Chronic feeding with protein-restricted diets affect ileal amino acid digestibility and the expression of nutrient-sensing, hormone secretion, gastrointestinal digestive enzyme, and nutrient transporter genes in young weaned pigs

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ABSTRACT

The aim of this work was to investigate the serum biochemical profile, ileal amino acid (AA) digestibility, and expression of nutrient-sensing, hormone secretion, gastrointestinal (GIT) digestive enzyme and nutrient transporter genes in pigs subjected to dietary protein restriction. Twenty-four weaned pigs were distributed into three treatments, and the animals in each treatment were fed crude protein (CP) diets at one of three levels (20, 17, and 14%). Our results showed that pigs fed the 20% CP diet had greater ($P < 0.05$) average daily gain and ratio of feed to gain than those fed the 14% CP diet, but there were no differences between the 20% CP and 17% CP diets. Additionally, the 20% CP diet tended to up-regulate ($P < 0.05$) the expression of nutrient-sensing-related genes, such as taste receptor type 1 member 1 (TasR1), calcium-sensing receptor (CaSR), and solute carrier family 1 (EAAC1), and the 17% CP diet tended to up-regulate ($P < 0.05$) the expression of digestive enzyme-related genes, hormone secretion-related genes, and nutrient transporter-related genes, such as pepsinogen, cholecystokinin type A receptor (CCK-1R), and dipeptide transporter 1 (PepT1), respectively. These results suggested that weaned pigs that were chronically fed a moderately restricted 17% CP protein diet could catch up to pigs fed a 20% CP diet in terms of growth performance and feed efficiency. In conclusion, the provision of 20% dietary CP was beneficial to the expression of nutrient-sensing and nutrient transporter genes in the GIT, and the provision of 17% dietary CP was beneficial to lipid metabolism and digestive enzymes in the GIT.

INTRODUCTION

Young mammals (including infant and weaned pigs) have often been reported to exhibit gastrointestinal tract (GIT) dysfunction, including alterations intestinal morphology, the immune system, and absorption functions, when fed a high-protein diet [1–5]. A reduction in dietary crude protein (CP) levels can reduce diarrhea and nitrogen (N) excretion and repair digestive and immune function by enhancing the gene expression levels of digestive enzymes and amino acid (AA) transporters in young pigs [4, 6]. For weaned pigs, it has been suggested that the CP levels in their diets can be reduced by 2–3 points without affecting average daily gain (ADG) [4, 6], feed efficiency, or gastrointestinal health when the diets are supplemented with AAs [7, 8]. However, reductions exceeding 3 points have produced no effects on ADG, feed efficiency and the mRNA levels of digestive enzymes (including trypsinogen, chymotrypsin B, and dipeptidase-II and III) and AA transporters [2, 4, 9–13], although these trends have not been observed in all studies [14–16]. These findings suggest that the reduction of dietary CP levels and supplementation with AAs may be an alternative method for decreasing N excretion and improving GIT digestive function while maintaining performance in young pigs. The level of dietary CP is a major issue in livestock production that impacts the economy and the environment. Therefore, the present work was principally focused on the impact of dietary CP levels on the regulation of the digestion, absorption, and metabolism of nutrients (for example, AAs, glucose, and lipids) in the GIT, along with feed intake.

