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Inclusion effect of ginger and turmeric mixture combined with *Lactobacillus* spp. isolated from rumen fluid of cattle on health status and growth of broiler

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ABSTRAK

Penelitian dilaksanakan untuk mengevaluasi pemberian kombinasi jahe, kunyit dengan *Lactobacillus spp.*(GTL) terhadap daya tahan tubuh dan produktivitas broiler. Sebanyak 576 broiler dengan bobot badan awal 36±0,99 g, digunakan dalam penelitian ini dan diamati selama 35 hari. GTL terdiri dari ekstrak jahe (0,2 g/liter), ekstrak kunyit (0,4 g/liter) dan *Lactobacillus spp.* (2,997 x 10^7 cfu/ml), diberikan sejak umur 1 hari. Parameter yang diamati adalah bobot bursa fabrisius, limpa, timus, kadar *gluthationine peroxidase* (GSH-Px), *superoksida dismutase* (SOD), bakteri usus meliputi *Lactobacillus spp., Coliform*, dan *Eschericia coli*, serta konsumsi pakan, pertambahan bobot badan dan konversi pakan (FCR). Penelitian disusun dalam rancangan acak lengkap (RAL) pola faktorial 3x4 (3 jenis pakan dan 4 level GTL), dengan 4 ulangan (@12 ekor). Faktor pertama, jenis pakan (A) yaitu pakan formulasi sendiri (A1), kombinasi pakan formulasi sendiri dan pakan komersial, masing-masing 50 bagian (A2), dan pakan komersial (A3). Faktor kedua, Level GTL (B) adalah 0 ml (B0), 2 ml (B2), 4 ml (B4) dan 6 ml (B6)/liter air minum. Data dianalisis ragam dan dilanjutkan dengan uji Duncan pada taraf 5%. Hasil penelitian menunjukkan bahwa pakan formulasi sendiri dengan level GTL 4 ml (A1B4) dapat meningkatkan daya tahan tubuh dan produktivitas broiler.

Kata kunci: broiler, daya tahan tubuh, jahe dan kunyit, Lactobacillus spp., produktivitas

ABSTRACT

This study was conducted to evaluate the combination of ginger and turmeric extracts added with *Lactobacillus* spp. (GTL) on body resistance and productivity of broilers. 576 broilers with an initial body weight of 36 ± 0.99 g were used and were observed for 35 days. GTL containing ginger extract (0.2 g/liter), turmeric extract (0.4 g/liter) and *Lactobacillus* spp. (2,997 x 107 cfu/ml), and was given since 1-day old**.** Parameters observed were bursa fabriscius, spleen, and thymus weights, gluthationine peroxidase (GSH-Px), superoxide dismutase (SOD), *Lactobacillus* spp., *Coliform*, and *Eschericia coli*, feed consumption, body weight gain, and feed conversion ratio (FCR). A completely randomized design (CRD) was assigned with 3x4 factorial pattern (3 types of feed, 4 levels of GTL), with 4 replications (12 birds each). First factor, types of feed (A): self-formulated feed (A1), combination of self-formulated and commercial feed with 50 portion each (A2) and commercial feed (A3). Second factor, levels of GTL (B): 0 ml (B0), 2 ml (B2), 4 ml (B4) and 6 ml (B6) per litre drinking water. Data were subjected to ANOVA and continued to Duncan test at 5% probability. Results indicated that self-formulated feed with

inclusion of GTL at 4 ml (A1B4) improve body resistance and increase production performance of broilers.
Keywords: body resistance, broiler, ginger and turmeric, Lactobacillus spp.*, productivity*

