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Morphological development of the small intestine in white Roman goslings

Bor-Ling SHIH^{1,2}, Yieng-How CHEN³ and Jenn-Chung HSU¹*

¹Department of Animal Science, National Chung Hsing University, 250 Kao-Kung Road, Taichung 402, Taiwan, Republic of China.

²Nutrition Division, Livestock Research Institute, Council of Agriculture, Hsinhua, Tainan 712, Taiwan, Republic of China.
³Department of Animal Science, Tung-Hai University, 181 Taichung Harbor Road, Section 3 Taichung 406, Taiwan, Republic of China.

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The objective of this study was to investigate the morphological development of small intestinal segments in white Roman goslings from hatching to 28 days of age. Forty day-old male goslings were used in this experiment. Eight goslings were selected randomly at hatching, 7, 14, 21 and 28 days of age, respectively. The small intestinal segments were sampled from six goslings for intestinal structure observation by light microscopy and from two male goslings for scanning electron microscopy (SEM). The villus height, width, perimeter, area, crypt depth, muscle thickness and height/width ratio significantly (P < 0.05) increased during the first four weeks post-hatching. The villus width of duodenum continued to increase (P < 0.05) until 28 days of age, whereas the jejunum and ileum villus width reached a peak at 21 days of age. The development of villus height, area and crypt depth increased two to three times in the small intestine of goslings from hatching to 28 days of age. The area of villi in the duodenum and jejunum were significantly larger (P < 0.05) than that in the ileum. The villus muscle depth of the ileum was greatest (P < 0.05) and villus height and perimeter of jejunum were greatest (P < 0.05) among the intestinal segments at 28 days of age. Duodenal villus width increased faster but crypt and muscle thickness became thinner than other intestinal segments (P < 0.05). The variables regressing the size and shape of villi in the smal intestine increased linearly (P < 0.05 to 0.001) in the small intestine. The surface of each segment villi of the small intestine exhibited different types. At hatching, the villi of duodenum and jejunum had a small and dense finger-like shape, followed by a more developed plate or tongue-like at four weeks of age. Ileal villi developed to finger-like with a round tip during the early growth period.

Key words: Geese, small intestine, villi, morphological development.

INTRODUCTION

Scanning electron microscopy (SEM) has given a new dimension to the study of gastrointestinal morphology in numerous mammalian species and chickens. Studies on pig intestines with the SEM revealed finger-like villi (Waxler, 1972), and plate-like shaped villi are observed in bovine and broiler intestines (Musgrave et al., 1973; Bayer et al., 1975). The intestinal villi of fowl vary in shape with age, from finger-like to leaf-like forms, and closely

resemble those found in mammals (Bayer et al., 1975). A study of the intestinal villus surface (Yamanchi and Isshiki, 1991) indicates that meat-type chickens develop more villus surface area as early as one day after hatching and have larger villi, wider microvilli and more activated epithelial cell extrusions on the duodenal and jejunal villus surface at 10 days of age than egg-type chickens. The greater absorptive area and intestinal cell activation of villi are related to the faster growth rate in the meat-type than egg-type chickens (Yamauchi et al., 1992). Consequently, studies on the fast movable morphological features of the villus surface that the fine structure of absorptive epithelial cells changes during post-hatching developmental period

^{*}Corresponding author. E-mail: jchsu@dragon.nchu.edu.tw. Tel: +886-4-22850315. Fax: +886-4-22870781.

in chicks (Noy et al., 2001).

The weight of the small intestine of birds increase more rapidly than the body weight (Nitsan et al., 1991). However, this process of rapid relative intestinal growth is maximal between six to eight days of age in poults and six to 10 days of age in chicks (Sell et al., 1991; Noy and Sklan, 1998). The small intestinal mucosa in chicks indicates that villus height of duodenum reaches a plateau at six to eight days of age, but only after 10 or more days of age in both the jejunum and ileum (Noy and Sklan, 1997). Geese have a greater digestive capability than other types of poultry and appear to digest dietary fiber more efficiently (Hsu et al., 2000). The fine structure of caecum (Chen et al., 2002) and the development of the gastrointestinal tract of geese (Shih et al., 2005) have been reported. However, there is relatively little information about the fine structural changes of the villi in small intestines of goslings. This study, therefore, was conducted to explore the villus distribution in the small intestine using the light microscope and scanning electron microscope in order to establish the post-hatch changes in morphology and structure of small intestinal segments in geese from hatching to 28 days of age.

