Histological Intestinal Recovery in Chickens Refed Dietary Sugar Cane Extract

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ABSTRACT Sugar cane extract (SCE), the residue after removing glucose, fructose, and sucrose from sugar cane juice, has growth-promoting, antistress, and immunostimulation effects. The objective of this study was to investigate the effects of refeeding dietary SCE on recovery of BW and intestinal histology after withdrawing feed from chickens. Forty-eight male Sanuki Cochin chickens were assigned randomly to 6 treatments and 8 replicates in a completely randomized design. The 6 treatments were intact control chickens fed ad libitum a basal commercial grower mash diet; 3 d of feed withdrawal; feed withdrawal followed by 1 d of ad libitum access to the same commercial mash diet (AFC); and free access to the commercial mash diet with 0.05, 1, or 3% SCE for 1 d. All SCE groups gained more weight in 1 d of refeeding than the AFC group (P < 0.05). Compared with the AFC group, the SCE groups increased cell mitosis (P < 0.05). On the villus apical surface, flat epithelial cells of the feed withdrawal group developed more protuberated cells than those of the intact control group in all refeeding groups. Compared with the AFC group, the SCE groups showed more protuberated cells. In addition, in the 0.05% SCE group, cell clusters aggregated by many cells were observed on the villus apical surface. The present histological intestinal alterations in chickens refed a SCE-containing diet demonstrate that the villi and epithelial cells might be hypertrophied because of some component in the SCE, resulting in quicker BW recovery in SCE-fed birds compared with those in the AFC group.

Key words: epithelial cell, histology, intestinal villus, sugar cane extract

INTRODUCTION

At the end of the laying cycle, hens have been induced to molt to improve decreased egg production (Bell, 2003) and egg quality (Al-Batshan et al., 1994). Generally, feed withdrawal has been widely used as the most effective method for forced molting (Alodan and Mashaly, 1999; Landers et al., 2005). Because the intestine is the digestive and absorptive site of feed, the intestinal absorptive epithelial cells might be closely affected by feed withdrawal and refeeding treatments during forced molting. The most important problem in layer production is the improvement of the refeeding method to induce a rapid recovery in intestinal function after feed withdrawal. After refeeding for 3 and 6 h (first recovery stage), the decreased villus height, cell area, and cell mitosis number in fasted chickens showed a faster recovery with a rice bran diet (powder; low nutrient) than with a grower mash diet (mash; nutritious and well-balanced nutrient; Shamoto et al., 1999). However, after refeeding for 24 h (later recovery stage), these light microscopic parameters more completely developed with the grower mash diet than with the rice bran diet. These results suggest that for the first recovery stage of villi after fasting, a quickly absorbable form, such as a powdered diet, is important rather than the nutrient content of it and that for the further complete recovery stage of villi, a nutritious and well-balanced diet is important. Necessarily, the refeeding of a nutritionally complete powdered diet ad libitum provides faster recovery of the duodenum than a nutritionally incomplete or coarsely textured diet (Shamoto and Yamauchi, 2000). In practice, these histological fundamental results have demonstrated that rapid recovery of postmolt egg production is observed after ad libitum refeeding of a ground formula diet (Shamoto and Yamauchi, 2002).

Recently, sugar cane extract (SCE), a byproduct of glucose, fructose, and sucrose removal from sugar cane juice produced from sugar cane in the raw sugar manufacturing process, has been reported to induce growth-promoting (El-Abasy et al., 2002, 2004) and other adjuvant (El-Abasy et al., 2003b) effects because of the immunostimulation in intact chickens. Furthermore, SCE has protective effects against Eimeria tenella infection (El-
Table 1. Chemical composition of sugar cane extract adsorbed to an oilcake of rice bran (DM basis; 1:4)

<table>
<thead>
<tr>
<th>Item</th>
<th>Composition (%) of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>19.8</td>
</tr>
<tr>
<td>Crude fat</td>
<td>4.8</td>
</tr>
<tr>
<td>Crude fiber (insoluble fiber)</td>
<td>7.5</td>
</tr>
<tr>
<td>Ash</td>
<td>18.8</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>49.1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Abasy et al., 2003a) and enhanced immune responses in sublethally x-ray-irradiated chickens (Amer et al., 2004). These facts indicate that the improved growth performance and immune responses induced by the SCE would accelerate the recovery of damaged intestinal function because of feed withdrawal stress. Thus, it was of great interest to observe the aspects of histological intestinal recovery in these SCE-fed birds.

