternative to antibiotics (Masoud-Moghaddam *et al*., 2021).

A natural feed additive known as a prebiotic has no residual effect but consumer's health friendly. Prebiotic inulin is usually obtained from a plant source, such as dahlia (*Dahlia variabilis*) tubers (Petkova *et al*., 2018). Chicory (*Cichorium intybus*) roots and jerusalem artichoke (*Helianthus tuberosus*) tubers (Redondo-Cuenca *et al*., 2021) are also other sources. Inulin, a soluble dietary fiber, cannot be digested or hydrolyzed by a digestive enzyme of poultry. However, inulin can be fermented by *Lactobacillus casei* together with endogenous LAB producing healthy intestinal conditions indicated by low coliform growth (Mangisah *et al*., 2020). A previous study indicated that the improvement of body health with better meat quality and low cholesterol content in crossbred local chicken was due to the modulation effect of inulin derived from dahlia tuber (Fajrih *et al*., 2014).

The molecular structure of inulin is a polymer of polysaccharide containing D-fructose that joined to the linkage of α-glycosidic bond (Apolinario *et al*., 2014; Petrovsky *et al*., 2015). A glycosidic bond is a factor that causes inulin to be resistant to the digestive enzymes of monogastric animals and poultry. However, concerning poultry production, especially in the tropical region, the improvement of antioxidant enzyme function and activity by feeding a diet supplemented with inulin is an important consideration. *In vivo* study in laying hen showed that dietary supplementation of inulin improved antioxidant status (Sang *et al*., 2018) through the indirect effect by suppressing the harmful endotoxin produced by Gram-negative bacteria. It was also specifically reported that the activity of endogenous antioxidant enzymes such as SOD and GSH-Px increased, and blood serum MDA concentration decreased. In addition to the indirect function of inulin, feeding nutritional antioxidants such as vitamin E, selenium, carotenoids, phytochemicals, etc., were known to contribute to the increase in SOD expression (Lee *et al*., 2017). Inulin and/or *Lactobacillus* sp. addition would support the effect of feeding a diet composed of microparticle protein sources through gut health improvement. The increase in nutrient digestibility, especially protein, is determined by the improvement of villi growth as an indication of better intestinal health. Supplementation of *Lactobacillus* mixture at a concentration of 1 g/kg diet during 42 days improved body weight gain and feed conversion ratio, also decreased cholesterol and triglyceride of broiler (Shokryazdan *et al*., 2017).

When the animal is in a state of aging or a hot climate environment, excessive reactive chemical species may produce and bring about the abnormality of the cell, either structure or function, finally causing a loss in production capability (Zhao *et al*., 2011; Liu *et al*., 2014). Profound stress also exerts a damaging effect on productive performance, and it does not rule out the possibility of causing diseases. Improvement of endogenous antioxidant activity is an important effort to increase or maintain stable performance. Therefore, it was necessary to evaluate antioxidant enzymes in relation to the growth performance of broiler due to the effects of additives (inulin source or *Lactobacillus acidophilus*) addition to a diet composed of microparticle protein, and compared to non-supplemented feed.

MATERIALS AND METHODS

The present experiment was conducted due to the approval of the Institutional Research Project under the inspection of the Institute of Research and Community Services, Diponegoro University number 474-84/UN7.P4.3/PP/2020, and rule of the Republic of Indonesia's law number 41, 2014, also reinforced the study.

Bird Management and Diet Preparation

Two hundred and forty (240) broilers (Cobb 500 strain) of 14-day old for treated animals and 10 birds for endogenous correction (average body weight was 268±30 g) were used in the current study. The birds since day-old until two weeks of age were provided a preliminary period that was divided into 2 steps as reported elsewhere (Suthama *et al*., 2021a). A commercial feed was fully given during the first week, and it was continued to a combination of commercial and experimental diets for another week thereafter. The birds were housed in brooder cage equipped with electric lamps for heating and lighting during the preliminary period. The preliminary period ended when the birds were 13-day old, and experimental diets were commenced at 14 days of age. The birds were divided into 3 groups of treatment by placing them in individual battery cages, and an experimental diet was given until 6-week old. In the last week of the experiment, one hundred and twenty birds (half of the total) were separated for blood sampling and digestibility trial (excreta collection). Experimental feeds were formulated with approximately similar protein and energy contents of 21% and 2900 kcal/kg, respectively (Table 1), according to the previous report (Suthama *et al*., 2019). The experimental diets were pelleted and were given *ad libitum* with free access to drinking water. Experimental feeds were composed of microparticle protein sources derived from fish and soybean meals. The microparticle protein processing method was based on the previous study of Suthama & Wibawa (2018) with an ultrasonic bath treatment system using an ultrasound transducer as the most recently reported (Suthama *et al*., 2021a).

