The Journal of Veterinary Medical Science

NOTE

Anatomy

Dietary carbohydrate effects on histological features of ileal mucosa in White Leghorn chicken

Md SALAHUDDIN[1)], Kohzy HIRAMATSU[2)]*, Kento TAMURA[3)] and Kazumi KITA[4)]

[1)]Department of Science and Technology, Graduate School of Medicine, Science and Technology, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan
[2)]Laboratory of Animal Functional Anatomy (LAFA), Faculty of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan
[3)]Department of Agriculture, Graduate School of Science and Technology, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan
[4)]Laboratory of Animal Nutrition, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

J. Vet. Med. Sci.
83(6): 952–956, 2021
doi: 10.1292/jvms.21-0157

Received: 15 March 2021
Accepted: 13 April 2021
Advanced Epub:
20 April 2021

ABSTRACT. White Leghorn chickens were divided into the control, low-carbohydrate (CHO), and CHO-free groups to investigate dietary CHO's significance on histological features of chicken ileal mucosa. Paraffin sections of distal ileum from each chicken were stained by periodic acid-Schiff reaction and subjected to morphometrical analysis. Most villi in the control group had a fingerlike shape but those of the experimental groups showed irregular shapes. Villus height, crypt depth and the number of mitotic cells per crypt were significantly lower in the CHO-free group than in the control group. The density of goblet cells also showed a significant decreasing trend with a reduction in dietary CHO level. In conclusion, dietary CHO positively affects the proliferation of epithelial cells in the chicken ileum.

KEY WORDS: carbohydrate, chicken, ileum, morphometry, mucosa

Carbohydrate (CHO) is the main energy source in the chicken diet for body maintenance and production. Ingested diet is digested and absorbed in the small intestine. The small intestine structure performs an important role in the digestion and absorption of nutrients [15], and nutritional manipulation can alter this function [26]. Several studies have reported that different dietary CHO sources such as fructo-oligosaccharide [19] and mannan-oligosaccharide [8], induce higher villus height and deeper crypt in the chicken intestine.

Villus height and crypt depth are two histological features regarded as indicators of intestinal health and development [7]. While villus height exhibits the absorptive area for nutrients and crypt depth indicates the turnover of the intestinal epithelium [2, 7], a higher villus enhances the absorptive surface of the lumen. This process increases the action of digestive enzymes, and speeds up nutrient transportation [22]. Deeper crypts alternatively indicate fast tissue turnover because various types of special cells are present in the crypt, including absorptive, secretory and regenerative cells [2]. These epithelial cells are differentiated in crypt by mitosis and migrated along the villus epithelium up to the tip [12], where they are exfoliated into the lumen.

Mucin is the main component of a mucus layer which covers the entire intestine's luminal surface and is produced in goblet cells located at the intestinal epithelium. This component is essential for facilitating many physiological functions including lubrication, enzyme production, nutrient absorption, and the reduction of bacterial attachment to the intestinal wall [3, 4, 20]. This mucin layer also acts as the first line of the intestinal immune defense by covering the epithelial surface [5]. Thus, many researchers report the effects of various CHO sources on morphological alteration and immunomodulation of the small intestine in different species including chicken.

Till date, however, it is unclear how dietary CHOs affect the structure of the small intestine in chickens. Therefore, this study aimed to clarify the effects of dietary CHOs on the histological features of the chicken ileal mucosa by morphometrical techniques.

Thirty male White Leghorn chicks at 1-day old were taken from a commercial farm and kept in the facility of the experiments for avian species of the Faculty of Agriculture, Shinshu University. Chicks were supplied with a commercial diet and water *ad libitum* for up to 6 weeks under continuous lighting conditions. The control diet as described below was given to chickens for 3 days to habituate with, before the experimental diet was administered during the experimental period.

*Correspondence to: Hiramatsu, K.: seitaik@shinshu-u.ac.jp

Fifteen healthy chickens were selected and randomly divided into three groups, the control, low-CHO, and CHO-free groups of five heads each based on their average body weight. Chickens were then fed in separate cages with an experimental diet for seven days. Experimental diets used in this study were produced in the Laboratory of Animal Nutrition, Faculty of Agriculture, Iwate University, and the composition of each diet is as shown in Table 1. The experimental diet for the low-CHO group contained 12.5% CHO of the control level. Corn oil was added to the experimental diets for all groups to maintain the same energy level among the three groups. Daily feed intake and body weight of each chicken were measured at the same clock time during the experimental period. The animal experiment protocol was reviewed by the Committee for Animal Experiments and finally approved by the president of Shinshu University (Approval Number 300090).

