JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.2025004

Histological Changes of the Mucosal Epithelium in the Chicken Intestine during Pre- and Post-Hatching Stages

Md. Al Amin[1], Md Badiul Alam[2] and Kohzy Hiramatsu[3]

[1]Department of Science and Technology, Graduate School of Medicine, Science and Technology, Shinshu University, Kami-ina, Nagano 399-4598, Japan.
[2]Department of Agriculture, Graduate School of Science and Technology, Shinshu University, Kami-ina, Nagano 399-4598, Japan.
[3]Laboratory of Animal Functional Anatomy (LAFA), Faculty of Agriculture, Shinshu University, Kami-ina, Nagano 399-4598, Japan

This study clarified the histological changes in the mucosal epithelium of the chicken intestine during the pre- and post-hatching stages. The duodenum, jejunum, ileum, and colorectum were collected from embryos at 15, 17, 18, 19, and 21 days of incubation and from chicks at 1 and 3 days after hatching. Paraffin sections prepared from tissue samples were stained with periodic acid-Schiff followed by alcian blue for histological analysis and to detect goblet cells. Villin and β-actin were detected using double immunofluorescence. Villi with finger-like shape were already observed in embryos after 15 days of incubation, and no obvious change in shape was observed even after hatching. Villous height increased in all intestinal regions as the developmental stage progressed, particularly a few days before and after hatching. Goblet cells first appeared in the epithelium of all intestinal regions after 18 days of incubation. The density of goblet cells rapidly increased from 18 to 21 days of incubation. Both villin and β-actin immunoreactivities were detected at the apical surface of the villous epithelium in all intestinal regions, and villin immunopositivity was stronger in the jejunum and ileum after hatching. These findings indicate that the villi and microvilli of the intestine of broiler chickens show histological changes during few days just before and after hatching. Additionally, the density of goblet cells rapidly increased for a few days before hatching.

Key words: chicken, development, epithelium, histological changes, intestine

J. Poult. Sci., 62: jpsa.2025004, 2025

Introduction

The intestinal tract, particularly the small intestine, has specialized villi morphology, which is suitable for the digestion and absorption of ingested feed. The cells that make up the mucosal epithelium of the small intestine are divided into three main groups: absorptive epithelial cells, goblet cells and endocrine cells. Absorptive epithelial cells form the majority of the constituent cells and have a structure that is conducive to the absorption of nutrients, which is the main function of the intestinal tract. There are numerous brush-like projections on the apical surface of these cells, which may be described as microvilli. The core of the microvilli is made up of β-actin, to which many proteins, including villin, bind to maintain the microvilli skeleton[1,2]. Microvilli are composed of 20-30 β-actin filaments and homogeneous extensions[3]. Actin has complex interactions with cellular membranes and mediates many functions, such as cell motility and maintenance of cell shape in chicks[4] and mice[5]. Villin is the first crucial actin-binding protein to locate to the apical surface of enterocytes[4,5]. These findings indicate that actin and actin-binding proteins play important roles in the functional expression of absorptive epithelial cells.

Numerous goblet cells are scattered throughout the epithelium of the intestinal tract and secret the glycoprotein mucin, which forms a protective mucus layer on the surface of the epithelium that protects intestinal tissues from foreign substances in the intestinal lumen. The lumen of the intestinal tract contains numerous bacterial florae, which are trapped in the mucus layer on the surface of the intestinal epithelium, protecting the organism from

Received: September 30, 2024, Accepted: December 4, 2024
Available online: January 17, 2025
Correspondence: Kohzy Hiramatsu, Ph.D., Laboratory of Animal Functional Anatomy (LAFA), Faculty of Agriculture, Shinshu University, Minami-minowa 8304, Kami-ina, Nagano 399-4598, Japan. (E-mail: seitaik@shinshu-u.ac.jp)

bacteria. Therefore, goblet cells are important for chicks to exhibit the same feeding habits as their parents after hatching.

Endocrine cells are also distributed in the mucosal epithelium of the intestine and secrete hormones such as glucagon-like peptide-1 and neurotensin to support digestive tract function[6,7]. These hormones exert important physiological effects on intestinal functions, including the regulation of motility and blood flow. Many types of endocrine cells are also in the chicken small intestine and are responsible for regulating function[7].

In the early days after hatching, chicks rely on the yolk sac to meet their nutritional needs[8–10], and peck their feed in a manner that resembles the feeding behavior of their parents. This feeding behavior differs significantly from that of mammals, which change gradually after birth. In addition, broilers show a rapid gain in body mass, reaching over 4,000g at 56 days of age. These results suggest that the functional morphology of broiler intestines contributes to the rapid increase in body mass. Although many studies have been conducted on the histological changes in the chicken gastrointestinal tract before and after hatching, there is no consensus on the process of microvilli formation and the characteristics of goblet cells. Few studies have focused on the morphological changes as the basis for rapid mass gain before and after hatching. The aim of this study was to determine the histological changes in the mucosal epithelium of the intestine before and after hatching, particularly in absorptive epithelial and goblet cells. The relationship between the rapid gain in body mass of broilers after hatching and the results obtained are also discussed.

Materials and Methods

The experimental protocol of this study was reviewed by the Shinshu University Committee for Animal Experiments and approved by the president of Shinshu University (approval number: 021028).

