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Effects of Dietary Bamboo Charcoal Powder Including Vinegar Liquid on Growth Performance and Histological Intestinal Change in Aigamo Ducks

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Abstract: To investigate effects of a mixture of bamboo charcoal powder and bamboo vinegar solution (SB) on growth performance and histological intestinal change, 48 mixed sex Aigamo ducks were fed the basal commercial diet supplemented with SB at 0, 0.1 and 1% *ad libitum* for 49 days. Although, feed intake, weight gain and feed efficiency were not significantly different, the growth performance tended to be improved with increasing dietary SB. In these birds, also the intestinal villus height, villus area, epithelial cell area and cell mitosis in all intestinal segments tended to be increased with increasing dietary SB and increased in 1% dietary group ($p<0.05$). Besides, protuberated cells were observed on the villus apical surface in SB groups. These histological intestinal alterations of the villi and epithelial cells suggest that the intestinal function would be hypertrophied by the dietary SB and that the dietary SB can use at 1% level for Aigamo duck diets.

Key words: Bamboo charcoal powder, bamboo vinegar liquid, villi, histological intestinal change, Aigamo duck

INTRODUCTION

A European has culture to eat broiler breast meat for cooking willingly, but an Asian likes broiler leg meat for roasting. Because, the taste of breast meat is plain due to little fat. As a bird in place of broiler, Aigamo ducks, which is a hybrid of the *kamo* (wild duck) and the *ahiru* (domestic duck; Furuno, 1992), have developed to use their red breast meat like a leg meat taste. On the other hand, nowadays, the animal production is shifting from an emphasis on productive efficiency to public security. Therefore, several efforts have been made also to produce high quality animal products without using medicines and to reduce environmental contamination by efficient utilization of natural substances. Some of these natural substances are not cited in the scientific literature, but are used locally. Such one application in poultry diets is wood charcoal powder including vinegar liquid inclusion to diets in Japan. Wood charcoal has a high adsorptive capacity to remove ingested toxicants due to many kinds of pore size (Chandy and Sharma, 1998). Bamboo charcoal is also activated charcoal made by dry distillation of a thick-stemmed bamboo and powder of which is known as a universal adsorbent, because it can bind with variety molecules since it contains a complex network of pores of various shapes and sizes (Zhao *et al*., 2008). Its powder has been used as an oral antidote to reduce the absorption of poison from the gastrointestinal tract, because activated charcoal acts as an insoluble carrier that non-specifically adsorbs molecules, thereby preventing their absorption (Anjaneyulu *et al*., 1993). Adsorption therapy with activated charcoal as a non-digestible carrier is one of the important methods of preventing the ingested toxicants or noxious substances formed in the gastrointestinal tract (Van *et al*., 2006). On the other hand, bamboo vinegar compound liquid has obtained after cooling smoke during making bamboo charcoal. The characteristic odors in bamboo vinegar are sour, smoky and medicinal note. It is believed that bamboo vinegar can act as insecticide, a bactericide, a deodorant for treating malodour from pets and also as a folk medicine (Akakabe *et al*., 2006). The main component of bamboo vinegar compound liquid is acetic acid. Acetic acid is one of the main short chain fatty acids produced by intestinal microbes, which can affect intestinal functions and metabolism (Bergman, 1990; Kishi *et al*., 1999; Lutz and Scharrer, 1991). In addition to these effects of activated bamboo charcoal and bamboo vinegar compound liquid, recently, a mixed powder (Super BOB®, SB) of both substances has been completed by Shikoku Tekuno Ltd (Kagawa-ken, Japan) for animal feed supplementation. However, no growth performance of animals fed SB has yet traced.

The intestine seems to be the most fundamental organ for improving animal products. Activation of intestinal function of Aigamo ducks might increase meat products in response to an increasing demand for animal protein. Therefore, it was interesting to investigate how intestinal histology would be affected after feeding the SB. In this study, effects of dietary SB on feed intake, body weight gain and feed efficiency were examined in Aigamo ducks. Then, villus height, villus area, cell area and cell mitosis number in each intestinal segment of these ducks were observed using light microscope. Besides,

fine structural alterations of the villus apical surface were compared using scanning electron microscope.

