Effects of Semi-purified Pellet Diet on the Chicken Intestinal Villus Histology

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To establish the basic histological data on intestinal villi in chickens fed a semi-purified pellet diet, birds were divided into the following groups: intact control (IC), 3-d fasting (F), ad libitum refeeding of a formula mash diet (AFM), or force-feeding of a formula pellet diet (FFP) or a semi-purified pellet diet (FSP) for one day after 3-d fasting. The intestinal histological recovery in these groups was compared with light (LM) and scanning electron microscopy (SEM).

The body weight recovery was similar in AFM and FFP, but they tended to be faster than that of FSP. In LM observations, the decreased values of villus height, cell area, cell mitosis, and villus area in F tended to recover to the IC level in AFM and FFP and but did not recover in FSP. All parameters of AFM and FFP were almost the same. In SEM observations, the dome-shaped cells, cell outlines, and cell protuberances seen in IC became faint in F, and the luminal surface area of the cells became small (P<0.05). After refeeding, the dome-shaped cells and the clear cell outline reappeared in AFM and FFP, and such a morphological recovery was clearer in FFP than AFM. The luminal surface area of the cells tended to increase in AFM and increased in FFP (P<0.05). In addition, cell clusters were frequently observed in FSP. The present histological observations after refeeding suggest that the formula diet can induce hypertrophic intestinal histological alterations at the villus and cell levels. However, although the semi-purified diet does not bring about the histological activation of the villi, it is quicker than the formula diet to activate the cell function. Therefore, it seems that the formula diet produces intestinal histological alterations related to intestinal function at LM and SEM levels, but such a relationship is limited to the SEM level in the semi-purified diet.

This demonstrates that nutritional and physiological data from the semi-purified diet cannot always be applied to feeding with a formula diet.

Key words: semi-purified diet, pellet, intestinal villi, absorptive epithelial cells, histological change

Introduction

Generally, nutritional and physiological feeding experiments have been carried out using mash and crumble formula diets and semi-purified pellet diets. In formula diets, light microscopic (LM) parameters, such as villus height, cell area, and cell mitosis, are known to show a rapid decrease after feed withdrawal but recover quickly after refeeding; the tips of the villus surfaces become smooth after feed withdrawal but
recover their characteristic rough surface after refeeding (Shamoto and Yamauchi, 2000; Tarachai and Yamauchi, 2000; Yamauchi, 2002; Yamauchi et al., 1995, 1996; Yamauchi and Tarachai, 2000). These data obtained using formula diets have been used to assess the enteral nutrient absorption of ingested feeds from a histological point of view (Mekbungwan et al., 2002; Yamauchi, 2002). Although the assessment of each nutrient using semi-purified diets has been applied to the formula diets in poultry production, no information regarding the effects of a semi-purified pellet diet on the histology of intestinal villi has ever been published. In addition, although feeding experiments using semi-purified diets have been carried out in pellet form and by force-feeding, a clear understanding of how such diets and feeding affect the histology of intestinal villi is still lacking.

In this study, to establish basic histological data on intestinal villi in chickens fed a semi-purified pellet diet, birds were reared in a formula mash diet or force-fed a formula pellet diet or a semi-purified pellet diet after fasting, and the intestinal histological recovery in these groups was compared by LM and scanning electron microscopy (SEM).

Materials and Methods

Animals and diets

Male Sonea Brawn layer chickens were provided a commercial formula finisher mash diet (Table 1: Nippon Haigoushiryou Co., Ltd., Japan). The experimental semi-purified diets were prepared by mixing each nutrient with water and kaolin (Table 2) and dried at 40°C for pelleting.

