Effect of extraction *Andrographis paniculata* and *Moringa oleifera* on microbiological jejunal of broiler chickens

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Abstract. This study aims to evaluate the effect of the combined extraction of *Andrographis paniculata* and *Moringa oleifera* leaves as phytobiotics on the digestive microbiology of broiler chickens. The number of chickens used in this study was one hundred twenty-eight (128) chickens divided into 4 treatments, 4 replications where each replication consisted of 8 chickens. The treatments in this study were T0 (control), T1 (basal diet + 0.25% *Andrographis paniculata* and *Moringa oleifera* leaves), T2 (0.50% *Andrographis paniculata* and *Moringa oleifera* leaves), and T3 (0.75% *Andrographis paniculata* and *Moringa oleifera* leaves). The method used in this research is in vivo. Data analysis used a completely randomized design. If there is a significant difference, it is analysed using the Duncan Multiple Range Test (DMRT). The results of the study showed that the use of *Andrographis paniculata* and *Moringa oleifera* leaves had a significant effect on the number of lactic acid bacteria, Escherichia coli and Salmonella in the digestion of broiler chickens. T3 showed the highest number of lactic acid bacteria compared to other treatments, while T3 showed the lowest number of E. coli and Salmonella. The use of *Andrographis paniculata* 0.75% and *Moringa oleifera* leaves had a positive influence on the number of lactic acid bacteria in the digestion of broiler chickens.

1 Introduction

Poultry farming has the potential to be developed to meet food needs for animal protein sources. However, the toughest challenges for breeders are the emergence of diseases that can cause losses and the prohibition of the use of Antibiotics Growth Promoter (AGP). So it becomes an obstacle for breeders to completely abandon the use of AGP. This is because AGP can have a negative effect on the poultry digestive system. Apart from that, the use of AGP can leave residue in the bird's body and the final product is meat or eggs which will be consumed by humans. This makes the product unsafe for consumption. Several researchers have presented previously related to the alternative for AGPs including the use of enzymes, lactic acid bacteria, or even the use of prebiotics [1-3].

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However, coccidiosis is a disease caused by parasites from the genera Eimeria and Isospora which are included in the phylum Apicomplexa with a complex life cycle [1]. This parasite will attach to the wall of the small intestine and affect the absorption of nutrients, resulting in damage to other organs [2]. This disease is transmitted through feces, especially in birds that live in groups in large populations and high densities [3]. So coccidia will reduce poultry productivity and require quite large treatment costs. Herbal plants that have the potential to act as anticocidiosis are Moringa leaves and bitter leaves. Chemical compounds identified in the Moringa plant include Alkaloids, Flavonoids, Saponins, Terpenoids, Tannins, Steroids [4]. At the same time, the pharmacological activities found in the Moringa plant are antioxidant, antihyperuricemic, analgesic, anticancer, antihyperglycemic, anti-inflammatory, antihyperlipidemic and antimicrobial activities [4]. In Addition, bitter leaves contain orthosiphon glucose, essential oils, saponins, polyphenols, flavonoids, sapophonins, potassium salts and myonositol [5]. Based on the 2 problems above, namely replacing AGP and coccidiosis and looking at the potential opportunities for the content of bitter and Moringa leaves, the aim of this research is to utilize herbal plants as an innovative replacement for AGP as well as anti-coccidiosis in poultry. The urgency or priority of this research is to prevent coccidiosis caused by parasites using active substances from herbal plants. This is also an effort to replace antibiotic growth promoter (AGP) in poultry. The use of herbal plants is expected to reduce disease and improve the quality of products in the form of chicken meat. Products produced from poultry in the form of healthy meat will support the availability of quality food. The novelty in research on NGP herbal products from a combination of *Andrographis paniculata* and *Moringa oleifera* leaves is 1) NGP products are produced which are able to prevent the emergence of coccidiosis, 2) NGP herbal products use crude herbal extract technology and nanoparticle technology, 3) NGP herbal products are used as chicken feed additives with 2 forms, namely extraction. The purpose of this research is to produce quality herbal products to prevent or treat coccidiosis and increase chicken productivity.

2 Materials and Methods

2.1 Experimental design

The materials used in this research were 128 broiler chickens obtained from PT JAPFA Comfeed. Other ingredients used are *Andrographis paniculata* extract and *Moringa* leaf extract obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine. The research was conducted at the Sumbersean field laboratory located in Dau District, Malang Regency. The method used in this research was in vivo using 4 treatments and 4 replicated consisting of 32 broiler chickens as follows:
T0= Control without additional treatment
T1= Use 0.25% mixture of Bitter and *Moringa* extracts in liquid form
T2= Use 0.50% mixture of Bitter and *Moringa* extracts in liquid form
T3= Use 0.75% mixture of Bitter and *Moringa* extracts in liquid form
Data was analysed using a completely randomised design. Completely randomised design is a type of experimental design where treatment is given randomly to all experimental units. This can be done because the environment where the experiment is held is relatively homogeneous so that the media or place of the experiment does not have a significant influence on the observed response. If there were a significant difference, then analysis with Duncan Multiple Range Test (DMRT).

