Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]

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ABSTRAK

Saragih HTSSG, Susanto A, Aditya NC, Damayanti SC, Firdaus ABI, Salsabila N, Nuriliani A. 2024. Performa pertumbuhan ayam broiler yang disuplementasi dengan ekstrak buah naga merah [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]. JITV 29(4):208-220. Doi: http://dx.doi.org/10.3443/jitv.v29i4.3445.

Salah satu keunggulan ayam broiler adalah masa panen yang singkat. Faktor yang mempengaruhi pertumbuhan ayam broiler salah satunya adalah kandungan nutrien pakan yang dikonsumsi. Namun, akan sulit untuk mendapatkan pertumbuhan yang optimal apabila hanya mengandalkan pakan konvensional. Oleh karena itu, diperlukan suplemen pertumbuhan yang dapat meningkatkan performa pertumbuhannya. Ekstrak buah naga merah memiliki kandungan flavonoid dan vitamin yang bertindak sebagai antioksidan, sehingga dapat digunakan sebagai suplemen untuk mendorong pertumbuhan pada ayam broiler. Penelitian ini bertujuan untuk mempelajari efek perlakuan ekstrak buah naga merah dalam air minum terhadap pertumbuhan ayam broiler. Penelitian ini menggunakan 300 DOC ayam broiler jantan yang dipelihara sampai umur 21 hari, dilakukan menggunakan Rancangan Acak Lengkap, dengan 5 kelompok dan 5 ulangan, tiap kelompok ulangan terdiri dari 12 ekor DOC. Lima kelompok terdiri dari kelompok kontrol (P0), P1 0,25%, P2 0,50%, P3 1%, dan P4 2%. Parameter yang diamati meliputi struktur histologis usus halus, otot pektoralis, dan lien, serta performa pertumbuhan ayam broiler. Hasil penelitian menunjukkan bahwa perlakuan ekstrak buah naga merah dapat meningkatkan panjang dan luas vili, rasio panjang vili dengan kedalaman kripte, jumlah dan luas sel goblet, luas fasikulus, luas serabut otot, luas pulpa putih, indeks organ limpa, serta meningkatkan performa pertumbuhan yang meliputi berat badan ayam dan efisiensi pakan. Oleh karena itu, dapat disimpulkan bahwa ekstrak buah naga merah dapat meningkatkan struktur histologis usus halus, otot pektoralis, dan limpa, serta meningkatkan performa pertumbuhan ayam broiler, terutama pada ekstrak buah naga merah dengan konsentrasi 2%.

Kata Kunci: Ayam Broiler, Performa Pertumbuhan, Struktur Histologis, Ekstrak Buah Naga Merah, Usus Halus

ABSTRACT

Saragih HTSSG, Susanto A, Aditya NC, Damayanti SC, Firdaus ABI, Salsabila N, Nuriliani A. 2024. Growth Performance of Broiler Chicken Supplemented with Water-Extracted Red Dragon Fruit [*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose]. JITV 29(4):208-220. Doi:http://dx.doi.org/10.3443/jitv.v29i4.3445.

One of the advantages of broiler chickens is their short harvest period. Factors that influence their growth include the nutrient content of the feed. However, it will be difficult to get optimal growth only with conventional feed. Therefore, growth supplements are needed. Red dragon fruit water-extract contains flavonoids and vitamins that act as antioxidants. This research aimed to study the effect of red dragon fruit water-extract treatment in drinking water on the growth of broiler chickens. This research used 300 DOC male broiler chickens which were reared until they were 21 days old. This research was conducted using a completely randomized design, with 5 groups and 5 replications, each replication group consisting of 12 DOC. The five groups consisted of the control group (P0), P1 0.25%, P2 0.50%, P3 1%, and P5 2%. Parameters observed included the histological structure of the small intestine, pectoralis muscle, and spleen, as well as growth performance. The results showed that red dragon fruit water extract could increase villi length and area, ratio of villi length to crypt depth, number and area of goblet cells, fasciculus area, muscle fiber area and white pulp area, splenic organ index, as well as improving growth performance including chicken body weight and feed efficiency. Therefore, it can be concluded that red dragon fruit water extract can improve the histological structure of the small intestine, pectoralis muscle, and spleen, as well as increase the growth performance of broiler chickens, especially in red dragon fruit extract with a concentration of 2%.

Key Words: Broiler Chicken, Growth Performance, Histological Structure, Red Dragon Fruit Water-Extracted, Small Intestine

INTRODUCTION

Broiler chickens make a major contribution to the availability of meat in Indonesia, because it has a soft texture and relatively large size (Umam et al., 2015). The optimal growth of broiler chickens is influenced by nutritional factors from the feed consumed. However, it is not easy to get optimal growth performance if only relying on conventional feed. According to Saragih et al. (2018), adding feed supplements with the right concentration could have a positive influence on the quality of broiler chickens including the digestive system, immunity, and growth performance. However, feed supplements that act as antibiotics on the market have been banned in most countries in the world because of concerns about the accumulation of residues in livestock products and resistance to these types of antibiotics (Ravindran 2013).

A safe source of feed supplement can come from plants. One of them was using the ethanolic extract of cashew leaves as a supplement to chicken feed (Jingga et al. 2019). Red dragon fruit can also be used as a natural ingredient for animal feed supplements because it could positively impact the digestive tract by increasing the optimization of absorption in the digestive tract (Yuniarti et al. 2015). Several studies have explored the benefits of dragon fruit- flesh on the growth of Wistar rats and quail (Cantika et al. 2019; Heryani 2016; Prakoso et al. 2017; Yuniarti et al. 2015). Meanwhile, so far, research on the growth of broiler chickens has only used red dragon fruit-skin (Simanjuntak et al. 2014).