MATERIALS AND METHODS

Animals and treatments

A total of 40 day-old male goslings, from Chang-Hua animal breeding station, Livestock Research Institute, Council of Agriculture (COA-LRI) was used as experimental animals. All birds were divided into four replicate groups of 10 goslings in floor-pens, with approximately 850 cm² of floor space per bird. The goslings were kept at 32 to 38°C by an electric heat of raise program during the first one week; afterwards there was no extra heat supplementation during the two to four weeks of age. Each pen floor was covered with rice-hull shavings. The birds were fed with the starter diet (crumbles containing 200 g protein/kg, 11.82 MJ MEn/kg and 38.00 g crude fiber/kg) (Table 1). Feed and water were supplied ad libitum from hatching to four weeks of age. At hatching and 7, 14, 21 or 28 days of age, six male for intestinal structure observation, otherwise, two male goslings for SEM, were selected and slaughtered. At necropsy, the small intestine was immediately removed and tissue samples (about 4 × 4 cm) divided into the duodenum, jejunum and ileum. Samples were taken from middle of small intestinal sections. The duodenal sample was taken 15 cm from the gizzard, while the jejunal sample was taken from the duodenum to the Meckel's diverticulum and the ileum sample was taken from the remaining part of the small intestine. All experimental procedures were approved by the Laboratory Animal Management Committee of our institute.

Samples preparation for histological detection

Intestinal samples were placed into 10% buffered neutral formaldehyde solution (pH 7.2) and shaken for 24 h for fixation according to the method of Chiou et al. (1996). Then, all samples were dehydrated gradually by increasing concentrations of ethyl alcohol (60 to 100%) for 1 to 1.5 h each. Two 1 mm thickness cross-sectional pieces were cut from each sample and embedded in a paraffin block. Six cuts of 6 μ m thickness sections were made of

each block. The specimens were embedded in paraffin and then sliced into 6 μ m. The sections were stained with hematoxylin and eosin, and mounted. Morphometric analyses were conducted with a light microscope connected to a video-based, computer-linked system that was programmed to perform morphometrical analysis (Yu et al., 1998). Values are means from five adjacent villi from six birds. Only vertically oriented villi and crypts were measured. The morphometric indices were determined using computer-assisted image analysis for the area of villi. Villus circumference, height, width and crypt depth, muscle thickness of intestine were evaluated. Variables are expressed as the means from 10 adjacent villi. The average datum for each variable from each section of each bird was used to calculate the mean and variability for that variable.

Samples preparation for SEM detection

Intestinal samples were prepared for SEM according to the method of Chen et al. (2002). All intestinal samples were first rinsed with 0.9% NaCl, and then fixed in 2.5% glutaric dialdehyde in 0.1 M phosphate buffer (pH 7.3) for 2 h (Tsai and Wang, 1993). The specimens were then fixed in 1% osmium tetroxide in 0.1 M phosphate buffer solution (pH 7.3) for 2 h. The samples were rinsed in phosphate buffer solution for duration of 10 min each. Thereafter, the samples were gradually dehydrated using increasing alcohol concentrations (30, 50, 70, 80, 90, and 95%) for 15 min each, and absolute alcohol for the final three rinses. The samples were dried in a critical point dryer (CPD), mounted on aluminum stubs with double adhesive tape, coated with gold to about 20 nm thickness, and observed with SEM (Topcon 150S) at 15 kv.

Statistical analysis

All data were analyzed using the General linear model procedures of Statistical Analysis System (SAS, 2002). Comparison of treatment means was using a least square means test. A significance level P < 0.05 was applied in all cases and orthogonal comparison was used in the multi-regression model (Steel and Torrie, 1960).

