

Effect of *Lactobacillus casei* and Garlic Powder Administration on Broiler Performance, Immune Response and Blood Profile

Mangisah I, Yunianto VD, Sumarsih S, Sugiharto S

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University
Semarang 50275, Central Java, Indonesia
E-mail: istnamangisah@yahoo.co.id

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ABSTRAK

Mangisah I, Yunianto VD, Sumarsih S, Sugiharto S. 2022. Pengaruh *Lactobacillus casei* dan tepung bawang putih terhadap performa, respon imun dan profil darah ayam broiler. JITV 27(3):152-158. DOI: <http://dx.doi.org/10/14334/jitv.v27i3.2961>.

Penelitian bertujuan untuk mengkaji pengaruh pemberian campuran *Lactobacillus casei* dan tepung bawang putih (LGP) terhadap performa, respon imun dan profile darah ayam broiler. Ayam broiler umur sehari sebanyak 140 ekor dengan BB rata-rata 43.70 ± 0.88 g ditempatkan secara acak dalam 20 unit percobaan. Penelitian didesain dengan rancangan acak lengkap dengan 4 perlakuan dan 5 ulangan. Perlakuan diberikan pada saat ayam umur 22-35 hari (fase finisher). Perlakuan yang diberikan adalah campuran *Lactobacillus casei* dan tepung bawang putih (LGP) dengan konsentrasi 0, 1, 2 dan 3%. Parameter yang diukur adalah profil darah dan bobot organ limfoid (bursa fabricus, limpa, dan thymus) serta produksi karkas. Data yang terkumpul dianalisis ragam dan jika terdapat pengaruh nyata maka dilanjutkan dengan uji wilayah ganda Duncan pada taraf 5%. Hasil penelitian menunjukkan bahwa pemberian LGP memperbaiki respon imun, bobot karkas dan meningkatkan bobot badan akhir ayam broiler. Pemberian LGP 3% adalah konsentrasi terbaik dalam memperbaiki respon imun dan performa ayam broiler.

Kata Kunci: Darah, Broiler, Bawang Putih, Imun, *Lactobacillus*

ABSTRACT

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This study aimed to examine effect of giving a mixture of *Lactobacillus casei* and garlic powder (LGP) on broiler performance, immune response and blood profile. One hundred and forty-day old broilers with an average body weight of 43.70 ± 0.88 g were placed randomly in 20 experimental units. Completely randomized design was used in this study, with 4 treatments and 5 replications. The treatment was conducted when the chickens were 22-35 days old (finisher phase). The chickens were given a mixture of *Lactobacillus casei* and garlic powder (LGP) at concentration levels of: 0, 1, 2, and 3%. Parameters measured were blood profile, weight of lymphoid organs (bursa of Fabricius, spleen, and thymus), and carcass production. Data were analyzed for variance and if there was a significant effect, then Duncan's multiple range test was followed at the 5% level. Results showed that the administration of LGP improved the immune response, carcass weight and final body weight of broiler chickens. The administration of 3% LGP improved immune response and achieved the best broiler performance.

Key Words: Blood, Broilers, Garlic, Immune, *Lactobacillus*

INTRODUCTION

In Indonesia, most of broiler chickens are kept in open cages. Indeed, chickens in open cages are very susceptible to stress due to changes in environmental temperature and humidity. The short harvest age of broilers with fast growth makes the chickens susceptible to stress and disease infections. Stress can lower the body's immunity so that it is susceptible to several diseases and reduce growth, and ultimately affect the profits of farmers.

There are stressors that come from outside the chicken body, including environmental temperature,

humidity, flock density, and transportation (Tamzil 2014). Causes of stress that come from within the body include infectious diseases and malnutrition (Das et al. 2012). An alternative to minimize the negative effect of stress and disease cases in broiler chickens is the administration of an antibiotic growth promoter. In many countries, the use of growth promoters in the form of antibiotics has been prohibited, because it makes microorganisms resistant and drug residues are found in livestock products.