MATERIALS AND METHODS

Birds and experimental design: Mixed sex Aigamo ducks were obtained from a commercial farm on the hatching day. They were housed in electrically heated brooder cages and had *ad-libitum* access to water and conventional starter mash diet for ducks (Table 1, Nippon Formula Feed MFG. Co., Ltd., Kanagawa-ken, Japan). At 7 days of age, the ducks were weighted and randomly divided into 3 groups with 4 replicates of 8 male and 8 female ducks. The means of weight of each group were arranged to be as uniform as possible and birds were maintained in cages at the density of 2 duck per cages (750 cm^2 per duck) in an environmentally controlled room under a photoperiod of 14 h light.

The basal commercial diet was supplemented with SB (Table 2-3) at 0, 0.1 and 1%. Commercial SB was produced in the company by as follows; bamboo vinegar compound liquid obtained after cooling smoke during making charcoal from a thick-stemmed bamboo by dry distillation at 700°C was kept for one year; then the skimmed solution was distilled to remove the harmful substances such as tar; this bamboo vinegar compounds of 3 liter were adsorbed into bamboo charcoal carbon powder of 8 kg. A conventional starter mash diet for ducks was fed to ducks up to 21 days of age and then the diet was changed to the finisher mash diet until 49 days old. Each ducks had free access to water and feed throughout the experiment. Feed intake and body weight gain were measured weekly.

Tissue sampling: At the end of the experiment, the remaining feed from each group was measured and returned to ducks. During tissue sampling, ducks were fully fed. Four ducks per group were selected at the mean body weight of each group and killed by decapitation after diethyl ether. All experimental treatments were performed according to the humane care guidelines provided by the Kagawa University. Small intestine was quickly removed and place in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The same fixative solution was also injected into the intestinal lumen. The intestine was divided into duodenum, jejunum and ileum. Intestinal part from the proventriculus to pancreatic and bile duct was regarded as duodenum; jejunum from the ducts to Meckel's diverticulum and ileum from the diverticulum to ileo-caeco-colic junction. The tissue sample was used from midpoint of each part.

Light microscopic examination: Each intestinal tissue was obtained from all groups. A 3 cm length of each intestinal segment was transversally cut and fixed in Bouin's solution and prepared for paraplast embedding. Four micrometer thick transverse sections were cut and every tenth 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: The highest 2 villi having a lamina propria were randomly selected per transverse section. The villus height was measured from the villus tip to the bottom, not including the intestinal crypt. 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 4 mean villus heights from 4 birds were expressed as a mean villus height for one treatment group.

Measurement of villus area: The width of villus was measured at the basal and apical parts and two villi were selected from each section. The width of 16 villi was measured from different sections in each bird. The apparent villus area was calculated from the villus height, basal width and apical width (Iji *et al*., 2001). A total 16 calculations of the villus area were made for each bird. The average of these was expressed as the mean for each bird. Finally, the 4 bird means were expressed as the mean villus area for one group.

Measurement of absorptive epithelial cell area: The area of the epithelial cell layer was randomly measured at the middle part of the villus and then the cell nuclei within this measured epithelial cell layer were then counted. Finally, the area of the layer was divided by the number of cell nuclei to obtain an epithelial cell area. A total of 16 samples per bird were counted in each group.

Measurement of cell mitoses in the crypt: Mitotic cells having homogenous, intensely stained basophilic nuclei with hematoxylin were counted. 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 mean cell mitosis for one treatment group.

Scanning electron microscopic examination: For scanning electron microscopic observations, a 2-cm tissue sample of each intestinal segment lying next to the light microscopic examination was transversely cut, slit longitudinally and intestinal contents were washed away with phosphate-buffered saline (pH 7.4). To prevent curling, the edges of tissue samples were pinned flat, serosa side up, to the paraplast-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) at room

temperature. Then, samples were cut into 4×7-mm^2 squares and fixed for 1 h more. The pieces were rinsed with a 0.1 M sodium cacodylate buffer and post-fixed with 1% osmium tetroxide in a 0.1 M ice-cold sodium cacodylate buffer for 2 h. These specimens were washed in distilled deionised water and dehydrated in graded ethanol solution. The specimens were kept in t-butyl alcohol 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 millitorr under 7 milliamperes for 15 min and observed using a scanning electron microscope (Hitachi S-4300SE/N, Hitachi Ltd, Tokyo, Japan) at 8 kV. Morphological alterations of the epithelial cells on the villus apical surface were compared between groups. Because the exfoliative zone around the central sulcus on the villus apical surface is a final goal of epithelial cell life after birth in the intestinal crypt. We observed the morphological alterations of epithelial cells around the central sulcus.