Inulin Extract and *Lactobacillus acidophilus* Preparation

Dahlia tuber extract as a source of inulin was prepared according to the simply modified procedure as previously reported (Krismiyanto *et al*., 2014). Clean tubers of approximately one-year-old dahlia plants were peeled and sliced to a thin and small size (0.5-1 cm). Sliced tubers were dried in an oven at 50 °C for 24 hours, ground into powder, dissolved in distilled water at a ratio of 1 : 10 (w/v), and then 70% alcohol was added with a ratio of 50 : 50 (v/v). The solution was destructed in a water bath at 80 °C for 30 min, and the filtrate was collected thereafter. The filtrate was placed in the refrigerator at 4 °C for one day until a white or yellow precipitate appeared, and then it was collected. The precipitate was dried in an incubator at 30 °C for 4-6 hours before being mashed as an inulin extract of dahlia

Table 1. Experimental diet composition and nutrient content

Ingredient (%)	Dietary treatment		
	Control	MP-DTE	MP-La
Ground yellow corn	48	48	48
Rice bran	14	14	14
Intact fish meal	10	–	–
Microparticle fish meal	–	10	10
Intact soybean meal	27	–	–
Microparticle soybean meal	–	27	27
$CaCO_3$	0.5	0.5	0.5
Vitamin and mineral mixture	0.5	0.5	0.5
Total	100	100	100
Nutrition content			
Metabolizable energy*** (kcal/kg)	2,926	2,961	2,961
Crude protein (%)	21.02	21.27	21.27
Ether extract (%)	3.02	3.05	3.05
Crude fiber (%)	5.1	5.16	5.16
Calcium total (%)	0.98	0.99	0.99
Phosphorus total (%)	0.53	0.54	0.54
Methionine + Cystine (%)	0.74	0.76	0.76
Lysine (%)	1.1	1.12	1.12
Methionine (%)	0.42	0.45	0.45

Note: MP-DTE and MP-La are the same composition but different additive supplementation.
Control = diet composed of intact protein; MP-DTE = diet composed of microparticle protein + 1.2% dahlia tuber extract; MP-La = diet composed of microparticle protein + 1.2 mL *Lactobacillus acidophilus*
***Calculated value based on the formula of Sibbald & Wolynetz (1985).

tuber. Probiotic *Lactobacillus acidophilus* was purchased from Biotechnology Laboratory, Universitas Gadjah Mada, Yogyakarta, as a bacterial culture. A rejuvenation process was performed to obtain ready-to-use bacteria as probiotic with a 108 cfu/mL density.

Treatments and Variables Measurement

Dietary treatments applied in the present study were Control: a diet containing 21% intact protein, MP-DTE: a diet containing 21% microparticle protein added with 1.2% dahlia tuber extract as an inulin source, and MP-La: a diet containing 21% microparticle protein added with 1.2 ml *L. acidophilus*. One mL *Lactobacillus acidophilus* is equal to 10^8 cfu. Dahlia inulin or *L. acidophilus* was mixed with a small portion of feed, approximately 25 g according to treatment level; the mixed diet was given in the morning until it was consumed all. An experimental diet without additives was fed thereafter to fulfill the daily requirement. Nitrogen (N) retention determination was performed according to a combination method of total collection and Fe_2O_3 as an indicator. Amino acid digestibility was determined based on the method of Ravindran *et al*. (1999).

Villi sample preparation was performed based on the hematoxylin-eosin (HE) staining method. The jejunal samples were cut 2 cm long and dissected at the midpoint position. After that, the tissue samples were sent to the Laboratory of Animal Health, Vocational Education, Universitas Gadjah Mada, Yogyakarta, for further processing. Digesta samples from duodenum and jejunum were diluted in media of deMan Rogosa Sharpe (MRS, Merck KGaA) and eosin methylene blue agar (EMBA, Levine formula) for determination of total LAB and *E. coli*, respectively. The respective samples were diluted until 10^{-5} and 0.1 mL diluted samples were poured into the test tube containing 0.9 MRS Broth and EMBA, respectively, to obtain 10^{-2} until 10^{-5} dilution. A 0.1 mL sample was taken from a test tube containing 10^{-5} diluted samples and then planted in MRS and EMBA media. Both media were incubated using an incubator at 37 °C for 48 hours. The total colony was calculated according to Fardiaz (1992). The bacterial population was measured using mixed digesta from duodenum and jejunum because most nutrients were effectively absorbed in those segments (Rinttila & Apajalahti, 2013), whereas the absorption rate depended on pathogenic bacterial interference.

