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Growth performance and histological intestinal alterations in piglets fed dietary raw and heated pigeon pea seed meal

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Summary. Histological intestinal villus alterations were studied in piglets fed raw (PM) or heated (HPM) pigeon pea seed meal. The trypsin inhibition rate was 99.15% in PM and 54.31% HPM. The PM and HPM were added into the basal diet (crude protein; 176.3 $g.kg^{-1}$, gross energy; 4.15 kcal/g, control) at 20% and 40% levels, respectively. The diets were formulated in order to adjust protein to 180 $g.kg^{-1}$ and gross energy to about 4.20 kcal/g. The feed intake was not different among groups. The daily body weight gain and feed efficiency tended to decrease with the increasing PM level, and they decreased significantly in the 40% PM group compared with the control group ($P < 0.05$). However, HPM groups showed a growth performance similar to the control. The villus height, cell area and cell mitosis tended to decrease with the increasing PM level, and they decreased significantly in the 40% PM group compared with the control group ($P < 0.05$). In HPM group, these villus height, cell area and cell mitosis were significantly higher than those of the 40% PM group ($P < 0.05$), and did not show a significant difference compared with the control. Compared with the duodenal villus surface of the control group, the PM groups had a smooth surface due to flat cells and the HPM group showed a rough surface due to protuberated cells.

The current histological alterations of intestinal villi demonstrate that the villi might be atrophied in the piglets fed raw PM due to anti-nutritional factors, resulting in the decreased growth performance, and that heating PM might abolish such a harmful effect of the anti-nutritional factors on the villus function, resulting in a similar growth performance to the control. Raw PM could be incorporated under a level of 40%, but heated PM increases the incorporation rate up to the 40% level.

Key words: Growth performance, Intestinal morphology, Pigeon pea seed meal, Piglets

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Introduction

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is harvested throughout the tropical and subtropical areas of the world, from about 30 °N to about 30 °S of the equator (Nwokolo, 1996). Pigeon pea seeds have a great potential as an important protein source (Cheva-Isarakul, 1992; Oshodi et al., 1993; Mekbungwan et al., 1999) for human nutrition, especially in protein-deficient diets such as those that are cereal-based or vegetarian. Pigeon pea seeds can be soaked in water, cooked and eaten alone or with rice, or various vegetables. With regard to animals, effective utilization of pigeon pea seed meal (PM) has been reported to avoid competition with human food in its raw state in broilers (Tangtaweewipat and Elliott, 1989; Mizubuti et al., 1995), pigs (Castro et al., 1987; Batterham et al., 1993; Mekbungwan et al., 1999) and lambs (Rao and Phillips, 2001). However, effective use of PM is limited by the presence of protease inhibitors such as trypsin and chymotrypsin inhibitors (Batterham et al., 1993; Visitpanich et al., 1985a). To improve the nutritional quality of PM, these anti-nutritional factors can be reduced by heating (Visitpanich et al., 1985b; Singh, 1988), 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). Higher growth rates have been reported in rats (Udayasekhara Rao and Belavady, 1978) and pigs (Visitpanich et al., 1985a) given heated PM than in those fed unheated PM. The weight gain of pigs varied in an inverse relationship to the measured trypsin inhibition levels (Simoongwe et al., 2001). As the decreased body weight was accompanied by the short villi in pigs (Zijlstra et al., 1997), while the pigs showing an increased body weight gain had the long villi (Zijlstra et al., 1996), the presence of anti-nutritional factors in PM also seems to affect the histological alterations of intestinal villi. Although most of the research surrounding PM has been concentrated on its nutrient composition, nutritive value and anti-nutritional factors, none has focused on the histological intestinal alterations

brought about by anti-nutritional factors in PM.

As pigs and humans have a similar gastrointestinal morphology and physiology (Miller and Ullrey, 1987; Cooper et al., 1997), the pig is a useful model for developing relevant information about potential effects of anti-nutritional factors in PM on intestinal atrophy. All of the above-mentioned literature has led us to investigate how histological alterations would be affected in intestinal villi after feeding both raw and heated PM. For this paper, raw (rich in anti-nutritive substance) and heated (poor in anti-nutritive substance) PM were fed to piglets, and growth performance, internal organs and histological intestinal alterations were compared using light and electron microscopy.

