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Research Article

THE ADDITION OF *Andrographis paniculata* IN THE DIET OF TEGAL DUCKS REARED IN DIFFERENT CAGE DENSITIES AND THE EFFECT ON GUT MICROFLORA, INTERNAL ORGANS, PROFILE LIPIDS, AND DUCK PERFORMANCE

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ABSTRACT

This study aims to examine the effect of adding levels of *Andrographis paniculata* leaf meal (ALM) on intestinal bacteria, intestinal growth, blood lipid profile, and performance of Tegal ducks kept in different cage densities. A total of 140 7-day-old male Tegal ducks with an average body weight of 130.83 ± 7.54 g/head were placed in 20 experimental units in stilt cages following a completely randomized design procedure, 5 treatments, and 4 replications. The research ration was prepared with an EM content of 2900 kcal/kg and 18% crude protein. The treatments tested included T0: density of 5 ducks/m^2 + basal ration, T1: density of 5 ducks/m^2 + 0.25% ALM, T2: density of 5 ducks/m^2 + 0.5% ALM, T3: density of 10 ducks/m^2 + 0.25% ALM, T4: density of 10 ducks/m^2 + 0.5% ALM. The parameters measured include intestinal bacterial populations (LAB and coliform), digestive organs, blood lipid profile (cholesterol levels, low density lipoprotein/LDL, high density lipoprotein/HDL and triglycerides) and duck performance. Data were processed with the SPSS version 22 using analysis of variance and Duncan's multiple area test at a significance level of 5%. The results showed that the addition of ALM to Tegal duck feed had a significant effect ($p<0.05$) on coliform, blood cholesterol and LDL levels, and duck body weight. Treatment had no significant effect on relative intestinal weight, HDL and blood triglycerides, and feed consumption. This study concluded that a dietary strategy providing *A. paniculata* decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

Key words: *Andrographis paniculata,* duck, gut microflora, profil lipid, digestive organ

INTRODUCTION

Tegal ducks are native to Indonesia and are often kept in villages and suburbs. In Indonesia, pastured Tegal ducks act as natural predators of insects and snails, while duck droppings contribute to soil fertility and crop yields (Ismoyowati and Sumarmono, 2019). Female Tegal ducks are kept for egg production. Specifically, male Tegal ducks are kept for their meat. The population of Tegal ducks is quite high and is spread across many provinces in Indonesia (Subiharta et al., 2013), in 2021, it was 58,651,838 heads (Statistics Indonesia 2021).

In general, ducks are traditionally kept by breeders in open cages with unselected seed quality, feed quality that does not meet standards, and maintenance with cage density that is not taken into account, resulting in low productivity. Tegal duck productivity in traditional reared is currently still low. The body weight gain and egg production of Tegal ducks are lower than that of Magelang ducks. Egg production Tegal duck had an average of 78 ± 19 eggs per duck within 4 months or 65%, and the egg weight was 66.60 ± 5.05 g / egg, lower than Magelang ducks, namely 70.4% with an egg weight of 69.6 g/item (Ismoyowati et al., 2018).

Efforts need to be made to increase Tegal duck production, including by regulating cage density and adding feed additives. Cage density is an important factor in the livestock production process because of its influence on the comfort level of livestock. Inappropriate density can affect temperature, humidity, and air circulation in the cage. High cage density can cause stress, which affects physiological, immunological, and microbiological changes, changes in behavior, increases corticosteroid hormones, and ultimately affects performance (Abudabos *et al.*, 2013 and Sugiharto 2022).

Another effort to improve the productivity of Tegal ducks can be done by adding feed additives, in the form of herbs. One of the herbal plants that grows widely in Indonesia is sambiloto. The sambiloto has the latin name *Andrographis paniculata.* Supplementation with unfermented and fermented *A. paniculata* across different treatments improved growth, immune status, intestinal morphology, and intestinal microbiota composition and structure in Muscovy ducks (Liu et al., 2023). *A. paniculata* leaves contain numerous compounds with anti-inflammatory, antibacterial, and antioxidant properties. In the leaves, *A. paniculata* contains the main compounds in the form of andrographolide and flavonoids. Other compounds contained in *A. paniculata*, but in small levels, include saponins, alkaloids, and tannins. The flavonoid content of *A. paniculata* extract is 0.022 mg/mL quercetin (Fardiyah *et al.*, 2020). The properties of andrographolide include antiviral, antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, antitumor, chemopreventive, spasmolytic, and uterorelaxant (Dai *et al*., 2019). In several previous experiments, *A. paniculata* was proven to have a strong impact on *Eimeria spp*., the causative agent of coccidiosis (Indrati and Titisari 2020) and inhibiting the growth of pathogenic bacteria thereby increasing the immune status and performance of poultry (Liu *et al*., 2023; Jahja *et al.*, 2023). The results of previous research showed that the administration of 0.4% *A. paniculata* leaf powder provided optimal psychobiotic effects on Mojosari laying ducks, without affecting feed consumption (Yulianti *et al.*, 2015).

Based on the description above, it is necessary to research the use of A. paniculata to overcome stress in ducks kept in open cages with different cage densities. Can the content of andrographolide and flavonoid compounds in A. paniculata if given to ducks kept in higher cage densities have an impact on intestinal health, especially the microbiota population, so that they can result in performance as well as at low densities?

Based on our knowledge, we have not found the study on administering A. paniculata to ducks kept in different cage densities. Therefore, this research was carried out to determine the effect of using feed containing A. paniculata on intestinal microbiota population, internal organ growth, blood lipid profile, and performance of Tegal ducks reared in different cage densities

MATERIALS AND METHODS

All activities carried out in this research have received approval from the Animal Research Ethics Committee, Faculty of Animal Science and Agriculture, Diponegoro University No. 60-02/A-06/KEP-FPP.

Animal and Diets

This study used 140 male Tegal ducks aged 7 days with an average body weight of 130.83 ± 7.54 g. Open-sided cages were used in this study. A total of 20 experimental units measuring 1 m × 1 m were prepared, using bamboo partitions. The rations are prepared using local feed ingredients, in the form of yellow corn, rice bran, soybean meal, fish meal, palm oil, premix, bentonite, monocalcium phosphate, limestone, methionine and NaCl, with a metabolizable energy content of 2,905.9 kcal/kg and a crude protein of 18.06 %. Treatment rations during the study for T0, T1, T2, T3 and T4 were the same. The composition and nutrient content of the research ration can be seen in Table 1.

Research Treatment and Design

This study was structured using a completely randomized design (CRD). Completely randomized design is the simplest type of randomized design, to look at just one factor, namely the level of *A. paniculata*, and no other factors influence the response in outside the factors studied. The material used is homogeneous so there is no need to group it. There were 5 treatments applied and each treatment had 4 replications.

T0: Density of 5 ducks/m^2 + Ration without ALM (control)

T1: Density of 5 ducks/m^2 + 0.25% ALM

T2: Density of 5 ducks/m^2 + 0.5% ALM

T3: Density of 10 ducks/m^2 + 0.25% ALM

T4: Density of 10 ducks/m^2 + 0.5% ALM

Ducks were adapted for 1 week, starting at 7-14 days of age. Feeding 100% of the treatment diet to the ducks was carried out for 4 weeks, starting at the age range of 15-42 days. Feed and water were given ad libitum. Duck weights were weighed weekly to monitor weight gain. Newcastle disease (ND) vaccination was conducted using Medivac ND La Sota vaccine through drinking water when the ducks were 10 days old.

Parameter measurement

Lactic acid bacteria (LAB) and coliform counts were measured using 20 ileal fluid samples from 20 ducks from each experimental unit. Ducks were slaughtered by cutting the neck. The cecal fluid was collected and placed in a sterile Eppendorf tube. LAB and coliform counts were calculated using the total plate count method. The calculation of LAB and coliform counts followed Fardiaz (2001).

The relative weight of the intestine was also measured using 20 ducks representing each replicate. The ducks were slaughtered, the abdomen dissected, and the internal organs removed. Duodenum, jejunum, ileum, and cecum were separated and weighed. The relative weight of the organs was obtained by dividing the weight of the organs by the live weight, then the result was multiplied by 100%.

The stage for taking blood and digestive organs was carried out on day 43. Blood was taken from the brachial vein and put into a non-EDTA vacutainer tube. The blood was centrifuged for 15 minutes at 3000 rpm to separate the blood serum. The blood serum was then put into a sample tube and stored in an ice box. Cholesterol, HDL, and LDL levels were analyzed using the enzymatic cholesterol oxidase-para aminophenazone method (CHOD-PAP KIT), while triglyceride levels (mg/dl) were measured using the GPO-PAP method using a spectrophotometer.

STATISTICAL ANALYSIS

Data were processed using analysis of variance and Duncan's multiple area test at a significance level of 5%, with the SPSS version 22.

RESULTS

Intestinal Bacteria

The total LAB and ileal coliforms of ducks are presented in Table 2. Statistical analysis showed that the addition of *A. paniculata* leaf meal (ALM) to the ration had a significant effect ($P<0.05$) on the coliform, but had no significant effect on total lactic acid bacteria in the ileum.

Relative Weight of Intestines

The relative weights of the duodenum, jejunum and ileum were not affected by ALM treatment (Table 3). The addition of ALM in the diet resulted in the relative weight of the small intestine (duodenum, jejunum, and ileum) being the same as the control.

Profile lipid

The addition of *A. paniculata* leaf meal had a significant effect ($p<0.05$) on blood cholesterol and LDL levels of Tegal ducks, but had no significant effect on HDL and triglycerides (Table 4).