INTRODUCTION

Feeding natural additive either live organisms or active compound derived form plant sources become an increasing interest and attention due to the use of antibiotic as growth promoter (AGP) for decades have indicated some problems for both host animals and consumers. Continuous feeding AGP in poultry is known to gain residue in the animal product and bring about the negative effect on consumer health. Nowadays, dietary inclusion of AGP in poultry production has been banned worldwild. The ban of using antibiotic growth promoter (AGP) in Indonesia has been initiated via the Low No. 18/2009 in conjunction with the Low No.41/2014 concerning Animal Husbandry and Animal Health. Therefore, the ban of using AGP in animal feed has been officialy enforced since January 1, 2018 based on article 16 of the Regulation of the Minister of Agriculture No. 14/2017 by refering to the Low as described above. By considering that the withdrawal of AGP can cause production and health problems in broilers, hence every alternative to replace AGP is important to maintain the productivity of broilers (Sugiharto and Ranjitkar, 2019). There are many alternatives to replace antibiotic have been studied using herbs alone or in combination with probiotics. In the absent of feeding AGP, dietary inclusion of probiotic either alone succesfully improved chickens health and produced higher meat quality in broiler (Cholis *et al*., 2018; Wulandari *et al*., 2018) or in combination with prebiotic in crossbred local chicken (Abdurrahman *et al*., 2016a; 2016b).

Lactobacillus spp. was previously isolated from the rumen fluid of cattle showed that probiotic activity could survive against gastric juice (unpublishsed data). In the present study, the herbs were combined with probiotic *Lactobacillus* spp. in order to improve the efficacy of the additive. It is well known that modern broiler strains are susceptible to stress and oxidative damage, a condition which can adversely affect the health and performance of broilers (Sugiharto *et al.*, 2016). However, other studies reported that health status improved, indicated by lower H/L ratio, with better productive performance, indicated by higher carcass percentage and body weight gain, in crossbred native birds fed natural prebiotic (Fajrih *et al*., 2014), and those given a combination of probiotic and prebiotic (Purbarani *et al*., 2019). The different results was assumed to be attributable to the respond of chickens strain.

Several study have shown that berbs, especially ginger, may improve the antioxidant status of broiler (Habibi *et al*., 2014) due to its potential antioxidant property (Stoilova *et al*., 2007) and finally was able to increase nutrients absorption and metabolism (Fakhim *et al.*, 2013). Other study indicated that broiler fed plant source additive in the form of inulin extract with no additional probiotic improved meat quality and body resistance supported by lower heterophil-lympocyte ratio and *Eschericia coli* counts (Suthama *et al*., 2019). Some previous studies also stated that ginger (Karangiya *et al*., 2016), and turmeric (Kafi *et al*., 2017; Johannah *et al*., 2018; and Shohe *et al*., 2019) at a given level improved growth performances of broilers, but no health or oxidative status was defined due to additional probiotics effect. Although probiotics, in other side, have the potential effect as an antioxidant which can be beneficial for broiler health and performance (Majidzadeh *et al*., 2011), nothing was dercribed concerning their combination with herb-originated additives such as ginger or turmeric, or combination of both. Probiotics are established as dietary additive and are known to have health beneficial effects. This phenomenon has been clarified through either *in vitro* or *in vivo* studies indicated that probiotics exhibit as potential antioxidant. Therefore, the present study was conducted to evaluate the effect of the combination of ginger and turmeric extract added with *Lactobacillus* spp. (GTL) on intestinal microbial population, body resistance, antioxidant status, and growth performance of broiler in relation to feeding different types of diet.

MATERIALS AND METHODS

Ginger, Turmeric Extract and *Lactobacillus* spp. (GTL) Preparation

Ginger and turmeric were extracted from their respective rhizomes using destilled water as a solvent. *Lactobacillus* spp. was isolated from the rumen fluid of cattle. Isolated *Lactobacillus* spp. was added into the mixture of ginger and turmeric extracts and *in vitro* trial against pancreatic juice was performed thereafter to evaluate their viability (unpublished data). Extracts of ginger and turmeric at 0.2 and 0.4 g/L, respectively, and *Lactobacillus* spp. at 2,997 x 10^7 cfu/ml were combined and used as main component of additive. The mixture of the three components namely, ginger and turmeric extracts, and *Lactobacillus* spp., was abbreviated as GTL, and it was stored in refrigerator (±5^{o}C) before being given to chicken.