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity were determined according to the assay kit of the manufacturer's instructions through xanthine oxidase and dithio-dinitrobenzoic acid methods, respectively. Both antioxidant enzymes were measured using a spectrophotometer at the absorbance of 550 nm and 412 nm for SOD and GSH-Px, respectively, as previously reported (Wang *et al*., 2011). The concentration of SCFA was analyzed as free acids by gas chromatography equipped with a glass column packed using 80/120 Carbopack B-DA. The instrument was also equipped with helium that serves as a carrier gas, and the result was monitored through a flame ionization detector (Gonzalez-Ortiz *et al*., 2020). The determination of meat protein and fat mass were performed based on a formula as previously reported (Suthama, 2003). Productive performances, including body weight gain (BWG), feed consumption, and feed conversion ratio (FCR), were measured according to the most recently reported (Suthama *et al*., 2021).

Design and Statistical Analysis

The current experiment was assigned in a completely randomized design with three treatments and eight replications (ten birds per replicate). Data were processed based on analysis of variance and continued to Duncan tests at 5% probability when treatment indicated a significant effect ($p<0.05$).

RESULTS

Protein and Amino Acids Digestibility

Broilers given diet composed of microparticle protein sources added with either inulin derived from dahlia extract (MP-DTE) or *L. acidophilus* (MP-La) significantly ($p<0.05$) indicated higher protein digestibility and nitrogen retention than those of control (Table 2). It was found that not all essential amino acid digestibilities were increased; however, only some, such as leucine, lysine, methionine, and tryptophan were significantly improved by both treatments, MP-DTE and MP-La.

Intestinal Physiology and Antioxidant Enzymes

Feeding diet using microparticle protein sources with the inclusion of either inulin of dahlia extract (MP-DTE) or *L. acidophilus* (MP-La) significantly ($p<0.05$) enhanced both villi height and population of lactic acid bacteria (LAB) as indicated in Table 3. On the other hand, pathogenic bacteria such as *E. coli* significantly ($p<0.05$) reduced in the MP-DTE as well as MP-La treatments. Intestinal short-chain fatty acid levels of both acetate and butyrate were significantly ($p<0.05$) increased by treatments of MP-DTE and MP-La, but propionate was not affected (Table 3). A similar pattern was found for the blood concentrations of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), indicating that both antioxidant enzymes significantly ($p<0.05$) increased in MP-DTE and MP-La treatments.

Growth Performances

Meat quality characteristics, namely, protein and fat meat mass (MPM and MFM), growth performances, including feed consumption, body weight gain (BWG), and feed conversion ratio (FCR), were variables measured (Table 4). Body weight gain (BWG) significantly ($p<0.05$) improved, and in contrast, feed conversion ratio (FCR) significantly reduced ($p<0.05$) by feeding a diet with the inclusion of either dahlia tuber extract as inulin source (MP-DTE) or *L. acidophilus* (MP-La). However, feed consumption was not affected by any treatments.

DISCUSSION

Protein and Amino Acids Digestibility

Diet that was added with dahlia tuber extract as inulin source (MP-DTE) or *L. acidophilus* (MP-La) resulted same protein digestibility, but that in MP-DTE was statistically not different compared to control group. Nitrogen retention and some essential amino acid digestibility increased due to MP-DTE or MP-La. Digestibility of some essential amino acids, such as leucine, lysine, methionine, and tryptophan, indicated the increasing value caused by feeding MP-DTE or MP-La. Protein digestibility was not significantly affected by MP-DTE, although its value slightly increased. However, MP-DTE treatment significantly increased meat protein mass (MPM), supported by the improvements of N retention and some essential amino acid digestibility. The increase in the number of particles and surface area due to the reduction of particle size impact the accessibility of digestive enzyme much more intensive on feed or nutrient as substrate. It was possible that broiler-fed microparticle protein sources of fish meal and soybean meal increased protein digestibility and N retention (Suthama & Wibawa, 2018). Other reports also supported that protein and amino acid digestibility and Ca and N retentions increased in broiler fed diet composed of microparticle protein source added with acidifier (Suthama *et al*., 2021a). The increase in protein utilization efficiency in particular and feed in general is an important key in the broiler management industry as an economical meat producer.

