JAMBURA JOURNAL OF ANIMAL SCIENCE

(Animal Science Department, Faculty of Agriculture, State University of Gorontalo)

https://ejurnal.ung.ac.id/index.php/jjas/index

PISSN: 2655-4356

e-ISSN: 2855-2280

Research Article

Patogen Bacteria and Visceral Organ Weight of Native Chicken in Feed Herbal Supplementation

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Jambura Journal of Animal Science. Volume 7 No 1 (2024)

Keywords:
Herbs,
Native chicken,
Clostridium perfringens,
Pasteurella multocida,
Mycobacterium avium

Abstract: A healthy gastrointestinal tract is the most critical assurance for optimal poultry production. The gut health of poultry is intimately linked to feeds and feeding. This study aims to determine the effect of herbal supplementation as a feed additive, through feeding, on the relative weight of visceral organs and the number of pathogenic bacteria in the small intestine. The method used was the experimental method, which consisted of 2 treatment groups, namely group T0 (chickens were only given basal feed, without the addition of herbs) and group T1 (chickens were given basal feed and additional of 1% herbs). Each group consisted of 50 cross-breeding native chicken, namely Joper and KUB. The variables measured were the relative weight of the visceral organs (heart, pancreas, liver, spleen and bursa of Fabricius) and the number of pathogenic bacteria in the small intestine (Clostridium, Staphylococcus, Mycobacterium, Pasteurella). Data on the relative weight of visceral organs and the number of pathogenic bacteria in the small intestine were analyzed using quantitative descriptive analysis. The results indicated that giving Joper and KUB 1% herbal supplementation had no effect on the relative weight of the visceral organs examined; nevertheless, no pathogenic bacteria were detected in the small intestine after giving Joper and KUB 1% herbal supplementation. This study concludes that no harmful bacteria are detected in the small intestine of native chicken, both Joper and KUB, and that 1% herbal supplementation as a feed additive has a good effect on the health of the heart, digestive glands, and lymphoid glands.

Citation APA Style:
Rahayu D I, Utari T, Widodo W, Sutanto A, Anggraini D.A., ***2024.*** *Patogen Bacteria and Visceral Organ Weight of Native Chicken In Feed Herbal Supplementation. Jambura Journal of Animal Science, 7 (1)1-10*

Received: 02.07.2023 | Accepted: 14.09.2024 | Online published: 08.11.2024

INTRODUCTION

Kampung chicken (native chicken) including cross-bred kampung chicken is a national treasure in the context of fulfilling food neccessity, especially as a source of animal protein. Super kumpung Chicken or Jowo Super (Joper) and Kampung Unggul Balitbangtan (KUB) have been widely cultivated at this time, i.e., extensively, semi-intensively and intensively. One of the inhibiting factors in the development of ayam kampung business is the aspect of disease, including bacterial diseases. There are several bacterial diseases in poultry that are not well known and paid attention to by breeders, even though these diseases are economic or strategic diseases, which are economically detrimental and can also disrupt human health, because they cause foodborne diseases. These bacterial diseases include Clostridial Necrotic Enteritis caused by *Clostridium perfringens* (Cl. perfringens), Pasteurellosis caused by *Pasteurella multocida* (P. multocida), Mycobacteriosis caused by *Mycobacterium avium* (M. avium) and one caused by *Staphylococcus aureus* (S. aureus).

Pathogenic strain Cl. *perfringens* has developed several virulence factors that contribute to the development of necrotic enteritis (NE) in chickens. These virulence factors are essential for the attachment of bacteria to the mucosa (adhesins) and for providing nutrients for their rapid proliferation (degradative enzymes), as well as for toxin production. Virulence genes including variants netB, pfoA, cpb2, tpeL, and cna are strongly associated with NE-induced C. *perfringens* isolates (Kiu et al., 2019).

Based on the post-mortem examination of 100 dead chickens, it was confirmed that there was a bacterial disease of 58%. Among these diseases, 52% were diagnosed as Colibacillosis, 4% Salmonellosis, and 2% avian cholera (Halder et al., 2021). M. *avium* infection in both humans and poultry is still rare, so it is not well known by breeders and the people of Indonesia. However, this disease needs serious attention, because it is feared that the increasing cases of M. *avium* infection will also increase the infection of M. avium complex (MAC) caused by M. *avium* and mycobacterium intracellular (M. intracellular), which are often found in AIDS patients (Crilly et al., 2021).

