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Full Length Research Paper

Developmental morphology of the small intestine in Yangzhou goslings

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The objective of this study was to investigate the development of the weight and the morphological development of the small intestine in Yangzhou geese. The weight, length and perimeter of the small intestine, height and width of the villi, depth of the crypts were measured when geese were 1, 14, 28, 42, 56 and 70 days of age. The results revealed that the weight of the duodenum, jejunum, and ileum (relative to the BW) increased, peaked on day 14 and tended to decline thereafter with age. The weight of the duodenum, jejunum, and ileum (relative to the BW) kept steady-going on 56, 42, 42 days, respectively. A 3-fold increase in length and 2-fold increase in perimeter for the three segments during the period were obtained. The duodenum increased little in length, whereas both jejunum and ileum increased 2 and 3-folds from 1 to 70 days examined. The increase in perimeter of the duodenum, jejunum and ileum was greater from day 1 to 14 than from day 14 to 28. The length and perimeter of the intestinal segments of the gastro-intestinal of Yangzhou geese (relative to the BW) peaked on day 1, then decreased with age and kept steady-going on 42 days, except the relative perimeter of the duodenum. The villus height, surface area and crypt depth in the small intestine were positively correlated with the age of the geese. The ratio of the villus height to the crypt depth (V/C) differed among the segments of the small intestine and at the different time points. The V/C in the ileum increased from day 1 to 70, whereas in the duodenum the ratio first decreased, then rose and descended. However, in the jejunum the ratio increased first, then dropped and rose again, and descended eventually.

Key words: Geese, small intestine, morphology.

INTRODUCTION

Geese are a special kind of herbivorous poultry with characteristics of low fat, high unsaturated fatty acids, high protein. The Yangzhou goose is a major breed in China. They are medium in body size and have a dual purpose for meat and egg production. With the development of animal husbandry in China, raising goose is becoming specialized and more widespread. It is essential to study regularity of animal growth and development.

The growth of an animal depends in part on its capacity to digest and assimilate ingested macromolecules, and any impairment of this is expected to constrain growth.

The gastrointestinal tracts (GITs) of the birds possess the functions of food content storage, secretion digestion juice and absorption of nutrients. Nutrient absorption is important at all stages of life. The small intestine, especially the crypts and villi of the absorptive epithelium, play significant roles in the final stages of nutrient digestion and assimilation. Numerous reports showed that the development of GIT by swine, turkeys, quail and young chicks (Adeola and King, 2006; Sell et al, 1991; Lilja et al., 1985; Lilja, 1983), but information about the develop-ment of GIT of geese is sparse. Adequate feeding in the first week post hatch of the chick played a relevant role in broiler chicken performance (Murakami et al., 2007). Sell (1996) found that the peak of development of the small intestine is shown to be between days 5 and 7 post hatch. Noy and Sklan (1998) reported that days 6 to 8 post hatch was the growth peak for the small intestine.

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Table 1. Ingredient and nutrient composition of trial diet.

Ingredient	Amount (%)	
	1~28 day-old	29~70 day-old
Corn	64	61.5
Soybean meal	27	13.6
Fish meal	3	3
Purple medic	2	16
Soybean oil	0	2
Calcium hydrogen phosphate	1.8	1.9
Limestone	0.9	0.8
Salt	0.3	0.2
Vitamin and trace mineral premix[1]	1	1
Nutrient composition		
AME (MJ/kg)	11.18	11.25
Crude protein (%)	19	15.1
Crude fiber (%)	3.1	5.2
Calcium (%)	1.03	1.05
Phosphorus (%)	0.50	0.49
Lysine (%)	1.02	0.74
Methionine (%)	0.45	0.43

[1] Supplied per kilogram of total diet: vitamin A, 20000IU; vitamin D3, 4500IU; vitamin E, 300IU; vitamin K3, 20 mg; vitamin B1, l0 mg; vitamin B2, 120 mg; vitamin B6, 20 mg; vitamin B12, 0.2 mg; nicotinic acid, 600 mg; pantothenic acid, 180 mg; folic acid, l0 mg; folate, l0 mg; biotin, 0.8 mg; choline, 7 g; Fe, 1.2 g; Cu, 0.2 g; Mn, 1.9 g; Zn, 1.8 g; I, l0 mg, Se, 6 mg.

In general, to understand or speculate on the capacity of the small intestines to absorb nutrients, it is important to examine the morphological changes occurring therein and the digestive enzymatic activity during development. Therefore, the present investigation was undertaken to determine the relationship between age and the morphological changes occurring in the small intestine of Yangzhou geese from 1 to 70 days of age.

