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Histological structure of *Pectoralis thoracicus*, small intestine, and growth performance of broiler chicken after supplementation of peanut hulls (*Arachis hypogaea* L.)

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ABSTRACT: The main factor that must be considered in achieving the success of the broiler industry is feed. Processed peanuts from home industries produce waste material in the form of peanut hulls. This study aims to determine the effect of peanut hulls supplementation (PHS) on feed on the histological structure of the *Pectoralis thoracicus* (PT) muscle, small intestine, and the growth performance of broiler chickens. This study used 360 chickens then divided into 4 treatment groups with each group amounting to 90 with 3 replications. The control group (K) was given basal feed, perlakuan 1 (P1) group with PHS 0.25% /kg basal feed, group P2 with PHS 0.5%/kg basal feed, and group P3 with PHS 1%/kg basal feed. The treatment was carried out until the chicken was 18 days old. The parameters observed were chicken body weight at post-hatch age, 3, 6, 9, 12, 15, and 18 days, feed conversion ratio (FCR), histological structure of pectoralis muscle, and small intestine. Data were analyzed using One-way ANOVA, significance ($P \leqslant 0.05$). The results showed that body weight and FCR values of groups P1, P2, P3 were significant towards the control. The results of the pectoralis muscle treatment group were increased significantly compared to the control. The morphological results of duodenum and jejunum groups P1, P2, P3 were significantly increased towards the control. The conclusions of this study indicate that PHS in feed shows improved body weight, the FCR value, muscle performance of PT, and structure of small intestine of broiler chickens with the addition of the most optimal supplement of peanut hulls, which is 0.5%/kg basal diet.

Keywords: Waste material; Peanut hulls; *Pectoralis thoracicus* muscle; Growth performance; Small intestinal

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INTRODUCTION

Feed is one of the important factors in achieving the success of broiler chicken productivity. The cost of feed is the largest cost component that reaches 60-70% of the total cost of poultry production (Anggitasari, sjofjan dan djunaedi, 2016). The feed provided by broiler chicken breeders is a type of commercial feed. The price of commercial feed varies according to the type of raw material used. Raw materials that are generally imported cause the price of commercial feed to be relatively expensive. Chicken muscle development is used as an indicator of meat quality. This muscle is used as a parameter of chicken development because of its low-fat content. *Pectoralis thoracicus* (PT) muscle has a good response to nutrient intake, namely in the growth phase of chickens, so it can be used as an indicator of meat and feed quality testing to see the correlation between growth and the effect of the feed used (Halevy *et al*., 2003).

The process of digestion and absorption of food is influenced by the morphology of the small intestine such as villi height and crypt depth. The higher the villi and the depth of the crypts, the wider the surface area for absorption of food nutrients. The villi height and the crypt depth can be influenced by the composition of the feed given. Good feed is feed that has high nutrition, which comes from protein. (Mescher, 2010).

Protein can be found in both plant and animal sources. One of the easiest ingredients for us to find is peanuts (Lindemann, Kornegay, and Moore 1986). Even though it has a high nutritional content, people are still not able to utilize all parts of peanuts, one of which is the hulls of peanuts. This causes the emergence of waste products.

Peanut hulls contain 9% crude protein, 14% crude fiber, and 84.2% dry matter (Lindemann, Kornegay, and Moore 1986). The content of peanut hulls is expected to be used as a supplementation in basal feed. However, information on the utilization of peanut hulls (*Arachis hypogaea* L.) epidermis for improvement of intestinal morphology, muscle, and growth performance of chickens has not been widely carried out. This study was conducted to examine the histological structure of the *Pectoralis thoracicus* muscle and small intestine, as well as the growth performance of broiler chickens after supplementation with peanut hulls (*Arachis hypogaea* L.).

MATERIALS AND METHODS

Ethical clearance

This study has obtained a code of ethics from the Faculty of Veterinary Medicine, Gadjah Mada University, and has been declared to meet the ethical requirements to carry out research with certification number 0094/EC-FKH/Ex./2019.

Proximate test and bomb calorimeter

The proximate and bomb calorimeter tests were carried out in a certified laboratory, which is the Center for Food and Nutrition Studies, Gadjah Mada University. The proximate test was carried out to determine the composition and nutrient content of the peanut husk, while the bomb calorimeter was carried out to determine the results of the energy-burning residue.

