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Influence of low protein diets on gene expression of digestive enzymes and hormone secretion in the gastrointestinal tract of young weaned piglets*

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Abstract: To investigate dietary protein level effects on digestive mechanisms, weaned piglets were fed for 45 d with diets containing 20%, 17%, or 14% crude protein (CP) supplemented to meet requirements for essential amino acids. This article describes the influence of dietary protein on gastrointestinal hormones and expression of an array of digestive enzymes in the gastrointestinal tract and pancreas. Results indicated that there were no significant differences in expression of enzymes involved in carbohydrate digestion, except for maltase in the duodenum. In the jejunum, amylase expression in pigs fed 20% CP was much higher than that in pigs fed other diets ($P<0.05$) and maltase expression in those fed 17% CP was higher than that in other treatments ($P<0.05$). Although there were no remarkable differences in expression of aminopeptidase in the small intestine or carboxypeptidase in the pancreas ($P>0.05$), there was a trend towards higher expression of various proteases in pigs fed 17% CP. The duodenal expression of enteropeptidase in diets with 14% and 17% CP was significantly higher than that with 20% CP ($P<0.05$), but treatment differences did not existed in jejunum ($P>0.05$). The expression of GPR93 as a nutrient-responsive G protein-coupled receptor in 14% and 17% CP diets was significantly higher than that in 20% CP diet in the small intestine ($P<0.05$). The expressions of genes for pancreatic enzymes, lipase and elastase, were significantly higher in pigs fed diets with low CP, while similar trends occurred for carboxypeptidase, chymotrypsin and amylase. Conversely, the gastric expressions of pepsinogen A and progastricsin were lower with the 17% CP diet. Differences between treatments were found in the gastric antral contents of cholecystokinin and somatostatin: both increased in pigs fed 17% CP, accompanied by decreased content of motilin, which was also seen in plasma concentrations. These patterns were not reflected in duodenal contents. In general, 17% dietary CP was beneficial to the digestion of nutrient substance in the gastrointestinal tract.

Key words: Dietary protein, Piglet, Gastrointestinal tract, Pancreas, Digestion, Hormone
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1 Introduction

Dietary proteins perform a wide range of nutritional and biological functions. In addition to their nutritional role in supplying amino acids for endogenous protein synthesis, they play a part in regulating feed intake and glucose and lipid metabolism in order

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to sustain growth, development, and multiplication of organisms (Len *et al*., 2009; Jahan-Mihan *et al*., 2011; Peng *et al*., 2016). Within the gastrointestinal tract (GIT), the level of protein has a major impact on digestion and absorption, and their digestive products affect several regulatory functions by interacting with receptors releasing hormones, affecting stomach emptying, gastrointestinal (GI) transport and absorption of nutrients (Leray *et al*., 2003; Jahan-Mihan *et al*., 2011; Yang *et al*., 2011; He *et al*., 2016). The amount of dietary protein is a major issue impacting the economy and environment, and therefore this study was principally focused on the importance of dietary protein levels on the regulation of amino acid, glucose and lipid metabolism in the GIT, along with feed intake.

The early stage of growth after weaning is considered to be most efficient in the conversion of nutrients to animal tissue (Kendall *et al*., 2008). Digestive enzymes and GI hormones play essential roles in nutritional digestion and absorption, and hence expressions of gut enzymes involved in carbohydrate, protein and fat digestion were examined here during this phase of maximal growth. Bikker *et al*. (2006) pointed out that dietary protein level did not affected maltase, sucrase or aminopeptidase N activity in jejunum, but Pinheiro *et al*. (2013) reported that dietary protein restriction increased disaccharidase activity such as lactase and sucrase in the small intestine. Yue and Qiao (2008) demonstrated that reducing dietary crude protein (CP) concentration from 23.1% to 17.2%, but not partial reduction to 18.9%, changed gut morphology and reduced disaccharidase activities in the small intestine. Some studies indicate that pancreatic enzymes were positively associated with dietary protein level and low dietary protein inhibited synthesis of pancreatic enzymes and pancreatic growth; little is known of the possible influence of low protein diets on pancreatic lipase (Lindemann *et al*., 1986; Makkink *et al*., 1994).