The color of red dragon fruit is caused by pigments that contain lots of anthocyanins. Anthocyanins are phenolic compounds in plants. One of the phenolic compounds in dragon fruit is flavonoids. Phenolic compounds in red dragon fruit flesh extract are the main contributors to antioxidant activity (Prakoso et al. 2017). Natural antioxidant compounds in plants are generally polyphenolic and phenolic compounds, including flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and others (Hossain et al. 2021). Flavonoids can play an active role as supplements in feed. The flavonoids in red dragon fruit-flesh are known to have anti-inflammatory, antioxidant, anticancer, antibacterial, antidiabetic, and hepatoprotective properties (Nuari et al. 2017; Fidrianny et al. 2017). The flavonoid content in red dragon fruit also has the potential to influence the chicken's immune system. Kamboh et al. (2015) stated that the epigallocatechin and cyanidin glycoside compounds in fruit can increase interleukin-2 secretion, lymphocyte proliferation, and NK (Natural Killer) cell activity. According to research by Jingga et al. (2019), the ethanolic extract of cashew leaves containing flavonoids can increase the organ index and splenic white pulp of super Javanese chickens, especially at a concentration of 2%. High immunity in chickens can reduce the risk of exposure to disease so that chickens can have optimal growth performance. Flavonoids in the form of glycosylation or methylation in plants have more stable structures, are easy to obtain, and have easy bioactivity. Flavonoids are belongs to the water-soluble polyphenol family (Arifin and Ibrahim 2018), so in this study, red dragon fruit was made as a water extract for broiler chicken supplements.

So far, there are limited studies that have been done on the growth of broiler chickens using water extract from red dragon fruit. The newest study was conducted by Darmawan et al. (2003) using drinking water containing dragon fruit peel juice in broiler chicken. If the chicken's digestive system works well, it is hoped that nutrient absorption from the intake provided will increase and improve the chicken's growth performance. Meanwhile, strong immunity will protect chickens from pathogens and can affect chicken growth. Therefore, this research was carried out to study the effect of red dragon fruit water-extracted on morphology of the small intestine, pectoralis muscle, spleen, and growth performance in broiler chickens.

MATERIALS AND METHODS

Ethics approval

Procedures for the care and use of test animals have been approved by the Ethics Committee for the Faculty of Veterinary Medicine, Gadjah Mada University with letter number 0024/EC-FKH/Eks./2020.

Red dragon fruit extract preparation

The red dragon fruit used in this study was aged 50 days after it had flowered. The fruit weighed 50 grams and was cut into cubes. The weighed dragon fruit was put in the jam bottle containing 100 mL of mineral water. The jam bottle was closed tightly and incubated at room temperature for 12 hours. After that time, the substance was filtered to extract the dragon fruit water. The protocol followed the preparation of an aqueous extract from Javed and Bashir (2012). The total flavonoid of red dragon fruit water-extracted (RDFWE) was examined at LPPT Unit II UGM.

Experimental design

Acclimation

DOC (day-old chicken) broiler obtained from PT Japfa Comfeed Indonesia Tbk. The chicks were acclimated for 3 days following movement to the cage at the Sawitsari Research Station, Faculty of Biology UGM.

Animal management

This study used 300 DOC which were divided into 5 groups. The group consisted of a control group (P0) which was given drinking water only. Whilst treatment groups P1, P2, P3, and P4 which were given drinking water mixed with water-extracted red dragon fruit at a concentration of 0.25%; 0.5%; 1%; and 2%. The research was carried out with 5 replications, each replication was containing 12 chicks.

Treatment with red dragon fruit extract

Red dragon fruit water-extracted treatment was carried out by mixing it with drinking water given at the same time as feeding with basal feed at 09.00 o'clock in the morning. Then, at 17.00 o'clock in the afternoon, the treated drink was replaced with plain water. The treatments and feeding begun at the age of 3 days and terminated at 21 days. The amount of drinking water was provided 30 mL/chick in the first week, 40 mL/chick in the second week, and 50 mL/chick in the third week. The provision of water extract of red dragon fruit based on the amount of drinking water consumed by broiler chickens is as follows (Table 1). The composition of the basal feed used is presented in Table 2.

Histological preparations of the small intestine, pectoralis muscle, spleen of broiler chickens, and spleen index measurements

DOC was euthanized using the decapitation method (PIC 2016). Three DOCs were taken per replication. Histological preparations of the small intestine, pectoralis muscle, and spleen were made using the paraffin method. The samples were fixed with Bouin's solution for 12 hours, dehydrated with graded alcohol, cleared with toluol, and embedded with paraffin at 57-60°C. Next, the preparation was sliced with a thickness of 5 µm. Small intestine preparations were stained with PAS-AB dye to measure villi length, crypt depth, and the number and area of goblet cells. Meanwhile, pectoral and splenic muscle preparations were stained with Hematoxylin-Eosin dye.

Observation of the fasciculus area, muscle fibers, and white pulp was carried out by examining 5 different fields of view in each coupe. Determining the area was done by finding the average value in each treatment and control group. The splenic organ index was calculated using the formula of Shin et al. (2020):

$$Organ\ index = \frac{OW\ (g)}{CBW\ (g)} \times 100\%$$

where OW is organ weight and CBW is chicken body weight.

Morphometry of the small intestine

Observation and measurement of the small intestine using ImageJ software consists of several stages.

Measurement of the length and area of the villi

The length, basal and apical width of the villi in the duodenum, jejunum, and ileum were measured from 5 fields of view on each preparation. The area of the villi (mm2) is calculated using the formula of Setiawan et al. (2018):

$$Villi\ area = \frac{VBW + VAW}{VAW} \times VH$$

where VBS is villi basal width, VAW is villi apical width and VH is villi height.

Table 1. The total volume of red dragon fruit water extract given to broiler chicken from the first to third week.

