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The Effect of Adding Fermented Garlic (*Allium sativum*) Peel Extract Through Drinking Water on Intestinal Histology, Meat Chemistry, and Digestive Tract Microbes of Broilers

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Abstract---The study aimed to determine the effect of additional fermented garlic peel extract through drinking water on intestinal histology, meat chemical quality, and microbes of the digestive tract of broilers aged 4 weeks. The research was conducted at Ssetan Farm, Faculty of Animal Science, Udayana University, Jalan Raya Ssetan, Gang Markisa Number 5, Ssetan Village, South Denpasar District, Denpasar City, Bali. The design used was a completely randomized design with 4 treatments and 4 replicates, each replicate containing 5 broilers so the total number of broilers used was 80. The treatments given were broiler chickens given drinking water without garlic peel extract P0 (Control), broiler chickens treated with 1.5% garlic peel extract in drinking water (P1), broiler chickens treated with 3% garlic peel extract in drinking water (P2), broiler chickens treated with 4.5% garlic peel extract in drinking water (P3). The variables observed were villi height, kript depth, ash content, water content, fat content, protein content, carbohydrate content, total plate count (TPC), lactic acid bacteria (BAL), *escherichia coli*, and Coliform. The results showed that treatment with a level of 1.5% in the drinking water of broiler chickens aged 4 weeks can significantly ($P < 0.05$) increase villi height, protein content, carbohydrate content, and total plate count (TPC). As well as the addition of fermented garlic peel with a level of 4.5% in drinking water showed a real effect ($P < 0.05$) on the depth of crypts, ash content, fat content, *escherichia coli*, and coliform in broiler chickens aged 4 weeks. It can be concluded that the addition of fermented garlic peel with a level of 4.5% to the drinking water of broiler chickens can improve intestinal histology, meat chemical quality, and digestive tract microbes.

Keywords---broiler, digestive tract microbes, fermentation, garlic peel, intestine histology, meat chemical quality.

Introduction

The need for animal protein is very high for the community and can be met quickly, especially in the poultry sector in Broiler products. It has characteristics as a meat producer, low ration conversion, can be slaughtered at a young age and produces meat with soft fibers (Bell & Weaver, 2012) and 4 to 6 weeks can be harvested (Ishantana, 2021).

Antibiotic Growth Promoters (AGPS) have been used by farmers for 40 years as additives in feed with relatively small amounts capable of increasing feed efficiency and livestock production. The issuance of the Animal Husbandry and Health Law No. 18 of 2009, article 22, paragraph 4c prohibits the use of AGPS, because it can cause pathogenic microbial contamination and leave residues in meat that are harmful to consumers (Saeid & Al-Nasry, 2010). The use of antibiotics as one of the feed additives mixed into livestock drinking water is closely related to productivity, livestock health, and the nutritional state of the livestock, one of which is antibiotics. The high use of

antibiotics is due to their practical use and shows fast and significant results. Long-term use will cause excessive residue buildup and chemicals contained in livestock products produced in the form of resistance to bacteria contained in the body of chickens (Leusink et al., 2010; Gao et al., 2021; Józefiak et al., 2007).

Fermentation is a processing technology that aims to improve the nutritional content of a material. Processing by fermentation is related to the work of enzymes produced by microbes that are able to break down complex components into simple ones that are easily digested by livestock (Pamungkas, 2011). Garlic peel fermentation aims to reduce the crude fiber of food ingredients before fermentation. High crude fiber can be an obstacle to nutrient absorption for the utilization of livestock productivity. The role of microorganisms in the fermentation process of garlic peel can add nutritional content for livestock growth. Syamsiah (2003), added that in addition to allicin garlic also contains scordinin compounds that function as antioxidants that can suppress cholesterol, increase endurance, spur growth, and prevent cell damage. Purwanti (2015), stated that the addition of rations with 2.0% garlic water extract is capable of being anti-bacterial and can be used as an alternative feed additive to replace antibiotics as a growth promoter. The results of research by Nuriyasa et al. (2022), found that the use of fermented banana peel at the level of 5% in feed can reduce cholesterol content and fat content of 0-10-week-old native chicken meat.

Broiler meat supplemented with garlic flour and temulawak flour is more tender because garlic contains several active compounds that are important for nutrient metabolic processes in the body (Shoetan et al., 1984). Prabayanti et al. (2023), stated that the effect of garlic peel extract through drinking water at the level of 1% - 3% had no significant effect on broiler external offal.