Dietary protein level also affects GI hormones, digestion and nutrient uptake (Chaudhri *et al*., 2006). High protein diets increased cholecystokinin (CCK) release and pancreatic protease secretion (Green *et al*., 1986), but Shi *et al*. (1997) and Leray *et al*. (2003) argued that a low-protein diet inhibited gastric emptying through modulation of CCK release and gastric muscle sensitivity to CCK, and postprandial plasma levels of CCK were increased significantly. Pancreatic amylase production was suppressed by endogenous CCK (Hara *et al*., 2001). Somatostatin (SS), a GI motility regulator, also inhibits the release of gut hormones including gastrin, insulin, CCK, and motilin (MLN) and slows gastric emptying, gallbladder contraction, and small intestinal segmentation (Ling *et al*., 1973; Patel, 1999; Morisset *et al*., 2000). The G protein-coupled receptor, GPR93, regulates mucosal cell proliferation and differentiation and the extent of hormonal secretion triggered by digestive products in the lumen. Lower levels of GPR93 occur in the gastric mucosa than in the intestinal mucosa (Choi *et al*., 2007). Pancreatic lipase, produced by pancreatic acinar cells, efficiently hydrolyzes triglycerides to free fatty acids and accounts for the bulk of fat digestion in animals (Lowe, 1994; Xiao *et al*., 2013). There appears to be little known about the effect of low protein diets on the secretion of MLN and SS, and mRNA expression of GPR93 and pancreatic lipase. According to previous researches, we presumed that decreasing adequately dietary CP was sufficient for the requirement of growth performance and more beneficial to a weaned piglet. The focus of the present study was to explore physiological and metabolic indices of GIT and pancreatic function, including expression of genes for digestive enzymes and secretion of selected hormones.

2 Materials and methods

2.1 Animals and experimental treatments

Cross-bred (Duroc×Landrace×Yorkshire) piglets were weaned at 28 d of age and the experiment started after a 7-d adaptation period. Eighteen piglets (initial body weight (BW) is (9.57±0.64) kg) were randomly assigned to three groups. Diets with 20%, 17%, and 14% CP were based on the National Research Council (NRC) recommendation (Committee on Nutrient Requirements of Swine, National Research Council, 2012), as shown in Table 1, and were supplemented with 4 essential amino acids (Lys, Met, Thr, and Trp) to meet the requirements of weaned piglets (Wu *et al*., 2015). Piglets were housed individually in cages and had free access to drinking water and their respective diets throughout the 45-d experimental period (35 to 80 d of age).

Table 1 Composition and nutrient levels of diets*

Component	Content (%)[a]		
	14% CP	17% CP	20% CP
Ingredient			
Corn	71.80	66.50	63.70
Soybean meal	13.40	18.80	19.80
Whey powder	4.40	4.30	4.30
Fish meal	1.50	4.00	9.00
Soybean oil	4.10	2.60	0.80
Lys	0.88	0.62	0.38
Met	0.27	0.19	0.10
Thr	0.33	0.21	0.09
Trp	0.08	0.04	0.01
Calcium hydrophosphate	1.15	0.74	0.00
Limestone	0.79	0.70	0.52
Salt	0.30	0.30	0.30
1% premix compound[b]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated nutrient content			
DE (MJ/kg)	14.60	14.60	14.60
CP	14.00	17.00	20.00
Total Ca	0.70	0.71	0.69
Total P	0.53	0.55	0.57
Arg	0.75	0.91	1.09
His	0.34	0.40	0.46
Ile	0.49	0.60	0.70
Leu	1.15	1.32	1.49
Lys	1.23	1.23	1.23
Met+Cys	0.68	0.68	0.68
Phe	0.59	0.69	0.80
Thr	0.73	0.73	0.73
Trp	0.20	0.20	0.20
Val	0.53	0.65	0.77
EAA/NEAA	0.90	0.80	0.70
Recommendation rate of NRC[c]	0.80	0.80	0.80

DE: digestible energy; CP: crude protein; EAA: essential amino acids; NEAA: non-essential amino acid; NRC: National Research Council. [a] The values are expressed as percentage (%), except for DE (MJ/kg), EAA/NEAA and recommendation rate of NRC. [b] Premix provided these amounts of vitamins and minerals per kilogram on an as-fed basis: vitamin A, 10800 IU; vitamin D_3, 4000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Fe, 100 mg as ferrous sulfate; Cu, 150 mg as copper sulfate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite. [c] Committee on Nutrient Requirements of Swine, National Research Council, 2012. * Reprinted from Wu *et al.* (2015)

2.2 Slaughter and tissue collection

Pigs were fasted overnight (about 12 h), and then blood was collected by jugular venipuncture using heparin-coated vacutainer tubes immediately before slaughter. Samples were centrifuged for 15 min at 1000*g* at 4 °C. The plasma was aliquoted into 2 plastic vials and stored at −80 °C. Pigs were electrostunned and then exsanguinated, and the small intestine and stomach were immediately dissected, emptied of contents and placed on ice. Segments (2 cm in length) of mid-duodenum and gastric antrum were fixed in 10% Bouin's solution and stored at 4 °C for 48 h. In addition, 3 cm segments from the mid-jejunum and mid-duodenum were also immediately flushed with phosphate buffer saline (PBS) at 4 °C and frozen in liquid nitrogen in sterile Eppendorf tubes then held at −80 °C.

2.3 Relative quantification of mRNA expression

Total RNA was extracted from tissues (100 mg) with TRIzol Reagent (TaKaRa, Otsu, Japan) and dissolved in RNase-free water. RNA (1 μg) was reverse-transcribed to complementary DNA (cDNA) using PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa) following the manufacturer's protocol (Chen *et al.*, 2013).

