International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Intestinal Microflora Status Changes in Broiler Fed Turmeric and Acidifier Supplemented Feed

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Abstract**—** *The study aims to examine the effect of turmeric powder (Curcuma domestica) and acidifier in broiler feed on intestinal microflora status. A total of unsexed 240-day-old commercial broiler chicks (Cobb strains) were used. This study was conducted for five weeks at a village farm in Buring, Kedungkandang, Malang. The experiment consisted 6 treatments and 4 replications. The treatments were T0: Basal feed without additive, T1: Basal feed + 2.0% turmeric powder + 0% acidifier, T2 : Basal feed + 1.5% turmeric powder + 0.5% acidifier, T3 : Basal feed + 1.0% turmeric powder + 1.0% acidifier, T4: Basal feed + 0.5% turmeric powder + 1.5% acidifier, T5: Basal feed + 0% turmeric powder + 2.0% acidifier. Then, the analysis of variance (ANOVA) was performed by using a Completely Randomized Design (CRD), and Duncan's New Multiple Range Test was used if a significant effect was detected. Results of the study revealed that the treatments show a highly significant response (P<0.01) on the number of microflora colonies (Lactic Acid Bacteria/LAB dan Escherichia coli), and a significant response (P>0.05) on the number of Salmonella sp. In conclusion, the mix of 0.5% turmeric powder and 1.5% acidifier gives the best result in increasing the LAB colony and inhibiting pathogenic bacteria (Escherichia coli dan Salmonella sp.) in broilers.*

Keywords**—** *Turmeric powder, acidifier, microflora, ileum, and digesta.*

I. Introduction

Due to their affordability, broiler chickens are highly consumed as animal proteins in Indonesia, especially compared to other kinds of meat. A variety of menus then could be found in different parts of the country. Therefore, ensuring the balance between supply and demand for broiler chicken meat is essential.

Broiler feed is one of the most important factors in the poultry management system. The high-quality one helps broilers meet their nutritional requirements in terms of quality and quantity. Furthermore, broilers' nutrient digestibility would not be optimal if feed additives are not included. Widodo [1] defines a feed additive is an extra nutrient outside the main substances (proteins, carbohydrates, fat, vitamins, and minerals) to enrich poultry nutrition.

Antibiotics are mostly-used feed additives to improve the performance of broiler chickens. However, the irresponsible use of antibiotics can cause residues, which are dangerous for the poultries, and particularly consumers. According to the Regulation of the Minister of Agriculture No.14/PERMENTAN/PK.350/5/2017 about the classification of veterinary drugs, the use of antibiotics in Indonesia has been prohibited since 2018. Phytobiotics and acidifiers are popular feed additive alternatives to Antibiotic Growth Promoters (AGP).

Turmeric is a plant herb grown commercially in the tropics. This plant can be used as a phytobiotic feed additive. Sjofjan et al. [2] reported that turmeric supplement in animal feed exhibits antifungal activities, immunomodulators, antioxidants, and antimutagen. Additionally, turmeric can also be an effective antibacterial to suppress pathogenic bacteria and increase beneficial bacteria in the poultry digestive system, which can optimize its gut health. So, turmeric can act as a natural antibiotic in poultry. Other functions of curcumin are reducing peristalsis, prolonging the digestion process, and improving nutrient absorption.

Acidifier is kinds of organic and inorganic acids intentionally used in the broiler feed as a feed additive. Wijayanti et al. [3] mentioned that the acidifier could reduce pH in avian digestive tracts and, therefore, might suppress pathogenic growth. Yoeman et al. [4] described that microorganisms in the avian digestive tracts play an important role in broilers' growth, and the development of intestinal morphology. The acid condition in the digestive tract can boost enzyme activities, lactic acid bacteria, and gut performance.

Considering the benefits, this research aims to evaluate the use of turmeric powder (*Curcuma domestica*) and acidifier could form an excellent combination to change the intestinal microflora benefit for productivity of broilers.

II. Material and Methods

The research was conducted from 12 November to 18 December 2022 in an open house farm in Buring, Kedungkandang, Malang. A total of 240 day-olds commercial Cobb broiler chicks were used without considering sex. The average body weight was 40.55±1.77 grams with a coefficient of variation (CV) of 4.35%. The chickens were raised for 35 days in an experimental unit with the sizes of 100×100×70 cm. The experiment was divided randomly into six treatment groups with four replications of 10 chickens in each. The basal feed used was a commercial complete feed produced by PT. Japfa Comfeed Tbk Indonesia. The feed was obtained from local poultry shop. The feed ingredients of basal feed consisted of local corn, wheat flour, soybean meal (SBM), meat and bone meal (MBM), corn gluten meal (CGM), wheat bran, palm oil (Palm olein), and amino acids. The nutritional content of the basal feed is presented in Table I.