Experimental Animal, Feed and Equipment

A total of 576 day-old broiler chick (COBB 500 strain) were used in the present study. There were three different types of feed tested namely, self-formulated feed (Table 1), combination of self-formulated and commercial feeds with 50 portion, respectively, and commercial feed with description of nutrient content only (Table 2). The birds were reared in open-sided housing system consisting of 48 pens with 12 birds each, and were provided GTL at the level of either 0, 2, 4 or 6 ml per 1 litre drinking water. The animal house was set at 31^{o}C during the first 7 days, and it was gradually decreased to 27^{o}C until the end of the experiment.

Data Collection and Parameters

Body weight and feed conversion ratio (FCR) were recorded on days 21 and 35 while the feed consumption was recorded daily. On day 35, blood was collected from the bird's wing vein and put into vacutainer without anticoagulants. The blood was maitained at room temperature and allowed to clot in order to obtain serum. Serum was stored in the freezer until antioxidant enzyme (gluthathionine peroxidase and superoxide dismutase) was analyzed. The same birds at the

Table 1. Composition and Nutrient Content of Self-formulated Feed

Ingredient	Starter (1-21 days)	Finisher (22-35 days)
	.... (%)	....
Yellow corn	57.50	62.60
Soybean meal	34.20	30.10
Fish meal	4.00	4.00
Wheat bran	1.00	1.00
Vegetable oil	3.00	2.00
Salt	0.30	0.30
Total	100.00	100.00
Nutrient Content (%)*		
Metabolizable energy (kcal/kg)**	2,916	3,122
Crude protein	22.58	20.12
Ether extract	4.10	4.60
Crude fiber	3.90	4.30
Calcium total	1.10	0.90
Phosphorus total	0.70	0.70
DL-Methionine***	0.05	0.05
L-Lysine***	0.15	0.10

*Chemical analysis at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University
**Calculated value based on the formula of Bolton (1967)
***Based on Table of National Research Council (1994)

Table 2. Nutrient Composition of Commercial Feed

Nutrient content*	Starter (1-21 days)	Finisher (22-35 days)
	.... (%)	
Metabolizable energy (kcal/kg)	3,025 – 3,125	3,125 – 3,225
Crude protein	21.50 – 23.80	19.50 – 21.50
Ether extract (max)	5.00	5.00
Crude fiber (max)	5.00	5.00
Calcium (max)	0.60	0.90
Phosphorus (max)	0.60	0.60

*Composition based on animal feed labels of PT. Charoen Pokphand Indonesia

same age (21 and 35 days old) were immediately decapitated, and lymphoid organs including bursa fabricius, thymus, and spleen were removed and weighed. For the microbiological measurement, digesta was collected from the small intestine of broiler. The numbers of intestinal *Lactobacillus* spp. was determined on Bromocresol Purple Agar (BCPA). The colonies were counted after anaerobic incubation at 38^{o}C for 48 hours. The number of *Escherichia coli* was counted on Eosin Methylene Blue Agar (EMBA) after aerobic incubation at 38^{o}C for 24 hours, while *Coliform* were determined on MacConkey Agar (MCA) after aerobic incubation at 38^{o}C for 24 hours.

Experimental Design and Statistical Analysis

The experiment was assigned in 3×4 factorial of a completely randomized design, with 4 replications. Three types of feed tested namely, self-formulated feed (A1), combination of self-formulated feed and commercial feed with 50 portion, respectively (A2) and commercial feed (A3) were the first factor. Four levels of GTL were the second factor (B) namely, 0 ml (B0), 2 ml (B2), 4 ml (B4) dan 6 ml (B6) per liter drinking water. Data were analyzed by analysis of variance, and when the treatment indicated significant effect (P<0.05) Duncan multiple range test was subsequently conducted.