Eliminating antibiotics in poultry feed leads to decreased growth performance and increased disease prevalence (Bilal et al. 2021). To overcome this

problem, many natural substances have been developed to maintain health and improve physiological functions, modulate the immune system, improve bacterial balance, modulate intestinal morphology, and increase growth rate (Sugiharto 2016). Among the alternative to antibiotic growth promoters are probiotics and prebiotics or a mixture of both (synbiotics). In this study, use of synbiotics was studied in broilers through a mixture of *Lactobacillus casei* (as probiotic) and garlic powder (as prebiotic).

Recent study showed that synbiotics can improve intestinal microflora and stimulate the growth of beneficial bacteria and improve broiler performance (Alagawany et al. 2021). The use of synbiotics has a better effect than the separate supplementation of probiotics and prebiotics on broiler (Sugiharto 2016). Several studies have shown the benefits of synbiotics in the improvement of the intestinal microbial ecosystem (Shanmugasundaram et al. 2019).

Use of synbiotics (*Lactobacillus* spp., *Saccharomyces cerevisiae* and inulin) for broilers increased lactic acid production, short chain fatty acids, and commensal bacterial population, while it decreased branched chain fatty acids and pathogenic population in the intestine (Markowiak-Kopeć & Śliżewska 2020). The following increased feed conversion ratio warranted the use of synbiotics as feed additives in broiler chicken production (Shanmugasundaram et al. 2019). Other studies have shown that synbiotic supplementation in feed improve performance of broiler chickens under stress (Sohail et al. 2010; Cengiz et al. 2015; Kridtayopas et al. 2019).

The often-used probiotic in synbiotics is *Lactobacillus casei*, which has potential as a probiotic. These commensal bacteria are capable of producing lactic acid and hydrogen peroxide which can suppress the growth of intestinal pathogenic bacteria, provide favorable conditions for improving digestion and utilization of nutrients, and increasing feed efficiency. Study also revealed that the use of *Lactobacillus casei* may compensate for the deficiency of the endogenous antioxidant enzyme superoxide dismutase (SOD) when chickens are under stress (Hill et al. 2018). *Lactobacillus casei* like other lactic acid bacteria (LAB) requires a "food" substrate to support its growth, which is called a prebiotic. Among the ingredients that are classified as prebiotics are fructooligosaccharides and inulin.

Garlic (*Allium sativum*) contains high levels of fructooligosaccharides (FOS) and inulin, and has been shown to be useful as a prebiotic (Sunu et al. 2019; Mudannayake et al. 2015). FOS compounds in garlic bulbs are 1-2% (Dixit et al. 2018), while according to Sunu et al. (2019), FOS content in garlic bulbs is 3.34%. According to (Mudannayake et al. 2015), content of fructan type inulin based on fresh weight in garlic is 18.62%. Garlic, in addition to containing FOS

and inulin as prebiotics, also contains various bioactive components, including phenolic compounds, organosulfur and saponins, which improve digestive tract health (Kothari et al. 2019). Garlic's organosulfur and phenolic compounds play a very important role as antioxidants to prevent cell and organ damage from the oxidation process (Kothari et al., 2019; Sunu et al. 2021).

Purpose of this study was to examine effect of synbiotics supplementation (*Lactobacillus casei* and garlic powder) on immune response, blood profile, and carcass production of broiler chickens during finisher period. This study is useful to provide information on synergistic work of *Lactobacillus casei* and garlic powder in improving health and productivity of broiler chickens in terms of immune response, blood profile, and carcass production.

MATERIALS AND METHODS

Production of a mixture of *Lactobacillus casei* and garlic powder (LGP)

The manufacture of synbiotic *Lactobacillus casei* and garlic powder begins with the process of making garlic powder. Garlic was peeled, washed, thinly sliced, and dried under the sun, then finely ground into garlic powder. Pure isolates of *Lactobacillus casei* were obtained from the Microbiology Laboratory of Gadjah Mada University. The isolates were rejuvenated using agar medium (MRSA) at the Animal Physiology Laboratory of the Faculty of Animal Husbandry and Agriculture. A loopful of bacteria was scratched into the medium so that it was slanted and then incubated for 48 hours at 38°C. *Lactobacillus casei* bacteria with a concentration of 10⁹ cfu/ml were then inoculated into 100 ml of 10% sterile skim milk solution and incubated for 24 hours at 37°C. The incubation results were mixed with 400 ml of sterile 10% skim milk solution and incubated again for 24 hours at 37°C. The next step, the bacteria were mixed with 1.5% garlic powder and then incubated again at the same temperature for 48 hours. Then, lactic acid bacteria concentration was calculated from the result using total plate count method. Furthermore, the mixture was called a mixture of *Lactobacillus casei* and garlic powder (LGP). LGP was then stored in refrigerator and ready to be applied as a treatment in this study.