General histology

Fertile eggs of White Cornish broiler were purchased from Hyogo Station of National Livestock Breeding Center (NLBC). Eggs were incubated in an incubator regulated at 37±1 °C and 58-60% humidity. Tissue samples were collected at pre- and post-hatching stages when significant morphological changes were expected. Intestinal segments were collected from embryos at 15, 17, 18, 19 and 21 days of incubation and chicks at 1 and 3 days after hatching. Six individuals were used in this study at each stage. The duodenum, jejunum, ileum, and colorectum were dissected out to obtain tissue samples. Tissue samples were rinsed with 0.75% sodium chloride solution and fixed in Bouin's fluid (code No.023-17361, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 4 °C for 24 h. Processed samples were embedded in paraffin wax according to conventional methods. Paraffin sections were cut at 5 μm thickness with a microtome. To observe the general histology of each intestinal regions and goblet cells, sections were stained with periodic acid-Schiff (PAS) and Alcian blue (AB) and counterstained with Mayer's hematoxylin solution (code No.131-09665, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Morphometrical analysis

Morphometrical analysis of the villous height and density of goblet cells was performed under a light microscope (Axio-Imager, Zeiss, Göttingen, Germany). For the villous height, 20 well-oriented villi were randomly selected from each intestinal region of each bird. The height of 400 villi was measured at each stage. The density of goblet cells was measured as previously described[11].

Double immunofluorescence

To detect immunoreactivities of villin and β-actin, the double immunofluorescence method was carried out by a previously described method[11]. Deparaffinized sections were treated with antigen retrieval solution (Immunosaver, Nisshin EM, Tokyo, Japan) at 98°C for 40 min and transferred to 10% normal goat serum. Sections were incubated with mouse anti-villin monoclonal antibody against chicken villin (diluted 1:20, sc-58897, Santa Cruz Biotechnology, Dallas, TX, USA) for 24 h followed by a 24 h incubation with rabbit antiserum against C-terminal of human β-actin (diluted 1:5,000, GTX109639, Gene Tex, Irvine, CA, USA), that has been demonstrated to be crossreactive with chicken β-actin by the manufacturer, for 24 hrs. After binding by primary antibodies, the sections were incubated with a mixture of DyLight 549-labeled goat anti-rabbit IgG serum (diluted 1:300, 611-142-122, Rockland Immunochemicals, Pottstown, PA, USA) and DyLight 488-labeled goat anti-mouse IgG serum (diluted 1:300, 610-141-121, Rockland Immunochemicals, Pottstown, USA) for 3 h. After coverslipping with an aqueous mounting medium, the preparations were observed under a fluorescence microscope (Zeiss). All incubations were performed in a moist chamber at room temperature. After each incubation step, sections were washed with phosphate buffered saline three times for 5 min each.

As negative controls, the primary antibodies against villin and β-actin were replaced by mouse anti-Ki-67 monoclonal antibody (1:20, MAB4190, Sigma-Aldrich, St. Louis, MO, USA) and rabbit anti-chicken IgG serum (1:5,000, A30-207A, Bethyl Laboratories, Montgomery, TX, USA), respectively, and the same procedure was used for staining. A negative control in which the primary antibody was omitted was also performed.

Statistical analysis

One-way analysis of variance was used for the statistical analysis of data using Minitab version 21.2.0 where Tukey's comparisons test was used to measure the significant difference among the intestinal segments on each incubation day. Data are presented as the mean ± standard deviation (SD). Statistical significance was set at $P < 0.05$.

Result

General histology

The villi showed a finger-like shape in all intestinal regions of the embryos after 15 days of incubation, and no obvious differences in shape were observed even after hatching (Fig. 1a-d). Crypts were observed in the epithelium of the whole intestine