The digestion and absorption of dietary CP involves complex mechanical disruption and enzymatic breakdown in the stomach, followed by further enzymatic breakdown by pancreatic proteases in the duodenum and jejunum, resulting in a substance from which hydrolysated oligopeptides and free AAs can be readily absorbed and, finally, the conversion of nutrients to animal tissue to maintain the early stage of growth after weaning [17, 18]. To efficiently digest and absorb nutrients from ingested food, these events are regulated by nutrient-sensing receptors and the secretion of the GIT hormones gastrin and cholecystokinin (CCK) following the secretion of digestive enzymes and the expression of AA transporters [4, 9–11, 19–21]. The identification of these receptors or their signaling elements in the GIT mucosa indicates that these receptors may play roles in nutrient-sensing and, potentially, hormone secretion [22]. Nutrient-sensing receptors such as members of the taste 1 receptor family (for example, TasR1, TasR2, and TasR3), gene and protein expression of the free fatty acid receptor family (for example, GPR40, GPR41, and GPR43, also known as FFAR1, FFAR2, and FFAR3, respectively), G protein-coupled receptor class C group 6 member A (GPRC6A), and calcium-sensing receptor (CaSR), hormones such as somatostatin (SST), ghrelin, and gastrin, and the insulin receptor (INR), leptin receptor (leptin-R), cholecystokinin receptor family (for example, CCK-1R, CCK-2R), and glucagon-like peptide receptor family (for example, GLP-1R, GLP-2R) have been identified [22]. Proteolytic enzymes are mainly produced by the stomach (pepsin), pancreas (trypsin, chymotrypsin, and elastase), and intestine (membranous and cytosolic enzymes) [4]. Several studies have shown that G protein-coupled receptor (GPCR)-related mechanisms involved in the direct nutrient-sensing of proteins, lipids, and carbohydrates result in hormone secretion, while little attention has been paid to the nutrient sensing involved in the direct activation of hormone secretion in response to dietary protein restriction [23, 24].

To gain insight into the variations underlying the adaptive response to a protein-restricted diet in weaned pigs, we devised an experiment to evaluate the effects of dietary protein restriction on growth performance, ileal AA digestibility and the variation in serum free AAs as well as the expression levels of nutrient-sensing, hormone and receptor genes and genes encoding gastrointestinal digestive enzymes and nutrient transporters after feeding weaned pigs protein-restricted diets with three different levels of CP.

RESULTS

Effect on growth performance

As shown in Table 1, the weaned pigs fed the 14% CP diet had a final BW ($P < 0.05$), ADG ($P < 0.05$) and F/G ($P < 0.05$) that were different from those observed in the pigs fed the 17% CP and 20% CP diets, while ADFI was significantly different between the 14% CP and 20% CP groups ($P < 0.05$).

Effect on the serum concentration of free AAs and biochemical parameters

As shown in Table 2, fed the weaned pigs the 20% CP diet had a significantly different serum free AA concentrations of Arg ($P < 0.05$), His ($P < 0.05$), Leu ($P < 0.01$), Val ($P < 0.01$), and Tyr ($P < 0.05$) than the pigs fed the 14% CP diet, while Ser ($P < 0.05$) was significantly different between the 14% CP and 17% CP groups. There was no significant difference ($P > 0.05$) in the serum free AA concentrations of other AAs between the three groups of weaned pigs (Table 2).

As shown in Table 2, the weaned pigs fed the 20% CP diet had significantly different serum biochemical parameters of UN ($P < 0.05$) than the 17% CP diet group, while cholesterol ($P < 0.05$) and triglycerides ($P < 0.05$) were significantly different among the 14% CP and 20%

Table 1: Protein-restricted diets affect growth performance in young weaned pigs

Items	14% CP	17% CP	20% CP	*P* value	SEM ±
Young weaning pigs[1]					
Initial BW[2], kg	9.54	9.56	9.48	0.972	0.142
Final BW, kg	26.21[b]	29.64[a]	32.23[a]	0.002	0.799
ADG[3], g/d	369.50[b]	446.60[a]	505.4[a]	0.003	18.494
ADFI[4], g/d	691.50[b]	766.80[ab]	837.60[a]	0.089	27.570
F:G[5]	1.86[a]	1.71[b]	1.65[b]	0.003	0.029

Data are means with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. [a,b]Mean values with different letters were considered to be significantly different ($P < 0.05$) from applying a one-way ANOVA followed by Duncan's multiple comparison test. [1] Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) (see Table 4) to provide the same total concentrations as in the 20% CP diet. [2] BW: body weight; [3] ADG: average daily gain; [4] ADFI: average daily feed intake; [5] F:G: the ratio of feed and gain. CP = crude protein.

