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Research article

Effect of dietary new sugar cane extract on growth performance and intestinal histology in broiler chickens

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Abstract

The effects were investigated of feeding dietary new sugarcane extract (SCE) on the growth performance and intestinal histology of broiler chicks using two treatments and four replicates in a completely randomized design. Thirty-two newly hatched male broilers (Marshall Chunky) were assigned randomly to the two treatments: a basal diet group (control) and a basal diet supplemented with 0.1% new SCE group. There were no significant differences between the two treatment groups in feed intake, body weight gain and feed efficiency. Compared with the control, the broilers fed new SCE tended to increase in carcass percentage. Bird mortality was two in the control, and one in the new SCE group. There were no significant differences between groups regarding villus height, villus area and crypt depth in all intestinal segments, excepting the ileal villus height which tended to increase in the 0.1% new SCE group. Protuberated cells were observed on the jejunal and ileal villus apical surface in the 0.1% new SCE group. These results demonstrated that 0.1% dietary new SCE can activate intestinal epithelial cell function, especially in the jejunum and ileum segments.

Introduction

The European Union has banned the use of antibiotics as growth promoters from 1 January 2006, resulting in substitution with natural substances as antibiotics. The new sugarcane extract (SCE) is a byproduct of the raw sugar production. SCE mainly consists of phenolic compound such as sinapic acid, chlorogenic acid, apigenin, tricin and gallic acid (Xia et al., 2017). One function of SCE is immunostimulation when administered orally to chickens at the 0.05% SCE level, for 3 d consecutively, producing increased antibody levels and body weight (BW) gain (El-Abasy et al., 2002), and resistance against *Eimeria tenella* (El-Abasy et al., 2003).

Histological intestinal alteration has been demonstrated to indicate the response of intestinal function from ingested feed ingredients (Yamauchi, 2007). The gastrointestinal tract development of the broiler chicken is rapid 2 d after hatching (Uni et al., 1998). In previous studies, the quicker recovery of BW in fasted chicks re-fed 0.05% dietary SCE diet was caused by activated epithelial cells and villi (Yamauchi et al., 2006). Based on these concepts, the objective of the present study was to assess the impact of 0.1% dietary new SCE on more effective growth performance and visceral organ and histological intestinal alteration in broiler chickens.

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Materials and Methods

Preparation of sugar cane extract

Sugar cane juice was produced from sugar cane (*Saccharum officinarum* L.) via the raw sugar manufacturing process used by Mitsui Sugar Co., Ltd. (Tokyo, Japan) as detailed in Yamauchi et al. (2006). Old SCE excludes sugar from the molasses using chromatographic separation, while the present new SCE excludes sugar and salt from the molasses using an ultrafiltration membrane (0.001–0.01μm pores), resulting in an abundance of high-molecular weight components derived from polysaccharides and lignin. The new SCE consists of 51.6% sugar, 22.3% crude fiber, 17.1% crude protein and 8.9% ash.

Animals and housing

Thirty-two newly hatched male broilers (Marshall Chunky) were provided from a commercial hatchery (Kyowa-Furan Co., Ltd., Mitoyo, Japan). The chickens were fed conventional mash starter diet (Nichiwa Sangyo Co., Ltd., Kagawa, Japan) from days 1–21 and finisher from days 22–49 as a basal diet (Table 1). At age 7 d, the chickens were individually weighed and allocated to two groups: a basal diet group (control group) and a group fed the basal diet supplemented with 0.1% new SCE. Each group consisted of four replicates, with four birds per replicate pen. The chickens were raised in floor pens under natural light during the day and under continuous fluorescent lighting at night in an environment at room temperature with rice hulls used as bedding. Feed and water were available *ad libitum* throughout the experimental period (to age 49 d). The protocol for this study was approved by the Animal Care and Use Committee of Kagawa University, Kagawa-ken, Japan and the birds were handled according to the humane care guidelines provided by Kagawa University.

Table 1 Composition (%) of basal diets

Item	Starter 1–21 d	Finisher 22–49 d
Ingredient (%)		
Corn	59.00	42.00
Milo	2.00	22.00
Corn gluten meal	29.00	–
Soybean meal (45% CP)	–	27.00
Rice bran	2.30	5.20
Fish meal (57% CP)	7.00	3.00
Tallow	0.50	0.60
Vitamin/mineral premix*	0.20	0.20
Total	100.00	100.00
Calculated composition		
ME (kcal/kg)	3,050.00	3,250.00
CP (%)	22.00	18.00
Crude fat (%)	4.00	6.00
Crude fiber (%)	4.00	4.00
Crude ash (%)	7.00	7.00
Ca (%)	0.80	0.70
Available phosphorus (%)	0.50	0.45

CP = Crude protein; ME = Metabolisable energy.