The chicks administered orally at the 0.05% SCE level showed the most effective results in growth promotion and immunostimulation (El-Abasy et al., 2002, 2003a, b). Also, the chickens fed 0.05% dietary SCE showed the most effective results in growth performance (Yamauchi et al., accepted). Therefore, the 0.05% dietary SCE was the supplementation rate used in this study. The 1 and 3% dietary SCE groups were used to produce a clearer morphological alteration in the intestine. In this study, fasted chicks were refed, and duodenal villus height, epithelial cell area, and cell mitosis number were compared using light microscopy. Fine structural alterations of the villus apex surface were also observed using scanning electron microscopy.

MATERIALS AND METHODS

SCE Preparation

Sugar cane juice was produced from sugar cane (Saccharum officinarum L.) via the raw sugar manufacturing process. Sugar cane extract (169 g of CP/kg, 5 g of fat/kg, 465 g of nitrogen-free extract/kg, and 361 g of ash/kg) was prepared by Shin Mitsui Sugar Co., Ltd. (Tokyo, Japan) as follows. Most sugar components, such as glucose, fructose, and sucrose from sugar cane juice, were separated by ion exchange column chromatography using synthetic adsorbent to produce SCE. Then, this SCE was adsorbed to an oilcake of rice bran (DM basis; 1:4) and dried for dietary supplement. Crude protein, fat,
and each refeeding day/initial BW of refeeding period, feed intake and BW were measured. All chickens were given free access to water. At the end of these 1-d refeeding period, each intestinal segment was also injected into the intestinal lumen. Immediately after finishing this injection step, each intestinal segment was prepared for light and scanning electron microscopy. For each intestinal segment, the segment from gizzard to pancreatic and bile ducts was regarded as duodenum; for jejunum, the area included segment from the ducts to Meckel’s diverticulum; and for ileum, the area included segment from the diverticulum to ileo-ceco-colic junction. The tissue samples were taken at the middle of each part.

**Light Microscopy**

A 2-cm segment of the duodenum, jejunum, and ileum was transversally cut in the beaker as just described, fixed in Bouin’s fixative solution, dehydrated, and embedded in paraplast. Five-micrometer transverse sections were cut, and every 10th section was collected. After staining with hematoxylin-eosin, the following values were measured using an image analyzer (Nikon Cosmozone 1S, Nikon Co., Tokyo, Japan).

**Measurement of Villus Height.** For measurement of villus height, 2 villi with 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 8 different sections in each segment per bird. An average of these 16 villi was expressed as the mean villus height for each bird. Finally, the 4 mean villus heights from 4 birds were expressed as a mean villus height for one treatment group.

**Measurement of Epithelial Cell Area.** One cell area from the longitudinally cut epithelial cells was conducted. Within one longitudinally cut villus, the area of columnar epithelial cell zone was counted at the middle level of this cell zone; then, the number of cell nuclei within this measured epithelial cell layer zone was counted. Finally, the cell area of the columnar cell zone was divided by the number of cell nuclei. A total of 16 samples per bird was counted in each group. An average of these 16 samples was expressed as the mean cell area for each bird. Finally, the 4 mean cell areas from 4 birds were expressed as the mean cell area for one treatment group.

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### Table 3. Average BW at the beginning and end of feed withdrawal, feed intake, and BW gain during 1 d of refeeding after a 3-d feed withdrawal in chickens

<table>
<thead>
<tr>
<th>Treatment¹</th>
<th>BW at the beginning of feed withdrawal (g/d)</th>
<th>BW at the end of feed withdrawal (g/d)</th>
<th>Feed intake (g per bird per d)</th>
<th>BW gain (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact control</td>
<td>1.22 ± 0.04</td>
<td>1.25 ± 0.02⁶</td>
<td>75.71 ± 6.76⁶</td>
<td>14.29 ± 4.14⁴</td>
</tr>
<tr>
<td>Feed withdrawal</td>
<td>1.15 ± 0.03</td>
<td>1.02 ± 0.03⁶</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AFC</td>
<td>1.16 ± 0.04</td>
<td>1.02 ± 0.03⁶</td>
<td>127.50 ± 24.37⁹</td>
<td>53.75 ± 16.36⁰</td>
</tr>
<tr>
<td>AFC with 0.05% SCE</td>
<td>1.17 ± 0.05</td>
<td>1.02 ± 0.04⁶</td>
<td>115.00 ± 5.22⁹</td>
<td>101.88 ± 6.61⁶</td>
</tr>
<tr>
<td>AFC with 1% SCE</td>
<td>1.17 ± 0.04</td>
<td>1.02 ± 0.04⁶</td>
<td>148.13 ± 16.90⁹</td>
<td>98.13 ± 11.49⁹</td>
</tr>
<tr>
<td>AFC with 3% SCE</td>
<td>1.17 ± 0.04</td>
<td>1.02 ± 0.03⁶</td>
<td>133.75 ± 10.47⁹</td>
<td>128.75 ± 11.41⁹</td>
</tr>
</tbody>
</table>

**Notes:**

- Means with varying superscripts differ significantly at *P* < 0.05 (mean ± SE; n = 8).
- The 6 treatments were intact control chickens fed ad libitum a basal commercial grower mash diet; 3-d feed withdrawal; 3-d feed withdrawal followed by 1 d of ad libitum free access to the same commercial mash diet (AFC), and free access to the commercial mash diet with 0.05, 1, or 3% SCE (sugar cane extract) for 1 d.