RESULTS AND DISCUSSIONS

Intestinal Bacteria Populations in 21 and 35-day Broiler Chicken

Intestinal bacteria of broiler in A1B4 and A1B6 groups both on days 21 and 35 indicated the highest (P<0.05) numbers of *Lactobacillus* spp., and the lowest (P<0.05) counts of either *Coliform or Escherichia coli* (Table 3). Similar patterns of *Lactobacillus* spp. and phatogenic bacteria populations were found in A3B4 and A3B6 as well. It seemed that *Lactobacillus* spp. in the GTL could increased the intestinal population of *Lactobacillus* spp. of broilers. Indeed, the self-formulated feed resulted in the highest colony of intestinal *Lactobacillus* spp. of broiler. AGP-free of the self-formulated feed seemed to be no interference to the growth of *Lactobacillus* spp. In relation to the lowest numbers of intestinal *Coliform, and Escherichia coli*, in the A3B6 group on days 21 and 35 (Table 3), it was most likely that the active compounds of herbs extract in GTL could inhibited the growth of these pathogenics bacteria. Similar phenomenon that the presence of AGP in the commercial feed can possibly inhibit the proliferation of *Coliform* and *Escherichia coli* in the intestine of broiler. The present results were consistent with the previous studies that the inclusion of single probioric *Lactobacillus* spp. (Cholis *et al*., 2014), and a combination of *Lactobacillus* spp. and prebiotic (Purbarani *et al*., 2019) into self-formulated diet increased ileal lactic acid bacteria and decreased total *Coliform.* Some previous studies were comparable that feeding plant components such as inulin dahlia in local chicken (Krismiyanto *et al*., 2014), and soybean meal extract in broiler (Suthama *et al*., 2018) increased lactic acid bacteria population. It is well known that fermentation activity of *Lactobacillus* spp. or lactic acid bacteria on low molecule weight carbohydrate of the feed produce short chain fatty acid which cause low intestinal pH. This

Table 3. Intestinal Microbes of Broiler Fed Additive Mixture of Ginger and Turmeric extracts, and *Lactobacillus* sp. with Different Types of Feed

Factor / Parameter (%)*	A_1				A_2				A_3			
	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
21-d old												
Lactobacillus sp. (x10^7)	1.12^{cd}	1.42^{b}	1.60^{ab}	1.90^{a}	0.52^{d}	0.90^{cd}	1.32^{bc}	1.60^{ab}	0.30^{de}	0.60^{d}	0.90^{cd}	1.10^{cd}
Escherichia coli (x10^5)	2.22^{a}	1.90^{b}	1.60^{c}	1.60^{c}	1.60^{c}	1.52^{c}	1.30^{d}	1.20^{d}	1.00^{de}	0.80^{e}	0.60^{f}	0.60^{f}
Coliform (x10^6)	1.10^{a}	0.90^{ab}	0.72^{c}	0.60^{d}	0.70^{c}	0.70^{c}	0.62^{d}	0.50^{d}	0.50^{d}	0.50^{d}	0.40^{e}	0.40^{e}
35-d old												
Lactobacillus sp. (x10^7)	1.72^{c}	2.22^{b}	2.40^{a}	2.52^{a}	1.72^{c}	2.22^{b}	2.40^{a}	2.52^{a}	0.60^{de}	0.90^{d}	1.20^{cd}	1.40^{cd}
Escherichia coli (x10^5)	3.10^{a}	2.80^{b}	2.40^{c}	2.42^{c}	3.10^{a}	2.80^{b}	2.40^{c}	2.42^{c}	1.50^{e}.	1.30^{e}	1.20^{f}	1.10^{f}
Coliform (x10^6)	1.60^{a}	1.50^{b}	1.20^{cd}	1.10^{cd}	1.60^{a}	1.50^{b}	1.20^{cd}	1.10^{cd}	0.92^{d}	0.80^{de}	0.60^{e}	0.60^{e}

