Animal (2008), **2:3**, pp 366–374 © The Animal Consortium 2008
doi: 10.1017/S1751731107001243

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Effects of a charcoal powder–wood vinegar compound solution in piglets for raw pigeon pea seed meal

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(Received 28 March 2007; Accepted 22 October 2007)

Histological intestinal villus alterations were studied in piglets fed a raw pigeon pea meal (PM) diet including a powder mixture of amorphous charcoal carbon and wood vinegar compound solution (CWVC). Twenty-eight male castrated piglets were divided into seven dietary groups of four piglets each. The control group was fed raw PM supplemented to the basal diet (178 g/kg crude protein, 4.23 kcal/g gross energy) at 0 g/kg (CONT), 200 g/kg (PM200) and 400 g/kg (PM400). The treatment groups were fed CWVC in both PM200 and PM400 diet groups at levels of 10 g/kg and 30 g/kg (PM200 + CWVC10, PM200 + CWVC30, PM400 + CWVC10 and PM400 + CWVC30). With increasing dietary PM levels, daily feed intake tended to increase. In contrast, daily body-weight gain tended to decrease, significantly in the PM400 group ($P < 0.05$), resulting in a significant decrease of feed efficiency in PM groups ($P < 0.05$). Body-weight gain and feed efficiency were higher in the CWVC groups compared with the PM groups. The duodenum and ileum were longer ($P < 0.05$) in the PM400 group than in CONT, but were similar to CONT in CWVC groups. The liver was heavier ($P < 0.05$), whereas the weights of the heart, kidney and stomach were decreased in the CWVC groups than in other groups. Most values for the intestinal villus height, cell area and cell mitosis number were lower in PM groups than those in CONT ($P < 0.05$) for each intestinal segment; however, these values were higher in CWVC groups than in PM groups ($P < 0.05$). The epithelial cells on the duodenal villus surface of the PM200 group showed cell morphology almost similar to CONT. However, the PM400 group had a smooth villus surface due to the presence of flat cells. The epithelial cells of the CWVC groups were protuberated, resulting in a much rougher surface than CONT. The current growth performance and histological intestinal alterations in piglets fed PM and PM + CWVC diets demonstrate that the intestinal features might be atrophied by feeding PM, resulting in decreased growth performance. CWVC might prevent the harmful effects of PM dietary toxins on intestinal function, resulting in a normal growth performance.

Keywords: charcoal powder–wood vinegar, intestinal histology, pigeon pea, pigs

Introduction

Pigeon pea (*Cajanus cajan* (L.) Millsp.) seeds have a great potential as an important protein source (Cheva-Isarakul, 1992; Oshodi *et al*., 1993) and every effort has been made to use pigeon pea seed meal (PM) for chickens (Mizubuti *et al*., 1995), pigs (Batterham *et al*., 1993; Mekbungwan *et al*., 1999) and lambs (Rao and Phillips, 2001). However, the efficacy of PM is limited by the presence of protease inhibitors such as trypsin and chymotrypsin inhibitors (Batterham *et al*., 1993). To improve the nutritional quality of PM, these antinutritional factors can be eliminated by heating (Singh, 1988; Mekbungwan and Yamauchi, 2004), boiling (Rani *et al*., 1996), roasting (Simoongwe, 1998), extraction (Benjakul *et al*., 2000) and cooking (Aarti *et al*., 2001), resulting in improved protein and starch digestibility (Rani *et al*., 1996). In our histological study (Mekbungwan and Yamauchi, 2004), the intestinal villi and epithelial cells of piglets fed raw PM showed an atrophic morphology owing to the presence of such antinutritional factors, resulting in decreased growth performance. Heating PM prevented the harmful effect of the antinutritional factors on villus histology, resulting in a growth performance comparable with the controls.

Additionally, charcoal powder has been used to reduce the adsorption of toxins from the gastrointestinal tract. Activated charcoal was reported to reduce the effects of dietary toxins by preventing intestinal absorption

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(Anjaneyulu *et al*., 1993). Also, a dietary 3 g/kg powder mixture of amorphous charcoal carbon and wood vinegar compound solution (CWVC) has been shown to produce more intestinal villus hypertrophy compared with the controls, resulting in improved feed efficiency in chickens (Samanya and Yamauchi, 2001) and pigs (Mekbungwan *et al*., 2004b). Therefore, it was interesting to investigate the possibility that CWVC could reduce the protease inhibitory effect of dietary PM in piglet nutrition and encourage villus hypertrophy.