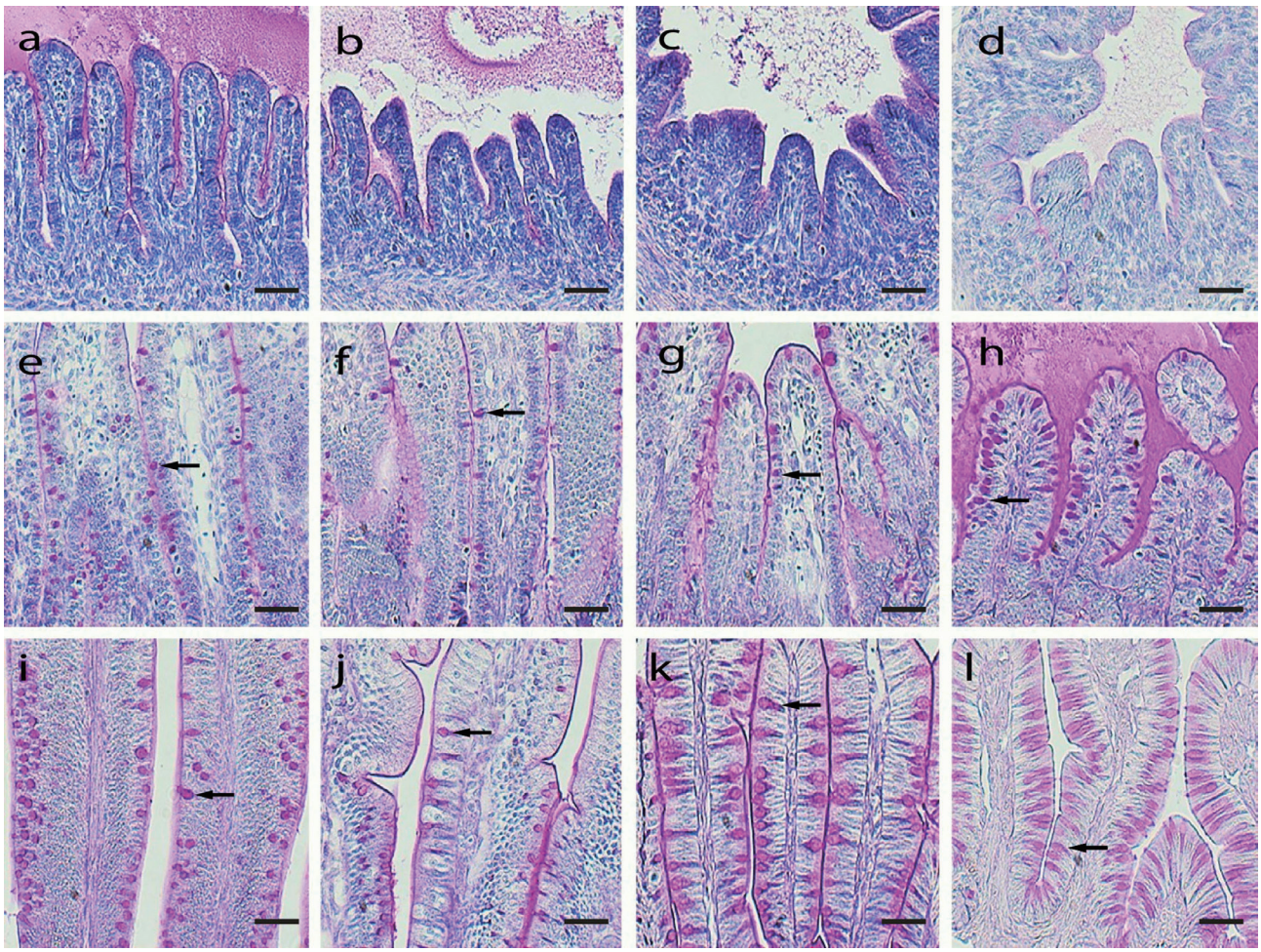

Fig. 1. **Light microscopic images of PAS reaction in duodenum (a, e, i), jejunum (b, f, j), ileum (c, g, k) and colorectum (d, h, l) from embryos at 15 days of incubation (upper columns) and 21 days of incubation (middle columns) and from chicks at 3 days after hatching (lower columns).** There are almost no PAS-positive cells in the entire intestine from embryos at 15 days of incubation. PAS-positive cells (arrows) increase in their number after hatching throughout the intestine. Bars indicate 20μm.

before hatching, but were not well developed. However, they were well-defined and contained several mitotic cells 3 days after hatching. PAS-positive cells were first observed after 18 days of incubation and were not detected before that stage. These cells were distributed in the villous epithelium of all intestinal regions. In the embryonic stages, most goblet cells in each intestinal region contained small and round mucigens showing PAS-positive staining; however, they became elongated after hatching (Fig. 1e-h). In preparations stained with AB following PAS staining, no goblet cell showing PAS-positive reaction only was observed (Fig. 2). All PAS-positive cells were also AB-positive.

Morphometrical analysis

Morphometric data for villous height are summarized in Table 1. At all developmental stages, villous height was significantly higher in the duodenum and lower in the colorectum than those in other intestinal regions. Villous height significantly increased in all intestinal regions as the developmental stage progressed. However, there were differences in villous elongation among the four intestinal regions. The degree of villous elongation in the jejunum was approximately 2 fold during the 3 days before hatching (18DI to 21DI), but approximately 1.5 times during the 3 days after hatching (21DI to 3DH). Villous elongation in the ileum and colon was approximately 1.3 and 1.5 fold during the 3 days before hatching, but were approximately 2 fold during the 3 days after hatching. The degrees of villous elongation in the duodenum 3 days before and after hatching were approximately 1.8 and 2.0 fold, respectively.

The densities of goblet cells are shown in Table 2. The density of goblet cells was highest in the duodenum and lowest in the jejunum among the four intestinal regions in all developmental stages. The density significantly increased in all intestinal regions as the developmental stage progressed, but increased more