MATERIALS AND METHODS

Animals and diets

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Yangzhou University. The experiment was conducted using a total of 108 1-day-old Yangzhou geese (male geese) with three replicate pens, 36 male geese per separate replicate pen on the basis of their BW and equalizing BW and the variance among groups. This experiment included two stages of 1 - 28 days (the first stage) and 29 - 70 days (the second stage) of age. Geese were raised on the net floor with 2 × 2 cm square hole wire rack 70 cm high from the ground and covering with plastic net (1 × 1 cm during 1 - 70) days of age in a similar indoor house (0.5 m^2/goose). Feed and water were made available *ad libitum*. Geese were fed the experimental diet shown in Table 1, which was formulated according to the specifications of Shi et al. (2007) and Zhou (2008).

Collection and assessment

After having a 12 h fasting, two male geese were weighted and killed by cutting the carotid arteries and were immediately bled on days 1, 14, 28, 42, 56, and 70. The empty weights of the small intestine and its regions, as defined by Branton et al. (1988) were obtained at different ages. Other visceral organs including the liver, pancreas, heart and gizzard were also weighed. The length of each intestinal segment was measured using the method described by Leopold (1953). The whole intestine was removed and the mesenteric tissue trimmed off. The duodenum, jejunum, ileum and caecum were resected and laid out in a straight line without loops and without stretching and the lengths were measured.

Histomorphometry

Segments were removed from the duodenum, jejunum, and ileum as follows: (1) intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum, the middle section of which was taken for microscopy; (2) midway was between the point of entry of the bile ducts and Meckel's diverticulum (jejunum); (3) 10 cm proximal to the ileo-cecal junction (ileum). The samples were handled as described (Onderci et al., 2008). The samples were placed in a 10% buffered neutral formaldehyde solution (pH 7.2 - 7.4) and were gradually dehydrated with increasing concentrations of ethyl alcohol (50 - 100%). The dehydrated specimens were embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (Gracia et al., 2009). The sections were analyzed under a light microscope (Nikon Eclipse E200) and the height and width of the villus were measured using a computer assisted image analysis (LY-WN-HP SUPPER CCD). A total of 15 intact well oriented crypt-villus units were selected randomly for each sample. The mean values attributed to individual geese were used in the statistical analysis. Villus height was measured from the tip of the villus to the crypt-villus junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi (Hu and Guo, 2006). The

Table 2. Changes in weights of small intestine of Yangzhou geese during 1 to 70 days.

intestine	Item	Age					
		1d	14d	28d	42d	56d	70d
Intestinal	Weight (g)	3.03 ± 0.53^{A}	21.30 ± 3.78^{B}	37.94 ± 4.50^{C}	45.52± 2.41^{D}	57.70 ± 3.40^{E}	58.41 ± 1.04^{E}
	Weight:BW (g/kg)	37.47 ± 6.65^{A}	57.10 ± 9.25^{B}	35.78 ± 4.16^{A}	22.03 ± 0.88^{C}	21.82 ± 0.92^{C}	18.42 ± 0.79^{C}
Duodenum	Weight (g)	0.59 ± 0.11^{A}	3.45 ± 0.48^{B}	6.02 ± 0.77^{Ca}	7.52 ± 1.40^{CEb}	8.04 ± 1.78^{DEb}	7.95 ± 1.12^{Deb}
	Weight:BW (g/kg)	7.30 ± 1.34^{A}	9.23 ± 1.02^{B}	5.67 ± 0.72^{C}	3.63 ± 0.59^{Dac}	3.03 ± 0.662^{Dbc}	2.50 ± 0.34^{Db}
Jejunum	Weight (g)	0.84 ± 0.25^{A}	8.2 7± 2.23^{B}	13.90 ± 2.09^{C}	16.72 ± 1.70^{D}	21.89 ± 1.14^{Ea}	19.93 ± 0.75^{Eb}
	Weight:BW (g/kg)	10.40 ± 3.19^{ACDa}	22.17 ± 5.85^{B}	13.12 ± 2.00^{D}	8.09 ± 0.74^{Cb}	8.28 ± 0.40^{Cb}	6.29 ± 0.46^{ACb}
Ileum	Weight (g)	0.94 ± 0.27^{A}	6.12 ± 0.86^{B}	11.18 ± 1.12^{Ca}	12.41 ± 0.95^{Cb}	17.43 ± 0.78^{D}	17.52 ± 0.90^{D}
	Weight:BW (g/kg)	11.68 ± 3.40^{A}	16.40 ± 2.00^{B}	10.54 ± 1.0^{A}	6.02 ± 0.60^{C}	6.60 ± 0.29^{C}	5.53 ± 0.50^{C}