Material and methods

Animals and housing

Twenty-eight 30-day-old male castrated hybrid (0.25 Large White × 0.25 Landrace × 0.50 Duroc; average body weight = 14 kg) piglets were used in this study. Each piglet was placed in a pen with a 1.5 × 2.0 m floor area and given *ad libitum* access to feed and water. Weight gain and total feed intake were recorded. At the end of the experiment, three piglets per treatment were taken to a slaughterhouse; each piglet sedated with a 2-ml Nembutal (60 mg/ml pentobarbital sodium) intravenous injection and euthanased with a saturated injection of $MgSO_4$ solution into the jugular vein. All the experiments were performed according to the humane care guideline for the care and use of laboratory animals established by the Faculty of Agriculture, Kagawa University (Miki, Japan).

Dietary treatment

CWVC (Nekkarich®; 25 g/kg CP, 3 g/kg crude fibre, 134 g/kg ash, 39 g/kg calcium, 2 g/kg phosphorus and 286 g/kg water; pH 7.8; Miyazaki Midori Seiyaku Co., Ltd, Miyazaki, Japan) was produced as described below. Wood vinegar liquid obtained after cooling charcoal smoke from broadleaf trees by dry distillation at 300 to 450°C was kept for 2 to 3 years. Then the skimmed solution was distilled to remove harmful substances such as tar. This wood vinegar compound liquid was adsorbed onto amorphous charcoal carbon powder (1 : 4). The experimental design consisted of seven dietary treatment groups with four piglets in each group: unsupplemented diet (CONT), supplemented raw PM with 200 g/kg (PM200) and 400 g/kg (PM400) to basal diet, supplemented CWVC with 10 and 30 g/kg to PM200 and PM400 (PM200 + CWVC10, PM200 + CWVC30, PM400 + CWVC10 and PM400 + CWVC30) (Table 1).

Tissue sampling

After the piglets were euthanased, a mid-line incision was made to open the abdominal cavity and all the visceral organs were excised. Samples consisting of 5-cm sections of small intestine were taken at 15 cm from the stomach (regarded as the duodenum), in the middle of the small intestine (regarded as the middle part of the jejuno-ileum) and at 1 m from the ileo–caeco–colonic junction (regarded as the caudal part of the jejuno-ileum). Each section was ligated with a thread at both ends and a mixture of 30 ml/l glutaraldehyde and 40 ml/l paraformaldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) was injected, taking care to avoid distension. Then, the sections were removed and kept in a bottle with the same fixative and prepared for light and scanning electron microscopy immediately.

Table 1 *The composition of experimental diets (g/kg) in 0, 200 and 400 g/kg pigeon pea seed meal (PM) (CONT, PM200 and PM400, respectively) diet, and 200 and 400 g/kg PM diets supplemented with 10 or 30 g/kg charcoal powder including wood vinegar compound liquid (CWVC) (PM200 + CWVC10, PM200 + CAVC30, PM400 + CWVC10 and PM400 + CWVC30, respectively)*

	Dietary PM supplemented with CWVC						
Items	CONT	PM200	PM200 + CWVC10	PM200 + CWVC30	PM400	PM400 + CWVC10	PM400 + CWVC30
Ingredients							
Corn	672.8	548.3	548.3	548.3	423.9	423.9	423.9
Rice bran	50	50	50	50	50	50	50
Soybean meal	207.2	131.7	131.7	131.7	56.1	56.1	56.1
Pigeon pea seed meal	0	200	200	200	400	400	400
Fish meal	50	50	50	50	50	50	50
Dicalcium phosphate	15	15	15	15	15	15	15
Premix† (vitamins + minerals)	5	5	5	5	5	5	5
CWVC	0	0	10	30	0	10	30
Chemical composition							
Dry matter	885.2	888.5	881.5	889.3	882.1	887.5	889
Protein	178.7	179.5	179.5	177.7	178.2	180.7	178.2
Fat	59.1	47.6	58.5	55.9	43.8	48.3	52.8
Crude fiber	47.9	36.7	45.5	48.6	42.6	42.2	48.5
Ash	51.7	50.3	53	53.9	49.8	53.7	55.8
Ca	8.8	7.9	8.6	8.9	7.6	9.2	9
P	7.5	8.1	7.7	7.8	7.3	8	7.6
GE, kcal/g	4.23	4.20	4.20	4.23	4.23	4.15	4.22

†Premix supplies (per kg diet): vitamin A (3.333IU), vitamin D (667IU), vitamin E (0.33 mg), vitamin K_3 (0.66 mg), vitamin B_2 (1.67 mg), vitamin B_{12} (0.003 mg), calcium pantothenate (6.67 mg), cobalt (3.47 mg), copper (27.60 mg), iodine (0.77 mg), manganese (18.47 mg), zinc (50.00 mg) and Fe (60.00 mg).