Table 2: Protein-restricted diets affect serum concentrations of free amino acids (AAs) and serum biochemical parameters in young weaned pigs

Items	14% CP	17% CP	20% CP	*P* value	SEM ±
Young weaning pigs[1]					
Serum free AAs concentration					
Arg	44.58[b]	53.01[ab]	58.16[a]	0.036	3.958
His	25.10[b]	28.23[ab]	34.17[a]	0.046	2.66
Ile	23.27	21.44	26.36	0.313	1.436
Leu	32.54[b]	40.25[a]	44.03[a]	0.003	3.381
Lys	67.35	74.73	73.92	0.076	2.337
Met	28.15	33.26	36.24	0.204	2.362
Phe	20.38	25.13	27.36	0.105	2.058
Thr	24.14	31.23	30.26	0.069	2.219
Trp	29.73	28.36	32.63	0.386	1.259
Val	29.18[b]	37.27[ab]	42.37[a]	0.004	3.84
Gly	130.08	142.92	148.43	0.351	5.436
Ser	28.32[b]	34.37[a]	31.28[ab]	0.033	1.747
Glu	50.27	54.46	58.73	0.203	2.442
Tyr	20.23[b]	29.16[a]	28.83[a]	0.012	2.923
Asn	25.26	24.12	31.53	0.202	2.304
Asp	6.72	7.92	7.32	0.369	0.346
Gln	213.37	242.53	248.23	0.349	10.796
Ala	123.67	136.25	153.24	0.242	8.568
Serum biochemical parameters					
Total Protein (g/L)	61.2	63.6	59.8	0.341	1.110
Urea Nitrogen (mmol/L)	2.66[b]	3.02[b]	4.13[a]	0.003	0.442
Glucose (mmol/L)	5.29	5.64	6.06	0.301	0.223
Cholesterol (mmol/L)	2.60[a]	2.31[ab]	1.98[b]	0.037	0.179
Triglyceride (mmol/L)	0.84[a]	0.58[ab]	0.49[b]	0.015	0.105

Data are means with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. [a,b]Mean values with different letters were considered to be significantly different ($P < 0.05$) from applying a one-way ANOVA followed by Duncan's multiple comparison test. [1]Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) (see Table 4) to provide the same total concentrations as in the 20% CP diet.

Table 3: Protein-restricted diets affect the ileal digestibility of DE, DM, and IDAA in young weaned pigs

Items	14% CP	17% CP	20% CP	*P* value	SEM ±
Ileal digestibilities of DE, DM, and IDAA[1]					
Energy	86.58	84.12	83.64	0.194	0.901
DM[2]	80.30	82.20	81.30	0.194	0.910
CP [3]	82.3[a]	81.40[ab]	79.90[b]	0.009	0.700
Arg	88.52[a]	86.83[a]	80.64[b]	0.006	2.400
His	83.39[a]	78.23[b]	78.32[b]	0.021	1.710
Ile	84.78[a]	82.26[ab]	78.21[b]	0.011	1.910
Leu	83.89	80.37	80.89	0.285	2.090
Lys	87.91[a]	83.46[a]	77.54[b]	<0.001	3.600
Met	87.73[a]	83.66[ab]	80.10[b]	0.043	1.640
Cys	84.43	83.98	80.26	0.136	1.320
Phe	83.91[a]	81.97[ab]	77.95[b]	0.029	2.890
Tyr	87.54	85.85	84.86	0.402	2.240
Thr	80.57[a]	77.27[ab]	74.64[b]	0.031	3.620
Trp	80.46[a]	77.12[ab]	73.63[b]	0.007	1.970
Val	79.36[a]	72.58[b]	72.28[b]	0.007	2.310
Ala	82.87	80.14	80.23	0.261	0.900
Asp	80.96[a]	78.52[ab]	74.22[b]	0.02	2.820
Glu	87.67[a]	86.68[a]	80.88[b]	0.005	2.120
Gly	77.52	76.54	74.43	0.518	0.910
Pro	76.30[a]	72.47[ab]	70.93[b]	0.038	1.600
Ser	82.10[a]	79.89[ab]	75.72[b]	0.012	3.640

Data are means with the pooled means ± standard error of the mean (SEM), n = 8/treatment group. [a,b]Mean values with different letters were considered to be significantly different ($P < 0.05$) from applying a one-way ANOVA followed by Duncan's multiple comparison test. [1]Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) (see Table 4) to provide the same total concentrations as in the 20% CP diet. [2]DM = dry matter; [3]CP = crude protein; IDAA = ileal digesta of amino acids.

