

Supplementation of garlic powder and *Lactobacillus casei* to improve nutrient digestibility, physiological conditions, and performance of broiler during starter phase

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ABSTRAK

Penelitian bertujuan untuk mengkaji pengaruh campuran tepung bawang putih dan *Lactobacillus casei* (GLC) terhadap perkembangan usus, parameter hematologi, pencernaan nutrisi dan performa ayam pedaging periode starter. Seratus empat puluh ekor ayam broiler umur 1 hari dibagi menjadi empat kelompok yaitu kontrol (tidak diberikan suplemen GLC) dan diberikan suplemen GLC pada level 1, 2 dan 3% dari total pakan. Ayam dipelihara selama 21 hari di kandang terbuka. Pada hari ke-21, dilakukan pengambilan darah, bobot organ limfoid, dan organ pencernaan, serta data performans. Konsumsi pakan akumulatif sampai hari ke 21 tidak berbeda ($P \leq 0,05$) antar kelompok perlakuan. Pada penelitian ini terjadi peningkatan ($P \leq 0,05$) tinggi vili di jejunum, dan keasaman di duodenum dan sekum, akibat peningkatan ($P \leq 0,05$) asam lemak rantai pendek (SCFA; asetat dan propionat). Pencernaan protein dan serat kasar pada ayam yang diberi GLC secara signifikan lebih tinggi ($P \leq 0,05$) dibandingkan dengan kelompok kontrol. Suplementasi GLC (1, 2, dan 3% dari total pakan) secara signifikan meningkatkan penambahan bobot badan dan memperbaiki ($P \leq 0,05$) konversi pakan (FCR). Kesimpulannya, penggunaan GLC selama fase starter dapat meningkatkan pencernaan nutrisi, kesehatan usus, dan performa ayam broiler.

Kata kunci: Ayam broiler, Bawang putih, Lactobacillus casei, Performa, Fase starter

ABSTRACT

This study aimed to assess the effect of a mixture of garlic powder and *Lactobacillus casei* (GLC) on intestinal development, hematological parameters, nutrient digestibility, and performance of broilers in the starter phase. One hundred forty 1-day-old broiler chicks were divided into four groups, namely control (not given GLC supplement) and given GLC supplement either 1%, 2%, and 3% of total feed. Chickens were reared for 21 days in an open cage. On the 21st day of the experiment, blood collection, lymphoid organ and digestive organ weight, and performance data were recorded. Accumu-

lative feed intake until day 21 did not differ ($P>0.05$) among treatment groups. In this study, there were improvements in the height of villi in the jejunum, and the acidity in the duodenum and caecum, due to the increase of short-chain fatty acids (SCFA: acetate and propionate). Protein and crude fiber digestibility in the GLC-given chickens was significantly higher than in the control group ($P\leq 0.05$). GLC supplementation (1%, 2%, and 3% of total feed) significantly increased body weight gain and improved feed conversion ratio (FCR) ($P\leq 0.05$). In conclusion, the use of GLC during the starter phase can improve the nutrient digestibility, intestinal health, and performance of broiler chickens.

Keywords: broiler chicks, garlic, Lactobacillus casei, performance, starter phase

INTRODUCTION

The starter phase is the initial period for the growth and development of chickens, as well as a critical phase for chickens (Alkie *et al.*, 2019). During the starter phase, the immune and organ systems of the chicken are not functioning optimally. On the other hand, chickens in the starter phase experience very fast growth. For this reason, the optimal nutritional intake is needed to support hyperplasia, the development of chicken organs, and the formation of muscle mass/meat. The body weight in the starter phase greatly determines the final weight of broiler chickens. Administration of antibiotic growth promoters (AGPs) in feeds had usually been applied to support the rapid growth of broiler chickens (Sugiharto, 2016). However, the negative effects of antibiotic resistance in both broiler chickens and broiler consumers have prompted nutritionists to replace AGPs with natural-based additives and immune boosters. Two of the various AGP alternatives, phytobiotics and probiotics drew great interest because they can improve growth, regulate the immune system of broiler chickens, and increase feed efficiency (Sugiharto, 2016; Rahman *et al.*, 2021). One of the phytobiotics that has the potential as a natural additive is *Allium sativum* or garlic.