Experiment design

At 144 days of age, 20 chickens of uniform body weight were selected and divided into five groups of four birds each: 1) intact control chickens fed ad libitum a

<table>
<thead>
<tr>
<th>Table 1. Composition of a commercial formular finisher mash diet</th>
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<tbody>
<tr>
<td>Ingredient</td>
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<td>Soybean meal, Rice bran</td>
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<tr>
<td>Fish meal</td>
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<td>Concentrate mixture</td>
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<table>
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<tr>
<th>Ingredient</th>
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<td>Phosphorus</td>
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commercial formula finisher mash diet (IC) ; 2) 3-d fasting with free access to water (F) ; 3) F followed by 1-d \textit{ad libitum} free access to the same formula mash diet (AFM) ; 4) F followed by 1-d force-feeding of a formula pellet diet (FFP) ; 5) F followed by 1-d force-feeding of a semi-purified pellet diet (FSP). During the refeeding period, all chickens were given free access to water. In the force-fed chickens, a total of 100 g of the diet was force-fed three times a day.

**Tissue sampling**

At the end of each experimental period, chickens under light anesthesia with diethyl ether were killed by decapitation. The part from the gizzard to the pancreatic and bile ducts was recognized as the duodenum, and a 5 cm length of the duodenum was quickly excised and placed in a mixture of 3\% glutaraldehyde and 4\% paraformaldehyde fixative solution on 0.1 M cacodylate buffer (pH 7.4). The same fixative was also injected into the intestinal lumen. A 3 cm length of the duodenum was used for LM observations, and the remaining 2 cm length was used for SEM observations.

**Light microscopy**

A 3-cm segment was transversally cut, fixed in Bouin’s fixative solution, and embedded in paraplast. Five-\mu m-thick transverse sections were cut, and every 10\textsuperscript{th} section was collected. After staining with hematoxylin-eosin, the following values were

<table>
<thead>
<tr>
<th>Ingredient</th>
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<tr>
<td>Milk casein</td>
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<tr>
<td>Alpha potato starch</td>
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<tr>
<td>Beta corn starch</td>
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<td>Tallow</td>
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<tr>
<td>Calcium carbonate</td>
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<td>Calcium phosphate tribasic</td>
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<tr>
<td>Sodium chloride</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Premix*</td>
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<tr>
<td>Kaolin</td>
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<td><strong>Total</strong></td>
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<th>Calculated chemical component (% W/W air dry basis)</th>
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<tr>
<td>Crude fiber</td>
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<tr>
<td>Dry matter</td>
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*Premix supplies (per kg premix) Vitamin A (600 IU), Vitamin D3 (120,000 IU), Thiamine nitrate 1,000 mg, DL-alpha Tocopherol acetate 2,000 mg, Nicotinamide 9,000 mg, Calcium pantothenate 6,000 mg, Chloride 20,000 mg, L-lysine hydrochloride 20,000 mg, Lactose 19,000 mg.

**Table 2. Composition of a semi-purified pellet diets**

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measured using an image analyzer (Nikon Cosmozone 1S Nikon Co., Tokyo, Japan).

**Measurement of villus height**

For the measurement of villus height, two villi having a lamina propria were randomly selected per transverse section. The length from the tip to the base, excluding the intestinal crypt, was measured. A total of 16 villi were counted from different sections in each bird. An average of these 16 villi was expressed as the mean villus height for each bird. Finally, the four mean villus height from four birds were expressed as a mean villus height for one treatment group.

**Measurement of cell area**

For the measurement of cell area, the area of the epithelial cell layer was randomly measured at the middle part of the villus, and the cell nuclei within this measured epithelial cell layer were then counted. Finally, the area of the epithelial cell layer was divided by the number of cell nuclei. A total of 16 samples per bird were counted in each group.

**Measurement of cell mitoses in the crypt**

For the measurement of cell mitoses in the crypt, mitotic cells having homogenous, intensely stained basophilic nuclei with hematoxylin (Tarachai and Yamauchi, 2000) were counted. In the case of cells in the late stages of division, the cell mitosis number was counted as one mitotic event. The total cell mitosis numbers were counted from four different sections for each bird, and these four values were used to calculate the mean cell mitosis for one bird. Finally, the four mean cell mitoses from four birds were expressed as a mean cell mitosis in one treatment group.

**Calculating the villus area**

For the villus area, the width of the villus was measured at the basal and apical parts, and two villi were selected for each section. The widths of 16 villi at the basal and apical parts were measured from different sections in each bird. The villus area was calculated from the villus height, villus basal width, and villus apical width according to method of Iji et al. (2001). A total of 16 calculations of the villus area were made for each bird. The average of these 16 villus areas was expressed as the mean villus area for each bird. Finally, the 4 mean villus areas from 4 birds were expressed as the mean villus area for one treatment group.

