

Protein deposition and carcass weight in broiler given noni fruit extract (*Morinda citrifolia* L.) compared to zinc bacitracin

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ABSTRACT: The purpose of the study was to examine the effect of giving noni fruit extract (*Morinda citrifolia* L.) compared to zinc bacitracin to broiler chickens on total lactic acid bacteria, coliform bacteria, small intestine pH, protein digestibility, meat calcium mass, meat protein mass and carcass weight. This research used 200 unsexed broiler chickens of Cobb MB 202 aged 15 days with an average body weight of 246.02 ± 11.31 g. The study used a completely randomized design with 5 treatments and 4 replicates (each 10 bird). The treatments include T₀: ration (control), T₁: T₀ + 0.04% zinc bacitracin, T₂: T₀ + 0.04% noni fruit extract, T₃: T₀ + 0.08% noni fruit extract, T₄: T₀ + 0.12% noni fruit extract. Parameters measured included total lactic acid bacteria, coliform bacteria, small intestine pH, protein digestibility, meat calcium mass, meat protein mass and carcass weight. Data were processed using analysis of variance and Duncan's test in the SPSS version 16 program. The results showed that the provision of noni fruit extract had a significant effect ($p < 0.05$) on all parameters measured. The study concludes that the feeding of noni fruit extract at 0.12% can be used as a substitute for zinc bacitracin by increasing total lactic acid bacteria, protein digestibility, calcium mass, meat protein, carcass weight but reduce pH and total small intestine coliform in broiler chickens.

Keywords: Broiler, carcass, noni fruit extract, protein, zinc bacitracin.

INTRODUCTION

Broiler are meat-producing birds that are efficient in converting rations so that they have fast growth. However, broilers are easily stressed and susceptible to disease, so they need feed additives that can increase endurance or at least maintain health. Fast growth must be balanced with the provision of good quality rations and is expected to produce highly nutritious meat, as a source of animal protein. However, feed ingredients with high protein content are relatively more expensive, resulting in increased production costs. In addition, the use of antibiotics as growth promoters has been banned, because it can lead to resistance of certain bacteria that

are unhealthy for the host livestock and cause residues that are not health-friendly for consumers.

The use of antibiotics in Indonesia has been banned since January 1, 2018, because it causes the problems mentioned above. Safe, healthy, whole and halal products are a guarantee for veterinary public health and the safety of food products from livestock (Widodo *et al.*, 2019). The presence of antibiotic residues in meat is a concern in the procurement of animal food products because it is feared that it can affect consumer health which has an impact in the long term (Ma'ruf *et al.*, 2019). The provision of noni fruit extract as a herbal plant that is rich in health benefits,

can be used as an alternative herbal feed additive to replace antibiotics.

Noni fruit (*Morinda citrifolia* L.) contains flavonoid compounds, which are bioactive substances that function as antioxidants (Rastini *et al.*, 2010). Flavonoids also act as antibacterials that inhibit the development of pathogens, thus implicating the health of the digestive tract (Lima *et al.*, 2009). A healthy digestive tract supports the process of digestion and absorption of nutrients, especially protein, to be maximized (Sudarman *et al.*, 2011). In addition to flavonoids, noni fruit contains acubin, alizarin and anthraquinone which are also antibacterial (Sudewi and Lolo, 2016). With these active substances, noni fruit has the opportunity to be developed as a feed additive for livestock. Feed additives that are antibacterial are needed, because the health of broiler is strongly influenced by the balance of microflora in the digestive tract (Sulfani *et al.*, 2018).

Quality rations with balanced nutrient content and according to the needs of chickens, become more efficient because they are supported by a healthy digestive tract due to the provision of noni fruit. A healthy digestive tract is associated with better nutrient digestibility, especially protein and calcium (Farida *et al.*, 2017). Protein and calcium play an important role in the growth process of an individual, as a substrate for the formation of new cells, repairing damaged body tissues and increasing meat protein synthesis (Suthama, 2003). Calcium is necessary for bone growth, and can also be deposited in meat, which in this study is called meat calcium mass. Calcium absorption is closely related to protein availability, because calcium is absorbed in the form of calcium binding protein (CaBP) (Fanani *et al.*, 2016). CaBP bonds are formed in the mucosa and facilitate the intestine in the process of calcium absorption (Syafitri *et al.*, 2015). If the concentration of calcium in the form of free ions is high in meat, it becomes an activator for protease enzyme activity in meat called calcium activated neutral protease. Free calcium ions are activators of enzymes in meat, called calcium activated neutral protease (CANP) which determines the high and low mass of meat protein (Bikrisima *et al.*, 2014). The provision of noni fruit extract is expected to modify calcium metabolism, so that it is not degradative to meat protein.

