Effect of Sugar Cane Extract, Commercial Probiotic and their Mixture on Growth Performance and Intestinal Histology in Broiler Chickens

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Abstract: Problem statement: As the intestinal function is intimately affected by fed diets, many kinds of natural substances and probiotics have been supplemented to broilers to raise poultry productivity due to activating intestinal function. Besides, the intestinal histology is clearly altered by intestinal functions. The aim of this study was to investigate whether Sugar Cane Extract (SCE) and commercial probiotic (SPB), either alone or in combination, could improve growth performance and how intestinal histological alterations would be observed in these birds. **Approach:** A total of 64, 7-d-old male broiler chicks were randomly assigned to 4 treatment groups, consisting of 4 replicates of 4 birds each. Commercial mash starter and finisher diets were supplemented with 0.05% SCE, 0.4% SPB, or a mixture of 0.05% SCE and 0.4% SPB (SCE + SPB). **Results:** Body weight gain was better in all the experimental groups than the control. The greatest improvement was observed in the SCE + SPB group. Most values of villus height, villus area, cell area and cell mitosis in all intestinal segments were higher (p<0.05) in the experimental groups than in the control group. Most epithelial cells on the villus apical surface of the experimental groups were composed of protuberant cells. In addition, cell clusters composed of these cells were observed in the duodenum of the SCE + SPB group and in the jejunum of the SCE group. In the ileum, the SCE + SPB group had the most protuberant cells. **Conclusion:** The present results of enhanced light microscopic parameters and protuberant epithelial cells in SCE and SPB groups suggest that the intestinal villi and epithelial cells might be hypertrophied by SCE and SPB. The fact that a synergistic effect was observed with regard to growth performance and intestinal histology in the SCE + SPB group suggests that SCE is a good supplement to probiotics.

Key words: Sugar cane extract, probiotics, chicken, villi, small intestine

INTRODUCTION

Various studies have demonstrated that probiotics can increase the growth performance and pathogen control of chickens. Living microorganisms have been used as probiotics, which induce health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Most probiotic microorganisms belong to the lactic acid bacteria, such as *Lactobacillus* spp., *Bifidobacterium* spp. and *Enterococcus* spp. (Klein *et al.*, 1998). The dietary supplementation of *Lactobacillus* has been manipulated to achieve *E. acervulina* reduction by altering intestinal intraepithelial lymphocyte subpopulations in chickens (Dalloul *et al.*, 2003) and reduced *Salmonella* in day-of-hatch broilers (Higgins *et al.*, 2007). A variety of yeast species are also used as probiotics. Supplementation with yeast such as *Saccharomyces* spp. can improve the immune functions of chickens by stimulating the secretion of secretory IgA in the intestine (Gao *et al.*, 2008). Spring *et al.* (2000) indicated that feeding dietary yeast to chickens could reduce the colonies of pathogenic microorganisms such as *Salmonella* in the ceca. Furthermore, yeast products could improve body weight gain and feed efficiency of broiler chickens (Zhang *et al.*, 2005). Another health-enhancing compound as a feed additive is Sugar Cane Extract (SCE). The main component of SCE is nitrogen-free extract (46.5%) and dietary SCE stimulates the immune system against *E. tenella* infection (El-Abasy *et al.*, 2003) and has growth promoting effects (El-Abasy *et al.*, 2002) by activating intestinal functions (Yamauchi *et al.*, 2006) in chickens.

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Based on the closely related effects of probiotic microorganisms and SCE on the intestinal immune system, it is possible that they could together enhance the resistance of the host to enteric pathogens. Therefore, the purpose of this study was to observe the effect of SCE and SPB, either alone or in combination on growth performance and histological intestinal alterations in broiler chickens.

MATERIALS AND METHODS

Sugar cane extract 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^{-1}, 5 g of fat kg^{-1}, 465 g of nitrogen free extract kg^{-1} and 361 g of ash kg^{-1}) was prepared by 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.

