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THE EFFECT OF BLACK CUMIN (*Nigella sativa*) SUPPLEMENTATION THROUGH DRINKING WATER ON THE HISTOLOGY OF SMALL INTESTINE AND LARGE INTESTINE OF BROILER CHICKENS

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ABSTRACT

This study aimed to determine the effect of black cumin (*Nigella sativa*) supplementation through drinking water on the histology of broiler chickens' small intestine and large intestine. The research was conducted from April-September 2020 in a cage facility of the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung. This research used a completely randomized design with four treatment groups and three replications (five broilers per replication) with a total of 60 male broilers. The treatments were drinking water without black cumin (P0, control); drinking water with black cumin 36 mg/kg BW/day (P1); 72 mg/kg BW/day (P2); and 144 mg/kg BW/day (P3). Three broilers from each group were randomly necropsied at 31 days old, and samples of the small intestine (duodenum, jejunum, and ileum) and large intestine were fixed with 10% formalin solution and sent to the Lampung Disease Investigation Center for histological preparations. The observation of preparations was carried out microscopically using the Leica DM500® Binocular Microscope to accurately calculate various parameter sizes. The results were analyzed statistically with one way analysis of variance at significant level 5% and if proven significant, then a Tukey test was conducted. The results of this study were that the supplementation of black cumin 72 mg/kg BW/day through drinking water could significantly increase ($P<0.05$) 1) the average sizes of villi height and villi area of small intestine (duodenum, jejunum, ileum) and large intestine (colon) of broiler chickens; 2) the average sizes of basal villi width, villi apex width, and gland diameter of broiler duodenum; and 3) the average size of apex villi width of broiler colon. The conclusion of this study was the supplementation of black cumin at dose of 72 mg/kg BW/day through drinking water could increase the histological sizes of the small intestine and large intestine of broilers.

Key words: broiler, histology, large intestine, *Nigella sativa*, small intestine

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh pengaruh suplementasi jintan hitam (Nigella sativa) melalui air minum terhadap histologi usus halus dan usus besar broiler. Penelitian dilakukan pada April-September 2020 di unit kandang Laboratorium Lapang Terpadu, Fakultas Pertanian, Universitas Lampung. Penelitian bersifat eksperimental menggunakan Rancangan Acak Lengkap dengan empat kelompok perlakuan dan tiga ulangan (lima ekor tiap ulangan) sehingga total 60 ekor broiler jantan. Perlakuan yang diberikan yaitu pemberian air minum tanpa jintan hitam (P0, kontrol); air minum dengan jintan hitam 36 mg/kg bobot badan/hari (P1); 72 mg/kg bobot badan/hari (P2); dan 144 mg/kg bobot badan/hari (P3). Tiga ekor dari tiap kelompok secara acak dinekropsi pada hari ke-31 dan diambil sampel organ usus halus (duodenum, jejunum, ileum) dan usus besar (colon) kemudian difiksasi dengan larutan formalin 10% dan dikirim ke Balai Veteriner Lampung untuk pembuatan preparat histologi. Pengamatan preparat dilakukan secara mikroskopis menggunakan Mikroskop Binokuler Leica DM500® untuk menghitung berbagai ukuran parameter secara akurat. Analisis hasil dilakukan secara statistik menggunakan analisis sidik ragam satu arah dengan taraf signifikansi 5% dan dilanjutkan dengan uji lanjut Tukey. Suplementasi jintan hitam 72 mg/kg bobot badan/hari dapat meningkatkan secara signifikan ($P<0,05$) terhadap 1) ukuran rata-rata tinggi vili dan luas vili usus halus (duodenum, jejunum, ileum) dan usus besar broiler; 2) ukuran rata-rata tinggi vili, lebar vili basal, lebar puncak vili, luas vili, dan diameter kelenjar duodenum broiler; dan 3) ukuran rata-rata lebar puncak vili usus besar broiler. Disimpulkan bahwa suplementasi jintan hitam (Nigella sativa) dosis 72 mg/kg bobot badan/hari melalui air minum dapat meningkatkan ukuran histologi usus halus dan usus besar broiler.