Animal and feed experimental

All procedures used in this experiment were approved by the Animal Research Ethics Committee, Faculty of Animal Science, Faculty of Animal and

Table 1. Relative weight of lymphoid organs of broiler chicks

Items (% live body weight; unless otherwise noted)	Dietary treatments			
	Control	LGP1	LGP2	LGP3
Bursa of Fabricius	0.06± 0.03 ^c	0.08±0.03 ^b	0.10±0.05 ^{ab}	0.13±0.02 ^a
Spleen	0.10±0.04	0.11±0.05	0.10±0.03	0.08±0.01
Thymus	0.29±0.07 ^b	0.40±0.07 ^a	0.34±0.10 ^a	0.40±0.04 ^a

^{a,b}Values with different superscript within the same row were significantly different. Control= broilers receiving ration without LGF; LGF1= broilers receiving 1% LGF; LGF2= broilers receiving 2% LGF; LGF3= broilers receiving 3% LGF

Table 2. Blood profile and performance of broiler chicks

Items	Dietary treatments			
	Control	LGP1	LGP2	LGP3
Erythrocytes (10 ⁶ /μL)	2.46±0.17 ^b	2.44±0.75 ^b	2.57±0.81 ^{ab}	2.79±0.64 ^a
Hemoglobin (g/dL)	7.62±0.16 ^b	8.34±0.75 ^a	8.42±0.81 ^a	8.70±0.64 ^a
Total Plasma Protein (g/dL)	2.16±0.09 ^b	2.52±0.27 ^{ab}	2.48±0.26 ^{ab}	2.72±0.44 ^a
Pack cell volume (%)	24.00±2.44 ^b	24.80±3.03 ^b	25.20±1.64 ^b	29.60±2.51 ^a
Leukocytes (10 ³ /μL)	8.62±2.12 ^b	9.54±1.52 ^{ab}	10.87±1.25 ^a	11.12±1.80 ^a
Heterophils (10 ³ /μL)	34.60±5.50 ^a	26.60±2.51 ^b	20.40±3.21 ^c	19.60±0.55 ^c
Lymphocytes (10 ³ /μL)	60.60±3.51	65.60±12.50	68.20±8.29	68.40±2.61
H/L	0.57±0.13 ^a	0.39±0.04 ^b	0.31±0.05 ^b	0.30±0.07 ^b
Weight of carcass (g)	1251.20±24.49 ^b	1381.40±72.54 ^a	1292.60±35.14 ^{ab}	1304.80±27.81 ^{ab}
Final body weight (g)	1846.32±58.23 ^b	1947.88±112.91 ^{bc}	1954.80±149.54 ^{bc}	2024.54±85.57 ^a

^{a,b}Values with different superscript within the same row were significantly different. Control= broilers receiving ration without LGF; LGF1= broilers receiving 1% LGF; LGF2 =broilers receiving 2% LGF; LGF3= broilers receiving 3% LGF

Agricultural Sciences, Diponegoro University, No. 57-01/A-4/KEP-FPP. One hundred and forty-day old broilers with average body weight of 43.70±0.88 g were used in this study. The chickens were obtained from commercial hatcheries in Salatiga, Indonesia, were randomly assigned to 20 experimental plots. The chickens were reared for 35 days in open cages with litter floors, and were given commercial feed and drinking water on an ad libitum basis. Chickens were given ND B1 vaccine on day-4 and Gumboro on day-14 through eye drops. Lasota ND vaccine was given on day- 21 through drinking water. On days 22 to 35, the chickens were divided into 4 groups (treatments), each consisting of 7 birds. Each treatment had 5 replications. A completely randomized design was used in this experiment. Broilers chicken were receiving treatment feed containing the synbiotic of LGP (0, 1, 2, and 3%), namely: (1) Control= broilers receiving ration without LGP, (2) LGP1= broilers receiving 1% LGP in the ration, (3) LGP2= broilers receiving 2% LGP in the ration, and (4) LGP3= broilers receiving 3% LGP in the ration. Synbiotics were added to 50% of the ration for that day. The next 50% of the ration was given when the feed containing LGP has finished.