Materials and methods

Animals, diets, and feeding

Twenty-five castrated male piglets of 13 kg average body weight, (about 56 days old, a crossbreed of Large White 25%, Landrace 25% and Duroc 50%) were transferred to individual pens and allotted to five groups (treatments), each group composed of five animals (replications). The PM was harvested in Thailand and crushed by a hammermill to pass through a 4 mm sieve. Heated pigeon pea seed meal (HPM) was prepared by heating PM by steam at 105 ºC for 2 h then dried in air before being used as feeds. Soybean meal (SM) was purchased from a commercial company (Porn Amnouy Thrup Co. Ltd, Sukothai, 64000, Thailand) after the seed oil had been extracted. All of the PM, HPM and SM were added into the basal diet (control) and mixed with other feed ingredients to produce five feed formulations that consisted of 0% (control), 20% PM, 40% PM, 20% HPM and 40% HPM, respectively. The diets were formulated in order to adjust protein to 180 $g.kg^{-1}$ and energy to about 4.20-kcal/ g gross energy (Table 1). Each piglet was allowed feed and water ad libitum for 30 days in the 1.5x2.0 m slot pen. At the end of the experiment, three piglets per group were randomly slaughtered (30 kg average body weight) at a slaughterhouse. All experiments were carried out according to the human care guidelines for the care and

Table 1. Composition ($g. kg^{-1}$) of experimental diets.

ITEMS	DIETS				
	Control	20%PM	40%PM	20%HPM	40%HPM
Ingredients					
Corn	672.8	548.3	423.9	548.3	423.9
Rice bran	50	50	50	50	50
Soybean meal	207.2	131.7	56.1	131.7	56.1
Pigeon pea meal	0	200	400	0	0
Heated pigeon pea	0	0	0	200	400
Fish meal	50	50	50	50	50
Dicalcium phosphate	15	15	15	15	15
Premix * (Vitamins+minerals)	5	5	5	5	5
Chemical composition					
Dry matter	884.1	888.5	882.1	886.5	887.4
Crude Protein	176.3	179.5	178.2	180.2	178.8
Crude Fat	50.7	47.6	43.8	60.5	57.8
Crude fiber	33.6	36.7	42.6	34.7	38.2
Ash	51.0	50.3	49.8	54.4	52.6
Ca	7.6	7.9	7.6	8.9	9.7
P	9.7	8.1	7.3	8.1	7.8
Gross energy, kcal/g	4.15	4.20	4.23	4.22	4.28
Amino acids**					
Lysine	10.2	10.5	10.8	10.5	10.8
Methionine + Cystine	6.4	6.0	5.7	6.0	5.7
Tryptophan	2.2	2.1	2.1	2.1	2.1
Threonine	7.4	7.1	6.8	7.1	6.8
Isoleucine	8.4	7.5	6.6	7.5	6.6
Leucine	17.5	16.1	14.6	16.1	14.6
Arginine	11.8	8.8	5.9	8.8	5.9
Phenylalanine+Tylosine	15.9	14.4	12.9	14.4	12.9
Histidine	4.9	5.2	5.5	5.2	5.5
Valine	9.7	9.0	8.4	9.0	8.4

PM: raw pigeon pea seed meal, HPM: steam heated pigeon pea seed meal at 105 °C for 2 h. *Premix supplies (per kg diet): vitamin A (3.333IU), vitamin D (667IU), vitamin E (0.33 mg), vitamin K3 (0.66 mg), vitamin B2 (1.6 7mg), vitamin B12 (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). Calculated values**.

use of laboratory animals established by the Faculty of Agriculture of Kagawa University.

The feeding trials and analysis of their chemical composition were done at the Department of Animal Technology, Maejo University, Thailand (Table 1, see Mekbungwan et al., 1999 for chemical composition, such as essential amino acids of PM and SM). The light and scanning electron microscopic observations were done at the Laboratory of Animal Science, Faculty of Agriculture, Kagawa University, Japan.