Week	Red dragon fruit extract (%)	Total volume of water- extracted dragon fruit (mL)/chick	Total Flavonoid concentration/chick/days (µg)
First week	P1 (0.25%)	0.075	50.71
	P2 (0.5%)	0.15	101.43
	P3 (1%)	0.3	202.86
	P4 (2%)	0.6	405.71
Second week	P1 (0.25%)	0.1	67.62
	P2 (0.5%)	0.2	135.24
	P3 (1%)	0.4	270.48
	P4 (2%)	0.8	540.95
Third week	P1 (0.25%)	0.125	84.52
	P2 (0.5%)	0.25	169.05
	P3 (1%)	0.5	338.01
	P4 (2%)	1	676.19

Crypt depth measurement

The depth of the crypts was observed from 5 fields of view on each histology preparation. The ratio between villi length and crypt depth is calculated using the formula of Fard et al. (2014):

$$\frac{Villi}{Crypt\ depth} ratio = \frac{villi\ height\ (\mu m)}{crypt\ depth\ (\mu m)}$$

Calculation of the number and area of goblet cells

The number of goblet cells was counted in small intestinal villi of 500 µm long. The area of goblet cells observed was measured from the edge of the membrane surrounding the goblet cells on a cross-section of the villi of the small intestine.

Calculation of Feed Conversion Ratio (FCR)

Feed Conversion Ratio is the comparison between the amount of feed consumed and the amount of weight produced. Feeding of broiler chicks was carried out from the age of 3 to 21 days. The feed that went into the cage and the remaining was weighed, to calculate the amount of feed consumed by chicks every day. The formula for calculating the Feed Conversion Ratio according to Umam et al. (2015) is as follows:

$$FCR = \frac{total\ feed\ consumed\ (gram)}{chicken\ weight\ gain\ (gram)}$$

Table 2. Basal feed formulation and nutrition content.

Composition of feed (%)	Single feed
Corn	49.0
Soybean meal	29.0
Rice bran	9.8
Full-fat soya	5.4
Crude palm oil	3.0
Dicalcium phosphate	2.37
Premix vitamin[a]	0.03
Premix mineral[b]	0.06
D, L-methionine	0.22
NaCl	0.32
Calcit	0.5
L-lysine HCl	0.1
L-threonine	0.04
Choline chloride 60%	0.16
Calculated composition[c]	
Metabolizable energy of poultry (kcal/kg)	2,904.02
Crude protein (%)	20.23
Fiber (%)	8.30
Lysine (%)	3.37
Methionine (%)	1.22
Methionine + cycteine (%)	0.53
Calcium (%)	0.86
Phosphorus, available (%)	1
Sodium (%)	0.95
Chloride (%)	0.5

[a]Premix vitamin provided the following per kilogram of diet (Vitamin A= 15000 IU, Vitamin D3= 3000 IU, Vitamin E= 22.5 mg, Vitamin K3= 3 mg, Vitamin B1= 3 mg, Vitamin B2= 9 mg, Vitamin B6= 4.5 mg, Vitamin B12= 30 mcg, biotin= 30 mcg, folic acid= 1.5 mg, niacin= 45 mg, pantothenic acid= 1.5 mg, Vitamin C= 0 mg, choline= 2090 mg and 1242 mg), [b]Premix mineral provided the following per kilogram of diet (Cu= 12 mg, Fe= 72 mg, Iodine= 0.9 mg, Mn= 84 mg, Se= 0.3 mg, Zn= 60 mg). [c]Proximate, amino acids, minerals, and metabolizable energy were obtained from calculated values of Haritadi et al. (2017)

Statistical analysis

The data obtained were quantitative data for variables of villi length, crypt depth, number and area of goblet cells, area of fascicles and muscle fibers, splenic index, and white pulp area. Each treatment was then analyzed using one-way ANOVA and Duncan's test to the level of $P<0.05$ significancy for each treatment.

RESULTS AND DISCUSSION

Total flavonoid examination

The examination result of total flavonoid could be seen in Table 3, with the concentration of total flavonoid is 676,19 µg/ml.

Histological structure of the duodenum, jejunum and ileum of broiler chickens

Based on the results of the research that has been carried out (Figure 1, Figure 2, and Table 4), it can be seen that the length of the villi for the duodenum and jejunum of broiler chickens increased significantly in the treatment group of red dragon fruit water-extracted (RDFWE) concentration of 0.25% and 1 % (P1 to P3) compared with the control group (P0), but there was no significant difference between the control group (P0) and the 2% RDFWE of treatment group 4 (P4). The length of the villi in the duodenum and jejunum showed the highest numbers in the RDFWE treatment group with a concentration of 0.5% (P2).

The depth of the crypts in the duodenum of broiler chickens showed a significant increase in the 0.25% RDFWE treatment group 1 (P1) compared to the control group (P0), which also differed significantly the group of 0.5% (P2) group of 1% (P3); and group of 2% (P4). The depth of the duodenal crypts was highest in the group of 0.25% (P1). Meanwhile, the depth of crypts in the jejunum of broiler chickens increased significantly in the group of 0.25% and 0.5% (P1 to P2) compared to the control group (P0), which was also significantly different from the group of 1% and 2%. (P3 and P4). The depth of the jejunal crypts was highest in the of 0.5% (P2). The ratio of villus length to duodenal crypt depth was highest in the group of 0.5% (P2) but was not significantly different from the control group (P0). The ratio in the jejunum was highest in the group of 0.25% (P1) and there was a significant difference between the control group (P0) and all RDFWE treatment groups.

The number of goblet cells in the duodenum and jejunum of broiler chickens showed a significant increase in the group of 0.25% (P1) to 0.5% (P2) compared to the control group (P0). In the duodenum, there was no significant difference between the number of goblet cells in the control group (P0) and in the group of 2% (P4), while in the jejunum it was significantly different from all treatment groups. The highest number of goblet cells in the duodenum and jejunum was found in the group of 0.5% (P2). The highest goblet cell area in the duodenum and jejunum of broiler chickens was found in the group of 0.5% (P2), and the control treatment (P0) was significantly different from all RDFWE treatment groups.