Feed preparation

The peanut hulls were mashed using a blender to make them smooth. Next, the finely chopped peanut hulls were mixed with basalt feed obtained from industrial partners. The mixture of peanut hulls and basal feed was made in 4 groups based on the percentage of peanut hulls mixed in the basal diet. The mixed feed groups included the control group (K) without the addition of peanut hulls, treatment group perlakuan1 (P1) with supplementation of peanut hulls (PHS) of 0.25%/kg basal feed, treatment group 2 (P2) with PHS of 0.5%/kg basal feed and treatment group 3 (P3) with PHS of 1%/kg basal feed.

Acclimation, feeding, and caring

A total of 360 DOC broiler chickens were grouped into 4, based on the type of

feed tested, namely K, P1, P2, and P3. Each group consisted of 90 chickens with 3 replications. Acclimation was carried out for 3 days from post-hatch to day 3 of age. The chicks were taught to eat with basal feed and drink with palm sugar water on the day of arrival.

After that, intensive care was carried out in containers equipped with bulbs, water, and feed supplemented with peanut hulls until the age of 18 days. The chickens were fed and watered in *ad libitum* procedure.

Measurement of feed conversion ratio (FCR)

The amount of feed given was calculated every day until the age of 18 days. Furthermore, the feed conversion ratio (FCR) was measured by calculating the amount of feed per kilogram needed (Feed Intake / FI) to increase body weight per kilogram (Weight Gain / WG). The FCR value according to (Panase and Mengumphan, 2015) can be obtained by the formula:

$$FCR = \frac{Total\ feed}{Weight\ gain}$$

Euthanasia and measurement of the length of the duodenum and jejunum

Nine (9) 18-day-old chickens from each group were euthanized and their muscles and intestines were taken. The left muscle tissue was collected to measure muscle weight and the muscle area, while the right muscle tissue was made for histological preparations. Meanwhile, the length of the duodenum and jejunum was measured using a medline.

Preparation of histology slides for the *pectoralis thoracicus*, duodenum, and jejunum muscles

The pectoralis thoracicus, duodenum, and jejunum muscles that have been collected will then be made histological preparations. The right thoracic pectoralis muscle was cut to a size of 1x1cm and stained with *hematoxylin-eosin* (HE) to see the area of the *fasciculus* and *myofibers*. Furthermore, observations were made under a Leica microscope with a magnification of 10x to observe *fasciculus* and 40x to observe *myofibers*. In each slide, 5 *fasciculus* fields of view were taken and 5 *myofibers* were observed in each *fasciculus*. In the preparation of histology slides for the duodenum and jejunum, the organs were cut transversely so that the villi length and the crypt depth could be observed, then stained with *Alcian blue* and *Schiff reagent*. Furthermore, villi, crypts, and goblet cells were observed and documented using a Leica microscope with 4x magnification. The results of the documentation in the form of *myofiber* area, *fasciculus* area, villi length, crypt depth, number, and area of goblet cells will be measured using *ImageJ* software.

Data analysis

The data obtained were body weight, feed intake, weight gain, FCR, muscle weight, muscle area, *myofiber* area, *fasciculus* area, duodenum and jejunum length, villi length, crypt depth, and the number and area of goblet cells. Furthermore, the data were analyzed using Oneway Anova and continued with the Tukey Test analysis at a significance of $P \leqslant 0,05$.

RESULT AND DISCUSSION

Based on the results of the proximate test that was carried out at the Center for Food and Nutrition Studies, Gadjah Mada University, the content of the peanut hulls used in this study was as follows Table 1.

From the results of the proximate analysis (Table 1), it is known that the fat content in the peanut hulls is 12.24%, the carbohydrate content is 64.67%, while the crude protein content is 12.97%. Protein from feed will be digested in the digestive tract with the help of protease enzymes produced by the pancreas into amino acid molecules. Amino acids will be absorbed by the small intestine and flowed by the blood to the liver and then distributed throughout the body's tissues. Amino acids enter the muscle through active transport assisted by

the hormone insulin and IGF. The peptide bonds formed will result in new proteins producing contractile proteins, namely actin and myosin (Corzo *et al*., 2006).