Trypsin inhibitor assay

The trypsin inhibitor was extracted by homogenizing 1 g of both raw and heated PM in 1 ml Tris buffer (0.1 M, pH 8.0) by a Physcotron homogenizer (NS-50; Nition Co. Ltd, Chiba, Japan) for 1 minute and then by centrifuging the homogenate at 10,000 g for 10 minutes. The supernatant of 0.2 ml of the PM extract solution was added into the cuvette including 1.6 ml Tris buffer and 0.2 ml Trypsin solution (porcine trypsin, Sigma T-0303, 10 mg/100 ml in 0.001 M HCl). The mixed solution was incubated for 20 minutes at 25 ºC in an incubator and finally 1 ml of synthetic substrate of N-benzoyl-L-arginine-p-nitroamide (Peptide Institute 3057, 10 mg/10ml in distilled water) was added and quickly mixed. This solution was then set in the spectrophotometer (Shimadzu, UV-160A, Kyoto, Japan), and absorbance changes were read at 405 nm every 60 seconds for eight minutes at 25 ºC. Trypsin inhibition percentage was calculated by the following formula; (1 - Trypsin activity with PM extract/Trypsin activity without PM extract) x 100.

Tissue sampling

At the moment of killing the piglets, a horizontal incision along the midline was made to open the abdominal cavity, and all the internal organs were excised. Samples of 5 cm length of the small intestine were taken at 20 cm from the stomach (duodenum), in the middle of the small intestine (jejunum), and at 1 m proximal to the large intestine (ileum). A 5 cm length of each intestinal part was ligated with a thread at both ends, and a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) was injected without distending. Then, each intestinal segment was removed and kept in a bottle with the same fixative. Immediately after finishing the removal of all intestinal segments, each one was prepared for light and scanning electron microscopy.

Light microscopic procedure

An 8x10 mm segment was cut from each 5-cm intestinal segment, fixed with Bouin's fixative solution for 1 week in room temperature, embedded in paraplast and cut at 5 μm cross section. Every 10th section was collected and stained with hematoxylin-eosin. For measurement of the villus height, the villi including the lamina propria were chosen and the length from the villus tip to the bottom, not including the intestinal crypt, was measured (Mekbungwan et al., 2003a). From one section three villi were selected under 10 x 10 magnification. A total of 30 villus heights were counted from 10 sections per piglet, and an average of these values was expressed as a mean villus height for each piglet. For measurement of one cell area on the 5 μm 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. Then, the area of the epithelial cell layer was divided by the number of cell nuclei. This measurement was carried out 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 a mean cell area for each piglet. For measurement of the cell mitosis number per crypt, five crypts with almost the same size within one microscopic field (10 x 40 magnification) were randomly selected, and then all cell mitoses were counted, expressed as cell mitosis per one crypt. One or 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 analyzer (Nikon Cosmozone lS, Nikon Co., Ltd., 100-8331 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 microscopic procedure

Each 2x3 cm segment was cut from a 5-cm intestine segment close to the light microscopic sample, and slit longitudinally along the non-mesenteric side for its entire length. The intestinal contents were washed away with 0.01 M phosphate-buffered saline (pH 7.4). The tissue samples were pinned flat to prevent curling and to fix them vertically with the mucosal face downwards in the mixture fixative of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 1 h (Mekbungwan et al., 2003b). The tissue block was further cut into a 3x10 mm rectangle and fixed for a further 1 h. The pieces were rinsed with 0.1 M sodium cacodylate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide in ice-cold buffer for 2 h. The specimens were dried in a critical-point drying apparatus (Hitachi HCP-1, Hitachi Ltd., Tokyo, 100-8220 Japan). The dried specimens were coated with platinum (RMC-Eiko® RE vacuum coater Eiko Engineering Co., Ltd., Tokyo, Japan), and observed with a scanning electron microscope (Hitachi S-800, Hitachi Ltd., Tokyo, Japan).

Gross anatomical protocol

After removing the tissue samples for microscopy,

the remaining small intestine was gross-anatomically divided into each intestinal segment according to surface structural features: about 5% of the first part caudal to the stomach was regarded as the duodenum, showing a smoother surface than the jejunum; about 18% of the final part, proximal to the large intestine, was the ileum, showing a larger haustra than the jejunum; and the remaining 77% middle part was the jejunum having a small haustra. Each part was cut and washed with 0.9% NaCl solution to remove the intestinal contents and the length and weight of each part was measured. Internal organs were washed in the same solution and weighed again.

