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≪**Review**≫

Review on Chicken Intestinal Villus Histological Alterations Related with Intestinal Function

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The nutritional value of diets fed to chickens has traditionally been evaluated by growth performance and nutrient digestibility. In addtion to nutritional physiological studies, research on intestinal structure is also important, as the intestine is the digestive and absorptive organ. In macroscopic anatomy, the guts of poultry differ relative to body weight (Thomas, 1984). Fowls produced for the meat purpose, such as broiler (BR) chickens and Peking ducks, have intestines of greater length, weight and area than those of egg-laying fowl, such as the White Leghorn (WL) chickens and wild ducks (Yamauchi *et al.,* 1990 b). Poultry innards are affected by diet (Moss, 1974 ; Miller, 1975 ; Langhout *et al.,* 1999 ; Yasar and Forbes, 1999). Feeding habits rather than individual body weight difference account for gross anatomical differences in the intestine (Yamauchi and Zhou, 1988). These reports suggest that the nutritional value of diets may produce microscopic alterations in the intestinal mucosa. Although the general histological features of the intestine are well known, the relationship between the structure and function of the intestine has not been established. The reason that no information on the histological alterations related with intestinal function has been reported is related to the fact that studies on the interaction between them are limited to morphological observations, which is due to lack of an immunocytochemical procedure using antibodies. Confirmation that intestinal histological alterations are indeed induced by intestinal functions is currently impossible and can be established only by comparison of morphological characteristics with intestinal functions.

Consequently, over the past decade, there has been much interest in establishing how intestinal histological alterations are related to intestinal function. The intent of this interest is to assess the enteral nutrient absorption of feed ingredients histologically. This review highlights recent studies on histological alterations related to intestinal function, and adds new information to the assessment of the nutritional values of various kinds of chicken feed.

Histology of intestinal villi

Small intestine is divided into duodenum, jejunum and ileum. In this review, intestinal part from the ventriculus to pancreatic and bile ducts is regarded as duodenum ; jejunum from the ducts to Meckel's diverticulum and ; ileum from the diverticulum to ileo-caeco-colic junction (Yamauchi, 2001). Intestinal villi are the

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protrusions of the lamina propria into the intestinal lumen to enlarge the digestive and absorptive area. The villus surface is covered with a simple columnar epithelial cell layer consisting of absorptive, goblet, and entero-endocrine cells. At the base of the villi, the cell layer lines the inside surface of tubular indentations of intestinal crypts, which reach the mucosal muscle layer. Near the smooth muscle layer, the inside layer of the crypt again lines the neighboring villus surface, resulting in blind ends in the crypt. All epithelial cells originated by mitosis in the stem-cell zone located in the lower portion of the crypt migrate along the villus surface upward to the villus tip within a few days for maturating (Imondi and Bird, 1966), where they are extruded into the intestinal lumen within 48 h after birth (Potten, 1998). In recent years, the different mechanisms of epithelial cell loss which are associated with intraepithelial lymphocytes and lamina propia macrophages have been re-investigated (Hall *et al.*, 1994 ; Mayhew *et al.*, 1999). Such a cellular migration is likely to be altered by intestinal function. In addition, since villi consist of epithelial cells, the villus height (length) is assumed to change according to the cell area and number. Consequently, intestinal function might be determined by measuring 1) villus height, cell area, and cell mitosis using light microscopy, 2) morphological observations of the villus surface using a scanning electron microscope, and 3) the ultrastructure of the epithelial cells using a transmission electron microscope.

Post-hatching development of villus height in White Leghorn

Male WL had the highest villi in duodenum followed by lower in jejunum and the lowest in ileum during starter stage (Yamauchi *et al.*, 1995). However, the jejunal villus height showed a marked growth rate up to the next developer stage follwed by ileum, while the duodenal villi slightly increased. This explains that the vigorous absorptive part would be mainly the duodenum in early life stage and then extend to the jejunum with increasing age, and while the intestinal histology is altered during post-hatching development.

