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Research Article

Combination of Gembili Tuber and *Lactobacillus plantarum* on the Performance, Carcass, Hematological Parameters, and Gut Microflora of Broiler Chickens

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Abstract | The purpose of the research to evaluate the combination of gembili tubers with *Lactobacillus plantarum* (CGLp) on the performance, carcass, hematological parameters, and gut microflora of broiler chickens. The research used 144 one-day-old broiler chicks (44.33±1.09 g). The research was conducted with a completely randomized design. The treatments included of T0: diet + 0% CGLp (control), T1: diet + CGLp 1.0%, T2: diet + CGLp 1.5%, T3: diet + CGLp 2.0%, T4: diet + CGLp 2.5% and T5: diet + CGLp 3.0%. The treatment of CGLp was significantly increased (p <0.05) of body weight gain, live body weight, carcass percentage, meat protein content and lactic acid bacteria. The treatment decreased (p<0.05) of feed conversion ratio (FCR), meat fat content, meat cholesterol and total coliform in the ileum and caecum. The treatments of CGLp were not affected (p> 0.05) of feed intake, immune organs, hematological and pH in the ileum and caecum. In conclusion, the CGLp treatments improve the performance, carcass, and gut microflora of broiler chickens.

Keywords | Gembili tuber, Broiler, Probiotic, Performance, Gut intestinal

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INTRODUCTION

The use of antibiotics growth promoters (AGP) in animal feed has been forbidden by the European Union, due to its detrimental effects such as residues at poultry product and antibiotic resistance (Abdurrahman et al., 2016). Therefore, synbiotics are one safe alternative as a replacement of AGP for enhancing livestock health at present. Synbiotics are probiotic and prebiotic combinations that work synergistically (Abdel-Hafeez et al., 2017). Utilization of synbiotic in broiler chickens has been shown to improve the performance growth, increasing the intestinal microflora population, meat quality and carcass characteristics (Hassanpour et al., 2013; Mohammed et al., 2021). Several studies have also demonstrated that the utilization of synbiotics in broiler chicken has a good impact on the carcass quality (Abdel-Wareth et al., 2019; Rehman et al., 2020). In recent years, various types of probiotic strains and prebiotics have been utilized in synbiotic study, with varying results.

Gembili tuber is one type of plants that acts as a prebiotic, because gembili tuber was known to contain of 14.77% inulin (Winarti et al., 2011). In addition, the extracts of inulin from gembili tuber had a beneficial influence on the *Lactobacillus plantarum* growth (Zubaidah and Akhadiana, 2013). Previous research by Setyaningrum et al. (2019) showed that the extracts of inulin from gembili tuber which was obtained through the extraction process with ethanol and combined with *Lactobacillus plantarum* as synbiotic can enhance the broiler chicken growth.

Therefore, current research will examine the use of prebiotics derived from gembili tuber flour without any process combined with *Lactobacillus plantarum* on the broiler chicken performance. It was supposed the synbiotic product can be easily applied by the farmers. Moreover, evidence on the effect combination of gembili tuber flour with *Lactobacillus plantarum* (CGLp) on the performance of broiler chickens has yet to be discovered. According to previous study, the utilization of gembili tuber on broiler chicken is only limited used as a prebiotic (Fajrih and Khoirudin, 2020). While the other study explained that the administration of gembili tuber combined with *Lactobacillus plantarum* at native chicken was effective (Setyaningrum et al., 2022). Based on that, as an alternative of AGP, the usage of CGLp is expected will have positive impact on the broiler chicken performance. The purpose of the research to evaluate the combination of gembili tubers with *Lactobacillus plantarum* (CGLp) on the performance, carcass, hematological parameters, and gut microflora of broiler chickens.