Duck Performance

Giving ALM 0.25 and 0.5% did not have a significant effect ($P>0.05$) on feed consumption in ducks kept in different cage densities (Table 5). However, treatment had a significant effect ($P<0.05$) on daily body weight gain. In ducks reared at a density of 5 birds/m^2, administration of 0.5% *A.paniculata* (T2) resulted in the highest daily body weight gain. Meanwhile, giving 0.25% *A. paniculata* (T1) to ducks did not increase daily body weight gain ($P>0.05$). In ducks reared at a density of 10 birds/m^2, administration of *A. paniculata* at a dose of 0.25 or 0.5% (T3 and T4) did not increase daily body weight gain.

DISCUSSION

Intestinal Bacteria

Suplementation of ALM significantly reduced the number of intestinal coliforms in ducks reared at a cage density of 5 ducks/m^2 (see Table 2). Andrographolide compounds in ALM proved to be able to reduce coliforms, resulting in coliform counts in T1 and T2 that were much lower than the control group (T0). This finding is consistent with the study of Liu *et al.* (2023), which showed that supplementation of unfermented and fermented *A. paniculata* (30 g/kg each) in Muscovy rations can reduce the number of harmful bacteria in the caeca, such as *Succinivibrio*, *Succinatimonas*, *Sphaerochaeta*, and *Mucispirillum*.

Other researchers have also reported similar results. For example, the addition of *A. paniculata* and *Origanum vulgare* aqueous extracts in broiler diets resulted in an improved microbiota profile compared to zinc bacitracin and the negative control. The profile included increased numbers of *Lactobacillus spp.* and *Bacillus spp.*, while lower numbers of *Escherichia coli* and *Salmonella spp.* isolated from the intestine (Jahja *et al*., 2023).

Keeping ducks with a cage capacity of 10 ducks/m^2 (T3 and T4) has the potential to cause stress that can alter the balance of intestinal bacteria, which is manifested in an increase in coliform counts, as shown in Table 2. Coliforms in T3 were recorded much higher than in T1 and T2. However, at T4 there was a more significant decrease in coliforms due to the administration of ALM (0.5%) compared to T3 (0.25%).

Andrographolide ($C_{20}H_{30}O5$) in ALM has a high hydrogen content, so it can dissociate into the bacterial cell wall and eventually cause the death of pathogenic bacteria. Indrati and Titisari (2020) showed that *A. paniculata* extract added to broiler feed can reduce the number of *Eimeria tenella* oocysts. The decrease in coliform count can also be caused by the presence of flavonoid compounds in ALM that function as antibacterials. Flavonoids inhibit bacterial growth by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and energy metabolism, so bacteria cannot grow or develop (Panche *et al.*, 2016).

Relative Weight of Intestines

The level of administration of 0.25 and 0.5% ALM to Tegal ducks with a cage density of 10 ducks/m^2 resulted in the relative weight of the small intestine being the same as ducks with a cage density of 5 ducks/m^2. Growth of duckling digestive organs occurs during embryonic development, reaching a peak when the ducklings are 3 days after hatching. Intestinal growth rate decreases after 3 weeks of age (Lilburn and Loeffler 2015). Treatments were given when the ducks were 3-6 weeks old, and the data in Table 3 were measured when the ducks were 6 weeks old, where the digestive organs had reached the peak of growth. The results showed that feeding *A. paniculata* Leaf Meal (ALM) did not have a significant effect on the relative weight of the intestine of ducks, both those reared at cage densities of 5 birds/m^2 and 10 birds/m^2.

Although there was no significant effect, T3 and T4, where ducks were reared at a density of 10 birds/m^2 and fed 0.25% and 0.5% ALM, respectively, showed the highest relative weights of duodenum, jejunum, and ileum. This is in line with T0, indicating that flavonoids in *A. paniculata* play a role in reducing stress in ducks. Flavonoids can bind free radicals and form new non-reactive compounds, so that the body's metabolism can run smoothly, including the growth of digestive organs (Panche *et al*., 2016).

Profile lipid

The addition of *A. paniculata* leaf meal reduces blood cholesterol levels in the Tegal ducks (Table 4). This finding is consistent with Bogusławska-Tryk et al. (2016) who state that total cholesterol, lipoprotein fractions, and triglycerides are influenced by factors such as age, sex, genetic type, as well as environmental and feed conditions.

Cholesterol and LDL levels in TI and T2 were significantly lower compared to the control. Meanwhile, cholesterol levels in T3 and T4 were significantly higher than those in T1 and T2. Ducks in T3 and T4, which were reared with higher cage density, faced higher stress levels and increased free radicals. However, the addition of *A. paniculata* leaf meal, which contains flavonoids as antioxidants, may help prevent the negative impact of increased free radicals.

According to Tan *et al*. (2022), flavonoid supplementation in feed can modulate metabolism and reduce cholesterol content. Flavonoids, as antioxidants, can prevent cell damage due to oxidative stress, and the enzyme HMG-CoA reductase can reduce the activity of cholesterol synthesis in the body. Flavonoids also have antibacterial properties that can reduce the number of pathogenic bacteria such as E. coli and increase the growth of lactic acid bacteria (LAB) in the digestive tract (Panche *et al.*, 2016).

Tsai *et al.* (2014) mentioned that LAB can produce the enzyme bile salt hydrolase (BSH), which affects cholesterol reduction by inhibiting the recycling of bile salts. Increasing

LAB can increase BSH activity, which in turn can inhibit cholesterol absorption in the gastrointestinal tract. As a result, cholesterol is excreted along with feces, contributing to the reduction of blood cholesterol (Kumar *et al.*, 2012). The importance of adding *A. paniculata* extract to feed is also reinforced by Sudarmi *et al.* (2018), who state that the addition of *A. paniculata* extract to feed can reduce cholesterol levels by 0.21%.

The T3 and T4 treatments were carried out with a cage density of 10 ducks/m^2, so the ducks were more likely to experience stress, which caused an increase in cholesterol and LDL levels compared to T1 and T2 (density of 5 ducks/m^2). In T3, *A. paniculata* was given at a low dose, with a high cage density of 10 ducks/m2, which increased the potential for stress in the ducks. Ducks under stress will produce stress hormones, such as corticosterone, whose synthesis requires cholesterol as a precursor. LDL levels act as a transporter of cholesterol and triglycerides to peripheral tissues and glands, resulting in an increase in LDL levels as the need for triglycerides and cholesterol by tissues and glands increases.

The addition of 0.5% ALM caused a decrease in cholesterol and LDL in T4. *A. paniculata*, in the form of leaf flour, contains flavonoid compounds. This study is consistent with the findings of Prihambodo *et al*. (2021), who stated that flavonoids in feed can reduce LDL levels due to delayed activity of cholesterol acyltransferase Acyl-CoA in liver hepatocellular carcinoma cells and a decrease in LDL constituent compounds, such as CHO compounds and glucose.

ALM was not able to increase HDL levels and also did not reduce blood triglycerides in Tegal ducks (P>0.05). Flavonoid levels as antioxidants at T1 to T4 did not contribute to the increase in HDL and decrease in triglycerides. Blood cholesterol, MDA, triglyceride, and lipoprotein levels were influenced by the amount of flavonoids consumed. The higher the amount of flavonoids consumed, the higher the activity of superoxide dismutase and HDL (Prihambodo *et al.*, 2021). It is suspected that in this study, flavonoid consumption was still low, although this was not measured in this study. This finding is not in line with the results of Tan *et al.* (2022), who state that flavonoids can inhibit free fatty acids which directly reduce the formation of fatty acids in the circulation, followed by a decrease in free fatty acids towards the portal vein, thereby reducing fat deposition in liver tissue.

Duck Performance

Feed consumption in this study was not influenced by ALM addition (Table 5). However, the addition of ALM to the ducks' rations significantly increased the ducks' body weight. It can be seen that giving 0.5% ALM resulted in the highest body weight of Tegal ducks in the group of ducks kept at a cage density of 5 ducks/m^2. This is thought to be related to the role of andrographolide as an antibacterial, which can reduce coliforms (Table 2). The main bioactive compound of A. paniculata, andrographolide, has been reported in several studies to have antibacterial activity. The main bioactive compound of *A. paniculata*, andrographolide, has been reported in several studies to have antibacterial activity. Andrographolide works by inhibiting bacterial growth through inhibition of DNA synthesis, almost equivalent to the effectiveness of fluoroquinolone antibiotics (Banerjee *et al.*, 2017). The performance of poultry, including ducks, is influenced by the morphology of the intestinal mucosa, the area of the intestinal villi, the balance of the intestinal microbiota and also the health of the poultry (Wang *et al.*, 2021). It is suspected that the increase in body weight at T2 was due to the role of *A. paniculata* supplementation, which significantly increased the height of the villi and the surface area of the duck's villi (although this data was not measured in this study). The research results of Liu *et al.* (2023) explained that supplementation of 0.3% *A. paniculata* had a significant effect ($P<0.05$) on increasing the intestinal thickness of Muscovy ducks, which in turn improved the function and health of the intestines in Muscovy ducks and ultimately improved duck performance. Intestinal thickness is important in maintaining the integrity of intestinal epithelial cells, protecting against pathogens and immune responses (Tian *et al*., 2021).The impact is to maintain the health of the digestive tract, improve the function of the small intestine in digestion, and optimize nutrient absorption. This causes the availability of raw materials for the synthesis of meat and bone tissue to be optimized, resulting in increased body weight. This finding is consistent with the results of Jahja *et al*., (2023), who stated that supplementary feeding in the form of *A. paniculata* leaves can improve broiler performance.