The use of Antibiotic Growth Promoter's (AGP) as a feed additive to treat bacterial diseases in poultry has several negative impacts, including bacterial resistance to antibiotics (Untari et al., 2021), antibiotic residues in broiler chicken meat, and is harmful to human health because it causes foodborne disease (Mutia et al., 2018). Some of these reasons demand the government to ban the use of AGP as a feed additive through the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 14/PERMENTAN/PK.350/5/2017. The addition of herbs as natural feed additives which can be given in single or mixed form is expected to replace the role of AGP, where herbs is assumed to be safer for poultry and public health.

Some herbal plants that are often used by farmers/breeders to maintain the health of poultry include turmeric (*Curcuma longa Linn*), kencur/aromatic ginger (*Kaempferia galanga L.*), garlic (*Allium sativum L.*), ginger (*Zingiber officinale Rosc*), galangal (*Alpinia galangal*), cinnamon (*Cinnamomum verum*), and temulawak (*Curcuma zanthorrhiza*). Herbs contain several active compounds that function as anti-bacterials, including allicin, essential oils, quercetin and curcumin (Tahalele et al., 2018). The presence of essential oils stimulates the walls of the gallbladder to secrete the bile and stimulates the release of pancreatic juice containing the enzymes of amylase, lipase and protease. The curcumin in *Curcuma zedoaria* acts as an anti-bacterial, anti-oxidant and anti-hepatoxic (Widodo et al., 2021; Shena et al., 2021). As an antioxidant, *Curcuma zedoaria* has the ability to reduce free radicals which is quite high, which is equal to 0.005 ± 1.1 g/ml. Even though it is still relatively low, the combination of various phytobiotic actions can stimulate the secretion of pancreatic digestive enzymes (amylase, lipase and protease), increase the palatability and flavor of feed, thereby increasing feed consumption, growth and productivity of poultry (Gheisar and Kim, 2018). It was further reported that the active substance has the ability to increase the growth of good bacteria, especially the lactic acid

bacteria (LAB) group, including Enterococcus, Lactobacillus, Pediococcus, Streptococcus, Lactococcus, Vagococcus, Leuconostoc, Oenococcus, Weissella, Carnobacterium, and Tetragenococcus species, in contrast, inhibits growth of Gram-positive pathogenic bacteria, including *Clostridium perfringens* and *Staphylococcus aureus*, as well as Gram-negative ones, including E. *coli* and *Salmonella* (Reuben et al., 2019). Several LAB are known to produce important metabolites, such as reuterin, bacteriocin, diacetyl, reutericyclin, organic acids, acetoin, and hydrogen peroxide, which are very strong biopreservative agents (Ibrahim et al., 2021).

The relative weight of lymphoid organs, namely spleen, thymus, and bursa of Fabricius reflects the overall health and immune status of poultry, because these organs are the sites of B and T cell differentiation in chickens. The addition of Oridonin, isolated result from traditional Chinese medicine (Rabdosia rubescens), has been shown to increase the relative weight of spleen and bursa of fabricius in broiler chickens (Wu et al., 2018).

Based on the problems mentioned above, research is urgently needed to examine the effect of herbal supplementation as a feed additive on the relative weight of visceral organs, namely the heart, digestive glands (pancreas and liver), lymphoid glands (lymph and bursa of Fabricius), and on the number of pathogenic bacteria in the small intestine of native chicken. The aim of this study was to determine the effect of administering herbal supplements as feed additives through feed on the relative weight of visceral organs and the number of pathogenic bacteria in the small intestine.

MATERIAL AND METHOD

This study employed 200 Day Old Chick (DOC) from 2 types of native chicken strains, namely Jowo Super (Joper) and Kampung Unggul Balitbangtan (KUB). The commercial super jowo chicken utilized is a hybrid between superior local chickens and laying breeds, whereas the KUB chicken is a genetically chosen native chicken produced by the Agricultural Research and Development Agency (Balitbangtan) in Indonesia.