This study aims to determine the effect of giving red dragon fruit water-extracted on the development of the small intestine, pectoralis muscle, spleen and growth performance of broiler chickens. Red dragon fruit was very famous for its good antioxidant activity that could provide various health benefits (Tenore et al. 2012). In this study, the chemical compound examination that used for red dragon fruit extract is total flavonoid concentration. Red dragon fruit is known to contain flavonoids and phenols as antioxidants, which can affect the growth of broiler chickens. According to Huyut et al. (2017), phenol and flavonoid compounds are known to have antioxidant properties. Apart from flavonoids, red dragon fruit also contains protein, carbohydrates, vitamin C, crude fiber, minerals (phosphorus, potassium, calcium, magnesium, iron, zinc, sodium), essential fatty acids (linoleic acid, oleic acid), betacyanin, and phytoalbumin (Nurul and Asmah 2014; Luu et al. 2021).

The advantage of broiler chickens compared to other types of chicken is their fast growth. Growth efficiency in broiler chickens is related to the nutrients from the feed absorbed and is strongly influenced by the development of the digestive tract. The small intestine is part of the digestive tract which has the function of absorbing food nutrients and consists of the duodenum, jejunum, and ileum (Nasrin et al. 2015). The use of feed with high nutrition is very important in the process of raising broiler chickens. According to Sitompul et al. (2016), nutritional factors in feed, especially protein, can increase the value of pectoralis muscle deposition in chickens. Apart from that, the feed given to chickens also influences their lymphoid organs. In chickens, splenic function is very important considering that the lymph nodes and vessels are not as well developed as in mammals (Davison et al. 2008).

In the intestine, villi and crypts are parts of important role in the absorption of nutrients from feed or supporting supplements (Wang and Peng 2008). The growth of the length of the villi is related to the potential of the small intestine to absorb food juices. The longer the villi, the more effective the absorption of food essences in the small intestinal epithelium () (Lenhardt and Mozeš 2003). This is an expression of the smooth nutrient transportation system throughout the body. Based on the results of research that has been carried out (Figures 1 and 2, Table 4), the length of the

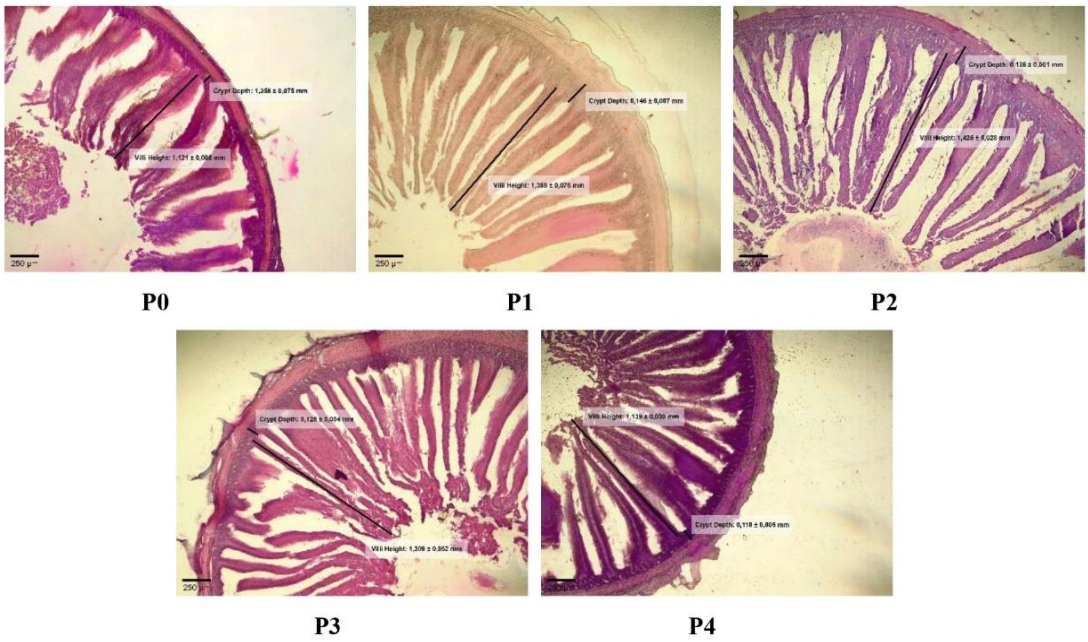

Figure 1. The images of villi length, crypt depth, and the number and area of goblet cells in the duodenum of 21 old broiler chicks treated with red dragon fruit water-extracted in their drinking water of Hematoxylyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