Treatment T3 and T4 resulted in the same increase in body weight as the control. At T3 and T4 the number of ducks per land area was 2 times higher than the control (10 birds/m2 vs 5 birds/m2). Much literature states that raising livestock with higher cage capacity causes stress and decreased production (Sugiharto 2022, Mangisah et al., 2022 and Mangisah et al., 2023). The research results in Table 5 are interesting, because in T3 and T4 there was no decline in production. This is thought to be due to the andrographolide and flavonoids contained in A. paniculata which act as antibacterials and antioxidants, so they can ward off free radicals and improve the condition of intestinal bacteria. This finding is very useful for duck breeders, because the use of ALM, which is easy to obtain and apply, has an impact on production efficiency. With the same land, farmers can raise more ducks, so that socio-economically you can increase the duck population and also have an impact on the farmer's income.

The findings in this research contribute to increasing the productivity of Tegal ducks and the economic value of Tegal duck breeders in rural and suburban areas. It is important to implement a duck health care strategy that focuses on optimizing duck gut health through herbal administration, optimal environmental conditions, and avoiding the use of chemical drugs that can cause residue. The density of the cage for ducks must also be considered, to minimize stress and prevent disease which can reduce duck production. Traditional livestock management by utilizing medicinal plants contribute significantly socio-economically to increasing the livestock population and the number of livestock farmers (Bhatt, 2015; Traore et al., 2020, Shahrajabian et al, 2021).

CONCLUSION

This study concluded that a dietary strategy providing A. paniculata decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

CONFLICT OF INTEREST

The authors declare that there is no any conflict of interest in the manuscript.

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Dear : AAVS Editor

We have made revisions according to your suggestions. Corrections are written in blue ink in the manuscript. The following is a list of revisions:

Your suggestions	Revised
The reasons why the focus is on Tegal ducks compared to other ducks or agricultural issues need to be stated explicitly	Lines 34 to 39 Tegal ducks are native to Indonesia and are often kept in villages and suburbs. In Indonesia, pastured Tegal ducks act as natural predators of insects and snails, while duck droppings contribute to soil fertility and crop yields (Ismoyowati and Sumarmono, 2019). Female Tegal ducks are kept for egg production. Specifically, male Tegal ducks are kept for their meat. The population of Tegal ducks is quite high and is spread across many provinces in Indonesia (Subiharta et al., 2013), in 2021, it was 58,651,838 heads (Statistics Indonesia 2021). Lines 42-49 Tegal duck productivity in traditional reared is currently still low. The body weight gain and egg production of Tegal ducks are lower than that of Magelang ducks. Egg production Tegal duck had an average of 78 ± 19 eggs per duck within 4 months or 65%, and the egg weight was 66.60 ± 5.05 g / egg, lower than Magelang ducks, namely 70.4% with an egg weight of 69.6 g/item (Ismoyowati et al., 2018).
Formulate a clear hypothesis or research question	Lines 73-82 Based on the description above, it is necessary to research the use of A. paniculata to overcome stress in ducks kept in open cages with different cage densities. Can the content of andrographolide and flavonoid compounds in A. paniculata if given to ducks kept in higher cage densities have an impact on intestinal health, especially the microbiota population, so that they can result in performance as well as at low densities? Based on our knowledge, we have not found the study on administering A. paniculata to ducks kept in different cage

	densities. Therefore, this research was carried out to determine the effect of using feed containing A. paniculata on intestinal microbiota population, internal organ growth, blood lipid profile, and performance of Tegal ducks reared in different cage densities
Ethical considerations regarding the use of animals	Lines 84-86 All activities carried out in this research have received approval from the Animal Research Ethics Committee, Faculty of Animal Science and Agriculture, Diponegoro University No. 60-02/A-06/KEP-FPP.
Composition and nutritional content of research rations	Lines 90-94 The rations are prepared using local feed ingredients, in the form of yellow corn, rice bran, soybean meal, fish meal, palm oil, premix, bentonite, monocalcium phosphate, limestone, methionine and NaCl, with a metabolizable energy content of 2,905.9 kcal/kg and a crude protein of 18.06 %. Treatment rations during the study for T0, T1, T2, T3 and T4 were the same.
Reasons for choosing a Completely Randomized Design	Lines 98-101 Completely randomized design is the simplest type of randomized design, to look at just one factor, namely the level of *A. paniculata*, and no other factors influence the response in outside the factors studied. The material used is homogeneous so there is no need to group it.
Results and Discussion: statistical details such as p values or confidence intervals have not been included. Results narrative improved.	Lines 149-155 Giving ALM 0.25 and 0.5% did not have a significant effect ($P>0.05$) on feed consumption in ducks kept in different cage densities (Table 5). However, treatment had a significant effect ($P<0.05$) on daily body weight gain. In ducks reared at a density of 5 birds/m^2, administration of 0.5% *A.paniculata* (T2) resulted in the highest daily body weight gain. Meanwhile, giving 0.25% *A. paniculata* (T1) to ducks did not increase daily body weight gain ($P>0.05$). In ducks reared at a density of 10 birds/m^2, administration of *A.*

	paniculata at a dose of 0.25 or 0.5% (T3 and T4) did not increase daily body weight gain.
comparison with similar studies	Lines 257-267 The performance of poultry, including ducks, is influenced by the morphology of the intestinal mucosa, the area of the intestinal villi, the balance of the intestinal microbiota and also the health of the poultry (Wang *et al.*, 2021). It is suspected that the increase in body weight at T2 was due to the role of *A. paniculata* supplementation, which significantly increased the height of the villi and the surface area of the duck's villi (although this data was not measured in this study). The research results of Liu *et al.* (2023) explained that supplementation of 0.3% *A. paniculata* had a significant effect ($P<0.05$) on increasing the intestinal thickness of Muscovy ducks, which in turn improved the function and health of the intestines in Muscovy ducks and ultimately improved duck performance. Intestinal thickness is important in maintaining the integrity of intestinal epithelial cells, protecting against pathogens and immune responses (Tian *et al.*, 2021). Lines 273 -283 Treatment T3 and T4 resulted in the same increase in body weight as the control. At T3 and T4 the number of ducks per land area was 2 times higher than the control (10 birds/m2 vs 5 birds/m2). Much literature states that raising livestock with higher cage capacity causes stress and decreased production (Sugiharto 2022, Mangisah et al., 2022 and Mangisah et al., 2023). The research results in Table 5 are interesting, because in T3 and T4 there was no decline in production. This is thought to be due to the andrographolide and flavonoids contained in A. paniculata which act as antibacterials and antioxidants, so they can ward off free radicals and improve the condition of intestinal bacteria. This finding is very useful for duck breeders, because the use of ALM,

	which is easy to obtain and apply, has an impact on production efficiency. With the same land, farmers can raise more ducks, so that socio-economically you can increase the duck population and also have an impact on the farmer's income.
Wider implications of this study for duck farming practices	Lines 284-292 The findings in this research contribute to increasing the productivity of Tegal ducks and the economic value of Tegal duck breeders in rural and suburban areas. It is important to implement a duck health care strategy that focuses on optimizing duck gut health through herbal administration, optimal environmental conditions, and avoiding the use of chemical drugs that can cause residue. The density of the cage for ducks must also be considered, to minimize stress and prevent disease which can reduce duck production. Traditional livestock management by utilizing medicinal plants contribute significantly socio-economically to increasing the livestock population and the number of livestock farmers (Bhatt, 2015; Traore et al., 2020, Shahrajabian et al, 2021).
Conclusion: inconsistency	Lines 294-295 This study concluded that a dietary strategy providing A. paniculata decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

We really hope that our article can be published in the next edition of AAVS.

Semarang, March 16, 2024

Best Regards,

Istna Mangisah

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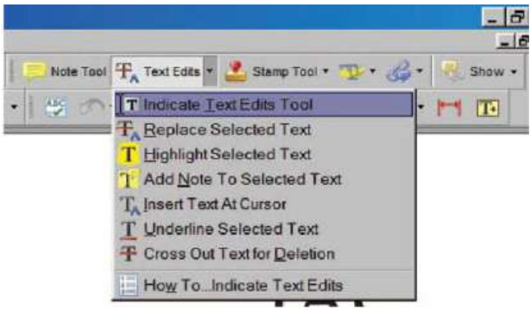

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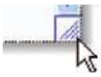

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		the article. Please provide these.	Indonesia, 2021 Correct : Badan Pusat Statistik, 2021 Page 5, column 2, paragraphs 2 and 3, Prihambodo is not listed in the references. Correct : Prihambodo TR, Sholikin MM, Qomariyah N, Jayanegara A, Batubara I, Utomo DB,and Nahrowi N)(2021). Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. Anim Biosci. 2021 Mar; 34(3): 434–442. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7961189/. doi: 10.5713/ajas.20.0379 Page 6, paragraph 2, written: Mangisah et al 2022, 2023 (False) Correct: Mangisah and Sugiharto, 2023; Mangisah et al 2024 The writing on page 6 has been corrected and the 2 pieces of literature are listed on References page 7, : Mangisah I, Sugiharto S (2023). Effect of synbiotic plus selenium or betaine on performance, intestinal health, nutrient digestibility, and carcass quality of Tegal ducks. J. Indonesian Trop. Anim. Agric., 48(3): 222–231. https://doi.org/10.14710/jitaa.48.3.222-231 Mangisah I, Ismadi VDYB, Sumarsih S, Sugiharto S (2024). Effect of synbiotic plus betaine and selenium supplementation on the behavior and performance of broilers stocked in highdensity pens. J. Anim. Behav. Biometeorol., 12(1): 2024007. https://doi.org/10.31893/jabb.2024007

AQ5	6	Please provide Novelty statement.	Novelty : *A. paniculata* has the potential to improve gut coliform and the performance of Tegal ducks reared in different cages densities.
AQ6	6	Please provide contents under “Authors Contribution” (how authors contributed in creating the article)	Authors Contribution : Research ideas and writing research proposals: Istna Mangisah (ISM) Data collection: Istna Mangisah (ISM), Lilik Krismiyamto (LLK), Fajar Wahyono (FW) Data analysis and interpretation: Istna Mangisah (ISM), Vitus Dwi Yunianto (VDY) Compiling articles: Istna Mangisah (ISM), Mulyono (MYN) Critical revision of the article:Nyoman Suthama (NST) Final approval of the version to be published: ISM
		Changes from the author	Results and discussion: Improvement: Deleted 1 paragraph about “duck performance,” which is located under Table 5. Reason for deletion: The discussion about “duck performance “ in the paper is written twice, namely: 1) under Table 5 Page 4; 2) last paragraph of page 5.