Table 1. Composition of diets used in the present study

Composition	Experimental group		
	Control	Low-CHO	CHO-free
Isolated soybean protein	217.4	217.4	217.4
L-Cysteine	0.7	0.7	0.7
L-Methionine	0.9	0.9	0.9
L-Threonine	0.4	0.4	0.4
Cornstarch	491.4	61.4	0.0
Cellulose	194.7	449.3	485.7
Corn oil	30.0	205.4	230.4
Mineral mixture	60.0	60.0	60.0
Vitamin mixture	2.0	2.0	2.0
Choline chloride	1.5	1.5	1.5
Inositol	1.0	1.0	1.0

Diets were formulated at the same energy density (metabolizable energy=2,850 kcal/kg) with the same carbohydrate (CHO) source (cornstarch). Unit=g.

At the end of the experimental period, chickens were sacrificed by decapitation under anesthetic conditions by an intravenous injection of sodium pentobarbital (64.8 mg/kg body weight) after measuring body weight. The distal part of ileum between ceca was taken as a tissue sample from each bird. Tissue samples were immediately washed with 0.75% sodium chloride solution and fixed in Bouin's fluid (Catalog No.16045, Polyscience, Warrington, PA, USA) at room temperature for 24 hr.

Samples were embedded in a paraffin wax according to the ordinary method, after which sections were cut at 5 µm thickness and stained by periodic acid-Schiff (PAS) reaction with counterstaining of Mayer's hematoxylin. Preparations were then subjected to histological observation and morphometrical analysis under a light microscope (AxioImager, Zeiss, Göttingen, Germany). Villus height was measured as well from the base to the tip (white line in Fig. 1a). Twenty well oriented villi were measured in each of the five sections from one chicken. A total of 500 villi were measured, and the average was calculated for each group. The crypt depth was determined as an invagination between the adjacent villi (red line in Fig. 1a) and measured for each group. Mitotic cells per crypt were counted. Twenty crypts were examined to count mitotic cells for each chicken. A total of 100 crypts were examined, and the average value was calculated for each group. The density of goblet cells was calculated in the following manner. Twenty surfaces of villus epithelium were randomly selected on photomicrographs from each chicken. The number of goblet cells was then counted on each surface, and then a surface length was measured. The density of goblet cells was computed from the number of goblet cell counts divided by the surface lengths of villus epithelia, and expressed as the number per 100 µm surface length. A total of 100 villus surfaces were examined for each group.

All data were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA), and significant differences among three groups were measured using the Turkey's multiple range test. Data were expressed as mean ± standard error (SE). Statistically significant differences were declared at $P<0.05$.

Daily feed intakes of the control, low-CHO and CHO-free groups were 70.0 ± 4.9, 68.9 ± 9.7 and 60.2 ± 7.8 (g/day/chicken, mean± SE) respectively. There was no significant difference in the daily feed intake among the three groups. Similarly, body weight gains during the experimental period of the control, low-CHO and CHO-free groups were 127.3 ± 34.4, 79.5 ± 31.0 and 90.6 ± 20.8 (g/chicken, mean± SE), respectively. Also, no significant difference in this parameter among the three groups was observed, but two experimental groups showed lower weight gain compared to the control group as expected.

Villus shapes from the control and the two experimental groups are given in Fig. 1a–c. As shown, various types of villus shapes (i.e., finger-, leaf-, tongue- and pear-like shape) were observed among the three groups. Fingerlike shapes, however, were most commonly found in the control group (Fig. 1a). Differently from the control group, villi in an irregular shape (i.e., leaf-, tongue- or pear-like shape) were observed in the low-CHO (Fig. 1b) and CHO-free (Fig. 1c) groups. However, villi in leaf-like shape were more dominant in the CHO-free group than in the low-CHO group. Alterations of villus shape also tended to change based on the dietary CHO level.