$^{a-d}$Values within a row with no common superscript differ significantly ($P < 0.05$); $^{A-E}$Values within a row with no common superscript differ significantly ($P < 0.01$).

villus width was defined as the distance from the outside epithelial edge along a line passing through the vertical midpoint of the villus. Villus area was calculated from the villus height and width at half height.

Statistical analysis

All data from the experiments were analyzed using a one-way ANOVA (SPSS Inc., 1993). When appropriate, differences among treatment means were compared by LSD's multiple-range test. Differences were considered significant at $P < 0.05$.

RESULTS

Total intestinal weights increased more rapidly from 1 to 28 days than from 28 to 56 days, the slowest rate of increase was from 56 to 70 days. Intestinal weight as a proportion of BW was the greatest ($P < 0.05$) at 14 d for the age groups studied. Weights of the duodenum, jejunum and ileum increased ($P < 0.05$) as geese grew from 1 to 56 days (Table 2). The jejunum increased in mass more rapidly than both duodenum and ileum, all three segments of the small intestine increased to a maximal proportion of BW at 14 days after hatching and decreased thereafter. The increases in duodenum, jejunum and ileum weight were greater from 1 to 28 days.

Tables 3 and 4 showed, respectively, the length and perimeter of the geese duodenum, jejunum and ileum from 1 to 70 days of age. The results showed a 3-fold increase in length and a 2-fold increase in perimeter for the three segments during the period considered. Whereas, the weight of these segments increased by 18-fold (Table 2). This increase was due to increased muscular and mucosal development. The total intestinal length increased more rapidly from day 1 to 14 than d 14 to 28. The duodenum increased little in length, whereas both jejunum and ileum increased 2 to 3-fold from 1 to 70 days examined. Further, the increase in perimeter of the duodenum, jejunum and ileum were greater from day 1 to 14 than from day 14 to 28.The regional analysis of the results indicated that the jejunum was the longest segment and the duodenum the shortest. Photos 1, 2 and 3 show the morphology of duodenum, jejunum and ileum at different developmental stages, respectively.

Changes in villus height, villus area and crypt depth with age were determined by computer-aided light microscope image analysis and presented in Table 5. The villus height in all the small intestinal segments increased with age ($P < 0.05$). The villus area in the duodenum and jejunum increased rapidly with age, which segments increased much more rapidly than in the ileum. Changes in crypt depth increased with age were smaller and were initially faster in the duodenum, ileum and the crypt depth developed much more rapidly than in the jejunum from day 1 to 14. The V/C in the ileum increased from day 1 to 70, whereas in the duodenum the ratio was dropped first, then rose and descended, but in the jejunum the ratio was rose first, then dropped and rose again and descended eventually.

DISCUSSION

Segments of the gastrointestinal tract (GIT) increase in size and weight more rapidly in relation to body weight during early post hatching than other organs and tissues of chickens (Lilja,1983), quail

Table 3. Changes in length of intestinal segments of the gastro-intestinal tract of Yangzhou geese during 1 to 70 days.