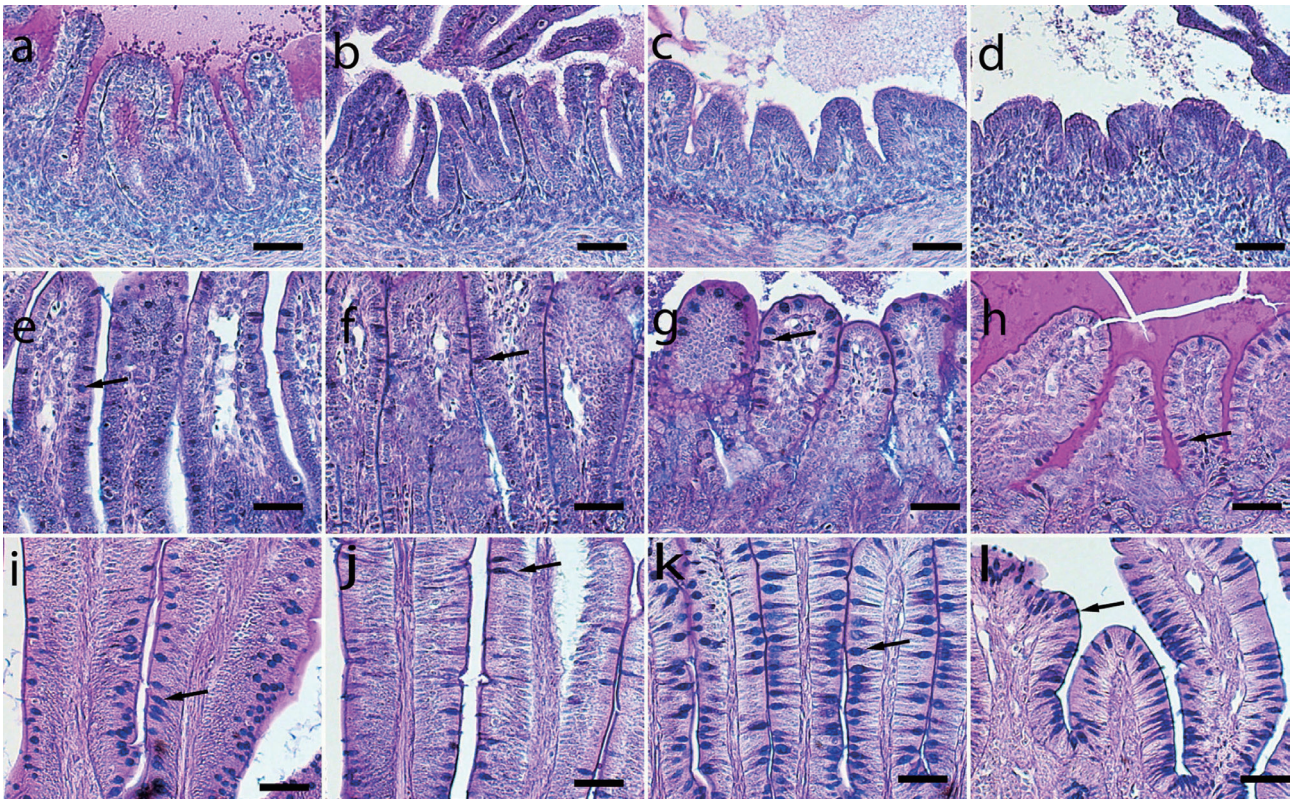

Fig. 2. **Light microscopic images of AB reaction following PAS reaction in duodenum (a, e, i), jejunum (b, f, j), ileum (c, g, k) and colorectum (d, h, l) from embryos at 15 days of incubation (upper columns) and 21 days of incubation (middle columns), and from chicks at 3 days after hatching (lower columns).** AB-positive cells (arrows) show similar expression and distribution patterns as PAS-positive cells in Fig. 1. There are no cells showing PAS-reaction only in all intestinal regions at all developmental stages. Bars indicate 20μm.

Table 1. **Villous height (μm) in the duodenum, jejunum, ileum, and colorectum at each developmental stage.**

Stage	Duodenum	Jejunum	Ileum	Colorectum
15DI	99.5 ± 13.2 $^{\alpha, g}$	79.0 ± 11.4 $^{\beta, f}$	63.9 ± 11.5 $^{\gamma, g}$	23.7 ± 7.6 $^{\delta, g}$
17 DI	119.8 ± 27.2 $^{\alpha, f}$	91.9 ± 16.0 $^{\beta, e}$	79.1 ± 13.8 $^{\gamma, f}$	63.9 ± 11.7$^{\delta, f}$
18 DI	141.4 ± 32.5 $^{\alpha, e}$	115.5 ± 21.7 $^{\beta, d}$	106.1 ± 16.1 $^{\gamma, e}$	82.6 ± 18.2 $^{\delta, e}$
19 DI	196.2 ± 44.7 $^{\alpha, d}$	132.4 ± 17.9 $^{\beta, c}$	120.4 ± 21.6 $^{\gamma, d}$	93.9 ± 27.1 $^{\delta, d}$
21 DI	293.4 ± 52.9 $^{\alpha, c}$	241.0 ± 40.6 $^{\beta, b}$	141.3 ± 24.7 $^{\gamma, c}$	124.0 ± 28.8 $^{\delta, c}$
1 DH	336.9 ± 55.6 $^{\alpha, b}$	251.1 ± 40.8 $^{\beta,b}$	186.7 ± 39.6 $^{\gamma, b}$	167.8 ± 40.7 $^{\delta, b}$
3 DH	534.6 ± 46.6 $^{\alpha, a}$	358.8 ± 48.5 $^{\beta, a}$	282.3 ± 37.9 $^{\gamma, a}$	250.3 ± 35.2 $^{\delta, a}$

Values are presented as mean ± standard deviation. α>β>γ>δ: compared among four intestinal regions at the same stage ($P< 0.05$). a>b>c>d>e>f>g: compared among developmental stages in the same intestinal regions ($P< 0.05$). DI: days of incubation. DH: days after hatching.

rapidly from 18 to 21 days of incubation compared to that after hatching.

Immunohistochemistry for β-actin and villin

Immunoreactivities for villin and β-actin were observed on the surface of villous epithelium at all intestinal regions (Figs. 3, 4). Immunoreactivity for β-actin was observed in all intestinal regions at 15 days of incubation and covered the apical surface of absorptive epithelial cells (Fig.3a-d). This distribution pattern did not change as the stage progressed (Fig.3). However, the distribution patter of villin immunoreactivity chaged as the stage progressed. After 15 days of incubation, villin immunoreactivity was weak in the duodenum, jejunum and ileum (Fig.4a-c), and relatively strong in the colorectum (Fig.4d). Immediately before hatching, immunoreactivity was strong at the apical surface of

Table 2. **Density of goblet cells (number of cells/100 μm) in the duodenum, jejunum, ileum, and colorectum at each developmental stage.**

Stage	Duodenum	Jejunum	Ileum	Colorectum
15DI	-	-	-	-
17 DI	-	-	-	-
18 DI	$4.2 \pm 1.3^{\alpha, e}$	$1.4 \pm 0.6^{\gamma, e}$	$1.9 \pm 0.9^{\beta, e}$	$2.0 \pm 0.9^{\beta, e}$
19 DI	$5.6 \pm 1.4^{\alpha, d}$	$2.9 \pm 1.0^{\gamma, d}$	$3.6 \pm 1.3^{\beta, d}$	$3.9 \pm 1.1^{\beta, d}$
21 DI	$9.0 \pm 1.8^{\alpha, c}$	$4.5 \pm 1.6^{\gamma, c}$	$5.1 \pm 1.3^{\beta, c}$	$5.6 \pm 1.8^{\beta, c}$
1 DH	$14.7 \pm 2.9^{\alpha, b}$	$8.2 \pm 2.0^{\gamma, b}$	$10.4 \pm 2.1^{\beta, b}$	$11.1 \pm 2.1^{\beta, b}$
3 DH	$20.7 \pm 3.2^{\alpha, a}$	$11.8 \pm 2.9^{\gamma, a}$	$13.1 \pm 2.7^{\beta, a}$	$14.0 \pm 2.6^{\beta, a}$

Values are presented as mean ± standard deviation. α>β>γ: compared among four intestinal regions at the same stage ($P< 0.05$). a>b>c>d>e: compared among developmental stages in the same intestinal regions ($P< 0.05$). DI: days of incubation. DH: days after hatching. -: not detected.