Partial or full-length gene coding sequences were amplified from cDNA using oligodeoxynucleotide primers (Table 2) designed from porcine sequences in GenBank with Primer Premier 5.0 (Applied Biosystems, Carlsbad, CA, USA). Real-time polymerase chain reaction (PCR; Bio-Rad System) was performed in a total volume of 20 μl containing 2 μl of cDNA, 10 μl SYBR Green 2× mix, 0.8 μl (100 nmol/L) each of the forward and reverse primers. An initial denaturation step of 95 °C for 2 min was followed by 39 cycles of 95 °C for 15 s, 30 s at the annealing temperature, and 72 °C for 30 s. The housekeeping gene *β-actin* was used as an internal control for efficiency of reverse transcription. Data were quantified using the comparative cycle threshold (C_T) method. For each sample, data were normalized to *β-actin*, and the relative expression values for each gene were calculated using the $2^{-\Delta\Delta C_T}$ method with efficiency correction and using one control sample as calibrator.

2.4 Relative quantification of mRNA expression

Freshly harvested tissue (100 mg) was rinsed with 1× PBS, homogenized in 1 ml of 1× PBS, and stored overnight at −20 °C. After further lysis with two freeze-thaw cycles, the homogenates were centrifuged for 5 min at 5000*g*, 2–8 °C and supernatants were collected and assayed immediately. Also, aliquots were stored at −80 °C, and re-centrifuged before assay. Concentrations of CCK, SS, and MLN in antral and duodenal extracts and plasma were determined using commercial enzyme linked immunosorbent assay (ELISA) kits (CUSABIO, Wuhan, China).

Table 2 Primers used for real-time PCR analysis

Gene	Primer sequence (5'→3')	Size (bp)	Accession No.	T_M (°C)
Sucrase	F: CGAGCAGATTCTTAGTGATGGAT R: GCATAGTTGTCTCCAAGCCAGT	189	XM_005657098.1	55
Glucosidase F	F: TGATGAAGTCTGGGTGGGTGTG R: ATGTAGGCGAGTGAGCGGAA	183	NM_001258385	65
Amylase	F: GGGCAGCGTTTATTCTCACTCA R: TCTCTTGCTTCTTCCCTGTTCC	252	NM_214195	65
Maltase	F: TGCCTTACCTCTACACGCTGATGC R: GATTCACTGCCAGATTCCGTGCTAT	113	XM_003475331	62
Lactase	F: GCTACCACCTAATACAGTAAACCTCCC R: CCCACAGAAAGTCATCCCGAAA	235	XM_003359430	55
Aminopeptidase	F: TCATCAATCGGGCTCAGGTC R: TCCGTTCAGGAAGAGGGTGTT	101	HQ824547.1	55
Enteropeptidase	F: GCCCTGGGTTGTTGCTCTGTAT R: ATGTCACTGTCCTTTCTCCGTCTG	243	D30799.1	55
GPR93	F: GGTGCTGATGGTGATGGT R: GAAGGAAGACAGAGAGTGGGA	175	XM_005652601	57
Lipase	F: GCTCACGGTCACCATTTCAC R: TGAGGTAAGGCTCGTGGGAT	150	NM_001177912.2	55
Carboxypepidase	F: CCAAGAACCGAATGTGGAGAAA R: TCAGGTATGCTTTGATGGAGGAG	222	NM_214169.1	60
Elastase	F: CTGCTGGTTGTGGACTATGC R: AGGGCTTGTGGTAGTAGTTGC	217	NM_214109.1	57
Chymotrypsin	F: CGGCACCTTAATCACCTCTACTCA R: CAAAGATGCTATCCACACCCACGA	146	NM_001244379.1	60
Pepsinogen A	F: TGTTTCCGTCGAGGGTTACTG R: CCGATGTCGCTCTGGATGTT	159	NM_213873.2	60
Progastricsin	F: TGGCAGATTGGCATTGAGG R: TGATGGCGACAGAGGGAACT	252	NM_001172999.1	59

F: forward; R: reverse; T_M: melting temperature

2.5 Immunohistochemistry

Fixed tissue samples were washed with PBS and then embedded in paraffin after dehydration with a graded series of ethanol concentrations followed by xylene. Paraffin-embedded tissue sections (4 μm) were de-waxed with xylene, ethanol-washed, and re-hydrated with deionized water. After treatment with 3% (v/v) H_2O_2 and three washings with 1× PBS, proteinase K was used for antigen retrieval followed by three rinses with 1× PBS and once with cold glycine. Nonspecific binding of antibody protein was blocked with bovine serum albumin. Sections were incubated overnight at 4 °C with antibodies (AbD, Bio-Rad) against SS and CCK diluted (1:200) in PBS, washed three times with 1× PBS, then incubated with secondary antibody (AbD, Bio-Rad) diluted (1:250) in PBS. After further washing three times with 1× PBS, diaminobenzidine (DAB) was used as the chromogen. Sections were counterstained with hematoxylin and examined microscopically. Relative intensity of antigen signals was quantified using Image-pro plus6.0 software. Statistical analysis of the antigen signals used a pool of at least 6 images.

