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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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[J.KED.HEWAN] Submission Acknowledgement

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

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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 small intestine and large intestine of broiler. The research was conducted from April September 2020 in cage facility of Integrated Field Laboratory, Faculty of Agriculture, University of Lampung. This research was using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) with a total of 60 male broilers. The treatment 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 necropsed at 31st days old, and samples of the small intestine (duodenum, jejunum, 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 analyze descriptively. The conclusion of this study was the supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increase the average sizes of villi height, villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

Key words : black cumin, broiler, Leica DM500 microscope, large intestine, small intestine.

ABSTRAK

Penelitian ini bertujuan untuk 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 (RAL) 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 BB/hari (P1); 72 mg/kg BB/hari (P2); dan 144 mg/kg BB/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. Analsis hasil dilakukan secara deskriptif. Kesimpulan penelitian ini yaitu suplementasi

Jintan Hitam (Nigella sativa) 36 mg/kg BB/hari meningkatkan rata-rata ukuran tinggi vili, lebar apeks vili, lebar basal vili, luas vili, dan diameter kelenjar pada organ saluran pencernaan yaitu usus halus (duodenum, jejunum, ileum) dan usus besar broiler.

Kata kunci : broiler, jintan hitam, mikroskop Leica DM500, usus besar, usus halus.

INTRODUCTION

The development of poultry farming in Indonesia is increasing. Broilers are one of the fastest growing poultry. The relatively short period of time and the relatively lower maintenance costs compared to ruminants make breeders prefer to cultivate broilers. In 2018, the broiler population in Indonesia reached 1.89 billion heads and an increase of 2.26% from the broiler population in 2017 of 1.85 billion heads (BPS, 2019).

Disease is a serious obstacle in the broiler farming industry. The high incidence of disease can cause a decrease in productivity and even death of livestock which causes significant losses for breeders. Administration of antibiotics in the livestock industry is used for the treatment of livestock so as to reduce the risk of death and restore the condition of the livestock to health, however, giving antibiotics for a long period of time can cause residual buildup which has negative effects if consumed by humans.

The use of antibiotics needs to be reduced to prevent negative effects by providing natural ingredients as immunomodulators. Immunomodulators can be defined as biological or synthetic substances that can stimulate the innate immune system, adaptive or both. One of the herbs that can act as an immunomodulator is Black Cumin (*Nigella sativa*). *Nigella sativa* is a plant that has the potential as an immunostimulant that can stimulate and strengthen the

system by increasing the number, quality and activity of the body's immune cells (Hendrik, 2009). *Nigella sativa* contains *thymoquinone*, saponins, zinc or zinc, *alpha-linolenic acid* (Omega 3) and *linoleic acid* (Omega 6) which functions in cell formation, maintains the immune system, and helps in the process of blood formation (Yusuf, 2014).

The solution needed for the above problems is to examine the effect of black cumin (*Nigella sativa*) supplementation through histological studies of the digestive organs of broilers which are expected to have the potential to increase the size of villi height, apex villi width, basal villi width, villi area, crypt depth and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine.

MATERIALS AND METHODS

Materials

This study used broiler cages, sprayer for cage disinfection, bamboo to make 12 cage plots, plastic tarpaulin for curtains, newspaper and used husks as litter, 12 bulbs of 15 bulbs watt as a heating source for thearea. *brooding*, *a hanging feeder* 12 pieces, *chick feeder tray* 12 pieces, 12 pieces of chicken drinking places; 1 bucket, 1 *hand spray*, 1 water tray for *dipping*, 1 electric scale, *thermohygrometer* for measuring temperature and humidity, sack and plastic. Organ sampling equipment, namely necropsy equipment, *object glass*, *cover glass*, *refrigerator*, microtome, light microscope, camera technology and *software* Optilab®along with a laptop for taking tissue images and measuring the parameters of each organ.

Materials used in the study were 60 Day Old Chicks (DOC) male broiler Cobb CP 707 strains kept for 30 days, rations, drinking water, extract of Black Cumin (*Nigella sativa*), vaccines of Newcastle Disease (ND), Avian Influenza (AI) and Infectious Bursal Disease (IBD), 10% formalin solution.

Methods

This research carried out for 6 months (April - September 2020) in the Integrated Field Laboratory enclosure unit, Faculty of Agriculture, University of Lampung. This research was experimental in nature using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) so a total of 60 male broilers. The treatment dose was according to the broiler body weight, namely 1) drinking water without black cumin (P0, control); drinking water with Jintan Hitam 36 mg / kg BW / day (P1); drinking water with Jintan Hitam 72 mg / kg BW / day (P2); and drinking water with cumin 144 mg / kg BW / day (P3). On the 31st day, three from each group were randomly necropsed and samples of the small intestine (duodenum, jejunum, ileum) and large intestine were then fixed with 10% formalin solution and sent to the Lampung Veterinary Center for making histological preparations. with Hematoxylin Eosin (HE) staining. The observation of preparations 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. Observation of histological preparations using 10x magnification objective lens. The calculation of each parameter was carried out as many as three villi (villiheight, villi apex width, basal villi width, villi area, crypt depth) and nine gland diameters in each digestive tract organ (duodenum, jejunum, ileum, large intestine). There were three replications per organ, so that the total for each organ was obtained an average of the nine villi and twenty-seven glands.

The calculation of the surface area of the intestinal villi using the method of Iji *et al.* (2001) 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.

$$\textbf{Surface area} = \frac{b + c}{2} \times a$$

Description:

a = height of intestine villi

b = width of apex of intestine villi

c = width of basal of intestine villi

Data analysis

Measurement data for various research parameters were calculated the average of all replications of each digestive tract organs (duodenum, jejunum, ileum, and large intestinum) then analyzed descriptively .

RESULTS AND DISCUSSION

Parameter Measurements

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, large intestine) are presented in Table 1. Calculation of each parameter Three villi were performed (height of villi, width of villi apex, basal width of villi, area of villi, depth of crypt) and nine gland diameters on histopathological preparations in each digestive tract organ (duodenum, jejunum, ileum, large intestine) in each treatment. There were three replications for each organ, so that the total for each organ from each treatment was obtained an average calculation of the nine villi and twenty-seven glands.

Based on Table 1, it is known that the treatment of giving black cumin (*Nigella sativa*) to the digestive organs of broilers through drinking water resulted in an increase in the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the diameter of the glands compared to the control (P0). Treatment P1 with a dose of 36 mg / kg of broiler body weight gave the highest effect on the increase in the average size of the villi height, villi apex width, basal villi width, villi area, and gland diameter in the broiler digestive tract organs, namely the small intestine (duodenum, jejunum, ileum) and large intestine.

Table 1. Average measurement of each parameter in each treatment, giving Black Cumin (*Nigella sativa*) to the digestive organs of broilers through drinking water (in milimeter)

	Villi Height	Basal Villi Width	Apex Villi Width	Vili Size	Crypt Depth	Gland Diameter
Duodenum						
P0	0.5438	0.1716	0.0696	0.0616	0.2872	0.0497
P1	0.8781	0.2489	0.1449	0.1746	0.2728	0.0576
P2	0.7096	0.2251	0.0979	0.1161	0.3312	0.0523
P3	0.6446	0.1498	0.0838	0.0757	0.2473	0.0513
Jejenum						
P0	0.3789	0.1571	0.1439	0.0540	0.2366	0.0561
P1	0.8741	0.1836	0.1325	0.1264	0.2651	0.0611
P2	0.5516	0.1055	0.0448	0.0422	0.1910	0.0560
P3	0.3423	0.1480	0.0864	0.0423	0.2312	0.0529
Ileum						

P0	0.2890	0.1916	0.1060	0.0454	0.1781	0.0511
P1	0.4572	0.2448	0.1623	0.0910	0.1743	0.0529
P2	0.3256	0.1371	0.0923	0.0370	0.1314	0.0461
P3	0.4234	0.1554	0.0656	0.0454	0.1403	0.0500
Large Intestinum						
P0	0.1914	0.1736	0.0666	0.0231	0.2051	0.0493
P1	0.3126	0.1936	0.1174	0.0493	0.1571	0.0511
P2	0.2266	0.1185	0.0510	0.0190	0.1381	0.0500
P3	0.2592	0.1328	0.0649	0.0251	0.1288	0.0602

P0 (drinking water without Black Cumin (*Nigella sativa*));

P1 (drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day);

P2 (drinking water with Black Cumin *(Nigella sativa*) 72 mg/kg BW/day);

P3 (drinking water with Black Cumin *(Nigellasativa)*144 mg/kg BW/day)

Highlight text (highest measurement for each treatment).

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) and also influenced by the height and surface area of the villi organs. digestive tract (Sugito et al., 2007; Ibrahim 2008). These villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). 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).

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 height of the villi can expand the absorption area of the villi. According to Asmawati (2014), the wider the villi the more food substances that will be absorbed in the end can have an impact on the growth of the body's organs and according to Rahmawati (2016) the higher the size of the villi, the wider the area of nutrient absorption by the small intestine wall so that it will trigger Increased growth, according to Guyton (1997), the more villous surface area indicates the more efficient absorption of nutrients that occurs. Efficiency of nutrient absorption cannot be separated from the work of hormonal, nervous and digestive glands in the digestive tract and its accessory glands.

Food, environment, and metabolic activity affects the number of intestinal glands. Chickens generally eat food consisting of granules and are hard, so that a more active secretion of intestinal glands is needed, to support the development of epithelial cells that make up the villi (Mardhiah, 1991). Crypts 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 black cumin in drinking water showed an increase in the size of the gland diameter in each organ teruta. However, in P1 treatment with the highest

increase 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.