Light microscopy
An 8 × 10-mm segment was cut from each 5-cm intestinal segment, fixed with Bouin's fixative solution for one week at room temperature, embedded in paraplast and cut into 5-μm cross-sections. Every 10th section was collected and stained with haematoxylin-eosin. For villus height measurement, the villi (including the lamina propria) were chosen and the length from the villus tip to the bottom excluding the intestinal crypt was measured. Three villi were selected under 10 × 10 magnification for each section. Thirty values of villus height were counted from 10 sections per piglet, and the average of these values was expressed as the mean villus height for each piglet. To measure one cell area on the 5-μm cross-section, the area of the epithelial cell layer was randomly measured in the middle of the villi and the number of cell nuclei within this layer was counted. The area of the epithelial cell layer was then divided by this number. This measurement was employed in one to two fields per section. Fifteen samples were counted from 10 sections per piglet, and the average of these values was expressed as the mean cell area for each piglet. To measure the cell mitosis number per crypt, five crypts with almost the same size as one microscopic field (10 × 40 magnification) were randomly selected, the mitosis numbers were counted and then expressed as cell mitosis per one crypt. One to two fields per section were measured and 15 cell mitosis numbers were counted from 10 sections per piglet, and an average of these values was expressed as a mean cell mitosis number for each piglet. These measurements were recorded using an image analyser (Nikon Cosmozone lS, Nikon Co., Ltd, Tokyo, Japan). Finally, the mean of each intestinal parameter from the respective three piglets was expressed as the mean villus height, cell area and cell mitosis for one group.

Scanning electron microscopy
A 2 × 3 cm segment was cut from the 5-cm duodenal segment close to the light microscopic sample and slit longitudinally along the non-mesenteric side for its entire length. The intestinal contents were washed with 0.01 mol/l phosphate-buffered saline (pH 7.4). The tissue samples were pinned flat to prevent curling and fixed vertically with the mucosal surface facing downwards in the fixative mixture of 30 ml/l glutaraldehyde and 40 ml/l paraformaldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) at room temperature for 1 h. The tissue block was further cut into a 3 × 10 mm rectangle and fixed for an additional 1 h. The pieces were rinsed with 0.1 mol/l sodium cacodylate buffer (pH 7.4) and post-fixed with 10 g/l osmium tetroxide in ice-cold buffer for 2 h. The specimens were dried in a critical-point drying apparatus. The dried specimens were coated with platinum and observed with a scanning electron microscope. After birth, as the exfoliative zone around the centre of the villus apical surface is the final stage of growth in epithelial cells of the intestinal crypt, we also observed the morphological alterations of epithelial cells around the central apical surface.

Gross anatomical protocol
After removing the tissue samples for microscopic examination, the remaining small intestine was divided into gross anatomical segments based on surface structural features: an intestinal part fixed to the abdominal cavity was prepared as the duodenum (showing thicker wall and slighter vessel networks on the serous surface than the jejuno-ileum; about 55 cm long), and a jejuno-ileal part of 2 m from the ileo–caeco–colonic junction to the rostral side was regarded as the caudal part of the jejuno-ileum (showing larger diameter but fewer aquare blood vessel networks than the rostral part of the jejuno-ileum). A remaining middle part was regarded as the middle part of the jejuno-ileum. Each part was cut, washed with 9 g/l NaCl solution to remove the intestinal contents and measured for length and weight. Visceral organs such as the liver, heart, spleen, kidney, stomach and lung were also weighed.

Statistical analysis
Data on growth performance, gross anatomy and light microscopic examination were statistically analysed by one-way analysis of variance (ANOVA), and significant differences among the treatments were determined with Duncan's multiple range test using the Stat View® program (Abacus Concepts, Inc., Berkeley, CA, USA). Differences were considered significant at $P < 0.05$.

Results

Growth performance
With increasing dietary PM levels, daily feed intake tended to increase. In contrast, daily body-weight gain tended to decrease, significantly in the PM400 group ($P < 0.05$), resulting in a significant decrease of feed efficiency in PM groups ($P < 0.05$) (Table 2). In general, body-weight gain and feed efficiency were higher ($P < 0.05$) in CWVC groups than in PM groups.

Gross anatomical observation
The duodenum and caudal part of the jejuno-ileum were longer ($P < 0.05$) in the PM400 group than in CONT (Table 3), but were similar to CONT in CWVC groups. Compared with CONT, PM200 and PM400 groups, the liver was heavier ($P < 0.05$), whereas the heart, kidney and stomach were lighter in CWVC groups; the weight of the heart in PM200 + CWVC10 and PM200 + CWVC30 groups was significantly decreased ($P < 0.05$).

Light microscopic observations
Villus height, cell area and cell mitosis number tended to decrease with increasing PM levels, and these values were lower ($P < 0.05$) in the PM400 group than in CONT for each intestinal segment (Figure 1). Most of these light microscopic parameters were higher in CWVC groups than in PM groups ($P < 0.05$).