Native chicken strains reared or 60 days in open house in Malang, East Java, Indonesia. The feed used in this study being factory-made feed, produced by New Hope, which was given when the chickens were 1-28 days old, and 28-60 days old use feed from PT. Charoen Pokphand Indonesia. Feed and drinking water was given ad-libitum. The herbs used are the form of *jamu*, which is a mixture of eight types of herbs, including temulawak, garlic, betel leaf, turmeric, ginger, galangal, *kencur* and cinnamon sticks, with the brand "Siyuna Organik", which is produced by the Animal Husbandry Study Program, Faculty of Agriculture-Animal Husbandry, Universitas Muhammadiyah Malang. Herbs were given when the chickens were 30 days to 59 days old.

This study used an experimental method. Joper and KUB strains of native chickens were raised in the same location. Both strains were divided into 2 groups, namely T0 (feed without herbal supplementation) and T1 (feed with the addition of herbal supplementation, 1% of the total feed). Each treatment consisted of 50 chickens. Data on the relative weight of visceral organs and the number of pathogenic bacteria in the small intestine were analyzed using quantitative descriptive analysis, by comparing the means of T0 and T1 treatments in Joper and KUB.

Measurement of the relative weight of the visceral organs (heart, pancreas, liver, spleen, and bursa of Fabricius) in Joper and KUB chickens was done when the chickens were 60 days old. The weight of the organs is determined by obtaining the digestive tract from the body of slaughtered chickens, then separating the digestive tract by cutting each organ and weighing it with an analytical scale.

The relative weight of the visceral organs was calculated based on the formula used by Pertiwi et al. (2017), which is as follows:

$$\text{The relative weight of the visceral organs} = \frac{\text{Organ weight}}{\text{Live weight}} \text{ X } 100\%$$

Calculation of the number of bacterial colonies was carried out based on the modification done by Azizah and Soestyaningsih (2020), namely testing small intestine samples based on the Total Plate Count (TPC) test, using the spread plate method, by inoculating 100 µl of samples (all series of dilutions) on chromogenic media that has been poured into the petri dish. The media is leveled by moving the petri dish. After the petri dish is solid, it is followed by incubation in an inverted position for 24 hours at 37°C. Then, the results of the growing colonies are observed. Colony color differences can be used to identify the individual bacteria tested.

RESULT AND DISCUSSION

The Percentage of Visceral Organ Weight in Joper and KUB Chickens

The digestive organs play a significant role in the absorption of nutrients ingested by animals. The growth of the digestive organs and all of their accessories impacts the intestines' ability to absorb nutrition. The herbs utilized are a variety of herbs, including galangal, garlic, ginger, betel leaves, cinnamon, galangal, turmeric, and ginger. Many prior studies have investigated the content of active compounds that provide numerous benefits.

Saponins, flavonoids, polyphenols, and essential oils are all components of kencur rhizome (KaempferiagalangaL.) (Winarto, 2007). Garlic contains organic sulfur and phenolic chemicals that act as antioxidants (Aisyah, 2020). Cinnamon contains essential oils, eugenol, safrole, sinamaldehyde, tannins, calcium oxalate, resin, and tanning agents (Maslahah and Nurhayati 2023); Ginger is rich in active components, including phenolic and terpene chemicals (Mao et al., 2019).

The majority of active chemicals found in green betel extract are phenolic groups with antibacterial properties (Pratiwi and Muderawan, 2016). Galangal rhizome (Alphinia purpurata k.schum) contains alkaloids, flavonoids, phenols, and terpenoids (Sangadji et al., 2021); turmeric rhizomes contain curcumin, essential oils, resins, oleoresins, desmetoksikurkumin, and bidesmetoksikurkumin (Sihombing, 2007); Temulawak contains xanthorrizol, curcuminoids, and essential oils (Aris et al.,2006).

Table 1 shows the results of the investigation on the provision of herbal mixtures in feed. Based on Table 1, the addition of herbal Joper and KUB chickens produces an average relative heart weight in the normal range of 0.55 - 0.60%. Timur et al. (2020), stated that the relative weight of the heart ranged from 0.49 - 0.63%. The use of herbal medicine (T1) in Joper produces a lower relative heart weight than without the use of herbal medicine (T0) with a difference of 0.04% while in KUB chickens the difference obtained between T0 and T1 is 0.01%.