Comparison of intestinal villi between White Leghorn and broiler chickens

The growth rate of selected heavy type BR was twice that from the nonselected light type during the first week after hatching (Marks, 1979) and selected BR consumed more feed than those of nonselected birds even on the hatched day (Marks, 1980). Selection of growth rate is positively associated with genetic increases in feed intake and efficiency of feed utilization (Proudman *et al.*, 1970). It may be assumed that such an increased feed intake and efficient feed utilization have also altered intestinal function, which could influence intestinal morphology. WL and BR are genetically selected for increasing of growth rate and egg production, respectively. Therefore, possible morphological differences in the small intestinal villi between WL and BR chickens are of considerable interest, and both kinds of chickens are most suitable natural chicken model for investigating the intestinal histological alterations related with function.

Comparison of post-hatching development of villus tip surface morphology using scanning electron microscope

Post-hatching developmental alterations of villi were observed in 1, 10 and 30-d-old WL and BR chickens (Yamauchi and Isshiki, 1991). At the hatching day, duodenal villi of each intestinal part showed a finger-like appearance with a dome-shaped tip surface in both breeds. Compared with WL (Fig. 1 A), in which villus surface was smooth with flat epithelial cells, BR had many more developed cell protrusions on villus tip surface, resulting in rough surface (Fig. 1 B). At 10 day of age, villi had enlarged to develop to plate-like ones in both breeds. However, BR had larger villi than those of WL. After 10 days of age, villi further increased in size in both breed. Based on their distribution pattern, two kinds of villi could be identified. One was the villus enlarging obliquely rostro-laterally at an angle of 20° to 30° along its transversal axis and the other was obliquely caudo-laterally at the same angle. Two types of villi were found, like an oblique T-shape having an angle of 40° to 60° and showing as a whole a zigzag arrangement. Such a characteristic zigzag arrangement of villi is thought to be a more effective structure for nutrient absorption than villi being located in parallel to each other, because the absorption of ingesta by villi should be increased by a long zigzag flow of ingesta rather than a straight feed flow. These morphological distinctions suggest a greater absorptive surface area and a more active intestinal function immediately after hatching in BR. Such characteristics are thought to be induced by genetic selection for rapid growth rate and should permit the faster growth rate by increased feed intake and more effective feed utilization in BR.

Comparison of post-hatching development of ultrastructure in absorptive epithelial cells using transmission electron microscope

Post-hatching developmental alterations in the ultrastructure of the absorptive epithelial cells were observed in 1, 10 and 60-d-old WL and BR chickens (Yamauchi *et al.,* 1992). Epithelial cells in 1-d-old WL and BR had a well-developed Golgi area and

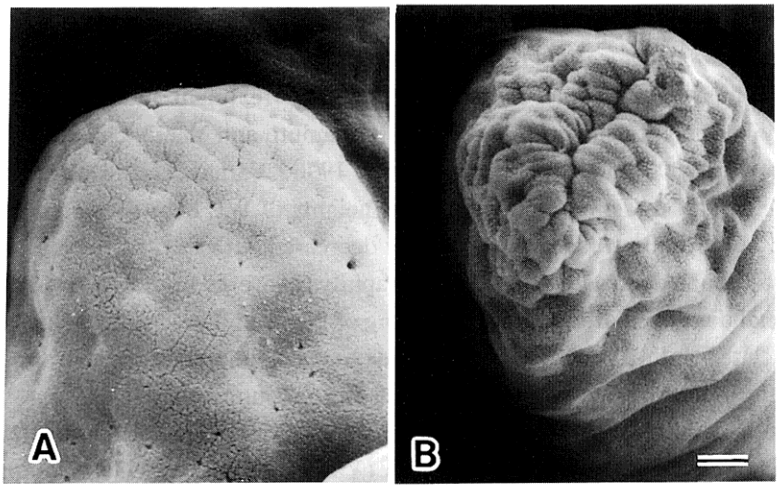

Fig. 1. Duodenal villi in 1-d-old WL (A) and BR (B) chicks. Note the more developed epithelial cell protuberances on the villus tip in BR than WL, resulting in a smooth surface in WL but a rough surface in BR. Bar ; 10μm ($\times$690).