Statistical analysis: All data collected for feed intake, body weight gain, feed efficiency and light microscopic examination were statistically analyzed by using the one-way Analysis of Variance (ANOVA) and significant differences between the treatments were determined with Duncan's multiple range test using the SAS® program (Statistical Analysis Systems Institute Inc., Cary, NC, USA). Differences at $p<0.05$ were considered as significant.

RESULTS

Growth performance: Table 4 shows feed intake, body weight gain and feed efficiency of ducks fed 0, 0.1 and 1% dietary SB diets. Although all values did not show a statistical difference, weight gain and feed efficiency tended to be improved with increasing dietary SB.

Villus height, villus area, absorptive epithelial cell area and cell mitosis: All light microscopic parameters showed a tendency to be increased with increasing dietary SB in all intestinal segments and they increased in 1% SB group ($p<0.05$) (Fig. 1).

Morphological alterations of the epithelial cells: On the duodenal villus apical surface of the control group (Fig. 2a), flat cells (arrows) were found. In the 0.1% SB group (Fig. 2b), flat cells from birds in the control group themselves became protruding large cells in the 0.1% SB group, which aggregated around the central sulcus (arrows). In the 1% SB group (Fig. 2c), most cells were much more protuberated (large arrows) and had formed large cell clusters (arrows with C).

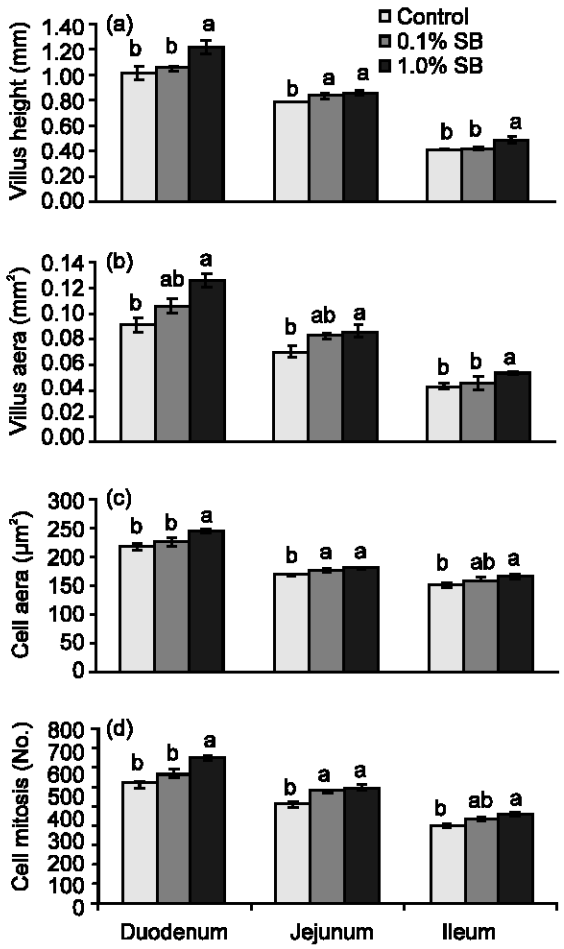

Fig. 1: Villus height, villus area, cell area and cell mitosis number in each intestinal segment of duck fed a commercial mash duck diet (control group), the commercial mash duck diet supplemented with 0.1% SB and 1% SB (n = 4, mean±SE). a,bMeans with different superscripts are significantly different from each other ($p<0.05$). Most values are higher in SB groups than control

On the jejunal villus apical surface of the control group (Fig. 3a), cells were also flat (arrows). In the 0.1% SB group (Fig. 3b), a part of faintly protuberated cells (arrows) were found. In the 1% SB group (Fig. 3c) was similar to the 0.1% SB group, except that large cell clusters (arrow with C) were observed in addition to the protuberated cells (large arrows).