Garlic contains 1 - 2% fructooligosaccharides (FOS) (Dixit *et al.*, 2018), fructan 45.8 to 54.4g/100 g (Lisciani *et al.*, 2017), and inulin 20.3 – 36.1 % (Moshfegh *et al.* 1999). Garlic, in addition to containing FOS, fructan and inulin as prebiotics, also contains phenolic compounds (70.24 mg / g), organosulfur, and saponins, which can improve the health of the digestive

tract (Kothari *et al.*, 2019). Organosulfur acts as an antibacterial, but is well known not to inhibit gram-positive bacteria such as lactic acid bacteria (LAB) (Hill *et al.*, 2018). The phenolic compounds in garlic play a very important role as antioxidants to prevent cell and organ damage from the oxidative process (Gawad *et al.*, 2014; Capasso, 2013). Garlic as a phytobiotic improved nutrient utilization by increasing the secretion of enzymes, antimicrobial, antioxidants, and anti-inflammatory activities (Rahman *et al.*, 2021; Wenk, 2003). In previous studies, phytobiotics have been identified as potential candidates that differentially stimulate favorable bacteria growth such as *Lactobacilli* and *Bifidobacteria* and decrease the growth of pathogenic bacteria (Jamroz *et al.*, 2003; Ferdous *et al.*, 2019). Considering all the above facts, a nutritional approach was carried out in the present study through a combination of phytobiotics (garlic powder) and probiotics (*Lactobacillus casei*) as an alternative to promoting the growth of broiler chicks during the starter phase. Previous studies have shown that as a probiotic strain, *Lactobacillus casei* can adhere to the intestinal mucosa and is able to colonize the gut, by attaching to the host gastrointestinal (GI) mucosa. It also has high efficiency of gut colonization, and promotes the host's epithelium and immune cells (Hill *et al.*, 2018). *Lactobacillus casei* has been extensively studied as a fermentation starter culture, since it has various functions such as increasing the expression of interleukin 12 in dendritic cell, and preventing the toxic effect induced by particulate matter (Jung *et al.*, 2021). To the best of our knowledge, studies on the combined use of garlic powder and *Lactobacillus casei* to improve the

growth and intestinal health of broiler chicks during the starter phase have never been found in the literature.

The purpose of this study was to determine the effect of a mixture of garlic powder and *Lactobacillus casei* on the growth rate, internal organ development, nutrient digestibility, and physiological conditions of broiler chicks during the starter phase.

MATERIALS AND METHODS

Preparation of a Mixture of Garlic Powder and *Lactobacillus casei*

Garlic was purchased from a local market in Semarang city. It was peeled, thinly cut, and dried in an oven at 40°C until dry. *Lactobacillus casei* (10^9 cfu/g) was obtained from the Microbiology Laboratory Gadjah Mada University Indonesia. The isolates were rejuvenated on a petri dish using de Man, Rogosa, and Sharpe (MRS) agar. *Lactobacillus casei* was incubated for 1 × 24 hours at 37°C in 100 ml of 10% skim milk. Then, it was added with 1.5% (weight/volume) of garlic powder. The mixture of garlic powder and *Lactobacillus casei* were incubated for 2 × 24 hours to make mixture of garlic powder and *Lactobacillucasei* (GLC). The total plate count method was applied to count the bacterial population in GLC (Usman *et al.*, 2018).

Experimental Design, Ration, and Management

A completely randomized design was used in this study. Two hundred 1-day old Lohmann broilers (MB-202) (mean body weight = 44.7 ± 0.52 g) were used in this study. Broiler chickens were reared in broiler houses with open sides, for 21 days. Chicks were randomly assigned to 20 experimental units, each unit was consisted of 10 birds. During the study period (1-21 days), the chicks were fed commercial feed containing 14% water, 21-23% crude protein, 5% crude fiber, 5% crude fat, and 7% crude ash (nutrient composition obtained from the feed mill company). Drinking water and feed were given *ad libitum*. Supplements in the form of a mixture of garlic

and *Lactobacillus casei* (GLC) were added at doses of 0 (control), 1% (GLC1), 2% (GLC2), and 3% (GLC3) of total feed.

On the 21st day, 20 birds (one chick per experimental unit) were randomly selected and weighed after fasting for 12 h. Blood was taken and was put into vacutainers containing Ethylene diamine tetra acetic (EDTA) to determine the hematological value. After blood sampling, birds were slaughtered according to Islamic law by cutting the neck of the chicken (Ahmed *et al.*, 2014). The intestinal organs including the duodenum, jejunum, ileum, and caeca were collected, then pH organs were observed and each organ was weighed. Caeca samples from both ceca were diluted by weight in buffered peptone water. Then, it was homogenized and was analyzed for volatile fatty acids (VFA). Lymphoid organs including *bursa of Fabricius*, spleen, and thymus were also weighed.

