Development of the Intestinal Villi Associated with the Increased Epithelial Cell Mitosis in Chickens

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Abstract To clarify whether or not an increase of the intestinal villi in size is induced by the increase of the cell proliferation, post-hatching developmental changes of the villous size and the cell mitotic numbers in the crypt were observed in the 1-, 10- and 20-day-old broiler chickens. Newly-hatched chicks were bred in the following nutritional conditions; conventional, high protein-low energy (HL) or low protein-high energy (LH) dietary groups. Besides, in the fasted group for 5 days after hatching morphological alterations of the villi were observed. The intestine of HL group increasing food intake and growing fast had well-developed large villi with marked extrusions of cells at the apex and showed much mitotic division of epithelial cells in the crypt even at 10-day-old. The LH group decreasing food intake and growing slow had small villi and showed few cell mitoses. Denuded apical surfaces were also frequently observed at 20-day-old. In fasted group, shrunk villi and denuded apical surfaces were found. The present findings indicate that the increase of villous size might be induced by the active cell proliferation in the intestinal crypts as well as by the decreased cell loss from the villous tips.


Key words: intestinal villi, mitosis, scanning electron microscopy, broilers

A gross morphology of the avian intestine is known to be different in each species of domestic fowls and to change with the kinds of fed diets. Even both in the fine structure of the chicken intestine and among the different breeds of chickens, intestinal villi show a morphological difference. Under the adequate nutritional conditions, broiler chickens (BR) showed the larger villi, the more developed protuberances of epithelial cells on the apical surface of villi and more activated fine structures than White Leghorn chickens (WL). These characterisitic features of the villous surface in BR are thought to be positively associated with activated functions of the intestine. Because genetic increases in food intake and efficient food utilization in BR due to an artificial genetic selection for rapid growth seem to alter the role of the gastrointestinal tract in food regulation, the intestinal tract is more important in BR than the hypothalamus in WL. In the intestine, epithelial cells originate by mitosis in the lower half of the crypt and migrate along the villous surface to the top, where they are extruded into the intestinal lumen. These cellular mitosis rate, migration speed and extrusion frequency seem to be associated with
the size of intestinal villi and to be affected by a change of nutritional functions of the intestine.

In spite of many morphological studies on the intestinal villi, relationships between the villous development and epithelial cell mitosis have not been studied in detail. In this study, how these villous development and cell mitosis were affected under the experimentally activated or inactivated functions of the intestine by feeding different levels of protein and energy in diets.

**Materials and Methods**

Newly-hatched male and female BR (Marshall Chunky) chicks (*Gallus gallus domesticus*) were divided into 3 groups of 14 birds, 7 birds of each sex, for subjecting to the following feeding experiments as shown in Table 1: Conventional dietary group; high protein-low energy dietary group (HL); low protein-high energy one (LH). Each diet was fed from 3 to 20 days of age. At 1-, 10- and 20-day-old, 2 chicks of each sex selected at random from each group were sacrificed by decapitation. Immediately after finishing each experiment, tissue samples were taken at the mid part of the duodenum, jejunum and ileum. Samples for light microscopic observations were fixed in Bouin’s fixative solution, embedded in paraplast, cut at 5 μm transversal sections and stained with Hematoxylin-Eosin. Five sections randomly selected in each group were used for counting the numbers of mitosis of epithelial cells in the intestinal crypt. Tissue samples for scanning electron microscopic observations were processed as described previously.[18] For counting the numbers of villi, photographs were taken at low magnification. Within a 20 × 20 mm square (corresponding to 833 × 833

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**Table 1. Composition of experimental diets**