CP diet groups. However, there was no difference in the serum parameters of total protein ($P > 0.05$) and glucose ($P > 0.05$) between the weaned pigs fed the 20% CP diet and those in the 14% CP and 17% CP diet groups.

Effect on the ileal digestibility of AAs

As shown in Table 3, the low-CP diet had no effect ($P > 0.05$) on DE and DM. Weaned pigs fed the 14% CP diet showed higher digestibility of CP ($P < 0.01$), Arg ($P < 0.01$), His ($P < 0.05$), Ile ($P < 0.05$), Lys ($P < 0.001$), Met ($P < 0.05$), Phe ($P < 0.05$), Thr ($P < 0.05$), Trp ($P < 0.01$), Val ($P < 0.01$), Asp ($P < 0.05$), Glu ($P < 0.01$), Pro ($P < 0.05$), and Ser ($P < 0.05$) than pigs fed the 20% CP diet (Table 3). Lys digestibility increased ($P < 0.001$) from 77.54% in weaned pigs fed the 20% CP diet to 87.91% in weaned pigs fed the 14% CP diet. There was no significant difference ($P > 0.05$) in the digestibility of other AAs between the three groups of weaned pigs (Table 3).

Effects on the gene expression levels of nutrient-sensing and hormone secretion genes, gastrointestinal digestive enzymes, and nutrient transporters

As shown in Figure 1, weaned pigs fed the 20% CP diet showed the highest mRNA levels of taste receptor type 1 member 1 (TasR1); however, TasR1 levels were different between the 17% and 20% CP diet groups ($P < 0.05$) but not the 14% CP diet group ($P > 0.05$). There was no significant difference ($P > 0.05$) in the mRNA levels of taste 1 receptor member 2 (TasR2), taste 1 receptor member 3 (TasR3), and G protein-coupled receptor, class C, group 6, member A (GPRC6A) among the three groups of weaned pigs (Figure 1A). The weaned pigs fed the 20% CP diet showed the highest mRNA levels of calcium-sensing receptor, transcript variant 1 (CaSR); however, there was no significant difference ($P > 0.05$) between the 14% CP and the 17% CP groups. There were no significant differences ($P > 0.05$) in the mRNA levels of

long-chain fatty acid receptor G-protein-coupled receptor 40 (GPR40), short-chain fatty acid receptor G-protein-coupled receptor 41 (GPR41), and short-chain fatty acid receptor G-protein-coupled receptor 43 (GPR43) among the three groups of weaned pigs (Figure 1B).

As shown in Figure 2A and Figure 2B, there were no significant differences ($P > 0.05$) in the mRNA levels of somatostatin (SST), ghrelin, glucagon-like peptide 1 receptor (GLP-1R), glucagon-like peptide 2 receptor (GLP-2R), insulin receptor (INR), leptin receptor (Leptin-R), gastrin, or cholecystokinin B receptor (CCK-2R) between the three groups of weaned pigs. As shown in Figure 2B, the weaned pigs fed the 17% CP diet showed higher mRNA levels of the cholecystokinin type A receptor (CCK-1R) than the pigs fed the 14% CP diet ($P = 0.031$); however, there was no significant difference ($P > 0.05$) between the 17 and 20% CP diet groups.

As shown in Figure 3, weaned pigs fed the 17% CP diet displayed higher mRNA levels of pepsinogen than pigs fed the 14% CP diet ($P = 0.021$). However,