RESULTS

Histological observation of villi by light microscopy

In general, feed intake increased with increasing body weight of gosling. The average feed intakes in goslings were 27.73, 69.49, 226 and 243g at 7, 14, 21 and 28 days of age, respectively. The development of villus height, width, perimeter and area in the small intestinal segments of goslings from hatching to 28 days of age are shown in Table 2. Villus morphologic variables increased rapidly in all three intestinal segments from hatching to four weeks of age. During this period, the means of height, width, perimeter and area of villi among the intestinal segments of goslings increased (P < 0.05) by 3.2, 1.7, 3.2 and 4.2 times, respectively. At hatching, the initial height of duodenal villi was longer (P < 0.05) than that of jejunal or ileal villi, thereafter; there was an increase (P < 0.05) in the villus height in all intestinal sections. The villus widths of duodenum continued to increase (P < 0.05) until 28 days of age, and the villus width of jejunum and ileum and the average width of intestinal segments reached a peak

Ingredient	g/kg
Yellow corn, ground	516.0
Wheat bran	300.0
Soybean meal, 44%	291.0
Fish meal, 60%	300.0
Alfalfa, 17%	700.0
Tallow	350.0
Dicalcium phosphate	132.0
Limestone, pulverized	50.0
Salt	40.0
D, L-methionine	20.0
Choline chloride, 50%	8.0
Vitamin-mineral premix ¹	30.0
Calculated value	
Crude protein	200.00
Metabolisable energy, MJ/kg	118.2
Crude fiber	38.0
Calcium	8.30
Available phosphorus	4.50
Analyzed value	
Crude protein	202.50
Calcium	8.50
Total phosphorus	6.80

Table 1. The composition of diet in goslings during 0

to 4 weeks of age.

¹Supplied per kg of diet: vitamin A, 10,000 IU; vitamin D₃, 1,000 IU; vitamin E, 25 IU; vitamin K, 3 mg; thiamin, 3 mg; riboflavin, 5 mg; pyridoxine, 3 mg; vitamin B₁₂, 0.03 mg; Ca-pantothenate, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 3 mg; Mn (MnSO₄.5H₂O), 60 mg; Zn (ZnO), 60 mg; Cu (CuSO₄.5H₂O), 5 mg; Fe (FeSO₄.7H₂O), 70 mg; Se (Na₂SeO₃), 0.1 mg.

at 21 days of age, then remained almost constant. The initial villus area of jejunum was less compared with that of the duodenum; it increased about five-times by 28 days of age, whereas villus area of ileum was the same as jejunum at hatching, but reached least among the three different intestinal sections throughout the experimental period. The results reveal that villus area of jejunum increased (P < 0.05) more rapidly with age than that of the duodenum and ileum. Table 3 shows the development of villus crypt depth, muscle depth and height/width ratio of the small intestine segments in goslings from hatching to 28 days of age. The crypt depth of intestinal segments increased two- to three-times with age. The crypt and muscle depths of villi generally were greatest in the ileum and least in the duodenum. Except at hatching, the villus height/width ratio was significantly (P < 0.05) greater in the jejunum than in the duodenum and ileum. The correlations of small intestine morphological variables with age are shown in Table 4. All variable increased

linearly (R² from 0.47 to 0.82 with P < 0.05 to 0.001). The highest correlation was between the villus height and age (R² = 0.82). The quadratic correlation of height of villi with age was nearly significant (P = 0.052); Y = 369.31 ± 15.54 ± 19.44 ± 2.48 X + 0.16 ± 0.08 X²; where Y is villus height (µm) and X is age in days with R² being 0.76.

Examination of villi by SEM

Figures 1 and 2 show scanning electron micrographs of the small intestine in geese at hatching and 28 days of age, respectively. Small intestinal villi from goslings had two morphological types of villi at hatching, finger-shaped villi or more narrow plate-like villi at hatching. The villi of the duodenum were more rounded at the apex and with a rough surface in the goslings; whereas, the villi of ileum was narrower than those of the duodenum and jejunum at hatching. At hatching, the villi of duodenum had a small and dense finger-like shape. During development to 28 days of age, the villi were transformed to plate-like shapes. The surface of the duodenum and jejunum in goslings was relatively smooth at hatching in comparison with that at 28 days of age. The villus surface of duodenum and jejunum in goslings at 28 days of age had folds and recesses to produce a convoluted surface (Figure 2, panel D-2 and J-2). Higher magnification SEM of the intestinal villi (Figure 2, panel D-2, J-2 and E-2) revealed the many goblet cells, and surrendered to the orifices along the villus tip at 28 days of age in goslings.