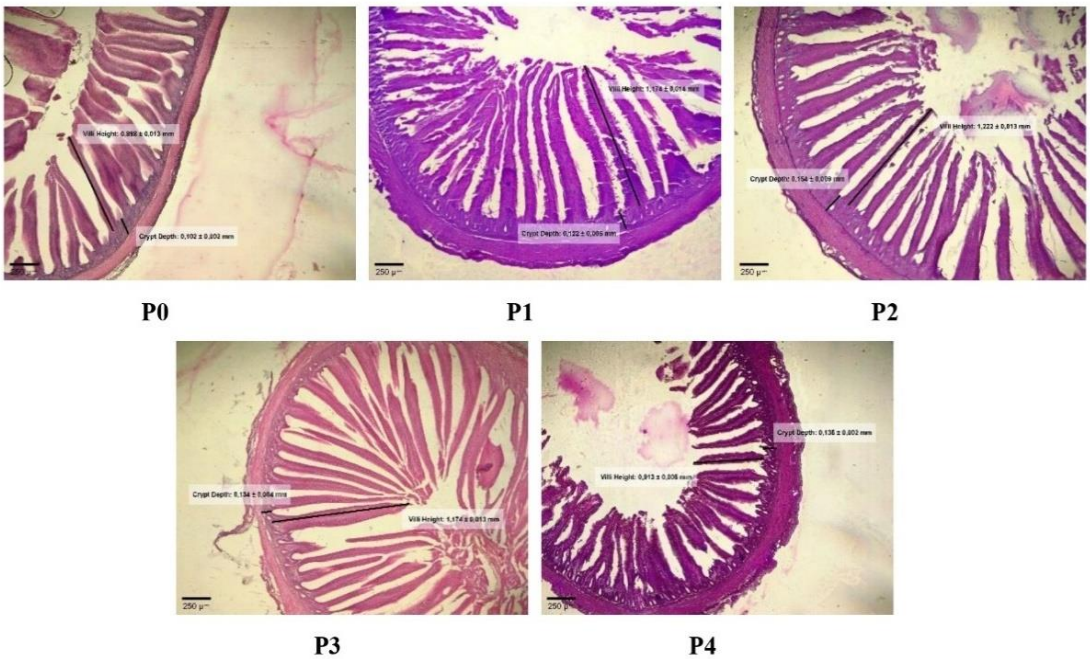

Figure 2. The image of villi length, crypt depth, and the number and area of goblet cells in the jejunum, of 21 old broiler chicks, treated with red dragon fruit water-extracted in their drinking water. Hematoxylyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

Table 3. Flavonoid total content of red dragon fruit water extract

Parameter	Result	Method
Flavonoid total	676.19 µg/mL	Spektrophotometry UV-vis

duodenal and jejunal villi of broiler chickens was highest in the red dragon fruit water-extracted (RDFWE) treatment group with a concentration of 0.5% (P2). The depth of the duodenal crypt was highest in the RDFWE treatment group with a concentration of 0.25% (P1), while in the jejunum was in the RDFWE treatment group of 0.5% (P2). The ratio of villous length and duodenal crypt depth was highest in the RDFWE treatment group at a concentration of 0.5% (P2) and in the jejunum was at the group of 0.25% (P1).

The red dragon fruit water-extracted used in this research had a flavonoid content of 676.19 µg/mL. Based on research conducted by Edi et al. (2018), flavonoid levels of 1286.9 µg/mL contained in teak leaf extract were able to improve growth performance in laying hens. The flavonoid content in red dragon fruit water-extracted has a role in increasing the length of the villi and the depth of the crypts in the duodenum of broiler chickens. Based on the measurement results, it shows that the length of the villi and the depth of the crypts in all treatment groups increased when compared with the control group. The flavonoid content in red dragon fruit water-extracted can stimulate the mitosis of duodenal villous epithelial cells. The longer villi in the treatment group compared to the control group were caused by the epithelial cells which is being active in mitosis. The depth of the crypt has a role in the center of villous cell regeneration due to the increase the length of the villi, which always requires new cells originating from crypt cell migration (Rajput et al. 2013). The ratio of villi length to crypt depth which increased in the treatment group, showing that the flavonoid content in red dragon fruit water-extracted makes the intestinal function healthier.

The goblet cells in the villi have the function of producing mucus. This mucus functions as a protector for the epithelial cells in the villi and can protect the brush border during the process of digestion of food. According to Wang and Peng (2008), goblet cells can increase the ability of nutrient absorption in chickens. The observation results (Table 4) showed that the number and area of goblet cells in the duodenum and jejunum were highest in the RDFWE treatment group with a concentration of 0.5% (P2). The highest number and area of goblet cells in P2 was probably to be caused by the flavonoid content in red dragon fruit water-extracted which acted as an antimicrobial (Tenore et al. 2012). Its role as an antimicrobial can protect epithelial cells from pathogenic bacteria found on mucosal surfaces. Epithelial cells in good and healthy condition will reduce goblet cell mucus secretion, so that the group treated with dragon fruit water-extract had a larger size and number of goblet cells than the control. When goblet cells release mucus in the lumen, glycoproteins from the mucus will be secreted form a gel that protects the epithelial cells. Goblet cells are cells that must always be renewed to prevent damage to epithelial cells (Setiawan et al. 2018).

Flavonoids as an antibiotic compound are more easily absorbed by the small intestine (Arifin and Ibrahim 2018). Flavonoids can also prevent damage to goblet cells. Apart from that, flavonoids function to protect the digestive tract from damage due to stress or pathogens and can protect and prevent erosion of epithelial cells due to pathogens on the surface of the villi. Based on research by Fard et al. (2014) and Setiawan et al. (2018), giving flavonoids to chickens can increase the length of the villi. In previous study, Albab et al. (2022) stated that flavonoid content in date water extract could protect the small intestine of broiler chicken and maintain its good condition. Flavonoids in red dragon fruit water-extracted play a role in maintaining intestinal health by stimulating the growth of beneficial bacteria and inhibiting the growth of pathogenic bacteria (Cardona et al. 2013).

Pectoralis muscle growth at the cellular level was determined by measuring the fasciculus area and muscle fiber area. The administration of red dragon fruit water-extracted in drinking water showed a significant difference between the fascicle area of the treatment and control. The results indicated that the fascicle area and pectoralis muscle fiber area of broiler chickens showed the highest numbers in the RDFWE treatment group with a concentration of 2% (P4). An increase in skeletal muscle mass is caused by an increase in the area of muscle fibers (Glass 2005; Saragih et al. 2018). Based on Saragih et al. (2024), the administration of marine macroalgae (*Chaetomorpha limun*) which has flavonoid content has positive effects on the *pectoralis major* muscle growth.

Area of fascicles and pectoralis muscle fibers

Based on the results of research that has been carried out (Figure 3 and Table 5), it can be seen that the area of the fasciculus in the pectoralis muscle of broiler chickens increased significantly in the treatment groups of 0.25% to 2% (P1 to P4) compared with the control group (P0). The fascicle showed the highest area in the group of 2% (P4). The area of pectoralis muscle fibers in broiler chickens showed a significant increase in the groups of 0.5% to 2% (P2 to P4) compared to the control group (P0), but there was no significant difference between control group (P0) and group of 0.25% (P1). The area of pectoralis muscle fibers was highest in the group of 2% (P4).

