OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

The Effect of Feeding *Lactobacillus casei* and Dahlia Tuber Extract Mixture on Intestinal Bacteria, Nutrient Digestibility, Immune Organ and Growth of Broilers

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Abstract | Maintaining a healthy digestive tract is important for poultry production. Intestinal microbial balance is beneficially influenced by either probiotics or prebiotics as well as their combination. This study aimed to investigate the feeding effect of *Lactobacillus casei* and dahlia tuber extract (LDTE) mixture on intestinal microbial, digestive and immune organs weights, nutrient digestibility, and productive performance of broiler starter. Day-old broiler chickens with an average body weight of 45.61 ± 3.46 g, a total of 160 birds, were placed evenly in 20 experimental plots. The treatment tested was the LDTE level in 100 grams of feed, LDTE0 = without LDTE, LDTE1 = 1 mL, LDTE2 = 2 mL, and LDTE3 = 3 mL. The treatment was given for 21 days, starting from d-1 until d-21. Intestinal microbial population, weights of digestive and immune organs, nutrient digestibility, and growth performance of broiler starter were the parameters observed. The research was arranged in a completely randomized design. Data were analyzed according to one-way ANOVA in a completely randomized design. Dietary supplementation of *Lactobacillus casei* and dahlia tuber extract mixture significantly increased ($p < 0.05$) nutrient digestibility and intestinal total lactic acid bacteria, but decreased coliform and improved growth performance of broilers. However, the treatment did not affect the digestive and immune organ weights of broilers. In conclusion, supplementation of LDTE2 improves intestinal bacterial balance, protein digestibility, and growth performance of broilers, but it did not affect the relative weight of digestive tract segment and immune organs of broilers.

Keywords | Broiler growth, Digestive tract, Feed additive, Intestinal bacteria, Lymphoid organ

Received | July 04, 2022; **Accepted** | September 30, 2022; **Published** | November 15, 2022
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Citation | Mangisah I, Rizqiati H, Suthama N (2022). The effect of feeding *Lactobacillus casei* and dahlia tuber extract mixture on intestinal bacteria, nutrient digestibility, immune organ and growth of broilers. Adv. Anim. Vet. Sci. 10(12): 2555-2561.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.12.2555.2561
ISSN (Online) | 2307-8316

INTRODUCTION

Feeding antibiotics as growth promoters (AGPs) and other medicinal products for the last decade have been widely used in the poultry production system to modify intestinal microbes in relation to disease control and to boost growth performance. However, continuous feeding of AGPs or other chemical substances has resulted in negative effects on either host animal or product for consumers (Alagawany et al., 2021). Due to this problem, nutritionists are encouraged to look for natural additives as alternative ingredients to improve the performance of poultry production while leaving no residue and being safe for consumers' health.

Various natural feed additives have been studied as alternative to antibiotics function as growth promoters (Sugiharto, 2016; Alagawany et al., 2021). When fed in sufficient

numbers, probiotics in the form of either a single or mixed culture of living microorganisms have been shown to have health benefits and improve immune responses (Das et al., 2012). Probiotics are made up of various microorganisms, the most common of which are lactic acid bacteria (LAB), which include *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus bulgaricus* (Sugiharto, 2016). *Lactobacillus sp.*, as a probiotic, could alter the gastrointestinal environment to promote the growth of nonpathogenic facultative anaerobic and Gram-positive bacteria that produce lactic acid and hydrogen peroxide, suppress the growth of intestinal pathogens, improve nutrient digestion and utilization, and improve feed efficiency (Markowiak and Śliżewska, 2018**).** Aside from probiotics, a prebiotic known as inulin is a type of substance that feeds colonies of beneficial bacteria in the gastrointestinal tract. Inulin derived from *Dahlia variabilis* tuber plants is a potential source of prebiotic in Indonesia, with a significant effect on chicken productivity. Based on total carbohydrate bases, the total inulin content of dahlia tuber ranged from 65 to 75% (Haryani et al., 2013). In poultry, inulin enhanced the growth of *Bifidobacterium* and *Lactobacillus*, thus improving the host's microbial balance**.** Inulin can influence the microbiota profile, gut morphology, and improve the body weight of broiler (Li et al., 2018).