3 Results and discussion

. Lactic acid bacteria (LAB) is non-pathogenic bacteria that has an important role in the digestive tract. The bacteriocin properties of herbal ingredients can have an antagonistic effect on the growth of pathogenic bacteria, thereby reducing bacterial growth and improving the microflora in the intestinal [6]. For instance, the lactic acid bacteria, while pathogenic bacteria in the poultry digestive tract that have high mortality are, for example, Escherichia coli. Lactic acid bacteria can be grouped as probiotic bacteria which can be mixed with locally formulated feed or commercial feed. Lactic acid bacteria are able to kill pathogenic bacteria, one of which is Escherichia coli which can cause colibacilosis.

Based on the results of the analysis of the variety of lactic acid bacteria (LAB) in table 1 in digesta broiler chickens, it shows that there is no significant effect (P>0.05). The differences in lactic acid bacteria were not significant, indicating that up to a dose of 0.75% the combination of bitter and moringa extracts had no effect on chicken digesta. This is thought to occur because broiler chickens have a good digestive system because there are many positive bacteria that support the chicken's digestive system to work optimally. Apart from that [8] stated that differences in total lactic acid bacteria were influenced by differences in age and the influence of probiotic administration. The highest average number of lactic acid bacteria was obtained from treatment T3, namely 39.15 x 10^7 CFU/ml and the lowest average number of lactic acid bacteria in treatment T0 was 6.6 x 10^7 CFU/ml. The average percentage of lactic acid bacteria in broiler chickens from the lowest respectively is T0 (6.6 x 10^7 CFU/ml), T1 (8.95 x 10^7 CFU/ml), T2 (11.95 x 10^7 CFU/ml) and T3 (39.15 x 10^7 CFU/ml). T3 is the best treatment because it has the highest average number of lactic acid bacteria (LAB) compared to other treatments.

Absorption of nutrients in feed requires good digestive tract function to optimise the digestive process [9]. Intestinal health is influenced by the population of microbes or bacteria that live in it [10]. Lactic acid bacteria can be grouped as probiotic bacteria which can be mixed with locally formulated feed or commercial feed. Lactic acid bacteria are able to kill pathogenic bacteria, one of which is Escherichia coli which can cause colibacilosis [7].

Table 1. The Results of analysis various effects of *Andrographis paniculata* and *Moringa oleifera* extraction on the microbiology of broiler chicken Digista

Treatment	*Lactic Acid Bacteria*	*Escherichia Coli*	*Salmonella*
T0	6.6 ± 0.282	53.5 ± 2.828^{cd}	26.95 ± 6.85^{cd}
T1	8.95 ± 1.626	30.95 ± 12.51^{b}	1645 ± 10.39^{bc}
T2	11.95 ± 3.88	41.5 ± 6.5^{bc}	6.40 ± 2.12^{ab}
T3	39.15 ± 17.88	13.35 ± 2.89^{a}	0 ± 0^{a}

Note: The different superscripts in columns indicate significant differences (P<0.05).

Escherichia coli (*E. coli*) is a bacterium that lives in the digestive tract of warm-blooded animals, including mammals and birds. This bacterium was first isolated by Theodor in 1885. E.coli is a rod-shaped Gram-negative bacteria. E. coli cells have a length of 2.6 – 6.0 µm and a diameter of 1.1 – 1.5 µm, singly or in pairs and are non-motile with *peritrichous flagella* [11]. Based on the results of the analysis of Escherichia coli bacteria in broiler chicken digesta, it showed a significant effect (P<0.05). The lowest average number of *E. coli* bacteria was obtained from treatment T3, namely 13.35 x 10^4 CFU/ml and the highest average number of E.coli bacteria in treatment P0 was 53.5 x 10^4 CFU/ml. The average percentage of broiler chicken carcasses from the lowest respectively is T3 (13.35 x 10^4 CFU/ml), T1 (30.95 x 10^4 CFU/ml), T2 (41.5 x 10^4 CFU/ml) and T0 (53.5 x 10^4 CFU/ml). T3 is the best treatment because it contains 0.75% bitter and Moringa leaf extracts.