Commercial probiotic: The commercial probiotic product (Super-BioLicks®; SPB) provided by Nippon Formular Feed MFG. Co., Ltd. (Tochigi, Japan). It mainly consisted of *Leuconostoc* spp. (10^7 CFU g^{-1}), *Pichia* spp. (10^7 CFU g^{-1}) and *Bacillus subtilis* (10^5 CFU g^{-1}).

Animal and feeding experiments: The experiment was carried out in accordance with the guidelines for regulation of the Laboratory of Animal Science, Kagawa University, Japan. A total of sixty-four, 7-day-old male Marshall Chunky broiler chickens were used in a growth performance trial with 4 treatments and 4 replicates of 4 chicks each. The birds were housed in wire pens under daily lighting regimen of 24 h of light and environmental room temperature. A conventional mash diet (Nichiwa Sangyo Co., Ltd., Kagawa, Japan) (Table 1) was provided as basal diet. Diets were supplemented with 0.05% SCE, 0.4% SPB and combination of SCE and SPB (SCE + SPB) for a total of four treatments. Birds were fed with experimental starter and finisher diets from 7-21 and 22-49-day-old, respectively. Feed and water were allowed *ad libitum* access throughout the feeding periods. Feed consumption and body weight were recorded weekly.

Table 1: Feed formulation and nutrient composition of commercial broiler starter and finisher mash diet (%)

Item	Starter 1-21 days	Finisher 22-49 days
Ingredients		
Ground maize	51.00	58.00
Milo	2.00	9.00
Soybean meal	37.00	22.00
Fish meal	4.00	4.00
Animal fat	4.00	4.60
Rapeseed meal	-	0.60
Calcium carbonate	1.00	0.85
Calcium phosphate tribasic	0.70	0.65
Sodium chloride	0.15	0.15
Vitamin/ mineral premix[1]	0.15	0.15
Calculated chemical component		
Crude protein	23.50	18.00
Metabolizable energy (kcal kg^{-1})	3100.00	3200.00
Crude fiber	4.00	4.00
Crude fat	4.50	6.00
Calcium	0.80	0.70
Phosphorus	0.60	0.55

[1]Concentrate mixture including (per kg of diet): vitamin A 10800 IU, Vitamin D3 2000 IU, vitamin E 25 mg, vitamin K3 2 mg, vitamin B1 5.40 mg, vitamin B2 7.20 mg, vitamin B6 10.20 mg, vitamin B12 8 μg, biotin 0.30 mg, pantothenic acid 17 mg, folic acid 1.10 mg, nicotinic acid 70.20 mg, choline 1,500 mg, zinc 80 mg, copper 16 mg.

Tissue sampling: At the end of feeding experiment, 4 birds from each group were weighed and killed by decapitation under light anesthesia with diethyl ether. The whole small intestine were quickly excised and placed in the mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The intestinal segment from the gizzard to the pancreatic and bile ducts was regarded as duodenum, jejunum from the ducts to Meckel's diverticulum and ileum from the diverticulum to ileo-cecal-colonic junction. Each section was injected with the same fixative solution into the intestinal lumen and the middle section of which was taken.

Light microscopic examination: The segments were transverse cut approximately 2 cm from the duodenum, jejunum and ileum, fixed in Bouin's fixative solution at room temperature, embedded in paraplast and cut into 4 μm thick cross section. Every tenth section was collected and stained with hematoxylin-eosin. The 4 light microscopic parameters were measured for each intestinal segment using an image analyzer (Nikon Cosmozone 1S, Nikon Co., Tokyo, Japan).