a dense cluster of rod type mitochondria. In BR, cells included well-developed profiles of endoplasmic reticulum, but fewer supranuclear vacuoles than those of WL, in which numerous free ribosomes were also found. Some mitochondria in BR showed a bud-like protrusion from the main body. These suggest that the fine structural maturation of the epithelial cells in WL is involved in the process of cell maturation but epithelial cells of BR have almost matured at hatching. In 10-d-old WL and BR, supranuclear vacuoles had disappered and mitochondria increased in number. In WL, in addition to a decrease in free ribosomes, mitochondria developed to the tadpole type and further to dumbbel type ones. In BR, mitochondria also aggregated at the perinuclear region and some of them had developed from dumbbel shapes to a thick doughnut type. These findings indicate that epithelial cells in both breeds are more developed ultrastructurally than those in 1-d-old and that epithelial cells in BR are more activated for digestive and absorptive functions than those in WL. In 60-d-old WL and BR, the cell structure and function had reached a stable state. BR revealed higher values of cell area and RNA content than WL at every age and RNA content of both breeds were maximal at 10 days, followed by 60 days. These observations suggest that epithelial cells of BR are more highly activated than WL cells at each age.

Intestinal villus histological alterations related with intestinal functional changes induced experimentally

In comparison of villi between WL and BR, BR has larger villi and much more matured ultrastructure in the epithelial cells than those in WL even at hatching. These villus morphological features correspond with increased feed intake and rapid growth rate of BR, suggesting a possibility of intestinal villus histological alterations related with intestinal function. Next, to certify this relationship seen in the intact chickens more certainly, studies on villus histological alterations related with intestinal functional changes induced experimentally have been carried out.

Duodenal villus histological alterations due to fasting and refeeding treatments

In the light microscopic observations, fasting treatment induced a decrease of duodenal villus height (Shamoto *et al.,* 1999 ; Shamoto and Yamauchi, 2000 ; Tarachai and Yamauchi, 2000 ; Yamauchi *et al.,* 1995, 1996 ; Yamauchi and Tarachai, 2000). Within the first 24-h-fasting, duodenal villus heights of WL hens showed a remarkable reduction, but after 36-h-fasting it gradually decreased with days of fasting (Yamauchi *et al.,* 1996). Such decreased villus heights rapidly recovered after only 1-d-refeeding of conventional diets even after long-term fasting of 20 days. These villus height variations were associated with a reduction of cell area and cell mitosis, demonstrating that the villus height mainly varied with the cell area and cell numbers.

In the scanning electron microscopic observations, fasting treatment depressed post-hatching development of intestinal villi (Yamauchi *et al.,* 1993). Compared with the villus size at hatching, duodenal villus size developed only slightly after fasting for 5 days, but jejunal and ileal villi did not showed further development. These villus sizes were increased by activated cell mitossis as well as by decreased cell loss from villus tip. Besides, morphology of epithelial cells distributing over the duodenal villus tip surface was affected by fasting (Shamoto *et al.,* 1999 ; Shamoto and Yamauchi, 2000 ; Tarachai and Yamauchi, 2000). The surface of duodenal villus apex of *ad libitum* fed birds revealed a rough surface due to a clear cell outline, cell protuberances, and cell extrusion into the intestinal lumen. After 3-d-fasting, cell outlines became faint and protuberances and extrusion of cells disappeared, resulting in smooth surface. After refeeding for 3 hours, clear cell outlines were again apparent.