Table 1. The Mean of Relative Weight of Visceral Organs of With and Without Herbal Addition

Response Variable	Joper Chicken		KUB Chicken	
	T0	T1	T0	T1
Relative Weight of Visceral Organs (%):				
Heart	0,59 ± 0,05	0,55 ± 0,05	0,59 ± 0,03	0,60 ± 0,05
Proventriculus and gizzard	5,13 ± 0,56	4,78 ± 0,99	4,81 ± 1,29	4,46 ± 0,72
Pancreas	0,24 ± 0,00	0,25 ± 0,01	0,30 ± 0,02	0,34 ± 0,02
Liver	2,47 ± 0,19	2,41 ± 0,20	2,06 ± 0,17	2,00 ± 0,20
Spleen	0,26 ± 0,07	0,23 ± 0,10	0,21 ± 0,05	0,23 ± 0,05
Bursa of Fabricius	0,33 ± 0,19	0,31 ± 0,05	0,30 ± 0,04	0,28 ± 0,05

Description: T0 (without the addition of herbs), T1 (with herbal addition 1%).

The usage of herbs in Joper and KUB resulted in a relative liver weight of 2.00-2.41%. The usage of herbs resulted in a reduced relative liver weight compared to T0. The difference between T0 and T1 in Joper and KUB was only 0.06%. The relative weight of the heart and liver produced within the normal range with the use of herbs demonstrates that the addition of herbs to Joper and KUB chickens is safe and does not interfere with the function of the heart and liver. This is

strongly related to the active compounds found in herbs, including gingerol, which improves blood channel relaxation and accelerates blood flow to the heart.

The weight of proventriculus and gizzard resulting from herbs is 0.35% lower than T0 in Joper and KUB chickens. The proventriculus is an organ that serves as the first site of enzymatic protein digestion; its weight promotes the maximum action of the enzyme pepsin in breaking down protein into peptone. Martinez et al. (2021) described the proventriculus as a glandular organ that produces hydrochloric acid and pepsinogen. It transports food boluses to the gizzard, where and crushed before being carried to the gut. According to Sari and Ginting (2012), the nutritional content of the feed can influence the size of the proventriculus, as the proventriculus produces HCL, pepsin, and enzymes that can break down protein and crude fiber in the feed.

The addition of herbs to Joper and KUB increased pancreas relative weights (0.24 - 0.34%) compared to T0 (0.24 - 0.30%), with a difference of 0.01 - 0.04%. According to Sulistyoningsih (2015), the relative weight of the liver in feed containing herbs ranged from 2.00 to 2.41%, which is still within the normal range of 1.88 to 3.01%. The addition of herbs to Joper and KUB increased the average relative weight of lymph and fabrisius bursa, which is higher than the research conducted by Arfanda et al. (2019). The relative weight of lymph in super free-range chickens is 0.20 - 0.22%, and the weight of the fabricius bursa is between 0.17 - 0.20%.

High lymphoid organ weights in Joper and KUB show that the chickens are healthy and resistant to infectious agents due to high antibody levels. This is consistent with the statement of Orakpoghenor et al. (2021), who indicate that the decrease in the relative weight of the bursa fabricius in experimental chickens is thought to be caused by bursa atrophy following severe damage from Infectious Bursal Disease (IBD) viral infection.

Number of Pathogenic Bacteria in the Small Intestine of Joper and KUB Chickens

The intestinal tract is the area of the digestive system where bacteria are most prevalent (Kurniawan, 2007). The balance of pathogens and non-pathogens in the microflora of the digestive tract is usually maintained by the body's defenses, but unfavorable environmental conditions can also interfere with the growth of both pathogenic and non-pathogenic microflora (Havenaar and Veld 1992). Pathogenic microflora can impede optimum production and have an impact on the growth of the gastrointestinal tract (Abun, 2008).