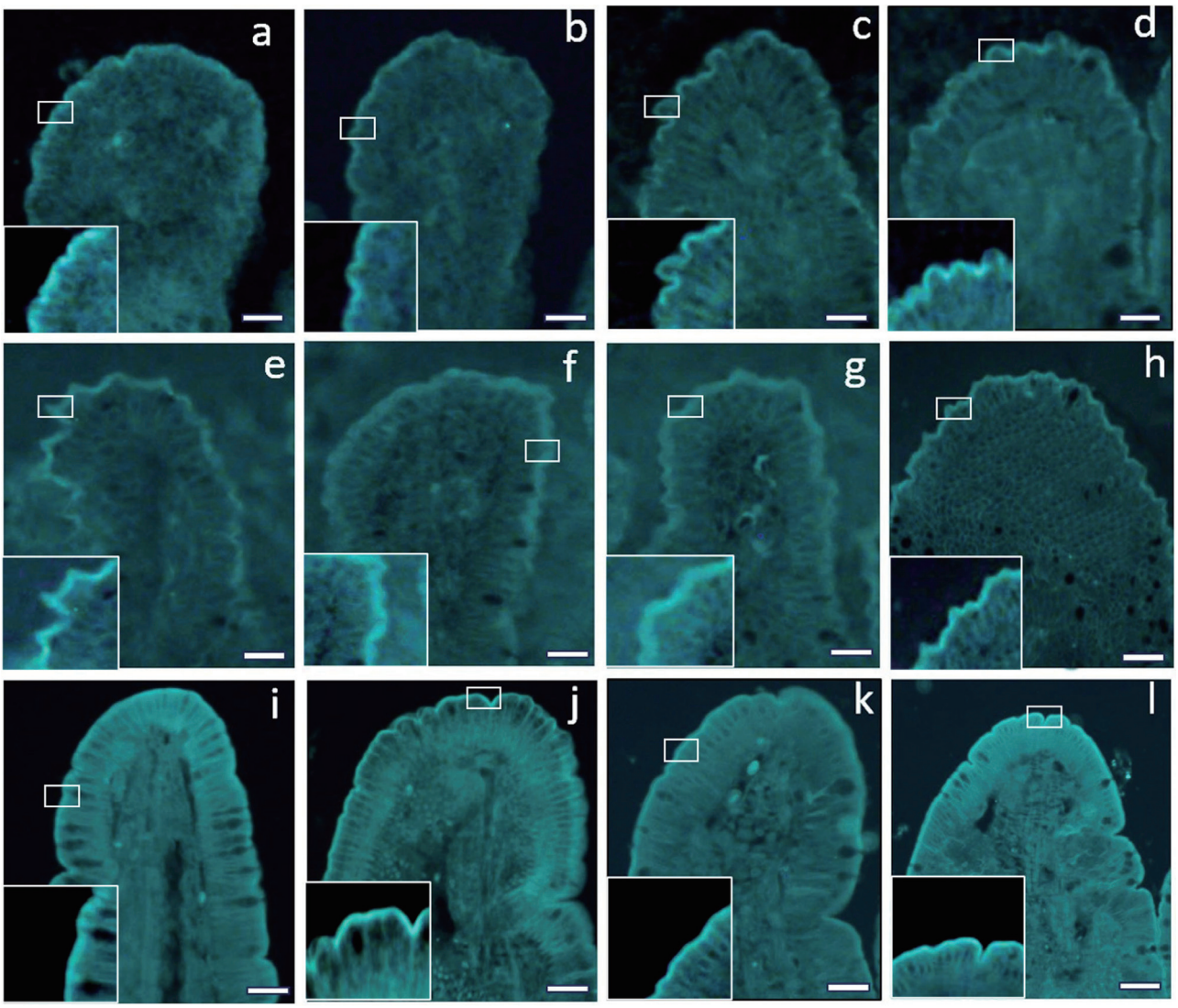

Fig. 3. **Distribution of β-actin immunoreactivity in duodenum (a, e, i), jejunum (b, f, j), ileum (c, g, k) and colorectum (d, h, l) from embryos at 15 days of incubation (upper columns) and 21 days of incubation (middle columns), and from chicks at 3 days after hatching (lower columns).** Immunoreactivity of β-actin is observed on the apical surface of the absorptive epithelial cells in all intestinal regions (insets). Bars indicate 20μm.