Histological parameters of each group are summarized in Table 2. As shown, the CHO-free group showed significantly lower villus height compared to the control and low-CHO groups ($P<0.05$). Although villus height of the low-CHO group was numerically higher than that of the control group, there was no significant difference between these two values. However, there were significant differences in the crypt depth between the control and the two experimental groups. Values of the crypt depth in the two experimental groups were also significantly lower than that of the control group ($P<0.05$). Results also showed that two experimental groups showed a significantly lower number of mitotic cells per crypt than the control group (Fig. 1d–f, $P<0.05$). There were significant differences in the density of goblet cells among the three groups ($P<0.05$). The control group revealed a higher density of goblet cells (Fig. 1g) compared to the two experimental groups (Fig. 1h and 1i). Similarly, the density of goblet cells showed a decreasing trend, with a decrease of dietary CHO level.

Thus, this study demonstrated that a CHO-free diet induced histological alterations, such as irregular villus shapes, reduction of villus height, crypt depth, reduced goblet cell density, and mitotic activity of the chicken ileal mucosa. These findings, therefore, indicate that dietary CHO plays an important role in the maintenance of intestinal mucosa.

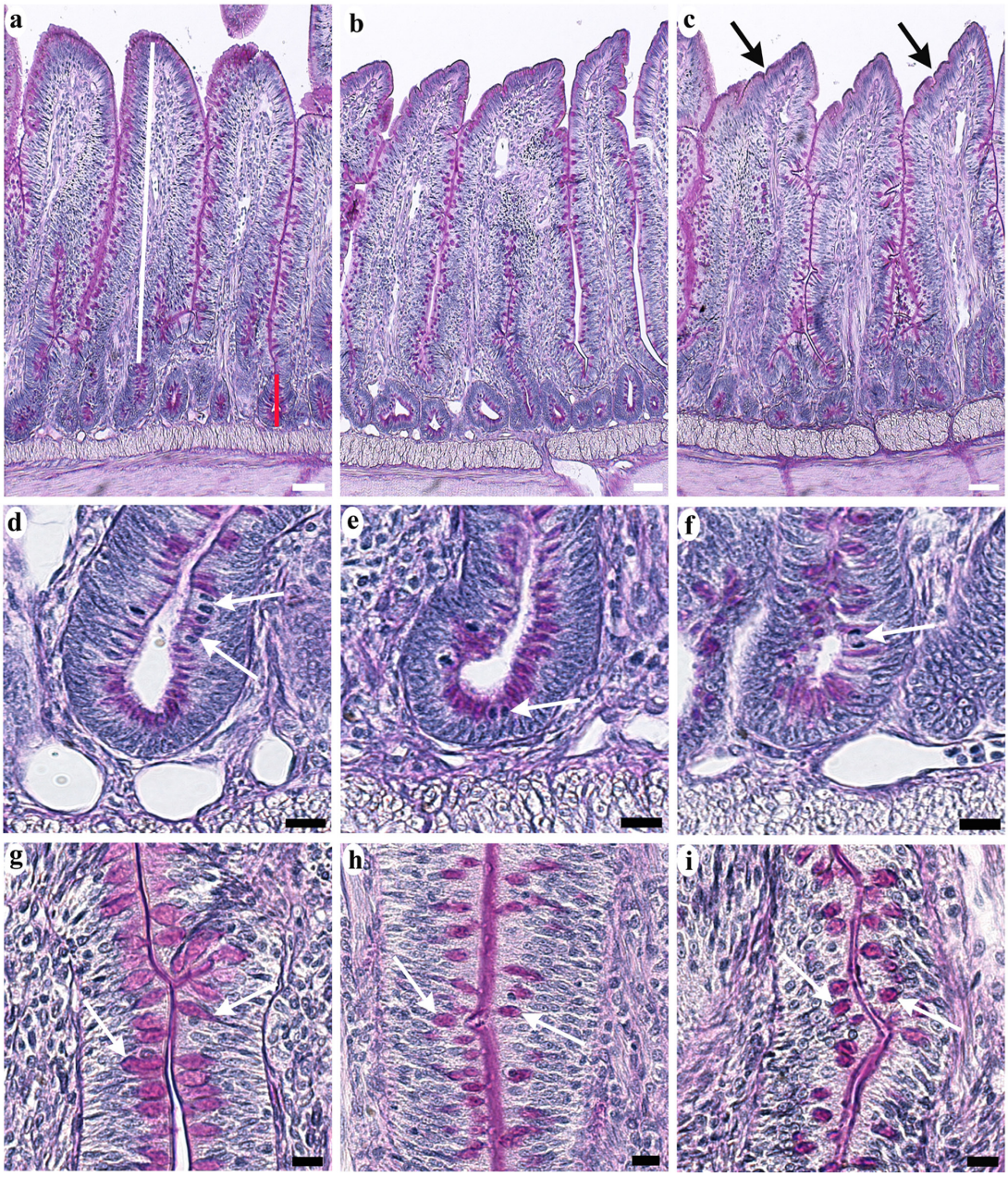

Fig. 1. Histological features of mucosa (a–c), crypts (d–f) and villus epithelium (g–i) from the control (a, d, g), low-carbohydrate (CHO) (b, e, h) and CHO-free (c, f, i) groups. Periodic acid-Schiff (PAS) reaction with Mayer's hematoxylin counterstaining. White line along villus in a denotes villus height and red line along crypt also in a denotes crypt depth. Most villi had a fingerlike shape in the control group (a), but villi showing a leaf-like shape were frequently observed in CHO-free group (c, arrows). Mitotic cells (d–f, arrows) were constantly observed in the control group (d), but the number of them were decreased in low-CHO (e) and CHO-free (f) groups. The density of goblet cells detected with PAS positive reaction (g–i, arrows) was lower in low-CHO (h) and CHO-free (i) groups than the control group (g). Bars in a–c and d–i indicate 50 µm and 10 µm, respectively.

Table 2. Histological parameters in the chicken distal ileum from the control, low-carbohydrate (CHO) and CHO-free groups

Parameters	Group		
	Control	Low-CHO	CHO-free
Villus height (µm)	457.80 ± 5.77 [a]	464.35 ± 8.35 [a]	428.67 ± 8.90 [b]
Crypt depth (µm)	97.72 ± 2.83 [a]	88.62 ± 2.19 [b]	84.37 ± 2.78 [b]
Mitotic cell number (cells/crypt)	1.04 ± 0.09 [a]	0.66 ± 0.06 [b]	0.54 ± 0.06 [b]