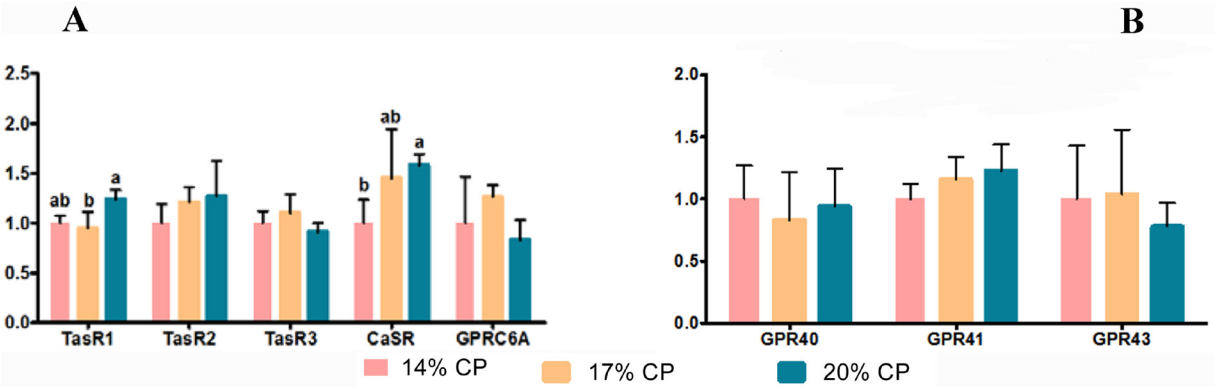

Figure 1: mRNA abundance of nutrient-sensing genes in the jejunum of young weaned pigs fed the protein-restricted diets. Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) (see Table 4) to provide the same total concentrations as in the 20% CP diet. CP = crude protein; TasR1= taste receptor type 1 member 1-like; TasR2 = taste 1 receptor member 2; TasR3, taste 1 receptor member 3; CaSR = calcium-sensing receptor, transcript variant 1; GPRC6A = G protein-coupled receptor, class C, group 6, member A; GPR40 = long-chain fatty acid receptor G-protein-coupled receptor 40 (also known as free fatty acid receptor 1, FFAR1); GPR41 = short-chain fatty acid receptor G-protein-coupled receptor 41 (also known as free fatty acid receptor 3, FFAR3); GPR43 = short-chain fatty acid receptor G-protein-coupled receptor 43 (also known as free fatty acid receptor 2, FFAR2). The data are means, with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. [ab] Mean values with different letters were considered to be significantly different ($P < 0.05$) according to a one-way ANOVA followed by Duncan's multiple comparison test. The mRNA abundance of TasR1, TasR2, TasR3, CaSR, and GPRC6A genes were shown in (**A**) and the mRNA abundance of GPR40, GPR41, and GPR43 genes were shown in (**B**).