**Scanning electron microscopy**

A 2-cm duodenal sample was slit longitudinally, opened, and washed with 0.1 M phosphate buffered saline (pH 7.4). To prevent the slit intestine from curling, the edges were pinned to the paraffin-covered bottom of a petri dish containing a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in a 0.1 M cacodylate buffer (pH 7.4). The slit duodenal sample was fixed in this flattened position at room temperature for 2 h and cut into a 4 × 10 mm squares, and the pieces were washed with a 0.1 M sodium cacodylate buffer and postfixed with 1% osmium tetroxide in a 0.1 M ice-cold sodium cacodylate buffer for 2 h. The specimens were washed in distilled deionized water and dehydrated in graded ethanol solutions. The specimens were kept in isoamyl acetate and dried in a critical point drying apparatus (Hitachi HCP-1, Hitachi, Ltd., Tokyo, Japan) using liquid carbon dioxide as the medium. The dried
specimens were mounted on aluminium stubs with electrically conducting cement (silver paste), sputter-coated with platinum (RMC-Eiko RE vacuum coater, Eiko Engineering Co., Ltd., Tokyo, Japan) at 100 mtorr, 7 mA for 15 min. The epithelial cells around the central sulcus on the surface of a duodenal villus tip were examined with a Hitachi S-800 SEM (Hitachi, Ltd., Tokyo, Japan) at 8 kV.

**Calculating the luminal surface area of the cells**

For the measurement of the luminal surface area of epithelial cells, the number of cells distributed near the central sulcus on the surface of the villus tip was counted within a $2 \times 2$ cm square on SEM photographs ($\times 1,000$). The $2 \times 2$ cm area was divided by the number of cells, and these calculated values were expressed as the real luminal surface area of cells ($\mu m^2$). A total of 10 squares were randomly selected for each group.

**Statistical analysis**

All LM parameters and the calculation of the luminal surface area of the cells were statistically analyzed using one-way ANOVA, and significant differences among the treatments were determined with Duncan’s multiple range test using the Stat View program (Abacus Concepts, Inc., HULINKS, Inc., Tokyo, 171-002 Japan) at the level of $P<0.05$.

**Results**

**Relative body weight**

After a 3-d-fasting, the relative body weight decreased by 7.7% relative to the initial body weight. Fig. 1 shows a relative body weight of AFM (feed intake ; 64 g per bird per day), FFP, and FSP during a 1-d refeeding after a 3-d fasting and of IC during the final 1-d without fasting. After refeeding, AFM and FFP groups showed a higher relative body weight than IC ($P<0.05$), in which AFM and FFP were of similar values but tended to be higher than FSP.

**Light microscopic data**

Fig. 2 shows the villus height, cell area, cell mitosis number, and villus area of IC, F, AFM, FFP, and FSP groups. After feed withdrawal, all parameters tended to decrease, and the villus height and villus area decreased significantly ($P<0.05$). After refeeding, the AFM and FFP groups showed increased villus height and villus area ($P<0.05$) and a tendency to increase in cell area and cell mitosis ; both groups had similar values for each parameter. However, in the FSP group, the villus height and villus area tended to increase, while the cell area and cell mitosis were not different from those of the F group ; all parameters tended to be lower than those of the AFM and FFP groups.