Measurement of Parameters

The performances of broilers included feed consumption, body weight gain, and feed conversion ratio (FCR) were calculated on the 21st day. The relative weight of immune and visceral organs was calculated by dividing wet organ weight and live body weight. The performance and relative weight of immune and visceral organs were calculated according to Sugiharto *et al.* (2016), while lactic acid bacteria and coliforms from the intestinal digesta were measured based on the method of Peng *et al.* (2016). pH values of internal organs were determined by a digital pH meter in each part of the small intestine following the method of Nabizadeh (2012). The midpoint of the ileum was removed 3 cm and was fixed in 10% buffered formalin. The ileum segments were embedded in paraffin, and a 5 µm section of each sample was placed on a glass slide and was stained with hematoxylin and eosin to be observed under light microscopy. Measurement of villi height of jejunum was done using a computer with Microsoft Office Picture Manager Program at 10× magnification. Each slide was measured at least three times. The villus height (VH) was measured from the tip of the

villus to the villus-crypt junction (Elmi *et al.*, 2020). Twenty replicates were used as the sample, and the average values VH were used in statistical analysis. Volatile fatty acids (VFA) were analyzed according to Wielen *et al.* (2000).

During days 21 to 24, chickens were fed by experimental diet, and all excreta were collected for each replicate. Pooled excreta were mixed, dried, grounded, and analyzed for their proximate. Sample of feed and excreta were analyzed for their proximate by AOAC (2007) for dry matter, crude protein (CP), crude fat, and crude fiber. Nutrient digestibility was determined using the nutrient of feed and excreta. Nutrients (protein, fat and crude fiber) digestibility was measured according to Kong and Adeola (2014), as follows:

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

Apparent metabolizable energy (AME) was calculated according to Wealleans *et al.* (2017):

$$\text{AME} = [(\text{FI} \times \text{GED}) - (\text{EO} \times \text{GEE})] / \text{FI}$$

FI = feed intake, GED = gross energy diet; EO = Excreta output; GEE = gross energy excreta

The N Retention or Ca Retention was calculated by substitution of N or Ca diet and N or Ca excreta in place of the respective gross energy. The total erythrocytes and leukocytes were calculated according to Sugiharto *et al.* (2016), using the dilution flask method (cell count using the Bürker chamber), while the differential leukocytes were analyzed using a light microscope. H/L ratio was calculated by dividing the numbers of heterophils and lymphocytes.

Statistical Analysis

Data were analyzed using analysis of variance by SPSS version 22. Significant differences among treatment groups were analyzed using Duncan's multiple range test ($P \leq 0.05$).

RESULTS

The Immune and Visceral Organs

The data on the relative weight of immune and visceral organs of broiler chickens were presented in Table 1. GLC administration had a significant effect on the relative weight of the *bursa of Fabricius* ($P \leq 0.05$), but it had not shown a significant effect ($P \geq 0.05$) on the relative weight of the spleen and thymus. Chickens in the GLC2 and GLC3 have a higher relative weight of the *bursa of Fabricius* compared to the control and GLC1. The addition of GLC had a significant effect ($P \leq 0.05$) on the relative weight of the ileum and caecum, but it had not shown a significant effect on the other viscera organs.

Production of VFA and pH Intestinal

A diet with supplementation of GLC affected the production of VFA in the caecum (Table 2). Acetate and propionate in the group of chickens that were given GLC diet was higher ($P \leq 0.05$) than those in the control group. A diet with GLC significantly decreased ($P \leq 0.05$) pH in the duodenum and ileum (Table 3), but pH of the jejunum, caecum, and colon were the same among treatments.

Nutrient Digestibility

Diet with GLC supplementation significantly affected nutrient digestibility in starter phase broilers (Table 4). GLC 1-3% to broiler chickens had a significant effect ($P \leq 0.05$) on the increase of protein and crude fiber digestibility, as well as Ca retention.

Blood Parameters

The effect of GLC supplementation data on blood parameters in broilers were presented in Table 5. Hematological variables of broiler chickens measured that showed a significant difference ($P \leq 0.05$) were the heterophil levels, lymphocytes, and the heterophil to lymphocytes (H/L) ratio. Regarding the H/L ratio, the control chickens had the highest H/L ratio ($P \leq 0.05$) compared to other treatments, while GLC3 showed the lowest H/L.