Based on the description above, this study aims to evaluate the level of administration of noni fruit extract (*Morinda citrifolia* L.) as a substitute for zinc bacitracin in broiler, on total small intestinal bacteria, protein and calcium utilization in relation to carcass weight.

MATERIALS AND METHODS

Materials

The livestock used were Cobb MB 202 unsexed broiler chickens aged 15 days as many as 200 heads with an average body weight of 246.02 ± 11.31 g. The supporting

Table 1. Composition and nutrient content of research rations.

Feed Stuff	Composition
Yellow Corn	44.80
Rice Bran	17.70
Soybean Meal	26.70
Meat Bone Meal	9.20
CaCO ₃	0.60
Premix	1.00
Total	100.00
Nutrient Content	
Metabolizable Energi (kcal/kg)**	3,001.96
Crude Protein (%)*	21.06
Crude Fat (%)*	3.82
Crude Fiber (%)*	5.63
Calcium (%)*	1.06
Phosphor (%)*	0.65

*Feed was analyzed for proximate and minerals in the laboratory of animal nutrition, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (2023). ** Based on the Bolton calculation formula (1967).

equipment in relation to the production of noni fruit extract is a grinder and oven to smooth and dry the noni fruit respectively. The research cage is equipped with feed and drinking water containers, as well as a thermohygrometer to measure the temperature and humidity outside and inside the cage. The research ration (basal) consisted of corn, rice bran, soybean meal, meat bone meal, CaCO₃ and premix (Table 1). Noni fruit extract and zinc bacitracin antibiotic were added to the basal ration as treatments.

Methods

The study began with cage sanitization, making brooder for DOC, preparing colony cages according to the number of treatments. Cages were labeled based on treatment and replication, and arranged randomly according to the experimental design. Chickens at the time of DOC were kept in a brooder, then at the age of 15 days were moved to colony cages, then began to be given research rations plus noni fruit extract or zinc bacitracin according to treatment, and drinking water *ad libitum*.

Noni fruit extract is made from clean noni fruit cut into thin slices, then dried using an oven at a temperature of <math><50^{\circ}\text{C}</math>. The dried slices of noni fruit were then ground with a grinder until they became flour. Noni fruit flour was macerated (soaking) using 70% ethanol solvent in a ratio of 1:20 (10 g of noni fruit flour plus 200 ml ethanol) for ± 72 hours. The results of maceration are filtered, the filtrate is collected and then evaporated using a rotary evaporator machine with a temperature of $45-50^{\circ}\text{C}$ until all the solvent evaporates, so that a thick extract of noni fruit is obtained.

The study was arranged in a completely randomized design (CRD) with 5 treatments and 4 replicates, each

consisting of 10 birds. The treatments were as follows: T₀: Standard ration with 21% protein T₁: T₀ with zinc bacitracin (antibiotic) 0.04% T₂: T₀ with noni fruit extract 0.04% T₃: T₀ with noni fruit extract 0.08% T₄: T₀ with noni fruit extract 0.12%.

Crude protein digestibility

Crude protein digestibility was calculated based on the total collection method combined with Fe₂O₃ indicator as much as 0.5% of the ration given for 3 days starting on day 39 to 41. Chickens used for total collection were 2 chickens in each experimental unit. Endogenous chickens as many as 6 chickens were fed (only given water) for 2 x 24 hours. Excreta collection was carried out for 24 hours. A plastic-lined tray was placed under the cage to collect excreta, then sprayed periodically with 0.2 N HCl to prevent nitrogen from evaporating. The collected excreta were then cleaned from ration scattering and feather shedding. Excreta was cleaned every day, then weighed fresh weight and dry weight. The dried excreta samples were weighed and mashed and then analyzed to calculate protein digestibility using the formula of Ensminger *et al.* (1990) as follows:

$$PD (\%) = \frac{[PI - (AexP - AenP)]}{PI} \times 100$$

Where: PD = Protein digestibility, PI = Protein intake, AexP = Amount of excrete protein, AenP = Amount of endogenous protein.

Intestinal Microflora

Lactic acid bacteria and Coliform of the small intestine, were the parameters measured on week 5 (35 days). Population of LAB and coliform were measured using media deman Rogosa Sharpe (MRS) and eosin methylene blue agar (EMBA). Testing was proceeded with total plate count method according to Fardiaz (1993) with the following formula:

Total colony (cfu/g) = total colony x 1/dilution factor per plate.