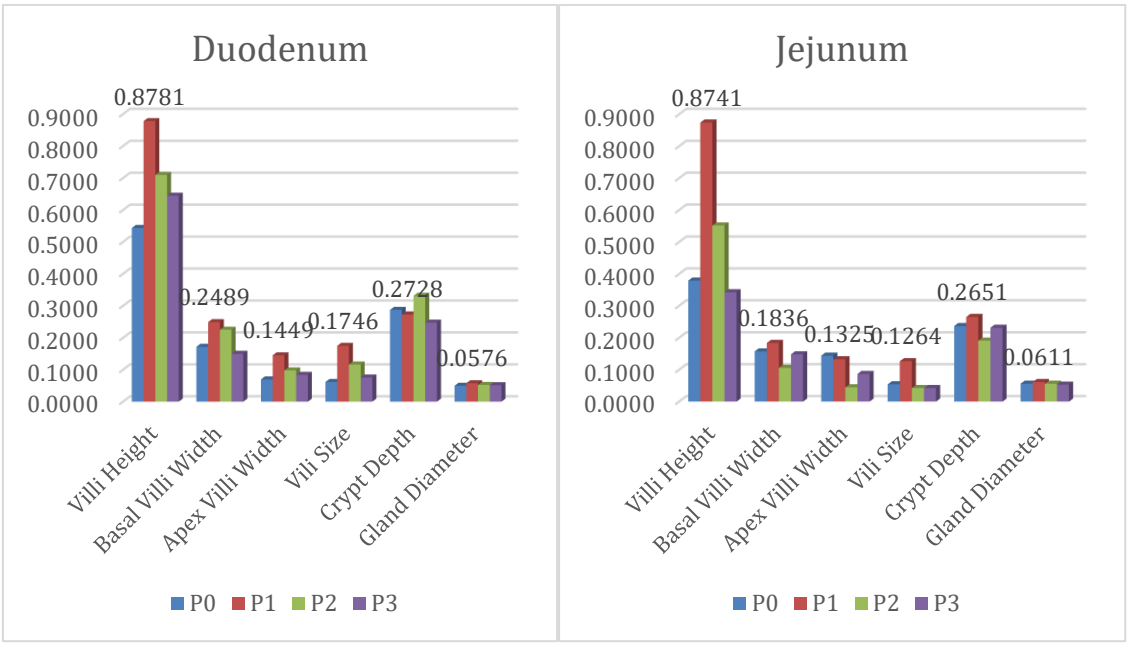
The highest mean measurement results for the depth of crypto varied in each treatment in each organ (Table 1). According to Sun et al. (2005) and Smirnov et al. (2005) that into crypto has no effect after broilers are more than 28 days old. Broilers in this study collected samples of digestive tract organs 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 Figure 1, supplementation of Black Cumin (*Nigella sativa*) could increase the size of the research parameters, namely the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the gland diameter of all digestive tract organs compared to controls. P1 treatment with a dose of 36 mg/kg of broiler body weight had the highest effect on increasing the size of each of these parameters.

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.

The digestive tract organs are supported by villi which are a special shape in the mucosa. The villi are finger-shaped protrusions of the mucosa and are characteristic of the small intestine. Increasing the number of villi will increase food absorption. Villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the

intestine and growth of the chicken (Sun, 2004). The increase in villi causes more villi surface area to absorb nutrients into the bloodstream (Mile *et al*. 2006; Rostinawati 2008).



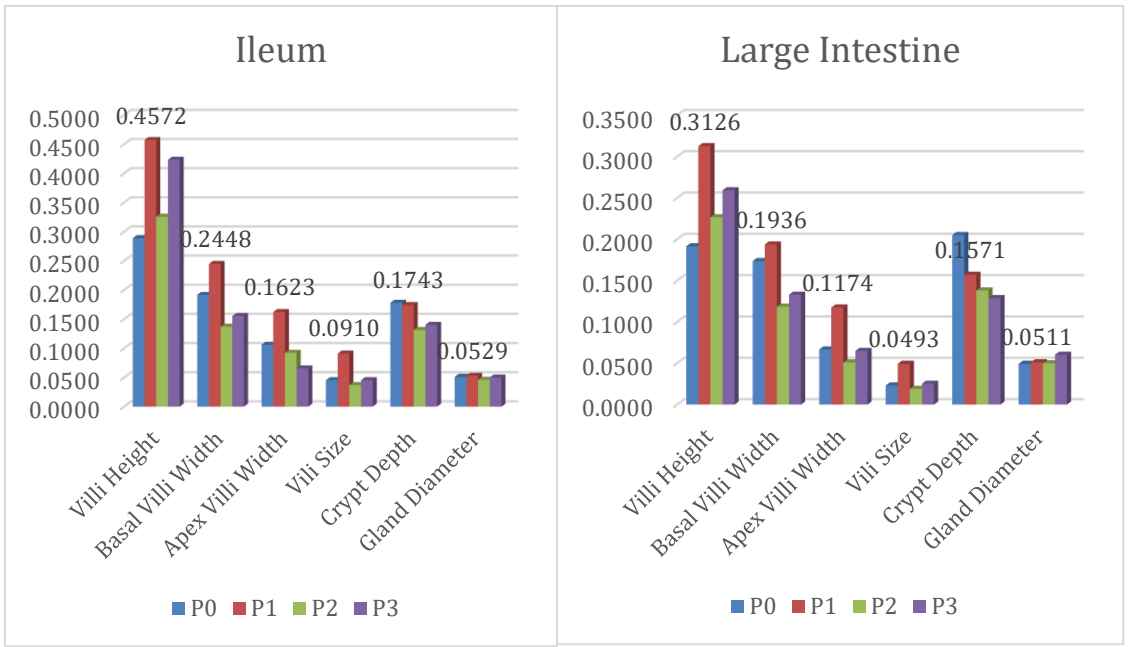


Figure 1. Drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day (P1) was the highest measurement of each research parameter in the small intestine (duodenum, jejunum, ileum) and large intestine

High villi indicate that the intestines are better off 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) states 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.

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 jejejnum. 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 simplek tubulose gland. 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 (Pfeiffer and Macpherson, 1990).

CONCLUSION

Supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increasing the average sizes of villi height, villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

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Author
2021-03-17 03:26 PM

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

Terimakasih atas kesempatan yang diberikan untuk kami dapat merevisi naskah.

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Rendy Sirat <m.mirandy@fp.unila.ac.id>

[J.KED.HEWAN] Editor Decision

2 messages

Drh. T Armansyah TR, M.Kes. <jurnal@unsyiah.ac.id> Wed, Mar 10, 2021 at 4:01 PM
To: "drh., M.Sc. Muhammad Mirandy Pratama Sirat" <m.mirandy@fp.unila.ac.id>
Cc: Madi Hartono <madi.hartono@fp.unila.ac.id>, Purnama Edy Santosa <purnama.edy@fp.unila.ac.id>, Ratna Ermawati <ratna.ermawati@fp.unila.ac.id>

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Rendy Sirat <m.mirandy@fp.unila.ac.id> Thu, Mar 11, 2021 at 1:16 PM
To: "Drh. T Armansyah TR, M.Kes." <jurnal@unsyiah.ac.id>

Kepada Yth. Editor JKH

Dengan hormat,
Teriimakasih kami ucapkan atas kesempatan yang diberikan. Kami akan segera melengkapi berdasarkan saran-saran yang telah diberikan.

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drh. Muhammad Mirandy Pratama Sirat, M.Sc.
Department of Animal Husbandry
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082226238837

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2.	drh. Filphin Adolfin Amalo, M.Sc.	Faculty of Veterinary Medicine University of Nusa Cendana	Jl. Adi Sucipto Penfui, Lasiana, Klp. Lima, Kota Kupang, Nusa Tenggara Tim.	085237940993	drh.filphin.amalo@gmail.com

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Our decision is: Revisions Required

Reviewer A: it is better if using OneWAY ANOVA statistic continue Tukey test to comparing among small intestine

Reviewer B: See in Online Journal JKH

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

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Regarding reviewers results of our article, could you help me how can I see the review results from reviewers as you said in email before.

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Reviewer B: See in Online Journal JKH

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Best regards

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Author
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Subject: EFFECT OF BLACK CUMIN (Nigella sativa) SUPPLEMENTATION THROUGH DELETE DRINKING WATER ON THE HISTOLOGY OF SMALL INTESTINE AND LARGE INTESTINE OF BROILER

Dear Mr. Tongku

I have been submitted revision of our article and have adjusted it according to the reviewers' suggestions.

Thank you for your attention and cooperation

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[J.KED.HEWAN] Editor Decision

1 message

Drh. T Armansyah TR, M.Kes. <jurnal@unsyiah.ac.id> Fri, Apr 16, 2021 at 2:31 PM
To: "drh., M.Sc. Muhammad Mirandy Pratama Sirat" <m.mirandy@fp.unila.ac.id>
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EFFECT OF BLACK CUMIN (*Nigella sativa*) SUPPLEMENTATION THROUGH DRINKING WATER ON THE HISTOLOGY OF SMALL INTESTINE AND LARGE INTESTINE OF BROILER

Authors

ABSTRACT

This study aimed to determine the effect of Black Cumin (*Nigella sativa*) supplementation through drinking water on the histology of small intestine and large intestine of broiler. The research was conducted from April September 2020 in cage facility of Integrated Field Laboratory, Faculty of Agriculture, University of Lampung. This research was using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) with a total of 60 male broilers. The treatment 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 necropsed at 31st days old, and samples of the small intestine (duodenum, jejunum, 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 analyze descriptively. The conclusion of this study was the supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increase the average sizes of villi height, villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

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descriptively but the results are quantitative variables including villi height, width

Please write the method for histomorphometry measurement of villi

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Key words : black cumin, broiler, Leica DM500 microscope, large intestine, small intestine.