In the transmission electron microscopic observations, the duodenal epithelial cells of BR chicks fed low protein-high energy diet showed a decrease in feed intake, growth rate and developed secondary lysosomes (Yamauchi and Isshiki, 1994). By fasting treatment, large lysosomal supranuclear vacuoles appeared in WL even after 3 days fasting (Yamauchi *et al.,* 1995). These vacuoles developed to larger lysosomal autophagous vacuoles in hens fasted for 20 days, and included residuary mitochondria (Yamauchi *et al.,* 1996). However, these were reduced in size by refeeding for only one day. These vacuoles may appear to obtain a deficient nutrient due to fasting by autophagic intracellular digestion. To investigate whether the vacuolar changes are related with fasting and refeeding treatments, and to obtain a useful index to judge the intestinal nutritional condition by means of these vacuolar changes, the fine structural alterations were examined in epithelial cells (Yamauchi and Tarachai, 2000). At 1 day of fasting, small electron-dense bodies appeared in the absorptive epithelial cells, and some of them were fusing each other. With increasing the fasting period, these small bodies developed to the moderate-sized nascent autophagic vacuoles containing various kinds of electron-dense contents in density and size, and finally become the large autophagic vacuoles with electron-lucent contents via the large degradative autophagic vacuoles with electron-dense contents. Some vacuoles showed positive acid phosphatase reactions, demonstrating that these are lysosomal autophagic vacuoles including hydrolytic enzyme. The large autophagic vacuoles with electron-lucent contents in 3 days fasting decreased to the small electron-dense bodies seen in 1 day fasting rapidly after only 1 day refeeding. These findings suggest that intestinal epithelial cells would have an ability to intracellularly digest their own cell components to supply the insufficient nutrients during fasting by means of lysosomal active autophagic transport mechanism and that after refeeding the epithelial cells returned to the absorption transport mechanisms of nutrients. The present results demonstrate that the autophagic vacuolar changes in the duodenal epithelial cells are correlated with changes of intestinal villus height, cell area and cell mitosis number induced by fasting and refeeding. This indicates that autophagic vacuoles are useful index judging the nutritional condition of chicken intestine ; the more large electron-lucent vacuoles in duodenal absorptive cells the lower the nutritional condition of chicken intestine is.

Difference of recovery responses of intestinal villus morphology in long-term fasted chickens to different refeeding procedures

Effects of form and nutritional level of refed diets on the recovery of villi in fasted chicks have been investigated. In the case of long fasting, WL chicks refed the rice bran for 3 and 6 hours after fasting for 10 days showed the faster revovery in villus height, cell area and cell mitosis than those refed grower mash (Shamoto *et al.,* 1999). Also smooth surface of the villus apex in fasted chicks changed to a much activated rough surface by many protuberated cells after refeeding rice bran. However, the refeeding of grower mash for 24 hours induced the much more activated morphology in villus height, cell area and cell mitosis than those refed rice bran. Villus apex showed the more developed protuberances of epithelial cells in grower mash. These morphological alterations indicate that the quickly absorbable form such as powdered diet is important rather than the nutrition of it for the first recovery stage of villi after fasting, and that the nutritious and well-balanced diet is important for further complete recovery stage of villi.

Difference of recovery responses of intestinal villus morphology in short-term fasted chickens to different refeeding procedures

In the case of short fasting, duodenal villus height in chicks fasted for 3 days was significantly increased after 3-h-refeeding rice bran or conventional grower mash diet, values of which were almost similar, and the chicks refed ground conventional grower mash diet was significantly higher than the former both groups (Shamoto and Yamauchi, 2000). Compared to villus height at 3-h-refeeding, rice bran-refed group showed no change after 24-h-refeeding but conventional grower mash diet and ground conventional grower mash diet had significantly increased villus height ; villus height in ground conventional grower mash diet was significantly higher than conventional grower mash diet. Dietary effects on cell area and cell mitosis numbers were similar to that observed for villus height. Besides, alterations of villus height, cell area and cell mitosis numbers due to fasting and different refeeding procedures were reflected in body weight gain after refeeding. Namely, birds refed the ground conventional grower mash diet gained significantly more weight than rice bran and conventional grower mash diet. Compared to body weight gain at 3-h-refeeding, rice bran-refed birds did not increase further after 24-h-refeeding. On the smooth surface of duodenal villus apex in 3-d-fasted birds, clear cell outlines were again apparent in all groups after refeeding for 3 hours. In ground conventional grower mash diet-refed chicks larger cell outlines and protuberated cells were found as a conspicuous morphological feature. Similar observations were made at 24-h-refeeding. These morphological findings suggest that fasted chickens benefit from *ad libitum* refeeding of a powdered diet that is nutritionally complete for rapid recovery of digestive function.