Table 1. The results of the proximate analysis of peanut hulls

Proximate analysis of peanut hulls (*Arachis hypogaea*)	
Water (%)	7.11
Ash (%)	3
Fat (%)	12.24
Protein (%)	12.97
Carbohydrate (%)	64.67

Table 2. The results of the bomb calorimeter analysis

Calorimeter Bomb Analysis *Arachis hypogaea* (%)				
	0	0,25	0,5	1
Calori (Kal/g)	2.553,14	2.559,52	2.565,90	2.578,67

The value of energy metabolism in each group in this study was not much different, ranging from 2,553.14-2,578.67 cal/g (Table 2), but the treatment group had a higher metabolic value than the control group. This shows that there is a good response in livestock because it is suspected that chickens can digest and utilize the nutritional value components of peanut hulls properly (Sugiyono, Hindratiningrum, and Primandini, 2015). The higher value of energy metabolism in the treatment group was due to the addition of peanut hulls supplements which would increase the protein content of the feed so that the energy supply for meeting basic life needs and production processes was higher (Amrullah, 2004).

Table 3. Effect of peanut hulls supplementation (PHS) on growth performance of broiler chickens from posthatch to 18 days of age.

		Treatmen			
Variable	Ages	K	P1	P2	P3
Body weigth (g)	*Posthatch*	47.90 ±4.99^{ns}	47.83±4.12^{ns}	48.19±3.01^{ns}	48.38±3.86^{ns}
	3	68.16±5.84^{ns}	69.88±6.46^{ns}	71.39±5.42^{ns}	72.20±4.40^{ns}
	6	112.50±8.27^{ns}	116.02±10.36^{ns}	115.02±6.81^{ns}	115.40±9.40^{ns}
	9	167.82±6.07^{ns}	167.60±8.34^{ns}	171.39±11.72^{ns}	168.19±5.28^{ns}
	12	236.02±5.58^{ns}	2039.37±16.11^{ns}	237.12±14.51^{ns}	239.29±10.75^{ns}
	15	337.34±11.19^{a}	342.14±13.90^{ab}	339.68±14.39^{ab}	351.08±14.76^{b}
	18	494.80±4.91^{a}	531.94±11.12^{b}	526.82±21.81^{b}	523.20±7.03^{b}
Feed Intake (g/day)		52.75±0.32^{ns}	52.95±0.88^{ns}	52.37±1.43^{ns}	53.34±1.10^{ns}
Weight gain (g/day)		28.35±0.32^{a}	30.81±0.89^{b}	30.56±1.00^{b}	30.19±0.24^{b}
FCR		1.8±0.01^{c}	1.72±0.03^{a}	1.71±0.02^{a}	1.76±0.01^{b}

Description: a, b, c values in the same line with different superscripts showed a significant difference (P≤0.05). ns = not significant. K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD.

Description: a, b, c values in the same line with different superscripts showed a significant difference (P≤0.05). ns = not significant. K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD.

The FCR results show (Table 3) that groups P1, P2, and P3 were significantly lower than the control group (P≤0.05), while P1 and P2 were significantly different from P3 (P≤0.05). The highest FCR value was in the control group, while the lowest FCR value was in the P2 group, which was 1.71±0.02. This shows that the addition of peanut hulls supplements in the feed can reduce the feed conversion value so that the chickens can convert their food into meat more efficiently. According to Lesson and Summer (2008), broiler chicken maintenance is still efficient if the feed conversion value is still below two. Based on the results of this study, the conversion value of broiler chicken feed was 1.71 – 1.8 so it was still within normal limits.

Weight gain will be positively correlated with feed intake. The high feed intake will usually increase the feed conversion value. A high FCR value indicates that the efficiency of feed utilization is not good, on the contrary, a low FCR value is assumed that livestock converts feed efficiently (Bently, 2003). Factors that affect the value of FCR according to Zuidhof *et al* (2014) are the amount of ration consumption and body weight gain, while according to Fontana *et al.* (1992) the value of FCR is influenced by DOC quality, nutritional quality, cage quality, and care management.

Table 4. Histological structure of the *Pectoralis thoracicus* muscle in broiler chickens after supplementation with peanut hulls (PHS) at the 18 days of age

Variable (µm)	K	P1	P2	P3
Weight of muscle (g)	29.95±0.05^{a}	35.67±1.79^{b}	35.27±2.10^{b}	35.22±2.66^{b}
Area of muscle (cm^2)	101.74±8.73^{ns}	95.59±4.64^{ns}	104.49±7.22^{ns}	98.22±7.82^{ns}
Area of *Fasciculus* (µm^2)	4.59±0.43^{a}	5.41±0.41^{bc}	5.46±0.39^{c}	4.63±0.50^{ab}
Area of *Myofiber* (µm^2)	249.46±23.97^{a}	330.18±20.41^{b}	367.22±34.05^{b}	342.90±48.20^{b}

Description: a, b, c values in the same line with different superscripts showed a significant difference (P≤0.05). ns = not significant. K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD.