$^{a-f}$ Values within the same row bearing different superscript differ significantly P<0.05)

A_1 : 100% self-formulated feed ; A_2 : 50% self-formulated feed+50% commercial feed ; A_3 : 100% commercial feed

B_0 : control; B_2 : 2 ml of GTL ; B_4 : 4 ml of GTL ; B_6 : 6 ml of GTL

GTL was a combination of ginger (0.02%), turmeric (0.04%), and *Lactobacillus* sp. (2,997 x 10^7 cfu/ml)

Table 4. Weight of Lymphoid Organs of Broiler Fed Additive Mixture of Ginger and Turmeric extracts, and *Lactobacillus* sp. with Different Types of Feed

Factor / Parameter (%)*	A_1				A_2				A_3			
	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
21-d old												
Bursa fabriscius weight	0.074^{d}	0.077^{b}	0.079^{a}	0.079^{a}	0.073^{d}	0.076^{bc}	0.077^{b}	0.078^{ab}	0.073^{d}	0.075^{c}	0.076^{bc}	0.077^{b}
Thymus weight	0.31^{c}	0.32^{bc}	0.34^{ab}	0.35^{a}	0.30^{cd}	0.319^{c}	0.32^{bc}	0.33^{b}	0.29^{d}	0.30^{d}	0.32^{bc}	0.32^{bc}
Spleen weight	0.118^{d}	0.121^{d}	0.123^{cd}	0.123^{cd}	0.122^{cd}	0.125^{c}	0.127^{bc}	0.127^{bc}	0.123^{cd}	0.128^{b}	0.132^{a}	0.133^{a}
35-d old												
Bursa fabriscius weight	0.82^{d}	0.84^{cd}	0.87^{b}	0.89^{a}	0.80^{de}	0.82^{d}	0.85^{c}	0.87^{b}	0.78^{e}	0.81^{de}	0.84^{cd}	0.86^{bc}
Thymus weight	0.36^{bc}	0.37^{b}	0.38^{ab}	0.39^{a}	0.34^{cd}	0.35^{c}	0.36^{bc}	0.38^{ab}	0.31^{e}	0.32^{de}	0.34^{cd}	0.36^{bc}
Spleen weight	0.122^{d}	0.124^{cd}	0.125^{c}	0.127^{b}	0.125^{c}	0.126^{bc}	0.128^{b}	0.128^{b}	0.126^{bc}	0.128^{b}	0.130^{a}	0.131^{a}

$^{a-e}$ Values within the same row bearing different superscript differ significantly P<0.05)

*Values were expressed as relative weight unit (% final body weight)

A_1 : 100% self-formulated feed ; A_2 : 50% self-formulated feed+50% commercial feed ; A_3 : 100% commercial feed

B_0 : control; B_2 : 2 ml GTL ; B_4 : 4 ml GTL ; B_6 : 6 ml GTL

GTL was a combination of ginger (0.02%), turmeric (0.04%), and *Lactobacillus* sp. (2,997 x 10^7 cfu/ml) phenomenon has been clarified by Cholis *et al*. (2018) that growth inhibition of pathogenic bacteria, such as *Coliform*, and the increase in beneficial bacteria, such as lactic acic bacteria, were corelated with the low intestinal pH. Active compounds of *gingerol* in ginger and *curcumin* in turmeric would be the concominatant effect in inhibiting the growth of pathogenic bacteria since GTL was composed of three components (ginger, turmeric, and *Lactobacillus* spp.).