Table 2 *Growth performance of piglets fed dietary 0, 200 and 400 g/kg raw pigeon pea seed meal (PM) (CONT, PM200 and PM400, respectively) diets, and 200 and 400 g/kg PM diet supplemented with 10 or 30 g/kg charcoal powder including wood vinegar compound liquid (CWVC)(PM200 + CWVC10, PM200 + CAVC30, PM400 + CWVC10 and PM400 + CWVC30, respectively), (*n = 4)

	Dietary PM supplemented with CWVC							
Items	CONT	PM200	PM200 + CWVC10	PM200 + CWVC30	PM400	PM400 + CWVC10	PM400 + CWVC 30	s.e.
Initial BW (kg)	13.6^{b}	14.8^{ab}	14.2^{ab}	14.2^{ab}	15.0^{a}	13.9^{ab}	14.1^{ab}	0.15
Final BW (kg)	30.3^{ab}	30.7^{ab}	31.5^{a}	30.4^{ab}	28.8^{b}	29.6^{ab}	29.9^{ab}	0.25
BW gain (kg)	16.7^{a}	15.9^{a}	17.2^{a}	16.2^{a}	13.8^{b}	5.7^{a}	15.7^{a}	0.26
Feed intake (kg)	33.7^{ab}	35.3^{ab}	33.3^{ab}	32.0^{b}	35.5^{a}	34.6^{ab}	33.0^{ab}	0.41
ADFI (g/d)	1125^{ab}	1178^{ab}	1112^{ab}	1066^{b}	1185^{a}	1154^{ab}	1101^{ab}	13.75
ADG (g/d)	558^{a}	530^{a}	575^{a}	540^{a}	462^{b}	523^{a}	525^{a}	8.90
BW gain(g)/feed(kg)	495^{ab}	450^{c}	516^{a}	506^{a}	390^{d}	453^{c}	476^{bc}	8.28

BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; s.e. = standard error of mean.
^{a,b,c,d}Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3 *Length of small intestine and weight of visceral organs of piglets fed dietary 0, 200 and 400 g/kg raw pigeon pea seed meal (PM) (CONT, PM200 and PM400, respectively) diets, and 200 and 400 g/kg PM diets supplemented with 10 or 30 g/kg charcoal powder including wood vinegar compound liquid (CWVC) (PM200 + CWVC10, PM200 + CAVC30, PM400 + CWVC10 and PM400 + CWVC30, respectively), (*n = 3)

	Dietary PM supplemented with CWVC							
Items	CONT	PM200	PM200 + CWVC10	PM200 + CWVC30	PM400	PM400 + CWVC10	PM400 + CWVC30	s.e.
Intestinal length (cm)								
Duodenum	49^{b}	48^{b}	49^{ab}	49^{ab}	52^{a}	50^{ab}	50^{ab}	0.39
Middle part of jejuno-ileum	1018	989	1041	1105	1001	946	994	19.84
Caudal part of jejuno-ileum	203^{b}	210^{ab}	209^{b}	211^{ab}	223^{a}	206^{b}	211^{ab}	1.82
Weight of visceral organs (g)								
Duodenum	50^{ab}	51^{ab}	53^{ab}	58^{a}	43^{b}	51^{ab}	52^{ab}	1.27
Middle part of jejuno-ileum	787^{ab}	832^{a}	800^{ab}	826^{a}	691^{b}	733^{ab}	709^{ab}	16.79
Caudal part of jejuno-ileum	175	170	191	176	170	159	169	4.79
Liver	707^{bc}	713^{bc}	784^{a}	731^{ab}	658^{c}	759^{ab}	727^{ab}	10.30
Heart	117^{ab}	125^{a}	111^{b}	107^{b}	118^{ab}	107^{b}	107^{b}	1.97
Spleen	57	55	55	54	55	52	53	1.01
Kidney	126^{ab}	134^{a}	124^{ab}	123^{ab}	121^{ab}	112^{b}	110^{b}	2.43
Stomach	228^{ab}	244^{a}	229^{ab}	224^{ab}	221^{ab}	201^{b}	203^{b}	4.13
Lung	295	353	343	293	341	261	296	11.12

s.e. = standard error of mean.
^{a,b,c}Within a row, means without a common superscript letter differ ($P < 0.05$).