2.6 Relative quantification of mRNA expression

Data are presented as mean±standard deviation (SD), with 6 piglets (n=6). Statistical analysis of the difference in mRNA levels was performed by one-way analysis of variance (ANOVA) with Tukey's post hoc test to compare the means. Statistical analyses were performed and the data were analyzed using Prism 6 (Graphpad Software, Inc.). Statistical significance was set at P<0.05, P<0.01, and P<0.001.

3 Results

3.1 Effect of dietary CP content on growth performance of piglets

The growth performance of piglets fed different diets was internal and unpublished data. The average daily gain (ADG) and average daily feed intake (ADFI) displayed a similar tendency, but ADG showed a significant increase following raising of the dietary CP level (P<0.01). ADFI in the 20% and 17% CP groups had no significant difference between them but was higher than that in 14% CP group. Feed to gain (F/G) ratio in the 20% CP group showed a lower level than that in the 14% and 17% CP groups.

3.2 Effect of dietary CP content on relative expression of genes of selected digestive enzymes in the duodenum, jejunum, and pancreas of piglets

There were no significant differences (P>0.05) between dietary CP contents in the expressions of sucrase, glucosidase, or aminopeptidase in the duodenum or jejunum (Figs. 1a, 1b, and 1f). Jejunal expression of amylase exceeded that in the duodenum and was significantly higher in pigs fed 20% CP than in either the 14% or 17% CP (Fig. 1c); intermediate levels of expression occurred in the pancreas but were unaffected by dietary CP. The expression of maltase showed inconsistent effects of dietary CP in the duodenum and jejunum; the expression in the 20% CP group exceeded that with 17% CP in duodenum and reached the highest expression in jejunum when compared with groups fed 14% or 20% CP (Fig. 1d). A similar inconsistency in the effect of dietary CP on expression of GPR93 was observed, and the highest expression levels occurred with 17% CP in the jejunum but the lowest expression levels in the group with 20% CP in duodenum (Fig. 1h). The duodenal expressions of lactase and enteropeptidase exceeded those in the jejunum (Figs. 1e and 1g). The lactase expression declined in the duodenum as the CP increased from 14% to 20%. The expression of enteropeptidase in the same tissue was greatest with the 17% CP diet; the highest jejunal expression of lactase occurred with 14% CP.