Duodenal difference of villus histological alterations compared to jejunum and ileum

The chicken duodenum is located near the pancreas between its proximal descending and distal ascending limbs. It is tightly connected to the pancreas by many arterial and venous ramus. However, the jejunal and ileal parts are not fixed to other tissues. This peculiar morphological feature might indicate that the intestinal lumen of the jejunum and ileum may have the ability to shrink during fasting and enlarge again when feed is consumed. This means that the height of the villus increases during a fast and decreases when feed is consumed. Therefore, fasting and refeeding have no preference effect on the villus height in jejunum and ileum. Consequently, during fasting and refeeding, the change in the height of intestinal villi is a powerful discriminator for judging the function of the duodenum, but it is an unsatisfactory discriminator for the jejunum and ileum.

Difference of recovery responses of intestinal villus morphology to luminal nutrient absorption, intraluminal physical stimulation and intravenous parenteral alimentation

Intestinal villus histological alterations due to fasting and refeeding described above are thought to be influenced by luminally absorbed nutrients. However, intraluminal stimulation is also correlated with these villus structural changes, and stimuli arising from factors such as dietary fibers within the intestine might also influence the structural integrity of the intestine. It is of interest, therefore, to determine which of the following three factors is most responsible for inducing villus morphological changes : luminal nutrient absorption, intraluminal physical stimulation, or intravenous parenteral alimentation for 2 days after 3-d-fasting. Compared with the WL fasted for 3 days, villus height, cell mitosis, and villus tip surface morphology of refeeding the conventional diet and force-feeding enteral hyperalimentation (drinking eutrophic) for 2 days exhibited rapid villus morphological recovery (Tarachai and Yamauchi, 2000). Villus morphological recovery of these enteral treatments appears to be caused by enteral nutrient absorption. However, villus morphology force-fed an indigestible (nonabsorbable) substance was not different from that in fasted birds, suggesting that intraluminal physical stimulation has no effect on villus morphological recovery. On the other hand, the birds intravenously dripped continuously by an amino acid solution including glucose and electrolytes showed no effect on villus morphological recovery, suggesting that the parenteral nutrient supplied to the villi via the blood could not induce villus morphological recovery ; the intestinal mucosal atrophy might be caused by the absence of enteral nutrients and would only be stimulated by enteral nutrient absorption. Alterations of these villus morphology due to fasting and different refeeding procedures were reflected in body weight gain after refeeding. These suggest that villus morphology is not governed either by intraluminal physical stimulation or parenteral alimentation but by enteral nutrient absorption.

Compensatory enlargement of ileal villi following jejunal resection

The ileal villi are smaller (Yamauchi and Isshiki, 1991 ; Yamauchi *et al.,* 1993) and lower (Yamauchi *et al.,* 1995, 1996) than those of the duodenum. This seems to relate to intestinal function. In chickens fed a conventional diet, very little nitrogen absorption was observed beyond the jejunum (Imondi and Bird, 1965), and ingested nutrients were absorbed in the upper part of the intestine (Isshiki *et al.,* 1989). This suggests that the absorptive function of ileal villi is less active than that of the intestinal proximal part. This may be due to the fact that nutrients have already been absorbed by the time intestinal contents reach the intestinal proximal part. Therefore, whether small ileal villi change to large villi due to intestinal contents rich in nutrients are most suitable experimental model to demonstrate the intestinal histological alterations related to intestinal function. Compared with intact control chickens, the chickens resected the jejunums showed almost similar body weight and protein-retention rate, suggesting an enhanced absorptive function of intestinal remnant (unpublished). The ileal remnant of these birds was much grater in size and height than the jejunal villi. An increased load of nutrients derived directly from the duodenum to the ileum may stimulate the ileal absorptive function, resulting in compensatory enlargement of the ileum. This finding demonstrates that even small villi might increase in size when the intestinal function is activated and that histological alteration is related to intestinal function.