ABSTRAK

Penelitian ini bertujuan untuk 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 (RAL) 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 BB/hari (P1); 72 mg/kg BB/hari (P2); dan 144 mg/kg BB/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. Analsis hasil dilakukan secara deskriptif. Kesimpulan penelitian ini yaitu suplementasi Jintan Hitam (Nigella sativa) 36 mg/kg BB/hari meningkatkan rata-rata ukuran tinggi vili, lebar apeks vili, lebar basal vili, luas vili, dan diameter kelenjar pada organ saluran pencernaan yaitu usus halus (duodenum, jejunum, ileum) dan usus besar broiler.

Kata kunci : broiler, jintan hitam, mikroskop Leica DM500, usus besar, usus halus.

INTRODUCTION

The development of poultry farming in Indonesia is increasing. Broilers are one of the fastest growing poultry. The relatively short period of time and the relatively lower maintenance costs compared to ruminants make breeders prefer to cultivate broilers. In 2018, the broiler population in Indonesia reached 1.89 billion heads and an increase of 2.26% from the broiler population in 2017 of 1.85 billion heads (BPS, 2019).

Disease is a serious obstacle in the broiler farming industry. The high incidence of disease can cause a decrease in productivity and even death of livestock which causes significant losses for breeders. Administration of antibiotics in the livestock industry is used for the treatment of livestock so as to reduce the risk of death and restore the condition of the livestock to health, however, giving antibiotics for a long period of time can cause residual buildup which has negative effects if consumed by humans.

The use of antibiotics needs to be reduced to prevent negative effects by providing natural ingredients as immunomodulators. Immunomodulators can be defined as biological or synthetic substances that can stimulate the innate immune system, adaptive or both. One of the herbs that can act as an immunomodulator is Black Cumin (*Nigella sativa*). *Nigella sativa* is a plant that has the potential as an immunostimulant that can stimulate and strengthen the system by increasing the number, quality and activity of the body's immune cells (Hendrik, 2009). *Nigella sativa* contains *thymoquinone*, saponins, zinc or zinc, *alpha-linolenic acid* (Omega 3) and *linoleic acid* (Omega 6) which functions in cell formation, maintains the immune system, and helps in the process of blood formation (Yusuf, 2014).

The solution needed for the above problems is to examine the effect of black cumin (*Nigella sativa*) supplementation through histological studies of the digestive organs of broilers which are expected to have the potential to increase the size of villi height, apex villi width,

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basal villi width, villi area, crypt depth and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine.

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MATERIALS AND METHODS

Materials

This study used broiler cages, sprayer for cage disinfection, bamboo to make 12 cage plots, plastic tarpaulin for curtains, newspaper and used husks as litter, 12 bulbs of 15 bulbs watt as a heating source for thearea. *brooding*, *a hanging feeder* 12 pieces, *chick feeder tray* 12 pieces, 12 pieces of chicken drinking places; 1 bucket, 1 *hand spray*, 1 water tray for *dipping*, 1 electric scale, *thermohygrometer* for measuring temperature and humidity, sack and plastic. Organ sampling equipment, namely necropsy equipment, *object glass*, *cover glass*, *refrigerator*, microtome, light microscope, camera technology and *software* Optilab®along with a laptop for taking tissue images and measuring the parameters of each organ.

Materials used in the study were 60 Day Old Chicks (DOC) male broiler Cobb CP 707 strains kept for 30 days, rations, drinking water, extract of Black Cumin (*Nigella sativa*), vaccines of Newcastle Disease (ND), Avian Influenza (AI) and Infectious Bursal Disease (IBD), 10% formalin solution.

Methods

This research carried out for 6 months (April - September 2020) in the Integrated Field Laboratory enclosure unit, Faculty of Agriculture, University of Lampung. This research was experimental in nature using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) so a total of 60 male broilers. The treatment dose was according to the broiler body weight, namely 1) drinking water without

black cumin (P0, control); drinking water with Jintan Hitam 36 mg / kg BW / day (P1); drinking water with Jintan Hitam 72 mg / kg BW / day (P2); and drinking water with cumin 144 mg / kg BW / day (P3). On the 31st day, three from each group were randomly necropsed and samples of the small intestine (duodenum, jejunum, ileum) and large intestine were then fixed with 10% formalin solution and sent to the Lampung Veterinary Center for making histological preparations. with Hematoxylin Eosin (HE) staining. The observation of preparations 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. Observation of histological preparations using 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 nine gland diameters in each digestive tract organ (duodenum, jejunum, ileum, large intestine). There were three replications per organ, so that the total for each organ was obtained an average of the nine villi and twenty-seven glands.

The calculation of the surface area of the intestinal villi using the method of Iji *et al.* (2001) 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.

$$\textbf{Surface area} = \frac{b + c}{2} \times a$$

Description:

a = height of intestine villi

b = width of apex of intestine villi

c = width of basal of intestine villi

Data analysis

Measurement data for various research parameters were calculated the average of all replications of each digestive tract organs (duodenum, jejunum, ileum, and large intestinum) then analyzed descriptively .

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RESULTS AND DISCUSSION

Parameter Measurements

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, large intestine) are presented in Table 1. Calculation of each parameter Three villi were performed (height of villi, width of villi apex, basal width of villi, area of villi, depth of crypt) and nine gland diameters on histopathological preparations in each digestive tract organ (duodenum, jejunum, ileum, large intestine) in each treatment. There were three replications for each organ, so that the total for each organ from each treatment was obtained an average calculation of the nine villi and twenty-seven glands.

Based on Table 1, it is known that the treatment of giving black cumin (*Nigella sativa*) to the digestive organs of broilers through drinking water resulted in an increase in the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the diameter of the glands compared to the control (P0). Treatment P1 with a dose of 36 mg / kg of broiler body weight gave the highest effect on the increase in the average size of the villi height, villi apex width, basal villi width, villi area, and gland diameter in the broiler digestive tract organs, namely the small intestine (duodenum, jejunum, ileum) and large intestine.

153 Table 1. Average measurement of each parameter in each treatment, giving Black Cumin
154 (*Nigella sativa*) to the digestive organs of broilers through drinking water (in
155 milimeter)

	Villi Height	Basal Villi Width	Apex Villi Width	Vili Size	Crypt Depth	Gland Diameter
Duodenum						
P0	0.5438	0.1716	0.0696	0.0616	0.2872	0.0497
P1	0.8781	0.2489	0.1449	0.1746	0.2728	0.0576
P2	0.7096	0.2251	0.0979	0.1161	0.3312	0.0523
P3	0.6446	0.1498	0.0838	0.0757	0.2473	0.0513
Jejenum						
P0	0.3789	0.1571	0.1439	0.0540	0.2366	0.0561
P1	0.8741	0.1836	0.1325	0.1264	0.2651	0.0611
P2	0.5516	0.1055	0.0448	0.0422	0.1910	0.0560
P3	0.3423	0.1480	0.0864	0.0423	0.2312	0.0529
Ileum						
P0	0.2890	0.1916	0.1060	0.0454	0.1781	0.0511
P1	0.4572	0.2448	0.1623	0.0910	0.1743	0.0529
P2	0.3256	0.1371	0.0923	0.0370	0.1314	0.0461
P3	0.4234	0.1554	0.0656	0.0454	0.1403	0.0500
Large Intestinum						
P0	0.1914	0.1736	0.0666	0.0231	0.2051	0.0493
P1	0.3126	0.1936	0.1174	0.0493	0.1571	0.0511

Commented [a110]: Please add ± Standar deviation (ad is it better used micrometer

P2	0.2266	0.1185	0.0510	0.0190	0.1381	0.0500
P3	0.2592	0.1328	0.0649	0.0251	0.1288	0.0602

P0 (drinking water without Black Cumin (*Nigella sativa*));
P1 (drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day);
P2 (drinking water with Black Cumin *(Nigella sativa*) 72 mg/kg BW/day);
P3 (drinking water with Black Cumin *(Nigellasativa)*144 mg/kg BW/day)
Highlight text (highest measurement for each treatment).

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) and also influenced by the height and surface area of the villi organs. digestive tract (Sugito et al., 2007; Ibrahim 2008). These villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). 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).

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 height of the villi can expand the absorption area of the villi.

According to Asmawati (2014), the wider the villi the more food substances that will be absorbed in the end can have an impact on the growth of the body's organs and according to Rahmawati (2016) the higher the size of the villi, the wider the area of nutrient absorption by the small intestine wall so that it will trigger Increased growth, according to Guyton (1997), the more villous surface area indicates the more efficient absorption of nutrients that occurs. Efficiency of nutrient absorption cannot be separated from the work of hormonal, nervous and digestive glands in the digestive tract and its accessory glands.

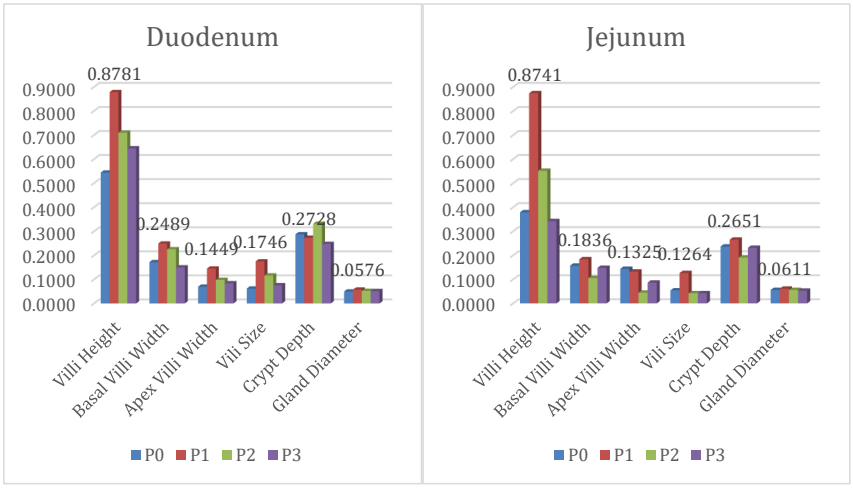
Food, environment, and metabolic activity affects the number of intestinal glands. Chickens generally eat food consisting of granules and are hard, so that a more active secretion of intestinal glands is needed, to support the development of epithelial cells that make up the villi (Mardhiah, 1991). Crypts 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 black cumin in drinking water showed an increase in the size of the gland diameter in each organ teruta. However, in P1 treatment with the highest increase 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.