A glycosidic bond is a factor that causes inulin to be resistant to the digestive enzymes of monogastric animals and poultry. However, inulin derived from dahlia tuber extract and *Lactobacillus acidophilus* are important for improving antioxidant enzyme functions in mediating the better balance of intestinal microflora found in the current study (Table 3). The present results were in accordance with the most recent report (Gurram *et al*., 2022) that the increased GSH-Px and SOD were associated with the improvement of bacterial balance (higher LAB, lower *E. coli* and *Salmonella* counts) due to

Table 2. Digestibility of protein and essential amino acid, and nitrogen (N) retention in broiler fed diet composed of microparticle protein added with dahlia inulin or *Lactobacillus acidophilus*

Variables	Dietary treatment		
	Control	MP-DTE	MP-La
Protein digestibility (%)	81.1 ± 8.2^{b}	82.8 ± 8.4^{ab}	85.6 ± 7.9^{a}
N retention (%)	55.6 ± 5.5^{b}	63.0 ± 7.0^{a}	62.7 ± 6.1^{a}
Amino acid digestibility			
Arginine (%)	81.4 ± 7.8^{b}	84.8 ± 8.7^{a}	83.2 ± 8.0^{ab}
Isoleucine (%)	80.1±7.8	81.4±7.9	81.1±8.1
Leucine (%)	79.2 ± 7.0^{b}	84.8 ± 8.8^{a}	85.3 ± 8.5^{a}
Lysine (%)	80.9 ± 7.7^{b}	86.7 ± 8.6^{a}	87.3 ± 8.9^{a}
Methionine (%)	62.2 ± 5.8^{b}	68.4 ± 6.3^{a}	69.1 ± 6.6^{a}
Phenylalanine (%)	78.8 ± 7.1^{b}	82.3 ± 8.0^{a}	80.0 ± 7.8^{ab}
Tryptophan (%)	75.5 ± 7.3^{b}	80.5 ± 8.1^{a}	81.3 ± 8.0^{a}
Valin (%)	78.8±7.4	77.2±7.1	78.7±7.9

Note: Control= diet composed of intact protein; MP-DTE= diet composed of microparticle protein + 1.2% dahlia tuber extract; MP-La= diet composed of microparticle protein + 1.2 mL *Lactobacillus acidophilus*

$^{a-b}$Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Villi height, intestinal physiology, and antioxidant enzymes in broiler fed diet composed of microparticle protein added with dahlia inulin or *Lactobacillus acidophilus*

Variables	Dietary treatment		
	Control	MP-DTE	MP-La
Jejunal villi height (μm)	1038.8 ± 50.4^{b}	1140.4 ± 51.2^{a}	1157.2 ± 59.8^{a}
Lactic acid bacteria (10^{8} cfu/g)	2.74 ± 1.8^{b}	4.85 ± 3.2^{a}	4.67 ± 3.7^{a}
Escherichia coli (10^{6} cfu/g)	4.90 ± 3.9^{a}	3.31 ± 2.8^{b}	3.49 ± 2.5^{b}
Short chain fatty acids			
Acetate (mmol/L)	31.9 ± 2.2^{b}	55.3 ± 4.3^{a}	54.6 ± 4.8^{a}
Propionate (mmol/L)	24.3± 1.9	25.9± 2.0	26.1± 2.2
Butyrate (mmol/L)	14.4 ± 0.98^{b}	19.2 ± 1.0^{a}	18.8 ± 1.1^{a}
Superoxide dismutase (SOD, U/mL)	59.7 ± 4.7^{b}	64.4 ± 5.6^{a}	66.5 ± 5.9^{a}
Glutathion peroxidase (GSH-Px, U/mL)	751.3 ± 44.2^{b}	862.4 ± 50.3^{a}	866.7 ± 49.9^{a}

Note: Control= diet composed of intact protein; MP-DTE= diet composed of microparticle protein + 1.2% dahlia tuber extract; MP-La= diet composed of microparticle protein + 1.2 mL *Lactobacillus acidophilus*

$^{a-b}$Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Meat quality and growth performance of broiler fed diet composed of microparticle protein added with dahlia inulin or *Lactobacillus acidophilus*