<table>
<thead>
<tr>
<th>Ingredient (%), Dietary Group</th>
<th>Conventional diet</th>
<th>High protein-low calorie</th>
<th>Low protein-high calorie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula feed for layer</td>
<td>83.4</td>
<td>9.5</td>
<td>57.7</td>
</tr>
<tr>
<td>Yellow corn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hulled rice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.7</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>4.3</td>
<td>15.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Defatted rice bran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat bran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley bran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tallow</td>
<td>3.6</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium phosphate, tribasic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.5</td>
<td>29.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Metabolizable energy (M cal/kg)</td>
<td>3.00</td>
<td>2.41</td>
<td>3.74</td>
</tr>
</tbody>
</table>
Results

Food intakes per bird per day during 3-10 days old were 15 g in control, 17 g in HL and 8.0 g in LH groups. Those during 11-20 days old showed 35 g in control, 45 g in HL and 8.0 g in LH groups. Compared with control, HL revealed faster growth but LH showed a markedly decreased body weight (Fig. 1). In the numbers of villi per measured area in these groups, each intestinal part showed a remarkable decrease from hatching to the first 10 days (Fig. 2); this means a rapid development of villi in size. HL showed the fewest numbers of villi in a great portion of the intestine and at every age except for the ileum at 10-day-old. The intestine of LH group had more numerous villi than that of HL group at the all stages.

In fine structural changes around the villous tips, fasted chicks (Fig. 3) showed slightly increased duodenal villi in size compared with those at hatching but villous tips showed smooth surfaces. Jejunal and ileal villi did not reveal a developmental increase and the jejunum had many damaged villi with denuded apical surfaces. Duodenal villi (Fig. 4) in HL developed to have wide apical surfaces with marked extrusions of cells within the first 10 days of age and increased in size with increasing age. However, chickens of LH had smaller and comparatively narrow villous surfaces than those of control but more activated villi than fasted birds. These morphological features were also found in jejunal villi (Fig. 5) in HL and LH. Atrophied villi having denuded apical surfaces were observed as a characteristic morphological feature in LH at 20-day-old. On the other hand, ileal villi (Fig. 6) in HL were smaller in size than those in the control, and LH showed extremely shrunk villi. Villi in HL were attached by filamentous bacteria.

All parts of the intestine remarkably increased in the numbers of mitosis in the crypt (Fig. 7) within the first 10 days of age. Then, in the duodenum and jejunum of HL and
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Fig. 2. The numbers of villi per 0.7 mm² at each intestinal part from 1 to 20-day-old broilers (Mean±SE, n=4-10; see Fig. 1 for abbreviations)

ab Means with different superscripts are significantly different among groups (P<0.05).

Discussion

In the previous study, we reported that BR had the larger villi, more developed epithelial cell protrusions on the tip of villi and more activated fine structural features than WL. These morphological differences are thought to be induced by activated intestinal functions in BR. HL showed the increased food intake, the rapid growth rate, the fewest numbers of villi and the most cell mitotic number per measured area. It is reasonable to think that the more villi decrease in the numbers, the more villous size increases. Consequently, HL had the largest villi, corresponding with direct overhead view of the intestinal luminal surfaces using scanning electron microscope, in which wide apical surfaces with marked cellular extrusions were also observed. LOEBRY et al. showed that the hypertrophied intestinal mucosa due to the increased food consumption resulted in elevated mucosal surfaces in lactating rats. ECKNAUER et al. reported that the villous surfaces were directly related to the cell production covering the villi in three-dimensional measurement method. From these studies, the increased villi in size of HL chickens are thought to be induced by activated nutritional absorption of the intestine. On the other hand, LH revealed the decreased food intake, the lowest body weight, the most villi, namely, the smallest villi and the fewest cell mitotic number. Villi in restricted cockerels for 8 days have been reported to show slightly shorter and thinner villi than normal. Taking this fact and the small villi seen in the present fasted chickens into consideration, the decreased villi in size of LH birds seem to be induced by...
Fig. 3. Scanning electron micrographs (×500) showing the intestinal villi in fasted chickens. Compared with the duodenum at hatching (A), duodenal villi (B) slightly increase but jejunal villi with denuded apical surfaces (C) and ileal villi (D) show no developmental growth.
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Fig. 4. Duodenal villi (×500) in control (A), HL (B) and LH (C) chickens at 10-day-old. One can see large villi in HL but small villi in LH. Villi in HL develop to the larger villi with marked cell extrusions on the tips at 20-day-old (D). (See Fig. 1 for abbreviations).
Fig. 5. Jejunum villi (×500) in control (A), HL (B) and LH (C) chickens at 10-day-old. Villi in HL develop to large villi but those in LH decrease in size and reveal the denuded apical tips at 20-day-old (D). (See Fig. 1 for abbreviations).
Fig. 6. Ileal villi (×500) in control (A), HL (B) and LH (C) chickens at 10-day-old. Villi in HL show smaller villi than control and have filamentous bacteria at 20-day-old (D). LH chickens have markedly decreased villi. (See Fig. 1 for abbreviations).
Fig. 7. The numbers of mitosis per 5 μm transversal sections at each intestinal part from 1 to 20-day-old broilers (Mean ±SE, n = 5; see Fig. 1 for abbreviations).