MATERIALS AND METHODS

Preparation of Combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp)

The CGLp was prepared in accordance with Setyaningrum et al. (2022). In this study, a nine-month-old gembili tuber was used. The gembili tubers were peeled, then sliced into small slices, dried and ground into flour. The *Lactobacillus plantarum* isolate (FNCC 0027) was derived from Laboratory of Biotechnology, Gadjah Mada University. The isolate of *Lactobacillus plantarum* was then cultured to population 10^9 cfu/ml at Experimental Garden and Animal Husbandry Laboratory of University of Pembangunan Panca Budi. The gembili tuber flour was then mixed with 5% of *Lactobacillus plantarum*, then that mixture was incubated at 37°C of temperature for 4 days.

Birds, diet, and treatments

The research was carried out in accordance with the standard procedures of rearing and treating for livestock stated in law of the Republic of Indonesia number 18, 2009 concerning animal husbandry and animal health. In this study used of 144 one-day-old chicks of broiler chicken (44.33±1.09 g) were obtained from local hatchery. All birds were randomly placed into 24 units, each of which contains of six chicks. All birds were kept in open sided housed with naturally ventilation. This research used rice husk as a litter material with litter thickness of 5 cm. Light was available along day. The feeding and drinking were practiced ad libitum. Feeding also drinking water were manually conducted in this study. The experimental diet was formulated according to NRC (1994) recommendation (Table 1). The treatments included of T0: diet + 0% CGLp (control), T1: diet + CGLp 1.0%, T2: diet + CGLp 1.5%, T3: diet + CGLp 2.0%, T4: diet + CGLp 2.5% and T5: diet + CGLp 3.0%. The treatment of CGLp was mixed into the experimental diet according to each treatment. The treatments were conducted for 42 d period.

Table 1: Ingredient and nutrient composition of experimental diet

Ingredient (%)	Starter (d one -21)	Finisher (d 22-42)
Corn	49.3	51.9
Rice bran	6	10
Soy bean meal	31.2	26
Fish meal	10	10
Palm oil	2.5	1.5
Premix*	1	0.6
Total	100	100
Composition of nutrient		
Crude protein (%)	22.37	20.62
Metabolizable Energy (kcal/kg)	3048.09	3020.19
Crude fiber (%)	5.64	6.79
Crude eter (%)	5.40	5.28
Calcium (%)	1.08	1.02
Phosphor (%)	0.75	0.60
Methionine (%)	0.52	0.50
Arginine (%)	1.76	1.62
Lysine (%)	1.66	1.51

*Each 10 kg contained vitamin A 12000000 IU; Vitamin D_3 2000000 IU; Vitamin E 8000 IU; Calcium-D-panthotenate 6000 mg; Fe 20000 mg; Cu 4000 mg; Co 200 mg; Zn 100000 mg

Data collecting

The body weight gain of broiler chicken was weighed every week. The weighed of feed intake was carried out daily by calculated the difference of feed given with the remaining feed and accumulated to calculated the weekly feed intake. The feed conversion ratio (FCR) was determined based on feed intake and body weight gain.

On day 42, one chicken was randomly selected for each replication for the measurements of carcass, immune organs and the intestinal microflora. The chicken from each replication was slaughtered and de-feathered. After eviscerated, carcass and immune organs (spleen, thymus, and bursa of fabricius) were weighed. The carcass and immune organ were calculated as a percentage of the live weight (Sugiharto et al., 2017). Carcass meat was skinned and deboned before the measurement of meat chemical parameters. Meat protein and fat levels were measured according to the AOAC (2007), and meat cholesterol levels were measured according to Abdurrahman et al. (2016). Sample digesta was collected from the ileum and cecum to determine of intestinal microflora (pH, coliforms and lactic acid bacteria (LAB)). Measurement of pH digesta on ileum also cecum based on Setyaningrum et al. (2019), digesta as much as 1 gram mixed with distilled water 10 ml, homogenized then measured with a digital pH meter. Measurement of total lactic acid and coliform bacteria based on Calik et al. (2017) with a slight modification, in which the fresh digesta 1 g was placed into sterile physiological saline solution 9 ml, homogenized and serially diluted up to 10^{-4} (coliform) and 10^{-6} (LAB). Measurement the total LAB was using MRSA (de Man Rogosa and Sharpe Agar) and anaerobically incubated for 48 hours at temperature 37 ºC. MacConkey Agar was used for determine coliforms and anaerobically incubated for 24 hours at temperature 37 ˚C. Data were presented as log 10 colony forming units per gram digesta.