For villus heights measurement, the villi having the lamina propria were chosen with the length from the villus tip to the base, except the villus crypt was measured. A total of 16 villi were expressed as the mean villus heights in each bird. The villus areas were calculated from the basal width, apical width and villus heights with 16 calculations of the villus area for each

bird. To measure one cell area, the epithelial cell layer was randomly measured at the middle of the villi and the number of cell nuclei within this layer was counted. The area of the epithelial layer was divided by this number, a total of 16 cell areas were counted for each bird. To measure the cells mitosis number in the villus crypt, four sections in each bird were randomly selected and counted mitotic cells having homogenous, basophilic nuclei intensely stained with hematoxylin-eosin. A total of cell mitosis numbers was counted from 4 different sections for each bird. Finally, the mean of each parameter of each bird was expressed as the mean for one group.

Scanning electron microscopic examination: Sections (approximately 2 cm in length) of duodenal, jejunal and ileal, which close to the light microscopic sample, were slit longitudinally. The intestinal contents were washed with 0.1 M phosphate buffered saline (pH 7.4). The tissue sample were pinned flat and fixed in this flattened position in the mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4) for 1 h at room temperature, cut into 4 mm x 4 mm squares and continued fixed for 1 h. The pieces were rinsed with 0.1 M sodium cacodylate buffer and were post-fixed with 1% osmium tetroxide in a 0.1 M ice-cold sodium cacodylate buffer for 2 h. The specimens were dried in a critical point drying apparatus. The dried specimens were coated with platinum and observed at 8 kV with Scanning Electron Microscope (SEM, Hitachi S-4300SE/N, Hitachi Ltd., Tokyo, Japan).

Statistical analyses: All data collected were analyzed by one-way Analysis Of Variance (ANOVA), supported by the Statistical Analysis System (SAS Institute, 2000). Statistical significant difference at $p < 0.05$ level due to treatments was separated by Duncan's multiple range tests.

RESULTS

Growth performance: Feed intake, body weight gain and the efficiency of feed utilization were not significantly different among groups (Table 2). However, feed intake was lower but body weight gain was better in all the experimental groups than that in the control. Among the experimental groups, the SCE + SPB group had the lowest feed intake, whereas the body weight gain was highest, resulting in higher feed efficiency.
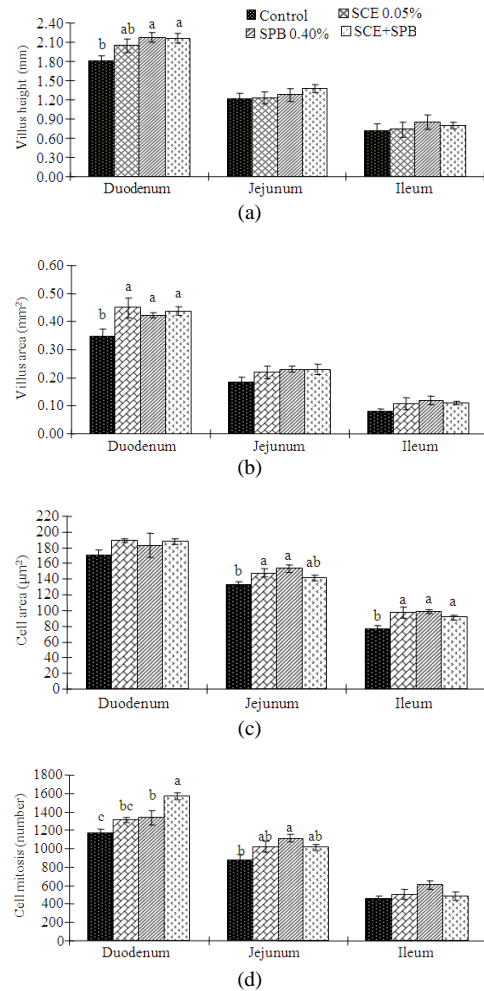

Fig. 1: Villus height, villus area, cell area and cell mitosis of duodenal, jejunal and ileal parts in the chickens fed dietary control, Sugar Cane Extract (SCE), Super-Biolicks (SPB) and the mixture of their (SCE + SPB) (n = 4). [a,b,c:] Means with different superscripts differ at $p < 0.05$

Histological analysis of light microscopic parameters: Compared with the control, most values of the intestinal villus height, villus area, cell area and cell mitosis number in each intestinal segment of the experimental groups were higher (Fig. 1). Values of duodenal villus height in SPB and SCE + SPB groups, duodenal villus area in all experimental groups, jejunal cell area in SCE and SPB groups and ileal cell area in all experimental groups were higher ($p < 0.05$). Mitosis in the duodenum of the SPB and SCE + SPB groups was improved and was increased ($p < 0.05$) in the jejunum of the SPB group.