Statistical analysis

Data for growth performance, gross anatomy and light microscopic examination were statistically analyzed by using the 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., HULINKS, Inc., Tokyo, Japan). Differences were declared significant at $P<0.05$.

Results

Trypsin inhibitor rate

The inhibition rate of the trypsin inhibitor was 99.15% in raw PM and 54.31% in heated PM.

Growth performance

Compared with the control group, the average daily feed intake did not show a significant decrease in all the dietary PM groups (Table 2). The average daily body weight gain tended to decrease with increasing raw PM

Table 2. Growth performance of piglets fed a control diet or a diet containing 20 or 40% of raw (PM) or heated (HPM) pigeon pea seed meal (PM) (n=5).

ITEMS	DIETS					SEM	P-value
	Control	20% PM	40% PM	20% HPM	40% HPM		
Initial BW, kg.	13.70	14.68	15.50	13.32	13.42	0.24	0.06
Final BW, kg.	31.20	31.10	29.46	29.78	29.72	0.31	0.23
Carcass weight, kg	22.37	22.70	22.62	22.52	22.42	0.16	0.98
BW gain, kg.	17.50[a]	16.42[a]	13.96[b]	16.66[a]	16.30[a]	0.29	0.001
ADFI, g d^{-1}	1300	1218	1174	1207	1236	0.09	0.33
ADG, g d^{-1}	583[a]	547[a]	465[b]	555[a]	543[a]	9.91	0.001
Gain (g)/feed (kg)	448[a]	449[a]	396[b]	460[a]	441[a]	0.006	0.008

BW: body weight, ADG: average daily gain, ADFI: average daily feed intake. a, b Within a row, means without a common superscript letter difference ($P < 0.05$).

Table 3. Relative length of small intestine and weight of internal organs in piglets fed a control diet or a diet containing 20 or 40% of raw (PM) or heated (HPM) pigeon pea seed meal (PM) (n = 3)

ITEMS	DIETS					SEM	P-value
	Control	20%PM	40%PM	20%HPM	40%HPM		
Killing weight, kg	29.83	30.26	30.16	30.03	29.90	0.22	0.98
Intestinal length, cm./kg body weight							
Duodenum	1.66	1.61	1.73	1.70	1.71	0.02	0.44
Jejunum	36.36	32.71	33.23	35.56	39.74	0.98	0.14
Ileum	7.24	6.96	7.42	6.87	6.87	0.09	0.24
Total	45.27	41.29	42.39	44.14	46.15	0.80	0.30
Weight of internal organs, g/kg body weight							
Duodenum	1.71 [a]	1.70[a]	1.44 [c]	1.71 [a]	1.59 [b]	0.03	0.001
Jejunum	25.06	27.57	22.90	24.31	23.20	0.67	0.17
Ileum	5.55	5.64	5.63	5.46	5.44	0.12	0.98
Total	32.32	34.92	29.98	32.13	30.54	0.73	0.23
Liver	23.21	23.60	21.84	22.58	23.72	0.44	0.71
Spleen	2.07	1.81	1.83	2.16	2.23	0.06	0.07
Stomach	7.78	8.07	7.33	7.43	7.51	0.14	0.51
Intestinal weight per length, g/cm	0.71	0.84	0.70	0.73	0.66	0.02	0.13

[a, b, c] Within a row, means without a common superscript letter difference ($P<0.05$).

levels, and it decreased significantly in the 40% PM group compared with the control group (P<0.05). However, heated PM groups did not show such a significant decrease. Feed efficiency was significantly decreased in the 40% PM group (P<0.05), but it was almost the same as that of the control group in the heated PM groups.