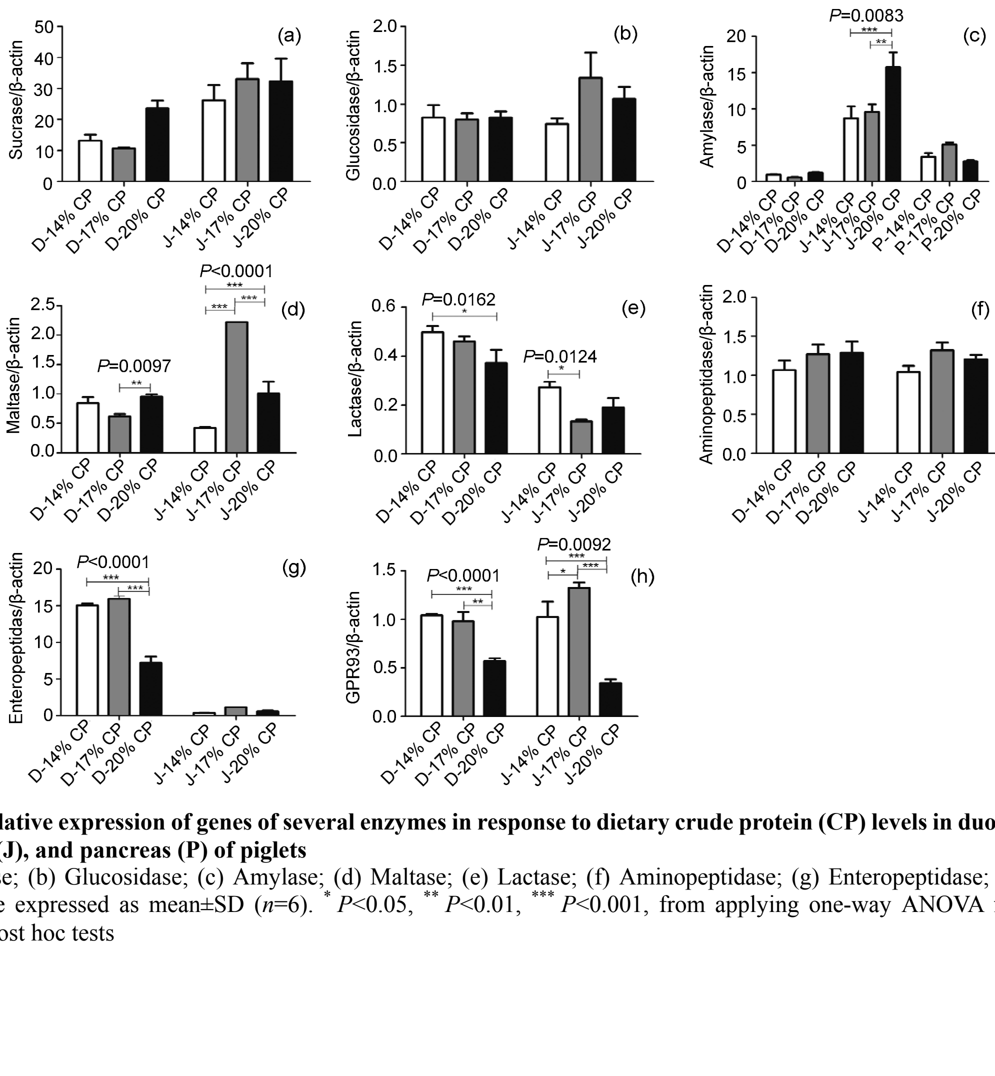


Fig. 1 Relative expression of genes of several enzymes in response to dietary crude protein (CP) levels in duodenum (D), jejunum (J), and pancreas (P) of piglets

(a) Sucrase; (b) Glucosidase; (c) Amylase; (d) Maltase; (e) Lactase; (f) Aminopeptidase; (g) Enteropeptidase; (h) GPR93. Values are expressed as mean±SD (n=6). * P<0.05, ** P<0.01, *** P<0.001, from applying one-way ANOVA followed by Tukey's post hoc tests

3.3 Effect of dietary CP content on relative expression of selected enzymes in the pancreas and stomach of piglets

In the pancreas, the low CP diets increased the expression of lipase and the expression of elastase was higher in the 14% CP group than in other groups (P<0.05). The lowest levels of expression of lipase, peptidases, carboxypeptidase, and chymotrypsin usually occurred with the 20% CP diet (Fig. 2). A reversal of the effect of dietary CP on gastric expression of pepsinogen A with 20% CP giving the highest level was observed (Fig. 2), and lowest expression occurred with 17% CP. The expression of progastricsin had a similar tendency to that of pepsinogen A, but it showed no significant difference among the CP diets.

3.4 Effect of dietary CP content on concentrations of CCK, SS, and MLN in piglets

The antral content of CCK in pigs fed 20% CP was significantly lower than that with other CP levels (P<0.05) but there were no differences between diets in CCK contents of the duodenum or in plasma (Fig. 3a). The concentrations of SS were significantly higher with the 17% CP in gastric antrum but were lower in the duodenum (P<0.05), and did not differ in plasma (Fig. 3b). Concentrations of MLN in the gastric antrum and plasma were higher in pigs fed 14% CP than in others and did not differ in the duodenal mucosa (Fig. 3c); there were no significant differences between pigs fed 17% or 20% CP.

3.5 Localization of CCK in the duodenum and SS in the antrum of the stomach

Immunohistochemical (IHC) localization was consistent with the ELISA data for CCK in the duodenum and SS in the gastric antrum. The quantity of duodenal CCK in pigs fed 17% CP exceeded that of the other CP levels, but not significantly so (P>0.05; Figs. 4a, 4b, 4c, and 4g). Signal intensity of SS in the gastric antrum fed the 17% CP diet was significantly greater than that observed in the group fed the 14% CP diet (P<0.05; Figs. 4d, 4e, 4f, and 4g).