The highest mean measurement results for the depth of crypto varied in each treatment in each organ (Table 1). According to Sun et al. (2005) and Smirnov et al. (2005) that into crypto has no effect after broilers are more than 28 days old. Broilers in this study collected samples of digestive tract organs 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 Figure 1, supplementation of Black Cumin (*Nigella sativa*) could increase the size of the research parameters, namely the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the gland diameter of all digestive tract organs compared to controls. P1 treatment with a dose of 36 mg/kg of broiler body weight had the highest effect on increasing the size of each of these parameters.

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.

The digestive tract organs are supported by villi which are a special shape in the mucosa. The villi are finger-shaped protrusions of the mucosa and are characteristic of the small intestine. Increasing the number of villi will increase food absorption. Villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). The increase in villi causes more villi surface area to absorb nutrients into the bloodstream (Mile *et al*. 2006; Rostinawati 2008).



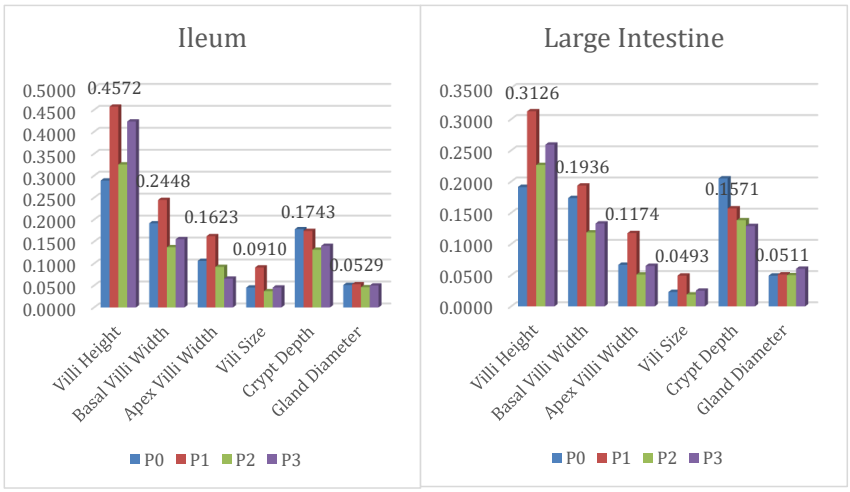


Figure 1. Drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day (P1) was the highest measurement of each research parameter in the small intestine (duodenum, jejunum, ileum) and large intestine

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High villi indicate that the intestines are better off 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) states 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.

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 jejejnum. 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 simplek tubulose gland. 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 (Pfeiffer and Macpherson, 1990).

CONCLUSION

Supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increasing the average sizes of villi height, villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

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

Authors

ABSTRACT

This study aimed to determine the effect of Black Cumin (*Nigella sativa*) supplementation through drinking water on the histology of small intestine and large intestine of broiler. The research was conducted from April September 2020 in cage facility of Integrated Field Laboratory, Faculty of Agriculture, University of Lampung. This research was using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) with a total of 60 male broilers. The treatment 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 necropsed at 31st days old, and samples of the small intestine (duodenum, jejunum, 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 analyze descriptively. The conclusion of this study was the supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increase the average sizes of villi height,

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villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

Keywords :black cumin, broiler, Leica DM500 microscope, large intestine, small intestine.

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ABSTRAK

Penelitianinibertujuanuntukmengetahuipengaruhpengaruhsuplementasi JintanHitam (Nigella sativa) melalui air minumterhadaphistologi usus halus dan usus besar broiler. Penelitiandilakukanpada April-September 2020 di unit kandangLaboratoriumLapangTerpadu, FakultasPertanian, Universitas Lampung. PenelitianbersifateksperimentalmenggunakanRancanganAcakLengkap (RAL) denganempatkelompokperlakuan dan tigaulangan (lima ekortiapulangan) sehingga total 60 ekor broiler jantan. Perlakuan yang diberikanyaitupemberianair minumtanpaJintanHitam (P0, kontrol); air minumdenganJintanHitam 36 mg/kg BB/hari (P1); 72 mg/kg BB/hari(P2); dan 144 mg/kg BB/hari(P3). Tigaekordaritiapkelompoksecaraacakdinekropsipada hari ke-31 dan diambilsampel organ usus halus (duodenum, jejunum, ileum) dan usus besar (colon) kemudiandifiksasidenganlarutan formalin 10% dan dikirimkeBalaiVeteriner Lampung untukpembuatanpreparathistologi.

PengamatanpreparatdilakukansecaramikroskopismenggunakanMikroskopBinokuler Leica DM500® untukmenghitungberbagaiukuran parameter secaraakurat. Analsishasildilakukansecaradeskriptif. Kesimpulan penelitianiniyaitusuplementasiJintanHitam (Nigella sativa)36 mg/kg BB/harimeningkatkan rata-rata ukurantinggivili, lebarapeksvili, lebar basal vili, luasvili, dan diameter kelenjar

pada organ saluranpencernaanyaitu usus halus (duodenum, jejunum, ileum) dan usus besar broiler.

Kata kunci :broiler, jintanhitam, mikroskopLeica DM500, usus besar, usus halus.

INTRODUCTION

The development of poultry farming in Indonesia is increasing. Broilers are one of the fastest growing poultry. The relatively short period of time and the relatively lower maintenance costs compared to ruminants make breeders prefer to cultivate broilers. In 2018, the broiler population in Indonesia reached 1.89 billion heads and an increase of 2.26% from the broiler population in 2017 of 1.85 billion heads (BPS, 2019).

Disease is a serious obstacle in the broiler farming industry. The high incidence of disease can cause a decrease in productivity and even death of livestock which causes significant losses for breeders. Administration of antibiotics in the livestock industry is used for the treatment of livestock so as to reduce the risk of death and restore the condition of the livestock to health, however, giving antibiotics for a long period of time can cause residual buildup which has negative effects if consumed by humans.

The use of antibiotics needs to be reduced to prevent negative effects by providing natural ingredients as immunomodulators. Immunomodulators can be defined as biological or synthetic substances that can stimulate the innate immune system, adaptive or both. One of the herbs that can act as an immunomodulator is Black Cumin (*Nigella sativa*). *Nigella sativa* is a plant that has the potential as an immunostimulant that can stimulate and strengthen the system by increasing the number, quality and activity of the body's immune

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cells (Hendrik, 2009). *Nigella sativa* contains *thymoquinone*, saponins, zinc or zinc, *alpha-linolenic acid* (Omega 3) and *linoleic acid* (Omega 6) which functions in cell formation, maintains the immune system, and helps in the process of blood formation (Yusuf, 2014).

The solution needed for the above problems is to examine the effect of black cumin (*Nigella sativa*) supplementation through histological studies of the digestive organs of broilers which are expected to have the potential to increase the size of villi height, apex villi width, basal villi width, villi area, crypt depth and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine (colon).

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MATERIALS AND METHODS

Materials

This study used broiler cages, sprayer for cage disinfection, bamboo to make 12 cage plots, plastic tarpaulin for curtains, newspaper and used husks as litter, 12 bulbs of 15bulbs watt as a heating source for thearea. *brooding*, *a hanging feeder* 12 pieces, *chick feeder tray* 12 pieces, 12 pieces of chicken drinking places; 1 bucket, 1 *hand spray*, 1 water tray for *dipping*, 1 electric scale, *thermohygrometer* for measuring temperature and humidity, sack and plastic. Organ sampling equipment, namely necropsy equipment, *object glass*, *cover glass*, *refrigerator*, microtome, light microscope, camera technology and *software*Optilab®along with a laptop for taking tissue images and measuring the parameters of each organ.

Materials used in the study were 60 Day Old Chicks (DOC) male broiler Cobb CP 707 strains kept for 30 days, rations, drinking water, extract of Black Cumin (*Nigella sativa*), vaccines of Newcastle Disease (ND), Avian Influenza (AI) and Infectious Bursal Disease(IBD), 10% formalin solution.

99

100 **Methods**

101 This research carried out for 6 months (April - September 2020) in the Integrated Field
102 Laboratory enclosure unit, Faculty of Agriculture, University of Lampung. This research was
103 experimental in nature using a completely randomized design (CRD) with four treatment
104 groups and three replications (five heads per replication) so a total of 60 male broilers. The
105 treatment dose was according to the broiler body weight, namely 1) drinking water without
106 black cumin (P0, control); drinking water with JintanHitam 36 mg / kg BW / day (P1);
107 drinking water with JintanHitam 72 mg / kg BW / day (P2); and drinking water with cumin
108 144 mg / kg BW / day (P3). On the 31st day, three from each group were randomly necropsed
109 and samples of the small intestine (duodenum, jejunum, ileum) and large intestine were then
110 fixed with 10% formalin solution and sent to the Lampung Veterinary Center for making
111 histological preparations. with Hematoxylin Eosin (HE) staining. The observation of
112 preparations was carried out microscopically using the Leica DM500® Binocular Microscope
113 Technology to accurately calculate various parameter sizes.

114 The research parameters were villi height, villi apex width, basal villi width, villi area,
115 crypt depth and gland diameter. Observation of histological preparations using 10x
116 magnification objective lens. The calculation of each parameter was carried out as many as
117 three villi (villiheight, villi apex width, basal villi width, villi area, crypt depth) and nine
118 gland diameters in each digestive tract organ (duodenum, jejunum, ileum, large intestine).
119 There were three replications per organ, so that the total for each organ was obtained an
120 average of the nine villi and twenty-seven glands.