Table 2: Growth performance in broiler fed dietary Sugar Cane Extract (SCE) and commercial probiotic (SPB) from 8-49 day old (n = 4)

	Control	SCE	SPB	SCE+SPB	p-value
Feed intake (g)	4855.4±75.90	4731.3±89.90	5142.2±297.50	4608.6±219.800	0.294
Body weight gain (g)	2733.0±112.9	2838.5±55.40	2790.8±54.700	2923.3±124.900	0.541
Feed efficiency	0.562±0.017	0.600±0.007	0.547±0.027	0.638±0.039	0.103

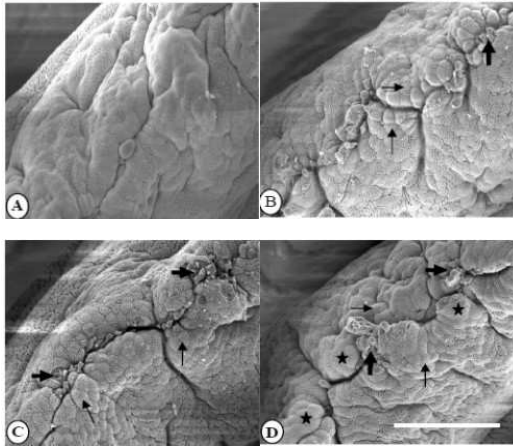There are no significant differences between each group (p>0.05)

Fig. 2: Epithelial cells on the duodenal villus apical surface in the chickens fed dietary commercial (control; A), Sugar Cane Extract (SCE; B), Super-Biolicks (SPB; C) and the mixture of their (SCE + SPB; D) (arrows = protuberant cells; large arrows = some cell devoid of any microvilli; stars = cell clusters). Scale bar, 50 μm
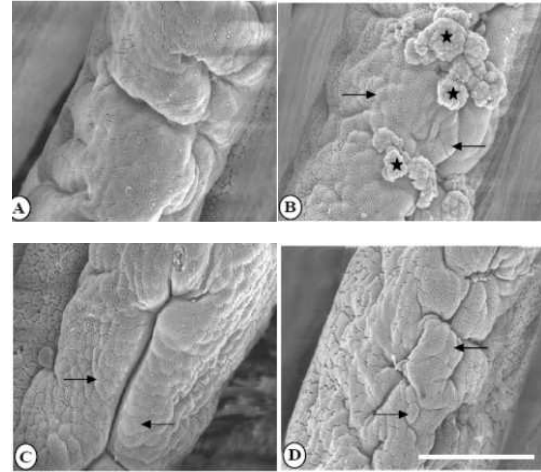
Morphology on the villus tip surface: Compared with comparatively flat epithelial cells of the control (Fig. 2A), the experimental groups had more protuberant cells (arrows) on the apical surface (Fig. 2B-D) and some cells around the central sulcus were devoid of any microvilli (large arrows). Cell clusters (stars), which were composed of many epithelial cells, were observed in the SCE + SPB group, resulting in a rough surface.

The jejunal villus tip surface of the control group was composed of flat cells, showing a smooth surface (Fig. 3A). However, in the experimental groups, most of the cells were slightly protuberant (Fig. 3B-D; arrows). In the SCE group, conspicuous cell clusters (stars) were observed.