Intestine	Item	Age					
		1d	14d	28d	42d	56d	70d
Intestinal	Length (cm)	58.03 ± 2.51^{A}	141.10 ± 16.29^{B}	176.37 ± 13.77^{CEa}	180.00 ± 6.26^{CDc}	191.67 ± 4.04^{DEb}	202.00 ± 7.07^{E}
	Length:BW (cm/kg)	718.13 ± 36.55^{A}	379.57 ± 45.72^{B}	166.28 ± 11.70^{C}	87.12 ± 1.53^{D}	72.54 ± 1.02^{D}	63.70 ± 3.54^{D}
Duodenum	Length (cm)	11.03 ± 0.75^{A}	21.88 ± 5.4^{Ba}	28.10 ± 3.07^{BCb}	35.67 ± 7.23^{Dc}	30.00 ± 4.26^{CDd}	29.17 ± 2.46^{CDd}
	Length:BW (cm/kg)	136.47 ± 8.84^{A}	59.16 ± 16.03^{B}	26.49 ± 2.66^{Ca}	17.29 ± 3.63^{CDb}	11.33 ± 1.40^{D}	9.20 ± 0.88^{D}
Jejunum	Length (cm)	20.92 ± 1.26^{A}	57.98 ± 13.51^{B}	72.37 ± 10.59^{CEac}	64.83 ± 6.28^{BCc}	76.83 ± 1.81^{CDd}	83.37 ± 7.04^{DEbd}
	Length:BW (cm/kg)	258.89 ± 18.27^{A}	155.91 ± 36.17^{B}	68.24 ± 9.96^{C}	31.37 ± 2.69^{D}	29.10 ± 1.43^{D}	26.31 ± 2.71^{D}
Ileum	Length (cm)	21.30 ± 1.87^{A}	52.58 ± 3.48^{B}	67.48 ± 6.17^{CDa}	65.67 ± 0.68^{C}	72.33 ± 1.16^{DEb}	76.17 ± 3.36^{E}
	Length:BW (cm/kg)	263.58 ± 24.47^{A}	141.50 ± 11.31^{B}	63.63 ± 5.52^{C}	31.81 ± 1.33^{D}	27.38 ± 0.86^{D}	24.00 ± 1.08^{D}

$^{a-d}$Values within a row with no common superscript differ significantly ($P < 0.05$); $^{A-E}$Values within a row with no common superscript differ significantly ($P < 0.01$).

Table 4. Changes in perimeter of the duodenum, jejunum and ileum during 1 to 70 days.

intestine	Item	Age					
		1d	14d	28d	42d	56d	70d
Duodenum	Perimeter(cm)	0.76 ± 0.05^{A}	1.72 ± 0.12^{Ba}	1.97 ± 0.22^{BDb}	2.32 ± 0.05^{C}	2.32 ± 0.30^{C}	2.18 ± 0.13^{CDc}
	Perimeter:BW (cm/kg)	9.39 ± 0.57^{A}	4.62 ± 0.34^{B}	1.86 ± 0.24^{C}	1.13 ± 0.05^{Da}	0.87 ± 0.10^{D}	0.69 ± 0.06^{Db}
Jejunum	Perimeter (cm)	0.77± 0.13^{A}	1.68 ± 0.17^{B}	2.00 ± 0.12^{C}	2.04 ± 0.05^{C}	2.09 ± 0.27^{C}	2.15 ± 0.15^{C}
	Perimeter:BW (cm/kg)	9.48 ± 1.64^{A}	4.51 ± 0.46^{B}	1.89 ± 0.12^{Ca}	0.99 ± 0.06^{CDbd}	0.79 ± 0.10^{CDcd}	0.68 ± 0.06^{D}
Ileum	Perimeter (cm)	0.79 ± 0.12^{A}	1.61 ± 0.13^{B}	1.86 ± 0.15^{Ca}	1.99 ± 0.10^{CD}	2.01 ± 0.11^{CDb}	2.10 ± 0.08^{D}
	Perimeter:BW (cm/kg)	9.83 ± 1.46^{A}	4.35 ± 0.48^{B}	1.76 ± 0.13^{Ca}	0.97 ± 0.09^{CDbd}	0.76 ± 0.04^{CDcd}	0.66 ± 0.05^{D}

$^{a-d}$Values within a row with no common superscript differ significantly ($P < 0.05$); $^{A-D}$Values within a row with no common superscript differ significantly ($P < 0.01$).

(Lilja et al., 1985), turkeys (Sell et al., 1991), and ducks (King et al., 2000). In this paper, total intestinal weights increased more rapidly from 1 to 28 days than from 28 to 56 days and the slowest rate of increase was from 56 to 70 days. Baranyiova and Holman (1976) noted that the mass of the small intestine increases nearly 600% within the first 7 days after hatching in fed chickens.

The intestinal length changes in the chicken small intestine during development in a study carried out by Mitjans et al. (1997) paralleled those found in this study. During the first two weeks of age, a more pronounced increase in jejunum and ileum than in the duodenum, followed by a slight reduction in the three segments, and then mass increase in the jejunum and ileum were observed. Uni et al. (1999) showed that length increased more rapidly in the jejunum and ileum from 0 to 12 d post hatch, whereas mass increased more in the duodenum and jejunum. The weight and the length of the small intestine increased simultaneously, but they differed in rate of speed. The length of the small intestine in 2 weeks old exceeded half of length in 10 weeks old indicating that the intestine increased in mass much more than in length over the 14 days period tested.