Lymphoid Organs in 21 and 35-day Broiler Chicken

There was significant interaction (P<0.05) between GTL dose and feed type on relative weights of bursa fabricius and thymus (Table 4). The birds in the treatment of A1B6 had the highest (P<0.05) bursa fabricius and thymus weights. The decreased *Coliform* and *Escherichia coli* populations, and on the contrary, the increased numbers of *Lactobacillus* spp. (Table 3) were the indication that the birds suffered less pathogenic microbial stress. The better microbial balance, and supported by the presence of active compounds in GTL, bring about the two lymphoid organs didn't work hard, thus the weights were maintained high. The results of the present study were in accordance with the findings of Al-Sultan (2003) and Abou-Elkhair (2014) that the treatment of turmeric had a positive effect on the weights of thymus and bursa fabriscius. Mohamed *et al.* (2012) also reported that ginger increased the weights of bursa fabricius and thymus of broilers. The mechanism of why the active compounds in turmeric and ginger can elevate the relative weight of lymphoid organ is largerly unknown. However, the capability of these active compounds in preventing the immunosuppression (negatively affecting the lymphoid organ development) may be the reason (Malekizadeh *et al*., 2012). In relation to the role of *Lactobacillus* spp., this bacteria may improve immune tissue as well as protecting it from the potential oxidative damage caused by the panthogenic bacteria (Bakari, *et al*., 2011). It has been previously described that the high weights of bursa fabricius and thymus was due to the synergistic effects of active compounds of ginger and turmeric, and *Lactobacillus* spp.

Glutathione Peroxidase (GSH-Px) and Superoxide Dismutase (SOD) Levels

Blood levels of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (Table 5) were consistent with the previous parameters. The present result showed that the highest (P<0.05) level of GSH-Px and SOD was found in A1B6 group. Endogenous antioxidant status in broiler given treatment of A1B6 would be started from the effect on improved balance of intestinal bacteria (Table 3) supported by the higher weight of lymphoid organs, sepecially bursa fabricius and thymus (Table 4). Therefore, the birds were assumed to have more comfortable live supported by the healthier gastrointestinal (high beneficial bacteria and low pathogen) which aided to the better digestibily process for the adequacy of nutrients supply, especially protein. The healthy gastrointestinal was certainly affected by bioactive compounds of ginger and turmeric and *Lactobacillus* spp. in GTL. Protein, in particular, is nedeed for oveall growth as reported by Purbarani *et al*. (2019) that probiotic *Lactobacillus* spp. combined with prebiotic increased villi height and protein digestibility.

The high levels of GSH-Px and SOD was the indication of the best antioxidant status found in birds of A1B6 group. It seemed that the bioactive contents in GTL derived from ginger, turmeric as well as *Lactobacillus* spp. played an important role in increasing the production of antioxidative enzyme. The active compound of ginger (*gingerol*) affected antioxidant activity in broiler, because it contains αβ-unsaturated ketone and had an antioxidant effect (Dugasani, 2010), and urcumin derived from turmeric also act as antioxidant that may sinergistically trigerring the activity of endogensus antioxidant (Cousins *et al*., 2007). The increased SOD and GSH-Px activity in broiler chickens treated with turmeric was in accordance with the research conducted by Daneshyar *et al*. (2012). The treatment of ginger in broilers also increased the SOD levels in broiler (Toghyani*, et al.,* 2015; Zhang *et al.*, 2009). In connection with the role of *Lactobacillus* spp., this probiotic bacteria may contribute to the increase in oxidative enzymes in broiler. Indeed, probiotic bacteria had an antioxidative activity as reported by Sugiharto *et al* (2018b). This may consequently increase the levels of antioxidant properties in broiler chicks. The present results were supported by the report of Abdurrahman *et al*. (2016a) that dietary inclusion of *Lactobacillus* spp. in combination with prebiotic increased endogenous antioxidant activity in the tissue. The increased endogenous antioxidant in the present study (Table 5) can be correlated with the reduced gastrointestinal pathogenic bacterial number

Table 5. Antioxidant Enzymes in Broiler Fed Additive Mixture of Ginger and Turmeric Extracts, and *Lactobacillus* sp. with Different Types of Feed