Potential Hydrogen

Potential hydrogen (pH) was measured from the digesta of each segment of small intestine using a pH meter. Rate of passage measurement was recorded based on the total collection method using indicator.

Protein mass and calcium mass

Chickens were slaughtered at the end of the study (day

35), one bird per replicate, to obtain meat samples which were then analyzed in the laboratory to calculate the protein mass and calcium mass of the meat. The empty carcass weight, meaning that it has been cleaned of feathers, and without viscera, head, legs and neck, was weighed before meat samples were taken. Next, the meat and bones of the breast and thigh were separated and weighed. Meat protein mass was calculated from the protein content multiplied by the overall meat weight. Meat was mixed and finely ground, then a composite sample of 20 g was taken to be analyzed for protein and calcium content. Meat protein mass was calculated according to Suthama (2003) as follows:

Meat protein mass (g) = meat protein content (%) x meat weight (g).

Meat calcium mass (g) = meat calcium content (%) x meat weight (g)

Data analysis

Data were analyzed using analysis of variance (ANOVA) applying SPSS 16.0 statistical software. Duncan's multiple range tests examined the differences between the treatments' means. The significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Total small intestinal bacteria

The results in Table 2 show that the provision of noni fruit extract had a significant effect ($p < 0.05$) on total lactic acid bacteria, coliforms and pH of the small intestine. Giving noni fruit extract as much as 0.12% (T₄) was able to increase total lactic acid bacteria compared to other treatments. This result is supported by a decrease in total coliform and pH of the small intestine which illustrates that a high level of 0.12% is able to act as an antibacterial, thus controlling the balance of bacteria in the small intestine. Lavanya and Brahmaprakash (2011) stated that noni fruit contains phytochemical compounds as antibacterial (flavonoids, anthraquinones and saponins). The antimicrobial properties of flavonoids can damage the protein structure in the cytoplasm, resulting in changes in the physical and chemical properties of the components that make up the peptidoglycan of bacterial cells, which results in death because the cell wall layer is damaged (Afrina *et al.*, 2018). Saponins work as antimicrobials through membranolytic properties in microbial cells, resulting in leakage of cell components that result in proteins, nucleic acids and nucleotides coming out of bacterial cells (Afiff and Amaliah, 2017). Anthraquinone compounds are responsible for inhibiting bacterial cell wall synthesis and membrane function, and inactivating microbial enzymes, but can be toxic, if too high (Narimani-

Table 2. Total small intestine bacteria in broilers.

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄
LAB (Log CFU/g)	3.43±2.12 ^b	1.83±3.09 ^c	3.73±2.52 ^b	4.20±3.41 ^b	5.42±4.09 ^a
Coliform (Log CFU/g)	4.18±1.11 ^a	3.64±1.04 ^a	1.84±0.87 ^b	0.99±0.78 ^c	0.79±1.14 ^c
pH	6.50±2.04 ^a	6.40±1.88 ^{ab}	6.30±2.18 ^{bc}	6.20±1.47 ^{bc}	6.10±1.18 ^c

Different superscripts on mean values indicate significant differences ($p < 0.05$).

Rad *et al.*, 2011).

The treatment of zinc bacitracin (T₁) which is low both on total lactic acid bacteria, coliform and pH of the small intestine can be caused by the lack of availability of low pH for lactic acid bacteria growth. Although zinc bacitracin given according to government recommendations. The results of giving low-high level noni extract (T₂-T₄) was able to increase total lactic acid bacteria and reduce total coliform and pH of the small intestine. Flavonoid compounds are acidic because they contain benzenol which can maintain intestinal pH in an acidic state, and have an impact on the digestive tract to be healthier (Syafitri *et al.*, 2015). A healthy digestive tract supports the process of digestion and absorption of nutrients, especially protein, to be maximized (Sudarman *et al.*, 2011). This phenomenon is very beneficial for chickens, so that the ration given can be maximally utilized for the production process. Therefore, noni fruit can be used as a cheap alternative natural feed additive for antibiotics to maximize nutrient use (Widoretno *et al.*, 2018).