In some chickens subjected to resection of the jejunum, intestinal stricture was frequently observed at the end-to-end anastomosis. In such case, the jejunal part just proximal to the anastomosis showed an abnormal enlargement due to the intestinal stricture. Villi in this part developed to long beltlike villi encircling the intestinal lumen (Fig. 2, Yamauchi, 2002). This might be induced by activated intestinal function due to long-term staying of the intestinal contents. This phenomenon suggests that the intestinal villi are adapted to the intestinal activated absorptive function not by increasing their numbers, but by fusing each other into the larger villi.

Intestinal histological alterations due to conventional feeding experiments

In additions to the intestinal histological alterations due to artificially induced intestinal functions, whether they also receive such a change by the conventional continued feeding of different feed ingredients is also important to assess an enteral

Fig. 2. Example of the villi at the abnormally enlarged jejunal part just proximal to the intestinal stricture due to end-to-end anastomosis in the chicken subjected to resection of the jejunum. Absorptive function of the enlarged part might be activated by long-term staying of the intestinal contents. One can see that the intestinal villi are adapted to the activated intestinal absorptive function not by increasing their numbers, but by fusing each other into the larger beltlike villi. Bar ; 200 μm ($\times$48) (Yamauchi, 2002).

nutrient absorption histologically. Various kinds of dietary feed ingredients have been fed on chickens.

Intestinal villus histological alterations after feeding the dietary charcoal powder including wood vinegar compounds

Charcoal is a solid fuel made by dry distillation of wood, and powder of which is traditionally scattered on the floor in chicken house to reduce the smell of feces by adsorbing ammonia. A mixed powder of wood vinegar compounds and amorphous charcoal carbon (CWVC) has been used as an oral antidote to produce high animal productions. The dietary addition of CWVC to diets induced a significant increase in hen-day egg production and feed conversion ratio (Sakaida *et al.,* 1987 a) and in broiler hatchability (Sakaida *et al.,* 1987 b). WL cockerels fed 1 and 3% dietary CWVC tended to be higher body weight gain and feed conversion ratio than controls (Samanya and Yamauchi, 2001). Values of the intestinal villus height, epithelial cell area and cell mitosis were higher in 1% dietary CWVC group but lower in 5% dietary CWVC group than those of another groups in each intestinal segment. The comparatively smooth surface of the duodenal villus apex in the control duodenum changed to the rough surface with a clear cell outline between each epithelial cell due to the conspicuous cell protuberances after feeding 1% dietary CWVC. However, such conspicuous cell protuberances disappeared and cells having no microvilli and deep cells at the sites of recently exfoliated cells were observed after feeding 3% dietary CWVC, and the latter was much increased after feeding 5% dietary CWVC. Fundamentally, the villus tip surface in the jejunum and ileum revealed an almost similar morphological alteration to that in the duodenum. However, in the intestinal distal part, cells having no microvilli and deeper cells due to recently exfoliated cells were not seen even in 5% dietary CWVC, and the cell protuberances of 1% dietary CWVC became faint with moving caudally. The present morphological changes of intestinal villi in chickens fed the dietary CWVC diets demonstrate that the villus function could be activated also in the ileum at 1% level, and that such an activated villus function in all small intestinal segmental parts might improve the feed conversion ratio.