Four genera and species of pathogenic bacteria were identified in the intestines of the hens under study: Clostridium perfringens, Staphylococcus aureus, Mycobacterium avium, and Pasteurella multocida. The bacteria belonged to the genera Clostridium, Staphylococcus, Mycobacterium, and Pasteurella. Table 3 displays information on the quantity of harmful bacteria found in the small intestines of KUB and Joper chickens. Poultry infections caused by the four pathogenic bacteria are frequently discovered in farms and can have a negative impact on producers. Poultry cholera, which can spread, is caused by Pasteurella multocida (Zainuddin, 2014; Winarsih at.,al). Bumble foot, or foot swelling, can result from an infection with pathogenic Staphylococcus aureus (S. aureus) germs (Schwartz, 1977; Cooper and Needham, 1981).

Clostridium perfringens is the pathogen that causes necrotic enteritis (NE). Although these bacteria are typical and found in the digestive tracts of healthy hens, they have the ability to multiply, generate toxins, and spread disease when there are variables that upset the delicate balance of the chicken's digestive system (Natalia 2004). Avian mycobacteriosis is a chronic infectious disease that birds contract from Mycobacterium avium complex (MAC). M. avium and M. intracellulare, two closely related species of mycobacteria, are the cause of MAC, which affects almost all bird species (Sattar at. al., 2021).

Table 2 Number of Pathogenic Bacteria in the Small Intestine of Chickens with and WithoutHerbal Addition

Response Variable	Joper		KUB	
	T0	T1	T0	T1
Number of small intestine pathogenic bacteria (cfu/ml):				
Clostridium	3,20 ± 0,62	0,00 ± 0,00	9,20 ± 3,67	0,00 ± 0,00
Staphylococcus	3,40 ± 0,73	0,00 ± 0,00	1,40 ± 0,33	0,00 ± 0,00
Mycobacterium	1,20 ± 0,05	0,00 ± 0,00	1,00 ± 0,24	0,00 ± 0,00
Pasteurella	0,00 ± 0,00	0,00 ± 0,00	2,60 ± 0,36	0,00 ± 0,00

Description: T0 (Joper ration without the addition of herbs), T1 (Joper ration withaddition of herbs 1%)

Table 2 indicates that, in comparison to the use of herbs (T0), the use of herbs (T1) in Joper and KUB chicken feed yields a good reaction. In the herbal therapy (T1), neither of the four small intestine pathogenic bacteria (Clostridium, Staphylococcus, Mycobacterium, and Pasteurella) was found in the small intestine of the chicken strains; with the T0 treatment, all four bacteria were present. Table 2 displays the quantity of harmful bacteria discovered in the small intestine. While Staphylococcus and Mycobacterium were more prevalent in Joper chickens under T0 therapy, Clostridium and Pasteurella were identified at higher concentrations in KUB chickens per cfu/ml than in Joper chickens.

The fact that the four different species of pathogenic bacteria are absent from the intestine at T1 suggests that the active ingredients in the herbs employed can effectively serve as an antibacterial in KUB and Joper chickens. Herbs of several kinds, including galangal, turmeric, betel leaf, ginger, garlic, and temulawak, are mixed together and used in feed. According to Agustina (2006), the presence of bioactive elements in herbal components is believed to have a sparing effect, which is the beneficial combination of ingredients that improves a number of performance indicators. Harlin (2013) states that curcumin, an essential oil, is the form of bioactive chemicals found in herbal compounds. Furthermore, the body uses quersetin and alicin from garlic in herbal components as antibacterials (Dharmawati et al., 2013).

Herbal substances have also been used in previous studies. The essential oil of ginger (Zingiber officinale Roscoe) has a high sensitivity to Staphylococcus aureus, as evidenced by a 24 mm inhibitory zone, according to research done by Tarfaoui et al. (2022). According to Nissa et al.'s (2018) research, nisin and red ginger essential oil combined, at concentrations of 62.5 IU/ml and 1%, respectively, were highly effective bacteriostatic agents against Salmonella typhimurium, E. coli, Bacillus cereus, Pseudomonas fluorescens, and Aspergillus niger. According to research by Haerazi, kencur extract (Kaempferia galanga L.) possesses antibacterial activity against Staphylococcus aureus by 15 mm and Streptococcus viridans by 16 mm when it is concentrated to 70%.

CONCLUSION

Based on the research that has been done, it can be concluded that 1% herbal supplementation as a feed additive has a positive effect on the health of the heart, digestive glands, lymphoid glands, and no pathogenic bacteria are found in the small intestine of Native chiken, both in Joper and KUB chickens.

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