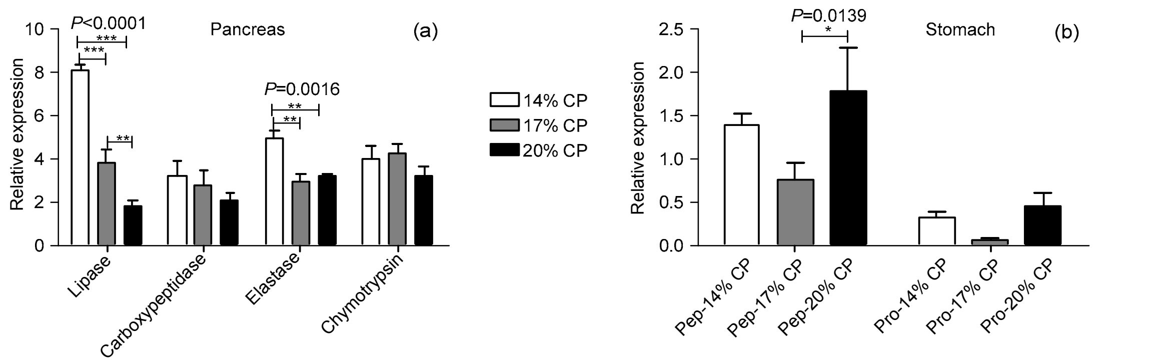


Fig. 2 Effect of dietary crude protein (CP) level on the relative mRNA expression of selected gastric and pancreatic enzymes
The pancreatic enzymes involved in fat and protein digestion examined were lipase, carboxypeptidase, elastase, and chymotrypsin (a) and gastric enzymes were pepsinogen A (pep) and progastricsin (pro) (b) among different dietary CP levels (14%, 17%, and 20%). Values are expressed as mean±SD (n=6). * P<0.05, ** P<0.01, *** P<0.001, from applying one-way ANOVA followed by Tukey's post hoc tests

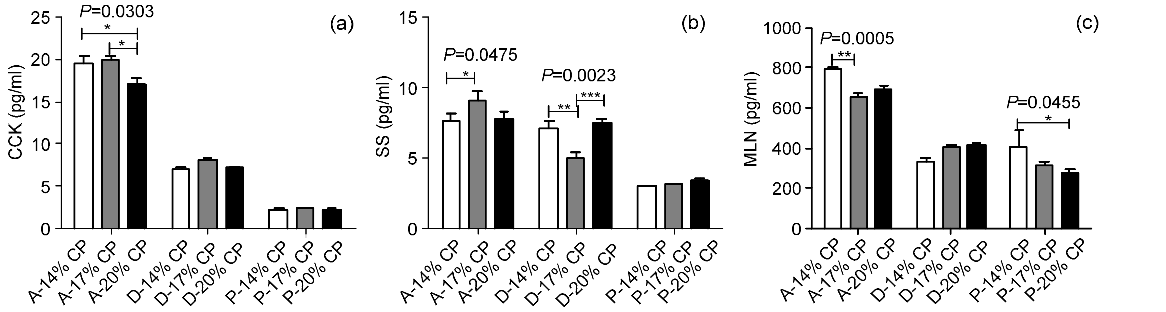
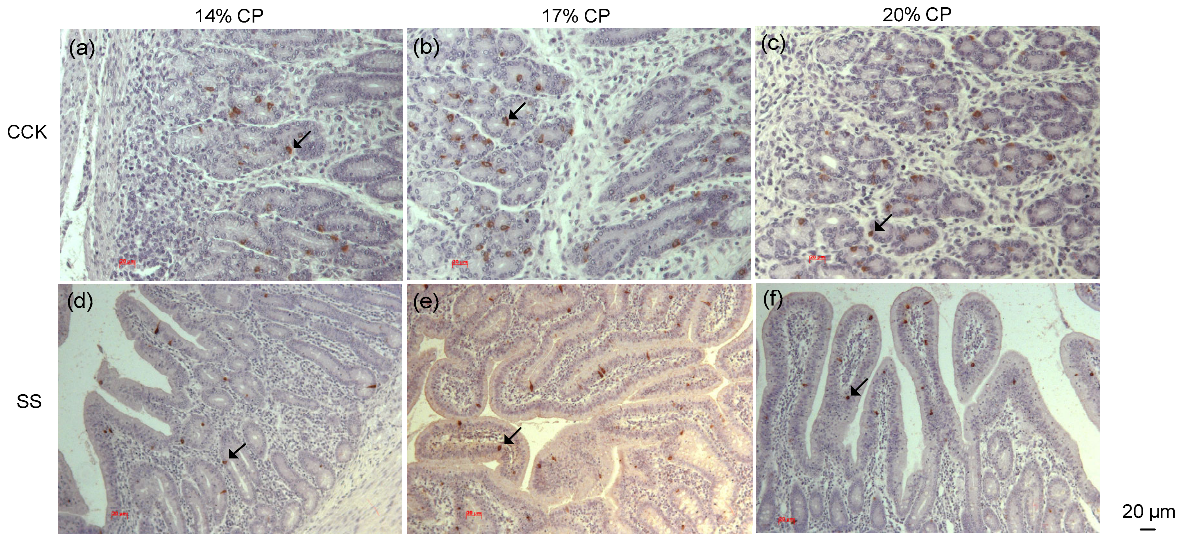


Fig. 3 Concentrations of hormones in plasma (P) and 1:10 supernatants of gastric antrum (A) and duodenum (D) of pigs fed different amounts of dietary crude protein (CP)
(a) Cholecystokinin (CCK); (b) Somatostatin (SS); (c) Motilin (MLN). Values are expressed as mean±SD (n=6). * P<0.05, ** P<0.01, *** P<0.001, from applying one-way ANOVA followed by Tukey's post hoc tests