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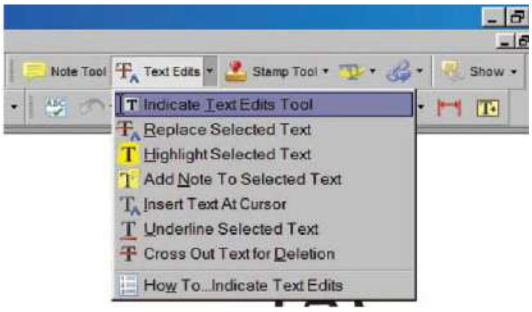

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Research Article

The Addition of *Andrographis paniculata* in the Diet of Tegal Ducks Reared in Different Cage Densities and the Effect on Gut Microflora, Internal Organs, Profile Lipids, and Duck Performance

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Abstract | This study aims to examine the effect of adding levels of *Andrographis paniculata* leaf meal (ALM) on intestinal bacteria, intestinal growth, blood lipid profile, and performance of Tegal ducks kept in different cage densities. A total of 140 7-day-old male Tegal ducks with an average body weight of 130.83 ± 7.54 g/head were placed in 20 experimental units in stilt cages following a completely randomized design procedure, 5 treatments, and 4 replications. The research ration was prepared with an EM content of 2900 kcal/kg and 18% crude protein. The treatments tested included T0: density of 5 ducks/m^2 + basal ration, T1: density of 5 ducks/m^2 + 0.25% ALM, T2: density of 5 ducks/m^2 + 0.5% ALM, T3: density of 10 ducks/m^2 + 0.25% ALM, T4: density of 10 ducks/m^2 + 0.5% ALM. The parameters measured include intestinal bacterial populations (LAB and coliform), digestive organs, blood lipid profile (cholesterol levels, low density lipoprotein/LDL, high density lipoprotein/HDL and triglycerides) and duck performance. Data were processed with the SPSS version 22 using analysis of variance and Duncan's multiple area test at a significance level of 5%. The results showed that the addition of ALM to Tegal duck feed had a significant effect ($p<0.05$) on coliform, blood cholesterol and LDL levels, and duck body weight. Treatment had no significant effect on relative intestinal weight, HDL and blood triglycerides, and feed consumption. This study concluded that a dietary strategy providing *A. paniculata* decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

Keywords | *Andrographis paniculata*, Duck, Gut microflora, Profil lipid, Digestive organ

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INTRODUCTION

Tegal ducks are native to Indonesia and are often kept in villages and suburbs. In Indonesia, pastured Tegal ducks act as natural predators of insects and snails, while duck droppings contribute to soil fertility and crop yields (Ismoyowati and Sumarmono, 2019). Female Tegal ducks are kept for egg production. Specifically, male Tegal ducks are kept for their meat. The population of Tegal ducks is quite high and is spread across many provinces in Indonesia (Subiharta *et al.*, 2013), in 2021, it was 58,651,838 heads (Statistics Indonesia, 2021).

In general, ducks are traditionally kept by breeders in open cages with unselected seed quality, feed quality that does not meet standards, and maintenance with cage density that is not taken into account, resulting in low productivity. Tegal duck productivity in traditional reared is currently still low. The body weight gain and egg production of Tegal ducks are lower than that of Magelang ducks. Egg production Tegal duck had an average of 78±19 eggs per duck within 4 months or 65%, and the egg weight was 66.60±5.05 g/egg, lower than Magelang ducks, namely 70.4% with an egg weight of 69.6 g/item (Ismoyowati *et al*., 2018).

Efforts need to be made to increase Tegal duck production, including by regulating cage density and adding feed additives. Cage density is an important factor in the livestock production process because of its influence on the comfort level of livestock. Inappropriate density can affect temperature, humidity, and air circulation in the cage. High cage density can cause stress, which affects physiological, immunological, and microbiological changes, changes in behavior, increases corticosteroid hormones, and ultimately affects performance (Abudabos *et al*., 2013; Sugiharto, 2022).

Another effort to improve the productivity of Tegal ducks can be done by adding feed additives, in the form of herbs. One of the herbal plants that grows widely in Indonesia is sambiloto. The sambiloto has the latin name *Andrographis paniculata*. Supplementation with unfermented and fermented *A. paniculata* across different treatments improved growth, immune status, intestinal morphology, and intestinal microbiota composition and structure in Muscovy ducks (Liu *et al*., 2023). *A. paniculata* leaves contain numerous compounds with anti-inflammatory, antibacterial, and antioxidant properties. In the leaves, *A. paniculata* contains the main compounds in the form of andrographolide and flavonoids. Other compounds contained in *A. paniculata*, but in small levels, include saponins, alkaloids, and tannins. The flavonoid content of *A. paniculata* extract is 0.022 mg/mL quercetin (Fardiyah *et al*., 2020). The properties of andrographolide include antiviral, antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, antitumor, chemopreventive, spasmolytic, and uterorelaxant (Dai *et al*., 2019). In several previous experiments, *A. paniculata* was proven to have a strong impact on *Eimeria spp*., the causative agent of coccidiosis (Indrati and Titisari, 2020) and inhibiting the growth of pathogenic bacteria thereby increasing the immune status and performance of poultry (Liu *et al*., 2023; Jahja *et al*., 2023). The results of previous research showed that the administration of 0.4% *A. paniculata* leaf powder provided optimal psychobiotic effects on Mojosari laying ducks, without affecting feed consumption (Yulianti *et al*., 2015).

Based on the description above, it is necessary to research the use of *A. paniculata* to overcome stress in ducks kept in open cages with different cage densities. Can the content of andrographolide and flavonoid compounds in *A. paniculata* if given to ducks kept in higher cage densities have an impact on intestinal health, especially the microbiota population, so that they can result in performance as well as at low densities?

Based on our knowledge, we have not found the study on administering *A. paniculata* to ducks kept in different cage densities. Therefore, this research was carried out to determine the effect of using feed containing *A. paniculata* on intestinal microbiota population, internal organ growth, blood lipid profile, and performance of Tegal ducks reared in different cage densities.

MATERIALS AND METHODS

All activities carried out in this research have received approval from the Animal Research Ethics Committee, Faculty of Animal Science and Agriculture, Diponegoro University No. 60-02/A-06/KEP-FPP.

Animal and diets

This study used 140 male Tegal ducks aged 7 days with an average body weight of 130.83±7.54 g. Open-sided cages were used in this study. A total of 20 experimental units measuring 1 m × 1 m were prepared, using bamboo partitions. The rations are prepared using local feed ingredients, in the form of yellow corn, rice bran, soybean meal, fish meal, palm oil, premix, bentonite, monocalcium phosphate, limestone, methionine and NaCl, with a metabolizable energy content of 2,905.9 kcal/kg and a crude protein of 18.06 %. Treatment rations during the study for T0, T1, T2, T3 and T4 were the same. The composition and nutrient content of the research ration can be seen in Table 1.

Research treatment and design

This study was structured using a completely randomized design (CRD). Completely randomized design is the simplest type of randomized design, to look at just one factor, namely the level of *A. paniculata*, and no other factors influence the response in outside the factors studied. The material used is homogeneous so there is no need to group it. There were 5 treatments applied and each treatment had 4 replications.

T0: Density of 5 ducks/ m^2 + Ration without ALM (control)

T1: Density of 5 ducks/m^2 + 0.25% ALM

T2: Density of 5 ducks/m^2 + 0.5% ALM

T3: Density of 10 ducks/m^2 + 0.25% ALM

T4: Density of 10 ducks/m^2 + 0.5% ALM

Table 1: Composition and nutrient content of basal rations.

Ingredient	Amount (%)
Yellow corn	53.15
Rice bran	15.00
Soybean meal	20.00
Fish meal	5.00
Palm oil	2.00
Premix	0.37
Bentonite	1.00
Mono calcium phosphate (MCP)	1.50
Limestone	1.25
Methionine	0.20
NaCl	0.28
Total	100
Nutrient content based on laboratory analysis (%, unless otherwise stated)	
ME (kkal/kg)	2,905.90
Crude protein	18.06
Extract ether	4.76
Crude fiber	3.69
Calsium	0.92
Phosphor	0.67

Ducks were adapted for 1 week, starting at 7-14 days of age. Feeding 100% of the treatment diet to the ducks was carried out for 4 weeks, starting at the age range of 15-42 days. Feed and water were given ad libitum. Duck weights were weighed weekly to monitor weight gain. Newcastle disease (ND) vaccination was conducted using Medivac ND La Sota vaccine through drinking water when the ducks were 10 days old.