**Scanning electron microscopic observations**

Fig. 3 is a dorsal view of the surface of a duodenal villus tip of IC, F, and AFM groups. Fig. 4 is an SEM micrograph of FFP and FSP groups. The luminal surface area of duodenal epithelial cells measured directly on the SEM micrographs is shown in Table 3. On the surface of the villus tip of the IC (Fig. 3 A), clearly defined round to pentagonal dome-shaped cells (arrowheads), clear cell outlines between them, cell protuberances into the intestinal lumen (arrows), and cell clusters (asterisk) composed
of dome-shaped cells were observed around the central sulcus, which had a rough surface. The cell clusters and protuberances disappeared or grew faint, appearing as flat cell and unclear cell outlines in F (Fig. 3 B). In addition, the luminal surface area of the cells became small (P < 0.05), resulting in a smooth surface. After a 1-d refeeding, the dome-shaped cells (arrowheads) and the clear cell outlines reappeared, and the luminal surface area of the cells tended to increase in the AFM group (Fig. 3 C). This morphological recovery was clearer in the FFP (Fig. 4 A) than it was in the AFM group, which had the more conspicuous dome-shaped cells (arrowheads) and larger luminal surface areas of cells (P < 0.05) than those of the AFM group. In addition, the cell clusters reappeared (asterisk). In the FSP group (Fig. 4 B), although the dome-shaped cells (arrowheads) and the luminal surface area of cells decreased more than in the FFP group (P < 0.05), various sizes of cell clusters (small asterisk, small cell cluster; large asterisk, large cell cluster) and deeper cells at the sites of recently exfoliated cells (small arrows) were observed in addition to the SEM morphological features seen in FFP, resulting in an unclear central sulcus. On the cell clusters, some cells devoid of any microvilli (large arrows) appeared among the intact cells covered totally with microvilli. These characteristic SEM features are illustrated in Fig. 5.

Discussion

During a 1-d refeeding after a 3-d fast, most groups showed a higher relative body weight than IC. The AFM and FFP groups had similar values and tended to be higher than the FSP group. This suggests an effective absorption of nutrients in each diet. Furthermore, the findings suggest that the formula diet has a more rapid effect on body growth.
weight gain than the semi-purified diet.

The main objective of this study was to obtain basic villus histological data regarding whether the semi-purified pellet diet induces histological alterations similar to those induced by the formula diet. Feed withdrawal treatment is known to induce atrophied LM and SEM histology, but these histological alterations are quickly recovered after a refeeding of the formula diet (Shamoto and Yamauchi, 2000; J. Poult. Sci., 40 (4)).

Fig. 2. Alterations of duodenal villus height, cell area, cell mitosis number in the crypt, and villus area in the intact control chickens (IC) and in 3-d fasted chickens (F) that were refeed *ad libitum* access to a formula mash diet (AFM) or force-feeding of a formula pellet diet (FFP) or force-feeding of a semi-purified pellet diet (FSP) for 1-d. The recovery of all parameters in FSP is slower than that of AFM and FFP. (mean ± SE ; n = 4).

*a, b* Means with different superscripts differ at *P* < 0.05.
In the present study, the villus area was observed to undergo alteration in a similar manner to the villus height, cell area, and cell mitosis. This indicates that the villus area would also be a suitable parameter by which to assess villus function. All LM parameters tended to increase in the AFM and the FFP groups, in which values were similar. To ascertain whether these villus histological alterations are related with intestinal function, digestive enzyme activities in the intestinal mucosa were measured to monitor intestinal function. The short villi were accompanied by reductions in the villus surface area and mucosal lactase and sucrase activities (Park et al., 2000), in the lactase and alkaline phosphatase activities (Zijlstra et al., 1997), in the alkaline phosphatase and disaccharidase activities (Lopez-Pedrosa et al., 1998), and in the total lactase phlorizin hydrolase enzyme activity and mucosal protein concentration (Dudley et al., 1998). On the other hand, long villi were observed in piglets that showed an increased body weight gain (Zijlstra et al., 1996), and increased villus size associated with activated cell proliferation (Lauronen et al., 1998). It has been suggested that long villi result in an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). It is understood that greater villus height and numerous cell mitoses in the intestine are indicators that the function of the intestinal villus is activated (Langhout et al., 1999; Yasar and Forbes, 1999). The intestinal structural and mucosal enzyme literature suggests that the present villi are histologically activated by the absorption of nutrients in both formula diets.