On the ileal villus apical surface of the control group (Fig. 4a), cells were also flat (arrows). In the 0.1% SB group (Fig. 4b), most cells were much more protuberated (arrows). In the 1% SB group (Fig. 4c), a conspicuous protuberance of each cell (large arrows) and cell clusters composed of many cells (arrows with C) were observed.

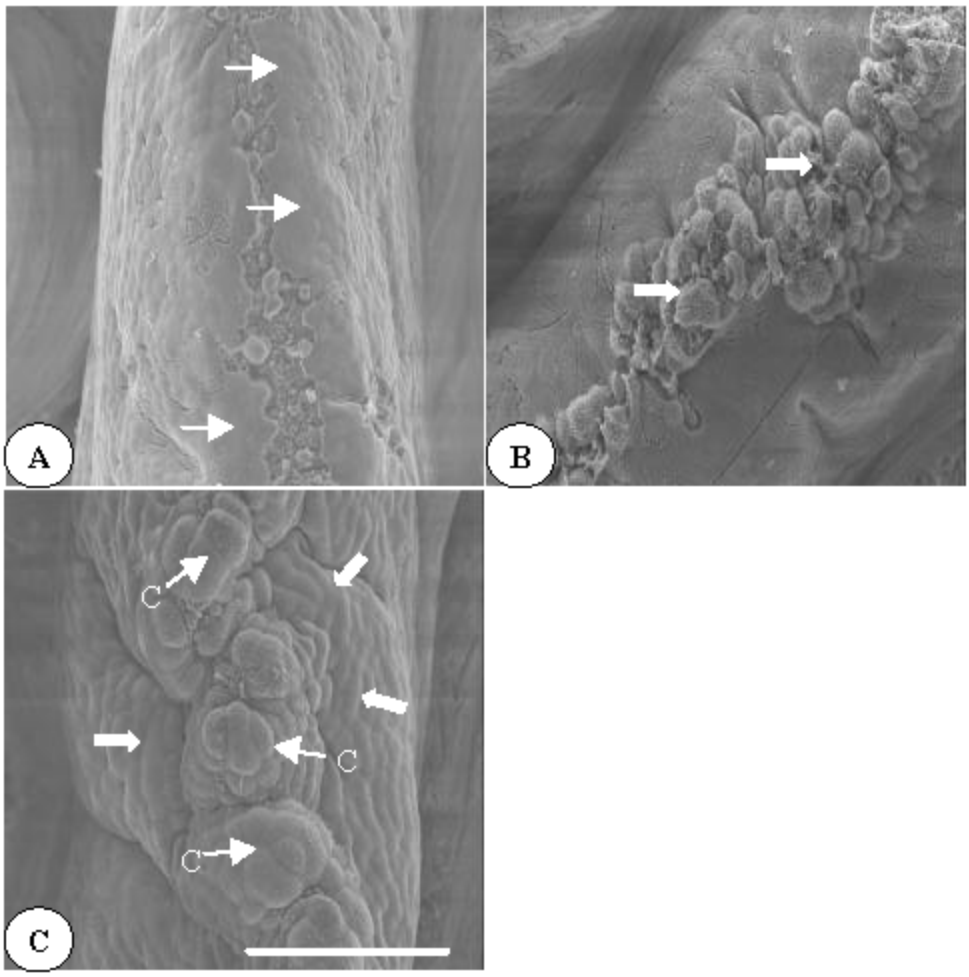

Fig. 2: Duodenal villus apical surface of ducks fed dietary SB at 0% (A; arrows, flat cell area; 0.1% (B; arrows, protruding large cells around the central sulcus) and 1% (C; large arrows, protuberated cells; arrows with C, cell clusters composed of many cells). Scale bar 50 μm (x1000).

DISCUSSION

The main purpose of this study was to investigate whether the growth performance of Aigamo ducks fed dietary SB could be much more improved and to investigate how alterations in intestinal histology could be observed in these ducks. We failed to obtain a significant increase of body weight gain in the SB-fed ducks. It might be related with that they are small type birds, because Aigamo duck is a crossbreed of wild duck and domestic duck (Tojo *et al*., 2007). However, the body weight gain of 1% SB group was 8% heavier than the control group. Besides, feed efficiency also tended to be higher in the SB groups than in the control group. As the present groups were studied in the same environmental conditions except the different dietary SB levels, the slightly increased growth performance of SB groups might be induced by the dietary SB levels.