*Vitamin and mineral premix included per kilogram of diet: retinyl acetate, 2,880 μg; cholecalciferol, 48 μg; DL-α-tocopherol acetate, 35 mg; menadione, 2.6 mg; thiamine, 5.8 mg; riboflavin, 7.3 mg; pyridoxine, 10.4 mg; cobalamine, 12.6 μg; biotin, 0.2 mg; folic acid, 1.0 mg; pantothenic acid, 16.1 mg; niacin, 69.1 mg; choline, 1,400 mg; manganese, 92.4 mg; zinc, 79.9 mg; copper, 12.8 mg.

Growth performance based on body weight and feed intake (FI) were measured weekly to calculate body weight gain (BWG); feed efficiency (FE) was calculated from BWG per FI. Each pen was used as the experimental unit.

Tissue sampling

At age 49 d, chickens with visually similar average body weights for each treatment from each pen were used for tissue sampling. The broiler chickens were killed by decapitation, then the whole small intestine was removed and immersed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The segment from the gizzard to the pancreatic and bile ducts was referred to as the duodenum, from the ducts to Meckel's diverticulum was referred to as the jejunum and from the diverticulum to ileo-cecal-colonic junction was referred to as the ileum.

Light microscopic examination

Intestinal samples (2 cm) were fixed using Bouin's fixative solution for 1 wk and then embedded in Paraplast Plus®, cut into 4 μm transverse sections, followed by staining with hematoxylin-eosin. Per bird, eight sections per intestinal segment were used to measure the villus height, villus area and crypt depth using an image analyzer (Nis-Element D, Nikon Co. Tokyo Japan). The villus area was calculated from the villus height, basal width and apical width according to the method of Iji et al. (2001). The crypt depth was measured from the base of the villus to the base of the crypt. The three villi with a similar height in each section were considered representative and used to measure the villus height, villus area and crypt depth.

Scanning electron microscopy

Samples (2 cm) of each segment were cut, opened and washed with 0.1 phosphate buffered saline. The samples were pinned on paraffin plates and fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4) at room temperature. These samples were cut into 2 × 10 mm square and fixed with the same solution. The pieces were then rinsed three times with 0.1 M cacodylate buffer and post-fixed with 1% osmium tetraoxide in 0.1 M cacodylate buffer for 2 hr. Then, the pieces were washed with cold deionized distilled water, dehydrated through an ethanol series and dried in a critical point drying apparatus (Hitachi HCP-1; Hitachi Ltd., Tokyo, Japan). The dried samples were fixed to aluminum stubs with electrically conducting cement and coated with platinum (Hitachi E-1030 Ion Sputter; Hitachi Ltd., Tokyo, Japan). The morphological alterations in the villus apical surface were observed and photographed using a scanning electron microscope at 500× magnification (SEM Hitachi S-4300SE/N; Hitachi Ltd., Tokyo, Japan).

Statistical analysis

The experiment was carried out using a completely randomized design with two treatments (0 and 0.1% new SCE) and four replicates, each containing four birds. Pens were used as the experimental units for growth performance. Growth performance and light microscopic parameters were analyzed with a one-way analysis of variance. Differences were considered to be significant at $p < 0.05$.

Results

Growth performance

There were no significant differences between groups regarding FI, BWG and FE (Table 2). However, values for the new SCE group were not significantly higher than for the control. In addition, the mortality of birds in the control was 12.5%, whereas in the new SCE it was 6.25%.

Table 2 Mean ± SD for feed intake (FI), body weight gain (BWG) and feed efficiency (FE) in broilers fed basal diet (control) and 0.1% new sugarcane extract (SCE) during 7–21 and 7–49 d ($n = 4$)

	Control	New SCE 0.1%	*p-value*
7–21 d			
FI (g)	991.875 ± 68.23	955.625 ± 52.18	0.431
BWG (g)	566.625 ± 38.66	588.063 ± 74.06	0.625
FE	0.573 ± 0.055	0.614 ± 0.054	0.330
7–49 d			
FI (g)	5780 ± 369.620	5778.54 ± 69.170	0.994
BWG (g)	3018.31 ± 114.71	3057.104 ± 356.56	0.843
FE	0.523 ± 0.019	0.529 ± 0.056	0.856
Carcass† (%)	53.468 ± 1.308	54.238 ± 1.313	0.293
Mortality (%)	12.50 ± 25	6.25 ± 12.50	0.670

†Carcass percentage = (carcass cutting yield) / (live weight) × 100.