Materials and Methods

Livestock, drinking water, and research draft

Broilers used were DOC (Day Old Chicken) broilers as many as 80 heads without distinguishing their gender (unsexed) DOC broilers obtained from UD. Setia Ternak, whose address is in Banjar Anyar, Kediri, Tabanan. With an average broiler DOC of 46 - 47 g. The chicken cages used were made of wood, bamboo, and iron wire with a size of 60 cm long × 60 cm wide × 50 cm high using a battery colony system of 16 units. This study used a completely randomized design (CRD) with 4 treatments and 4 replications, with each experimental unit consisting of 5 broilers, with a total of 80 broilers used with a homogeneous weight. The treatments in this study are as follows: P0: Broilers given drinking water without fermented garlic peel extract, P1: Broilers given drinking water with 1.5% fermented garlic peel extract, P2: Broilers given drinking water with 3% fermented garlic peel extract, P3: Broilers were given drinking water with 4.5% fermented garlic peel extract. The method of making fermented garlic peel extract is by collecting peeled garlic peel and still clean white, the garlic peel is then washed with clean water and then cut into small pieces, then the garlic peel is blended with water in a ratio of 1: 1, meaning that in 100g of garlic peel is filled with 100ml of water. Then the garlic peel is blended and squeezed to get garlic peel extract water. Next, it is put into a bottle, and fermentation of garlic peel extract is done by mixing EM4 as much as 10ml. fermented garlic peel extract is stored closed and fermentation is carried out for 5 days. After the fermented garlic peel extract can be given according to the treatment. Rations and drinking water are given at 08.00 WITA with ad libitum administration. Feed is given to broilers as much as 75% of the feed bin with the aim that the ration is not scattered because it is too full. Drinking water is given to broilers ad libitum in the morning at 08.00 WITA. Sampling of villi and crypts was done by slaughtering one broiler in each experimental unit. Villi samples were taken by cutting 4-5 cm in the ileum and then stored in a film pot containing formalin with a concentration of 10%. The villous samples were then brought to the laboratory for preparation. Sampling of meat chemical analysis was carried out by slaughtering chickens at the neck by cutting the carotid artery and jugular vein. Broiler digestive tract microbes namely *Escherichia coli*, coliform, Lactic Acid Bacteria (BAL), and Total Plate Count (TPC) can be calculated based on Laboratory Standbio (2011).

Table 1
Ration nutrient content 511 Bravo

Nutrient content (%)	Level
Water	Max 13
Protein	21 - 23
Fat	Min 5
Fiber	Max 5
Ash	Max 7
Calcium	Min 0,9
Phosphor	Min 0,6
ME (Kcal/kg)	2900 - 3000

Source: Feed brochure of PT Charoen Pokphan Indonesia, Tbk.

Table 2
Results of proximate analysis of garlic peel

Nutrient (%)	Result
Ash	2,331
Protein	1,501
Fat	14,982
Carbohydrates	67,871
Crude fiber	7,117

Source: (Prabayanti et al., 2023)

Table 3
Crude fiber yield, phytochemical content and lactic acid bacteria of unfermented and fermented garlic peel extracts

Content	Analysis Result	
	Without Fermentation	Fermentation EM4
Crude Fiber (%)	0,57	0,31
IC 50 (ppm)	8882,94	7631,06
Antioxidant capacity (mg/L GAEAC)	46,12	70,94
Fennol (mg/ 100g)	15,25	14,50
Flavanoid (mg/ 100g)	16,18	16,53
Tannin (mg/ 100g)	1526,15	1278,84
Lactic Acid Bacteria (CFU/ml)	3,88×10 ⁴	4,96×10 ⁴

Source: Pelayanan Terintegrasi Laboratory, Faculty of Agricultural Technology, Udayana University (2024)

Laboratory analysis

Villi samples were taken by cutting 4-5 cm in the ileum and then stored in a film pot containing formalin at a concentration of 10%. The villous samples were then taken to the laboratory for preparation. Sampling of meat chemical analysis is done by slaughtering chickens on the neck by cutting the carotid artery and jugular vein. After slaughtering and the blood has come out completely then the chicken is put into hot water with a temperature of 50-55°C for 10-15 minutes. After that, feather removal, removal of internal organs, and cutting of the neck and legs are carried out. The meat analyzed was taken from the breast and stored in airtight plastic. The meat was then placed in a refrigerator. Broiler digestive tract microbes namely *Escherichia coli*, coliform, Lactic Acid Bacteria (BAL), and Total Plate Count (TPC) can be calculated based on Laboratory Standbio (2011). Samples were cecal fluid in the small intestine taken on day 35 of rearing. Sampling was done randomly at each experimental level. Samples taken ± 5g were put into a clip bag, and then taken to the laboratory for testing. Sterilization of tools, medium, and test tubes containing 0.1% peptone solution as much as 9 ml. Sterilize the tools using an oven at 170°C for 1 hour, while sterilizing the medium and test tubes using an autoclave for 15 minutes at 121°C.