In these birds, light microscopic parameters were increased with increasing SB levels. It has been suggested that long villi result in increased surface area capable of greater absorption of available nutrients (Caspary, 1992) and long villi were reported in Aigamos (Khambualai *et al*., 2008) and in piglets (Zijlstra *et al*., 1996) that showed an increased body weight gain. It is understood that greater villus height and numerous cell mitoses in the intestine are indicators that the function of the intestinal villi is activated (Langhout *et al*., 1999; Yasar and Forbes, 1999). Increased villus size induces cell proliferation in the crypt (Lauronen *et al*., 1998). Besides, protuberated cells and cell clusters were observed on the villus apical surface of the SB groups. Such cells were reported in higher body weight in chickens (Yamauchi *et al*., 2006) and in Aigamos (Khambualai *et al*., 2008). Flat cells were observed in

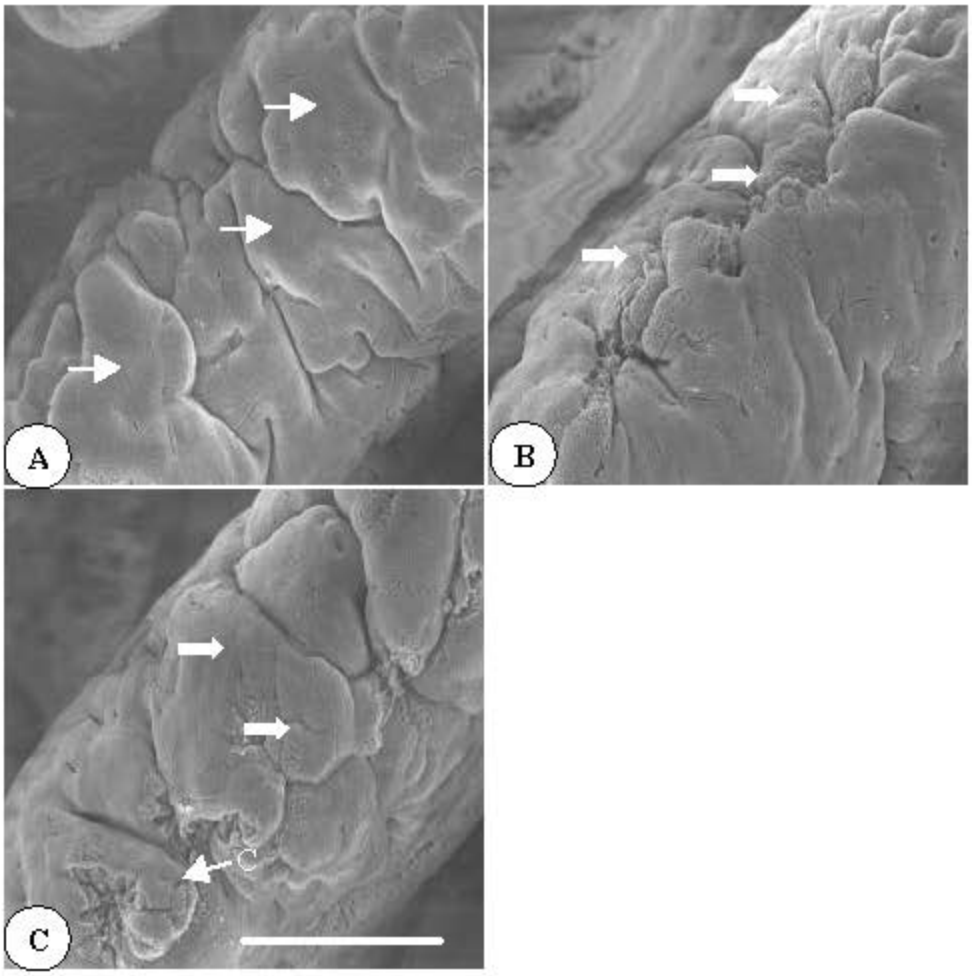

Fig. 3: Jejunal villus apical surface of ducks fed dietary SB at 0% (A; arrows, flat cell area), 0.1% (B; arrows, faintly protuberated cells) and 1% (C; large arrows, protuberated cells; arrow with C, cell clusters). Scale bar 50 μm (x1000).

the piglets fed diets including trypsin inhibitors, but protuberated cells appeared after feeding diets abolished such trypsin inhibitors, body weight gain was higher in the former than the latter (Mekbungwan and Yamauchi, 2004). From these studies, the present increased light microscopic parameters and protuberated cells suggest that the function of villi and epithelial cells might be hypertrophied after feeding dietary SB.