Scanning electron microscopic observations

The duodenal villus apical surface in CONT (Figure 2a) was covered with comparatively protuberated epithelial cells, resulting in an inflated rough surface. On the villus tip of the PM200 group (Figure 2b), sunken parts (arrows) were frequently observed. However, in PM200 + CWVC10 (Figure 2c) and PM200 + CWVC30 groups (Figure 2d), clear cell protuberances developed, covering the villus apical surface and resulting in an inflated rough surface. In particular, the dome shaped epithelial cells of the PM200 + CWVC10 group protruded further into the lumen compared with CONT. In the case of the PM400 group (Figure 3a), epithelial cells changed to flat cells with unclear outlines, resulting in a smooth villus apical surface. These flat cells developed some protuberances in the PM400 + CWVC10 group (Figure 3b, arrows), which became clear on the whole villus apical surface in the PM400 + CWVC30 group (Figure 3c).

Discussion

In this study, with increasing dietary PM levels, daily feed intake tended to increase. In contrast, daily body-weight gain tended to decrease, significantly in the PM400 group, resulting in a significant decrease of feed efficiency in PM groups. Nevertheless, the values of body-weight gain and feed efficiency were higher in CWVC groups compared with PM groups. Charcoal has been reported to reduce the effects of toxins in diets through adsorption (Anjaneyulu *et al.*, 1993). When activated 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). The present CWVC would also eliminate the harmful effects of antinutritional factors in PM by adsorbing the toxins and thereby preventing their absorption by the