Result and Discussion

Intestine histology, meat chemical quality, digestive tract microbes

Statistically, the results of the P1 and P3 treatments showed a significant effect (TABLE 4) on the variables of villi height, kript depth, ash content, protein content, fat content, carbohydrate content, TPC, E.Coli and Coliform. This condition occurs due to the allicin compound contained in garlic which is able to eliminate pathogenic bacteria so that the feed consumed can be digested and absorbed perfectly. The height of the small intestinal villi reflects the area of nutrient absorption (Sukada et al., 2016; Widyantara & Sukaatmadja, 2019; Ao et al., 2011). Furthermore, it is explained that increasing the height of the small intestine villi in broilers can affect the improvement of digestion and absorption functions due to the expansion of the absorption area and is an expression of the smooth transportation system of nutrients throughout the body (Awad et al., 2008). There is flavanoid content in garlic peel that can reduce the percentage of body fat by inhibiting its absorption process in the intestine. The higher the level of garlic peel, the lower the absorption of fat and the higher the absorption of protein nutrients in the intestine. Natural antioxidants can reduce the growth of many pathogenic intestinal bacteria, thereby reducing intestinal colonization and infectious processes, lowering the inflammatory process in the intestinal mucosa, and this increases the length of the villi, the depth of the crypta, and the function of secretion, digestion and absorption of nutrients (Iqbal et al., 2016).

Tabel. 4
Application of fermented garlic skin extract in drinking water on broiler intestinal histology

Variabel	Perlakuan ¹⁾				SEM ³⁾
	P0	P1	P2	P3	
Tinggi Vili(μm)	818,30 ^{c2)}	1167,40 ^a	969,15 ^{bc}	984,45 ^b	31,38
Kedalam Kript(μm)	159,62 ^b	119,48 ^d	152,90 ^c	207,88 ^a	14,42

Description:

1. Drinking water treatment

P0: Drinking water without fermented garlic skin extract (Control)

P1: 1.5% fermented garlic skin extract in drinking water

P2: 3% fermented garlic skin extract in drinking water

P3: 4.5% fermented garlic skin extract in drinking water

2. Values with the same letter in the same row indicate significantly different (P<0.05).

3. SEM: standard error of treatment means

This is due to some of the minerals contained therein such as alicin (organic sulfur) a mineral content present in garlic peel. The high ash content is determined by the content of insoluble minerals in the meat. Ash content is a component of inorganic substances that do not burn in the combustion process. According to Tamzil (2014), fresh chicken meat contains an ash content of 1.14%. Ash content is a determining factor of nutritional content related to mineral content in chicken meat. Ash is an inorganic substance left over from the combustion of an organic material. Ash content is a mixture of inorganic or mineral components found in food. Food consists of 96% inorganic material and water, while the rest is mineral elements (Andrée et al., 2010; Krystallis & Arvanitoyannis, 2006; Engberg et al., 2004).

It can be seen that the addition of fermented garlic peel, the higher the level of provision can increase the protein content of broiler meat. This is due to the antimicrobial content of garlic which serves to prevent the process of protein degradation carried out by microbes. This is in accordance with the statement of Meilani et al. (2014), that antimicrobials in garlic can inhibit microbes from hydrolyzing proteins which results in protein content being maintained, so that the higher the addition of garlic, the better the antimicrobial activity in inhibiting the protein hydrolysis process. According to Nurohim et al. (2013), allicin compounds function to inhibit microbial growth so that microbes cannot use energy sources, especially proteins perfectly, which results in less protein damage to meat.

The increase in broiler meat fat content in the treatment of the 4.5% addition of fermented garlic peel to drinking water is due to the effect of garlic on reducing fat content in chickens related to inhibiting the function of the main enzymes involved in fat synthesis so that onion supplementation affects the process of fat metabolism. Soeparno

(2009), states that, variations in chemical composition between fat and protein levels in meat reflect each other where if protein levels are higher then fat levels will be low and vice versa.

Carbohydrates are mostly in the form of glycogen, so meat is not a source of carbohydrates. The carbohydrate content of meat from this study is slightly higher than the general carbohydrate content of meat, which is 0.5% -1%. This range of carbohydrate content in meat is due to the ability to store glycogen in the body and the treatment before cutting which affects the glycolysis process. The glycolysis process will slowly stop and anaerobic glycolysis will take place by converting glycogen into lactic acid (Jiang et al., 2005).