121 The calculation of the surface area of the intestinal villi using the method of Iji*et al.*
122 (2001) modified with the assumption that the villi model is an analogue of the trapezium
123 shape so that the average number of the apical widths of the villi plus the average basal width

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of the villi is divided by two then multiplied by the height of the villi by the following formula.

Surface area= $\frac{b + c}{2} xa$

Description:

a = height of intestine villi

b = width of apex of intestine villi

c = width of basal of intestine villi

Data analysis

Measurement data for various research parameters were calculated the average of all replications of each digestive tract organs (duodenum, jejunum, ileum, and large intestinum) then analyzed descriptively .

RESULTS AND DISCUSSION

Parameter Measurements

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, large intestine) are presented in Table 1. Calculation of each parameter Three villi were performed (height of villi, width of villi apex, basal width of villi, area of villi, depth of crypt) and nine gland diameters on histopathological preparations in each digestive tract organ (duodenum, jejunum, ileum, large intestine) in each treatment. There were three replications for each organ, so that the total for each organ from each treatment was obtained an average calculation of the nine villi and twenty-seven glands.

Based on Table 1, it is known that the treatment of giving black cumin (*Nigella sativa*) to the digestive organs of broilers through drinking water resulted in an increase in the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the diameter of the glands compared to the control (P0). Treatment P1 with a dose of 36 mg / kg of broiler body weight gave the highest effect on the increase in the average size of the villi height, villi apex width, basal villi width, villi area, and gland diameter in the broiler digestive tract organs, namely the small intestine (duodenum, jejunum, ileum) and large intestine.

Table 1. Average measurement of each parameter in each treatment, giving Black Cumin (*Nigella sativa*) to the digestive organs of broilers through drinking water (in milimeter)

Commented [A14]: The average

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	Villi Height	Basal Villi Width	Apex Villi Width	Vili Size	Crypt Depth	Gland Diameter
Duodenum						
P0	0.5438	0.1716	0.0696	0.0616	0.2872	0.0497
P1	0.8781	0.2489	0.1449	0.1746	0.2728	0.0576
P2	0.7096	0.2251	0.0979	0.1161	0.3312	0.0523
P3	0.6446	0.1498	0.0838	0.0757	0.2473	0.0513
Jejenum						
P0	0.3789	0.1571	0.1439	0.0540	0.2366	0.0561
P1	0.8741	0.1836	0.1325	0.1264	0.2651	0.0611
P2	0.5516	0.1055	0.0448	0.0422	0.1910	0.0560
P3	0.3423	0.1480	0.0864	0.0423	0.2312	0.0529

Ileum						
P0	0.2890	0.1916	0.1060	0.0454	0.1781	0.0511
P1	0.4572	0.2448	0.1623	0.0910	0.1743	0.0529
P2	0.3256	0.1371	0.0923	0.0370	0.1314	0.0461
P3	0.4234	0.1554	0.0656	0.0454	0.1403	0.0500
Large Intestinum						
P0	0.1914	0.1736	0.0666	0.0231	0.2051	0.0493
P1	0.3126	0.1936	0.1174	0.0493	0.1571	0.0511
P2	0.2266	0.1185	0.0510	0.0190	0.1381	0.0500
P3	0.2592	0.1328	0.0649	0.0251	0.1288	0.0602

P0 (drinking water without Black Cumin (*Nigella sativa*));

P1 (drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day);

P2 (drinking water with Black Cumin *(Nigellasativa*) 72 mg/kg BW/day);

P3 (drinking water with Black Cumin *(Nigellasativa)*144 mg/kg BW/day)

Highlight text (highest measurement for each treatment).

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) and also influenced by the height and surface area of the villi organs digestive tract (Sugito et al., 2007; Ibrahim 2008). These villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). 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).

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 height of the villi can expand the absorption area of the villi. According to Asmawati (2014), the wider the villi the more food substances that will be absorbed in the end can have an impact on the growth of the body's organs and according to Rahmawati (2016) the higher the size of the villi, the wider the area of nutrient absorption by the small intestine wall so that it will trigger Increased growth, according to Guyton (1997), the more villous surface area indicates the more efficient absorption of nutrients that occurs. Efficiency of nutrient absorption cannot be separated from the work of hormonal, nervous and digestive glands in the digestive tract and its accessory glands.

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Food, environment, and metabolic activity affects the number of intestinal glands. Chickens generally eat food consisting of granules and are hard, so that a more active secretion of intestinal glands is needed, to support the development of epithelial cells that make up the villi (Mardhiah, 1991). Crypts 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 black cumin in drinking water showed an increase in the size of the gland diameter in each organ teruta. However, in P1

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treatment with the highest increase 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.

The highest mean measurement results for the depth of crypto varied in each treatment in each organ (Table 1). According to Sun et al. (2005) and Smirnov et al. (2005) that into crypto has no effect after broilers are more than 28 days old. Broilers in this study collected samples of digestive tract organs 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 Figure 1, supplementation of Black Cumin (*Nigella sativa*)could increase the size of the research parameters, namely the size of the villi height, the width of the villi apex, the basal width of the villi, the area of the villi, and the gland diameter of all digestive tract organs compared to controls. P1 treatment with a dose of 36 mg/kg of broiler body weight had the highest effect on increasing the size of each of these parameters.

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.

The digestive tract organs are supported by villi which are a special shape in the mucosa. The villi are finger-shaped protrusions of the mucosa and are characteristic of the small intestine. Increasing the number of villi will increase food absorption. Villi function to expand the surface of the intestine which affects the process of absorption of food

(Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). The increase in villi causes more villi surface area to absorb nutrients into the bloodstream (Mile *et al*. 2006; Rostinawati 2008).

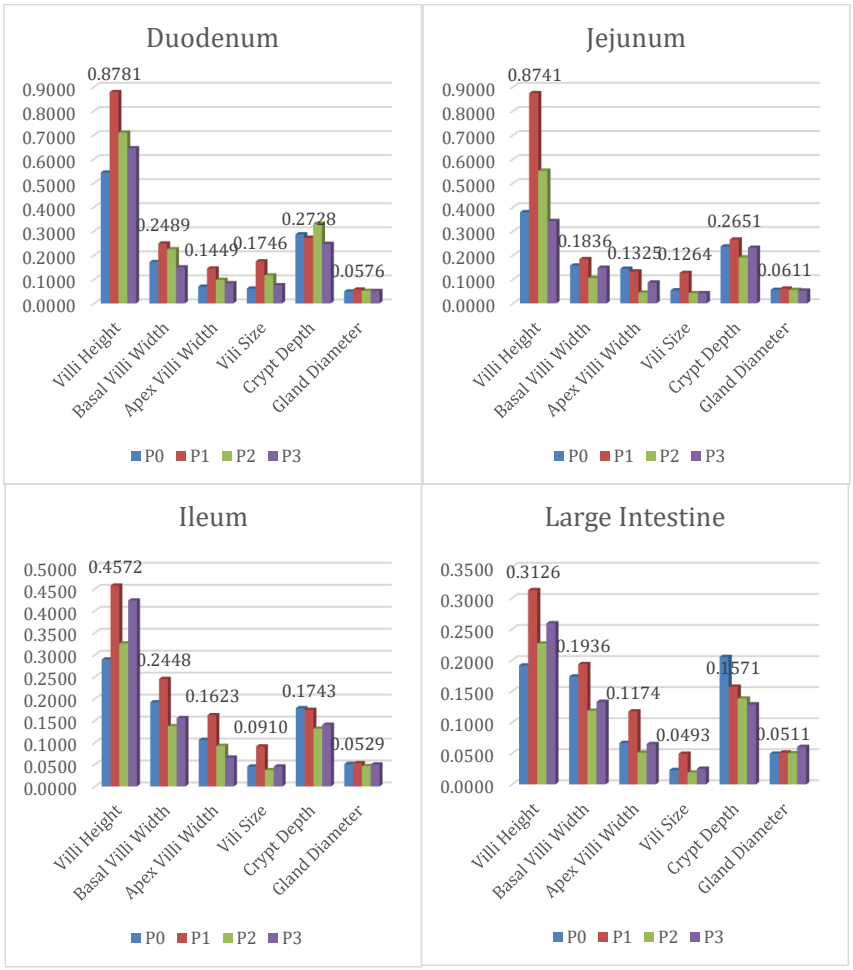

Figure 1. Drinking water with Black Cumin (*Nigella sativa*) 36 mg/kg BW/day (P1) was the highest measurement of each research parameter in the small intestine

(duodenum, jejunum, ileum) and large intestine

High villi indicate that the intestines are better off 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) states 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.

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 jejejnum. One of the parameters used to measure the quality of growth is the intestinal morphological structure (Wang and Peng, 2008).

Commented [A18]: jejunum

The intestinal gland (Lieberkuhn's gland) has a small hole that becomes the mouth of the simplektubulose gland. 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 (Pfeiffer and Macpherson, 1990).

CONCLUSION

Supplementation of Black Cumin 36 mg/kg BW/day through drinking water could increasing the average sizes of villi height, villi apex width, basal villi width, villi area, and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine of broiler.

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Commented [A20]: Pustaka yang belum ada :
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3.Guyton, 1997
4.Mardhiah, 1991
5.Aughey & Friye, 2001
6.Wang et al, 2008
7.Ningtias, 2013
8.Rofiq, 2003
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EFFECT OF BLACK CUMIN (*Nigella sativa*) SUPPLEMENTATION THROUGH DRINKING WATER ON THE HISTOLOGY OF SMALL INTESTINE AND LARGE INTESTINE OF BROILER

Authors

ABSTRACT

This study aimed to determine the effect of Black Cumin (*Nigella sativa*) supplementation through drinking water on the histology of small intestine and large intestine of broiler. The research was conducted from April September 2020 in cage facility of Integrated Field Laboratory, Faculty of Agriculture, University of Lampung. This research was using a completely randomized design with four treatment groups and three replications (five heads 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 31st days old, and samples of the small intestine (duodenum, jejunum, 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 significant then continue with Tukey test. The conclusions of this study were the supplementation of Black Cumin (*Nigella sativa*) 72 mg/kg BW/day through drinking water could increase significantly ($P<0.05$) to 1) the average sizes of villi height and villi area of small intestine (duodenum, jejunum, ileum) and large intestine

(colon) of broiler; 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 widht of broiler colon.