Goblet cell density (cells/100 µm)	22.88 ± 0.75 [a]	20.18 ± 0.68 [b]	17.42 ± 0.52 [c]

Values are expressed as mean ± standard error. Significant differences are detected between different alphabets in the same parameter column. $P<0.05$. a>b>c.

The small intestine plays an important role in the digestion and absorption of ingested feeds, and high performance of poultry is attributed to a well-conditioned function of the small intestine. Mucosa of the small intestine is characterized by numerous projections termed villi [15]. Chicken ileum villi have a finger- or tongue-like appearance under normal conditions [15, 24], but these vary in their appearance according to condition or dietary formation [23]. Intestinal villi having a fingerlike shape, contain a comparatively larger cell population than other villus shapes [9], resulting in an increased intestinal mucosal surface area. Increased surface area is then capable of more efficient absorption of available nutrients [7, 27]. In this study, most of the ileal villi of the control group showed a fingerlike shape, but those of the two experimental groups presented irregular shapes. This finding indicated that the efficient absorption of nutrients was lower in the experimental groups because of its decreased surface area. Average weight gains were also lower in the two experimental groups than the control group, despite the similar daily feed intake among the three groups.

Villus height and crypt depth are frequently used as indicators of intestinal health [7]. Thus, an increase in villus height indicates an increased surface area of the intestine and higher capability of absorbing available nutrients as mentioned above [7]. An increase in crypt depth suggests fast tissue turnover, which is necessary for villus renewal [2]. In this study, villus height and crypt depth of the CHO-free group was significantly decreased compared to those in the control group. Moreover, the number of mitotic cells per crypt was also significantly decreased in CHO-free group compared to the control group. Decrease of crypt depth and the number of mitotic cells demonstrate low proliferation levels in crypts of the CHO-free group. Several studies have revealed that dietary CHO induced mitosis of epithelial cells in the mammalian and chicken intestine [17, 21, 25]. Therefore, this study also demonstrated that dietary CHO level affected mitosis in epithelial cells of the chicken ileum. Thus, dietary CHO should be an important factor in maintaining the formation of the intestinal mucosa. Our data in this study indicate that the tissue turnover was slower, and not enough to maintain mucosal formation normal in the CHO-free group, which suggests that the irregular villus form of the CHO-free group depends on an imbalance between the proliferation and loss of epithelial cells.