DISCUSSION

The intestine as the major interface between an organism and its nutritional environment plays a critical role in postnatal development of a newborn animal (Noy and Sklan, 1995). In chicken, profound growth, morphological changes and functional maturation occur in the intestine during the early post-hatching period (Bayer et al., 1975). The physiological intestinal functions develop along with the morphological changes in mucosal structure especially villus height to increase absorptive efficiency (Hill, 1971). In the goslings (Table 2), there was significant increase in the height, surface and size of individual villi of all intestinal segments during the hatching and 28 days of age. The variable to measure size of the villi, especially villi height was linearly correlated with age (Table 4). These results are consistent with the findings of Crompton and Walters (1979) indicating major changes in the digestive tract, including an increase villus growth of the small intestine during early growth stages. The growth, faster in structure and size of villi in goslings, specify two times height compared with the fifty percentage increase height of villi in chickens during the hatching and four weeks of age (Uni et al., 1995). The villus height in the duodenum increased about 80% from hatching to seven

Days of age	Duodenum	Jejunum	lleum	Mean
Villus height, μm				
HA ^X	342.5 ^{Ad} ±25.57 ^Y	277.9 ^{Bd} ±12.09	297.8 ^{Bd} ±11.88	333.2 ^d ±13.18
7	550.4 ^{Bc} ±28.46	651.1 ^{Ac} ±12.74	473.8 ^{Cc} ±23.95	559.7 ^c ±14.84
14	723.7 ^{Ab} ±14.49	831.5 ^{Ab} ±14.79	628.9 ^{Bb} ±17.01	758.1 ^b ±14.38
21	772.7 ^{Bb} ±22.88	837.9 ^{Ab} ±18.61	753.1 ^{Bb} ±1001	748.1 ^b ±11.46
28	1029.3 ^{Ba} ±15.88	1170.6 ^{Aa} ±11.00	1025.5 ^{Ba} ±13.24	1075 ^a ±10.85
Villus width, μm				
HA	82.7 ^{Ac} ±4.88	77.2 ^{Ac} ±3.18	60.7 ^{Bc} ±3.40	74.35 ^c ±7.80
7	90.1 ^c ±6.49	98.4 ^b ±4.20	94.9 ^b ±2.47	99.02 ^b ±8.05
14	111.8 ^b ±5.21	116.2 ^b ±2.99	114.1 ^{ab} ±3.72	114.2 ^{ab} ±7.80
21	125.8 ^b ±11.58	126.3 ^a ±3.88	120.5 ^a ±2.69	122.7 ^a ±6.21
28	143.0 ^{Aa} ±1015	121.1 ^{Bab} ±2.72	114.9 ^{Bab} ±2.39	126.6 ^ª ±5.88
Villus perimeter, μ	m			
HA	923.2 ^{Ad} ±45.59	670.2 ^{Bd} ±23.24	735.1 ^{Bd} ±23.99	764.5 ^d ±33.21
7	1368.4 ^{Bc} ±42.73	1556.0 ^{Ac} ±24.02	1208.3 ^{Cc} ±50.66	1379.2 ^c ±34.26
14	1932.2 ^{Ab} ±41.89	1948.0 ^{Ab} ±28.28	1507.0 ^{Bb} ±39.53	1793.0 ^b ±34.01
21	1933.6 ^{Ab} ±67.36	1981.0 ^{Ab} ±37.81	1528.8 ^{Bb} ±23.48	1814.2 ^b ±28.41
28	2362.3 ^{Ba} ±39.90	2683.2 ^{Aa} ±48.94	2365.6 ^{Ba} ±27.96	2471.2 ^a ±25.05
Villus area, μm ² × 1	10 ³			
HA	53.0 ^{Ad} ±2.96	31.7 ^{Bc} ±0.89	32.7 ^{Bd} ±0.89	35.0 ^d ±9.14
7	70.3 ^{ABc} ±6.03	79.0 ^{Ad} ±2.79	61.2 ^{Bc} ±3.79	70.2 ^c ±5.42
14	121.2 ^{Ab} ±7.29	124.5 ^{Ab} ±6.30	81.1 ^{Bb} ±3.83	107.8 ^b ±5.25
21	125.7 ^{Ab} ±14.01	132.2 ^{Ac} ±4.01	86.7 ^{Bb} ±2.19	114.8 ^b ±4.18
28	150.5 ^{Aa} ±5.27	173.3 ^{Aa} ±3.52	138.0 ^{Ba} ±2.62	147.3 ^a ±3.96

Table 2. The development of villus height, width, perimeter and area in the small intestine segments of white

 Roman goslings from hatching to 28 days of age.