Light microscopic villus measurements

Light microscopic parameters are shown in Fig. 1. There were no significant differences between groups for the villus height, villus area and crypt depth in all intestinal segments. Compared with the control, the ileal villus height tended to increase in the 0.1% new SCE group.

Scanning electron microscopic observations of epithelial cells on the apical surface of the villus

In the duodenum (Fig. 2), both the control and the 0.1% new SCE groups had flat cells and deeper cells at the sites of recently exfoliated cells. In the jejunum (Fig. 3), some cells without microvilli and deeper cells at the sites of recently exfoliated cells were observed around the central sulcus in the control while the 0.1% new SCE group had protuberated cells. In the ileum (Fig. 4), the control had flat cells, but the 0.1% new SCE group had protuberated cells, resulting in a rough surface. Segment filamentous bacteria (SFB) were observed in both groups as a characteristic feature.

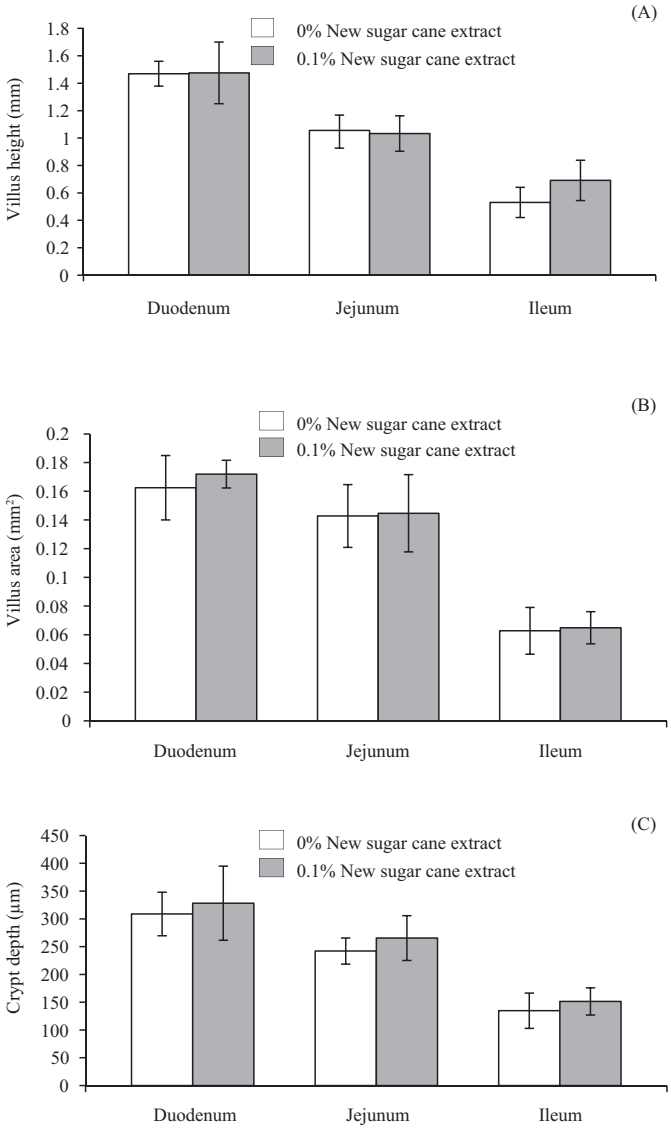

Fig. 1 Light microscopy results for duodenum, jejunum and ileum in broilers fed 0 (control) and 0.1% new sugarcane extract (mean ± SD, $n = 4$): (A) villus height; (B) villus area; (C) crypt depth

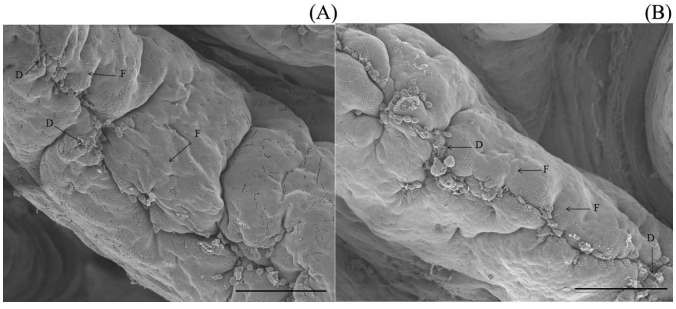

Fig. 2 Duodenal villus apical surface in broilers fed: (A) 0 % new sugarcane extract; (B) 0.1% new sugarcane extract, where F = flat cells, D = deeper cells at the sites of recently exfoliated cells and scale bar = 50 µm