In the present study, the villus height and villus area in all segments of the small intestine increased with age and these results were similar to those of previous studies (Wang and Peng, 2008). It is assumed that an increased villus height is paralleled by an increased digestive and

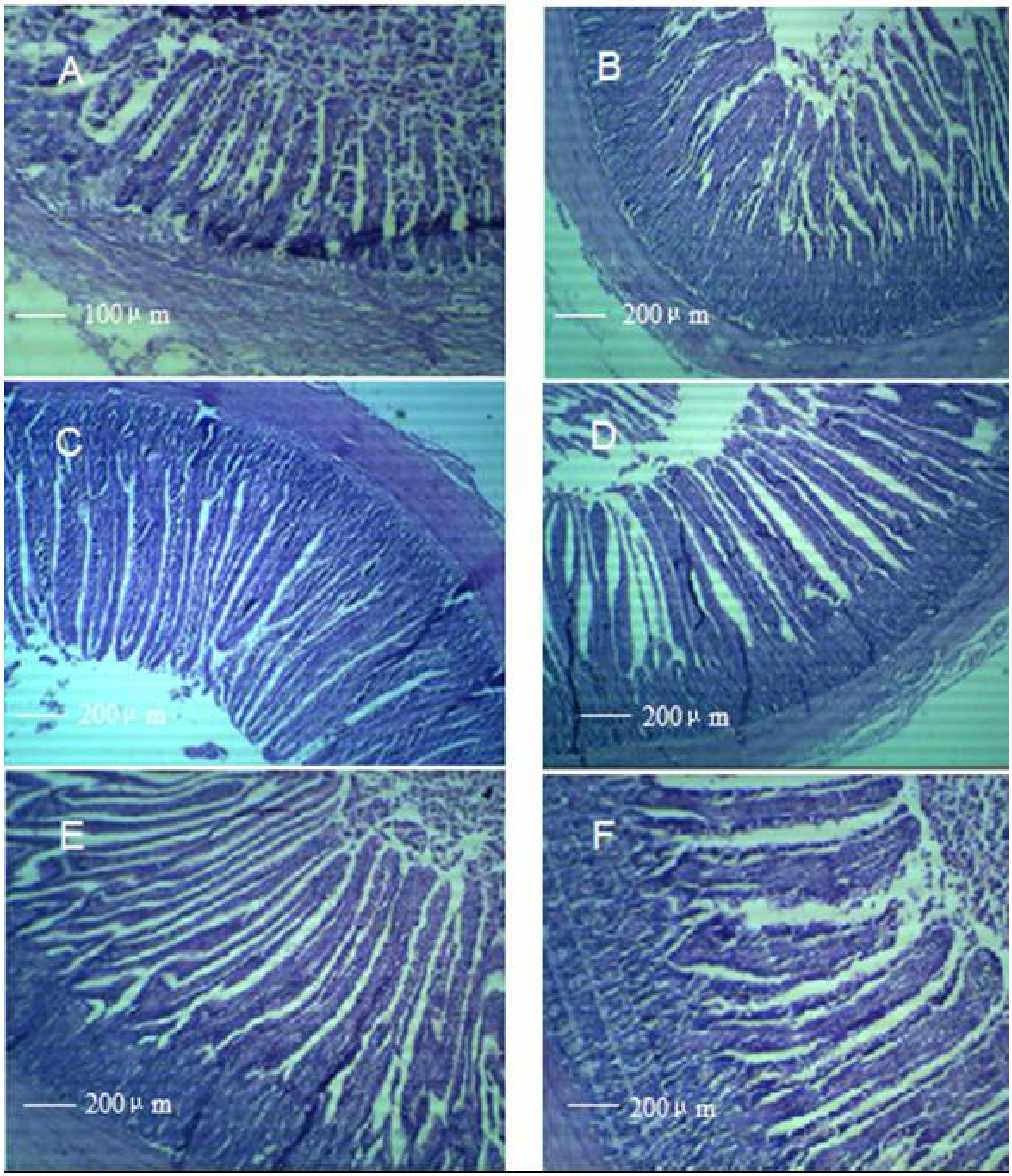

Photo 1. Morphology of duodenum at different developmental stages.

absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Noy et al., 2001; Moran, 1985). It has been shown that intestinal villus volume and crypt depth increase greatly from 4 to 21 days of age. Uni et al. (1995) reported that in chicks, villus height and crypt depth increased rapidly after hatch and reached a plateau after 6 days in the duodenum and 10 days in the jejunum and ileum. Examination of the morphology of the small intestine showed that the villus height and area increased several fold in the duodenum, jejunum and ileum from 1 to 14 days. However, Uni et al. (1999) found that villus size increased in parallel in the duodenum and jejunum and more slowly in the ileum, but these changes are of smaller magnitude than the initial mass increase observed in the jejunum.