Escherichia coli lives in the digestive tract of poultry, especially in the jejunum, ileum and cecum. Apart from that, it is also often found in the trachea and oesophagus. Several clinical symptoms caused by pathogenic E. coli are grouped into *E. coli* which causes diarrhea, septicemia and Avian Pathogenic Escherichia coli (APEC). In broiler chickens, *colibacillosis* has a negative impact and causes death during the rearing period so that body weight at harvest is below standard with varying morbidity rates and mortality reaching 5-20% [12]. The addition of bitter and moringa extracts can reduce the presence of E. coli bacteria in the digestive tract. Moringa leaves contain flavonoids, sterols, triterpenoids, alkaloids, saponins, tannins and phenols while bitter leaves contain flavonoid chemical compounds [13-14]. Apart from that, bitter also contains alkanes, ketones, aldehydes and minerals [15].

Moringa leaves as additive feed for broiler chickens is reported to be a strong antioxidant that can protect and maintain the condition of chickens against oxidative stress, thereby providing results in the form of better growth rates and carcass quality [16]. Moringa also contains amino acids such as scordinine, methionine, lysine, and cystine which can stimulate chicken growth, increase body weight and increase energy [17]. Sambiloto has activity in maintaining beneficial gastrointestinal microflora, including lactobacilli, and inhibiting harmful bacteria, namely E. coli and Staphylococcus [18]. The flavonoid compounds in bitter and moringa act as antioxidants and can support the immune system. The flavonoids and tannins in bitter have potential as an antelemintic [19].

Broiler chickens can be contaminated with Salmonella sp. starting from livestock which is influenced by the cage and livestock environment [20]. Salmonella sp. rod-shaped, perithtic flagella for movement, has no spores, is gram negative, measures 0.5-0.8 μm in diameter and 1-3.5 μm in length. Salmonella sp. grows easily on simple media and almost never ferments lactose or saccharose and forms acid and sometimes produces gas from glucose and mannit and gives negative results in the indole reaction. The average colony size is 2-4 mm [21]. Infection with *Salmonella sp.* called Salmonellosis. Salmonellosis is an infection that can disrupt the digestive tract and can cause death in broiler chickens [22].

Based on the results of the analysis of *Salmonella sp.* in broiler chicken, digesta showed a significant effect ($P<0.05$). The average number of *Salmonella spp bacteria*. The lowest was obtained from treatment T3, namely 0.00 x 10^3 CFU/ml and the average number of *Salmonella sp.* The highest in treatment T0 was 26.95 x 10^3 CFU/ml. The total average of *Salmonella spp* bacteria. from the lowest respectively, namely T3 (0.00 x 10^3 CFU/ml), T2 (6.4 x 10^3 CFU/ml), T1 (16.45 x 10^3 CFU/ml) and T0 (26.95 x 10^3 CFU/ml). ml). T3 was the best treatment with the addition of 0.75% bitter and Moringa leaf extract. P3 has an average number of Salmonella sp bacteria. lowest compared to other treatments. This is caused by the influence of compounds contained in bitter extract and Moringa leaves.

Moringa leaf extract has antioxidant activity which is known to scavenge free radicals and provides significant protection against oxidative damage, so Moringa leaves can be used as an additive to animal feed [23]. The most prominent content of the Moringa plant is antioxidants, especially in the leaves which contain high antioxidants. Based on phytochemical tests, Moringa leaves contain tannins, steroids, triterpenoids, flavonoids, saponins, interquinones and alkaloids, all of which are antioxidants [24]. Sambiloto (*Andrographis paniculata*) is a plant that can prevent increases in cholesterol levels. Sambiloto contains phybiotics and contains an active substance known as andrographolide, which gives a bitter taste and has anti-toxic, cancer-preventing, anti-allergic, anti-inflammatory and anti-bacterial [25].

Moringa contains nutrients and compounds that are important for the body, Moringa leaves also contain phytochemical substances such as tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones and alkaloids. This compound has the ability to act as an antibiotic, anti-inflammatory, detoxification and antibacterial drug, [26]. Compounds,

saponins, flavonoids, tannins and terpenoids can inhibit bacterial growth [27]. Flavonoids which are classified as phenols are antibacterial and are able to denature proteins and damage bacterial cell membranes [28].

Antimicrobial active ingredients can destroy bacterial cell membranes and break down bacteria by increasing cell wall permeability. With the presence of antimicrobials, the nutrients in the ration can be optimally absorbed by the digestive tract, thereby increasing the efficiency of ration use [29]. Apart from that, according to [30] the addition of Moringa leaf extract can increase protein levels in the digestive tract so that lactic acid bacteria can also increase and suppress pathogenic bacteria. Phytogenic can inhibit pathogenic bacteria such as *escherichia coli* and *Salmonella sp*. [31].

4 Conclusion

In conclusion, this study underlines the significance of carefully managing egg storage durations for Arab Chickens, with a particular emphasis on minimising adverse effects on growth-related parameters. The hatching eggs of Arab chickens can be safely stored for up to 4 days without any detrimental effect on their performance during the first week post-hatch. *Andrographis paniculata* and *Moringa oleifera* extract 0.75 % has a positive effect on the amount of microbiology in the digesta of broiler chickens.

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