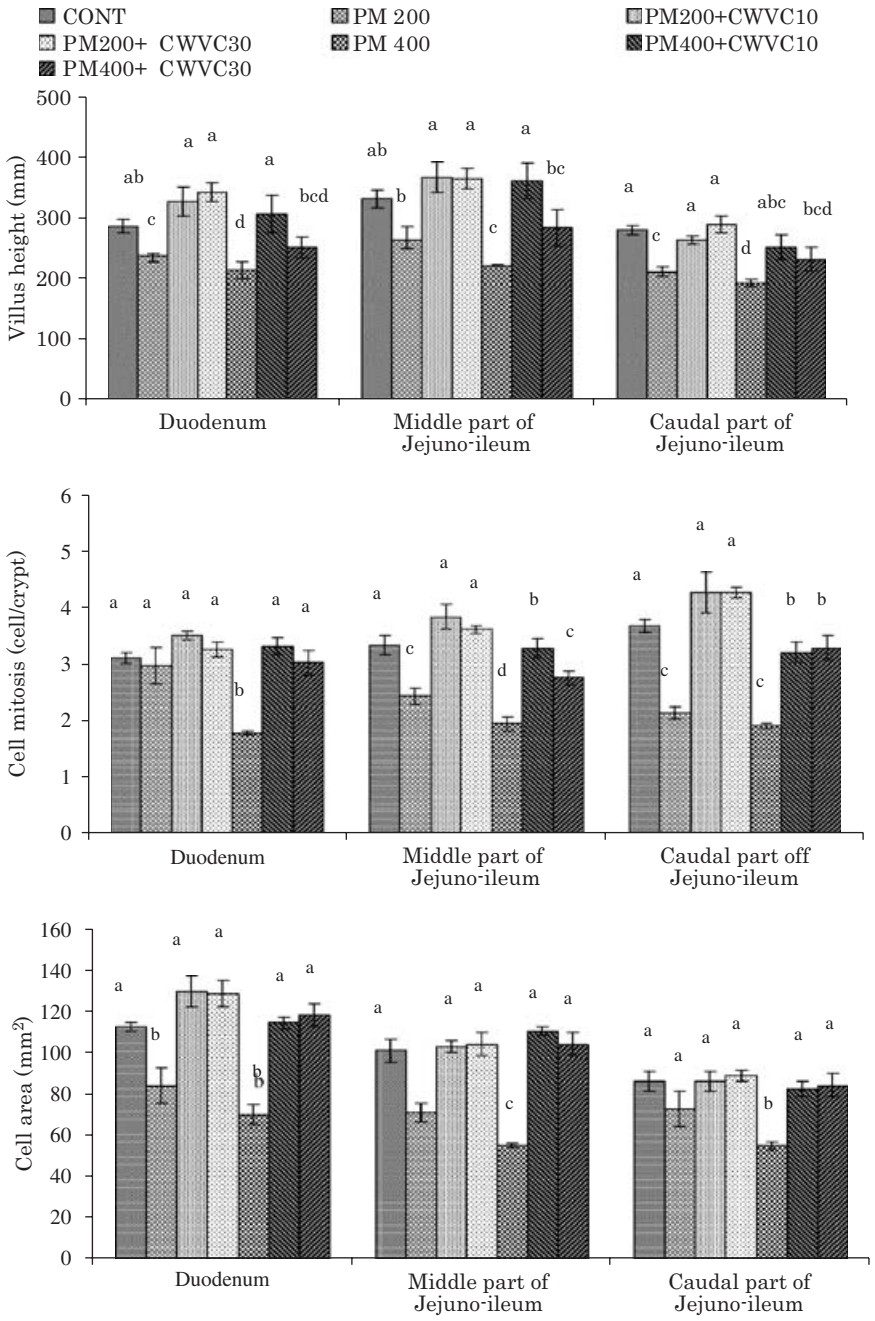

Figure 1 Villus height, cell area and cell mitosis number in duodenum, middle part of the jejuno-ileum and caudal part of the jejuno-ileum in piglets fed dietary 0, 200 or 400 g/kg of raw pigeon pea seed meal (PM) (CONT, PM200 and PM400, respectively) diets, and 200 and 400 g/kg PM diets supplemented with 10 or 30 g/kg charcoal powder including wood vinegar compound liquid (CWVC) (PM200 + CWVC10, PM200 + CAVC30, PM400 + CWVC10 and PM400 + CWVC30, respectively), ($n = 3$). Values of light microscopic parameters are higher in CWVC groups than in PM groups. [a,b,c,d]Means with different superscripts differ ($P < 0.05$) from each other.

intestine. These growth performances correspond with the histological intestinal alterations described later.

The PM400 group showed longer duodenum and caudal part of the jejuno-ileum, but decreased liver weight compared with CONT. Enlargement of the gastrointestinal tract was also observed in association with the various dietary arrangements (Iji *et al.*, 2001; Brenes *et al.*, 2002; Olkowski *et al.*, 2005). PM includes protease inhibitors such as trypsin and chymotrypsin inhibitors, which decreased the activities of the pancreatic and small intestinal trypsin and chymotrypsin (Yen *et al.*, 1977). The duodenum was reported as the site where the stimulation of pancreatic enzyme secretion is regulated (Corring, 1974; Yen *et al.*, 1977). The digestibility of crude protein and amino acids (Herkelman *et al.*, 1992) was lowered by the trypsin inhibitors. A hyponutrient diet induced an increase in the length of all the intestinal sections not due to submucosal and serosal components, but the mucosa (Olkowski *et al.*, 2005).

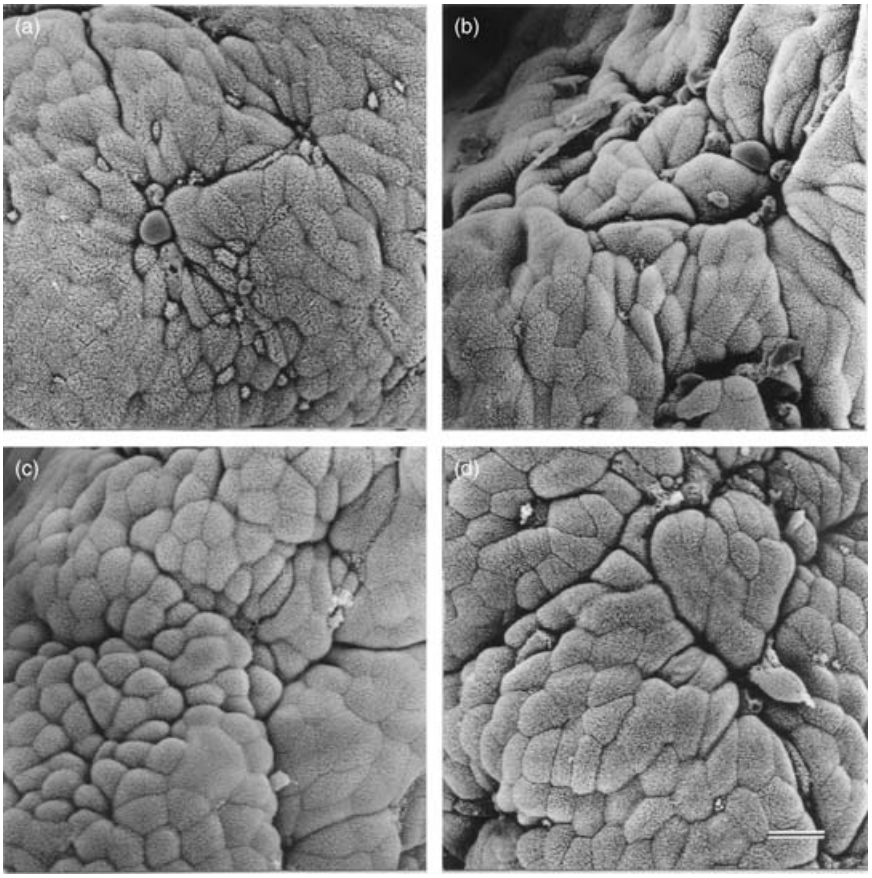

Figure 2 Duodenal villus apical surface of piglets fed dietary 0 (a) and 200 (b) g/kg raw pigeon pea seed meal (PM) diets, and fed dietary 200 g/kg PM diet supplemented with 10 (c) or 30 (d) g/kg charcoal-wood vinegar compounds (CWVC). Much more protuberated epithelial cells are seen in CWVC groups than in PM group. Scale bar = 32 μm, ×1058.