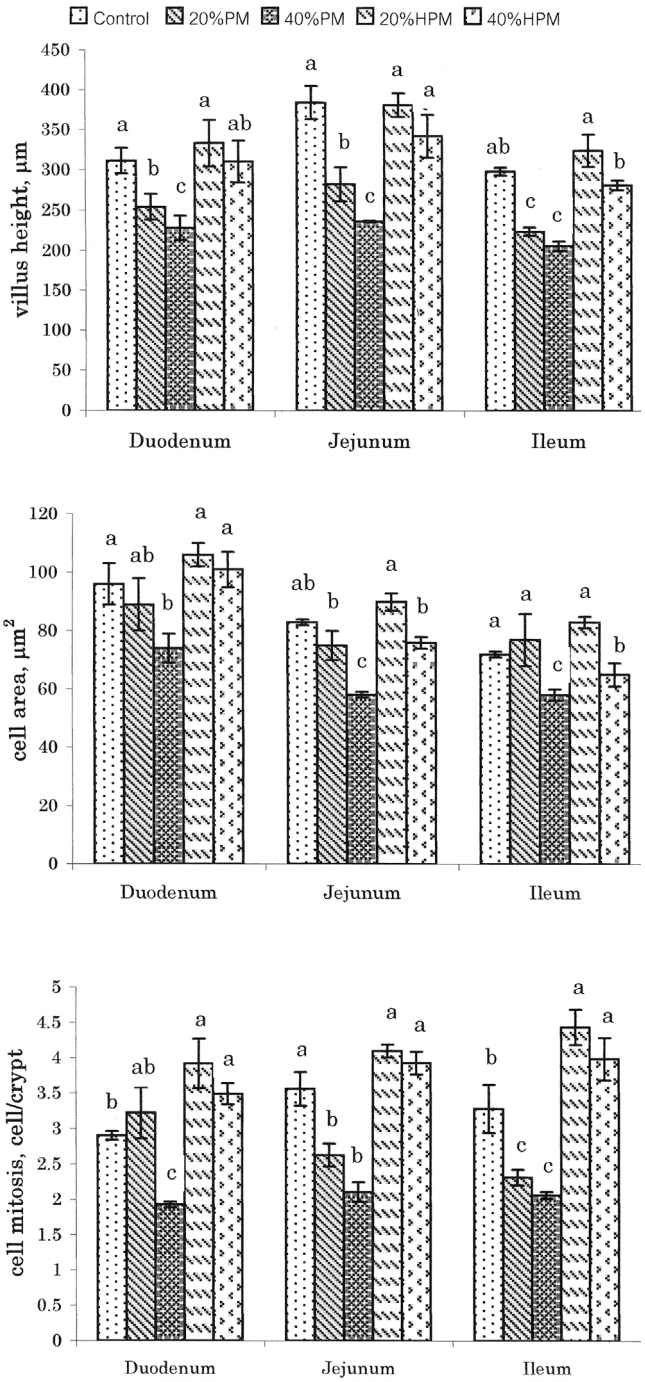

Fig. 1 Villus height, cell area and cell mitosis number in duodenum, jejunum and ileum of piglets fed a control diet or a diet containing 20% or 40% of raw (PM) or heated (HPM) pigeon pea seed meal (mean ± SEM, n=3). Light microscopic parameters tend to decrease with increasing PM, but are not affected by HPM. a, b, c.: means with different superscripts differ (*P*<0.05) from each other.

Gross morphological observations

Relative intestinal length, weight of internal organs and intestinal weight per length did not differ among each group except for the weight of the duodenum (Table 3). The duodenum was lighter in the 40% PM and 40% HPM groups than in another groups (P< 0.05), and in the 40% PM group it was again lighter than in the 40% HPM group (P< 0.05).

Light microscopic observations

In the raw PM group, the villus height, cell area and cell mitosis in each intestinal part tended to decrease with increasing PM levels (Fig. 1). A significant decrease was observed in the 40% PM group (P < 0.05). However, in HPM groups these light microscopic parameters were significantly higher than those of the 40% PM group, and did not show a significant difference compared with the control group except for the significantly increased cell mitosis of the 20% HPM group in the duodenum and ileum (P<0.05). Compared with the 20% HPM group, the 40% HPM group tended to have lower values in all light microscopic parameters. The 40% HPM group had a lower villus height in the ileum, the cell area in the jejunum and ileum was also lower than in the 20% HPM group (P<0.05).

Scanning electron microscopic observations

The flat cell protuberances (arrows in Fig. 2A) and faint cell outline between each cell on the duodenal villus tip surface of the control group were smaller in the 20% PM group (Fig. 2B), and disappeared in the 40% PM group, resulting in a smooth surface (Fig. 2C). However, in the 20% HPM group, clearer cell protuberances developed covering the villus tip surface (arrow in Fig. 2D). In the 40% HPM group, enlarged cell protuberances were much more developed, and the outline between each epithelial cell was clearer, resulting in a rough surface (Fig. 2E). At the higher magnification, intestinal epithelial cells showed a pentagonal dome-like shape and protuberance into the intestinal lumen (Fig. 2F).