Adi Nurbiantoro Munthe, Rositawati Indrati and Eko Widodo, "Intestinal Microflora Status Changes in Broiler Fed Turmeric and Acidifier Supplemented Feed," *International Research Journal of Advanced Engineering and Science*, Volume 8, Issue 2, pp. 145-147, 2023.

International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

TABLE I. Nutritional content of basal feed

Nutrient Content	Feed code (Period)	
	BR-1 (Starter)	BR-2 (Finisher)
Water (%)	max. 12	max. 12
Ash (%)	max. 7	max. 7
Crude protein (%)	21-23	19-20
Metabolizable energy (Kcal/kg)	min. 3.000	min. 3.100
Crude fat (%)	min. 5	min. 5
Crude fiber (%)	max. 5	max. 5
Calcium (Ca) (%)	0,8-1,1	0,8-1,1
Phosphor (P) (%)	min. 0,50	min. 0,45
Aflatoxin (µg/kg)	max. 50	max. 50

Source: PT. Japfa Comfeed Tbk.

A feed additive given to the chicks was a mix of turmeric powder (*Curcuma domestica*) and an acidifier. The turmeric powder was from UPT. Materia Medica, Batu, while the acidifier was from Poultry Shop. The acidifier contained formic acid 350g, lactic acid 58 g, propionic acid 78 g, citric acid 99.5g, silicon dioxide 18.5g and carrier to make 1kg. This study uses an experimental method with a Completely Randomized Design (CRD) using 6 treatments and 4 replications:

T0 : Basal feed without additive.
T1 : Basal feed + 2% turmeric powder + 0% acidifier
T2 : Basal feed + 1,5% turmeric powder + 0.5% acidifier
T3 : Basal feed + 1% turmeric powder + 1.0% acidifier;
T4 : Basal feed + 0,5% turmeric powder + 1.5% acidifier;
T5 : Basal feed + 0% turmeric powder + 2.0% acidifier.

On day 35, one bird from each experimental unit was taken, slaughtered and the visceral organ was removed, ileum part of the small intestine was taken. The digesta sampling was carried out from the ileum by gently squeezing intestinal content and transferring it into a petri dish. The sample was put into a pill bottle and taken to the laboratory for pH measurement, viscosity, and microbiological tests. The collected digesta was then analyzed for the number of Lactic Acid Bacteria (LAB), *Escherichia coli*, and *Salmonella sp*. based on Gariga, et al. [5].

The data were analyzed by using analysis of variance (ANOVA). The significant treatment ($P<0.05$) would then be continually analyzed using the Duncan's New Multiple Range Test.

III. Results and Discussions

The effect of feed additives (turmeric powder and acidifier) supplements in the broiler feed on the microflora of ileal digesta in the broiler is presented in Table II.

TABLE II. The effect of treatment ileal microflora in broiler.

Treatment	Bacterial Colony (CFU/ml)		
	LAB**	***Escherichia coli****	***Salmonella sp.***
T0	6.88 ± 0.48^{A}	4.63 ± 0.85^{b}	4.00 ± 0.91
T1	7.13 ± 0.63^{AB}	3.63 ± 0.63^{a}	3.75 ± 0.50
T2	8.50 ± 1.08^{BC}	3.38 ± 0.48^{a}	3.63 ± 0.75
T3	8.88 ± 0.75^{C}	3.13 ± 0.48^{a}	3.50 ± 0.41
T4	9.25 ± 0.87^{C}	3.00 ± 0.41^{a}	2.81 ± 0.38
T5	7.25 ± 0.29^{AB}	3.50 ± 0.58^{a}	3.75 ± 0.50

Note:
(*) Significant effect (P<0.05) indicated by different superscripts in the same column (a-c)
(**) Highly significant effect (P<0.01) indicated by different superscripts in the same column (A-C)

a). The treatment effect on the number of LAB colonies

Lactic acid bacteria (LAB) under the Lactobacillus genus are Gram-positive, facultatively anaerobic, rod-shaped (0.5-1.5 s/d 1.0-10 µm), and non-motile [6]. The growth of LAB in the digestive tract of the broiler depends on environmental factors, including temperature, pH, and nutritional contents. The optimal temperature range for LAB is 37–42 ºC [7].

The supplementation of turmeric powder and acidifier in broiler feed highly significantly affected ($P<0.01$) the number of LAB. Syukur [6] found that LAB could potentially keep the digestive system healthy and live well in a low pH (2-4). In this experiment, the gradual increase in acidifier level replacing the amount of turmeric would be expected to decrease the pH of ileal digesta. This finding is in accordance with such theory, except that removing turmeric from the supplement causes the number of LAB to decrease.