Previous reports regarding *Lactobacillus* as a probiotic and inulin as a prebiotic for poultry were available elsewhere (Wu et al., 2019; Yuanita et al., 2019). However, there was limited information on the feeding effect of *L. casei* and inulin derived from dahlia tuber extract in broiler starter. The effect of feeding a mixture of *L. casei* and dahlia tuber extract on broiler productivity should be investigated further. Therefore, the present study was conducted to evaluate the effect of feeding a mixture of *L. casei* and inulin derived from dahlia tuber extract on relative weight of digestive organ weight, intestinal microbial population, nutrient digestibility, and productivity of broilers during the starter phase.

MATERIALS AND METHODS

Experimental Animals and Diets

This research was supervised and was approved by the Animal Research Ethics Committee, Faculty of Animal and Agricultural Sciences, Diponegoro University. A total of 160 Lohmann MB-202, 1-day old broiler, with an average body weight of 45.61 ± 3.46 g were obtained from a commercial hatchery of Charoen Pokphand in Indonesia. Basal feed was composed of soybean meal, maize, rice bran, CaCO3 and meat bone meal (MBM). Feed nutritional content was in accordance with the Indonesian National Standards No 01-3930-2006 as described in Table 1.

Table 1: Ingredients composition and nutrients content of experimental diet

Ingredients	Composition (%)
Maize	45.00
Rice bran	19.00
Soybean meal	26.20
Meat bone meal	8.50
$CaCO_3$	0.30
Vitamin-mineral premix[1]	1.00
Total	100
Nutrient contents:	
Metabolizable energy (kcal/kg)[2]	3001.15
Crude protein (%)[3]	21.24
Crude Fiber (%)[3]	5.71
Ether extract (%)[3]	3.85
Calcium (%)[3]	1.12
Available Phosphorus (%)[3]	0.63
Methionine (%)[4]	0.42
Lysine (%)[4]	1.31

[1] Ingredient in 10 kg =Vitamin A 12.500.000 IU, Vitamin D3 2.500.000 IU, Vitamin E 10.000 mg, Vitamin K3 2.000 mg, Vitamin B1 2.000 mg, Vitamin B2 4.000 mg, Vitamin B6 1.000 mg, Vitamin B12 12.000 mg, Vitamin C 30.000 mg, Niacin 4.000 mg, Ca-d-pantothenate 200 mg, Biotin 200 mg, L - Arginine 10.000 mg, L - Threonine 15.000 mg, DL-Methionine 50.000 mg, L-Lysine 125.000 mg, Choline 20.000 mg, Folic Acid 500 mg, Zinc 70.000 mg, Ferros 30.000 mg, Manganese 60.000 mg, Copper 5.000 mg, Iodida 200 mg, Selenium 150 mg, Cobalt 200 mg, Antioxidant, carrier 10 mg

[2]Metabolizable energy was calculated based on formula of Bolton (1967) as follows: 40.81 [0.87 (Crude Protein + 2.25 crude fat + nitrogen free extract) + 2.5]

[3]Analisis results of the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University

[4]The values were calculated based on table of National Research Council (1994)

Experimental Design and Treatments

A completely randomized design with four treatments, each treatment involved five replications. The treatments were as follows:

LDTE0: basal diet without *Lactobacillus casei* and dahlia tuber extract mixture (LDTE) addition

LDTE1: basal diet + 1 mL LDTE /100 g feed

LDTE2: basal diet + 2 mL LDTE /100 g feed

LDTE3: basal diet + 3 mL LDTE /100 g feed

Preparation of *Lactobacillus casei* and Dahlia Tuber Extract Mixture

The preparation of the mixture began with the maceration of dahlia tuber extract in ethanol and progressed to the rejuvenation of *L. casei*. One loop of *L. casei* isolate was

inoculated in 50 mL of 10% skim milk and then incubated for 2 × 24 h. Incubation product was re-inoculated in 450 mL of skim milk (total mixed solution was 500 mL). Furthermore, 500 mL of liquid mixture was added with 1.5% sterilized dahlia tuber extract, and then it was re-incubated for 2 × 24 h. The bacterial population was finally counted using total plate count method (Yunita et al., 2015). Mixture of *L. casei* (10^9 cfu/mL) and dahlia tuber extract (1.5%) was used for the treatments.