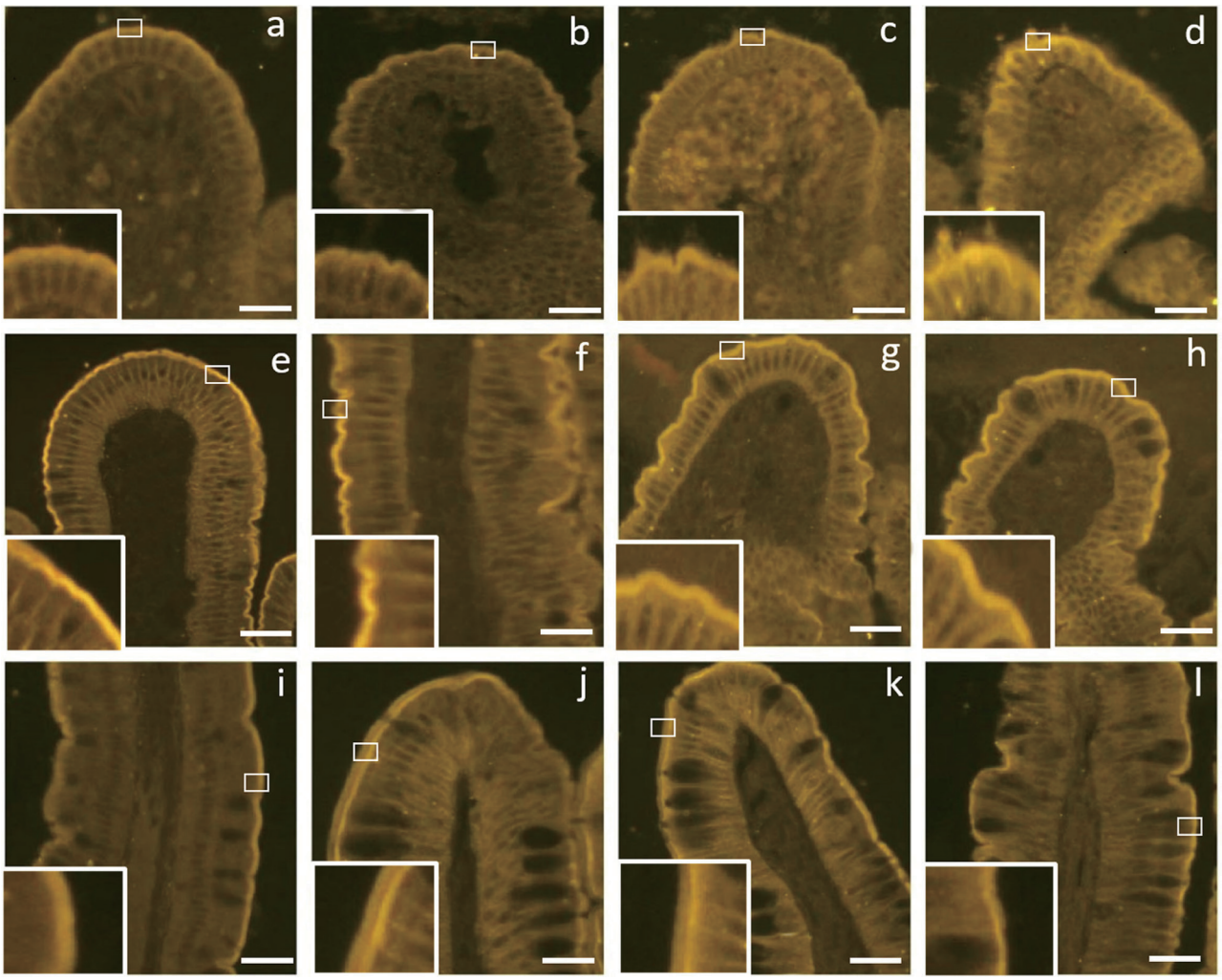

Fig. 4. **Distribution of villin immunoreactivity in duodenum (a, e, i), jejunum (b, f, j), ileum (c, g, k) and colorectum (d, h, l) from embryos at 15 days of incubation (upper columns) and 21 days of incubation (middle columns) and from chicks at 3 days after hatching (lower columns).** Villin-immunoreactivity is weakly observed on the apical surface of the absorptive epithelial cells throughout all intestinal regions at 15 days of incubation (insets of a-d), but is strongly found over the entire apical surface of the absorptive epithelial cells in all intestinal regions at 21 days of incubation (insets of e-h). At 3 days after hatching, villin immunoreactivity show similar distribution pattern to that at 21 days of incubation (insets of i-l), but is strongly observed at the base of the microvilli of the absorbing epithelial cells in jejunum and ileum (insets of j and k). Bars indicate 20μm.

the absorptive epithelial cells in all intestinal regions (Fig.4e-h). Three days after hatching, villin immunoreactivity was not observed on the outermost surface but was observed at the base of the microvilli of absorptive cells (Fig.4i-l). The localization of villin immunoreactivity in microvilli was marked in the jejunum (Fig.4j) and ileum (Fig.4k).

No immunoreactivity was observed on the surface of the villous epithelium when primary antibodies were replaced with other antibodies (Fig.5). Similar results were obtained when primary antibodies were omitted.

Discussion

The present study demonstrated that the villi of chick intestine show histological changes before and after hatching. This suggested that the microvillous structure of absorptive epithelial cells in the jejunum and ileum was also completed at the same time. Intestinal villus is an anatomical structure that projects into the intestinal lumen[12], increasing surface area and allowing for efficient absorption of nutrients. Chicks are supplied with nutrients from the yolk sac remnants for the first few days after hatching, while they are fed directly with exogenous diet. The immediate post-hatching period is a time of transition from a lipid-rich

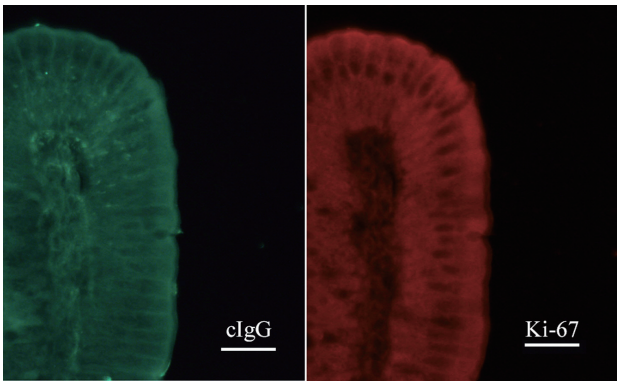

Fig. 5. **Fluorescence microscopic images of negative controls. Immunoreactivities are not observed on the surface of the villous epithelium in the ileum at 3 days after hatching.** cIgG: chicken immunoglobulin G. Bars indicate 20μm.

yolk as a source of nutrition to a carbohydrate- and protein-rich exogenous diet[13]. This transition is complemented by rapid gastrointestinal tract development[14]. During the first week after hatching, the weight of the small intestine increases more rapidly than body weight[13,15,16]. Growth of villi in the duodenum, jejunum, and ileum occurs at different ontogenetic times, and rapid morphological changes occur after hatching[13]. However, the present study demonstrated that regional differences in the degree of intestinal villi elongation occured before and after hatching in chickens. In the duodenum, significant elongation of the villi was observed 3 days before and 3 days after hatching. In the jejunum, significant elongation of villi was observed 3 days before hatching, and in the ileum and colorectum, it was observed 3 days after hatching. These results indicated that, even after hatching, there were differences in histological changes in the epithelium depending on the intestinal region. These differences may be due to functional differences in each region, residual yolk sacs, or feeding effects; however, the reason for these differences remains to be determined.