The best treatment to increase the populations of LAB was T4 and T3, it might suggest the best treatment might be achieved by adding 1.0% turmeric and 1.0% acidifier or 0.5% turmeric and 1.5% acidifier. Acidifier used in this experiment contains a mixture of formic acid, lactic acid, propionic acid and citric acid was effective to stimulate growth of LAB if combined with turmeric.

b). The treatment effect on the number of Escherichia coli colonies

Escherichia coli bacteria was discovered by Theodor Escherich in 1885 and was named after its founder. *Escherichia coli* is a rod-shaped, Gram-negative bacterium with a length of about 2 µm and a diameter of about 0.5 µm. This bacterium can live in a temperature range of 20–40ºC with an optimum temperature of 37ºC [8]. Moreover, Rositawati [9] mentioned that the shape of *Escherichia coli* varies from short rods (cocci) to elongated or filamentous rods. *Escherichia coli* cells can be motile and nonmotile and usually are not encapsulated. It also can be either aerobic or facultatively anaerobic.

Escherichia coli is generally safe and lives inside the digestive tract. *Escherichia coli* is initially nonpathogenic, but it will turn into a pathogenic bacterium if it obtains additional virulence genes from other microorganisms through the mechanism of gene transfer (transformation), plasmid transfer (conjugation) or gene transfer via bacteriophage (transduction). Pathogenicity is the ability of a microorganism to cause disease. *Escherichia coli* can cause symptoms of a certain disease if it can enter, adapt and survive in its host's body, which will then attack the immune system and eventually cause disease. This pathogenesis mechanism is carried out through several stages like other pathogenic bacteria: colonization at particular points in the intestine's surface sells (mucous cells), cell division, destruction of intestinal cells, crossing intestinal cells and entering the bloodstream, landing to a target organ and finally causing organ damage [7].

The supplementation of turmeric powder and acidifier treatments significantly affected ($P<0.05$) the number of *Escherichia coli*. In contrast to LAB, *Escherichia coli* could not grow in an acidic environment (pH below 6) [3]. This experiment demonstrated that the supplementation of turmeric powder and acidifier in broiler feed could decrease the colonies of *Escherichia coli*. The active substances in turmeric and the

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role of acidifier in lowering the pH value can synergize in suppressing the number and growth of pathogenic *Escherichia coli* bacteria. Febriza et al. [10] found that the curcumin in turmeric in the intestine could trigger intestinal epithelial cells to stimulate more Anti-Microbial Peptides (AMP), which were peptide proteins used for intestinal defense from pathogenic bacteria's harm. Ahmed et al. [11] added that turmeric could control and limit the growth of various species of pathogenic and nonpathogenic bacteria in the broilers' intestines, resulting in an intestinal microbial ecosystem balance that leads to feeding utilization efficiency in terms of live weight and body weight gain.

The decline of *Escherichia coli* colonies in each treatment was adversely correlated with the increase of LAB colonies in the broiler ileum. Santoso et al. [12] explained that LAB could produce acid in large quantities and quickly, as well as develop other anti-microbial components in addition to an acidic environment that is effective in inhibiting pathogenic bacteria.

c). The treatment effect on the number of Salmonella sp.

Salmonella sp. is a Gram-negative stem-shaped facultative which can cause typhoid, paratyphoid, and Foodborne disease [13]. *Salmonella sp.* was first discovered (examined) in patients in 1880 by Eberth and confirmed by Robert Koch in 1881. *Salmonella sp.* is a stem-shaped bacterium that turns pink on Gram staining (Gram-negative). The main habitat of *Salmonella* is the intestinal tract of humans and animals [14]. *Salmonellosis* in humans can cause various diseases, including fever, sepsis, tissue infection, and gastroenteritis. Poultry is a major source of infection by pathogenic agents in humans [15].

The turmeric powder and acidifier supplementation in broiler feed had no significant effect (P>0.05) on the number of *Salmonella sp.* colonies. It might be due to the dietary treatments had not been able to make a more acidic environment, so *Salmonella sp.* still grew in the generated environment. Putra [16] explained that *Salmonella sp.* could grow and thrive at a minimum pH of 3.5. Ahmed et al. [11] stated that turmeric could control the balance between various pathogenic and non-pathogenic bacteria in the intestines, resulting in better feed utilization in terms of live weight and body weight gain.

IV. Conclusion

The supplementation of turmeric powder and acidifier on broiler feed could increase the number of Lactic Acid Bacteria (LAB), and decrease the number of *Escherichia coli*, but unable to change the number of *Salmonella sp*. Therefore, the combination of turmeric and acidifier might be used to be proposed as an AGP replacer.