Parameter measurement

Lactic acid bacteria (LAB) and coliform counts were measured using 20 ileal fluid samples from 20 ducks from each experimental unit. Ducks were slaughtered by cutting the neck. The cecal fluid was collected and placed in a sterile Eppendorf tube. LAB and coliform counts were calculated using the total plate count method. The calculation of LAB and coliform counts followed Fardiaz (2001).

The relative weight of the intestine was also measured using 20 ducks representing each replicate. The ducks were slaughtered, the abdomen dissected, and the internal organs removed. Duodenum, jejunum, ileum, and cecum were separated and weighed. The relative weight of the organs was obtained by dividing the weight of the organs by the live weight, then the result was multiplied by 100%.

The stage for taking blood and digestive organs was carried out on day 43. Blood was taken from the brachial vein and put into a non-EDTA vacutainer tube. The blood was centrifuged for 15 minutes at 3000 rpm to separate the blood serum. The blood serum was then put into a sample tube and stored in an ice box. Cholesterol, HDL, and LDL levels were analyzed using the enzymatic cholesterol oxidase-para aminophenazone method (CHOD-PAP KIT), while triglyceride levels (mg/dl) were measured using the GPO-PAP method using a spectrophotometer.

Statistical analysis

Data were processed using analysis of variance and Duncan's multiple area test at a significance level of 5%, with the SPSS version 22.

RESULTS AND DISCUSSION

Intestinal bacteria

The total LAB and ileal coliforms of ducks are presented in Table 2. Statistical analysis showed that the addition of *A. paniculata* leaf meal (ALM) to the ration had a significant effect ($P<0.05$) on the coliform, but had no significant effect on total lactic acid bacteria in the ileum.

Table 2: Total ileal bacteria in Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
LAB (log cfu/g)	7.81	7.52	8.02	6.95	6.73	1.23	0.56
Coliform (log cfu/g)	5.10[a]	4.36[b]	3.74[b]	5.57[a]	4.62[b]	0.86	0.01

Relative weight of intestines

The relative weights of the duodenum, jejunum and ileum were not affected by ALM treatment (Table 3). The addition of ALM in the diet resulted in the relative weight of the small intestine (duodenum, jejunum, and ileum) being the same as the control.

Table 3: The relative weight of the small intestine of Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Duodenum (%)	0.45	0.47	0.46	0.49	0.41	0.05	0.35
Jejunum (%)	0.91	1.09	1.09	0.96	1.04	0.13	0.29
Ileum (%)	0.90	0.97	0.99	0.94	0.94	0.08	0.65

Profile lipid

The addition of *A. paniculata* leaf meal had a significant effect ($p<0.05$) on blood cholesterol and LDL levels of Tegal ducks, but had no significant effect on HDL and triglycerides (Table 4).

Table 4: Blood lipid profile of Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Cholesterol (mg/dl)	85.97[a]	63.25[c]	70.09[c]	83.37[a]	73.24[b]	10.72	0.01
HDL (mg/dl)	28.44	20.87	25.99	23.51	35.09	8.91	0.20
LDL (mg/dl)	66.26[a]	45.59[c]	50.93[c]	62.01[ab]	51.75[bc]	9.90	0.01
Triglycerides (mg/dl)	54.76	47.58	44.65	57.65	58.55	8.59	0.06

Table 5: Duck performance.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Daily feed intake (g)	149.35	147.74	151.15	151.90	147.16	3.69	0.29
Daily weight gain (g)	29.30[b]	28.57[b]	31.42[a]	28.32[b]	29.29[b]	1.30	0.01

Duck performance

Giving ALM 0.25 and 0.5% did not have a significant effect (P>0.05) on feed consumption in ducks kept in different cage densities (Table 5). However, treatment had a significant effect (P<0.05) on daily body weight gain. In ducks reared at a density of 5 birds/m², administration of 0.5% *A. paniculata* (T2) resulted in the highest daily body weight gain. Meanwhile, giving 0.25% *A. paniculata* (T1) to ducks did not increase daily body weight gain (P>0.05). In ducks reared at a density of 10 birds/m², administration of *A. paniculata* at a dose of 0.25 or 0.5% (T3 and T4) did not increase daily body weight gain.

Intestinal bacteria

Suplementation of ALM significantly reduced the number of intestinal coliforms in ducks reared at a cage density of 5 ducks/m² (Table 2). Andrographolide compounds in ALM proved to be able to reduce coliforms, resulting in coliform counts in T1 and T2 that were much lower than the control group (T0). This finding is consistent with the study of Liu *et al.* (2023), which showed that supplementation of unfermented and fermented *A. paniculata* (30 g/kg each) in Muscovy rations can reduce the number of harmful bacteria in the caeca, such as *Succinivibrio*, *Succinatimonas*, *Sphaerochaeta*, and *Mucispirillum*.

Other researchers have also reported similar results. For example, the addition of *A. paniculata* and *Origanum vulgare* aqueous extracts in broiler diets resulted in an improved microbiota profile compared to zinc bacitracin and the negative control. The profile included increased numbers of *Lactobacillus* spp. and *Bacillus* spp., while lower numbers of *Escherichia coli* and *Salmonella* spp. isolated from the intestine (Jahja *et al.*, 2023).

Keeping ducks with a cage capacity of 10 ducks/m² (T3 and T4) has the potential to cause stress that can alter the balance of intestinal bacteria, which is manifested in an increase in coliform counts, as shown in Table 2. Coliforms in T3 were recorded much higher than in T1 and T2. However, at T4 there was a more significant decrease in coliforms due to the administration of ALM (0.5%) compared to T3 (0.25%).

Andrographolide ($C_{20}H_{30}O5$) in ALM has a high hydrogen content, so it can dissociate into the bacterial cell wall and eventually cause the death of pathogenic bacteria. Indrati and Titisari (2020) showed that *A. paniculata* extract added to broiler feed can reduce the number of *Eimeria tenella* oocysts. The decrease in coliform count can also be caused by the presence of flavonoid compounds in ALM that function as antibacterials. Flavonoids inhibit bacterial growth by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and energy metabolism, so bacteria cannot grow or develop (Panche *et al.*, 2016).

Relative weight of intestines

The level of administration of 0.25 and 0.5% ALM to Tegal ducks with a cage density of 10 ducks/m² resulted in the relative weight of the small intestine being the same as ducks with a cage density of 5 ducks/m². Growth of duckling digestive organs occurs during embryonic development, reaching a peak when the ducklings are 3 days after hatching. Intestinal growth rate decreases after 3 weeks of age (Lilburn and Loeffler, 2015). Treatments were given when the ducks were 3-6 weeks old, and the data in Table 3 were measured when the ducks were 6 weeks old, where the digestive organs had reached the peak of growth. The results showed that feeding *A. paniculata* Leaf Meal (ALM) did not have a significant effect on the relative weight of the intestine of ducks, both those reared at cage densities of 5 birds/m² and 10 birds/m².

Although there was no significant effect, T3 and T4, where ducks were reared at a density of 10 birds/m² and fed 0.25% and 0.5% ALM, respectively, showed the highest relative weights of duodenum, jejunum, and ileum. This is in line with T0, indicating that flavonoids in *A. paniculata* play a role in reducing stress in ducks. Flavonoids can bind free radicals and form new non-reactive compounds, so that the body's metabolism can run smoothly, including the growth of digestive organs (Panche *et al*., 2016).

Profile lipid

The addition of *A. paniculata* leaf meal reduces blood cholesterol levels in the Tegal ducks (Table 4). This finding is consistent with Bogusławska-Tryk *et al.* (2016) who state that total cholesterol, lipoprotein fractions, and triglycerides are influenced by factors such as age, sex, genetic type, as well as environmental and feed conditions.

Cholesterol and LDL levels in TI and T2 were significantly lower compared to the control. Meanwhile, cholesterol levels in T3 and T4 were significantly higher than those in T1 and T2. Ducks in T3 and T4, which were reared with higher cage density, faced higher stress levels and increased free radicals. However, the addition of *A. paniculata* leaf meal, which contains flavonoids as antioxidants, may help prevent the negative impact of increased free radicals.

According to Tan *et al.* (2022), flavonoid supplementation in feed can modulate metabolism and reduce cholesterol content. Flavonoids, as antioxidants, can prevent cell damage due to oxidative stress, and the enzyme HMG-CoA reductase can reduce the activity of cholesterol synthesis in the body. Flavonoids also have antibacterial properties that can reduce the number of pathogenic bacteria such as *E. coli* and increase the growth of lactic acid bacteria (LAB) in the digestive tract (Panche *et al*., 2016).

Tsai *et al.* (2014) mentioned that LAB can produce the enzyme bile salt hydrolase (BSH), which affects cholesterol reduction by inhibiting the recycling of bile salts. Increasing LAB can increase BSH activity, which in turn can inhibit cholesterol absorption in the gastrointestinal tract. As a result, cholesterol is excreted along with feces, contributing to the reduction of blood cholesterol (Kumar *et al*., 2012). The importance of adding *A. paniculata* extract to feed is also reinforced by Sudarmi *et al.* (2018), who state that the addition of *A. paniculata* extract to feed can reduce cholesterol levels by 0.21%.