Parameter measurement

Parameters measured included performance, blood profile, immune response and carcass weight of broilers. A sample of 20 individuals was taken from the experimental unit. Blood was taken on day 35 from the wing vein with a 3 ml syringe in the morning, using tube containing an anticoagulant (EDTA). The blood samples were then analyzed for the number of red blood cells (RBC), white blood cells, hemoglobin (Hb), heterophils, and lymphocytes using a hematology analyzer referring to the instructions from PT. Prima Alkesindo Nusantara, Indonesia.

After the blood was drawn, the chicken was slaughtered. The abdomen was opened, and the physical condition of the digestive organs was observed. The intestines were pink, there were no fluid and lesions, and blood spots were not found. The liver was not fragile and the liver was red. The digestive organs were then removed and the lymphoid organs were weighed. Carcass weight was measured and recorded. The collected data was tested for variance and if there was a significant effect, then Duncan's multiple region test followed.

RESULTS AND DISCUSSION

Immune response

The mixture of LGP in the ration significantly affected the relative weight of bursa of Fabricius and thymus of broiler chickens (Table 1).

The weight of the lymphoid organs (bursa of Fabricius, thymus, and spleen) is an indicator of immune which is closely related to productivity. Result showed that the weight of bursa of Fabricius and thymus was significantly ($P < 0.05$) affected by the administration of the synbiotics. Bursa of Fabricius weight in this study was in the normal range. According to Sugiharto et al. (2018), the range of thymus relative weights was due to the administration of multi-strain probiotics plus vitamins and minerals, which is 0.13–0.20% of body weight, while the thymus is 0.44–0.54%. Increase in weight of bursa of Fabricius and thymus reflects an increase in body's resistance. Size of thymus varies greatly, in which the relative size is greatest in newborn animals, while the absolute size is greatest at puberty. After maturity, thymus undergoes atrophy of the parenchyma and the cortex is replaced by fatty tissue. Thymus that undergoes rapid atrophy is due to a reaction to stress (Cowan et al. 2020; Sunu et al. 2021). Thymus is a T cell regulator that acts on primitive cells originating from the bone marrow and makes them immunologically capable of acting as an antibody-forming body (Cowan et al. 2020).

Increasing of the immune response is caused by an increase in lactic acid bacteria in digestive tract, which affects balance of the microbiota population in the digestive tract. Changes in the microbiota affect the morphology of intestinal wall and cause immune reactions (Rehman et al. 2020), which in turn affect development of immune organs. Commensal microbiota has been recognized as an important inductor for maturation and development of innate defense mechanisms and adaptive immune response of chickens (Brisbin et al. 2008). Immune response is also influenced by T and B lymphocytes, which may be associated with stimulating lymphatic tissue. Thymic atrophy can be caused by acute stress, proinflammatory cytokines, steroid and hormone disorders (Cowan et al. 2020). Recent research shows that the synbiotic (combination of *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, *Lactobacillus reuteri*, and fructooligosaccharides) can be used as a growth promoter to reduce fear response and stress state of heat-stressed on broilers chicken (Mohammed et al. 2021).

Inclusion of LGP synbiotic leads to changes in intestinal microbial function and this is thought to affect synthesis of B vitamins. Immune regulation is influenced by specific B vitamins (Yoshii et al. 2019).

Deficiency of this vitamin can cause hyper homocysteinemia which suppress immune system (Ahmad et al. 2019).