Performance of Broiler Chicks

The results of our study in Table 6 showed

that giving a combination of garlic powder and *Lactobacillus casei* (GLC) significantly improved ($P \leq 0.05$) the performance of broiler chickens ($P \leq 0.05$). The increase in consumption in the GLC group was followed by an increase in body weight gain and a decrease in FCR ($P \leq 0.05$), but the carcass percentage of all treatments showed the same result ($P > 0.05$).

DISCUSSION

Our results showed that GLC supplementation in broiler rations can improve growth performance, nutrient digestibility and FCR of broilers. The results showed that the growth performance of broiler chickens given a mixture of garlic powder and *Lactobacillus casei* (GLC) at the level of 1-3% in the rations increased body weight gain compared to control. The results of this study were supported by Lukanov *et al.* (2018) that garlic powder and *Lactobacillus spp.* supplementation had a significant effect on broiler's growth performance. Ferdous *et al.* (2019) also reported that probiotics and phytobiotics improved performance, hematological parameters, and gut microbial load in broiler chicken. Likewise, the combination of the *Lactobacillus acidophilus* and natural antibacterial (medicinal plant aqueous extracts/MPAE) in broiler diet increased body weight gain and villus surface area of the jejunum (Elmi *et al.*, 2020). The growth enhancement mecha-

nism in broilers is still unclear, but was thought to be influenced by intestinal health, changes in intestinal microbes, immune organs, and increasing nutrient digestibility. This bodyweight gain was inferred to be related to the role of symbiotic GLC, which supported the growth of probiotic bacteria because, in GLC, specific substrates were available for fermentation. Therefore, the host obtained better benefits from this combination of phytobiotics and prebiotics. The increase in probiotic bacteria in the gut leads to increase production of lactic acid and SCFA, which in turn can increase the acidity in the intestine. This can be seen from the intestinal pH where there is a lower pH in the duodenum and ileum. The decreased intestinal pH in GLC group in the present study resembles to the result of Jamroz *et al.* (2003) and Ferdous *et al.* (2019), who reported that dietary supplementation of plant extracts in the diet significantly reduced the intestinal pH of broilers. The decreased pH in GLC treatment indicates that SCFA found in caecum reduce the number of pathogenic bacteria in the gut, while increasing nutrient digestibility. High acidity reduces the growth of pathogenic bacteria (Song *et al.*, 2014) so that the intestine becomes healthier. In newly hatched chicks in commercial hatcheries, the SCFA concentration and pH are not sufficient to chemically suppress pathogens (Al-Khalafah, 2020). Therefore, supplementation of probiotics and phytobiotics will be very beneficial.

Table 1. Relative weight of internal organs and villi height of jejunum of broiler chicks

Items (% live body weight; unless otherwise noted)	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Spleen	0.10	0.94	0.11	0.11	<0.01	0.48
<i>Bursa of Fabricius</i>	0.17 ^b	0.15 ^b	0.26 ^a	0.27 ^a	0.01	0.01
Thymus	0.29	0.29	0.30	0.36	0.01	0.23
Duodenum	0.59	0.56	0.64	0.58	0.01	0.10
Jejunum	1.27	1.22	1.32	1.40	0.03	0.14
Ileum	0.67 ^b	1.11 ^a	1.23 ^a	1.16 ^a	0.02	<0.01
Caecum	0.35 ^b	0.34 ^b	0.41 ^{ab}	0.42 ^a	0.01	0.04
Colon	0.13	0.18	0.24	0.23	0.02	0.06
Villi height of jejunum (µm)	794	859	872	941	19.9	0.12

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC; GLC2= broilers receiving 2% GLC; GLC3= broilers receiving 3% GLC; SEM= standard error of mean

The improvement of intestinal health is closely related to the growth of digestive tract. In this study, the relative weight of ileum and ceca at day 21 on broiler chickens also increased. Intestinal growth had increased due to GLC supplementation in the ration. Concomitant with this, Sugiharto *et al.* (2016) reported that feeding probiotic *Bacillus* plus vitamins and minerals on broiler during starter phase increased the growth and development of ileum. In general, the increased growth of ileum and caeca is associated with the improved digestive function of broilers. Protein and crude fiber digestibility in this study increased, followed by Nitrogen and Calcium retention. *Lactobacillus acidophilus* supplementation improved nutrient digestibility in broilers (Zhang and Kim, 2014), because it could stimulate digestive enzyme activity (Jin *et al.*, 2000). The increase in digestibility was followed by an increase in absorption because GLC administration tended to increase the height of the intestinal villi. According to Awad *et al.* (2010) and Song *et al.* (2014), *Lactobacillus* supplementation in the diet increased nutrient absorption, which is associated with improved intestinal architecture. Improvements in some of these parameters lead to increase body weight gain of broilers in the starter phase.