Discussion

The present decreased growth performance with the increasing level of raw PM but not with the heated PM seems to be induced by the complex mode of nutritional quality, quantity and availability of PM. Qualitatively, the crude protein and essential amino acid contents in the raw PM were almost half of those in the SM (Mekbungwan et al., 1999). However, as amino acids in

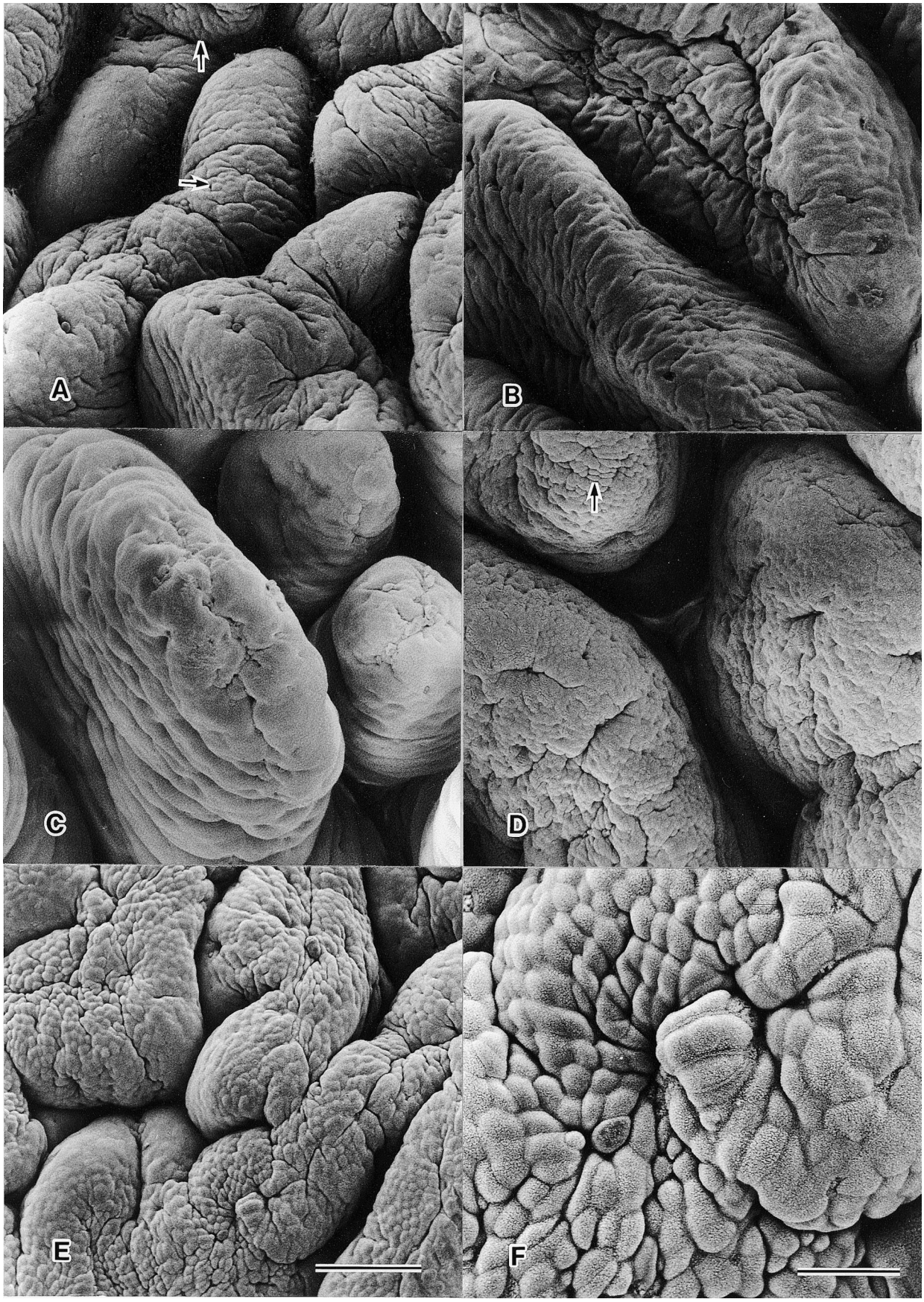

Fig. 2 Duodenal villus surface of piglets fed a control diet **(A)** or a diet containing 20% or 40% of raw (**B** and **C**, respectively) or heated (**D** and **E**, respectively) pigeon pea seed meal (PM). **F** is a higher magnification of **E** . One can see no clear cell outline in raw PM but clear cell protuberances in heated PM. Arrows in A: flat cell protuberances; arrow in D: developed clearer cell protuberances; A-E -= scale bar, 108 μm, x 287.; F = scale bar, 32.6 μm, x 958.

all diets were quantitatively enough for NRC (1998) essential amino acid requirements, and the chemical composition of the present diets was adjusted to an almost similar value in each diet, it seems that the reduced body weight was not induced by the nutrients in PM. On the other hand, raw PM was reported to contain anti-nutritional factors such as amylase inhibitors, protease inhibitors, phytolectins, polyphenols and oligosaccarides (Singh, 1988). In protease inhibitors, the trypsin and chymotrypsin inhibitors were demonstrated in PM (Jambunathan and Singh, 1980; Godbole et al., 1994; Rani et al., 1996), and were higher in PM (4.8 and 2.7 mg per g, respectively) than in SM (0.26 and 0.00 mg per g, respectively) (Batterham et al.,1993). The trypsin inhibitor activity was 10 times higher in PM (26 unit per mg) than in SM (Visitpanich et al., 1985a). As trypsin is a pancreatic enzyme (proteinase) necessary for protein digestion, trypsin inhibitors inhibit pancreatic enzyme