References

[1] Widodo. E. 2017. Ilmu Bahan Pakan Ternak dan Formulasi Pakan Unggas. Universitas Brawijaya Press. Malang.
[2] Sjofjan, O., Danung N. A, Muhammad H. N dan Ajeng K. 2020. Pengaruh Kombinasi Tepung Kunyit (*Curcuma Domestica VAL*) dan Probiotik Terhadap Penampilan Usus Ayam Pedaging. Jurnal Nutrisi Ternak Tropis dan ilmu Pakan. 2(1): 19-25.
[3] Wijayanti, D. A., I. H. Djunaidi and O. Sjofjan. 2019. Effect of Probiotic and Acidifier Combination as an Alternative to Antibiotic Growth Promoters on Digesta pH And Intestinal Microflora of Laying Hen. International Research Journal of Advanced Engineering Science. ISSN (Online): 2455 - 9024.
[4] Yoeman, J., C. Nicholas, J. Patricio, S. Maksim, D. Nigel, Goldenfel, Bryan and A. Welington. 2012. The Microbiome of the Chicken Gastrointestenial Tract. Cambridge University Press. Animal Health Research Reviews. 13(1): 89–99.
[5] Garriga, G., Pascual, P., Monfort, M., and Hugas, H. 1998. Selection of Lactobacilli for Chicken Probiotic Adjuncts. Journal of Applied Microbiology, 84(1), 125-132.
[6] Syukur, S. 2017. Bioteknologi Dasar Dan Bakteri Asam Laktat Antimikrobial. Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas: Padang, Sumatra Barat.
[7] Rahayu, K. S., F. Manin, Mairizal dan Akmal. 2020. Total Bakteri Asam Laktat dan *Escherichia coli* pada Broiler yang diberi Ransum Mengandung Bungkil Inti Sawit Fermentasi dengan *Bacillus cereus V9*. Prosiding Hasil Penelitian dan Pengabdian Kepada Masyarakat. ISBN: 978-602-50946-7-5. Pp: 307 – 314.
[8] Sutiknowati, L. I. 2016. Bioindikator Pencemar, Bakteri *Escherichia coli*. *Oseana.* 41(4): 63 – 67.
[9] Rositawati, T. 2009. Aktivitas Antibakteri Ekstrak Etanol Bunga Rosella (*Hibicus sabdariffa* L.) Terhadap *Escherichia coli*, *Salmonella Thypi* dan *Staphylococcus Aureus* dengan Metode Difusi Agar. Penelitian Mandiri. Universitas Padjajaran. Bandung.
[10]Febriza, A., V. N. A. Kasim, H. H. Idrus and M. Hatta. 2019. The Effect of Curcumin and Vitamin D-Combination as Inhibitor Toward *Salmonella Typhi* Bacteria Growth In Vivo. International Journal of Applied Pharmaceutics. 11(5): 1-5.
[11]Ahmed, I. T. El-Rayes and A. I. Ahmed. 2018. Assessment of Dietary Supplementation of Turmeric (*Curcuma Longa*) As a Phytobiotic on Broiler Performance and Bacterial Count. Egyptian Journal Nutrition and Feeds. 21(2): 519-528.
[12]Santoso, E. P., E. Fitasari dan N. Thiasari. 2021. Penggunaan Limbah Sayur Pasar dalam Pakan Crumble dan Pengaruhnya terhadap Penampilan Produksi dan Mikroflora Usus Ayam Broiler. Journal of Tropical Animal Production. 22(1): 1-11.
[13]Apriani, L., Rahmawati dan R. Kurniatuhadi. 2019. Deteksi Bakteri *Salmonella sp.* dan *Shigella* Pada Makanan Burger di Sungai Raya Dalam Pontianak. Protobiont. 8(3): 53-57.
[14]Kasim, V. N. A., 2020. Peran Imunitas Pada Infeksi *Salmonella Typhi*. C. V. Athra Samudra: Bone Bolango, Gorontalo.
[15]Nakphaichit M, Sobanbua S, Siemuang S, Vongsangnak W, Nakayama W, Nitisinprasert S. 2018. Protective effect of *Lactobacillus reuteri* KUB-AC_5 against *Salmonella Enteritidis* challenge in chickens. Beneficial Microbes. 10:43–54.
[16]Putra, H. P. 2015. Deteksi Bakteri *Salmonella sp* pada Es kelapa Muda yang di Jual di Wilayah Kecamatan Sekarbela, Kota Mataram. Jurnal Penelitian dan Kajian Ilmiah Kesehatan. 1(2): 51–55.

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