Blood profile

Blood profile of broiler chickens due to the administration of the mixture (LGP) in the finisher phase is presented in Table 2. The addition of 3% LGP gave the best effect on hemoglobin, erythrocyte, TPP, PCV, and leukocyte levels in finisher phase broilers reared in open cages. This result was in line with the findings of Beski and Al-Sardary (2015) that the administration of probiotics and synbiotics increased Hb, erythrocytes, and PCV, and decreased H/L in broiler blood. According to Sunu et al. (2021), hemoglobin value in broiler chickens given synbiotic *Lactobacillus acidophilus* and garlic extract resulted in an increase in Hb, by 7.91–9.45 g/dL. Sugiharto et al. (2018), erythrocytes in broiler given probiotic was 1.94–2.07 ($10^6/\mu\text{L}$). Garlic powder contained in synbiotics is known to contain fructooligosaccharides (FOS). This can be useful as prebiotics to stimulate the growth of *Lactobacillus casei* probiotic bacteria in the digestive tract (Kothari et al. 2019), resulting in better digestive tract conditions. This is supported by the population of lactic acid bacteria (LAB) in the cecum of broiler chickens in the LGP treatment particularly at 3%, which was 6.4×10^{11} CFU/g, and has the highest number compared to other treatments. An increase in LAB caused an increase in of lactic acid pruction, which altered pH of digestive tract more acidic and suppressed growth of pathogenic bacteria in the digestive tract. According to Shanmugasundaram et al. (2019), synbiotic supplementation consisting of *Lactobacillus reuteri*, *Bifidobacterium animalis*, *Enterococcus faecium*, *Pediococcus acidilactici* and *fructooligosaccharide* reduced *Salmonella* colonization in the intestines in poultry. Recently, the use of synbiotics (*Lactobacillus spp.*, *Saccharomyces cerevisiae* and inulin) in broiler chickens, increased lactic acid, short chain fatty acid, and beneficial bacteria populations, while decreasing branched chain fatty acids and gut pathogen populations (Śliżewska et al. 2020). The increase in LAB due to LGP supplementation was also caused by LAB being able to stimulate development of intestinal epithelial cells, so that it is more optimal in process of absorption of nutrients in feed including protein and Fe or iron, which are raw materials for hemoglobin and erythrocyte synthesis. Beski & Al-Sardary (2015) stated that giving synbiotics increases blood hemoglobin levels, because production of lactic acid from synbiotics makes pH condition of the small intestine more acidic so that absorption of Fe as a raw material for hemoglobin increases. Increased hemoglobin levels can also be

caused by increased production of vitamin B complex by bacteria in the small intestine (Yoshii et al. 2019). Vitamin B12 deficiency in livestock can cause weakness and anemia because vitamin B12 is involved in the formation of hemoglobin (Ahmad et al. 2019).

Total plasma protein increased with the increasing levels of LGP. This means that LGP synbiotics not only improve the condition of the digestive tract, but also increase the absorption of nutrients, especially protein as a raw material for blood synthesis. With the increase in total plasma protein due to administration of 1-3% LGP, the synthesis of immune organs and production of antibodies also increases. Blood plasma proteins play a role in maintaining colloid osmotic pressure, as a very fast substitute for amino acids, play a role in gluconeogenesis, transport minerals and hormones, and play a role in the formation of enzymes and the immune system in the body (Filipović et al. 2007).

The hematocrit value in the 3% LGP treatment was significantly higher than the other treatments. Packed cell volume (PCV) are very sensitive to the level of protein intake and protein digestibility by poultry (Saki et al. 2018). Administration of 1-3% LGP improves gut microbiota, lowers pH, and increases protein digestibility so that the availability of protein for red blood synthesis increases, which is indicated by an increase in PCV. Giving a combination of probiotics and prebiotics in feed significantly increased the PCV, RBC, and Hb of guinea fowl (Habibu et al. 2016). It was reported that hematocrit and red blood cell volume have a very large correlation with each other. If the average hematocrit decreases, the hemoglobin level becomes lower (Ahmad et al. 2019, Habibu et al. 2016).