Keywords : Broiler, Histology, Large intestine, *Nigella sativa*, Small intestine.

ABSTRAK

Penelitian ini bertujuan untuk 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 BB/hari (P1); 72 mg/kg BB/hari (P2); dan 144 mg/kg BB/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. Analsis hasil dilakukan secara statistik menggunakan analisis sidik ragam satu arah dengan taraf signifikansi 5% dan jika hasilnya signifikan maka dilanjutkan dengan uji lanjut Tukey. Kesimpulan penelitian ini yaitu suplementasi Jintan Hitam (Nigella sativa) 72 mg/kg BB/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.

Kata kunci : Broiler, Histologi, Nigella sativa, Usus besar, Usus halus.

INTRODUCTION

The development of poultry farming in Indonesia is increasing. Broilers are one of the fastest growing poultry. The relatively short period of time and the relatively lower maintenance costs compared to ruminants make breeders prefer to farm broilers. In 2018, the broiler population in Indonesia reached 1.89 billion heads and an increase of 2.26% from the broiler population in 2017 of 1.85 billion heads (BPS, 2019).

Intensive maintenance of broiler can make it easy to experience stress, resulting in a decrease in the ability of the immune system, it will be easy to contract various kinds of diseases. The disease is a serious obstacle in the broiler farming industry. The high incidence of disease can cause a decrease in productivity and even death of livestock which causes significant losses for breeders. The diseases can lead to decrease in the function of body organs, one of which is the digestive organ and the administration of antibiotics for a long period of time can cause residual buildup, antibiotic resistance in bacteria and could be has negative effects if consumed by humans (Marshall and Levy, 2011). One of effort to maintain the quality of the digestive tract to function properly and to reduce the residual of antibiotics in broiler by providing natural herbs such as Black cumin (*Nigella sativa*) that is believed can improve organ function digestion such as research that has been carried out in mice (Rostika, 2012).

This study was conducted to determine the effect of black cumin supplementation through histological studies of the digestive organs of broilers which are expected to have the potential to increase the size of villi height, apex villi width, basal villi width, villi area, crypt depth and gland diameter of small intestine (duodenum, jejunum, ileum) and large intestine (colon).

MATERIALS AND METHODS

Materials

This study used broiler cages, sprayer for cage disinfection, bamboo to make 12 cage plots, plastic tarpaulin for curtains, newspaper and used husks as litter, 12 bulbs of 15 bulbs watt as a heating source for the area brooding, 12 pieces hanging feeder, 12 pieces chick feeder tray, 12 pieces of chicken drinking places; 1 bucket, 1 hand spray, 1 water tray for dipping, 1 electric scale, thermohygrometer for measuring temperature and humidity, sack and plastic. Organ sampling equipments, namely necropsy equipment, object glass, cover glass, refrigerator, microtome, Leica DM500® Binocular Microscope Technology connected with a computer for taking tissue images and measuring the parameters of each organ.

Materials used in the study were 60 Day Old Chicks (DOC) male broiler Cobb CP 707 strains kept for 30 days, rations, drinking water, extract of Black Cumin (*Nigella sativa*), vaccines of Newcastle Disease (ND), Avian Influenza (AI) and Infectious Bursal Disease (IBD), 10% formalin solution.

Methods

This research was carried out for 6 months (April - September 2020) in the Integrated Field Laboratory enclosure unit, Faculty of Agriculture, University of Lampung. This research

was experimental in nature using a completely randomized design (CRD) with four treatment groups and three replications (five heads per replication) so a total of 60 male broilers. The treatment dose was according to the broiler body weight, namely 1) drinking water without black cumin (P0, control); drinking water with Jintan Hitam 36 mg / kg BW / day (P1); drinking water with Jintan Hitam 72 mg / kg BW / day (P2); and drinking water with cumin 144 mg / kg BW / day (P3). On the 31st day, three from each group were randomly necropsied 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 making histological preparations. with Hematoxylin Eosin (HE) staining. The observation of preparations 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. Observation of histological preparations using 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, colon). There were three replications per organ, so that the total for each organ was obtained 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) 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.

$$\textbf{Surface area} = \frac{\mathbf{b + c}}{\mathbf{2}} \times \mathbf{a}$$

Description:

a = height of intestine villi

b = width of apex of intestine villi

c = width of basal of intestine villi

Data analysis

Measurement data for various research parameters were calculated the average of all replications of each digestive tract organs (duodenum, jejunum, ileum, and colon) then analyzed with one way *Analysis of Variance* then continue with Tukey test. Different supercripts with letters in the same column indicate significant differences (P<0.05).

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) presented in each table. Calculation of each parameter nine villi were performed (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 that supplemented by black cumin (*Nigella sativa*) through drinking water presented in Table 1. Supplementation of black cumin (*Nigella sativa*) with a dose of 72 mg/kg of broiler body weight (P2) gave the significant effect (Figure 1) on the increase in the average size of villi height, basal villi width, villi apex width, villi area, and gland diameter of duodenum.

Table 1. The average measurement of each parameter in broiler duodenum supplemented by black cumin (*Nigella sativa*) 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}

Information : Drinking water without *Nigella sativa* (P0), Drinking water with *Nigella sativa* 36 mg/kg BW/day (P1), 72 mg/kg BW/day (P2), 144 mg/kg BW/day (P3); A1 (villi height of duodenum), B1 (basal villi width of duodenum), C1 (apex villi width of duodenum), D1 (vili area of duodenum), E1 (crypt depth of duodenum), F1 (gland diameter of duodenum). Different supercripts with letters in the same column indicate significant differences (P<0.05).

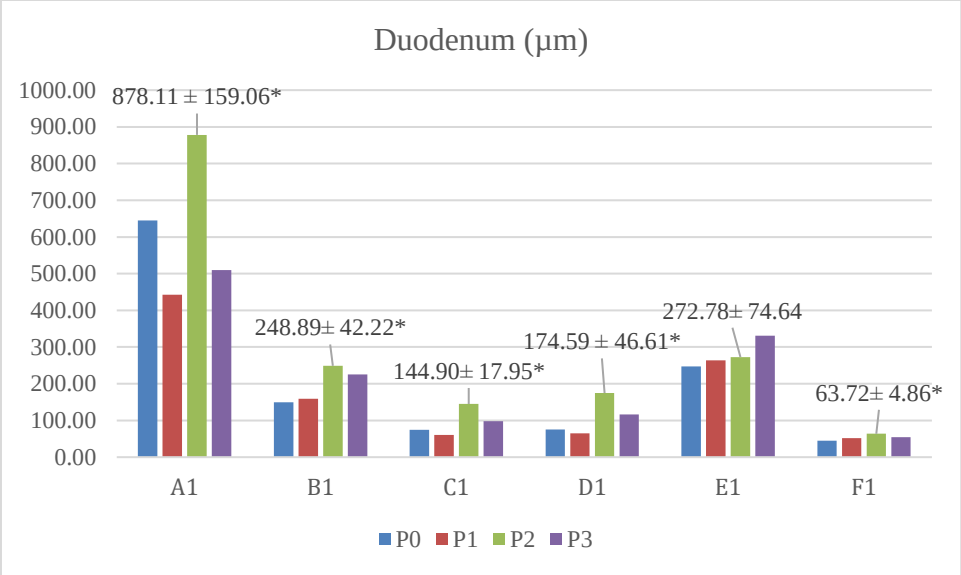

Figure 1. The supplementation of black cumin (*Nigella sativa*) 72 mg/kg BW (P2) in drinking water increasing average sizes of villi height (A1), basal villi width (B1), villi apex width (C1), villi area (D1), and gland diameter (F1) of broiler duodenum. Asterisk (*) indicates the most significant effect (P<0.05).

The average measurement of each parameter in broiler jejunum that supplemented by black cumin (*Nigella sativa*) through drinking water presented in Table 2. Supplementation of black cumin (*Nigella sativa*) with a dose of 72 mg/kg of broiler body weight (P2) gave the significant effect on the increase in the average size of villi height and villi area of jejunum.

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]

Information : Drinking water without *Nigella sativa* (P0), Drinking water with *Nigella sativa* 36 mg/kg BW/day (P1), 72 mg/kg BW/day (P2), 144 mg/kg BW/day (P3); A1 (villi height of jejunum), B1 (basal villi width of jejunum), C1 (apex villi width of jejunum), D1 (vili area of jejunum), E1 (crypt depth of jejunum), F1 (gland diameter of jejunum). Different supercripts with letters in the same column indicate significant differences (P<0.05).