Chicken muscle weight at the age of 18 days (Table 4) is known to have a significant increase in the P1, P2, and P3 groups compared to the control group (P≤0.05). The highest muscle weight was in the P1 group with a value of 35.67±1.79, while the muscle area in the control group was not significant to the treatment group (P≤0.05).

The muscle area with the highest value was in the P2 group with a value of 104.49±7.22. The results of the *fasciculus* area of the P1 and P2 groups significantly increased against the control (P≤0.05), but the P3 group was not significantly different from the control (P≤0.05). Based on the results of the *myofiber* area, it was found that P1, P2, and P3 groups significantly increased against the control (P≤0.05), where between P1, P2, and P3 groups did not differ significantly (P≤0.05). Based on these results, it is known that peanut hulls supplementation affects increasing the histological structure of PT muscle in broiler chickens which is indicated by the increase in muscle weight and *fasciculus* and *myofiber* area.

Muscle growth begins with hypertrophy which causes an increase in muscle weight, which usually occurs in chicks after hatching. Muscle hypertrophy occurs due to increased protein synthesis activity of *myofibers* supported by additional *myonuclei* (Petracci and Berri,

2012). In newborn chicks, muscle growth does not show an increase in the number of *myofibers*, but the size of the *myofibers*. This happens because DNA duplication is not followed by cell division. The increase in DNA coincides with the growth of *myofibers* through the transfer of *nuclei* from mitotically active satellite cells. According to Daughtry *et al*. (2017), muscle growth is supported by the proliferation of satellite cells in muscle tissue. Satellite cells proliferate and fuse with *myofibers* to increase in number and replicate DNA in favor of hypertrophy.

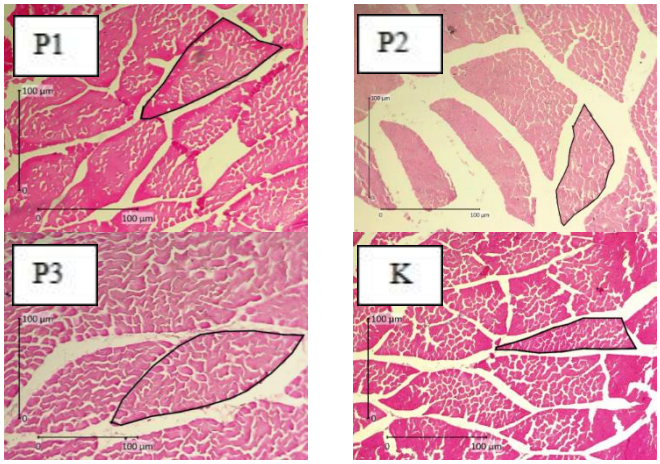

Figure 1. Histological structure of *fasciculus* of *Pectoralis thoracicus* muscle in broiler chickens aged 18 days after supplementation of peanut hulls with the magnification 10x. Description: K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD. The value of the *fasciculus* area in group P2 is higher than the other group.

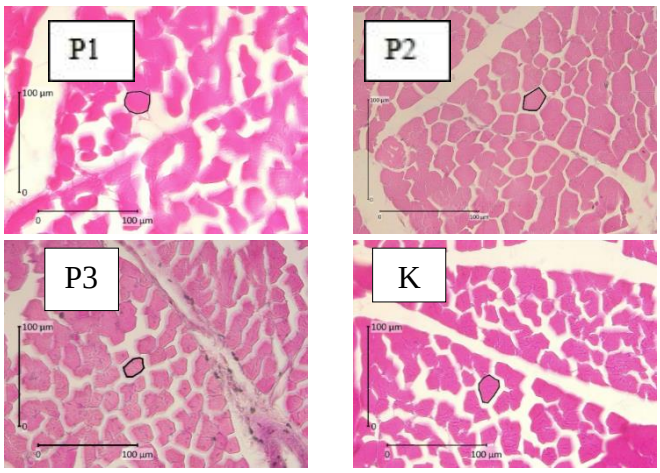

Figure 2. Histological structure of *myofiber* of *Pectoralis thoracicus* muscle in broiler chickens aged 18 days after supplementation of peanut hulls with the magnification 10x. Description: K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD. The value of the *myofiber* area in group P2 is higher than the other group.