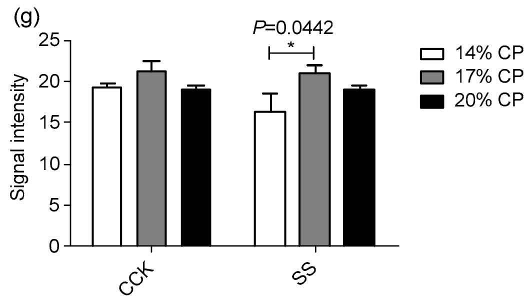


Fig. 4 Immunohistochemical (IHC) localization of cholecystokinin (CCK) in duodenum and somatostatin (SS) in gastric antrum in pigs fed different levels of crude protein (CP)

(a–c) IHC staining of duodenal CCK; (d–f) IHC staining of antral SS; the brown points represented positive stain (black arrows). (g) Quantification of IHC staining. Relative intensity of antigen signals was quantified by randomly choosing 5 fields per section for image capture and statistical analysis of the number of antigen signals used data from 6 pigs. Values are expressed as mean±SD (n=30). * P<0.05, from applying one-way ANOVA followed by Tukey's post hoc test (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

4 Discussion

In the context of pig production, maximizing amino acid utilization needs to be balanced with minimizing dietary protein input for environmental, economic, and sanitary reasons. The objective of the present study was to determine an appropriate low protein diet for piglets after weaning. To achieve this objective, diets of different protein content were fed to recently-weaned pigs for 45 d and selected hormones and transcript abundance of critical enzymes involved in digestion and absorption were investigated in the GIT and pancreas.

Diets with low CP compared with high CP content did not affect performance (le Bellego *et al*., 2002). It is known that diets with high CP may disturb the intestinal microbiota as a consequence of protein fermentation (Nyachoti *et al*., 2006; Hermes *et al*., 2009) and reduced dietary CP, balanced with required amounts of essential amino acids, should reduce N excretion and ammonia emission from swine manure due to fermentation and formation of microbial metabolites (Nyachoti *et al*., 2006; Htoo *et al*., 2007). Alternatively, Awati *et al*. (2006) have proposed reducing protein fermentation in the small intestine by the inclusion of fermentable carbohydrates in the diet. As for carbohydrate digestion, the expressions of sucrase, glucosidase, and amylase had no significant difference among the three dietary CP levels, but the expression of maltase in the 20% CP diet was higher than that in the 14% and 17% CP diets in the duodenum. In the jejunum, expression of maltase was higher in the 17% CP group than in the others, whereas amylase expression in the jejunum was highest in the piglets fed the 20% CP diet. The expression levels of these four enzymes were much higher in the jejunum than in the duodenum and were affected by dietary CP. Jin *et al*. (1998) reported that low CP diets improved the digestive health of swine, provided that the amino acid requirements are satisfied.

Hermes *et al*. (2009) pointed out that low CP diets, supplemented to meet amino acid requirements, can improve the integrity of the gut barrier and reduction in dietary CP while maintaining the levels of essential amino acids was an effective strategy to minimize post-weaning diarrhea (PWD) in piglets (Barea *et al*., 2007). A reduction in CP here from 20% to 17% enhanced expression of intestinal disaccharidases, presumably favoring more complete digestion and absorption of carbohydrates instead of leaving fermentation substrates in the intestinal lumen.

Barea *et al*. (2007) showed that decreasing the dietary CP increased the digestibility and metabolizability of energy. In the present study, reducing the dietary CP promoted significantly the expression of genes encoding pancreatic digestive enzymes including lipase, enteropeptidase, and elastase, but had no significant effect on the mRNA expression of chymotrypsin, amylase, or carboxypeptidase. However, it did not affect expressions of pepsinogen A and progastricsin in the gastric fundus and, consistent with an earlier finding (Bikker *et al*., 2006), intestinal expression of aminopeptidase was not affected. Taken together, the results obtained here showed that expression of pancreatic enzymes had an increasing tendency, but expressions of carboxypeptidase and chymotrypsin had no significant difference between pigs fed the 17% CP diet and those given the 20% CP diet, and expression of lipase in pigs fed 14% and 17% CP diets was significantly higher than that in the 20% CP diet. Enteropeptidase, from duodenal intestinal glands, cleaves trypsinogen to generate active trypsin, and then activates other pancreatic zymogens including pro-carboxypeptidases, chymotrypsinogen, pro-elastase, and some pro-lipases. The expression of enteropeptidase was highest with the 17% CP diet.