Variables	Dietary treatment		
	Control	MP-DTE	MP-La
Meat protein mass (MPM, g/bird)	109.8±24.5^{b}	122.1±33.7^{a}	120.9±37.2^{a}
Meat fat mass (MFM, g/bird)	52.3±4.6^{a}	46.8±3.8^{ab}	41.1±4.0^{b}
Feed consumption (g/bird)	2,265±55.1	2,281±49.9	2,308±50.4
Body weight gain (BWG, g/bird)	1,198±36.7^{b}	1,326±41.6^{a}	1,319±40.2^{a}
Feed conversion ratio (FCR)	1.89±0.14^{a}	1.72±0.11^{b}	1.75±0.02^{b}

Note: Control= diet composed of intact protein; MP-DTE= diet composed of microparticle protein + 1.2% dahlia tuber extract; MP-La= diet composed of microparticle protein + 1.2 mL *Lactobacillus acidophilus*
$^{a-b}$Means in the same row with different superscripts differ significantly ($p<0.05$).

the feeding effect of probiotic and inulin from chicory root. This physiological condition could be considered to have a potential effect on poultry production on one side. Feed particle size, on the other hand, provides many advantages for productive aspect of poultry via the improvement of gastrointestinal growth, nutrient utilization, and performance (Amerah *et al*., 2008). Particle size is closely related to digestive enzyme accessibility within the feed and thus increases nutrient digestibility (Jambrak *et al*., 2014; Huang & Stein, 2016), especially protein and some essential amino acids, as it was found in the present study (Table 2). This phenomenon was supported by the function of dahlia inulin as "feed source" for the fermentation activity of endogenous beneficial bacteria, such as LAB in MP-DTE, and *L. acidophilus* in MP-La work together with endogenous LAB (Table 3).

Intestinal Physiology and Antioxidant Enzymes

The increase in LAB population can be postulated to be able to compete with harmful or pathogenic bacteria via the mechanism known as "competitive exclusion". The previous study indicated that several extracts from herbs origin positively support beneficial microbial growth, especially *Lactobacillus* sp. (Zhou *et al*., 2016). The improved nutrient digestibility can be correlated with the health improvement of the intestinal lumen due to the feeding inulin extract as a "feed source" that can be fermented by beneficial endogenous bacteria, like LAB. Higher LAB and lower *E. coli* populations were due to the feeding effect of MP-DTE or MP-La, and this better balance of intestinal bacteria brought about improved villi growth (Table 3). Inulin derived from dahlia tuber can be fermented by beneficial endogenous bacteria, producing a higher LAB population (Krismiyanto *et al*., 2014). Also, the addition of *Lactobacillus* sp. to the microparticle protein composed-feed increased LAB count and short-chain fatty acids/SCFA (Cholis *et al*., 2018). The highest LAB population and SCFA were achieved with dahlia tuber extract at 1.2% as an inulin source and *L. acidophilus* at 1.2 mL, respectively.

These fermentation products bring about the acidic condition of the microenvironment of the intestine and exert an impact on the reduced intestinal pH. Acidic conditions of the intestine challenge the life cycle of pathogenic bacteria and depress their population and growth; on the other hand, low pH-resistant beneficial bacteria can favorably grow up and even speed up their growth. The improvement of intestinal bacterial balanced improved villi growth (villi height) and resulted in higher protein digestibility in up-graded local chicken (KUB chicken) given inulin combined with *Lactobacillus* sp. (Purbarani *et al*., 2019). The increased *Lactobacillus* and *Bifidobacteria* populations were previously found in either ileum or caeca of Haidong chicken fed with inulin inclusion at 0.7 or 1 g/kg diet (Ding *et al*., 2021). The improvement of microflora balance brings about better health condition of the intestinal villi and boost its digestive function.

The interaction of inulin and endogenous LAB in the present study were similar to the previous results that inulin combined with probiotic improved intestinal condition as indicated by better villi growth, and thus resulted in higher nitrogen retention and energy availability (Julendra *et al*. 2021). Therefore, it suggests that feeding MP-DTE or MP-La had a meaningful contribution to the improvement of gastrointestinal health, as shown by higher villi growth (Table 3). This condition was closely related to the increase in nutrient digestibility and absorption. The present results were similar to the previous report that intestinal bacterial balance and body resistance improved with slightly better growth in broilers fed prebiotic glucomannan derived from porang tuber extract at 0.1% (Perdinan *et al*., 2019). The healthy gastrointestinal tract due to feeding dahlia inulin extract or *L. acidophilus,* which brought about the increase in nutrients supply, such as nitrogen retention and essential amino acid, would serve as an important effector for the enzyme formations, especially SOD and GSH-Px (Table 3).