Karcher and Applegate[17] have reported that the microvillous length changes throughout the incubation period and that the villous tip increases from just after hatching to 3 days. Microvilli are fine finger-like projections that cover apical surface of absorptive epithelial cells and are at the forefront of intestinal absorptive function. The core of microvilli is composed of a bundle of β-actin and many other proteins that bind to it[18]. Villin is one of the β-actin-binding proteins related to the assembly of microvilli[19] and its expression increases with the developmental stage[20]. In this study, the mode of villin expression varied according to the developmental stage. Before hatching, weak villin immunoreactivity was observed on the surface of the mucosal epithelium, but it was stronger just before hatching. Three days after hatching, villin immunoreactivity was observed throughout the microvilli in the jejunum and ileum but was absent at their tips. However, insulin receptor tyrosine kinase substrate (IRTKS) localizes to the distal tips of actively growing epithelial microvilli[21,22], which may explain the lack of villin immunoreactivity at the microvillous tips, as described above.

According to the results of the negative controls, the immunoreactivity for villin and β-actin observed in this study was neither nonspecific nor hyperreactive.

Goblet cells, which line the intestinal mucosal epithelium, synthesize and secrete mucins, a glycoprotein. Mucins form a mucus layer that covers the surface of the intestinal epithelium[23]. The mucus layer functions as a barrier that protects the organism from toxic substances in the intestinal lumen[24]. Several studies have been conducted on goblet cell development in chickens, however, the results have been inconsistent. Uni et al.[23] have that goblet cells of Ross broilers were first observed after 18 days of incubation. Yu et al.[25] revealed that goblet cells of Arbor Acres broilers first appeared after 15 days of incubation. Reinolds et al.[26] have indicated that goblet cells are present in Cobb broilers after 19 days of incubation; however, they did not make any experiments on earlier developmental days. In this study using White Cornish broilers, similar to the results of Uni et al.[23], goblet cells first appeared after 18 days of incubation in all intestinal segments. Although there were discrepancies between these results, they were consistent in that goblet cells differentiated and developed in the days before hatching. Additionally, the density of goblet cells increased with stage progression. The density of goblet cells increased by 3.2 fold in the jejunum and 2.7 fold in the ileum from 18 days of incubation to just before hatching (21 days of incubation). Yu et al.[25] have shown that the number of goblet cells in the jejunum and ileum increases by 4.5 and 7.1 fold, resspectivly, from 18 to 21 days of incubation. Thus, during the period between the initial appearance and hatching, goblet cells rapidly increase in number and establish a defense system in the mucus layer against various pathogens.

Furthermore, almost all goblet cells showed AB-PAS positive staining, which did not vary according to developmental stage. AB (pH 2.5) binds acidic mucins and PAS agents bind neutral mucins. Generally, mucins can hold large amounts of water within their molecules. Acidic mucins are charged negatively by their sulfate groups and can hold a lot of water in their molecules. This creates a physical barrier on the surface of the intestinal mucosa, thereby blocking invasion of bacteria and other organisms. Thus, in the broiler intestine, the number of goblet cells increases during the pre-hatching period, creating a defense system against infection composed of acid mucins. Moreover, the pH of the contents in the chicken gastrointestinal tract is mostly acidic and differs from that of other domestic animals.. The percentage of acidic and neutral types of goblet cells in broilers varies according to the developmental stage[27,28]. This discrepancy with our results may be due to differences in strains or feeding methods used. Further research is required to determine the factors responsible for this phenomenon.

This study concluded that the villi and microvilli of the intestine of broiler chickens showed histological changes during the few days before and after hatching to prepare for the diges-

tion and absorption of exogenous nutrients. In addition, during the days prior to hatching, goblet cells rapidly increased in density and establish a defense system comprising the mucus layer, which was composed of acidic mucins.

Author Contributions

Md. Al Amin contributed to the conceptualization, methodology, formal analysis, investigation, resources, and writing the original draft. Md Badiul Alam contributed to the investigation and resources. Kohzy Hiramatsu contributed to the conceptualization, methodology, formal analysis, investigation, writing review, editing, and supervision.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Ferrary E, Cohen-Tannoudji M, Pehau-Arnaudet G, Lapillonne A, Athman R, Ruiz T, Boulouha L, Marjou FE, Doye A, Fontaine J-J, Antony C and Babinet C. Louvard, Jaisser F and Robine S. In vivo, villin is required for Ca-dependent F-actin disruption in intestinal brush borders. J Cell Biol, **146**: 819–829. 1999. https://doi.org/10.1083/jcb.146.4.819, PMID:10459016

[2] Shyer AE, Tallinen T, Nerurkar NL, Wei Z, Gil ES, Kaplan DL, Tabin CJ and Mahadevan L. Villification: how the gut gets its villi. Science, **342**: 212–218. 2013. https://doi.org/10.1126/science.1238842, PMID:23989955

[3] Markovic MA and Brubaker PL. The roles of glucagon-like peptide-2 and the intestinal epithelial insulin-like growth factor-1 receptor in regulating microvillus length. Sci Rep, **9**: 13010. 2019. https://doi.org/10.1038/s41598-019-49510-5, PMID:31506583

[4] Shibayama T, Carboni JM and Mooseker MS. Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. J Cell Biol, **105**: 335–344. 1987. https://doi.org/10.1083/jcb.105.1.335, PMID:2956268

[5] Ezzell RM, Chafel MM and Matsudaira PT. Differential localization of villin and fimbrin during development of the mouse visceral endoderm and intestinal epithelium. Development, **106**: 407–419. 1989. https://doi.org/10.1242/dev.106.2.407, PMID:2686960

[6] Monir MM, Hiramatsu K, Yamasaki A, Nishimura K and Watanabe T. The influence of restricted feeding on glucagon-like peptide-1 (GLP-1)-containing cells in the chicken small intestine. Anat Histol Embryol, **43**: 153–158. 2014. https://doi.org/10.1111/ahe.12062, PMID:23651280