The ileal villus apical surface in the control group had comparatively flat epithelial cells (Fig. 4A). In the SCE group (Fig. 4B), although each epithelial cell showed a similar morphology to the control, they formed cell clusters (stars) in some areas. In the SPB (Fig. 4C) and SCE + SPB (Fig. 4D) groups, conspicuous protuberances (arrow) of each epithelial cell and cell clusters (stars) appeared, with more greatly developed clusters in the latter.

Fig. 3: Epithelial cells on the jejunal villus apical surface in the chickens fed dietary commercial (control; A), Sugar Cane Extract (SCE; B), Super-Biolicks (SPB; C) and the mixture of their (SCE + SPB; D) (arrows = faintly protuberated cell; stars = cell clusters). Scale bar, 50 μm
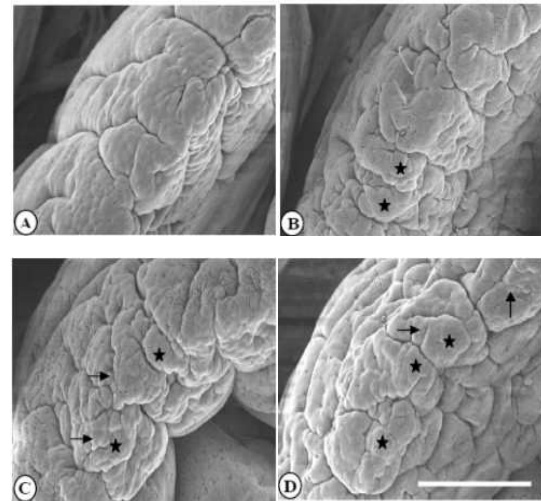

Fig. 4: Epithelial cells on the ileal villus apical surface in the chickens fed dietary commercial (control; A), Sugar Cane Extract (SCE; B), Super-Biolicks (SPB; C) and the mixture of their (SCE + SPB; D) (arrow = conspicuous protuberances; stars = cell clusters). Scale bar, 50 μm

DISCUSSION

In addition to nutritional-physiological studies on increasing poultry production with high quality of feeds, the exploitation of supplements to basal diets for maintaining the condition of the gut environment is also important. Recently, functional feeds such as probiotics and prebiotics have been used to enhance intestinal health and to obtain safe, reliable and high quality animal products without any medication and antibiotics. Probiotics are a live microbial feed additive that beneficially affects the host animal by improving its intestinal bacterial balance (Fuller, 1989). Chickens fed dietary probiotics showed improved body weight gain (Torres-Rodriguez *et al.*, 2005), reduced mortality (Vicente *et al.*, 2007) and enhanced feed conversion, ultimately resulting in an increase of broiler production (Willis *et al.*, 2007). Also, in this study, the SPB group showed a 2% higher body weight gain than the control. Alternatively, SCE has displayed a wide range of biological effects including immunostimulation (El-Abasy *et al.*, 2002), anti-inflammatory activity (Ledon *et al.*, 2003), activity as a vaccine adjuvant (El-Abasy *et al.*, 2003) and anti-stress effects (Brekhman *et al.*, 1978). Studies on chickens indicate that SCE acts as an adjuvant and has a protective effect against *Eimeria tenella* infection (El-Abasy *et al.*, 2003). Other effects of SCE including antibacterial activities and superoxide anion scavenging activity (Takara *et al.*, 2002) have been reported.

The combination of probiotics and other dietary substances has been the subject of renewed interest in health promotion due to their ability to enhance intestinal health. In piglets fed prebiotics, probiotics or synbiotics (a combination of the two) the population of bifidobacteria in the ileum increased and prebiotics and synbiotics increased their body weight gain (Shim *et al.*, 2005). The dietary combination of probiotics and Bio-mannan oligosaccharides improved the feed conversion ratio in broiler vaccinated against coccidiosis (Sun *et al.*, 2005). Also, in results described by Rowghani *et al.* (2007), a combination of probiotic and commercial feed additives had the most positive effect on performance in broilers. These results contrast with the result that broilers fed on either probiotics or mushroom extract were more likely to gain weight than those fed a mixture of them (Willis *et al.*, 2007). Such conflicting results might depend on the type of substances mixed with probiotics. The present SCE and SCE + SPB induced a 3.8 and 6.9% higher body weight gain than the control, respectively. As SCE had growth-promoting effects (El-Abasy *et al.*, 2002), the effects of dietary SCE + SPB on body weight gain might be due to the synergistic effect of SCE and SPB. This demonstrates that SCE would also be a good supplement with probiotics.