Profitability of wood charcoal was reported in reducing the effects of toxin in diets by adsorbing it and thereby preventing its absorption from the intestine (Anjaneyulu *et al*., 1993). When wood charcoal was added to diets containing aflatoxins or T-2 toxins, reductions in feed intake and body weight gain of chickens were ameliorated (Dalvi and McGowan, 1984; Anjaneyulu *et al*., 1993; Edrington *et al*., 1997). Wood charcoal carbon powder adsorbed much more selectively *Salmonella Enteritidis* than conventional *Enterococcus faecium* and wood vinegar liquid inhibited the growth of *S. Enteritidis* but accelerated the growth of *E. faecium* and *Bifidobacterium thermophilum* (Tana *et al*., 2003). Bamboo charcoal is considered to have a higher adsorption capacity than wood charcoal because of the special structure of micro pores of the bamboo stem (ChungPin *et al*., 2004). Bamboo charcoal is known to have about 4 times more cavities, 3 times more mineral content and 4 times better absorption rate (Zhao *et al*., 2008). On the other hand, the acetic acid is also main component of bamboo vinegar liquid. Acetic acid is one of the main short chain fatty acids produced by intestinal microbes, which can affect intestinal functions and metabolism (Bergman, 1990; Kishi *et al*., 1999; Lutz and Scharrer, 1991). Acetic acids were reported to control the balance of intestinal microflora and pathogen (Sorrells and Speck, 1970). These reports suggest that the