At the age of 42 days, one chicken was used for each replication for the measurement of hematological parameter. Measurement of hematological parameters according of Al-Khalaifa et al. (2019). Blood was taken from the wing veins and was put in EDTA (ethylene diamine tetra acetic acid) tube in order to measurement of leukocyte count, packed cell volume (PCV), erythrocytes, and hemoglobin (Hb).

Statistical Analysis

The research was conducted with completely randomized design. All data was obtained were analyzed by analysis of variance (ANOVA). If there were differences among the treatments, Duncan's multiple range test was applied.

RESULTS

The potential of gembili tuber flour as a prebiotic

The content of inulin, sucrose, raffinose and mannose of gembili tuber flour was presented in Table 2. The inulin content of this result was 1.78% w/w.

Table 2: Inulin, sucrose, raffinose and mannose content of gembili tuber flour

Parameters	Method	Level
Inulin (% w/w)	HPLC	1.78
Sucrose (%)	HPLC	1.11
Raffinose (%)	HPLC	0.02
Mannose (%)	HPLC	0.01

Growth performance

The average of body weight gain (BWG), feed intake, FCR and mortality were showed in Table 3. The CGLp treatments had no significant different ($p>0.05$) on feed intake, although the numerically treatments of CGLp (1.0%, 1.5%, 2.0%, 2.5% and 3.0%) increased the feed intake of broiler compared to control (CGLp 0%). Based in this study, CGLp treatments raised the BWG of broiler chickens compare to control. Supplementation of CGLp 1.5% produced the greatest BWG, followed by CGLp 2.5%, CGLp 2.0%, CGLp 3.0% and CGLp 1% treatments. The treatments of CGLp 0% CGLp had the lowest of BWG. The CGLp supplementation significantly reduced ($p<0.05$) the FCR of broiler chickens. FCR in chickens treated with 1.5% CGLp was lower ($p<0.05$) compared to those treated with 0% CGLp and 1.0% CGLp. The FCR value at 1.5% CGLp treatment was the same in chickens at 2%, 2.5%, and 3% CGLp. There was no mortality of broiler during the study period.

Carcass and meat chemical parameters

The CGLp treatments affected ($p<0.05$) live body weight (LBW) of broilers (Table 4). Live body weight of broiler without the CGLp supplementation (T0) was lower than that of the chickens supplemented with CGLp (T1, T2, T3, T4 and T5 treatments). Live body weight of broilers was highest at the 1.5% CGLp supplementation (T2). According to this study (Table 4) the supplementation of CGLp had been significantly ($p<0.05$) improved the carcass percentage of broiler chicken. The carcass percentage of broiler chicken was supplemented with CGLp was higher than of the control (CGLp 0%). This study showed that CGLp treatment increased ($p<0.05$) the meat protein content also decreasing ($p<0.05$) the meat fat and cholesterol levels in broilers (Table 4).

Immune organ

Based on Table 5, the treatments of CGLp did not affect ($p>0.05$) on the relative weight of spleen, bursa of fabricius, and thymus of broiler chickens. This condition indicated that the administration of CGLp had not been able to affect the broiler chickens of immune system. Similarly, Hidayat et al. (2020), supplementation of *Lactobacillus paracasei* was not influenced on the thymus, bursa of fabricius and spleen of broilers.

Intestinal microflora

The treatments of CGLp were not affected ($p>0.05$) on the ileum and cecum pH of broiler (Table 6). The results showed that the treatments of CGLp had no effect on the ileum or cecum pH, but the pH was decreased in the ileum and cecum compare with control. The treatments of CGLp significantly increased ($p<0.05$) LAB and significantly ($p<0.05$) reduced coliform in both at the ileum also cecum (Table 6).