White spleen pulp index and area

Based on the results of research that has been carried out (Figure 4 and Table 6), the splenic index of broiler chickens showed that there was no significant

Table 4. The average villi length, crypt depth, ratio of villi length to crypt depth, number of goblet cells, and goblet cell area in the intestines of broiler chicks given red dragon fruit water-extracted in drinking water at the age of 3 to 21 days

Variable	Treatment Groups									
	Duodenum					Jejunum				
	P0	P1	P2	P3	P4	P0	P1	P2	P3	P4
Villi Length (mm)	1.121±0.008^{a}	1.358±0.075^{bc}	1.425±0.028^{c}	1.309±0.052^{b}	1.139±0.030^{a}	0.898±0.013^{a}	1.174±0.014^{b}	1.222±0.013^{c}	1.174±0.013^{b}	0.913±0.005^{a}
Crypt Depth (mm)	0.107±0.001^{a}	0.146±0.007^{c}	0.126±0.001^{b}	0.125±0.004^{b}	0.110±0.005^{a}	0.102±0.002^{a}	0.122±0.005^{b}	0.154±0.009^{d}	0.134±0.004^{c}	0.135±0.002^{c}
Villi: Crypt Ratio	10.443±0.077^{b}	9.296±0.725^{a}	11.283±0.285^{b}	10.453±0.427^{b}	10.503±0.734^{b}	8.780±0.104^{a}	9.663±0.447^{d}	7.980±0.555^{b}	8.790±0.216^{c}	6.750±0.079^{c}
Total Goblet Cells/250 µm	33 ± 0.882^{a}	42.667±0.665^{b}	55.556±1.389^{c}	43.890±3.083^{b}	34.110±0.840^{a}	28.667±1.527^{a}	36.113±3.501^{b}	58.223±5.013^{c}	36.446±1.072^{b}	36.446±2.267^{b}
Goblet Cell Area (μm^2)	261.680±6.206^{a}	366.976±10.601^{c}	522.123±8.916^{d}	333.126±10.916^{b}	371.576±2.378^{c}	256.266±7.951^{a}	357.380±3.474^{t}	576.723±16.201^{d}	432.353±17.255^{c}	343.340±8.726^{b}

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Table 5. The average area of fascicles and muscle fibers of broiler chickens given red dragon fruit water-extracted at the age of 3 to 21 days.

Groups	Fascicle Area (µm2)	Muscle Fibers Area (µm2)
P0	46611.55±8683.09^{a}	310.02±42.35^{a}
P1	75010.85±12052.18^{c}	339.10±45.49^{a}
P2	65888.85±12233.24^{b}	454.62±39.37^{c}
P3	60704.45±8324.49^{b}	406.25±40.57^{b}
P4	86961.67±9154.03^{d}	598.35±75.88^{d}

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

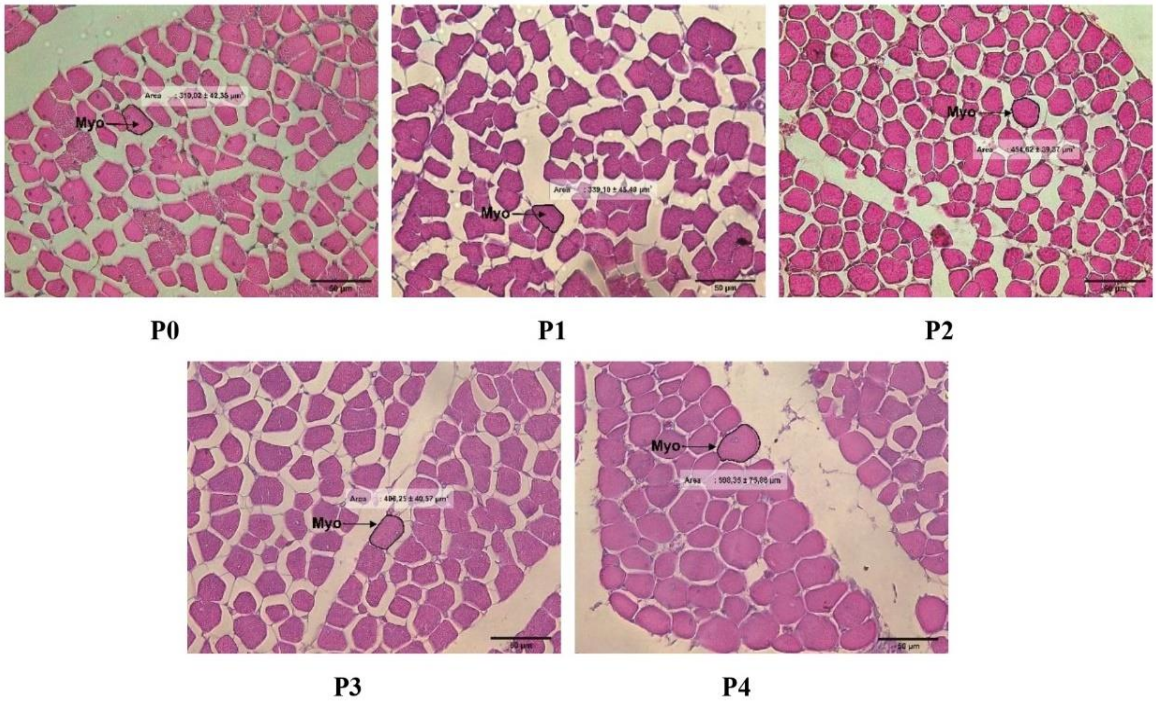

Figure 3. Cross section of pectoralis muscle fibers under magnification of 40 x 10 of 21-day-old broiler chicks treated with red dragon fruit water-extracted in their drinking water, Hematoxilyn-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract, Myo = muscle fiber. Scale bar: 250 µm.