We have previously demonstrated that dietary CHO activated glucagon-like peptide (GLP)-2 secretion from L cells in the chicken ileum [18]. GLP-2 is a 33-amino acid peptide derived by specific post-translational proteolytic cleavage and other enzymatic modifications of proglucagon [6, 10, 14], and is secreted from "open-type" endocrine L-cells in response to feed ingestion [13]. This gut hormone has various actions, such as stimulation of intestinal growth, up-regulation of villus height, concomitant with crypt cell proliferation and reduction of enterocyte apoptosis [6, 11, 14]. GLP-2 secretion from intestinal L cells is reduced by the lack of dietary CHO. Therefore, proliferation of epithelial cells is suppressed and histological alterations of mucosa are induced in chickens fed by CHO-free feed.

Goblet cells are specialized for the production of mucin which is the main component of the mucus layer in an intestine. Some investigators showed that supplementation with various CHOs in the diet, induced an increase of goblet cells in mammals and chickens [1, 8, 16, 19]. This study showed a decrease in the number of goblet cells in the CHO-free group. This finding strongly indicates that dietary CHO is necessary for goblet cell proliferation in the chicken ileum. Bar-Shira and Friedman [3] also reported that goblet cells had a unique role in chicken intestinal immunity. The present data on goblet cells were significant based on the involvement of dietary CHO in the maintenance of intestinal immunity.

In conclusion, dietary CHOs can influence the proliferation of intestinal epithelial cells, enterocytes and goblet cells. It is necessary for maintaining mucosal formation and the barrier layer of the chicken intestine. Digestion and absorption of nutrients occur from the intestinal lumen by direct contact with the intestinal mucosa. Therefore, the structural pattern influences the rate of nutrient absorption and protection against unwanted threats of the small intestine. The intestinal structure plays important roles and closely associated with its function, but is altered by manipulating various dietary nutrients.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

REFERENCES

1. Adebowale, T., Shunshun, J. and Yao, K. 2019. The effect of dietary high energy density and carbohydrate energy ratio on digestive enzymes activity, nutrient digestibility, amino acid utilization and intestinal morphology of weaned piglets. *J. Anim. Physiol. Anim. Nutr. (Berl.)* **103**: 1492–1502. [Medline] [CrossRef]
2. Awad, W. A., Ghareeb, K., Abdel-Raheem, S. and Böhm, J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poult. Sci.* **88**: 49–56. [Medline] [CrossRef]
3. Bar Shira, E. and Friedman, A. 2018. Innate immune functions of avian intestinal epithelial cells: Response to bacterial stimuli and localization of responding cells in the developing avian digestive tract. *PLoS One* **13**: e0200393 [CrossRef]. [Medline]
4. Bar-Shira, E., Cohen, I., Elad, O. and Friedman, A. 2014. Role of goblet cells and mucin layer in protecting maternal IgA in precocious birds. *Dev. Comp. Immunol.* **44**: 186–194. [Medline] [CrossRef]
5. Baurhoo, B., Ferket, P. R. and Zhao, X. 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poult. Sci.* **88**: 2262–2272. [Medline] [CrossRef]
6. Burrin, D. G., Stoll, B. and Guan, X. 2003. Glucagon-like peptide 2 function in domestic animals. *Domest. Anim. Endocrinol.* **24**: 103–122. [Medline] [CrossRef]
7. Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. *Am. J. Clin. Nutr.* **55** Suppl: 299S–308S. [Medline] [CrossRef]
8. Cheled-Shoval, S. L., Amit-Romach, E., Barbakov, M. and Uni, Z. 2011. The effect of in ovo administration of mannan oligosaccharide on small intestine development during the pre- and posthatch periods in chickens. *Poult. Sci.* **90**: 2301–2310. [Medline] [CrossRef]
9. Creamer, B. 1964. Variations in small-intestinal villus shape and mucosal dynamics. *BMJ* **2**: 1371–1373. [Medline] [CrossRef]