The T3 and T4 treatments were carried out with a cage density of 10 ducks/m^2, so the ducks were more likely to experience stress, which caused an increase in cholesterol and LDL levels compared to T1 and T2 (density of 5 ducks/m^2). In T3, *A. paniculata* was given at a low dose, with a high cage density of 10 ducks/m^2, which increased the potential for stress in the ducks. Ducks under stress will produce stress hormones, such as corticosterone, whose synthesis requires cholesterol as a precursor. LDL levels act as a transporter of cholesterol and triglycerides to peripheral tissues and glands, resulting in an increase in LDL levels as the need for triglycerides and cholesterol by tissues and glands increases.

The addition of 0.5% ALM caused a decrease in cholesterol and LDL in T4. *A. paniculata*, in the form of leaf flour, contains flavonoid compounds. This study is consistent with the findings of Prihambodo *et al.* (2021), who stated that flavonoids in feed can reduce LDL levels due to delayed activity of cholesterol acyltransferase Acyl-CoA in liver hepatocellular carcinoma cells and a decrease in LDL constituent compounds, such as CHO compounds and glucose.

ALM was not able to increase HDL levels and also did not reduce blood triglycerides in Tegal ducks (P>0.05). Flavonoid levels as antioxidants at T1 to T4 did not contribute to the increase in HDL and decrease in triglycerides. Blood cholesterol, MDA, triglyceride, and lipoprotein levels were influenced by the amount of flavonoids consumed. The higher the amount of flavonoids consumed, the higher the activity of superoxide dismutase and HDL (Prihambodo *et al.,* 2021). It is suspected that in this study, flavonoid consumption was still low, although this was not measured in this study. This finding is not in line with the results of Tan *et al.* (2022), who state that flavonoids can inhibit free fatty acids which directly reduce the formation of fatty acids in the circulation, followed by a decrease in free fatty acids towards the portal vein, thereby reducing fat deposition in liver tissue.

Duck performance

Feed consumption in this study was not influenced by ALM addition (Table 5). However, the addition of ALM to the ducks' rations significantly increased the ducks' body weight. It can be seen that giving 0.5% ALM resulted in the highest body weight of Tegal ducks in the group of ducks kept at a cage density of 5 ducks/m^2. This is thought to be related to the role of andrographolide as an antibacterial, which can reduce coliforms (Table 2). The main bioactive compound of *A. paniculata*, andrographolide, has been reported in several studies to have antibacterial activity. The main bioactive compound of *A. paniculata*, andrographolide, has been reported in several studies to have antibacterial activity. Andrographolide works by inhibiting bacterial growth through inhibition of DNA synthesis, almost equivalent to the effectiveness of fluoroquinolone antibiotics (Banerjee *et al*., 2017). The performance of poultry, including ducks, is influenced by the morphology of the intestinal mucosa, the area of the intestinal villi, the balance of the intestinal microbiota and also the health of the poultry (Wang *et al*., 2021). It is suspected that the increase in body weight at T2 was due to the role of *A. paniculata* supplementation, which significantly increased the height of the villi and the surface area of the duck's villi (although this data was not measured in this study). The research results of Liu *et al.* (2023) explained that supplementation of 0.3% *A. paniculata* had a significant effect ($P<0.05$) on increasing the intestinal thickness of Muscovy ducks, which in turn improved the function and health of the intestines in Muscovy ducks and ultimately improved duck performance. Intestinal thickness is important in maintaining the integrity of intestinal epithelial cells, protecting against pathogens and immune responses (Tian *et al.*, 2021). The impact is to maintain the health of the digestive tract, improve the function of the small intestine in digestion, and optimize nutrient absorption. This causes the availability of raw materials for the synthesis of meat and bone tissue to be optimized, resulting in increased body weight. This finding is consistent with the results of Jahja *et al.* (2023), who stated that supplementary feeding in the form of *A. paniculata* leaves can improve broiler performance.

Treatment T3 and T4 resulted in the same increase in body weight as the control. At T3 and T4 the number of ducks per land area was 2 times higher than the control (10 birds/m^2 vs 5 birds/m^2). Much literature states that raising livestock with higher cage capacity causes stress and decreased production (Sugiharto, 2022; Mangisah *et al.*, 2022, 2023). The research results in Table 5 are interesting, because in T3 and T4 there was no decline in production. This is thought to be due to the andrographolide and flavonoids contained in *A. paniculata* which act as antibacterials and antioxidants, so they can ward off free radicals and improve the condition of intestinal bacteria. This finding is very useful for duck breeders, because the use of ALM, which is easy to obtain and apply, has an impact on production efficiency. With the same land, farmers can raise more ducks, so that socio-economically you can increase the duck population and also have an impact on the farmer's income.

The findings in this research contribute to increasing the productivity of Tegal ducks and the economic value of Tegal duck breeders in rural and suburban areas. It is important to implement a duck health care strategy that focuses on optimizing duck gut health through herbal administration, optimal environmental conditions, and avoiding the use of chemical drugs that can cause residue. The density of the cage for ducks must also be considered, to minimize stress and prevent disease which can reduce duck production. Traditional livestock management by utilizing medicinal plants contribute significantly socio-economically to increasing the livestock population and the number of livestock farmers (Bhatt, 2015; Traore *et al.*, 2020; Shahrajabian *et al.*, 2021).

CONCLUSIONS AND RECOMMENDATIONS

This study concluded that a dietary strategy providing *A. paniculata* decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

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NOVELTY STATEMENT

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Conflict of interest

The authors have declared no conflict of interest.

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Research Article

The Addition of *Andrographis paniculata* in the Diet of Tegal Ducks Reared in Different Cage Densities and the Effect on Gut Microflora, Internal Organs, Profile Lipids, and Duck Performance

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Abstract | This study aims to examine the effect of adding levels of *Andrographis paniculata* leaf meal (ALM) on intestinal bacteria, intestinal growth, blood lipid profile, and performance of Tegal ducks kept in different cage densities. A total of 140 7-day-old male Tegal ducks with an average body weight of 130.83 ± 7.54 g/head were placed in 20 experimental units in stilt cages following a completely randomized design procedure, 5 treatments, and 4 replications. The research ration was prepared with an EM content of 2900 kcal/kg and 18% crude protein. The treatments tested included T0: density of 5 ducks/m² + basal ration, T1: density of 5 ducks/m² + 0.25% ALM, T2: density of 5 ducks/m² + 0.5% ALM, T3: density of 10 ducks/m² + 0.25% ALM, T4: density of 10 ducks/m² + 0.5% ALM. The parameters measured include intestinal bacterial populations (LAB and coliform), digestive organs, blood lipid profile (cholesterol levels, low density lipoprotein/LDL, high density lipoprotein/HDL and triglycerides) and duck performance. Data were processed with the SPSS version 22 using analysis of variance and Duncan's multiple area test at a significance level of 5%. The results showed that the addition of ALM to Tegal duck feed had a significant effect ($p<0.05$) on coliform, blood cholesterol and LDL levels, and duck body weight. Treatment had no significant effect on relative intestinal weight, HDL and blood triglycerides, and feed consumption. This study concluded that a dietary strategy providing *A. paniculata* decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

Keywords | *Andrographis paniculata*, Duck, Gut microflora, Profil lipid, Digestive organ

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INTRODUCTION

Tegal ducks are native to Indonesia and are often kept in villages and suburbs. In Indonesia, pastured Tegal ducks act as natural predators of insects and snails, while duck droppings contribute to soil fertility and crop yields (Ismoyowati and Sumarmono, 2019). Female Tegal ducks are kept for egg production. Specifically, male Tegal ducks are kept for their meat. The population of Tegal ducks is quite high and is spread across many provinces in Indonesia (Subiharta *et al*., 2013), in 2021, it was 58,651,838 heads (Badan Pusat Statistik, 2021).

In general, ducks are traditionally kept by breeders in open cages with unselected seed quality, feed quality that does not meet standards, and maintenance with cage density that is not taken into account, resulting in low productivity. Tegal duck productivity in traditional reared is currently still low. The body weight gain and egg production of Tegal ducks are lower than that of Magelang ducks. Egg production Tegal duck had an average of 78±19 eggs per duck within 4 months or 65%, and the egg weight was 66.60±5.05 g/egg, lower than Magelang ducks, namely 70.4% with an egg weight of 69.6 g/item (Ismoyowati *et al*., 2018).

Efforts need to be made to increase Tegal duck production, including by regulating cage density and adding feed additives. Cage density is an important factor in the livestock production process because of its influence on the comfort level of livestock. Inappropriate density can affect temperature, humidity, and air circulation in the cage. High cage density can cause stress, which affects physiological, immunological, and microbiological changes, changes in behavior, increases corticosteroid hormones, and ultimately affects performance (Abudabos *et al*., 2013; Sugiharto, 2022).