Means with different superscripts are significantly different among groups (P<0.05).

atrophied absorptioanl function of the intestine. These explain the fact that the larger villi contribute to induce a faster growth by elevating an absorptive area of villi.

The size of villi in BR rapidly increased within the first 10 days of age and then remained almost constant in size, while cell area in a longitudinally sectioned epithelial cell did not show a marked increase from 1 to 10-day-old. These findings suggest a possibility that the size of villi is not increased by an enlargement of cell area but developed by activated cell renewal. Noda observed that the width of one epithelial cell showed an almost similar value from 20th day embryo to newly hatched chicks and described that the marked development of villi in height during the chick embryo was owing to the increased numbers of cells. In rats, the villous surfaces were directly related to the cell production covering the villi; elevated mucosal surfaces in hypertrophied intestinal mucosa in lactating rats and about one-half of the normal renewal rate and decreased cell migration rate to the villous tips in starved mice were observed. In the present study, increased cell proliferation in larger villi of HL and decreased cell proliferation in atrophic villi of LH and fasted birds demonstrate that the villous development in size has been induced by activated cell mitosis and might be closely related to the functional states of the intestine. Marked decrease of mitosis of ileal epithelial cells in HL after 10 days old seems to be related to the filamentous bacteria adhering to the cells. In addition to mitosis, marked extrusions of epithelial cells at the villous apical tips in the duodenum in HL seem to induce an increase of villous size. Reversely, the excessive rates of death and loss of cells from the villous tips such as seen at villi with denuded apical surfaces are thought to cause a
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The results of the present study indicate that the activated developmental increase of villous size might be induced by cell proliferation in the crypts as well as by the decreased cell loss from the villous tips and that each intestinal part has a different role in intestinal functional development with increasing age.

References

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鶏腸障害における上皮細胞の細胞分裂増加
に伴う絨毛の発達

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プロイラー専用雑菌生産に通常飼料、高蛋白質・低カロリー飼料（HL）、または低蛋白質・高カロリー飼料（LH）を 20 日間給与し、腸管絨毛の生後発達と腸障害における上皮細胞の分裂像を 1，10 および 20 日齢で観察した。更に、孵化後 5 日間絶食した鶏における絨毛の形態学的変化についても調べた。HL 鶏は、摂食量の増加や急速な発育を示し、10 日齢で著しく発達した絨毛。活発な細胞分裂ならびに絨毛頂部における顕著な細胞隆起が認められた。LH 鶏では、著しい摂食量の減少や緩慢な体重増加が見られ、未発達な絨毛や細胞分裂の減少が認められ、更に絨毛頂部の割裂現象も 20 日齢で時折観察された。また、絶食鶏では萎縮した絨毛や絨毛頂部の割離がしばしば観察された。今回の観察結果から、絨毛の大きさの増加は絨毛頂部における細胞脱落が少なく、腸障害における上皮細胞数の増加によるものであると思われる。

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