10. Drucker, D. J. 2005. Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat. Clin. Pract. Endocrinol. Metab.* **1**: 22–31. [Medline] [CrossRef]
11. Guan, X., Karpen, H. E., Stephens, J., Bukowski, J. T., Niu, S., Zhang, G., Stoll, B., Finegold, M. J., Holst, J. J., Hadsell, D., Nichols, B. L. and Burrin, D. G. 2006. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. *Gastroenterology* **130**: 150–164. [Medline] [CrossRef]
12. Imondi, A. R. and Bird, F. H. 1966. The turnover of intestinal epithelium in the chick. *Poult. Sci.* **45**: 142–147. [Medline] [CrossRef]
13. Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K. and Herzig, K. H. 2008. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul. Pept.* **149**: 70–78. [Medline] [CrossRef]
14. Lovshin, J. and Drucker, D. J. 2000. New frontiers in the biology of GLP-2. *Regul. Pept.* **90**: 27–32. [Medline] [CrossRef]
15. McLelland, J. 1979. Digestive system. pp. 69–181. In: Form and Function in Birds, Volume 1 (King, A. S. and McLelland, J. eds), Academic Press, London.
16. Munyaka, P. M., Echeverry, H., Yitbarek, A., Camelo-Jaimes, G., Sharif, S., Guenter, W., House, J. D. and Rodriguez-Lecompte, J. C. 2012. Local and systemic innate immunity in broiler chickens supplemented with yeast-derived carbohydrates. *Poult. Sci.* **91**: 2164–2172. [Medline] [CrossRef]
17. Sakata, T. 1989. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation of isolated and denervated jejunal segment of the rat. *Scand. J. Gastroenterol.* **24**: 886–890. [Medline] [CrossRef]
18. Salahuddin, M., Hiramatsu, K. and Kita, K. 2021. Effects of dietary carbohydrate level on glucagon-like peptide-immunoreactive cells in the chicken small intestine. Proceedings of 26th World's Poultry Congress in Paris (in press).
19. Shang, Y., Regassa, A., Kim, J. H. and Kim, W. K. 2015. The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology, and immune responses in broiler chickens challenged with Salmonella Enteritidis lipopolysaccharides. *Poult. Sci.* **94**: 2887–2897. [Medline] [CrossRef]
20. Smirnov, A., Sklan, D. and Uni, Z. 2004. Mucin dynamics in the chick small intestine are altered by starvation. *J. Nutr.* **134**: 736–742. [Medline] [CrossRef]
21. Thomsen, L. E., Knudsen, K. E., Hedemann, M. S. and Roepstorff, A. 2006. The effect of dietary carbohydrates and *Trichuris suis* infection on pig large intestine tissue structure, epithelial cell proliferation and mucin characteristics. *Vet. Parasitol.* **142**: 112–122. [Medline] [CrossRef]
22. Tufarelli, V., Desantis, S., Zizza, S. and Laudadio, V. 2010. Performance, gut morphology and carcass characteristics of fattening rabbits as affected by particle size of pelleted diets. *Arch. Anim. Nutr.* **64**: 373–382. [Medline] [CrossRef]
23. van Leeuwen, P., Mouwen, J. M. V. M., van der Klis, J. D. and Verstegen, M. W. A. 2004. Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. *Br. Poult. Sci.* **45**: 41–48. [Medline] [CrossRef]
24. Yamauchi, K. and Isshiki, Y. 1991. Scanning electron microscopic observations on the intestinal villi in growing White Leghorn and broiler chickens from 1 to 30 days of age. *Br. Poult. Sci.* **32**: 67–78. [Medline] [CrossRef]
25. Yamauchi, K., Samanya, M., Seki, K., Ijiri, N. and Thongwittaya, N. 2006. Influence of dietary sesame meal level on histological alterations of the intestinal mucosa and growth performance of chickens. *J. Appl. Poult. Res.* **15**: 266–273. [CrossRef]
26. Yamauchi, K., Yamamoto, K. and Isshiki, Y. 1995. Morphological alterations of the intestinal villi and absorptive epithelial cells in each intestinal part in fasted chickens. *Jpn. Poult. Sci.* **32**: 241–251. [CrossRef]
27. Yasar, S. and Forbes, J. M. 1999. Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *Br. Poult. Sci.* **40**: 65–76. [Medline] [CrossRef]