The relative weight of *bursa of Fabricius* was higher in the GLC group than those in the control group. The mixture of GLC can increase the relative weight of immune tissues as also reported by Zhang *et al.* (2013) and Mangisah *et al.* (2020). In some cases, the relative weight of immune organs of broiler chickens can reflect responses of immune and functionality (El-Katcha *et al.*, 2014). The positive effect of a diet containing probiotics and phytobiotics (GLC) on the im-

mune response is associated with an increase in commensal bacteria in the digestive tract and the response to lymphatic tissue that produces T and B lymphocytes (Kabir *et al.*, 2004). The commensal bacteria contribute to the growth of the intestinal immune organs and also help in the stimulation of innate and adaptive immune responses (Adil and Magray, 2012). Alkhalf *et al.* (2010) reported that chickens treated with 1.6 g, 1 g, and 0.8 g of probiotic per kg of feed during 42 days showed an increase of the bursa of Fabricius weight. An increase in the relative weight of bursa may be attributed to an increase in the number of immune cells. The study showed that H/L ratio of chickens fed GLC2 and GLC3 was lower than those of chickens fed control diet or diet containing 1% of GLC. This finding suggests that diets with GLC, especially as much as 2 and 3% of the total rations, may preclude the chickens from infections, inflammation, or stress. These phenolic compounds in garlic powder could act as antimicrobial agents, and prevent the broiler chickens from infections and inflammation (Omer *et al.*, 2019). Garlic contains allicin, alliin, ajoene, diallyl sulfide, dithiin, S-allylcysteine. Garlic as a natural feed additive in poultry nutrition may be of great benefit, because it has antibacterial, anti-inflammatory, antiseptic, antiparasitic, and immune-modulatory properties (Rehman and Munir, 2015). In addition, the high antioxidant activity in the garlic combined with *Lactobacillus casei* may cope with the oxidative stress that can compromise the immune system. The decreased H/L ratio may indicate that chickens were healthy, while the increased H/L ratio indicated that chickens suffer from infections, inflammation, or stress (Davis *et al.*, 2008). The H/L ratio has recently been used as

Table 2. VFA concentrations of caecal content of broiler chicks

Items (mmol/kg)	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Acetate	128 ^b	192 ^a	170 ^a	170 ^a	3.47	<0.01
Propionate	13.1 ^b	22.5 ^a	17.9 ^{ab}	21.7 ^a	1.05	0.02
Butyrate	36.9	31.1	36.9	29.9	2.14	0.51

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC;

GLC2=broilers receiving 2% GLC; GLC3= broilers receiving 3% GLC; SEM= standard error of mean

Table 3. pH values of intestinal segments of broiler chicks

Items	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Duodenum	6.00 ^a	5.74 ^b	5.84 ^{ab}	5.96 ^a	0.03	0.03
Jejunum	5.92	6.00	5.90	6.18	0.04	0.10
Ileum	6.62 ^a	6.10 ^b	6.20 ^b	6.34 ^b	0.04	<0.01
Caecum	6.60	6.58	6.58	6.64	0.07	0.98
Colon	6.86	6.88	6.88	6.94	0.03	0.70

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC;

GLC2=broilers receiving 2% GLC; GLC3= broilers receiving 3% GLC; SEM= standard error of mean

Table 4. Nutrient digestibility of broiler chicks

Items	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Fat digestibility (%)	81.3	85.1	86.2	87.9	4.11	0.21
Protein digestibility (%)	79.9 ^b	83.9 ^a	84.3 ^a	86.0 ^a	0.38	<0.01
Crude fiber digestibility (%)	20.9 ^b	24.4 ^a	25.7 ^a	26.7 ^a	0.43	<0.01
AME (kcal/kg)	2971	3044	3164	3090	22.8	0.05
N Retention (g)	2.26	2.05	2.41	2.29	0.08	0.42
Ca Retention (g)	0.16 ^b	0.19 ^b	0.28 ^a	0.20 ^b	0.01	<0.01

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC; GLC2=broilers receiving

2% GLC; GLC3= broilers receiving 3%GLC; SEM= standard error of mean

an indicator of stress conditions in broiler chickens.