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**Birds and Housing**

Male Sanuki Cochin chickens from the animal science farm of Kagawa prefecture were placed into individual cages in an environmentally controlled room with a 14-h photoperiod at an average environmental temperature of 29°C. Birds were given ad libitum access to water and a basal commercial grower mash diet (18% CP, 2.85 kcal of ME/g; Nippon Formula Feed MFG., Co., Ltd., Kagawa, Japan; Table 2). The experimental diets were prepared by adding the SCE to the basal commercial mash diet.

**Experimental Design**

At 72 d of age, 48 birds were assigned randomly to 6 treatments and 8 replicates in a completely randomized design. The treatments were intact control chickens fed ad libitum a basal commercial grower mash diet; 3-d feed withdrawal; 3-d feed withdrawal followed by 1 d of ad libitum access to the same commercial mash diet (AFC), and free access to the commercial mash diet with 0.05, 1, or 3% SCE (sugar cane extract) for 1 d.

**Tissue Sampling**

At the end of the 1-d refeeding period, chickens under light anesthesia with diethyl ether were killed by decapitation. The entire small intestine was quickly excised from the longitudinally cut villus in the beaker as just described, placed in a beaker with a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The same fixative was also injected into the intestinal lumen. Immediately after finishing this injection step, each intestinal segment was bedded in paraplast. Five-micrometer transverse sections in each segment per bird. An average of these 16 samples was expressed as the mean villus height for each bird.

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**Light Microscopy**

A 2-cm segment of the duodenum, jejunum, and ileum was transversally cut in the beaker as just described, fixed in Bouin’s fixative solution, dehydrated, and embedded in paraplast. Five-micrometer transverse sections were cut, and every 10th section was collected. After staining with hematoxylin-eosin, the following values were measured using an image analyzer (Nikon Cosmozone 1S, Nikon Co., Tokyo, Japan).

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Measurement of Cell Mitoses in the Crypt. Mitotic cells with 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. Total mitosis numbers were counted from 4 different sections for each bird, and these 4 values were used to calculate the mean for one bird. Finally, these 4 means from 4 birds were expressed as the mean number of mitotic cells for one treatment group.

Scanning Electron Microscopy

A 2-cm duodenal sample, which was immediately distal to the segment collected for light microscopy, was slit longitudinally, opened, and washed with 0.1 M phosphate buffered saline (pH 7.4). To prevent 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 sample was fixed in this flattened position at room temperature for 2 h, cut into 4 × 10-mm rectangles, 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, dehydrated in 45 to 80% graded ethanol solutions, and kept in 80% ethanol. Just before drying of the specimens, the specimens were moved to 90 to 100% (each, 15 min) graded solutions followed by a mix solution of 100% ethanol and t-butyl alcohol (1:1; 15 min) and t-butyl alcohol (15 min; 2 times). Then, these specimens were freeze-dried (Hitachi freeze dryer, Hitachi E-1030 ion sputter, Hitachi Ltd., Tokyo, Japan). The dried specimens were mounted on aluminium stubs with electrically conducting carbon paste, sputter-coated with platinum, and examined with a scanning electron microscope (Hitachi S-4300SE/N, Hitachi Ltd.) at 8 kV.

All experiments were carried out according to the human care guidelines for the care and use of laboratory animals established by the Kagawa University.

Statistical Analysis

Chicken performance and all light microscopic data obtained in the experiments were statistically analyzed using one-way ANOVA, and significant differences among the treatments were determined with Duncan’s multiple range test using the Start View program (Abacus Concepts, Inc., HULINKS, Inc., Tokyo, Japan) at the level of \( P < 0.05 \).

RESULTS

After a 3-d feed withdrawal, BW of all birds decreased. After a 1-d refeeding, feed intake increased in all refeeding groups except for the 0.05% SCE group compared with the intact control group (Table 3). Body weight gain in all SCE groups showed higher values than those of the AFC and control groups.

Light Microscopic Observations

The villus height, cell area, and cell mitosis of the duodenum decreased after feed withdrawal, but these
light microscopic parameters increased after refeeding, except for the villus height of the AFC group (Figure 1). Compared with the AFC group, the SCE groups increased in cell mitosis.