Trypsin inhibitors induced pancreatic enlargement (Grant *et al*., 1993). From these cited references, the increased length of the intestine and decreased weight of the liver might be induced by the decreased use of nutrients in dietary PM diets due to the presence of trypsin and chymotrypsin inhibitors. Enlargement of the intestinal tract can be explained as an adaptive mechanism wherein by increasing the size of the intestine, the host increases its absorptive capacity in an attempt to extract maximum nutritional benefit from a poor-quality diet. However, these structural dissimilarities recovered to the CONT levels with inclusion of CWVC. From a nutritional and physiological standpoint, the mechanisms of lowered weight of heart, kidney and stomach associated with dietary CWVC are not completely understood in this study. However, it is speculated that these changes can also be explained as resulting from the adaptive mechanisms of the gastrointestinal tract in response to PM and CWVC diets.

Histology of the intestinal villi and epithelial cells on the villi apical surface in piglets (Mekbungwan *et al*., 2003) and chickens (Yamauchi *et al*., 2006b) are well known to be affected by dietary feed components. Values of the present villus height, cell area and cell mitosis numbers were lower in the PM groups compared with CONT. Compared with a commercial diet, short villi were reported in pigs showing decreased body weight (Zijlstra *et al*., 1996) and low nutrient digestibility (Mekbungwan *et al*., 2004a), and in those fed a diet containing a higher percentage of soya beans (Nabuurs *et al*., 1993). These short villi correspond with reductions in activities of enzymes such as mucosal lactase and sucrase (Park *et al*., 1998), lactase and alkaline phosphatase (Zijlstra *et al*., 1996), alkaline phosphatase and disaccharidase (Lopez-Pedrosa *et al*., 1998), and the total lactase phlorizin hydrolase and mucosal protein concentration (Dudley *et al*., 1998). The short villi were accompanied by reductions in the villus surface area (Park *et al*., 1998) resulting in a reduced absorptive function. Furthermore, with increasing dietary PM levels, the villus apical surface changed from being inflated and rough to smooth via the presence of sunken parts. As the cell area measured using light microscopy was reduced after feedings of PM200, these partially sunken parts are thought to be a consequence of reduced cell area. A further decrease in cell area in all epithelial cells might produce flat cells on the villus apical surface, resulting in a smooth surface seen in the PM400 group. Such flat cells were also reported in pigs undergoing a 3-day feed withdrawal (Mekbungwan *et al*., 2002). These studies suggest that the present decreased