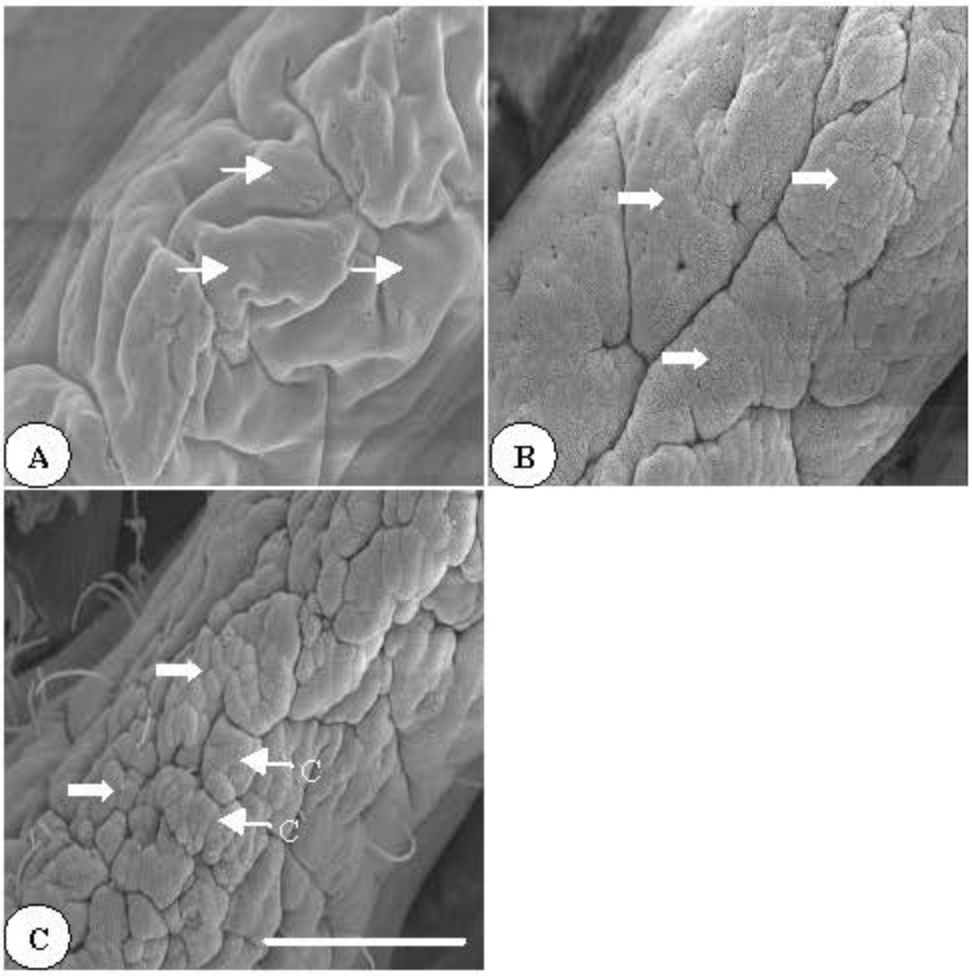

Fig. 4: Ileal villus apical surface of ducks fed dietary SB at 0% (A; arrows, flat cell area), 0.1% (B; arrows, protuberated cells) and 1% (C; large arrows, protuberated cells; arrows with C, cell clusters composed of many cells). Scale bar 50 μm (x1000).

Table 1: Feed formulations and nutrient composition of commercial duck starter and finisher mash diets (g/kg)

Item	Starter	Finisher
Ingredients		
Ground Corn	530.0	650.0
Soybean meal	370.0	250.0
Fish meal	50.0	40.0
Rice bran	-	1.0
Concentrate mixture"	50.0	59.0
Chemical component (g/kg)		
Crude protein	230.00	180.00
Metabolizable energy (MJ/kg)	12.77	13.19
Crude fiber	40.00	55.00
Crude fat	45.00	50.00
Calcium	8.00	7.00
Phosphorus, available	6.00	4.50

"Concentrate mixture including (per kg of diet): Vitamin A 9,600 IU, vitamin D_3 1,920 IU, vitamin E 35 mg, vitamin K_3 2.6 mg, vitamin B_1 5.8 mg, vitamin B_2 7.3 mg, vitamin B_6 10.4 mg, vitamin B_{12} 12.6 μ, biotin 0.2 mg, pantothenic acid 16.1 mg, folic acid 1.0 mg, niacin 69.1 mg, choline 1,400 mg: minerals; zinc 79.9 mg, copper 12.8 mg, manganese 92.4 mg

Table 2: Composition of bamboo vinegar compound solution

Item	(%)
Acidity	2.90
Total organic carbon	2.38
Acetic acid	2.72
Phosphate	0.10
Methanol	0.07
Formaldehyde	0.003
Phenol	0.134
Cresol	0.051
Tar	1.10
pH	2.70

present hypertrophied villi and epithelial cells in SB birds might be multiplicatively induced by both effects of bamboo charcoal and bamboo vinegar liquid, because the circumstance of intestinal lumen would be improved due to many good intestinal flora.

Table 3: Composition of bamboo charcoal powder

Nutrients	(%)
Ash	6.35
Nitrogen	0.57
Phosphate	1.06
Potassium	2.10
Silicon dioxide	1.20
pH	10.20

Table 4: Effects of bamboo charcoal powder including vinegar liquid (SB) levels on feed intake, body weight gain and feed efficiency in ducks (n = 4, mean±SE)

Items	Control	0.1% SB	1% SB
Initial body weight (kg)	0.133±0.001	0.132±0.002	0.132±0.001
Final body weight (kg)	1.215±0.03	1.229±0.05	1.302±0.03
Feed intake (kg/bird)	4.301±0.11	4.220±0.07	4.230±0.08
Body weight gain (kg/bird)	1.082±0.03	1.109±0.04	1.170±0.02
Feed efficiency	0.252±0.005	0.263±0.01	0.276±0.005

Conclusion: In conclusion, the present histological intestinal alterations in Aigamo ducks fed the dietary SB demonstrate that the function of villi and epithelial cells could be hypertrophied in all intestinal segments, resulting in improved growth performance. These results of this experiment are confirmed that the dietary SB can use as natural substance to supplement in Aigamo duck diets for alternative to antibiotics.

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