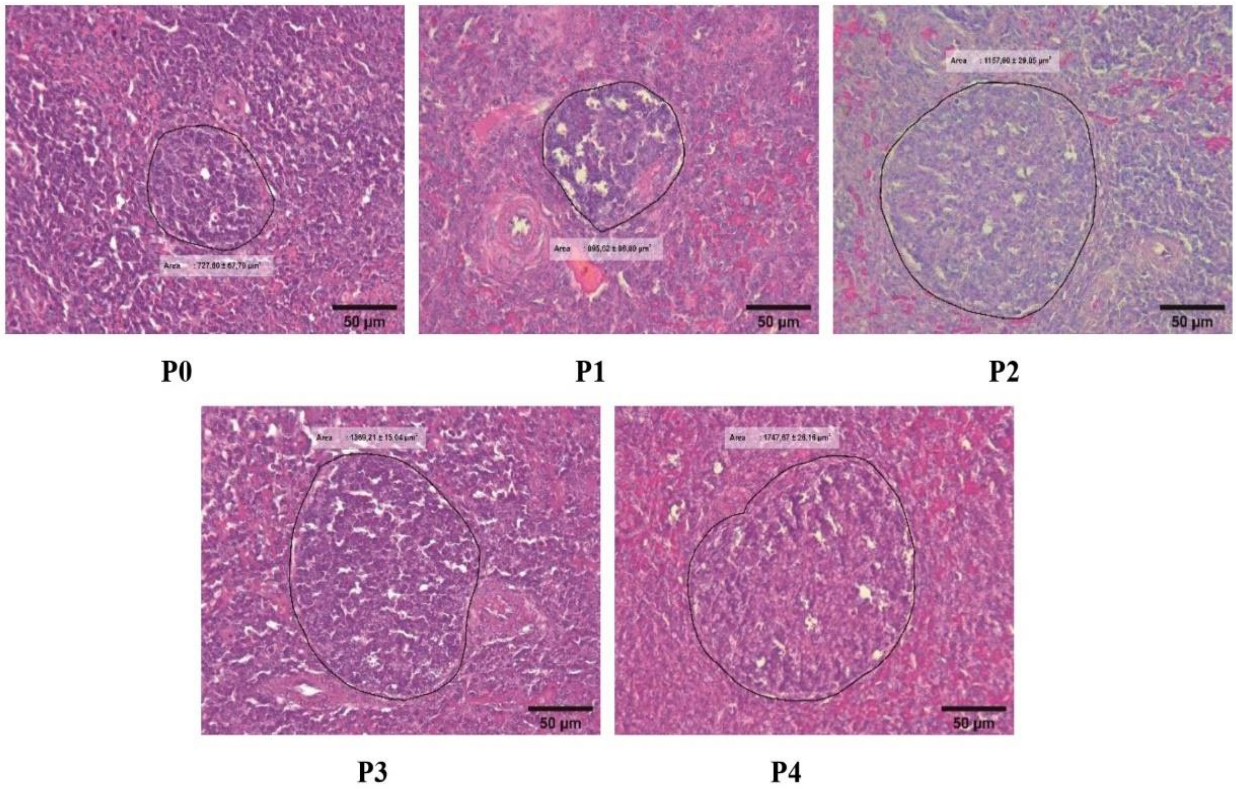

Figure 4. Structure of the white splenic pulp of 21-day-old broiler chicks treated with dragon fruit water-extracted in their drinking water. Hematoxylin-Eosin (HE) staining. P0: without RDFWE; P1: RDFWE 0.25%; P2: RDFWE 0.5%; P3: RDFWE 1.0%; P4: RDFWE 2.0%. P = Treatment, RDFWE = Red Dragon Fruit Water Extract. Scale bar: 250 µm

difference between the control group (P0) and all groups (P1 to P4). Meanwhile, the white pulp area showed a significant increase from group of 0.25% (P1) up to 2% (P4), which was also significantly different from the control group (P0). The highest white pulp area of the splenic organ of broiler chickens were shown in the RDFWE treatment group with a concentration of 2% (P4).

In Table 6 it can be seen that the splenic index of broiler chickens that were given red dragon fruit water-extracted tended to be the same as chickens that were not given water-extracted dragon fruit. Based on statistical analysis, the results obtained did not show any significant differences between treatment and control. According to Jingga et al. (2019) intake of flavonoids from ethanol extract of cashew leaves can increase the splenic index and bursa Fabricius of super Javanese chickens as the concentration increases. Zhou et al. (2019) revealed that intake of the flavonoid baicalein in broiler chicken diets significantly increased the splenic index and bursa of Fabricius compared to controls. However, this statement is at odds with research conducted by Goliomytis et al. (2014) who stated that administration of the flavonoid quercetin did not have a significant effect on the splenic index compared to controls. This statement is also supported by Martínez et al. (2021) who stated that consumption of flavonoids from cashew leaf flour did not affect the relative weight (index) of the bursa of Fabricius, thymus, and spleen of broiler chickens. Research by Yang et al. (2019) also strengthens the statement above which states that the combination of cinnamon oil intake with bamboo leaf flavonoids did not affect the weight of the spleen, thymus, and bursa of Fabricius of broiler chickens on days 21 and 42. There was no significant difference in the splenic index between the treatment and the control caused by an increase in chicken body weight accompanied by an increase in organ weight.

The data in Table 6 shows that the administration of red dragon fruit water-extracted increases the area of white splenic pulp. Based on the results of statistical tests, administering red dragon fruit water-extracted had a significant effect on increasing the area of white pulp in the treatment group. The 2% RDFWE group (P4) had the largest white pulp area and the control group (P0), which had the smallest area. Based on the results obtained, it can be assumed that there is an increase in the proliferation of immune cells in the spleen (Jingga et al. 2019). In Figure 4 it can be seen that the splenic white pulp area in the treatment group has a larger area compared to the control.