Crypts can be regarded as the production site where stem cells divide to permit renewal of the villus. Deeper

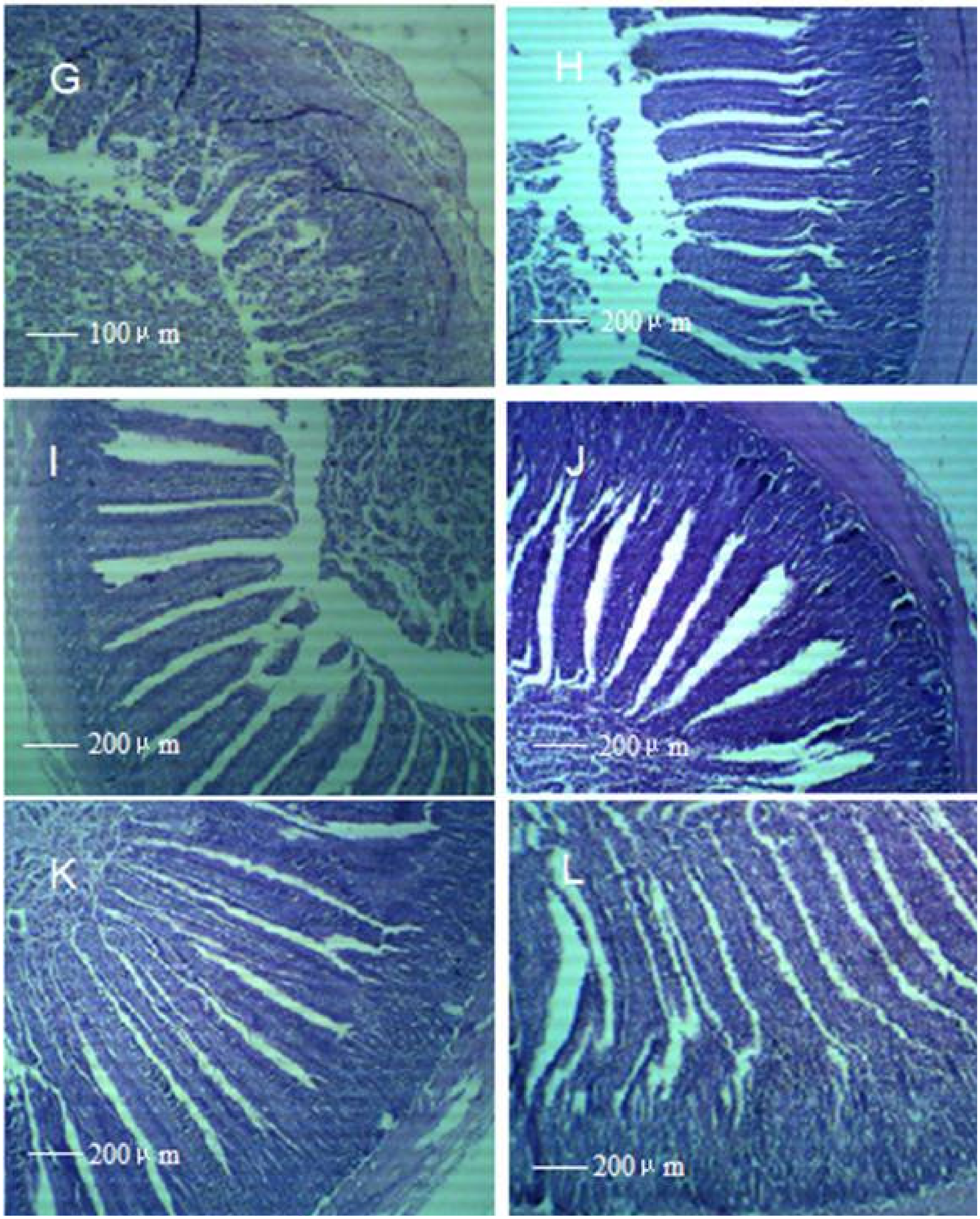

Photo 2. Morphology of jejunum at different developmental stages.