^{A, B}Means within the same row without the same superscript differ significantly (P < 0.05). For each age group, n = 6. ^{a, b}

^{b, c, d}Means within the same column without the same superscript differ significantly (P < 0.05). For each age group, n = 6. ^xHA, Day of hatching. ^YEach value is the mean ± SE for histological observation of villi.

days of age in the goslings. The villus area of the small intestinal segments enlarged three to five-fold during the first four weeks (Table 2). In general, the development of villi of small intestine by feed intake increased. The result is consistent with the rapid expansion in size and complexity of plica (internal folds) of villi during the development period of chicks (Uni et al., 1998). Crypt depth which reflects enterocyte-differentiating activity increased linearly in all intestinal segments. At 28 days of age, the crypt depth of jejunum and ileum were greater than in the duodenum. These results agree with those from microscopic examination of growth of the small intestine in chicks (Noy and Sklan, 1997).

The intestinal morphology of villi in geese at hatching with two morphological types of villi, finger-shaped or narrow cylinder-like is similar to those of duodenal and jejunal villi of mice (Rao and William, 1972) and chickens (Bayer et al., 1975). The micrographs of villi in duodenum and jejunum of day-old goslings showed slender finger-like shape, afterwards developed slowly to broad, leaf-like or tongue-like villi at 28 days of age in gosling. These data are in agreement with Yu et al. (1998). In the present study, the rough areas of villi showed epithelial disruptions with apparent folds at 28 days of age.

Examination of mucosa in the duodenum and jejunum revealed a large absorptive surface area, especially the area was enlarged with more apparent folds from an epithelial disruption of villi in the intestinal mucosa of chicks, indicating that villus shape are similar to the goslings seen in the chicks (Bayer et al., 1975). In the end experiment, villi of ileum in the goslings had a thickrounder finger-shaped tip; it showed developing thinner and narrower column villi than those in the duodenum and jejunum. The result is consistent with the ileum of pigs

Days of age	Duodenum	Jeiunum	lleum	Mean
Crypt depth_um		,		
	68 3 ^{Bb} +6 54 ^Y	64 4 ^{Bb} +2 34	06 2 ^{Ac} +5 40	74 28 ^d +3 27
7	$1210^{Aa} + 162$	124 9 ^{Aa} +2 04	105 0 ^{Bc} +2 40	14.20 ± 0.27
	121.9 ± 4.03	124.0 ± 3.94	105.9 ± 3.40	110.0 ± 4.37
14	125.1 ⁵ ±5.90	139.1 ^m ±3.42	125.1 ⁵ ±4.28	129.7°±4.24
21	137.3 ^a ±10.73	137.8 ^a ±4.60	156.6 ^b ±3.08	143.9 ^a ±3.58
28	142.9 ^{Ba} ±8.46	170.3 ^{Aa} ±0.50	189.1 ^{Aa} ±5.68	166.8 ^a ±3.20
Muscle thickness	s, μm			
HA	174.4 ^{Bd} ±4.30	154.0 ^{Cc} ±3.54	205.6 ^{Ac} ±8.47	174.2 ^d ±3.97
7	218.5 ^{Bc} ±7.47	214.9 ^{Bb} ±5.95	234.9 ^{Ac} ±6.24	223.5 ^c ±9.59
14	260.3 ^{Bb} ±10.17	267.1 ^{Bb} ±7.01	338.8 ^{Ab} ±10.04	291.3 ^b ±9.29
21	271.7 ^{Bb} ±4.60	278.7 ^{Bb} ±7.98	353.9 ^{Ab} ±5.24	305.8 ^b ±7.40
28	313.4 ^{Ca} ±4.78	386.8 ^{Ba} ±14.68	386.8 ^{Ba} ±14.68 488.7 ^{Aa} ±11.66	
Villus height/wid	th ratio			
HA	4.17 ^c ±0.40	4.11 ^c ±0.28	4.97 ^b ±0.38	4.74 ^d ±0.21
7	6.11 ^{ABb} ±0.39	6.62 ^{Ab} ±0.35	4.53 ^{Bb} ±0.28	5.89 ^c ±0.24
14	6.47 ^{Bb} ±0.33	7.15 ^{Ab} ±0.26	5.51 ^{Bb} ±0.23	6.78 ^b ±0.23
21	6.12 ^{Bb} ±0.59	6.63 ^{Ab} ±0.24	6.27 ^{Bb} ±0.14	6.51 ^b ±0.18
28	7.20 ^{Ba} ±0.47	9.16 ^{Aa} ±0.22	8.93 ^{Aa} ±0.25	9.45 ^a ±0.17