Another effort to improve the productivity of Tegal ducks can be done by adding feed additives, in the form of herbs. One of the herbal plants that grows widely in Indonesia is sambiloto. The sambiloto has the latin name *Andrographis paniculata*. Supplementation with unfermented and fermented *A. paniculata* across different treatments improved growth, immune status, intestinal morphology, and intestinal microbiota composition and structure in Muscovy ducks (Liu *et al*., 2023). *A. paniculata* leaves contain numerous compounds with anti-inflammatory, antibacterial, and antioxidant properties. In the leaves, *A. paniculata* contains the main compounds in the form of andrographolide and flavonoids. Other compounds contained in *A. paniculata*, but in small levels, include saponins, alkaloids, and tannins. The flavonoid content of *A. paniculata* extract is 0.022 mg/mL quercetin (Fardiyah *et al*., 2020). The properties of andrographolide include antiviral, antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, antitumor, chemopreventive, spasmolytic, and uterorelaxant (Dai *et al*., 2019). In several previous experiments, *A. paniculata* was proven to have a strong impact on *Eimeria spp*., the causative agent of coccidiosis (Indrati and Titisari, 2020) and inhibiting the growth of pathogenic bacteria thereby increasing the immune status and performance of poultry (Liu *et al*., 2023; Jahja *et al*., 2023). The results of previous research showed that the administration of 0.4% *A. paniculata* leaf powder provided optimal psychobiotic effects on Mojosari laying ducks, without affecting feed consumption (Yulianti *et al*., 2015).

Based on the description above, it is necessary to research the use of *A. paniculata* to overcome stress in ducks kept in open cages with different cage densities. Can the content of andrographolide and flavonoid compounds in *A. paniculata* if given to ducks kept in higher cage densities have an impact on intestinal health, especially the microbiota population, so that they can result in performance as well as at low densities?

Based on our knowledge, we have not found the study on administering *A. paniculata* to ducks kept in different cage densities. Therefore, this research was carried out to determine the effect of using feed containing *A. paniculata* on intestinal microbiota population, internal organ growth, blood lipid profile, and performance of Tegal ducks reared in different cage densities.

MATERIALS AND METHODS

All activities carried out in this research have received approval from the Animal Research Ethics Committee, Faculty of Animal Science and Agriculture, Diponegoro University No. 60-02/A-06/KEP-FPP.

Animal and diets

This study used 140 male Tegal ducks aged 7 days with an average body weight of 130.83±7.54 g. Open-sided cages were used in this study. A total of 20 experimental units measuring 1 m × 1 m were prepared, using bamboo partitions. The rations are prepared using local feed ingredients, in the form of yellow corn, rice bran, soybean meal, fish meal, palm oil, premix, bentonite, monocalcium phosphate, limestone, methionine and NaCl, with a metabolizable energy content of 2,905.9 kcal/kg and a crude protein of 18.06 %. Treatment rations during the study for T0, T1, T2, T3 and T4 were the same. The composition and nutrient content of the research ration can be seen in Table 1.

Research treatment and design

This study was structured using a completely randomized design (CRD). Completely randomized design is the simplest type of randomized design, to look at just one factor, namely the level of *A. paniculata*, and no other factors influence the response in outside the factors studied. The material used is homogeneous so there is no need to group it. There were 5 treatments applied and each treatment had 4 replications.

T0: Density of 5 ducks/ m^2 + Ration without ALM (control)
T1: Density of 5 ducks/m^2 + 0.25% ALM
T2: Density of 5 ducks/m^2 + 0.5% ALM
T3: Density of 10 ducks/m^2 + 0.25% ALM
T4: Density of 10 ducks/m^2 + 0.5% ALM

Table 1: Composition and nutrient content of basal rations.

Ingredient	Amount (%)
Yellow corn	53.15
Rice bran	15.00
Soybean meal	20.00
Fish meal	5.00
Palm oil	2.00
Premix	0.37
Bentonite	1.00
Mono calcium phosphate (MCP)	1.50
Limestone	1.25
Methionine	0.20
NaCl	0.28
Total	100
Nutrient content based on laboratory analysis (%, unless otherwise stated)	
ME (kkal/kg)	2,905.90
Crude protein	18.06
Extract ether	4.76
Crude fiber	3.69
Calsium	0.92
Phosphor	0.67

Ducks were adapted for 1 week, starting at 7-14 days of age. Feeding 100% of the treatment diet to the ducks was carried out for 4 weeks, starting at the age range of 15-42 days. Feed and water were given ad libitum. Duck weights were weighed weekly to monitor weight gain. Newcastle disease (ND) vaccination was conducted using Medivac ND La Sota vaccine through drinking water when the ducks were 10 days old.

Parameter measurement

Lactic acid bacteria (LAB) and coliform counts were measured using 20 ileal fluid samples from 20 ducks from each experimental unit. Ducks were slaughtered by cutting the neck. The cecal fluid was collected and placed in a sterile Eppendorf tube. LAB and coliform counts were calculated using the total plate count method. The calculation of LAB and coliform counts followed Fardiaz (2001).

The relative weight of the intestine was also measured using 20 ducks representing each replicate. The ducks were slaughtered, the abdomen dissected, and the internal organs removed. Duodenum, jejunum, ileum, and cecum were separated and weighed. The relative weight of the organs was obtained by dividing the weight of the organs by the live weight, then the result was multiplied by 100%.

The stage for taking blood and digestive organs was carried out on day 43. Blood was taken from the brachial vein and put into a non-EDTA vacutainer tube. The blood was centrifuged for 15 minutes at 3000 rpm to separate the blood serum. The blood serum was then put into a sample tube and stored in an ice box. Cholesterol, HDL, and LDL levels were analyzed using the enzymatic cholesterol oxidase-para aminophenazone method (CHOD-PAP KIT), while triglyceride levels (mg/dl) were measured using the GPO-PAP method using a spectrophotometer.

Statistical analysis

Data were processed using analysis of variance and Duncan's multiple area test at a significance level of 5%, with the SPSS version 22.

RESULTS AND DISCUSSION

Intestinal bacteria

The total LAB and ileal coliforms of ducks are presented in Table 2. Statistical analysis showed that the addition of *A. paniculata* leaf meal (ALM) to the ration had a significant effect ($P<0.05$) on the coliform, but had no significant effect on total lactic acid bacteria in the ileum.

Table 2: Total ileal bacteria in Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
LAB (log cfu/g)	7.81	7.52	8.02	6.95	6.73	1.23	0.56
Coliform (log cfu/g)	5.10^{a}	4.36^{b}	3.74^{b}	5.57^{a}	4.62^{b}	0.86	0.01

Relative weight of intestines

The relative weights of the duodenum, jejunum and ileum were not affected by ALM treatment (Table 3). The addition of ALM in the diet resulted in the relative weight of the small intestine (duodenum, jejunum, and ileum) being the same as the control.

Table 3: The relative weight of the small intestine of Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Duodenum (%)	0.45	0.47	0.46	0.49	0.41	0.05	0.35
Jejunum (%)	0.91	1.09	1.09	0.96	1.04	0.13	0.29
Ileum (%)	0.90	0.97	0.99	0.94	0.94	0.08	0.65

Profile lipid

The addition of *A. paniculata* leaf meal had a significant effect ($p<0.05$) on blood cholesterol and LDL levels of Tegal ducks, but had no significant effect on HDL and triglycerides (Table 4).

Table 4: Blood lipid profile of Tegal ducks.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Cholesterol (mg/dl)	85.97[a]	63.25[c]	70.09[c]	83.37[a]	73.24[b]	10.72	0.01
HDL (mg/dl)	28.44	20.87	25.99	23.51	35.09	8.91	0.20
LDL (mg/dl)	66.26[a]	45.59[c]	50.93[c]	62.01[ab]	51.75[bc]	9.90	0.01
Triglycerides (mg/dl)	54.76	47.58	44.65	57.65	58.55	8.59	0.06

Table 5: Duck performance.

Variable	Treatments						
	T0	T1	T2	T3	T4	SEM	P-value
Daily feed intake (g)	149.35	147.74	151.15	151.90	147.16	3.69	0.29
Daily weight gain (g)	29.30[b]	28.57[b]	31.42[a]	28.32[b]	29.29[b]	1.30	0.01

Intestinal bacteria

Suplementation of ALM significantly reduced the number of intestinal coliforms in ducks reared at a cage density of 5 ducks/m^2 (Table 2). Andrographolide compounds in ALM proved to be able to reduce coliforms, resulting in coliform counts in T1 and T2 that were much lower than the control group (T0). This finding is consistent with the study of Liu *et al.* (2023), which showed that supplementation of unfermented and fermented *A. paniculata* (30 g/kg each) in Muscovy rations can reduce the number of harmful bacteria in the caeca, such as *Succinivibrio*, *Succinatimonas*, *Sphaerochaeta*, and *Mucispirillum*.

Other researchers have also reported similar results. For example, the addition of *A. paniculata* and *Origanum vulgare* aqueous extracts in broiler diets resulted in an improved microbiota profile compared to zinc bacitracin and the negative control. The profile included increased numbers of *Lactobacillus* spp. and *Bacillus* spp., while lower numbers of *Escherichia coli* and *Salmonella* spp. isolated from the intestine (Jahja *et al.*, 2023).

Keeping ducks with a cage capacity of 10 ducks/m^2 (T3 and T4) has the potential to cause stress that can alter the balance of intestinal bacteria, which is manifested in an increase in coliform counts, as shown in Table 2. Coliforms in T3 were recorded much higher than in T1 and T2. However, at T4 there was a more significant decrease in coliforms due to the administration of ALM (0.5%) compared to T3 (0.25%).

Andrographolide ($C_{20}H_{30}O5$) in ALM has a high hydrogen content, so it can dissociate into the bacterial cell wall and eventually cause the death of pathogenic bacteria. Indrati and Titisari (2020) showed that *A. paniculata* extract added to broiler feed can reduce the number of *Eimeria tenella* oocysts. The decrease in coliform count can also be caused by the presence of flavonoid compounds in ALM that function as antibacterials. Flavonoids inhibit bacterial growth by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and energy metabolism, so bacteria cannot grow or develop (Panche *et al.*, 2016).