Leukocytes in this study showed a significant increase ($P < 0.05$). This means that giving LGP is able to modulate the number of white blood cells, which means that the production of antibodies increases to fight foreign objects in the body. The administration of LGP can increase the digestibility of nutrients, especially protein which is the material for the synthesis of blood components, including leukocytes. The increase in the number of leukocytes is also related to the immune system in reducing pathogen attacks. If the attack of pathogens increases, it will further increase the production of leukocytes in the blood (Sugiharto et al. 2018; Rehman et al. 2020). The response of broilers to stressors depends on the type of stress experienced. The increase in the number of leukocytes illustrates the presence of a humoral and cellular resistance response to pathogenic agents that cause disease. An increase in the number of leukocytes indicates an improvement in the body's defenses/ immune system. (Saki et al. 2018). Efficient activation of host defense and timely restoration of immune homeostasis are closely related to success against bacterial pathogens (Bayona et al. 2017). This research results indicate that the range of

broiler chicken leukocytes was normal. According to Sunu et al. (2021), the range of leukocytes in broiler chickens treated with synbiotics (garlic extract and *L. acidophilus*) was $7.43 \times 10^3/ \text{ml}$ – $11.60 \times 10^3/ \text{ml}$.

The administration of LGP in finisher phase broilers significantly reduced blood heterophil levels. Heterophils are a type of granulocyte from leukocytes that act as the first leukocyte defense that appears during acute inflammation and infection. Lymphocytes which are agranulocytes of leukocytes have a role as advanced defense cells that play a role in lysing toxic cells (T lymphocytes) and forming immunity (B lymphocytes). The heterophil-lymphocyte ratio (H/L ratio) can be referred to as an indicator of stress in chickens (Saki et al. 2018). In this study, the number of lymphocytes did not decrease. This means that the administration of LGP is able to capture free radicals and suppress the hormone corticosterone so that the production of lymphocytes remains normal and does not decrease (Kridtayopas et al. 2019). The decrease in the H/L ratio in the group of chickens treated with LGP synbiotic may be influenced by the increase in the number of beneficial microbes in the digestive tract, which stimulates afferent neurons through a cytokine neurohumoral route and lead to reduced levels of corticosterone in the bloodstream (Sohail et al. 2010).

The H/L ratio in the 1-3% synbiotic treatment decreased significantly, a lower H/L ratio indicated an increase in body resistance. The number of lymphocytes is most commonly found in white blood cells and is considered an indicator of the health level of livestock because lymphocytes are the body's most important defense cells (Saki et al. 2018). Some researchers report that the H/L ratio is more acceptable as an indicator of mild or severe stress when compared to measuring corticosterone hormone levels (Sunu et al. 2019; Saki et al. 2018). The decrease in the H/L ratio in this study may be due to the effect of synbiotic LGP in inhibiting stress caused by nutrition and the environment. (Kleniewska & Pawliczak 2017) stated that synbiotics containing *Lactobacillus casei* with inulin are effective compounds to protect the body from oxidative stress damage. Synbiotics may have a positive effect on the blood plasma antioxidant capacity and the activity of selected antioxidant enzymes. Stress can stimulates the adrenal glands to secrete hormones such as direct effect estrone for has a direct effect to analyze a lymphatic cell which causes an increase in H/L ratio (Beski et al. 2015).

Administration of LGP synbiotic significantly ($P < 0.05$) increased carcass production and final body weight. Giving 1-3% LGP resulted in the same carcass weight. Giving 3% LGP resulted highest final body weight. This was due to the role of *Lactobacillus casei* and garlic in the digestive tract, which causes an

increase in lactic acid bacteria, and improves microbial balance and better gut health. The gut microbiota plays a decisive role in the manipulation of intestinal epithelial proliferation, vitamin synthesis, and host energy metabolism. Changes in the microbiota can affect the morphology of the intestinal wall and cause immune reactions which in turn can affect the energy expenditure and development of chickens (Rehman et al. 2020). Administration of *Lactobacillus casei* DBN023 to chicks significantly increased their jejunum villi height, villi-to-crypt depth (V/C) ratio, and muscle thickness, enhanced gut mucosal immunity, regulates cytokine balance, and effectively reduces gut inflammation (Wang et al. 2019). The increase in gut villi height causes an increase in nutrient absorption for the synthesis of body tissue, and ultimately increases carcass weight and final body weight.

CONCLUSION

The administration of 3% LGP improved the weight of bursa fabricius, thymus, H/L ratio and yielded the best broiler performance.

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