In the chickens fed the experimental diets, all values of light microscopic parameters were higher than those of the control. There is a strong relationship between intestinal mucosal histology and body weight change induced by intestinal function (Awad *et al.*, 2006). Increases in villus length (Adibmoradi *et al.*, 2006), villus width (Johnson and Jee, 1986), villus surface (Awad *et al.*, 2006) and cell mitosis (Yamauchi *et al.*, 2006) provide a greater surface area for higher nutrient absorptive potential and thus improve nutrient digestibility (Onderci *et al.*, 2006). Conversely, short and narrow villi are associated with a decrease in body weight (Batal and Persons, 2002) and a reduction in the specific activity of the brush-border enzymes such as lactase and sucrase (Pluske *et al.*, 1996) and mucosal enzymes such as peptidase and aminopeptidase (Hedmann *et al.*, 2006). From these reports, the present higher values of light microscopic parameters in the SCE and SPB groups suggest that the intestinal villi might be hypertrophied by feeding SCE and SPB.

In the experimental groups, many kinds of morphological changes such as protuberant cells and cell clusters were found on the villus tip. Epithelial cells produced by proliferation within the crypts migrate up to the villus tip and are shed into the lumen. Therefore, many kinds of morphological steps such as cell apoptosis and cell protuberances are observed on the villus tip. These cell morphologies are altered by feeding (Hooper, 1956) and could be evaluated by scanning electron microscopy (Yamauchi *et al.*, 2006). In rat intestinal villi, cell proliferation was depressed (Tessitore, 2000) and the number of apoptotic cells was increased (Boza *et al.*, 1999; Tessitore, 2000) by starvation. Such cell proliferation was increased (Tessitore, 2000) and apoptotic cells were decreased after refeeding (Boza *et al.*, 1999). These results suggest that protuberant cells indicate hypertrophy. As SCE induced growth-promoting (El-Abasy *et al.*, 2002) and immunostimulatory effects (El-Abasy *et al.*, 2003) and the probiotics could inhibit adhesion of pathogens to the chicken intestinal wall (Jin *et al.*, 1996) by binding to intestinal mucus (Bernet *et al.*, 1994), the observed protuberant cells may indicate hypertrophy as a result of feeding SCE or SPB. This corresponds with the fact that the SCE + SPB group showed the most conspicuously rough surface on the duodenal villus apical surface and that duodenal cell mitosis numbers were the highest in the SCE + SPB group.

However, the SPB group had many conspicuous protuberant cells, corresponding with the result that all values of the light microscopic parameters were the highest in the SPB group. This agrees with the observations that probiotic strains could exert beneficial effects in the lower small intestine (Mottet and Michetti, 2005) and that dietary whole yeast or yeast cell wall could improve ileal mucosal development by promoting a greater ileal villus height in broilers (Zhang *et al*., 2005). A consideration of the higher values of light microscopic parameters in the SPB group than other groups and of the findings of similar studies in the literature, leads to the general conclusion that the present higher values of villi and protuberant cells in the ileum might be hypertrophied by SPB.

CONCLUSION

The results show that dietary SCE and SPB could induce hypertrophied intestinal villi and epithelial cells, resulting in improved growth performance. The fact that a synergistic effect was observed with regard to growth performance and intestinal histology in the SCE + SPB group suggests that SCE is a good supplementary partner for probiotics.

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