function and hinder protein digestion and absorption. The digestibility of crude protein in PM (49.8%) was lower than that in SM (80.6%) ($P<0.05$), and crude fiber digestibility was 43.2% in PM and 52.4% in SM (unpublished data). These anti-nutritional factors appear to induce low feed digestibility by inhibiting trypsin and chymotrypsin. The weight gain of pigs varied in an inverse relationship to the measured trypsin inhibition levels (Simoongwe et al., 2001). In this study, the trypsin inhibition rate of the raw PM was 99.15%, and at the 20% and 40% dietary PM levels, 36.4% and 72.9% of SM in the control diet was substituted by PM, respectively. Therefore, it is reasonable to think that the present decreased growth performance with the increasing level of raw PM would be induced by the volume of anti-nutritional factors substituted for SM in the diet. However, the trypsin inhibitor was decreased by roasting (Simoongwe, 1998) and cooking (Sharma and Sehgal, 1992) PM. The trypsin inhibitor activity in PM was lowered after boiling (Rani et al., 1996), and was completely destroyed by pressure cooking (Duhan et al., 2001). In these heated PM, the in vitro digestibility of protein and starch was increased (Rani et al., 1996). Actually, the net protein utilization of casein was improved in rats fed cooked PM (Nageswara Rao and Narasinga Rao, 1978), resulting in increased body weight gain in rats (Nageswara Rao and Narasinga Rao, 1978; Udayasekhara Rao and Belavady, 1978), and in pigs (Visitpanich et al., 1985a) given heated PM than in comparison with those fed unheated PM. In the present study, the trypsin inhibition rate was decreased from 99.15% in raw to 54.31% in heated PM. From these papers and the present result, it can be hypothesized that the growth performance similar to the control in HPM groups might be induced by the decreased anti-nutritional factors in PM.