Morphological demonstration of the stimulative effects of charcoal powder including wood vinegar compound solution on growth performance and intestinal villus histology

In a previous study, improved feed conversion ratio and activated morphological changes of intestinal villi were observed in chickens fed a 1% CWVC diet (Samanya and Yamauchi, 2001). As CWVC is a mixture of charcoal and wood vinegar compound solution, it is not yet clear whether these improved results were due directly to an effect of one of them or to a combined effect of both. To try and resolve this question, and then to investigate which substances of them induce such growth performance and morphological changes of intestinal villi, cockerels were fed wood vinegar compound solution, charcoal or CWVC (Samanya and Yamauchi, 2002). Feed intake and body weight gain tended to be increased in all experimental groups than those of control after feeding each experimental substance. In the feed conversion ratio, CWVC group showed the lowest value. Intestinal villus height, epithelial cell area and cell mitosis number also showed a tendency to be more increased in all experimental groups than those of control. In CWVC group, cell area of the jejunum and ileum were significantly elevated. On the duodenal villus surface of wood vinegar compound solution and charcoal groups, some cells devoid of microvilli were observed. In the CWVC group, such damaged cells were not found, and more remarkable cell protuberances than those of the control group appeared, suggesting that duodenal villus function might be activated. Such an activated morphology on the villus tip surface was found in all experimental groups in the jejunum, but in the charcoal and CWVC groups in the ileum. The present villus morphological findings demonstrate that among wood vinegar compound solution, charcoal and CWVC, the dietary supplement of CWVC might be the most effective substance for activating the intestinal absorptive function, and that the functional activation of whole intestine including the ileum may induce a slight elevation of chicken growth performance.

Intestinal villus histological alterations in chickens fed dietary dried Bacillus subtilis var. natto

Continued use of dietary antibiotics to improve poultry production have resulted in common problems such as the development of drug-resistant bacteria (Sorum and Sunde, 2001) and the imbalance of normal microflora (Andremont, 2000). A probiotic is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989), and is recommended as an effective alternative to antibiotics (Sissons, 1989 ; Tournut, 1989). Natto is a traditional Japanese health food made by fermentation of boiled soybeans in rice straw containing probiotics (Tamura, 1989 ; Tonouti *et al.,* 2000). The *Bacillus subtilis var. natto* (*B. subtilis natto*) (Ashiuchi *et al.,* 1998) cultured from natto is a Gram-positive sporeforming bacterium. In WL cockerels fed the dietary *B. subtilis natto* at 0, 0.5, 1 and 3% levels for 3 or 28 days, growth performance was not different amongst the groups, except that feed efficiency in all chickens fed dietary *B. subtilis natto* for 28 days tended to be improved (Samanya and Yamauchi, 2002). In these birds, blood ammonia concentration was decreased. These results suggest that the *B. subtilis natto* depressed ammonia concentration. On the other hand, in birds fed 0, 0.2, 0.5 and 1% dietary *B. subtilis natto* for 28 days, growth performance tended to be higher in the 0.2 and 0.5% groups than the 0% group. Intestinal villus height, cell area and cell mitosis of these birds were also higher than those of the controls. Flat cell outline on the duodenal villus surface in controls developed large, protruded cell clusters and cell protuberances after feedings of dietary *B. subtilis natto.* These results indicate that intestinal function was activated by the depressed blood ammonia concentration in the body of the chicken. The present results may suggest that the *B. subtilis natto* has potential to be a beneficial microorganism in chickens.

Segmented filamentous bacteria in the ileum of young chicks

Segmented filamentous bacteria are autochthonous bacteria colonizing the ileum of many young animals by attaching to intestinal epithelial cells. These non-pathogenic bacteria strongly stimulate the mucosal immune system by elevating immunoglobulin A-secreting cells (Klaasen *et al.,* 1993), and have a possible protective role against infection with *Salmonella enteritidis* (Garland *et al.,* 1982). Studies on segmented filamentous bacteria have been carried out morphologically, because these bacteria cannot be cultivated but can be detected by light and electron microscopy.