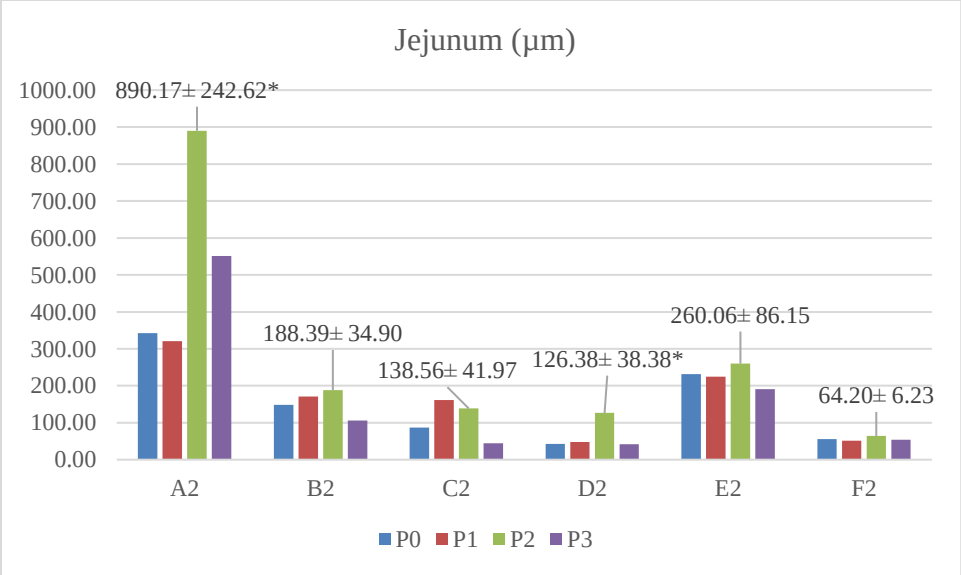

Figure 2. The supplementation of black cumin (*Nigella sativa*) 72 mg/kg BW (P2) in drinking water increase average sizes of villi height (A2) and villi area (D1) of broiler jejunum. Asterisk (*) indicates the most significant effect (P<0.05).

The average measurement of each parameter in broiler ileum that supplemented by black cumin (*Nigella sativa*) through drinking water presented in Table 3. Supplementation of black cumin (*Nigella sativa*) with a dose of 72 mg/kg of broiler body weight (P2) gave the significant effect on the increase in the average size of villi height and villi area of ileum.

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]	47.60 ± 12.7 [a]

Information : Drinking water without *Nigella sativa* (P0), Drinking water with *Nigella sativa* 36 mg/kg BW/day (P1), 72 mg/kg BW/day (P2), 144 mg/kg BW/day (P3); A1 (villi height of jejunum), B1 (basal villi width of jejunum), C1 (apex villi width of jejunum), D1 (vili area of jejunum), E1 (crypt depth of jejunum), F1 (gland diameter of jejunum). Different supercripts with letters in the same column indicate significant differences (P<0.05).

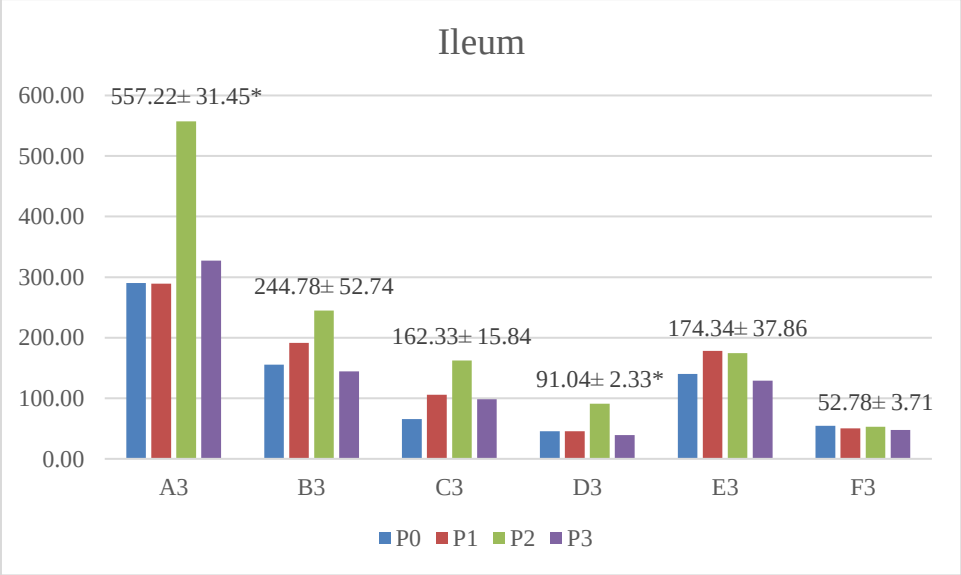

Figure 3. The supplementation of black cumin (*Nigella sativa*) 72 mg/kg BW (P2) in drinking water increase average sizes of villi height (A2) and villi area (D1) of broiler ileum. Asterisk (*) indicates the most significant effect (P<0.05).

The average measurement of each parameter in broiler colon that supplemented by black cumin (*Nigella sativa*) through drinking water presented in Table 4. Supplementation of black cumin (*Nigella sativa*) with a dose of 72 mg/kg of broiler body weight (P2) gave the significant effect on the increase in the average size of villi height, apex villi widht, villi area of colon and in parameter of crypt depth showed that dose of P1 (36 mg/kg BW) had a significant effect but was not different from the dose of P2.

Table 4. 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}

Information : Drinking water without *Nigella sativa* (P0), Drinking water with *Nigella sativa* 36 mg/kg BW/day (P1), 72 mg/kg BW/day (P2), 144 mg/kg BW/day (P3); A1 (villi height of colon), B1 (basal villi width of colon), C1 (apex villi width of colon), D1 (vili area of colon), E1 (crypt depth of colon), F1 (gland diameter of colon). Different supercripts with letters in the same column indicate significant differences (P<0.05).

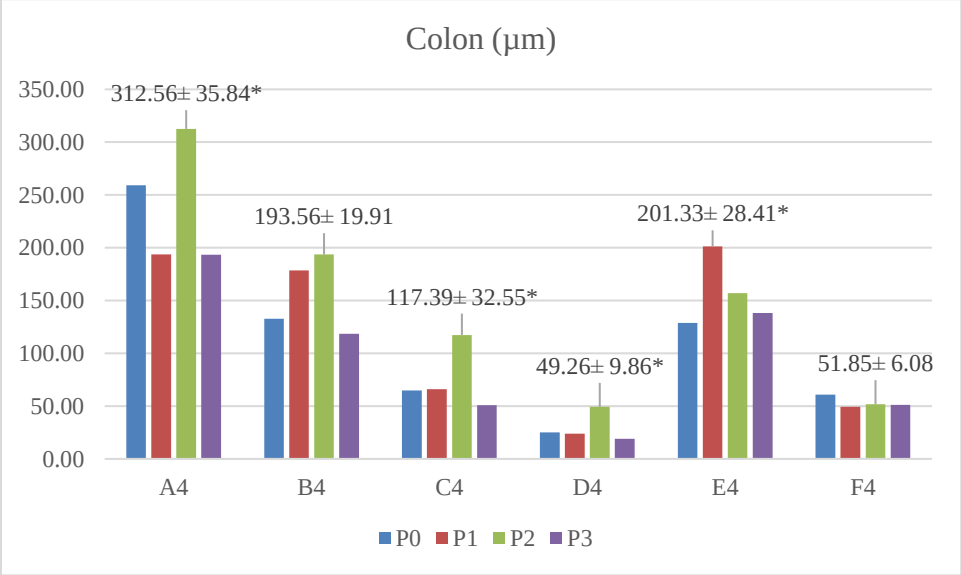

Figure 4. The supplementation of black cumin (*Nigella sativa*) 72 mg/kg BW (P2) in drinking water increasing average sizes of villi height (A1), villi apex width (C1), villi area (D1), and crypt depth (E1) of broiler colon.
Asterisk (*) indicates the most significant effect (P<0.05).

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) and also influenced by the height and surface area of the villi organs. digestive tract (Sugito et al., 2007;

Ibrahim 2008). These villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). 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) gave the most significant increase ($P<0.05$) in villi height and villi area of all broiler digestive organs (duodenum, jejununum, 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 more food substances that will be absorbed in the end can have an impact on the growth of the body's organs and according to Rahmawati (2016) the higher the size of the villi, the wider the area of nutrient absorption by the small intestine wall so that it will trigger increased growth, according to Guyton (1997), the more villous surface area indicates the more efficient absorption of nutrients that occurs. 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.

Food, environment, and metabolic activity affects the number of intestinal glands. Chickens generally eat food consisting of granules and are hard, so that a more active secretion

of intestinal glands is needed, to support the development of epithelial cells that make up the villi (Mardhiah, 1991). Crypts 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 gland diameter in duodenum. P2 treatment with the 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 (*Nigella sativa*) 36 mg/kg BW/day (P1) gave the most significant increase ($P<0.05$) in parameter of crypt depth of colon but was not different from the dose of P2 (Table 4). Supplementation of black cumin (*Nigella sativa*) had no significant effect for other organs (duodenum, jejunum, and ileum) that presented in other tables. According to Sun et al. (2005) and Smirnov et al. (2005) that into crypt has no effect after broilers are more than 28 days old. Broilers in this study collected samples of digestive tract organs 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 Figure 1, Figure 2, Figure 3, Figure 4, supplementation of Black Cumin (*Nigella sativa*) 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 controls. 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.

The digestive tract organs are supported by villi which are a special shape in the mucosa. The villi are finger-shaped protrusions of the mucosa and are characteristic of the small intestine. Increasing the number of villi will increase food absorption. Villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). The development of the intestinal villi in broiler chickens is related to the function of the intestine and growth of the chicken (Sun, 2004). The increase in villi causes more villi surface area to absorb nutrients into the bloodstream (Mile *et al*. 2006; Rostinawati 2008).

High villi indicate that the intestines are better off 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) states 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.

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 conclusions of this study were the supplementation of Black Cumin (*Nigella sativa*) 72 mg/kg BW/day through drinking water could increase significantly ($P<0.05$) to 1) the average sizes of villi height and villi area of small intestine (duodenum, jejunum, ileum) and large intestine (colon) of broiler; 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 widht of broiler colon.