In those pigs from the raw PM groups which showed a low growth performance, although specific gross morphological alterations were not observed, the villus height, cell area and cell mitosis were decreased. Villus atrophy has been investigated largely from the feed intake (Kelly et al., 1991; Pluske et al., 1996; Van Beers-Schreuers et al., 1998; Spreeuwenberg et al., 2001) and nutritional (Hampson and Smith, 1986) standpoints. In regard to the feed intake, the villus height of weaned pigs was influenced principally by the feed intake level (Van Beers-Schreuers et al., 1998), and low feed intake induced a decrease in villus height in weaning pigs (Spreeuwenberg et al., 2001). In this study, however, feed intake was not significantly different between the dietary treatments. This suggests no significant relationship between feed intake and villus height in pigs fed PM. On the other hand, light microscopic parameters as well as epithelial cells on the tip surface of the villi are well known to be rapidly altered by feed withdrawal and refeeding treatments, and to be affected by dietary feed components (Shamoto and Yamauchi, 2000; Tarachai and Yamauchi, 2000; Samanya and Yamauchi, 2002). Short villi were also reported in pigs showing decreased body weight as studied by Zijlstra et al. (1997), and in pigs fed the diet containing higher soybeans than that of a commercial diet (Nabuurs et al., 1993). Lectins in soybeans (Tsien and Schmidt, 1981) caused such a reduction in the villus length (Kik, 1991). These short villi correspond with reductions of enzyme activities such as the mucosal lactase and sucrase (Park et al., 1998), the lactase and alkaline phosphatase (Zijlstra et al., 1997), the 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 the reduced absorptive functions. Besides, on the tip surface of the short villi of PM groups, flattened epithelial cells were observed, showing a smooth surface. Such flat cells were also reported in the pigs of 3-day feed withdrawal (Mekbungwan et al., 2002). These studies suggest that the present decreased light microscopic parameters and flat cells in the PM groups might suggest atrophy, and might have consequences in term of low function of villi, as suggested by the above-mentioned literature. Conversely, in the pigs which showed no significantly decreased growth performance in HPM groups the light microscopic parameters were significantly higher than those of the 40% PM group, and were almost the same as those of the control. 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). Increased villus size was associated to activated cell proliferation (Lauronen et al., 1998). It has been suggested that long villi results in an increased surface area capable of greater absorption of available nutrients (Caspary, 1992), and that greater villus height and 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). Besides, on the tip surface of HPM groups,

protuberated cells were observed, showing rough surface. Such protuberated cells were also reported in the piglets that showed an increased feed efficiency after feeding dietary charcoal powder including wood vinegar compound liquid (Mekbungwan et al., 2003). This literature suggests that light microscopic parameters and protuberated cells in the HPM groups might show that these morphologies are not as atrophied as the control, but much more hypertrophied than PM groups. This would demonstrate that the intestinal histology is not affected after heating the raw PM. It is not clear at present why much greater cell protuberances developed in the 40% HPM group than in the 20% HPM group, but it is possibly related to the difference in the increased heated PM volume. The protein and starch digestibilities in raw PM showed a 23% and 32% increase after boiling of unsoaked seed, respectively (Rani et al., 1996). Net protein utilization of casein diet was improved from 0.49 in raw PM to 0.57 in cooked PM (Nageswara Rao and Narasinga Rao, 1978). The semi-purified diet composed of casein and starch did not bring about the elevation of light microscopic parameters, but it was quicker than the conventional diet to induce cell protuberances (Maneewan and Yamauchi, 2003). Cells protuberating further into the lumen than those of intact control were found in chickens force-fed an enteral hyperalimentative solution after 3-d feed withdrawal (Tarachai and Yamauchi, 2000). These studies suggest that absorptive epithelial cells themselves would be directly stimulated by such an easily absorbable feed ingredient in the heated PM.

In our previous study, we demonstrated that raw PM could be incorporated up to the 30% level in a piglet diet and up to the 20% level in a growing pig diet (Mekbungwan et al., 1999). Castro et al. (1987) also reported that the mean daily gain was 0.96 kg, 0.85 kg, 0.65 kg, 0.49 kg and 0.32 kg for the 0%, 25%, 50%, 75% or 100% dietary raw PM diets, suggesting that PM can replace up to 50% of SM in pigs. The present decreased growth performance with the increasing level of raw PM but not with the heated PM leads to the general conclusion that the raw PM could be incorporated under the 40% level, but the heated PM increased the incorporation rate up to the dietary 40% level.

In conclusion, histological intestinal villus demonstrate that the villi might be atrophied in the piglets fed raw PM due to anti-nutritional factors, resulting in the decreased growth performance. Heating PM might abolish such a harmful effect of the anti-nutritional factors on the villus function, resulting in a similar growth performance to the control.

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