**Scanning Electron Microscopic Observations**

On the villus apical surface of the intact control group (Figure 2A), faintly protuberated cells (arrows) and cell outlines between each epithelial cell were observed. After a 3-d feed withdrawal, the protuberances of cells and cell outlines disappeared, resulting in smooth surfaces (Figure 2B). After refeeding, cells protuberating further into the intestinal lumen than those of the control group were found in all SCE groups (arrows in Figure 3), resulting in clear cell outlines. Compared with the AFC group, the SCE groups showed more protuberated cells. Furthermore, in the 0.05% SCE group, cell clusters of many cells (stars) were observed.
DISCUSSION

Sugar can extract has been reported to induce growth-promoting (El-Abasy et al., 2002, 2004; Yamauchi et al., accepted) and immunostimulating (El-Abasy et al., 2002, 2003b) effects in intact chickens. This study has also shown that the relative BW of all feeding groups increased following 1 d of refeeding after a 3-d feed withdrawal. This is thought to have been induced by effective enteral absorption of nutrients in each diet.

In birds refed the SCE, most values of intestinal villus height and cell mitosis were higher than those of the AFC group. In addition, protuberated cells were observed in the SCE groups. Morphologically, it has been suggested that long villi result in an increased surface area that is capable of greater absorption of available nutrients (Caspar, 1992) and that greater villus height and more numerous cell mitosis in the intestine are indicators that the function of the intestinal villi is activated (Langhout et al., 1999; Yasar and Forbes, 1999). Actually, longer villi were reported in chickens showing a high activity of amylase in the intestinal content (Samanya and Yamauchi, 2002). Such longer villi were observed in heavy piglets fed milk replacer (Zijlstra et al., 1996) and in heavy turkeys fed dietary amylase (Ritz et al., 1995). Increased villus size has been associated with activation of cell proliferation (Lauronen et al., 1998). Conversely, short villi are known to be accompanied by reductions in the villus surface area (Park et al., 1998), resulting in reduced absorptive functions. Shorter villi have been reported in pigs (Zijlstra et al., 1997) and in chickens (Meneewan and Yamauchi, 2004; Mekbungwan et al., 2004) with decreased BW. Physiologically, shorter villi have corresponded with reductions in enzyme activities, such as mucosal lactase and sucrase (Park et al., 1998), lactase and alkaline phosphatase (Zijlstra et al., 1997), alkaline phosphatase and disaccharidase (Lopez-Pedrosa et al., 1998), and the total lactase phlorizin hydrodrolase and mucosal protein concentrations (Dudley et al., 1998) in pigs. In nutritional digestion trials and histological intestinal data, the higher values of the light microscopic parameters and the much more protuberated cells were observed in piglets showing a high nutrient digestibility than in piglets showing a low nutrient digestibility (Mekbungwan et al., 2004). These reports demonstrate that the high values of the light microscopic parameters and the protuberated cells are much more hypertrophied than the low values of the light microscopic parameters and the flat cells, respectively. In our previous refeeding experiments, more rapid recovery of these intestinal histologies were found in chickens refed an easily absorbable enteral hyperalimentative solution (Tarachai and Yamauchi, 2000) and a nutritionally complete powdered diet (Shamoto and Yamauchi, 2000) than in those refed nutritionally incomplete or coarsely textured diets. Such fundamental histological results related to intestinal function were actually demonstrated by an improvement in postmolt egg production in laying hens (Shamoto and Yamauchi, 2000). These studies indicate that the present increased light microscopic parameters and protuberated cells in the SCE groups might suggest hypertrophy and might have consequences relative to hypertrophy of villi, as demonstrated by the previously mentioned literature.

It is unclear at present why much greater recoveries of the BW, villi, and cells were observed in the SCE groups than in the control group, but it is possibly related to the composition of the SCE. The SCE has growth-promoting (El-Abasy et al., 2002, 2004; Yamauchi et al., accepted), immunostimulating (El-Abasy et al., 2002, 2003b), and antistress (Brekhman et al., 1978) effects and antioxidative activity (Nakasone et al., 1996; Takara et al., 2002). The SCE-treated pigs raised innate immunity to infections and showed a 7.87% growth enhancement compared with the controls (Lo et al., 2005). Therefore, the present rapid recovery of BW and intestinal histology in the SCE groups following damaged intestinal histology caused by feed withdrawal would be induced by a complex interrelationship of a wide range of biological effects. Conversely, further studies are being carried out to demonstrate which components induce the present rapid recovery in chickens fed SCE.

In conclusion, histological intestinal alterations in chickens reared a SCE diet demonstrate that the villi and epithelial cells might be hypertrophied because of the SCE, resulting in the quicker recovery of BW in SCE-fed birds than those in the AFC group.

REFERENCES