Kata kunci: broiler, histologi, usus besar, Nigella sativa, usus halus

INTRODUCTION

Intensive maintenance of broiler can often induce stress for the animal, resulting in a decreased responsiveness in the immune system, which increases the likelihood for disease and illness. Disease outbreaks are a serious obstacle in the broiler farming industry. The high incidence of disease can decrease productivity and even kill livestock which results in significant losses for breeders. Diseases can lead to decrease in body organ function, particularly the digestive organ. While antibiotics might prevent these diseases, the administration of antibiotics for long periods might cause residual buildup and antibiotic resistance in bacteria, which could have negative effects if consumed by humans (Marshall and Levy, 2011).

One strategy to maintain digestive tract quality and function, and to reduce the residual antibiotics in broilers is to provide them with natural herbs such as black cumin (*Nigella sativa*) as immunomodulation (Sulistiawati and Radji, 2014) and antibiotic growth promoter substitution (Miraghaee *et al*., 2011) with a high content of antioxidants thymoquinone as anti-oxidant that could eliminate free radicals (Kruk *et al*., 2000), anti-inflammatory effect s of several inflammation (Salem, 2005). *Nigella sativa* also has the ability to protect potentially different tissues and organs including liver, kidney, heart, blood, brain, lungs, reproductive system and gastrointestinal against chemical poison (Tavakkoli *et al*., 2017) and administration of *Nigella sativa* also can minimize heat stress in broilers by reducing cortisol and levels

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minimize histopathological changes in the liver (Hasan *et al*., 2019). This study was conducted to determine the effect of black cumin supplementation through histological studies of the digestive organs of broiler chickens which are expected to potentially increase the villi height, apex villi width, basal villi width, villi area, crypt depth, and gland diameter of small intestine (duodenum, jejunum, and ileum) and large intestine (colon).

MATERIALS AND METHODS

This research was carried out for six months (April-September 2020) in the Integrated Field Laboratory enclosure unit of the Faculty of Agriculture, University of Lampung. This research was designed using a completely randomized design (CRD) with four treatment groups and three replications with a total of 60 male broilers were used (five broilers per replication). The treatment dose was according to the broiler body weight, namely 1) drinking water without black cumin (P0, control); drinking water with black cumin at dose of 36 mg/kg BW/day (P1); drinking water with black cumin at dose of 72 mg/kg BW/day (P2); and drinking water with black cumin at dose of 144 mg/kg BW/day (P3). On the 31st day, three broilers from each group were randomly sacrificied and samples of the small intestine (duodenum, jejunum, ileum) and large intestine (colon) were then fixed with 10% formalin solution and sent to the Lampung Veterinary Disease Investigation Center for histological preparations with Hematoxylin Eosin (HE) staining. The preparation observation was carried out microscopically using the Leica DM500® Binocular Microscope Technology to accurately calculate various parameter sizes.

The research parameters were villi height, villi apex width, basal villi width, villi area, crypt depth, and gland diameter. The observation of histological preparations was done with a 10x magnification objective lens. The calculation of each parameter was carried out as many as three villi (villi height, villi apex width, basal villi width, villi area, crypt depth) and three gland diameters in each digestive tract organ (duodenum, jejunum, ileum, and colon). There were three replications per organ, so that the total for each organ was obtained through an average of the nine villi and nine gland diameters.

The calculation of the surface area of the intestinal villi using the method of Iji *et al*. (2001) which was slighly modified with the assumption that the villi model is an analogue of the trapezium shape so that the average number of the apical widths of the villi plus the average basal width of the villi is divided by two then multiplied by the height of the villi by the following formula.

$$\text{Surface area} = \frac{b + c}{2}\ x\ a$$

a= height of intestine villi
b= apex width of intestine villi
c= basal width of basal of intestine villi

Data Analysis

The average measurement data for various parameters of each digestive tract organ (duodenum, jejunum, ileum, and colon) were analyzed using analysis of variance, and followed by Tukey test.

RESULTS AND DISCUSSION

The average measurements of villi height, villi apex width, basal villi width, villi area, crypt depth, and gland diameter of the digestive tract organs (duodenum, jejunum, ileum, colon) are presented in each table. Each shows the calculation of each parameter from the nine villi (villi height, basal villi width, apex villi width, villi area, crypt depth) and nine gland diameters on histopathological preparations in each digestive tract organ (duodenum, jejunum, ileum, large intestine) in each treatment.