The GLC supplementation can decrease intestinal pH values. Inulin and FOS from garlic powder provides a specific substrate as a “nutrition source” that can be fermented by *Lactobacillus casei* and increases the growth of *Lactobacillus* in general in terms of lactic acid bacteria (LAB). The increased growth of LAB is

correlated with the higher production of lactic acid and SCFA (acetate, propionate, and butyrate), which correlates with the decrease of intestinal pH. The high concentration of lactic acid affected the decrease in pH and reduce the growth of harmful bacteria (Nkukwana, *et al.*, 2015). Acidic intestinal conditions affected the growth of intestinal commensal bacteria (LAB). The increase in LAB can increase the production

Table 5. Blood parameters of broiler chicks

Items	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Erythrocytes ($10^6/\text{mm}^3$)	53.6	2.52	2.66	2.57	0.08	0.71
Leukocytes ($10^9/\text{l}$)	9.13	10.3	10.5	10.4	0.36	0.51
Hemoglobin (g/dl)	7.36	7.28	7.66	7.66	0.09	0.32
Hematocrit (%)	22.8	24.2	24.0	26.0	0.74	0.52
Total protein plasma (g/dl)	1.66	2.12	2.48	2.16	0.17	0.37
Heterophils (10^8)	42.6 ^a	35.0 ^b	35.0 ^b	29.4 ^c	0.83	<0.01
Lymphocytes (10^8)	53.6 ^c	59.6 ^b	57.8 ^b	64.6 ^a	0.59	<0.01
H/L ratio	0.79 ^a	0.59 ^b	0.61 ^b	0.46 ^c	0.02	<0.01

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC;

GLC2=broilers receiving 2% GLC; GLC3= broilers receiving 3% GLC; SEM= standard error of mean

Table 6. Performances of broilers chicks

Items	Dietary treatments				SEM	P value
	Control	GLC1	GLC2	GLC3		
Feed intake (g/bird)	1111	1040	1185	1140	12.7	0.08
Body weight gain (g/bird)	660 ^b	730 ^a	704 ^b	714 ^a	8.08	0.04
FCR	1.69 ^b	1.42 ^a	1.69 ^b	1.60 ^b	0.03	0.01
Carcass (%)	57.6	58.1	59.9	58.5	0.30	0.07

^{a,b}Values with different superscript within the same row were significantly different

Control= broilers receiving ration without GLC; GLC1= broilers receiving 1% GLC;

GLC2= broilers receiving 2% GLC; GLC3= broilers receiving 3% GLC; SEM= standard error

of volatile fatty acids and bacteriocins, which can inhibit pathogens. The balancing intestinal microbiota of broiler chickens affected the health of gut and growth performance of broiler chickens (Sugiharto, 2016), and its influence was particularly relevant when chickens were young and their microbiota was still in the process of development. The interaction of microbiota, intestinal growth, digestive and nutrient utilization is critical during starter phase.

In this study, supplementation of GLC in the diet did not significantly effect on the number of erythrocytes, leukocytes, hemoglobin, hematocrit, and total protein plasma. Sugiharto *et al.* (2017) reported that three-week-old broilers had erythrocyte values of $2.15-2.98 \times 10^{12}/L$. Hematocrit in this study was in line with the number of erythrocytes and hemoglobin levels. The GLC supplementation in chicken rations significantly affected heterophils, lymphocytes, and H/L ratio. The results of this study were supported by Hanieh *et al.* (2010), Ao *et al.* (2011), and Rehman and Munir (2015), who also found that garlic supplementation increased the white blood cells, lymphocytes, and immunoglobulin G in broilers. The enhanced lymphocyte proliferation by garlic treatment along with the possible protection of the cells from oxidative stress seemed to contribute to the increasing of white blood cells (Rehman and Munir, 2015). Hematological variables in this study showed that giving GLC improved the health of broiler chickens, it is seen from the low value of the H/L ratio and the chickens did not experience stress. Hematological variables have commonly been used as indicators of physiological conditions, health,

and nutritional deficiency in broiler chickens. The changes in blood profile (hemoglobin, erythrocyte, and differential leukocytes) may indicate stress (Sugiharto *et al.*, 2016).

As a consequence of these improvements in intestinal health, immune organs, nutrient digestibility, and blood parameter, this study found a beneficial effect of GLC supplementation on growth performance, which is in line with previous findings reported by Mangisah *et al.* (2020) and Wealleans *et al.* (2017).

CONCLUSION

Supplementation of GLC on commercial diet improved growth rate, internal organ development, nutrient digestibility, and physiological conditions of broiler chicks during starter phase.

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