In the case of the present study, feeding MP-DTE or MP-La would be much more beneficial to improve broiler productivity. It seems like there was a synergistic effect between a microparticle protein-composed diet and additives in stimulating the healthy condition of the host animal and supporting productivity. The increased SOD and GSH-Px due to supplementation of either dahlia tuber extract or *Lactobacillus acidophilus* (Table 3) to the diet composed of microparticle protein source ingredients was evidence of a positive relationship. It can be confirmed that the positive impact of a microparticle protein diet with a higher supply of protein and/or amino acids was supported by the role of inulin or *L. acidophilus* in modulating the host animal healthy. These results were comparable to the previous study (Hunt *et al*., 2019) that the activity of SOD and CAT in rainbow trout was higher in a diet supplemented with 1% inulin than those in control. The report of Hosseinifard *et al*.

(2020) provided clarification that rats given inulin in combination with probiotic *Lactobacillus plantarum* or the inclusion of probiotic bacteria alone demonstrated the reduced oxidative MDA level and increased SOD concentration as the oxidative enzyme marker. The high availability of protein and minerals, especially Ca, due to the use of feed composed of microparticle protein source (Suthama *et al*., 2021) would be greatly possible supported by prebiotic inulin or *L. acidophilus* supplementation that acts as a positive impact on oxidative enzyme function. However, more scientific description is needed to clarify the mechanism clearly through the advanced study concerning the relationship between inulin or *Lactobacillus* sp. functions and oxidative enzyme-related minerals in the coming year.

Meat Quality and Growth Performance

Supplementation of either micronutrients or natural additives is important in high-growth rate broilers for meat production in which a sufficient supply of nutrients, especially protein and/or amino acids, is needed to support their productivity. The increased digestibility of protein and some essential amino acids in MP-DTE and MP-La treatments (Table 2) were the factors that support the productive performances proved by higher MPM and BWG (Table 4). In relation to the healthy intestinal tract indicated by the higher LAB and low *E. coli* counts followed by better villi growth (Table 3) is possible to build a systemic immunity supported by the increased circulating levels of antioxidant enzyme, GSH-Px, and SOD, which would be a humoral immunity indication. Protein and some amino acids digestibility and N retention increased (Table 2) and positively impacted both systemic and humoral immunities improvement, resulting in higher MPM and BWG but lower FCR with unchanged feed consumption (Table 4). It was the same as that found in up-graded local chicken (KUB) given inulin combined with *Lactobacillus* sp. improved villi growth and increased protein digestibility (Purbarani *et al*., 2019). Similar results were also reported in crossbred local chicken fed dietary inclusion of inulin and *Lactobacillus* sp. improved meat characteristics (Abdurrahman *et al*., 2016a) and performance (Abdurrahman *et al*., 2016b). Feeding either MP-DTE or MP-La diets improved growth performances supported by higher body resistance and defense, as indicated by higher circulating levels of GSH-Px and SOD. The present results were consistent with the previous study that nutrient digestibility was beneficial for the improvement of growth performances in a broiler-given diet with an organic acid-palm fat mixture (Goh *et al*., 2020). A result that would be important in the present study was that feed consumption was not affected by any treatment, but BWG increased by the feeding effect of either dahlia inulin or *L. acidophilus*. This phenomenon was postulated to be due to the increased protein and amino acid digestibility that was able to cover protein deposition to improve MPM and BWG. Antioxidant enzymes increased (Table 3), MPM and BWG improved with reduced MFM (Table 4) due to the addition of additives found in this study was assumed more profit could be achieved with healthy meat. The current results were supported by a previous report that broilers fed herbal additives improved immune response and decreased feed cost per kg carcass yield (Masoud-Moghaddam *et al*., 2021) because the herbal component has a similar effect as commercial growth promoters used for broiler production (Gharechopogh *et al*., 2021).

CONCLUSION

Supplementation of *Lactobacillus acidophilus* to the microparticle protein composed-diet (MP-La) improves intestinal physiology, antioxidant enzymes, and growth performance with high meat protein and lower meat fat mass.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with other institutions in relation to financial support as well as the material used during the experiment until submitting the manuscript.

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