The average measurement of each parameter in broiler duodenum, jejunum, ileum, and colon were presented in Table 1, Table 2, Table 3, Table 4 respectively. Supplementation of black cumin with a dose of 72 mg/kg of broiler body weight (P2) had a significant effect on the increase in the average villi height, basal villi width, villi apex width, villi area, and gland diameter of the duodenum; the average villi height and villi area of jejunum; the average villi height and villi area of the ileum; and the average size of villi height, apex villi width, villi area of colon.

The ability of digestion and absorption of food substances could be affected by the surface area of the intestinal epithelium, the number of folds, and the number of villi and microvilli that expand the absorption field (Austic and Nesheim, 1990; Ibrahim, 2008). It could also be influenced by the height and surface area of the villi organs or digestive tract (Sugito *et al*., 2007; Ibrahim, 2008). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun *et al*., 2005). Villi are places for absorption of nutrients, the wider the villi, the more food substances that will be absorbed, in the end it can have an impact on the growth of organs and increased carcass (Asmawati, 2014).

Treatment with a dose of 72 mg/kg BW/day (P2) showed the most significant increase ($P<0.05$) in villi height and villi area of all broilers digestive organs (duodenum, jejunum, ileum, colon) compared to other treatments. The increase in villi height in the broiler intestine is closely related to an increase in digestive function and absorption function due to the expansion of the absorption area and is an expression of the smooth transportation system of nutrients throughout the body (Awad *et al*., 2008). One of the parameters that can be used to measure the quality of growth is the morphological structure of the intestine. The height of villi in all parts of the small intestine (duodenum, jejunum, ileum) and large intestine in general increases (Ningtias, 2013). Increasing the villi width and the villi height can expand the absorption area of the villi. According to Asmawati (2014), the wider the villi, the

Table 1. The average measurement of each parameter in broiler duodenum supplemented by black cumin through drinking water

Treatment	Duodenum					
	A1	B1	C1	D1	E1	F1
	Mean±SD (µm)					
P0	644.55±71.73^{ab}	149.78±7.72^{a}	74.96±8.11^{a}	75.70±1.35^{ab}	247.33±37.83^{a}	44.44±4.67^{a}
P1	442.78±2.67^{a}	158.89±32.35^{a}	60.81±20.43^{a}	65.24±33.66^{a}	263.78±51.42^{a}	52.09±3.37^{ab}
P2	878.11±159.06^{b}	248.89±42.22^{b}	144.90±17.95^{b}	174.59±46.61^{b}	272.78±74.64^{a}	63.72±4.86^{b}
P3	509.55±135.72^{a}	225.07±26.34^{ab}	97.93±19.65^{a}	116.13±50.90^{ab}	331.22±112.74^{a}	54.1±7.02^{ab}

$^{a, b, ab}$Different superscripts in the same column indicate significant differences (P<0.05). P0= Drinking water without *Nigella sativa*, P1= Drinking water with *Nigella sativa* 36 mg/kg BW/day, P1= Drinking water with *Nigella sativa* 72 mg/kg BW/day, P2= Drinking water with *Nigella sativa* 144 mg/kg BW/day, A1= Villi height of duodenum, B1= Basal villi width of duodenum, C1= Apex villi width of duodenum), D1= Villi area of duodenum, E1= Crypt depth of duodenum, F1= Gland diameter of duodenum)

Table 2. The average measurement of each parameter in broiler jejunum supplemented by black cumin (*Nigella sativa*) through drinking water

Treatment	Jejunum					
	A1	B1	C1	D1	E1	F1
	Mean±SD (µm)					
P0	342.34±111.40^{a}	148.01±19.47^{a}	86.43±39.58^{a}	42.28±22.93^{a}	231.22±134.32^{a}	55.83±2.87^{a}
P1	320.67±176.51^{a}	170.63±58.27^{a}	161.23±93.12^{a}	47.71±32.24^{a}	224.44±95.06^{a}	51.52±3.82^{a}
P2	890.17±242.62^{b}	188.39±34.90^{a}	138.56±41.97^{a}	126.38±38.38^{b}	260.06±86.15^{a}	64.20±6.23^{a}
P3	551.53±128.51^{ab}	105.5±20.63^{a}	44.80±8.44^{a}	42.15±17.44^{a}	191.00±38.40^{a}	54.16±7.41