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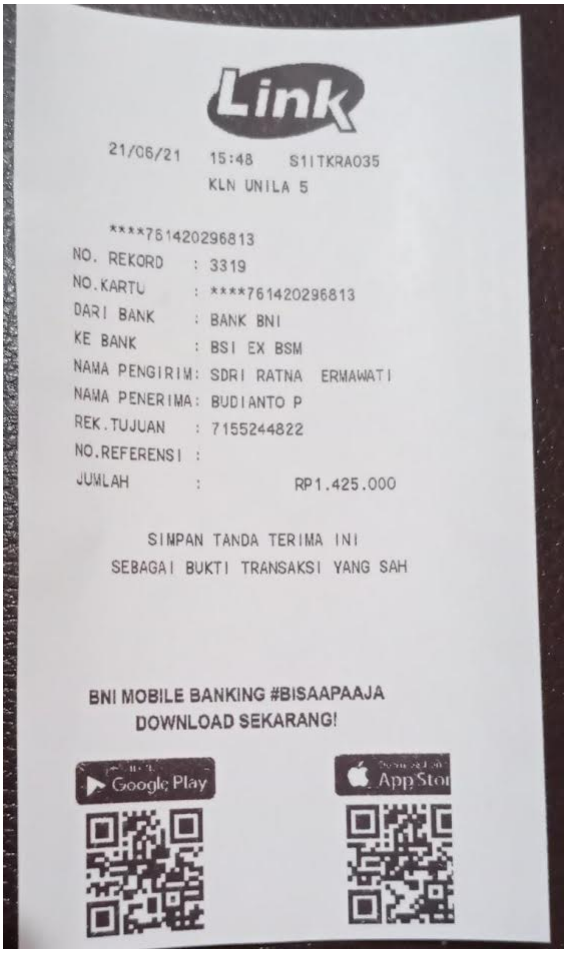

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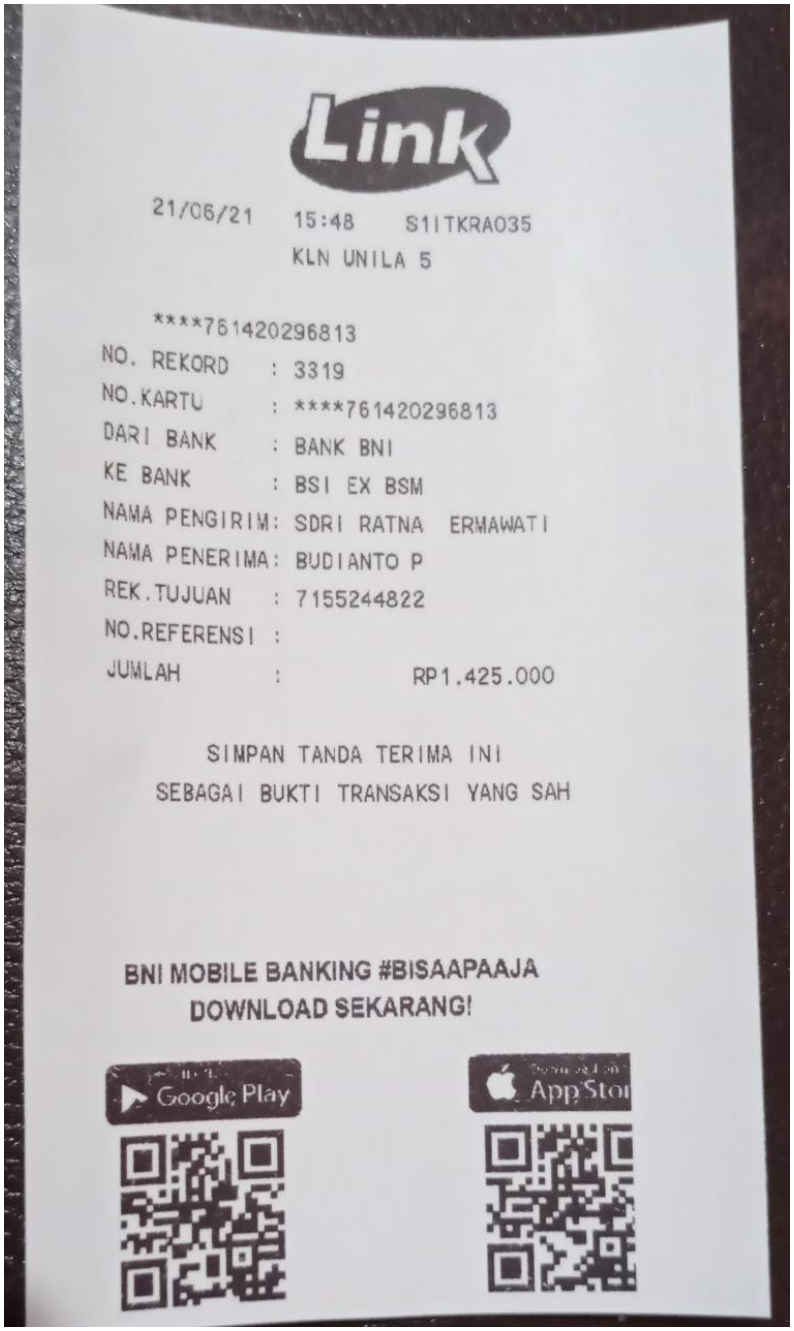

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THE EFFECT OF BLACK CUMIN (*Nigella sativa*) SUPPLEMENTATION THROUGH DRINKING WATER ON THE HISTOLOGY OF SMALL INTESTINE AND LARGE INTESTINE OF BROILER CHICKENS

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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, 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 (*Nigella sativa*) 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 (*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.

Key words: broiler, histology, large intestine, *Nigella sativa*, poultry, 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. Analsis hasil dilakukan secara statistik menggunakan analisis sidik ragam satu arah dengan taraf signifikansi 5% dan dilanjutkan dengan uji lanjut Tukey. Hasil penelitian ini yaitu 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. Kesimpulan penelitian ini bahwa suplementasi jintan hitam (Nigella sativa) dosis 72 mg/kg bb/hari melalui air minum dapat meningkatkan ukuran histologi usus halus dan usus besar broiler.

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

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INTRODUCTION

The development of poultry farming in Indonesia is increasing. Broilers are one of the fastest growing poultry. Many Indonesian breeders prefer to farm broilers because of their relatively short growth period and low maintenance costs compared to ruminants such as cattle, sheep, or goats. In 2018, the broiler population in Indonesia reached 1.89 billion increasing 2.26% from the 2017 broiler population of 1.85 billion heads (BPS, 2019).

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 immunomodulator (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 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, 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.

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This study used broiler cages, a sprayer for cage disinfection, bamboo to make 12 cage plots, plastic tarpaulin for curtains, newspaper and used husks as litter, 12 15-watt bulbs used as a heating source for the brooding area, 12 hanging feeders, 12 chick feeder trays, 12 chicken drinking apparatuses; 1 bucket, 1 hand spray, 1 water tray for dipping, 1 electric scale, thermohydrometer for measuring temperature and humidity, sack, and plastic. Organ sampling equipment was also used, namely necropsy equipment, object glass, cover glass, a refrigerator, a microtome, and a Leica DM500® Binocular Microscope Technology connected with a computer for taking tissue images and measuring the parameters of each organ. ¶ Materials used in the study were 60 Day Old Chicks (DOC) male broiler Cobb CP 707 strains kept for 30 days, food rations, drinking water, Black Cumin extracts (*Nigella sativa*), vaccines for Newcastle Disease (ND), Avian Influenza (AI) and Infectious Bursal Disease (IBD), and a 10% formalin solution. (Delete)¶
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$$\text{Surface area} = \frac{b + c}{2}\ x\ a$$

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 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

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The average measurement of each parameter in broiler jejunum supplemented by black cumin through drinking water is presented in Table 2. Supplementation of black cumin with a dose of 72 mg/kg of the broiler's body weight (P2) had a significant effect on the increase of the
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The average measurement of each parameter in broiler ileum supplemented by black cumin through drinking water is presented in Table 3. Supplementation of black cumin with a dose of 72 mg/kg of broiler body weight (P2) had a significant effect on the increase in …
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The average measurement of each parameter in broiler colon supplemented by black cumin through drinking water is presented in Table 4. Supplementation of black cumin with a dose of 72 mg/kg of broiler body weight (P2) had a significant effect on the increase in …
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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.

The digestive tract organs are supported by villi which are a special shape in the mucosa. The villi are finger-shaped protrusions of the mucosa and are characteristic of the small intestine. Increasing the number of villi will increase food absorption. Villi function to expand the surface of the intestine which affects the process of absorption of food (Alfiansyah, 2011). 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 off 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) 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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Authors would like to thank University of Lampung for providing research funding from the University of Lampung Grants in 2020.

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}

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). $^{a, b, ab}$Different superscripts with letters in the same column indicate significant differences (P<0.05)

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}

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). $^{a, b, ab}$Different superscripts with letters in the same column indicate significant differences (P<0.05)

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}	47.60 ± 12.7 ^{a}

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). $^{a, b, ab}$Different superscripts with letters in the same column indicate significant differences (P<0.05)

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}

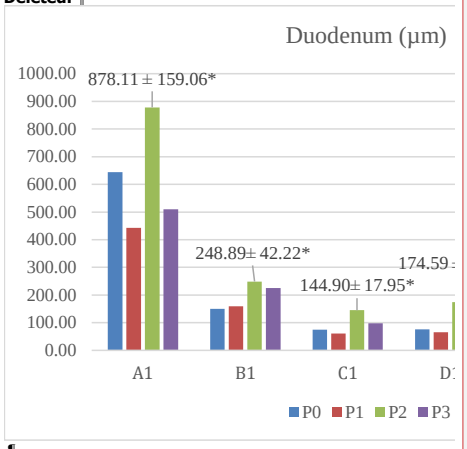

Figure 1. The supplementation of black cumin (*Nigella sativa*) 72 mg/kg BW (P2) in drinking water increased the average villi height (A1), basal villi width (B1), villi apex width (C1), villi area (D1), and gland diameter (F1) of broiler duodenum.
Asterisks (*) indicate the most significant increase (P<0.05)
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Treatment	Colon					
	A1	B1	C1	D1	E1	F1
	Mean ± SD (µm)					
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]

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). [a, b, ab]Different superscripts with letters in the same column indicate significant differences (P<0.05)

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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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