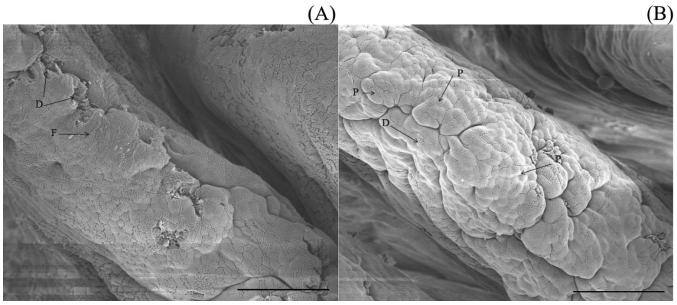

Fig. 3 Jejunal villus apical surface in broilers fed: (A) 0 % new sugarcane extract; (B) 0.1% new sugarcane extract, where F = flat cells, D = deeper cells at the sites of recently exfoliated cells, P = protuberated cells and scale bar = 50 µm

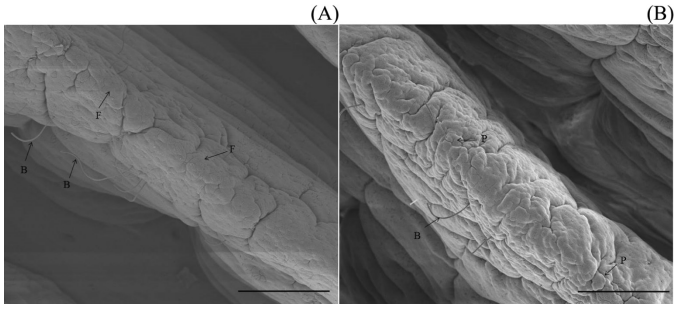

Fig. 4 Ileal villus apical surface in broilers: (A) 0 % new sugarcane extract; (B) 0.1% new sugarcane extract, where F = flat cells, P = protuberated cells, B = segment filamentous bacteria and scale bar = 50 µm

Discussion

The consecutive oral administration of SCE for 3 d or 6 d in chicks aged 1 wk was reported to improve BWG (El-Abasy et al., 2002). In the present study, the 0.1% dietary new SCE produced did not significantly increase BWG and FE throughout the experiment.

The light microscopic parameters did not show any clear improvement after feeding the 0.1% dietary new SCE. However, Khambualai et al. (2010) reported the higher values for light microscopic parameters in broilers fed dietary 0.05% SCE. The lack of any such response in the present study may have been related to the higher supplementation value of 0.1% compared to 0.05% used by Khambualai et al. (2010). Further studies will be necessary to determine more exactly the addition rate of the new SCE that is the most effective on BWG.

The birds fed dietary 0.1% new SCE had protuberated cells on the jejunal and ileal villus apical surfaces. The alteration of epithelial cells on the villus apical surface probably was affected by feeding. Flat cells of the villus apical surface were observed in 3 d feed withdrawal chickens and became protuberated after re-feeding (Yamauchi et al., 2006). On the villus apical surface, protuberated cells were more commonly observed with chickens fed a high protein diet compared to a low protein diet (Buwjoom et al., 2010). Furthermore, chickens fed dietary lysolecithin had higher body weight and their villus apical surface had more protuberated cells than for the control group (Khonyoung et al., 2015). These reports suggested that the presence of protuberated cells on the villus tip indicated activated intestinal function due to feeding dietary new SCE.

In addition, the mortality of birds fed the dietary new SCE was 6.25%, while for the control it was 12.5%. Chickens fed SCE had an enhanced antibody response (El-Abasy et al., 2002), as well as resistance against *Eimerie tenella* infection (El-Abasy et al., 2003). Wang et al. (2017) reported that SCE protected dextran sulfate sodium-induced colonic inflammation in mice via anti-inflammatory behavior and activated the superoxide dismutase 1 and Nrf2, which enhanced the anti-oxidative stress function. In the present study there were very few SFB in the chickens at age 49 d. However, many SFB were observed in chicks at age 10 d, and the ileal epithelial cells were the first triggering step for the immunological response to SFB by phagocytization (Yamauchi and Snel, 2000). In conclusion, the 0.1% dietary new SCE could activate the intestinal epithelial cell function, especially in the jejunum and ileum segments.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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