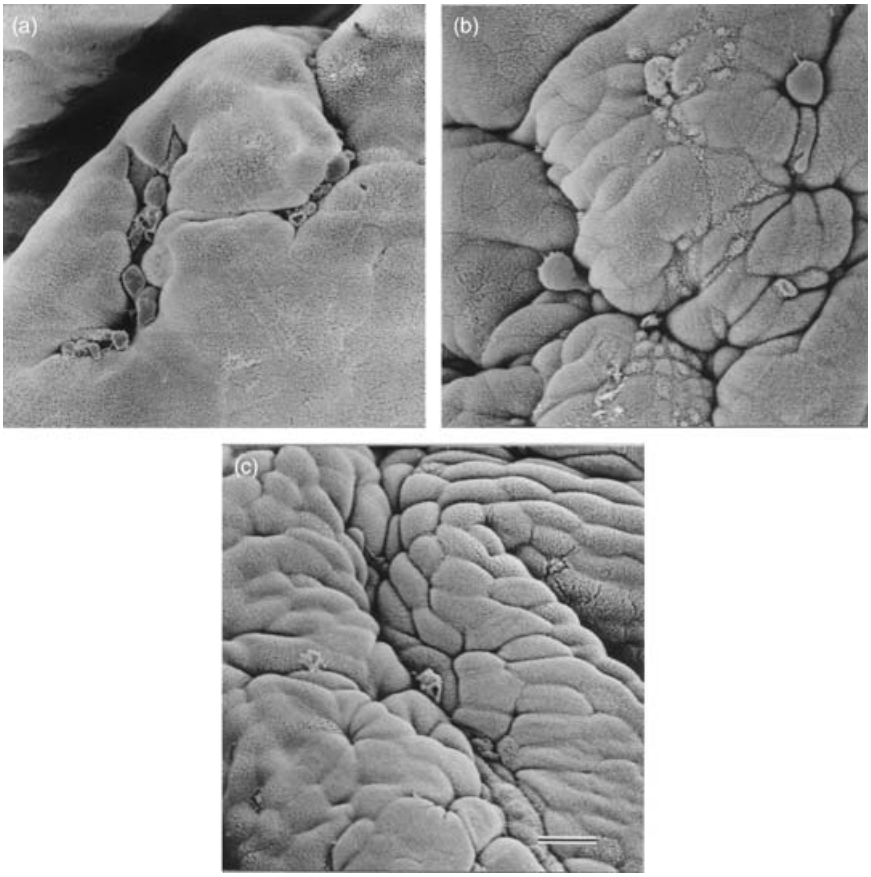

Figure 3 Duodenal villus apical surface of piglets fed dietary 400 g/kg raw pigeon pea seed meal (PM) diet (a), and fed dietary 400 g/kg PM diet supplemented with 10 (b) or 30 (c) g/kg charcoal-wood vinegar compounds (CWVC). One can see atrophied flat cells in PM group but hypertrophied protuberated cells in CWVC supplemented groups. Scale bar = 32 μm, ×1058.

light microscopic parameters and the flat cells in PM groups might suggest histological atrophy. Raw PM has been reported to contain antinutritional factors such as trypsin and chymotrypsin inhibitors (Jambunathan and Singh, 1980; Godbole *et al*., 1994; Rani *et al*., 1996), resulting in decreased growth performance with increasing levels of raw PM (Mekbungwan and Yamauchi, 2004). The main reason for the present histologically atrophied features is thought to be the presence of antinutritional factors in PM. Conversely, the atrophied histological alterations seen in PM groups were found to be improved in CWVC groups. Long villi were reported in piglets that showed an increased body-weight gain (Zijlstra *et al*., 1996), in turkeys fed dietary amylase (Ritz *et al*., 1995) and in chickens showing a high activity of amylase in the intestinal content (Samanya and Yamauchi, 2002). It has been suggested that long villi result in increased surface area capable of greater absorption of available nutrients (Caspary, 1992), and that greater villus height and increased cell mitosis in the intestine are indicators of the activated function of the intestinal villi (Langhout *et al*., 1999; Yasar and Forbes, 1999). In addition, on the villus apical surface of CWVC groups, protuberated cells were observed. Such cells were also reported in chickens showing a quicker recovery of body weight after refeeding sugar cane extract (Yamauchi *et al*., 2006a) and in piglets showing high nutrient digestibility (Mekbungwan *et al*., 2004a). These studies suggest that the present high light microscopic parameters and the protuberated cells are much more hypertrophied in CWVC than in PM groups. In our previous studies, such histological intestinal recovery from the atrophied intestinal histology in raw PM groups was observed after feedings of heated PM diet in piglets (Mekbungwan and Yamauchi, 2004), and of the CWVC diet in piglets (Mekbungwan *et al*., 2004b) and chickens (Samanya and Yamauchi, 2001). In the case of heated PM, the histological intestinal recovery was caused by a 99.15% decrease in the inhibition rate of trypsin in raw PM compared with 54.31% in heated PM (Mekbungwan and Yamauchi, 2004). The trypsin inhibitor in the raw PM has also been known to decrease by roasting (Simoongwe, 1998), cooking (Sharma and Sehgal, 1992) and heating (Mekbungwan and Yamauchi, 2004) PM. In the case of CWVC, the histological intestinal recovery was caused by the adsorbing effect of CWVC. Activated charcoal was reported to reduce the effects of dietary toxins by adsorbing them and thereby preventing their absorption from the intestine (Anjaneyulu *et al*., 1993). Amorphous charcoal carbon powder in CWVC adsorbed *Salmonella enteritidis*

much more selectively than conventional *Enterococcus faecium*, and the wood vinegar compound liquid in CWVC inhibited the growth of *S. enteritidis* but accelerated the growth of *E. faecium* and *Bifidobacterium thermophilum* (Tana *et al*., 2003). Although we did not measure the inhibition rate of trypsin inhibitor in this study, trypsin activity in the jejunal content was significantly decreased after feedings of 300 g/kg dietary PM ($P < 0.05$) but tended to improve after supplementation of 10 g/kg CWVC to this 300 g/kg dietary PM in chickens (unpublished). These observations and the findings of similar studies in the literature lead to a general conclusion that the CWVC might eliminate the harmful effects of the antinutritional factors in PM on the villus and cellular functions, thereby activating intestinal function, resulting in a growth performance similar to CONT. The raw PM can be used by adding CWVC without heating.

In conclusion, histological intestinal villus alterations in piglets fed a PM or PM + CWVC diet demonstrate that the villi might be atrophied in the piglets fed PM due to the presence of antinutritional factors, resulting in decreased growth performance. CWVC might eliminate the harmful effects of the antinutritional factors on the villus function, resulting in normal growth performance.

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