Result of the cross-section of the muscle, the skeletal muscle fibers are polygonal in shape and have many nuclei located in the peripheral area. A collection of these muscle fibers forms the *fasciculus*. One *fasciculus* to another is separated by connective tissue *perimysium*, which has a larger size than the endomysium. *The*

fasciculus in the control group (Figure 1) were smaller in size than the other groups and the *fasciculus* was separated by *perimysium* connective tissue. In P2 and P3 groups, the *fasciculus* was larger and tightly packed. In the histological observation of *myofiber* (Figure 2), it was found that the treatment group had a larger size and the arrangement of *myofiber* with denser perimisal connective tissue spacing than the control group. From these results, it is known that the addition of PHS to feed can improve muscle structure which is indicated by an increase in the area of *fasciculus* and *myofiber*. Feeding with high nutrition can increase the size of muscle fibers, whereas if the nutrition is low, it will cause a decrease in the size of muscle fibers. The number and size of *myofiber* also affect muscle performance. According to research conducted by Saragih et al. (2017), an increase in muscle mass is directly proportional to an increase in *myofiber*. Based on the results of Table 5, it is known that the duodenum length showed no significant difference to the control (P≤0.05), with the highest value in the P3 group of 21.33±1.05 cm. The histological structure of the duodenum (Figure 3) showed that the villi height in P1, P2, P3 groups had a significant difference between the controls (P≤0.05), where the P2 group had the highest value, which was 1061.07±38.00. m. At the crypt depth (Figure 3), the treatment group showed a significant increase compared to the control group (P≤0.05), where the P2 group was more significant than the other groups, with a value of 116.28±4.12 μm. The goblet cell area and count (Figure 4) also showed that the treatment group increased significantly against the control (P≤0.05), while the V/C ratio showed no significant difference against the control (P≤0.05).

From these results (Table 5), it can be seen that the addition of peanut hulls supplements increased the histological structure of the duodenum as indicated by the addition of villi height, crypt depth, goblet cell area, and goblet cell count.

Table 5. Histological structure of the duodenum of broiler chickens aged 18 days after administration of peanut hulls supplements

Variable	Treatments			
	K	P1	P2	P3
Lenght of Duodenum (cm)	20.33±0.82 ^{ns}	20.50±2.17 ^{ns}	20.50±1.87 ^{ns}	21.33±1.05 ^{ns}
Villus Height(μm)	636.38±90.27^{a}	801.14±29.06^{b}	1061.07±38.00^{d}	915.53±54.53^{c}
Crypt Depth (μm)	68.18±2.94^{a}	97.37±5.13^{b}	116.28±4.12^{c}	103.92±5.79^{b}
V/C ratio	9.33±1.25^{ns}	8.25±0.56^{ns}	9.14±0.59^{ns}	8.81±0.31^{ns}
Area of Goblet cells (μm^2)	6.79±1.99^{a}	9.51±1.47^{ab}	13.66±2.27^{c}	10.66±2.96^{bc}
Number of Goblet cells	26.20±3.96^{a}	35.00±5.29^{b}	48.80±6.57^{c}	41.20±2.77^{bc}

Description: a, b, c values in the same line with different superscripts showed a significant difference (P≤0.05). ns = not significant. K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD.

Based on Table 6, it is known that the length of the jejunum did not show a significant difference to the control (P≤0.05) with the highest results found in the P2 group of 53.33±4.46 cm. The villi height showed a significant difference against the control (P≤0.05), where the P2 group had the largest value, which was 971.62±92.59 μm. The results of the crypt depth showed a significant difference between the controls (P≤0.05), where the P2 group was more significant than the other groups with a

value of 131.78±12.57. For the value of the goblet cell area, the results showed a significant increase compared to the control, but the goblet cell count did not show a significant difference, while the results for the V/C ratio showed a significant difference.

The additional protein content in the feed will provide a source of nutrition and a source of energy for the cells of the digestive tract to proliferate. The increase in villi height in the duodenum and jejunum is thought to be due to amino acids that can trigger protein synthesis and the proliferation of intestinal cells (Wang *et al*., 2008). The increase in the histological structure of the duodenum and jejunum in this study is thought to be due to the additional protein content of the peanut hulls supplement which affects increasing the number of villi so that it will optimize the absorption of food substances.