Segmented filamentous bacteria adhering to the ileal epithelial cells

In scanning electron microscopy, segmented filamentous bacteria adhering to the ileal epithelial cells were observed around the ileal villus apical area in 10–20-d-old chicks (Yamauchi *et al.,* 1990 a). Transmission electron microscopic investigations showed that bacteria had a cytoplasmic membrane, cell wall and nucleus but no nuclear membrane and organella, were compartmentalized. At the attachment zone to the epithelium, many mitochondria were observed in the epithelial cells ; the bacterial membrane did not fuse to the epithelial membrane except at the apex of the attachment end where the bacterial membrane seemed to undergo lysis, suggesting a possibility that some bacterial components were transferred to the epithelial cells.

Phagocytosis and intracellular processing of segmented filamentous bacteria by the ileal epithelial cells

In scanning electron microscopy, no, few, medium and dense segmented filamentous bacterial colonization levels were classified. In transmission electron microscopy from animals with medium or dense segmented filamentous bacteria colonization levels, we could observe extracellular particles ranging from those only indenting the cell membrane to particles found in the cytoplasmic area beyond the terminal web. These particles had a structural similarity with segmented filamentous bacteria floating freely in the intestinal lumen. Furthermore, we observed unlacing of the membrane and septum surrounding the extracellular particles, and melting of them into host cytoplasmatic components. This strongly suggests that these particles are phagocytized and intracellularly processed segmented filamentous bacteria. This is supported by transmission electron microscopic analysis of samples with no or few segmented filamentous bacteria, where we failed to find these characteristic morphologies. The phagocytosis process described here could be an important trigger for the stimulating effect of segmented filamentous bacteria on the mucosal immune system.

Increased postmolt egg reproduction rate after *ad libitum* refeeding the ground formula diet in layers

To improve the decreased egg production (Bell and Adams, 1992) and eggshell quality (Al-Batshan *et al.,* 1994), hens have been induced to molt using fasting (Baker *et al.,* 1983 ; Alodan and Mashaly, 1999). Generally, restricted feeding treatment is employed as a refeeding method after fasting. To improve the refeeding method to induce a rapid recovery in egg production after fasting, an understanding of the fine structural alterations of the intestinal epithelium during fasting and refeeding would be important, because histology of the intestinal epithelium might be intimately affected by fasting and refeeding treatments. Histologically, we have demonstrated that the refeeding of a nutritionally complete powdered diet *ad libitum* provides faster recovery of the duodenum than nutritionally incomplete or course textured diets (Shamoto and Yamauchi, 2000). To demonstrate whether this histological results influence postmolt performances in laying hens, WL hens were fasted until 31% body weight loss. Then, the chickens were randomly assigned into 4 groups and re-fed as follows for 10 days, 1) restricted fed from 15 g of a commercial finisher diet on the first post molting day to 90 g of the same diet on post molting 10 days (restricted group), 2) *ad libitum* fed of a ground formula diet of starter (starter group), 3) *ad libitum* fed of a ground formula diet of grower (grower group) and 4) *ad libitum* fed of a ground formula diet of finisher (finisher group). After 10 days treatment from post molting, all chickens could access freely to a commercial diet for layer. Feed efficiency was higher in the all *ad libitum* fed groups than in restricted fed group. Among *ad libitum* fed groups, the highest feed efficiency was observed in starter group, and followed by finisher group. Egg production was started on 11 th day after refeeding in starter and finisher groups, and hens started laying on 12 th day after refeeding in grower and restricted groups. Furthermore, the day egg production rate was recovered to 80 % in starter, grower, finisher and restricted on 14, 15, 15 and 18 th day, respectively. The egg production rate did not differ significantly among all groups after 18 days of refeeding. During the experimental period, all hens survived, and diarrhea feces and reduction of egg production rate were not observed. These results suggest that rapid recovery of egg production after forced molting would be achived by improvement of nutritional condition on early stage of post molting, and ad libitum feeding method is the most effective to improve egg production after forced molting.

This review demonstrates that intestinal histological alterations could possibly be used as an index for judging intestinal function and adds information to the assessment of the nutritional value of various kinds of chicken feed.

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