Protein digestibility

The provision of noni fruit extract (*Morinda citrifolia* L.) had a significant effect ($p < 0.05$) on protein digestibility in broilers (Table 3). Giving noni fruit extract of 0.12% (T₄) can increase protein digestibility in broilers with the highest flavonoid content associated with the condition of the digestive tract. Akbar and Rosyidin (2016) stated that noni juice contains active substances that can help the digestive process and nutrient absorption. The digestive process is supported by the improvement of microflora balance between lactic acid bacteria and Coliform (Table 2). Lactic acid bacteria can ferment carbohydrates from the ration, especially low molecular weight carbohydrates, to produce short chain fatty acids (SCFA) including acetic, propionic and butyric acids. Perdinan and Larasati (2019) stated that under anaerobic conditions low molecular weight carbohydrates can be broken down into oligosaccharides and monosaccharides by glycoside hydrolase to produce short chain fatty acids. Similarly, Kubena *et al.* (2001) reported that short-chain fatty acids can also inhibit the colonization of pathogenic bacteria. Short-chain fatty acids, including butyrate and propionate, act as a source of energy and stimulate the growth of the small intestinal epithelium (Guilloteau *et al.*, 2010). The influence of butyrate and propionate can maximize the

digestion and absorption of nutrients, especially protein.

Treatments T₀, T₁, T₂ and T₃ showed lower protein digestibility compared to T₄ (Table 3). Associated with higher Coliform bacteria population (Table 2). Faradila *et al.* (2016) reported that low colonies of pathogenic bacteria affect villi growth, thus maximizing nutrient absorption, especially protein. Based on the description above, it can be stated that an increase in the number of Coliform causes inhibition of villi development and disruption of the digestive process, resulting in low protein digestibility. Giving zinc bacitracin antibiotics to broiler is less effective in supporting lactic acid bacteria growth so that it does not support increased protein digestibility. According to Kurniawan *et al.* (2016), the use of antibiotics as a ration supplement can kill bacteria in the digestive tract, both pathogenic and non-pathogenic bacteria. Antibiotics that can reduce the population of lactic acid bacteria are not effective in maintaining the health of the digestive tract, especially in relation to the process of digestion and nutrient absorption.

Meat calcium and protein mass

The administration of noni fruit extract (*Morinda citrifolia* L.) significantly affected the mass of calcium and meat protein in broiler (Table 3). Treatments T₃ and T₄ produced higher calcium mass and meat protein than T₀, because the provision of noni fruit extract with flavonoid levels of 0.04 and 0.08% was able to acidify the pH of the digestive tract. Low potential hydrogen (pH) can suppress the growth of pathogenic bacteria which causes the digestive tract to be healthier, resulting in better digestion and absorption of nutrients. Farida *et al.* (2017) stated that flavonoids, which include alcohols with acidic properties, have a positive impact on the absorption of nutrients, especially protein and calcium. As it is known that in an acidic atmosphere, calcium can be absorbed well so that calcium retention increases. The potential hydrogen (pH) in the T₄ treatment was lower than the others, so the balance of Lactic acid bacteria and Coliform populations was better (Table 2). Fluctuations in the number of Lactic acid bacteria and Coliform indicate competition between bacteria in the digestive tract for space and nutrients (Halimatunnisroh *et al.*, 2017). The balance of the population of non-pathogenic and pathogenic bacteria in the digestive tract can maximize the function of the digestive tract in digesting and absorbing nutrients, especially protein and calcium.

Table 3. Protein deposition and carcass weight in broiler.

Treatment	T ₀	T ₁	T ₂	T ₃	T ₄
Protein Digestibility (%)	73.02±5.71 ^b	75.36±7.17 ^b	76.30±6.19 ^b	77.58±5.82 ^b	80.70±6.17 ^a
Meat Protein Mass (g)	148.17±4.41 ^c	166,55±6.09 ^{bc}	160.06±5.5 ^{bc}	172.31±4.19 ^{ab}	180.29±6.12 ^a
Meat Calcium Mass (g)	0.49±0.17 ^b	0.64±0,21 ^{ab}	0.58±0.11 ^{ab}	0.85±0.18 ^a	0.84±0.15 ^a
Carcass Weight (g)	775.50±7.09 ^{bc}	736.00±8.19 ^c	784.00±6.68 ^{bc}	819.25±8.01 ^{ab}	856.00±7.14 ^a

Different superscripts on mean values indicate significant differences ($p < 0.05$).

Factors other than pH, ration nutrient content, especially protein and carbohydrates, are also important supporters in increasing the population of lactic acid bacteria in the digestive tract (Widodo *et al.*, 2015).