Factor / Parameter (U/ml)	A_1				A_2				A_3			
	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
35-d old												
GSH-Px	609.0^{j}	855.4^{f}	964.2^{b}	986.9^{a}	616.3^{j}	840.6^{g}	945.8^{c}	964.2^{b}	648.3^{i}	827.3^{h}	917.2^{e}	930.0^{d}
SOD	76.7^{g}	89.2^{e}	105.9^{bc}	109.2^{a}	77.5^{g}	86.0^{f}	103.4^{cd}	106.7^{b}	78.3^{g}	84.3^{fh}	100.6^{d}	104.2^{bc}

$^{a-h}$ Values within the same row bearing different superscript differ significantly P<0.05)

A_1 : 100% self-formulated feed ; A_2 : 50% self-formulated feed+50% commercial feed ; A_3 : 100% commercial feed

B_0 : control; B_2 : 2 ml GTL ; B_4 : 4 ml GTL ; B_6 : 6 ml GTL

GTL was a combination of ginger (0.02%), turmeric (0.04%), and *Lactobacillus* sp. (2,997 x 10^7 cfu/ml) |

Table 6. Performance Parameters of Broiler Fed Additive Mixture of Ginger and Turmeric extracts Combined with *Lactobacillus* sp.

Factor / Parameter (%)	A_1				A_2				A_3			
	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
21-d old												
Feed Intake (g)	$1,153^{de}$	$1,157^{cd}$	$1,159^{cde}$	$1,160^{cd}$	$1,150^{e}$	$1,162^{bcd}$	$1,165^{abc}$	$1,166^{abc}$	$1,140^{f}$	$1,154^{de}$	$1,169^{ab}$	$1,170^{a}$
Body weight (g)	881^{g}	953^{c}	972^{a}	973^{a}	888^{h}	937^{d}	956^{bc}	961^{b}	901^{f}	915^{e}	935^{d}	944^{c}
FCR	1.31^{ab}	1.21^{e}	1.19^{f}	1.19^{f}	1.29^{b}	1.24^{d}	1.22^{e}	1.21^{e}	1.26^{c}	1.26^{c}	$1,25^{d}$	1.24^{d}
35-d old												
Feed Intake (g)	$3,455^{e}$	$3,458^{e}$	$3,466^{de}$	$3,469^{de}$	$3,451^{f}$	$3,467^{de}$	$3,473^{cd}$	$3,476^{bc}$	$3,442^{g}$	$3,473^{cd}$	$3,479^{ab}$	$3,482^{a}$
Body Weight (g)	$2,041^{e}$	$2,282^{bc}$	$2,402^{a}$	$2,409^{a}$	$2,075^{f}$	$2,229^{c}$	$2,360^{b}$	$2,364^{b}$	$2,158^{d}$	$2,189^{d}$	$2,225^{c}$	$2,232^{c}$
FCR	1.69^{a}	1.51^{d}	1.44^{e}	1.44^{g}	1.66^{a}	1.55^{c}	1.47^{f}	1.47^{f}	1.59^{b}	1.59^{b}	1.56^{bc}	1.56^{bc}

$^{a-g}$ Values within the same row bearing different superscript differ significantly (P<0.05)

A_1 : 100% self-formulated feed ; A_2 : 50% self-formulated feed+50% commercial feed ; A_3 : 100% commercial feed

B_0 : control; B_2 : 2 ml GTL ; B_4 : 4 ml GTL ; B_6 : 6 ml GTL. GTL was a combination of ginger (0.02%), turmeric (0,04%), and *Lactobacillus* sp. (2,997 x 10^7 cfu/ml)

(Table 3). This was due to the supplementation of probiotic *Lactobacillus* spp. combined with active compound of *gingerol* and *curcumin* derived from extracts of ginger and turmeric, respectively. Probiotic can release antioxidant, such as GSH-Px enzyme, that prevents oxidative damage by pathogenic bacteria. The enzymatic mechanism as described above is consistent with the report of Murry *et al*. (2013) that probiotic, by releasing an antioxidant enzyme called GSH-Px, could prevent oxidative damage of the cell.