Table 3. The development of crypt depth, muscle depth and villus height / width ratio in the small intestine segments of white Roman goslings from hatching to 28 days of age.

^{A, B, C}Means within the same row without the same superscript differ significantly (P < 0.05). For each age group, n = 6. ^{a, b, c}Means within the same column without the same superscript differ significantly (P < 0.05). For each age group, n = 6. ^XHA, Day of hatching. ^YEach value is the mean \pm SE for histological observation of villi.

Table 4. The correlation of small intestinal morphology variables with age.

ltem	Intercept	Slope	R ²	Significance	Orthogonal comparison ¹
Villus height	441.41±21.09	20.12±1.05	0.82	P < 0.001	L
Villus width	79.49±20.44	2.18±1.02	0.47	P < 0.05	L
Villus perimeter	99.66±49.99	47.83±2.50	0.77	P < 0.001	L
Villus area	65793.81±12127.84	2427.29± 607.42	0.52	P < 0.001	L
Crypt depth	84.66±7.95	2.29±0.39	0.62	P < 0.001	L
Muscle thickness	180.46±5.33	4.73±17.69	0.73	P < 0.001	L

¹Orthogonal comparison of the mean of variables on days of age in goslings. L, Linear effect.

which had a finger-like villi rather than plate-like with the SEM observation (Waxler, 1972). At 4 weeks of age, the jejunal villi grew faster compared with the duodenum and ileum. The result is similar to the findings of Noy and Sklan (1997), who observed more crevices of jejunal villi for absorptive volume enlargement of chicks. In the present study, we observed the rapid morphologic development of each segment in the small intestine; with subsequent increase in the weight gain from hatching to 28 days of age. The growth of villi was initially faster in the

duodenum, and subsequently there were rapid enlargement in the jejunum from 14 to 28 days of age. Ingested nutrients are mostly absorbed in the upper part of intestine (Yamauchi et al., 1993), suggesting the conclusion that morphology and function are closely related. These results are consistent with Shih et al. (2005) indicating that the development of gastrointestinal tract specify the villus morphology of small intestine more rapidly than body weight, through the early growing period of goslings.



Figure 1. Scanning electron micrograph of small intestinal segments of goslings at hatching. D-1, Finger-like villi in the middle zone of the duodenum, x100; D-2, partially magnified duodenal villi, x750; J-1, finger-like villi in the middle zone of the jejunum, x100; J-2, magnified jejunal villi, x750; E-1, finger-like villi in the middle zone of the ileum, x100; E-2, magnified ileal villi, x750.



Figure 2. Scanning electron micrograph of small intestinal segments of goslings at 28 days of age. D-1, Finger-like villi in the middle zone of the duodenum, x100; D-2, partially magnified duodenal villi, x750, the villus surface showing numerous folds and recesses; J-1, finger-like villi in the middle zone of the jejunum, x100; J-2, **p**artially magnified jejunal villi, x750 showing goblet cell pores and cell extrusion zone in the circular frame; E-1, finger-ileal villi, x100 (the villi of the ileum had fewer creases and folds, compared to the duodenum and jejunum); E-2, magnified ileal villi, x750.

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