Table 3: Effects of combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp) on growth performance

Parameters	Treatments						SEM	p value
	T0	T1	T2	T3	T4	T5		
FI (g/bird)	2340	2402.6	2342.2	2382.4	2353.7	2356.2	47.3	0.57
BWG (g/bird)	1170[b]	1246.5[ab]	1337[a]	1308[a]	1316.5[a]	1280.8[a]	52.2	0.03
FCR	2.01[a]	1.93[ab]	1.76[c]	1.82[bc]	1.79[bc]	1.85[bc]	0.06	0.02
Mortality (%)	0	0	0	0	0	0	-	-

[abc] Means within the same row with different letter are significantly different ($p<0.05$), FI (feed intake), BWG (body weight gain), FCR (feed conversion ratio), T0 (control), T1 (diet + CGLp 1.0%), T2 (diet + CGLp 1.5%), T3 (diet + CGLp 2.0%), T4 (diet + CGLp 2.5%), T5 (diet + CGLp 3.0%), SEM (standard error of mean)

Table 4: Effects of combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp) on carcass and meat chemical parameters

Parameters	Treatments						SEM	p value
	T0	T1	T2	T3	T4	T5		
Live body weight (g)	1086.3[b]	1236.8[a]	1310.3[a]	1289[a]	1300.8[a]	1237[a]	37.6	0.001
Carcass percentage (%)	61.7[b]	64.4[a]	66.3[a]	65.2[a]	65.4[a]	61.8[b]	1.55	0.004
Meat protein content (%)	18.0[c]	18.9[bc]	19.7[ab]	19.0[bc]	19.5[ab]	20.5[a]	0.70	0.003
Meat fat content (%)	4.26[a]	3.26[b]	3.44[b]	3.30[b]	3.47[b]	3.54[b]	0.21	0.0004
Meat cholesterol (mg/100g)	105[a]	89.6[b]	78.3[c]	73[e]	75.3[d]	67.2[f]	0.37	<0.0001

[abc] Means within the same row with different letter are significantly different ($p<0.05$), T0 (control), T1 (diet + CGLp 1.0%), T2 (diet + CGLp 1.5%), T3 (diet + CGLp 2.0%), T4 (diet + CGLp 2.5%), T5 (diet + CGLp 3.0%), SEM (standard error of mean)

Table 5: Effects of combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp) on immune organ

Parameters	Treatments						SEM	p value
	T0	T1	T2	T3	T4	T5		
Spleen (%)	0.13	0.12	0.12	0.11	0.11	0.13	0.01	0.32
Bursa of fabricius (%)	0.11	0.12	0.12	0.13	0.13	0.14	0.01	0.37
Thymus (%)	0.22	0.23	0.28	0.26	0.23	0.29	0.03	0.14

T0 (control), T1 (diet + CGLp 1.0%), T2 (diet + CGLp 1.5%), T3 (diet + CGLp 2.0%), T4 (diet + CGLp 2.5%), T5 (diet + CGLp 3.0%), SEM (standard error of mean)

Table 6: Effects of combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp) on pH and intestinal microflora ($\log_{10}$ cfu/g)

Parameters	Treatments						SEM	p value
	T0	T1	T2	T3	T4	T5		
Ileum								
pH	6.46	6.03	6.13	6.24	6.00	6.27	0.22	0.39
LAB	6.29[c]	7.20[b]	7.92[a]	7.37[ab]	7.40[ab]	7.38[ab]	0.27	0.001
Coliform	6.12[a]	5.26[b]	5.22[b]	5.42[b]	5.45[b]	5.41[b]	0.35	0.01
Caecum								
pH	6.73	6.18	6.46	6.47	6.43	6.33	0.22	0.40
LAB	6.56[b]	7.23[a]	7.45[a]	7.57[a]	7.09[ab]	7.42[a]	0.30	0.04
Coliform	5.39[a]	5.08[ab]	4.88[b]	5.22[a]	5.14[ab]	5.15[ab]	0.16	0.04