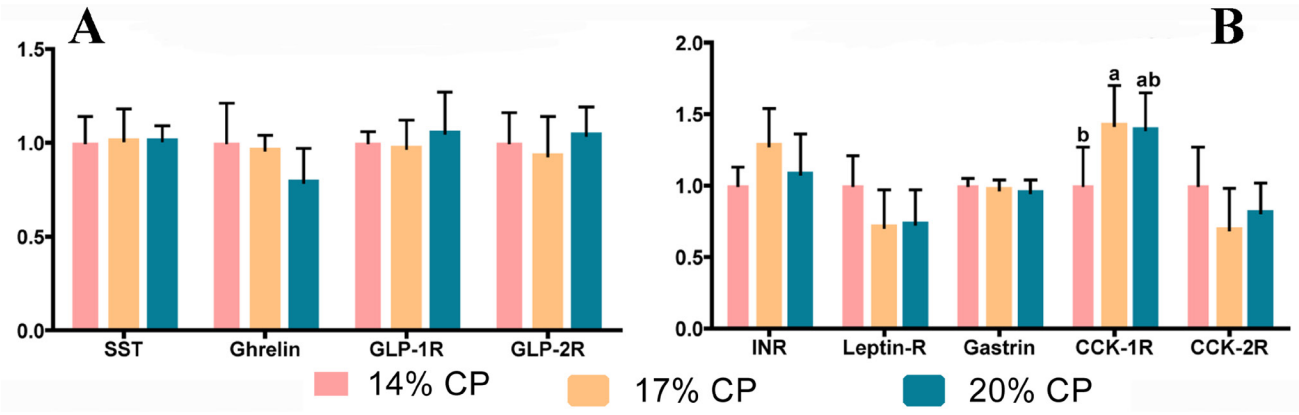

Figure 2: mRNA abundance of hormone secretion genes in the jejunum of young weaned pigs fed protein-restricted diets. Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) (see Table 4) to provide the same total concentrations as in the 20% CP diet. CP = crude protein; SST = somatostatin; GLP-1R = glucagon-like peptide 1 receptor; GLP-2R = glucagon-like peptide 2 receptor; INR = insulin receptor; Leptin-R = leptin receptor; CCK-1R = cholecystokinin type A receptor; CCK-2R = cholecystokinin B receptor. Data are means, with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. [ab]Mean values with different letters were considered to be significantly different ($P < 0.05$) according to a one-way ANOVA followed by Duncan's multiple comparison test. The mRNA abundance of SST, Ghrelin, GLP-1R, and GLP-2R genes were shown in (**A**), and the mRNA abundance of INR, Leptin-R, Gastrin, CCK-1R, and CCK-2R genes were shown in (**B**).

there was no significant difference ($P > 0.05$) between the 17% and 20% CP diet groups. There was no significant difference ($P > 0.05$) in the mRNA levels of trypsinogen, chymotrypsin, carboxypeptidase A (CPA), carboxypeptidase B (CPB), amino peptidase A (APA), amino peptidase B (APB), pancreatic lipase (P-lipase), sucrose, maltase, and α-amylase between the three groups of weaned pigs (Figure 3).

As shown in Figure 4A, the weaned pigs fed the 20% CP diet showed higher mRNA levels of solute carrier family 1 (EAAC1) than pigs fed the 14% CP and 17% CP diets ($P = 0.038$); however, there was no significant difference ($P > 0.05$) between the 14% and 17% CP diet groups. Weaned pigs fed the 14% CP diet showed a higher mRNA levels of Na^+-dependent neutral amino acid exchanger 2 (ASCT2) than pigs fed the 20% CP diet ($P = 0.041$), but there was no significant difference ($P > 0.05$) between the 17% CP and 20% CP diet groups (Figure 4A). The dipeptide transporter1 (PepT1) levels between the weaned pigs fed the 17% CP diet and the pigs fed the 14% CP diet showed a significant difference ($P = 0.027$), but there was no significant difference ($P > 0.05$) between the 17% CP and 20% CP diet groups (Figure 4C). There were no significant differences ($P > 0.05$) in the

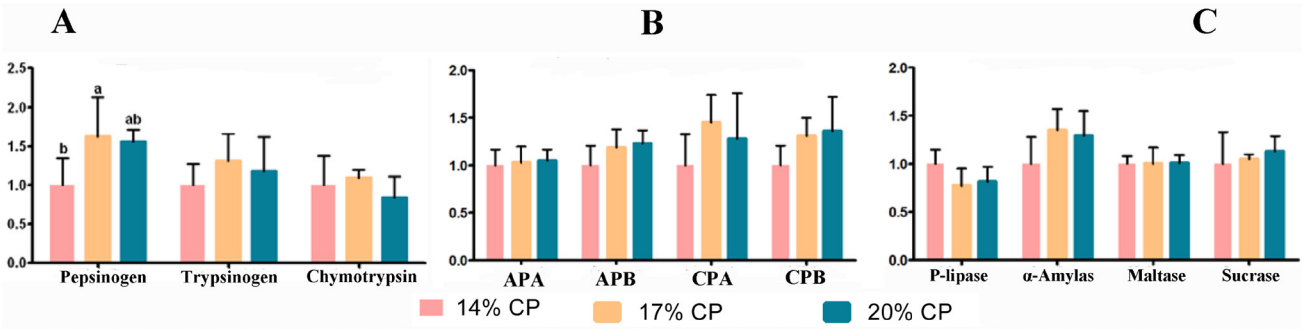

Figure 3: mRNA abundance of gastrointestinal digestive enzyme genes in the duodenum, jejunum, pancreas, and stomach of young weaned pigs fed the protein-restricted diets. Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) to provide the same total concentrations as in the 20% CP diet. CP = crude protein; APA = amino peptidase A; APB = amino peptidase B; CPA = carboxypeptidase A; CPB = carboxypeptidase B; P-lipase = pancreatic lipase. Data are means, with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. ^{ab}Mean values with different letters were considered to be significantly different ($P < 0.05$) according to a one-way ANOVA followed by Duncan's multiple comparison test. The mRNA abundance of Pepsinnogen, Trypsinnogen, and Chymotrypsin genes were shown in (**A**), the mRNA abundance of APA, APB, CPA, and CPB genes were shown in (**B**), and the mRNA abundance of P-lipase, α-Amylase, Maltase, and Sucrase genes were shown in (**C**).