Broiler chicken growth

Broiler chickens are a type of superior breed of chicken resulting from cross-breeding, selection, and genetic engineering from chicken breeds that have high productivity in meat production (Tamalluddin 2014). The growth of broiler chickens depends on several factors, namely food, environmental temperature, and rearing methods (Rasyaf 2012). Based on the research that has been carried out, the following results were obtained.

Results presented in Table 7 showed that the body weight of broiler chickens at the 21st day old after RDFWE treatment of 0.25% up to 2% (P1 up to P4) was t shown highest in group of 2% (P4) and was significantly different from control group (P0). Feed intake in the control group (P0) was not significantly different compared to all RDFWE treatment groups. The highest weight gain was shown in the of 2% (P4), which was significantly different from the control group (P0). The highest figure of FCR was in the control group (P0) and was significantly different from the group of 0.25%; 0.5%; and 2% (P1, P2, and P4). The results of the one-way ANOVA feed intake test did not show any significant differences between treatment and control. Meanwhile, one-way ANOVA weight gain test analysis showed that there were significant differences between the treatment and control groups.

The total flavonoid in red dragon fruit water-extracted can increase the proliferation activity of immunocompetent cells in the spleen organ (Grigore 2017; Martínez et al. 2019; Zhou et al. 2019). Flavonoids such as quercetin, chrysin, and wogonin can modulate proliferation and induce B-cell differentiation and antibody production (Martínez et al. 2019). The flavonoid baicalein can increase the production of gamma interferon (IFN-γ) from cytotoxic and helper T cells thereby inducing the proliferation of surrounding immune cells (Zhou et al. 2019). Proliferation of lymphocyte cells is very important as the initial step of the immune response to produce effector lymphocytes and memory lymphocytes (Desforges et al. 2016).

The growth performance of broiler chickens can be seen through body weight and FCR. The results of the study showed that giving red dragon fruit water-extracted in drinking water could increase the growth of broiler chickens (Table 7). The difference in growth between treatment groups and control group began to occur at the 10th day and lasted until the 21st day. The feed used in this research was basal. The basal feed used has a crude protein of 20.58% (Table 3), the composition of the crude protein is not much different from research by Ahmed et al. (2015) which used a crude protein of 20.89% for chickens aged 0-21 days. Providing feed with sufficient crude protein content is useful for protein synthesis and deposition in muscles (Susbilla et al. 2003).

Providing red dragon fruit water-extracted containing antioxidant has the benefit of suppressing the production of free radicals from the body's metabolic processes in broiler chickens. According to

Table 6. Organ index and splenic white pulp area (x10^2 µm^2) in broiler chickens after treatment with red dragon fruit water-extracted in drinking water at the age of 3 to 21 days

Groups	Spleen Index	White Pulp Area (µm^2)
P0	0.127±0.004^a	727.60±67.70^a
P1	0.121±0.012^a	895.62±86.00^b
P2	0.126±0.011^a	1157.80±29.05^c
P3	0.118±0.024^a	1369.21±15.04^d
P4	0.113±0.009^a	1747.67±26.16^e

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. $^{a-d}$ Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Table 7. Average growth of broiler chickens given red dragon fruit water-extracted at the age of 3 to 21 days.

Parameter	Age (Days)	Treatment Groups				
		P0 (0%)	P1 (0.25%)	P2 (0.5%)	P3 (1%)	P4 (2%)
Body live weight (g/chick)	1	48.00±1.52^a	48.50±1.07^a	47.44±1.64^a	47.39±1.72^a	48.67±0.81^a
	4	67.00±1.53^a	66.11±3.27^a	65.72±1.96^a	67.78±1.51^a	67.67±1.17^a
	7	107.61±3.22^a	106.67±3.10^a	106.89±3.96^a	106.94±1.39^a	108.55±2.49^a
	10	155.05±7.87^a	161.22±6.83^a	163.61±14.05^{ab}	163.78±4.87^{ab}	173.06±5.42^b
	13	247.45±7.05^a	252.50±7.37^a	247.61±8.42^a	250.50±8.49^a	261.50±1.50^b
	16	338.94±9.52^b	353.72±6.99^c	337.67±11.03^{ab}	327.83±7.44^a	359.55±5.88^c
	19	406.83±7.83^a	442.61±17.03^{bc}	429.05±9.49^b	444.78±13.59^c	468.72±11.48^d
	21	443.44±19.73^a	507.33±17.69^b	498.22±7.59^b	501.06±18.62^b	551.50±12.2^c
Feed Intake (g/chick/day)		30.67±2.96^a	29.66±2.30^a	29.19±1.04^a	31.22±2.63^a	32.57±1.54^a
Body Weight Gain (g/chick/day)		18.83±2.16^a	22.56±1.41^b	21.47±1.98^{ab}	21.60±1.27^b	23.95±2.45^b
FCR		1.63±0.06^b	1.32±0.08^a	1.37±0.15^a	1.45±0.13^{ab}	1.36±0.11^a

P0= without red dragon fruit water extract; P1= red dragon fruit water extract 0.25%; P2= red dragon fruit water extract 0.5%; P3= red dragon fruit water extract 1.0%; P4= red dragon fruit water extract 2.0%. a-d Differences in number notation followed by different letters in the same line show significant differences (P<0.05)

Zhang & Kim (2020), using quercetin to supplement the growth of broiler chickens can improve growth performance. Based on research by Shilov et al. (2020), feed that is high in antioxidants has been proven to increase the appetite of broiler chickens. The addition of red dragon fruit water-extracted increased the appetite of broiler chickens.

According to Saeed et al. (2017) the use of flavonoids in poultry can cause various biological activities, including growth promoter, anti-inflammatory, antioxidant and antibacterial. So, broiler chickens in the treatment group are thought to have more optimal nutritional absorption compared to the control.

CONCLUSION

Water-extracted red dragon fruit at a concentration of 2% has the effect of improving the histological structure of the intestine, pectoralis muscle, spleen, and growth performance of broiler chickens.

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