Relative weight of intestines

The level of administration of 0.25 and 0.5% ALM to Tegal ducks with a cage density of 10 ducks/m^2 resulted in the relative weight of the small intestine being the same as ducks with a cage density of 5 ducks/m^2. Growth of duckling digestive organs occurs during embryonic development, reaching a peak when the ducklings are 3 days after hatching. Intestinal growth rate decreases after 3 weeks of age (Lilburn and Loeffler, 2015). Treatments were given when the ducks were 3-6 weeks old, and the data in Table 3 were measured when the ducks were 6 weeks old, where the digestive organs had reached the peak of growth. The results showed that feeding *A. paniculata* Leaf Meal (ALM) did not have a significant effect on the relative weight of the intestine of ducks, both those reared at cage densities of 5 birds/m^2 and 10 birds/m^2.

Although there was no significant effect, T3 and T4, where ducks were reared at a density of 10 birds/m^2 and fed 0.25% and 0.5% ALM, respectively, showed the highest relative weights of duodenum, jejunum, and ileum. This is in line with T0, indicating that flavonoids in *A. paniculata* play a role in reducing stress in ducks. Flavonoids can bind free radicals and form new non-reactive compounds, so that the body's metabolism can run smoothly, including the growth of digestive organs (Panche *et al.*, 2016).

Profile lipid

The addition of *A. paniculata* leaf meal reduces blood cholesterol levels in the Tegal ducks (Table 4). This finding is consistent with Bogusławska-Tryk *et al.* (2016) who state that total cholesterol, lipoprotein fractions, and triglycerides are influenced by factors such as age, sex, genetic type, as well as environmental and feed conditions.

Cholesterol and LDL levels in TI and T2 were significantly lower compared to the control. Meanwhile, cholesterol levels in T3 and T4 were significantly higher than those in T1 and T2. Ducks in T3 and T4, which were reared with higher cage density, faced higher stress levels and increased free radicals. However, the addition of *A. paniculata* leaf meal, which contains flavonoids as antioxidants, may help prevent the negative impact of increased free radicals.

According to Tan *et al.* (2022), flavonoid supplementation in feed can modulate metabolism and reduce cholesterol content. Flavonoids, as antioxidants, can prevent cell damage due to oxidative stress, and the enzyme HMG-CoA reductase can reduce the activity of cholesterol synthesis in the body. Flavonoids also have antibacterial properties that can reduce the number of pathogenic bacteria such as *E. coli* and increase the growth of lactic acid bacteria (LAB) in the digestive tract (Panche *et al.*, 2016).

Tsai *et al.* (2014) mentioned that LAB can produce the enzyme bile salt hydrolase (BSH), which affects cholesterol reduction by inhibiting the recycling of bile salts. Increasing LAB can increase BSH activity, which in turn can inhibit cholesterol absorption in the gastrointestinal tract. As a result, cholesterol is excreted along with feces, contributing to the reduction of blood cholesterol (Kumar *et al.*, 2012). The importance of adding *A. paniculata* extract to feed is also reinforced by Sudarmi *et al.* (2018), who state that the addition of *A. paniculata* extract to feed can reduce cholesterol levels by 0.21%.

The T3 and T4 treatments were carried out with a cage density of 10 ducks/m^2, so the ducks were more likely to experience stress, which caused an increase in cholesterol and LDL levels compared to T1 and T2 (density of 5 ducks/m^2). In T3, *A. paniculata* was given at a low dose, with a high cage density of 10 ducks/m^2, which increased the potential for stress in the ducks. Ducks under stress will produce stress hormones, such as corticosterone, whose synthesis requires cholesterol as a precursor. LDL levels act as a transporter of cholesterol and triglycerides to peripheral tissues and glands, resulting in an increase in LDL levels as the need for triglycerides and cholesterol by tissues and glands increases.

The addition of 0.5% ALM caused a decrease in cholesterol and LDL in T4. *A. paniculata*, in the form of leaf flour, contains flavonoid compounds. This study is consistent with the findings of Prihambodo *et al.* (2021), who stated that flavonoids in feed can reduce LDL levels due to delayed activity of cholesterol acyltransferase Acyl-CoA in liver hepatocellular carcinoma cells and a decrease in LDL constituent compounds, such as CHO compounds and glucose.

ALM was not able to increase HDL levels and also did not reduce blood triglycerides in Tegal ducks (P>0.05). Flavonoid levels as antioxidants at T1 to T4 did not contribute to the increase in HDL and decrease in triglycerides. Blood cholesterol, MDA, triglyceride, and lipoprotein levels were influenced by the amount of flavonoids consumed. The higher the amount of flavonoids consumed, the higher the activity of superoxide dismutase and HDL (Prihambodo *et al.*, 2021). It is suspected that in this study, flavonoid consumption was still low, although this was not measured in this study. This finding is not in line with the results of Tan *et al.* (2022), who state that flavonoids can inhibit free fatty acids which directly reduce the formation of fatty acids in the circulation, followed by a decrease in free fatty acids towards the portal vein, thereby reducing fat deposition in liver tissue.

Duck performance

Feed consumption in this study was not influenced by ALM addition (Table 5). However, the addition of ALM to the ducks' rations significantly increased the ducks' body weight. It can be seen that giving 0.5% ALM resulted in the highest body weight of Tegal ducks in the group of ducks kept at a cage density of 5 ducks/m^2. This is thought to be related to the role of andrographolide as an antibacterial, which can reduce coliforms (Table 2). The main bioactive compound of *A. paniculata*, andrographolide, has been reported in several studies to have antibacterial activity. The main bioactive compound of *A. paniculata*, andrographolide, has been reported in several studies to have antibacterial activity. Andrographolide works by inhibiting bacterial growth through inhibition of DNA synthesis, almost equivalent to the effectiveness of fluoroquinolone antibiotics (Banerjee *et al.*, 2017). The performance of poultry, including ducks, is influenced by the morphology of the intestinal mucosa, the area of the intestinal villi, the balance of the intestinal microbiota and also the health of the poultry (Wang *et al.*, 2021). It is suspected that the increase in body weight at T2 was due to the role of *A. paniculata* supplementation, which significantly increased the height of the villi and the surface area of the duck's villi (although this data was not measured in this study). The research results of Liu *et al.* (2023) explained that supplementation of 0.3% *A. paniculata* had a significant effect (P<0.05) on increasing the intestinal thickness of Muscovy ducks, which in turn improved the function and health of the intestines in Muscovy ducks and ultimately improved duck performance. Intestinal thickness is important in maintaining the integrity of intestinal epithelial cells, protecting against pathogens and immune responses (Tian *et al.*, 2021). The impact is to maintain the health of the digestive tract, improve the function of the small intestine in digestion, and optimize nutrient absorption. This causes the availability of raw materials for the synthesis of meat and bone tissue to be optimized, resulting in increased body weight. This finding is consistent with the results of Jahja *et al*. (2023), who stated that supplementary feeding in the form of *A. paniculata* leaves can improve broiler performance.

Treatment T3 and T4 resulted in the same increase in body weight as the control. At T3 and T4 the number of ducks per land area was 2 times higher than the control (10 birds/m^2 vs 5 birds/m^2). Much literature states that raising livestock with higher cage capacity causes stress and decreased production (Sugiharto, 2022; Mangisah and Sugiharto, 2023; Mangisah *et al* 2024). The research results in Table 5 are interesting, because in T3 and T4 there was no decline in production. This is thought to be due to the andrographolide and flavonoids contained in *A. paniculata* which act as antibacterials and antioxidants, so they can ward off free radicals and improve the condition of intestinal bacteria. This finding is very useful for duck breeders, because the use of ALM, which is easy to obtain and apply, has an impact on production efficiency. With the same land, farmers can raise more ducks, so that socio-economically you can increase the duck population and also have an impact on the farmer's income.

The findings in this research contribute to increasing the productivity of Tegal ducks and the economic value of Tegal duck breeders in rural and suburban areas. It is important to implement a duck health care strategy that focuses on optimizing duck gut health through herbal administration, optimal environmental conditions, and avoiding the use of chemical drugs that can cause residue. The density of the cage for ducks must also be considered, to minimize stress and prevent disease which can reduce duck production. Traditional livestock management by utilizing medicinal plants contribute significantly socio-economically to increasing the livestock population and the number of livestock farmers (Bhatt, 2015; Traore *et al*., 2020; Shahrajabian *et al*., 2021).

CONCLUSIONS AND RECOMMENDATIONS

This study concluded that a dietary strategy providing *A. paniculata* decreases gut coliform and improves the performance of Tegal ducks reared in different cage densities.

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NOVELTY STATEMENT

A. paniculata has the potential to improve gut coliform and the performance of Tegal ducks reared in different cages densities.

AUTHOR'S CONTRIBUTION

Research ideas and writing research proposals: Istna Mangisah (ISM) Data collection: Istna Mangisah (ISM), Lilik Krismiyamto (LLK), Fajar Wahyono (FW) Data analysis and interpretation: Istna Mangisah (ISM), Vitus Dwi Yunianto (VDY) Compiling articles: Istna Mangisah (ISM), Mulyono (MYN) Critical revision of the article:Nyoman Suthama (NST) Final approval of the version to be published: ISM

Conflict of interest

The authors have declared no conflict of interest.

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