Animals Rearing Management

All chickens were firstly weighed to determine initial body weight, and subsequent weighing was performed once a week. Chickens were fed experimental diets *ad libitum*, and provided 24 h access to drinking water, from one-day old until 21 days of rearing period (during the starter period).

Sampling and Parameters Measurement

Digestive and lymphoid organs: Sampling for parameters measurement were performed at day 21. One bird per replicate with the mean body weight close to the average group was selected, weighed, and then sacrificed by cervical dislocation. The gastrointestinal tract (GIT) from the proventriculus until the end of the large intestine was separated, and the digesta was removed. The empty proventriculus, ventriculus, duodenum, jejenum, ileum, seca, and colon were weighed and were expressed as a percentage of live body weight. Lymphoid organs were weighed and were expressed as a percentage of live body weight according to the previously described method (El-Deep et al., 2016).

Microbial population: Total LAB and coliform bacteria counts were determined from digesta samples of the ileum of 20 chickens based on the method described by Setyaningrum et al. (2019). One gram of the sample was transferred to 9 mL of sterile physiological saline, homogenized, diluted up to 10^{-8}, and then plated in de Man Rogosa and Sharpe agar, and finally anaerobically incubated at 37 °C for 48 h to calculate LAB. Coliform counts calculation were carried out by homogenizing digesta samples, diluted up to 10^{-6}, plated in MacConkey agar, and then anaerobically incubated at 37 °C for 24 h.

Nutrient digestibility: Nutrient digestibility was measured on 21-day-old chickens with total collection. The excreta were collected, weighed, dried, and analyzed for nutrients content. Nutrient digestibility including crude protein, extract ether, and crude fiber were measured with slightly modified methods of Kong and Adeola (2014), as follows:

$$\text{Nutrient digestibility} = \left[\frac{(\text{nutrient consumed} - \text{nutrient in excreta})}{\text{nutrient consumed}}\right] \times 100$$

Growth performance: Growth performances, including feed intake, weight gain, and feed conversion ratio were measured. Feed intake was recorded daily by subtracting the total amount of feed given with the remaining feed. Feed conversion ratio was calculated based on the ratio between feed consumption and body weight gain.

Statistical Analysis

Data were analyzed according to one-way ANOVA and Duncan's multiple range test by SPSS program.

RESULTS

Digestive Organs

Supplementation levels of LDTE in broiler diet on gastrointestinal weights are presented in Table 2. Dietary additions of LDTE indicated no significant effect on all parts of gut segments including relative weights of esophagus, crop, proventriculus, ventriculus, intestine, seca, and colon.

Microbial Population

Dietary supplementation of LDTE during the starter phase significantly increased ($p<0.05$) LAB population. On the other hand, supplementation of LDTE significantly decreased ($p<0.05$) coliform counts, but it did not affect intestinal pH (Table 3). The LAB populations in either LDTE2 or LDTE3 were the same, and were significantly ($p<0.05$) higher than those in LDTE1 and the control group. In contrast, the total coliform in both treatments, LDTE2 and LDTE3, were significantly ($p<0.05$) lower than those in LDTE1 and the control (LDTE0).

Nutrient Digestibility

Supplementation a combination of *L. casei* and dahlia tuber extract significantly affected nutrient digestibility in broiler starter (Table 4). Protein digestibility in LDTE1, LDTE2, and LDTE3 treatments was higher ($p<0.05$) than that in the control group (LDTE0). In contrast, lipid digestibility in the treatments of LDTE1, LDTE2, and LDTE3 was lower ($p<0.05$) than that in the control group, but there was no difference among those of three treatments (LDTE1, LDTE2, and LDTE3). However, the LDTE treatment did not affect digestibility of dietary crude fiber.