$^{a, b, ab}$Different superscripts in the same column indicate significant differences (P<0.05). P0= Drinking water without *Nigella sativa*, P1= Drinking water with *Nigella sativa* 36 mg/kg BW/day, P1= Drinking water with *Nigella sativa* 72 mg/kg BW/day, P2= Drinking water with *Nigella sativa* 144 mg/kg BW/day, A1= Villi height of jejunum, B1= Basal villi width of jejunum, C1= Apex villi width of jejunum), D1= Villi area of jejunum, E1= Crypt depth of jejunum, F1= Gland diameter of jejunum)

Table 3. The average measurement of each parameter in broiler ileum supplemented by black cumin (*Nigella sativa*) through drinking water

Treatment	Ileum					
	A1	B1	C1	D1	E1	F1
	Mean±SD (µm)					
P0	290.11±25.06^{a}	155.40±9.59^{a}	65.64±16.48^{a}	45.43±18.92^{a}	140.26±18.97^{a}	54.43±9.23^{a}
P1	289.00±88.88^{a}	191.56±37.02^{a}	106.00±59.13^{a}	45.41±26.65^{a}	178.11±52.14^{a}	50.56±3.02^{a}
P2	557.22±31.45^{b}	244.78±52.74^{a}	162.33±15.84^{a}	91.04±2.33^{b}	174.34±37.86^{a}	52.78±3.71^{a}
P3	327.05±87.56^{a}	144.68±52.87^{a}	98.26±43.50^{a}	39.33±18.74^{a}	128.93±22.06^{a}	47.6 ±12.7^{a}

$^{a, b, ab}$Different superscripts in the same column indicate significant differences (P<0.05). P0= Drinking water without *Nigella sativa*, P1= Drinking water with *Nigella sativa* 36 mg/kg BW/day, P1= Drinking water with *Nigella sativa* 72 mg/kg BW/day, P2= Drinking water with *Nigella sativa* 144 mg/kg BW/day, A1= Villi height of ileum, B1= Basal villi width of ileum, C1= Apex villi width of ileum), D1= Villi area of ileum, E1= Crypt depth of ileum, F1= Gland diameter of ileum)

Table 4. The average measurement of each parameter in broiler colon supplemented by black cumin (*Nigella sativa*) through drinking water

Treatment	Colon					
	A1	B1	C1	D1	E1	F1
	Mean±SD (µm)					
P0	259.22±33.07^{ab}	132.82±44.40^{a}	64.95±4.74^{ab}	25.15±3.85^{a}	128.81±28.46^{a}	60.80±11.19^{a}
P1	193.56±25.20^{a}	178.39±38.25^{a}	66.10±27.36^{ab}	23.76±5.23^{a}	201.33±28.41^{b}	49.29±7.77^{a}
P2	312.56±35.84^{b}	193.56±19.91^{a}	117.39±32.55^{b}	49.26±9.86^{b}	157.11±12.36^{ab}	51.85±6.08^{a}
P3	193.22±45.30^{a}	118.53±13.63^{a}	50.97±8.34^{a}	19.03±7.77^{a}	138.07±29.86^{ab}	51.14±7.58^{a}

$^{a, b, ab}$Different superscripts in the same column indicate significant differences (P<0.05). P0= Drinking water without *Nigella sativa*, P1= Drinking water with *Nigella sativa* 36 mg/kg BW/day, P1= Drinking water with *Nigella sativa* 72 mg/kg BW/day, P2= Drinking water with *Nigella sativa* 144 mg/kg BW/day, A1= Villi height of colon, B1= Basal villi width of colon, C1= Apex villi width of colon), D1= Villi area of colon, E1= Crypt depth of colon, F1= Gland diameter of colon)

more food substances will be absorbed, which can have a long-term impact on the growth of the body's organs. Similarly, according to Rahmawati (2016) the higher the size of the villi, the wider the area of nutrient absorption by the small intestine wall, thus will trigger the growth improvement. Guyton (1997) added that a more villous surface area leads to a more efficient nutrient absorption. The efficiency of nutrient absorption cannot be separated from the work of hormonal, nervous, and digestive glands in the digestive tract and its accessory glands.

Crypts are contained in the intestinal villi, which are composed of inline cylindrical epithelial cells. These glands produce mucus and several enzymes for the metabolism of peptides, fats, carbohydrates, and intestinal juices (mucin) which function to protect the intestinal mucosa (Aughey and Frye, 2001). The increase in the average size of the gland diameter in the treatment with supplementation of black cumin in drinking water showed an increase in the size of the duodenum's gland diameter. The P2 treatment showed a significant increase (P<0.05) in size compared to other treatments, it can support the development of epithelial cells that make up the villi, which will increase the absorption of nutrients in the digestive tract.