For the SEM observation of epithelial cell alterations, the exfoliative zone in the central sulcus seems to be the most suitable area due to its changeable features. Concurrently, epithelial cells originate by mitosis in the crypt and migrate along the villus surface upward to the villus tip within a few days (Imondi and Bird, 1966), where they are extruded into the intestinal lumen within 48 h of birth (Potten, 1998) or there is cell loss, associated with intraepithelial lymphocytes and lamina propria macrophages.
Epithelial cells reached the villus tip more quickly (within 3.0-3.1 days) in weaned lambs than in suckling animals (Attaix and Meslin, 1991). From these studies, the exfoliative zone is thought to show a much more dramatic alteration than the lateral surface. The present smooth surface of the villus tip in the F group recovered its rough surface in the AFM and FFP groups, and the luminal surface area of the cells increased in these groups more than it did in the F group. These findings correspond to our previous results (Shamoto and Yamauchi, 2000; Tarachai and Yamauchi, 2000; Yamauchi, 2002; Yamauchi et al., 1995, 1996; Yamauchi and Tarachai, 2000). Ono et al. (1987) reported that the number of epithelial cells per villus and the villus surface area measured directly on the SEM micrograph correlated well with the villus height and the number of epithelial cells of the same villus measured on the histological sections under an LM. Taking these results into consideration, the
formula diet can induce intestinal histological activation at the villus level as well as at the cell level, resulting in increased body weight.

Alternatively, all LM parameters of the FSP group tended to be lower than those of the AFM and the FFP groups, and the cell area and cell mitosis did not increase more than those in the F group. This indicates that a semi-purified diet might not be sufficient to recover LM parameters. However, in the SEM morphological results, various sizes of cell clusters and deeper cells at the sites of recently exfoliated cells were observed in the exfoliative zone in the FSP. On the cell clusters, some cells devoid of any microvilli appeared among the intact cells covered totally with microvilli. Epithelial cells reached the villus tip more quickly in weaned lambs than in suckling animals (Attaix and Meslin, 1991). Epithelial cells are known to be extruded into the intestinal lumen within 48 h (Potten, 1998) and 3.0–3.1 days (Attaix and Meslin, 1991) after birth and to be processed by intraepithelial lymphocytes and lamina propria macrophages (Hall et al., 1994; Mayhew et al., 1999). The present findings that many developmental steps were observed in cells in the FSP group suggest that the SEM features might be much more activated in the semi-purified diet than in the formula diet. The present results correspond with the finding that chickens that were refed the soluble hyperalimentative enteral solution had atrophic LM parameters but more activated cell features, such as those observed with SEM, than those that were refed the formula diet (Tarachai and Yamauchi, 2000). As the first villus recovery stage following refeeding, the particle size of the diet was a more important factor than the nutrient content (Shamoto et al., 1999); nutrients in the powdered semi-purified diet are more easily absorbed by cells than those

Fig. 4. Dorsal view of the surface of the duodenal villus tips of chickens treated with 1-d force-feeding of a formula pellet diet (A) or a semi-purified pellet diet (B) after 3-d fasting. Cells of chickens force-fed a semi-purified pellet diet show a more activated recovery than those force-fed a formula pellet diet. Arrowheads, dome-shaped cells; small asterisk, small cell cluster; large asterisk, large cell cluster; small arrows, deeper cells at the sites of recently exfoliated cells; large arrows, cells devoid of microvilli. Scale bar = 10μm (×1000).
of the formula diet, resulting in more activated features in cells, as observed by SEM. However, in the later villus recovery stage, nutrient content was the most important factor (Shamoto et al., 1999). The nutrients in the semi-purified diet may not be sufficient to induce the recovery of the LM parameters. This corresponds with the lowered recovery of body weight in the FSP group than in the AFM and FFP groups. These results suggest that a semi-purified diet does not induce intestinal histological activation at the villus level. Therefore, the body weight is lower than it would be with a formula diet. However, the semi-purified diet activates cell function more quickly than the formula diet.

In conclusion, the formula diet induces hypertrophic intestinal histological alterations at the villus and cell levels. However, although the semi-purified diet does not induce the villus histological activation, it activates the cell function more quickly than
the formula diet. These findings demonstrate that the formula diet produces intestinal histological alterations related to intestinal function at LM and SEM levels. However, the relationship is limited at the SEM level with a semi-purified diet. This suggests that nutritional data from the semi-purified diet cannot always be applied to the feeding of a formula diet.

References


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