Immune Organs

Statistical analysis indicated that dietary supplementation of LDTE did not affect the relative weight of lymphoid organs (bursa of fabricius, thymus, and spleen) in broiler starter (Table 5). The administration of LDTE at 1 to 3 mL/100g feed apparently had no effect on the relative weight of immune organs.

Growth Performance

Statistical analysis showed that dietary supplementation of

Table 2: Digestive organs relative weight of broiler chicks at the end of starter period

Items (% final body weight)	Treatments					
	LDTE0	LDTE1	LDTE2	LDTE3	SEM	*p-value*
Esophagus	0.14	0.15	0.14	0.19	0.02	0.17
Crop	0.31	0.51	0.43	0.39	0.03	0.11
Proventriculus	0.68	0.71	0.69	0.63	0.08	0.22
Ventriculus	2.61	2.79	3.01	2.48	0.03	0.39
Intestine	2.75	2.78	3.00	2.91	0.18	0.10
Seca	0.58	0.54	0.52	0.55	0.09	0.56
Colon	0.15	0.15	0.16	0.20	0.03	0.27

LDTE0, 0 mL/100 g feed, LDTE1, 1 mL/100 g feed, LDTE2, 2 mL/100 g feed and LDTE3, 3 mL/100 g feed. SEM, standard error of mean

Table 3: Total bacteria and pH in intestinal of broiler chicks at the end of starter period

Items	Treatments					
	LDTE0	LDTE1	LDTE2	LDTE3	SEM	*p-value*
Total LAB (10^9 cfu/g)	4.0^{b}	4.5^{b}	5.1^{a}	5.2^{a}	0.57	0.03
Coliform (10^8 cfu/g)	5.09^{a}	3.62^{b}	4.22^{b}	3.84^{b}	0.75	0.02
pH of intestine	5.75	5.5	5.5	5.25	0.45	0.13

^{ab} Mean values in the same row without common superscript differ at p<0.05; LDTE, 0 mL/100 g feed, LDTE1, 1 mL/100 g feed, LDTE2, 2 mL/100 g feed and LDTE3, 3 mL/100 g feed. SEM, standard error of mean

Table 4: Nutrient digestibility of broiler chicks at the end of starter period

Items	Treatments					
	LDTE0	LDTE1	LDTE2	LDTE3	SEM	*p-value*
Protein Digestibility (%)	76.61^{b}	82.08^{a}	78.04^{a}	78.43^{a}	2.32	0.003
Lipid digestibility (%)	87.49^{a}	75.73^{b}	72.04^{b}	75.95^{b}	3.45	0.002
Crude fiber digestibility (%)	23.95	26.56	26.04	24.41	2.24	0.06

^{ab} Mean values in the same row without common superscript differ at p<0.05; LDTE, 0 mL/100 g feed, LDTE1, 1 mL/100 g feed, LDTE2, 2 mL/100 g feed and LDTE3, 3 mL/100 g feed. SEM, standard error of mean

Table 5: Immune organs of broiler chicks at the end of starter period

Items (% final body weight)	Treatment					
	LDTE0	LDTE1	LDTE2	LDTE3	SEM	*p-value*
Bursa fabricius	0.27	0.31	0.29	0.32	0.06	0.20
Thymus	0.21	0.18	0.15	0.22	0.02	0.45
Spleen	0.12	0.09	0.12	0.11	0.02	0.48

LDTE, 0 mL/100 g feed, LDTE1, 1 mL/100 g feed, LDTE2, 2 mL/100 g feed and LDTE3, 3 mL/100 g feed. SEM, standard error of mean

Table 6: Performance of broiler at the end of starter period

Items	Treatment					
	LDTE0	LDTE1	LDTE2	LDTE3	SEM	*p-value*
Feed intake (g/bird)	693.42^{b}	725.55^{a}	694.52^{b}	706.55^{b}	8.96	0.05
Body weight gain (g)	403.50^{b}	414.13^{a}	415.88^{a}	416.89^{a}	7.24	0.01
FCR	1.72^{b}	1.75^{b}	1.67^{a}	1.69^{a}	0.14	0.01

LDTE, 0 mL/100 g feed, LDTE1, 1 mL/100 g feed, LDTE2, 2 mL/100 g feed and LDTE3, 3 mL/100 g feed. SEM, standard error of mean