crypts indicate fast cellular turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue (Yason et al., 1987). In the present study, the crypt depth in the small intestine increased with age, which indicates increased activity (Moran, 1985). The intestinal villi increase significantly in diameter and length during the first 7 to 10 d after hatching (Sklan, 2001). But changes in crypt depth with age were smaller and were initially faster in the duodenum and ileum and

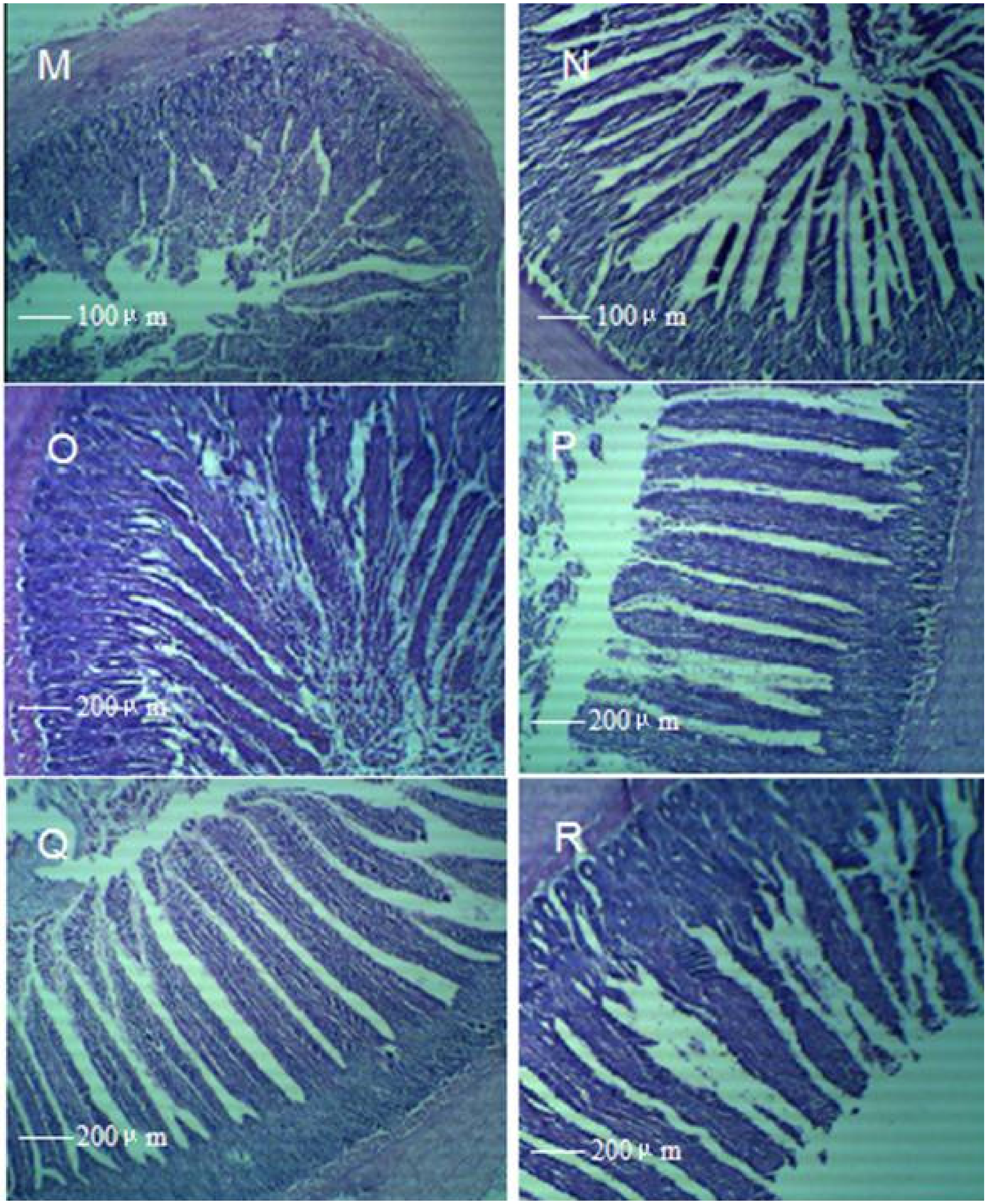

Photo 3. Morphology of ileum at different developmental stages.

the crypt depth much more rapidly than in the jejunum from d 1 to 14. Batal and Parsons (2002) found that crypt depth decreased in the chicks fed the corn-soybean meal and the crystalline amino acid diet as age increased from 7 to 21 days, indicating that the reduced crypt depth of chicks fed and a crystalline AA diet are due in part to slower development of the gastrointestinal tract.