The reduction of dietary CP to 17% resulted in more CCK in the gastric antrum of the stomach, duodenum, and plasma than the other two diets. CCK can stimulate gallbladder contraction, SS release, and pancreatic growth, and its release of enzymes (Wank, 1995), and is the basis for its older name, pancreozymin. The present research confirmed earlier reports (Green *et al*., 1986; Leray *et al*., 2003) that low protein diets enhance CCK release but did not support views (Shi *et al*., 1997; Hara *et al*., 2001) that low protein diets inhibited pancreatic enzymes including amylase and protease. However, 17% dietary CP stimulated gastric generation of SS and CCK while weakening expression of pepsinogen A and progastricsin and reducing intestinal SS release. SS released from the pyloric antrum enters the systemic circulation to reach locations where it will exert its inhibitory effects. In the animals studied here, 17% dietary CP decreased the quantity of SS in the duodenum and increased that of CCK in the stomach. Williams (2001) explained that CCK appears to be the main hormonal drive for increased zymogen secretion and lipase secretion in response to the presence of fats and amino acids in the intestinal lumen while increased CCK influenced feed intake and decreased SS, effectively accelerating the digestive process (Moran and Kinzig, 2004; Chaudhri *et al*., 2006), consistent with the present changes in duodenal rather than gastric CCK. Green *et al*. (1986) discovered that high protein diets increased CCK release and pancreatic protease secretion sufficient to match protein digestive requirement, whereas it was found here that high protein diets decreased CCK and pancreatic protease. It was inferred that CCK likely had a different mechanism in the stomach and intestine, and CCK may mediate food digestion in the stomach by decreasing MLN instead of SS. On the basis of measured hormones and expression of enzymes especially in maltase, enteropeptidase, and lipase, the diet with 17% CP appeared to be more beneficial to digestion than 20% or 14% CP in these pigs. MLN plays a physiological role as a regulator of gut motility between meals, and there was no significant difference in MLN content between pigs fed 17% and 20% dietary CP, though higher levels occurred in the gastric antrum and plasma of pigs fed 14% CP. Although there was no significant difference in expression of CCK in the small intestine, the expressions of GPR93 and CCK showed a similarity at different dietary CP levels. It is consistent with the known role of GPR93 in inducing CCK transcription and secretion. GPR93 was activated by dietary protein hydrolysate and was subsequently involved in inducing expression of CCK. Expression of CCK in the gastric antrum showed a significant difference from and was higher than that in the small intestine. This demonstrated that dietary CP level mainly affects the expression of CCK in the gastric antrum rather than in the small intestine. GPR93 is a nutrient-responsive, especially to proteins, G protein-coupled receptor (Nilaweera *et al*., 2010), and the present results showed higher expression in pigs fed the 17% CP than in pigs fed the 20% CP diets. Additionally, peptidases like carboxypeptidase, chymotrypsin, and aminopeptidase in the 17% CP group had higher expression than those in other groups, although there were no significant differences. Enteropeptidase also displayed higher expression in the 17% CP diets compared with 20% CP diets. The present results are consistent with GPR93 being important in the signaling mechanisms involved in the nutrient induction of the transcription and secretion of bioactive peptidases (Choi *et al.*, 2007). The diet with 17% CP was sufficient to meet the requirements for growth, total N excretion and ammonia emissions, digestion and absorption of nutrient substances, and appeared to be more beneficial than higher or lower CP to the weaned piglet.

5 Conclusions

Considering growth performance, 14% dietary CP was not sufficient for meeting the requirement for growth of wean piglet. On the basis of several indices of endocrines and digestive enzymes, this study has shown that, in young weaned pigs, 17% dietary CP, rather than 14% or 20% CP, was beneficial to processes likely to be involved in the digestion of three major nutrient classes: carbohydrates, proteins, and lipids.

Compliance with ethics guidelines

Zhi-mei TIAN, Xian-yong MA, Xue-fen YANG, Qiu-li FAN, Yun-xia XIONG, Yue-qin QIU, Li WANG, Xiao-lu WEN, and Zong-yong JIANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：低蛋白日粮对断奶仔猪胃肠道消化酶表达及激素分泌的影响
目　的：研究蛋白日粮水平对断奶仔猪消化的影响机制。
创新点：从营养物质消化及激素变化方面研究低蛋白日粮对仔猪胃肠道消化吸收的影响，探究低蛋白日粮饲养的可行性。
方　法：18 头 28 日龄断奶仔猪经过 7 天适应期后平均体重为(9.57±0.64) kg。仔猪随机分为三组，每组 6 头，分别饲喂 20%、17%和 14%粗蛋白日粮并自由采食 45 天。根据国家研究委员会（NRC）2012 年的标准，通过添加赖氨酸、蛋氨酸、半胱氨酸、苏氨酸及色氨酸等理想蛋白模型满足仔猪生长需求。实验结束后，进行颈静脉采血并屠宰取样。
结　论：结果表明基于蛋白日粮水平对内分泌及消化酶影响，与 14%及 20%粗蛋白组相比，17%蛋白日粮更有利于胃肠道和胰腺等组织中三种主要营养物质的消化。因此，17%粗蛋白水平更适合断奶仔猪的营养需求，证实了低蛋白日粮对断奶仔猪饲养的可行性。
关键词：日粮蛋白；仔猪；胃肠道；胰腺；消化；激素