Production Performance of 21 and 35-day Broiler Chickens

There was significant interaction between feed type and GTL level on either day 21 or day 35 (Table 6). Feed consumption of broilers in A3B6 treatment indicated significantly (P<0.05) highest value as compared to those in A1B4 or A1B6 groups. Broilers in A1B4 and A1B6 treatments consumed lower feed but they had significantly (P<0.05) higher body weight gain. Lower feed consumption with higher body weight gain is the indication of the birds that can more efficiently utilize nutrients for growth due to feeding effect of GTL. The higher efficiency of feed/nutrient utilization was proved by the lowest FCR value in birds of A1B4 and A1B6 treatments (Table 6). The active compounds of *gingerol* and c*urcumin* in GTL aided the stimulating effect on the secretion of digestive enzymes that caused the digestive process maximally take place to provide adequate nutrients supply, and resulted the greater weight gain, especially in A1B4 or A1B6 . The active herbs compound stimulated the pancreas to release digestive enzymes in greater amounts and leading to the increased nutrients digestibility and absorption to support growth (Rajput *et al.*, 2013). In the present study, the absent of AGP in A1 feed can be postulated to increase population growth and activity of *Lactobacillus* spp. in the intestine of broiler. The improvement microbial balance in the intestine indicated by the increasing amount of *Lactobacillus* spp. can further increased the ability of digestive tract to digest and absorb nutrients (Conway and Wang, 2000).

In case of the present study, *Lactobacillus* spp. in the GTL component was another positive effect that could be able to improve gastrointestinal health and further brought about the increased digestive activity. Probiotic *Lactobacillus* spp. had a stimulating effect on broiler weight gain because of its ability in balancing the intestinal microflora to optimize nutrients digestion and absorption in broilers (Huang, *et al*., 2013). The improved health condition due to the inclusion of *Lactobacillus* spp. was further able to increase feed/nutrient digestibility and absorption via the increasing secretion and activity of digestive enzyme especially pepsin, a protein hydrolyzing-enzyme (Shabani *et al*., 2012). The nature of digestive enzym secretion was supported by the improved intestinal health due to the better microbial balance (Table 3), and higher endogenous antioxidant (Table 5) triggered by the addition of GTL. Purbarani *et al*. (2019) proved that *Lactobacillus* spp.–fortified diet combined with prebiotic given to the chickens from one-day-old increased jejunal villi height, protein digestibility, carcass percentage, and finally improved body weight. In other side, it was reported that addition of ginger through drinking water in broiler chicken can reduced FCR (Arkan *et al*., 2012). The addition of GTL increased the number of *Lactobacillus* spp. (Table 3) that can regulate the balance of the intestinal microflora through a mechanism of competitive exclusion, and optimize intestinal absorption of nutrients. The increased weights of bursa fabricius and thymus (Table 4), as humoral and systemic responses, respectively, also contributed to the overall health condition and supported well-work of digestive tract. Therefore, the healthy gastrointestinal, due to the better microbial balance, enhance the activity of digestive enzymes and improve nutrients digestibility. This digestive physiological condition give an impact on the enhanced weight gain and on the decreased FCR of broilers given self-formulated feed (A1) combined with GTL both B4 and B6 (A1B4 and A1B6), but lower GTL level (B4) is more economically efficient.

CONCLUSION

Combination of ginger and turmeric extracts (4 mL per 1 litre drinking water), and *Lactobacillus* spp. increases body resistance, supported by lower pathogenic bacteria counts and higher antioxidant status (GSH-Px and SOD), and improves growth performance of broiler fed self-formulated diet (A1B4).

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