Conclusions

The weight of the duodenum, jejunum and ileum (relative

Table 5. Changes in histological measurements of the intestinal wall of Yangzhou geese during 1 to 70 days.

Intestine	Item	Age					
		1d	14d	28d	42d	56d	70d
Duodenum	Villus height(um)	247.82 ± 37.12^{A}	485.36 ± 31.18^{B}	724.43 ± 29.71^{C}	952.68 ± 32.02^{Da}	992.24 ± 21.10^{DEb}	1002.67 ± 30.82^{E}
	Crypt depth(um)	50.33 ± 8.33^{A}	157.33 ± 26.78^{B}	207.24 ± 20.61^{Ca}	211.40 ± 18.00^{C}	227.21 ± 15.05^{C}	235.11 ± 26.69^{Cb}
	Villus height/ crypt depth(V/C)	4.94 ± 0.35^{Aa}	3.16 ± 0.52^{B}	3.52 ± 0.29^{B}	4.54 ± 0.53^{Ad}	4.38 ± 0.26^{Abd}	4.31 ± 0.53^{Acd}
	Mucosal surface area ($\mu m \times 10^3$)	27.06 ± 5.12^{A}	120.03 ± 8.44^{B}	225.06 ± 30.42^{C}	310.24 ± 37.83^{D}	327.03 ± 36.57^{D}	343.03 ± 68.00^{D}
Jejunum	Villus height(um)	171.11 ± 28.42^{A}	521.24 ± 71.90^{B}	748.37 ± 67.39^{C}	875.41 ± 55.27^{D}	894.37 ± 84.50^{D}	947.38 ± 50.80^{D}
	Crypt depth(um)	70.74 ± 9.63^{A}	116.08 ± 11.22^{B}	191.27 ± 15.03^{Ca}	200.16 ± 14.33^{C}	209.90 ± 3.73^{CDb}	226.55 ± 12.84^{Dc}
	Villus height/ crypt depth(V/C)	2.45 ± 0.50^{A}	4.54 ± 0.83^{B}	3.95 ± 0.59^{B}	4.38 ± 0.35^{B}	4.26 ± 0.40^{B}	4.18 ± 0.26^{B}
	Mucosal surface area ($\mu m \times 10^3$)	15.18 ± 3.34^{A}	137.03 ± 23.44^{B}	237.06 ± 30.52^{C}	296.60 ± 44.43^{D}	307.42 ± 33.19^{D}	363.70 ± 36.06^{E}
Ileum	Villus height(um)	149.61 ± 16.93^{A}	440.97 ± 25.84^{B}	617.85 ± 27.18^{C}	665.74 ± 40.96^{CDa}	735.67 ± 28.47^{Db}	846.23 ± 101.45^{E}
	Crypt depth(um)	71.22 ± 5.65^{A}	176.15 ± 8.36^{B}	196.35 ± 26.30^{BCac}	196.74 ± 8.13^{BCc}	210.09 ± 19.32^{C}	220.35 ± 17.44^{Cb}
	Villus height/ crypt depth(V/C)	2.11 ± 0.35^{A}	$2.51 \pm .017^{A}$	3.19 ± 0.47^{B}	3.38 ± 0.18^{BCa}	3.53 ± 0.41^{BC}	3.86 ± 0.54^{Cb}
	Mucosal surface area ($\mu m \times 10^3$)	12.42 ± 0.88^{A}	86.99 ± 8.28^{A}	183.92 ± 22.44^{B}	198.68 ± 16.71^{BCa}	222.17 ± 26.73^{BC}	286.85 ± 69.05^{Cb}

$^{a\text{-}d}$Values within a row with no common superscript differ significantly ($P < 0.05$), $^{A\text{-}E}$Values within a row with no common superscript differ significantly ($P < 0.01$).

to the BW) increased, peaked on day 14 and tended to decline thereafter with age. The weight of the duodenum, jejunum and ileum (relative to the BW) kept steady-going on days 42 and 56, respectively. The length and perimeter of intestinal segments of the gastro-intestinal of Yangzhou geese (relative to the BW) peaked on day 1, then decreased with age and kept steady-going on 42 days, except relative perimeter of the duodenum. The ratio of the villus height to the crypt depth (V/C) differed among the segments of the small intestine and at the different time points.

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