The presence of calcium in the form of free ions has an impact on the degradative properties of calcium activated neutral protease (CANP). Calcium in ionic form is an activator of CANP enzymes or muscle proteolytic enzymes that can trigger high rates of protein degradation (Bikrisima *et al.*, 2014). Low protein synthesis and high rates of protein degradation result in low protein deposition in meat. Suthama (2003) stated that the presence of calcium ions affects the degradative activity of CANP enzymes, however, the high mass of meat calcium is not necessarily an activator. The phenomenon in this study showed that the addition of noni fruit extract containing flavonoid active substances resulted in high meat calcium mass (Table 3). This condition can be assumed that the presence of calcium in the form of free ions is low, so CANP is not degradative and has an impact on increasing the mass of meat protein. High meat protein mass is due to low degradation rate and high protein synthesis. Modification of calcium metabolism that results in high meat calcium mass but low presence of calcium in the form of ions are the effect of flavonoid active substance activity (Farida *et al.*, 2017). Meat protein mass is used as an indicator to determine calcium availability and its relationship with protein deposition in meat through the process of protein synthesis and degradation.

Treatments in T₁ and T₃ statistically showed meat protein mass that was not different from T₄. These results lead to the assumption that zinc bacitracin and active substances of noni fruit extract at not too high levels, have the same effect in the digestive tract. As discussed in the previous parameters, the administration of zinc bacitracin at T₁ not only inhibited pathogens (Coliform) but also suppressed the growth of lactic acid bacteria (Table 2). Bacterial balance conditions that are less favourable to the health of the digestive tract so that nutrient absorption, especially protein (Table 3), is not enough to contribute to the protein deposition process. The negative effects of long-term use of antibiotics, besides causing bacterial resistance, also have an unfavorable impact on the life of beneficial bacteria. Kurniawan *et al.* (2016) stated that antibiotics have a spectrum that not only kills pathogenic bacteria, but also non-pathogenic bacteria, so that the growth of lactic acid bacteria is also suppressed.

Carcass weight in broiler

The provision of noni fruit extract (*Morinda citrifolia* L.) has a significant effect ($p < 0.05$) on the carcass weight of broiler. The production ability of broiler is the accumulation effect of meat protein mass which is influenced by meat calcium mass, resulting in carcass weight as the final product. Carcass weight is an indication of high or low protein deposition in meat. The T₄ treatment was able to increase the highest carcass weight, although statistically the same as T₃. The addition of 0.12% noni fruit extract (T₄) can be categorized as having higher antioxidant activity. Flavonoid activity as an antioxidant is very effective for improving protein digestibility (Table 3) which is significantly different and supports high protein deposition in the form of meat protein mass (Table 3), resulting in high carcass weight. Bikrisima *et al.* (2014) stated that the effectiveness of flavonoid compounds and vitamin C works synergistically in increasing protein synthesis, resulting in better carcass weight. The same statement by Sukoco and Andri (2018) that giving noni leaf extract to broiler chickens can improve the balance of microflora and have an impact on the health of the digestive tract. Healthier digestive tract conditions cause better nutrient absorption, thereby increasing carcass weight.

The T₃ treatment produced carcass weights that were not different from T₄. As explained earlier, even with the provision of noni fruit extract at a moderate level, antioxidant activity is still effective in increasing protein utilization efficiency. Carcass weight in the T₃ treatment was supported by an increase in meat weight due to higher protein utilization efficiency due to a healthier digestive tract due to low Coliform population (Table 2). Carcass weight in the T₁ treatment was the lowest numerically, although statistically similar to T₀ and T₂ (Table 3). Changes in carcass weight originate from unbalanced microflora populations in the digestive tract, leading to low digestion and absorption of nutrients, especially protein. Low protein absorption due to an unhealthy digestive tract is characterized by low lactic acid bacteria and high Coliform populations (Table 2), so that protein deposition in the form of meat protein mass is also low (Table 3) which ultimately reduces carcass weight. Antibiotics given can cause resistance of pathogenic bacteria and accumulation of residues in the body of livestock (Fenita, 2011). Bacterial imbalance may occur in the T₁ treatment

because antibiotics are not selective to the presence of bacteria in the digestive tract. Kurniawan *et al.* (2016) stated that the use of antibiotics as a ration supplement can kill bacteria in the digestive tract, including non-pathogenic bacteria.

Conclusion

Feeding of noni fruit extract (*Morinda citrifolia* L.) at 0.12% can be used as a substitute for zinc bacitracin by increasing total lactic acid bacteria, protein digestibility, calcium mass, meat protein, and carcass weight but reduce pH and total small intestine coliform in broiler chickens.

CONFLICT OF INTEREST

The authors declare no competing interests.

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