LDTE at 1 to 3 mL significantly ($p<0.05$) affected growth performance, including feed intake, body weight gain, and FCR in the broiler starter (Table 6). Feed intake in LDTE1 was the highest value and significant ($p<0.05$) as compared to other treatments (LDTE0, LDTE2, and LDTE3), but no differences was observed among those the three treatments. Body weight gain was significantly higher ($p<0.05$) due to feeding effect of all LDTE levels (1 to 3 ml/100 g feed) than that of control (LDTE0). However, the significantly ($p<0.05$) decreased FCR was found only in two level of dietary LDTE supplementation at 2 ml (LDTE2) and 3 ml (LDTE3) per 100 g feed, but FCR between two other treatments (LDTE0 and LDTE1) was the same (Table 6).

DISCUSSION

In this study, supplementation of a mixture of *L. casei* and inulin from dahlia tuber extract did not affect the weight of the digestive tract organs in starter period of broiler. Supplementation of LDTE until 3ml/100kg feed did not exert the synergistic work in stimulating overall parts of digestive organs, which could be attributed to a lack of promoting level, despite affecting bacterial populations. Inulin cannot be hydrolyzed by intestinal enzyme of monogastric animal, but it can be fermented by selected bacteria. However, although it was not determined in the current study, the fermentation products in the form of short chain fatty acids (SCFA) were presumably insufficient to stimulate the development of gut organs. This result contradicted to the report of Liu et al. (2021), who found that a high concentration of SCFA improved gut morphology, maintained intestinal barrier function, and had a positive effect on gut development. The present study was in line with the results of Olnood et al. (2019) that feeding probiotic *Lactobacillus johnsonii* did not affect the relative weight of internal organs (liver, spleen, bursa, duodenum, jejunum, and ileum) of broilers.

Feed supplemented with LDTE, especially at the level of 2 and 3 ml/100 g feed (LDTE 2 and LDTE3) significantly increased LAB population and with all levels reduced coliform counts in the intestine of broilers. Inulin from dahlia tuber extract provides a specific substrate as "nutrition source" for the growth of *L. casei*, reflected by the increase in BAL population in the intestine. Inulin was shown to have function in promoting the growth of *Lactobacillus* and *Bifidobacteria* (Moens and de Vuyst, 2017). Feeding dietary inclusion of LDTE2, and LDTE3 brought about the improvement of intestinal microenvironment indicated by the increased LAB and the decreased coliform, although intestinal pH was unchanged. Kridtayopas et al. (2019), found that feeding a broiler diet supplemented with *Lactobacillus* could reduce *E. coli* numbers in the intestine and ceca. Probiotics stimulated beneficial microorganisms in the gut surface through the mechanism of competitive exclusion with pathogens, competed for nutrients, and produced antimicrobial compounds that inhibited pathogen growth (Neveling et al., 2017).

It was similar to the digestive tract weight that dietary combination of *L. casei* and inulin from dahlia tuber extract (LDTE) exerted no effect on the relative weights of lymphoid organs (bursa of fabricius, thymus, and spleen). The lack of LDTE supplementation effect on lymphoid organs was most likely due to no significant change in bacterial composition. Dietary inclusion of LDTE increased intestinal LAB and decreased coliform populations, but coliform counts was higher than total LAB in the control group. Antibacterial properties of *L. casei* can strongly counteract toxin substance produced by the pathogen (coliform) in such population range because of combining effect with inulin extract as "nutrition source". Therefore, it is possibly had little effect on the function of lymphoid organs, and thus, no change in weight was observed. Antibacterial compounds produced by probiotic bacteria was known to have function in overcoming the harmful effects of pathogens such as *E.coli* and other pathogenic bacteria (Wang et al., 2020: Husain et al., 2020). Several studies (Pourakbari et al., 2016, Hidayat et al., 2020) showed similar results with this study that probiotic supplementation had no effect on lymphoid organs.