[ab] Means within the same row with different letter are significantly different ($p<0.05$), LAB (lactic acid bacteria), T0:(control), T1 (diet + CGLp 1.0%), T2 (diet + CGLp 1.5%), T3 (diet + CGLp 2.0%), T4 (diet + CGLp 2.5%), T5 (diet + CGLp 3.0%), SEM (standard error of mean)

Table 7: Effects of combination of gembili tuber flour and *Lactobacillus plantarum* (CGLp) on Hematological parameters

Parameters	Treatments						SEM	*p* value
	T0	T1	T2	T3	T4	T5		
Leukocyte (10^3/µL)	22.1	19.6	23.2	20.5	23.2	22.0	2.13	0.34
Erythrocytes (10^6/ µL)	2.79	2.73	2.62	2.73	2.79	2.71	0.25	0.96
Haemoglobin (g/dL)	12.3	12.3	11.6	12.4	12.0	12.2	1.45	0.97
Packed cell volume (%)	30.4	30.9	29.4	30.3	31.9	30.5	2.80	0.95

T0 (control), T1 (diet + CGLp 1.0%), T2 (diet + CGLp 1.5%), T3 (diet + CGLp 2.0%), T4 (diet + CGLp 2.5%), T5 (diet + CGLp 3.0%), SEM (standard error of mean)

Hematological parameter

CGLp supplementation had no effect (p>0.05) on leukocytes, erythrocytes, packed cell volume (PCV) and hemoglobin (Hb) of broiler chickens. According to the findings (Table 7), the hematological level was within the normal range (Al-Khalaifa et al., 2019; Zanu et al., 2020). These results were similar with Setyaningrum et al. (2019), synbiotic supplementation did not affect the leukocytes, erythrocytes, hemoglobin and PCV of broiler chickens.

DISCUSSION

Table 2 indicates that gembili tuber flour can act as a prebiotic. This study was in line with Buclaw (2016), inulin is a natural oligosaccharide found in plants, such as chicory root (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) tubers. Petkova et al. (2018), inulin from dahlia tubers was 42%. Inulin can maintain the viability of *Lactobacillus, Bifidobacterium, Streptococcus* and others (Narala et al., 2022).

In this study, CGLp treatment did not influence (p>0.05) on the feed intake of broilers. Previous research showed that the administration of ampicillin, organic acid and synbiotic did not affected on the feed intake of broiler (Roth et al., 2019; Sobotik et al., 2021). In contrast to other studies, in which the administration of synbiotics has an influence on feed intake of broilers (Sunu et al., 2021). This was due to the different types of prebiotics and probiotics utilized in this study. Dev et al. (2020), the method of administration, type, source, and dose of probiotics, prebiotics, synbiotics, and chicken genetics all influenced the performance results.

The improved of the BWG of broiler chicken affected the CGLp treatments was similar with Abdel-Hafeez et al. (2017), dietary of probiotics, prebiotics, and synbiotic affected on broiler chickens body weight. The increased growth of lactic acid bacteria (LAB) in the ileum and cecum supported the body weight gain. This was due to a microbe balance that stimulates increased activity of digestive enzymes to improve digestion and nutrient absorption. Rehman et al. (2020), probiotic supplementation will stimulate of the digestive tract for the secretion of endogenous enzymes, therefore enhancing the digestive process and nutrient absorption. Sobolewska et al. (2017), the use of synbiotics has a substantial effect on intestinal structure, which has a positive effect on nutrient absorption.

Increased feed intake and BWG due to CGLp treatment supported the decreased FCR value. Al-Sultan et al. (2016), improving the broiler performance by the administration of prebiotic, probiotic, synbiotic and organic acid salt leads to more efficient utilization of nutrients such as protein, energy, minerals and vitamins.