Table 5
Application of fermented garlic skin extract in drinking water on chemical analysis of broiler meat

Variabel	Perlakuan ¹⁾				SEM ³⁾
	P0	P1	P2	P3	
Kadar Air (%)	63,07 ^{a2)}	60,04 ^b	63,45 ^a	62,98 ^a	0,79
Kadar Abu (%)	0,96 ^b	1,17 ^a	1,15 ^a	1,19 ^a	0,05
Kadar Protein (%)	21,03 ^b	22,42 ^a	22,15 ^a	22,38 ^a	0,31
Kadar Lemak (%)	4,00 ^a	3,64 ^a	3,32 ^{ab}	2,82 ^{bc}	0,17
Kadar Karbohidrat (%)	3,15 ^{cd}	5,56 ^a	3,64 ^c	4,63 ^b	0,26

Description:

1. Drinking water treatment

P0: Drinking water without fermented garlic skin extract (Control)

P1: 1.5% fermented garlic skin extract in drinking water

P2: 3% fermented garlic skin extract in drinking water

P3: 4.5% fermented garlic skin extract in drinking water

2. Values with the same letter in the same row indicate significantly different (P<0.05).

3. SEM: standard error of treatment means

Total plate count (TPC) increased due to the growth of lactic acid bacteria and decreased the growth of escherichia coli and coliform bacterial microflora (Table 5.3) because the provision of fermented garlic peel extract has phytochemical compounds in the form of phenols, tannins, flavonoids, and antioxidant capacity (Table 4.2) which act as antibacterial and anti-fungal so that lactic acid bacterial microflora has increased growth which is directly proportional to the decrease in escherichia coli and coliform bacterial microflora. This is in accordance with the statement of Bulu et al. (2020), that compounds in fermented clove leaf extract in the form of alkaloids, steroids, terpenoids, phenolics, saponins, flavonoids and tannins which are antibacterial in nature can suppress escherichia coli and coliform microflora and increase lactic acid bacteria with the highest total plate count (TPC) of 7.21 Log CFU/ml. Giving fermented garlic peel water extract at levels of 1.5, 3, and 4.5% was able to reduce the microflora of escherichia coli and coliform bacteria (Table 5.3). Fermented garlic peel extract has phytochemical compounds in the form of; phenols, tannins, flavonoids, and antioxidant capacity (Table 4.2) which are antibacterial and antifungal can interfere with the growth and even kill some bacteria by interfering with the metabolic processes of bacteria (Choi et al., 2010; Adjei-Mensah & Atuahene, 2023; Ogbuwu et al., 2021).

This is in accordance with the statement of Khera & Bhargava (2013), that flavonoids are secondary metabolites of plants that have antibacterial, antifungal and anti-inflammatory properties. Most secondary metabolites include isoprene derivatives, flavonoids and glucosinolates and a large number of these compounds have acted as antioxidants (Suganya et al., 2016). Plants contain phytochemical compounds as antioxidants with the ability to suppress the growth of pathogenic bacteria, provide immunity and endurance (Natsir, 2016). Aksu & Bozkurt (2009), reported that the addition of antibiotics had a significant effect on reducing Escherichia coli microflora. Furthermore, Khosravifar et al. (2014), reported that supplementation of 1.5% turmeric flour, 1.5% black pepper, 1.5% black cumin and 1 and 1.5% coriander can reduce the microflora of quail coliform bacteria.

Table 6
Feeding fermented garlic peel extract to drinking water on microbes in the gastrointestinal tract of broilers

Variabel	Perlakuan ¹⁾				SEM ³⁾
	P0	P1	P2	P3	
<i>Total Plate Count</i> (TPC) (CFU/g)	2,5×10 ^{8a2)}	2,8×10 ^{8b)}	1,2×10 ^{8d)}	1,4×10 ^{8cd)}	0,06
Bakteri Asam Laktat(BAL) (CFU/g)	1,4×10 ^{3a)}	2,2×10 ^{4a)}	9,2×10 ^{3b)}	8,7×10 ^{3c)}	4,27
<i>Escherichia Coli</i> (CFU/g)	13,5×10 ^{2a)}	10,5×10 ^{2b)}	10×10 ^{2c)}	9,5×10 ^{2d)}	1,06
<i>Coliform</i> (CFU/g)	1,7×10 ^{3a)}	1,6×10 ^{3b)}	1,5×10 ^{3c)}	1,5×10 ^{3d)}	86,60

Description:

- Drinking water treatment
 - P0: Drinking water without fermented garlic skin extract (Control)
 - P1: 1.5% fermented garlic skin extract in drinking water
 - P2: 3% fermented garlic skin extract in drinking water
 - P3: 4.5% fermented garlic skin extract in drinking water
- Values with the same letter in the same row indicate significantly different ($P < 0.05$).
- SEM: standard error of treatment means

Conclusion

It can be concluded from this study, that the addition of fermented garlic peel with a level of 1.5% in drinking water for broiler chickens aged 4 weeks significantly increases villi height, protein content, carbohydrate content, and TPC. The addition of fermented garlic peel with a level of 4.5% in drinking water showed the best effect on kripita depth, ash content, fat content, e.coli, and coliform in broiler chickens aged 4 weeks.

Suggestion

It can be suggested to farmers by utilizing fermented garlic peel with a level of 4.5% added to broiler drinking water can provide tangible results in improving intestinal histology, meat chemical quality and digestive tract microbes.

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