It was already stated in the previous paragraph that dietary inclusion of LDTE at the levels of 1 to 3 ml/100 kg feed improved intestinal health by inhibiting pathogenic bacteria and stimulating LAB growth. The healthier intestinal tract condition can be facilitated the improvement of nutrients utilization, which was proven by the higher protein digestibility in LDTE added-feed compared to control. Previous studies supported the present result that supplementation of *L. acidophilus* in broilers ration increases nutrient digestibility (Wu et al., 2019). On the other hand, the control group (LDTE0) had the highest lipid digestibility when compared to the other three treatments, LDTE1, LDTE2, and LDTE3.

Inulin derived from dahlia tuber extract is a specific substrate as a "nutrition source" for *L. casei* that can be fermented to result SCFA, as it has been previously discussed. The present of organic acids, component of SCFA, is possible to reduce potential hydrogen (pH) of the gut, and promotes the growth of *Lactobacillus* in general and LAB in particular. Organic acids had propound effect on the increased LAB population in small intestine, and improved bile salts deconjugation ability of that beneficial bacteria (Loh et al., 2013). The increased growth of LAB correlates with the higher production of bile salt hydrolase (BSH). This BSH enzyme is able to deconjugate bile salts, mak-

ing the lipid difficult to emulsify, and thus, inhibits lipid hydrolysis by the lipase enzyme. Therefore, lipid digestibility was lower in LDTE-added feed (LDTE1, LDTE2, and LDTE3) than that in LDTE0 (control). Kirana et al. (2017) found that the BSH enzyme produced by LAB could deconjugate bile salts into free cholic acid, which is less absorbed by the small intestine and thus reduces lipid absorption. Sharifi et al. (2012) support the current study's results that adding probiotics containing LAB reduced lipid digestibility in broilers (81.6% vs. 82.7%).

The improved gastrointestinal health, as indicated by higher LAB population and lower coliform count, brought about the increase in protein digestibility, which has a positive impact on broiler performance during the starter phase. Feed consumption in LDTE1 was significantly higher ($p<0.05$) than in all other treatments, but that in LDTE2 and LDTE3 showed a tendency to increase ($p=0.05$). Feed consumption was significantly higher only in LDTE1 and that in two other LDTE treatments (LDTE2 and LDTE3) tended to increase, as previously described. However, protein digestibility increased in all LDTE treatments is an indicator of the availability of nutrients, especially protein and amino acids, for the synthesis of meat tissue and contributes to growth. This phenomenon is indicated by the increased BWG due to the dietary inclusion of LDTE that was better than control. On the contrary, control group showed lower feed intake and BWG than LDTE treatments, especially at the level of 1 mL/100g feed. Feed conversion ratio (FCR) in the control group did not differ with LDTE1. Supplementation of LDTE1 increased nutrient digestibility but did not improve FCR. The increased protein digestibility in LDTE1 may be used for completing organ development in birds at the starter phase. Several studies have shown that a mixture of probiotics and prebiotics could improve growth performance in poultry (Yuanita et al., 2019; Setyaningrum et al., 2019).

CONCLUSION

Supplementation of LDTE in 100 g feed at the level of 2 and 3 ml (LDTE2 and LDTE3) indicate same results in term of the improvement of intestinal bacterial balance, nutrient, especially protein digestibility, and broiler growth performance, but no effect is observed for relative weight of gastrointestinal tract segments and immune organs. The conclusion is focused on the level of LDTE at 2 ml/100g feed (LDTE2) because it is considered to be more efficient since smaller amount is needed.

ACKNOWLEDGEMENT

This research was supported by "Program Riset Pengembangan dan Penerapan (RPP) Univeritas Diponegoro Indonesia".

CONFLICT OF INTEREST

The authors declare that there is no conflict related to the material used and described in the manuscript.

NOVELTY STATEMENT

The novelty of this research is the use of a mixture of *Lactobacillus casei* and dahlia tuber extract which is given to broiler chickens in the starter phase and their parameters are observed at the end of the starter phase.

AUTHORS CONTRIBUTION

Istna Mangisah compiled the research concept, collected and processed research data and drafted manuscript. Heni Rizqiati analyzed the data and supervised this research. Nyoman Suthama supervised and responsible for finalizing manuscript.

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