Supplementation of black cumin 36 mg/kg BW/day (P1) showed the most significant increase (P<0.05) in parameter of crypt depth of colon but was not different from the dose of P2 and P3 (Table 4). According to Sun *et al*. (2005) and Smirnov *et al*. (2005) that crypt depth has no effect after broilers are more than 28 days old. In this study, samples of broilers digestive tract organs were collected at the age of 31 days. It is assumed that the development of intestinal morphology is closely related to the role of micronutrients in line with the increasing age of broilers (Harimurti and Rahayu, 2009).

Based on the data presented in Table 1, Table 2, Table 3, and Table 4, the supplementation of black cumin at dose of 72 mg/kg BW/day had most significant effect to the size of the villi height and villi area of all digestive tract organs compared to the control group. One of the parameters that can be used to measure the quality of growth is the morphological structure of the intestine (Wang *et al*., 2008; Ningtias, 2013). According to Suprijatna *et al*. (2008), the small intestine is the main organ for digestion and absorption of digestive products. Various enzymes that enter this channel function to accelerate and streamline the breakdown of carbohydrates, proteins and fats to facilitate the absorption process. In adult chickens, the length of the small intestine is about 62 inches or 1.5 meters.

One of the parameters that can be used to measure the quality of growth is the morphological structure of the intestine (Wang and Peng, 2008). The carrying capacity of the digestive process for the given feed and nutrient absorption can be influenced by the surface area of the intestinal epithelium, the number of folds in it, the height of the villi, the number of villi and microvilli that expand the absorption area (Ruttanavut *et al*., 2009). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun *et al*., 2005). The increase in villi causes more villi surface area to absorb nutrients into the bloodstream (Mile *et al*., 2006).

High villi indicate that the intestines are better than short villi. Awad *et al*. (2008) stated that the increase in the height of the villi in the intestine with digestive and absorption functions occurs because of the intact villi form which is a smooth expression of the nutrient transport system throughout the body. Rofiq (2003) stated that the absorption of nutrients in the intestine is influenced by the inner surface area of the intestine (folds, villi and microvilli) and the length of transit of the digesta in the intestine. Based on the research of Khedr and Abdel-Fattah (2007) showed that administration of *Nigella sativa* can increase broiler body weight, it is possible because *Nigella sativa* is rich in essential fatty acids such as oleic, linoleic, and linolenic acids which are essential to help growth and the presence of the active substance thymoquinone which has activities of antimicrobial and antifungal so as to prevent the growth of fungi and inhibit the formation of aflatoxins thereby increasing the efficiency of nutrients in feed.

The surface area of the intestine such as the height of the villi describes the area for absorption of nutrients. Villi are small finger or leaf-like protrusions found on the mucous membrane, 0.5 to 1.5 mm long and found only in the small intestine. The villi in the ileum are finger-like in shape and shorter than the villi found on the duodenum and jejunum. One of the parameters used to measure the quality of growth is the intestinal morphological structure (Wang and Peng, 2008).

The intestinal gland (Lieberkuhn's gland) has a small hole that becomes the mouth of the glandular simplex tubule. The intestinal glands are scattered between the villi attached to the mucous membrane. The intestinal glands and intestinal villi are covered by an epithelium, consisting of goblet cells and enterocytes, among others. Goblet cells secrete mucus to lubricate and protect the surface of the intestine, while the enterocytes in crypt secrete large amounts of water and electrolytes. Goblet cells secrete a kind of mucus, namely mucin which functions to coat the intestinal tract and protect pathogens that can damage intestinal epithelial cells so that the number of goblet cells is important for the health of broilers (Forder *et al*., 2007). The presence of goblet cells in the broiler duodenum was available in sufficient numbers in the body of broilers since before hatching, but the number of goblet cells in the jejunum and ileum was only reached after the broilers have hatched (Reynold *et al*., 2020).

CONCLUSION

The supplementation of black cumin (*Nigella sativa*) at dose of 72 mg/kg BW/day through drinking water could increase the histological sizes of the small intestine and large intestine of broilers.

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