This study showed that CGLp supplementation could increase live body weight of broiler. That was due to synbiotic supplementation enhances intestinal repair thereby increasing nutrient utilization (Kridtayopas et al., 2019). In accordance with Dev et al. (2020), the combination of mannan-oligosaccharide (MOS) and *Lactobacillus acidophilus* increased the LBW of broiler chickens. In contrast with Sarangi et al. (2016), dietary of probiotic, prebiotic, and synbiotic in broiler chickens was not affected on live body weight.

CGLp supplementation increased the carcass percentage of broiler chicken. This was because the synergy between probiotics and prebiotics will modulate the population of microbial in the gastrointestinal tract and increase the villi height, resulting in a larger surface area for nutrient absorption, which will affect feed utilization by increasing digestibility and nutrient absorption (Abdel-Hafeez et al., 2017). Similar result with Rehman et al. (2020), dietary of prebiotic and probiotic affected on percentage of carcass. In contrast with Tavaniello et al. (2019) and Li et al. (2019), administration of probiotic, prebiotic, and synbiotic was not affected on carcass.

CGLp treatment increased meat protein content and reduced the meat fat content and levels of meat cholesterol in broilers. In accordance with Sugiharto et al. (2017) and Suryadi et al. (2019), the dietary of cassava pulp fermented with *Acremonium charticola* and supplementation of probiotics was improved the content of meat protein and reduced the content of meat fat of broiler chickens. A decrease in meat cholesterol was also found by supplementation of Dayak onion extract and *Lactobacillus acidophilus* (Yuanita et al., 2019). *Bacillus licheniformis* supplementation can improve the breakdown of food to make it easier to digest, hence enhancing the efficiency of the digestive process and influencing the chemical composition of muscles (Liu et al., 2012). According to Shang et al. (2020), a reduction in meat cholesterol levels in broiler chickens supplemented with a fermentation concentrate of *Hericium caput-medusae* (Bull.:Fr.) Pers. in feed was caused by a decrease in cholesterol production in the liver. Abdurrahman et al. (2016), the amount of bile salt hydrolase (BSH) in the gut will increase with probiotic supplementation, this will minimize the absorption of cholesterol for metabolic process and which is deposited in meat.

In this study, CGLp treatment did not change the pH in either the ileum or cecum, but the ileum and cecum pH decreased compare with control. Gashemi et al. (2020), decline of pH was a positive effect of an increase in lactic acid bacteria, thereby increasing the lactic acid and acetic acid concentration which lowered the pH in the intestine. The total of lactic acid bacteria in the ileum and cecum by the CGLp treatment was higher compare the control. This finding demonstrated an improvement in intestinal health caused by CGLp treatments compare with control. Probiotic and prebiotic treatment increased the growth of *Lactobacillus* (Yun et al., 2017). Wang et al. (2018), microencapsulated probiotics and prebiotics increased the number of *Lactobacillus* bacteria in the caecum. Synbiotics supplementation on the diet of broiler chicken were able to enhance the total of LAB and reduce the total of coliforms in the ileum and cecum (Setyaningrum et al., 2019). Khalid et al. (2021), supplementation of probiotics and synbiotics significantly improved the amount of LAB and reduced the number of pathogenic bacteria. It was due to the colonization of pathogenic bacteria in the intestine can be inhibited. Hassanpour et al. (2013), prebiotics was able to provide the substrate needed by probiotics for the fermentation process, allowing them to survive and compete with pathogenic bacteria in the digestive tract.

CONCLUSION

In conclusion, the CGLp treatments improve the performance, carcass, and gut micloflora of broiler chicken.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This study was found that the use of a combination of gembili tuber and *Lactobacillus plantarum* could improve the performance of broiler chickens

AUTHORS CONTRIBUTION

SS designed and performed the research, analyzed data, wrote and revised the manuscript. DJS and TGP conducted data collection, laboratory analysis, edited and revised the manuscript.

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