[7] Hiramatsu K. Chicken intestinal L cells and glucagon-like peptide-1 secretion. J Poult Sci, **57**: 1–6. 2020. https://doi.org/10.2141/jpsa.0190003, PMID:32174759

[8] Moran ET Jr. Nutrition of the developing embryo and hatchling. Poult Sci, **86**: 1043–1049. 2007. https://doi.org/10.1093/ps/86.5.1043, PMID:17435045

[9] Şahan U, Ipek A and Sozcu A. Yolk sac fatty acid composition, yolk absorption, embryo development, and chick quality during incubation in eggs from young and old broiler breeders. Poult Sci, **93**: 2069–2077. 2014. https://doi.org/10.3382/ps.2013-03850, PMID:24931969

[10] Ravindran V and Abdollahi MR. Nutrition and digestive physiology of the broiler chick: state of the art and outlook. Animals, **11**: 2795. 2021. https://doi.org/10.3390/ani11102795, PMID:34679817

[11] Salahuddin M, Hiramatsu K, Al-Amin M, Imai Y and Kita K. Low dietary carbohydrate induces structural alterations in enterocytes of the chicken ileum. Anim Sci J, **95**: e13919. 2024. https://doi.org/10.1111/asj.13919, PMID:38287469

[12] Chin AM, Hill DR, Aurora M and Spence JR. Morphogenesis and maturation of the embryonic and postnatal intestine. Semin Cell Dev Biol, **66**: 81–93. 2017. https://doi.org/10.1016/j.semcdb.2017.01.011, PMID:28161556

[13] Sklan D. Development of the digestive tract of poultry. Worlds Poult Sci J, **57**: 415–428. 2001. https://doi.org/10.1079/WPS20010030

[14] Uni Z, Noy Y and Sklan D. Posthatch development of small intestinal function in the poult. Poult Sci, **78**: 215–222. 1999. https://doi.org/10.1093/ps/78.2.215, PMID:10051034

[15] Sell JL, Angel CR, Piquer FJ, Mallarino EG and Al-Batshan HA. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poult Sci, **70**: 1200–1205. 1991. https://doi.org/10.3382/ps.0701200, PMID:1712968

[16] Katanbaf MN, Dunnington EA and Siegel PB. Allomorphic relationships from hatching to 56 days in parental lines and F1 crosses of chickens selected 27 generations for high or low body weight. Growth Dev Aging, **52**: 11–21. 1988. PMID:3170062

[17] Karcher DM and Applegate T. Survey of enterocyte morphology and tight junction formation in the small intestine of avian embryos. Poult Sci, **87**: 339–350. 2008. https://doi.org/10.3382/ps.2007-00342, PMID:18212379

[18] Mooseker MS, Bonder EM, Conzelman KA, Fishkind DJ, Howe CL and Keller TC III. Brush border cytoskeleton and integration of cellular functions. J Cell Biol, **99**: 104s–112s. 1984. https://doi.org/10.1083/jcb.99.1.104s, PMID:6378918

[19] Fath KR and Burgess DR. Microvillus Assembly: not actin alone. Curr Biol, **5**: 591–593. 1995. https://doi.org/10.1016/S0960-9822(95)00117-5, PMID:7552163

[20] Heintzelman MB and Mooseker MS. Structural and compositional analysis of early stages in microvillus assembly in the enterocyte of the chick embryo. Differentiation, **43**: 175–182. 1990. https://doi.org/10.1111/j.1432-0436.1990.tb00444.x, PMID:2387484

[21] Zhao H, Pykäläinen A and Lappalainen P. I-BAR domain proteins: linking actin and plasma membrane dynamics. Curr Opin Cell Biol, **23**: 14–21. 2011. https://doi.org/10.1016/j.ceb.2010.10.005, PMID:21093245

[22] Postema MM, Grega-Larson NE, Neininger AC and Tyska MJ. IRTKS (BAIAP2L1) elongates epithelial microvilli using EPS8-dependent and independent mechanisms. Curr Biol, **28**: 2876–2888.e4. 2018. https://doi.org/10.1016/j.cub.2018.07.022, PMID:30197089

[23] Uni Z, Tako E, Gal-Garber O and Sklan D. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poult Sci, **82**: 1747–1754. 2003. https://doi.org/10.1093/ps/82.11.1747, PMID:14653469

[24] Smirnov A, Perez R, Amit-Romach E, Sklan D and Uni Z. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. J Nutr, **135**: 187–192. 2005. https://doi.org/10.1093/jn/135.2.187, PMID:15671211

[25] Yu Y, Wang Z, Cao J, Dong Y, Wang T and Chen Y. Effects of monochromatic light stimuli on the development and Muc2 expression of goblet cells in broiler small intestines during embryogenesis. Poult Sci, **93**: 1801–1808. 2014. https://doi.org/10.3382/ps.2013-03805, PMID:24864285

[26] Reynolds KL, Cloft SE and Wong EA. Changes with age in density of goblet cells in the small intestine of broiler chicks. Poult Sci, **99**: 2342–2348. 2020. https://doi.org/10.1016/j.psj.2019.12.052, PMID:32359569

[27] Proszkowiec-Weglarz M, Schreier LL, Kahl S, Miska KB, Russell B and Elsasser TH. Effect of delayed feeding post-hatch on expression of tight junction– and gut barrier–related genes in the small intestine of broiler chickens during neonatal development. Poult Sci, **99**: 4714–4729. 2020. https://doi.org/10.1016/j.psj.2020.06.023, PMID:32988506

[28] Duangnumsawang Y, Zentek J and Goodarzi Boroojeni F. Development and functional properties of intestinal mucus layer in poultry. Front Immunol, **12**: 745849. 2021. https://doi.org/10.3389/fimmu.2021.745849, PMID:34671361