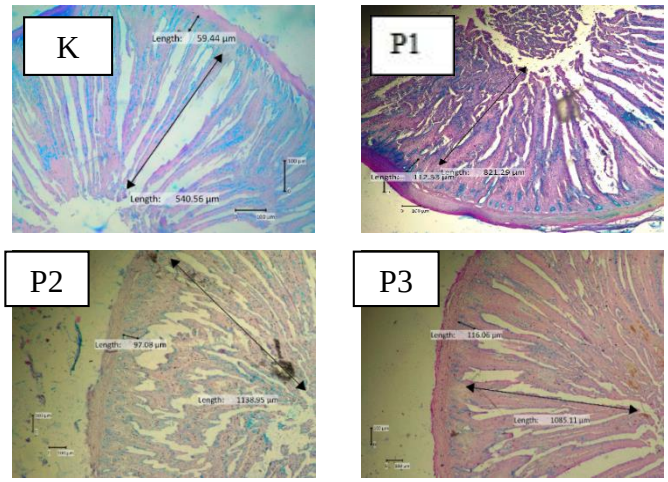

Figure 3. Histological structure of duodenum of broiler chickens aged 18 days after supplementation of peanut hulls with the magnification 4x10. Description: K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD. The villus height and crypt depth of the duodenum in the P2 group is higher than the other group.

Table 6. Histological structure of the jejunum of broiler chickens aged 18 days after administration of peanut hulls supplements

Variable	Treatments			
	K	P1	P2	P3
Lenght of Jejunum (cm)	49.67±1.97 ^{ns}	48.83±4.40 ^{ns}	53.33±4.46 ^{ns}	51.33±3.27 ^{ns}
Villus Height (µm)	743.86±100.74^{a}	758.43±75.73^{a}	971.62±92.59^{b}	838.09±71.73^{ab}
Crypt Depth (µm)	65.50±3.09^{a}	103.96±10.88^{b}	131.78±12.57^{c}	110.09±9.59^{b}
V/C ratio	11.37±1.62^{b}	7.30±0.24^{a}	7.44±1.16^{a}	7.69±1.19^{a}
Area of Goblet cells (μm^2)	13.31±3.32^{ns}	16.04±3.35^{ns}	19.21±4.34^{ns}	18.29±3.87^{ns}
Number of Goblet cells	32.00±4.36^{a}	40.60±4.83^{b}	50.60±3.65^{c}	48.60±6.80^{c}

Description: a, b, c values in the same line with different superscripts showed a significant difference (P≤0.05). ns = not significant. K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD.

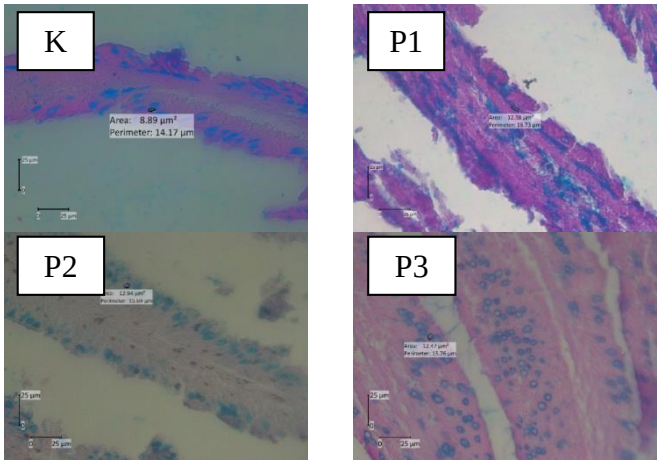

Figure 4. Histological structure of goblet cells of broiler chickens aged 18 days after supplementation of peanut hulls with the magnification 4x10. Description: K: Basal feed, P1: Peanut hulls supplementation (PHS) of 0.25%/kg basal feed, P2: PHS of 0.50%/kg basal feed, P3: PHS of 1%/kg basal feed. Mean ± SD. The value of goblets cell area of the duodenum in group P2 is higher than the other group.

The difference in the histological structure of the small intestine (Figs. 3 and 4) between the control group and the P1, P2, and P3 groups was due to the effect of the addition of peanut hulls into the diet. The crude fiber contained in the peanut hulls can increase the length of the duodenum and jejunum. Crude fiber is a component of plant cell walls that is difficult to digest by poultry and does not contain nutritional value, but its presence in the ration is very essential because crude fiber has physiological and nutritional functions for poultry. Increased levels of crude fiber in the ration tend to lengthen the intestines where the higher the crude fiber in the ration, the slower the rate of digestion and absorption of food substances (Siri *et al*., l992).

CONCLUSIONS

Supplementation of peanut hulls (*Arachis hypogaea L.*) on a basal diet can improve the histological structure of the *Pectoralis thoracicus* muscle, small intestine, and growth performance of broiler chickens with the addition of the most optimal supplement of peanut hulls, which is 0.5%/kg basal diet.

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