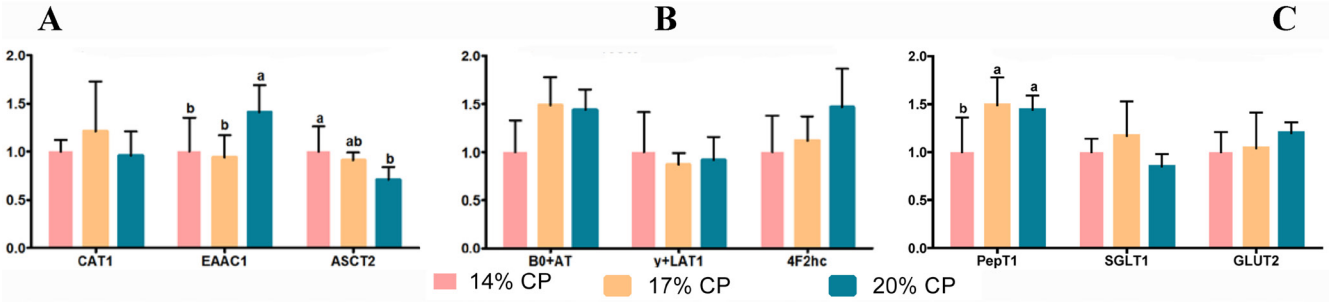

Figure 4: mRNA abundance of nutrient transporter genes in the jejunum of young weaned pigs fed the protein-restricted diets. Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) to provide the same total concentrations as in the 20% CP diet. CP = crude protein; CAT1 = cationic amino acid transporter 1; EAAC1 = solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 (SLC1A1); ASCT2 = Na^+-dependent neutral amino acid exchanger 2; $B^{0+}AT = b^{0,+}$amino acid transporter; $y^+LAT1 = y^+$L-type amino acid transporter 1; 4F2hc = solute carrier family 7 member 9 (SLCA9); PepT1 = dipeptide transporter 1; SGLT1 = sodium-glucose transporter 1; GLUT2 = glucose transporter 2. Data are the means with the pooled means ± standard error of the mean (SEM), $n = 8$/treatment group. ^{ab}Mean values with different letters were considered to be significantly different ($P < 0.05$) according to a one-way ANOVA followed by Duncan's multiple comparison test. The mRNA abundance of CAT1, EAAC1, and ASCT2 genes were shown in (**A**), the mRNA abundance of B0+AT, y+LAT1, and 4F2hc genes were shown in (**B**), and the mRNA abundance of PepT1, SGLT1, and GLUT2 genes were shown in (**C**).

mRNA levels of cationic amino acid transporter1 (CAT1), $b^{0,+}$amino acid transporter ($B^{0+}AT$), y^+L-type amino acid transporter1 (y^+LAT1), solute carrier family 7 member 9 (4F2hc), sodium-glucose transporter 1 (SGLT1), and glucose transporter 2 (GLUT2) among the three groups of weaned pigs (Figure 4A, 4B, and 4C).

DISCUSSION

Several reports have shown that a diet containing low CP supplemented with AAs may reduce diarrhea in weaned pigs, boost feed economy, repair digestive function by increasing the digestion and absorption of nutrients (such as AAs), and reduce environmental pollution [4, 7, 12–15]. Previous studies have shown that a CP reduction of 2–3 points or more than 3 points can be implemented without affecting ADG or feed efficiency when diets are supplemented with AA [4, 12, 15, 31, 32]; however, these trends were not always observed in other studies [14–16]. In the present study, the results indicated that a reduction of dietary CP by 3 points with a concomitant addition of essential AAs supported similar growth performance and feed efficiency. However, a substantial reduction in dietary CP by 6 points had a negative impact on the growth performance of weaned pigs (Table 1). These results were consistent with previous results obtained by other researchers [13, 32, 33].

AAs play important roles as metabolic intermediates in nutrition and immune function and boost growth performance [9, 10]. Many studies have shown that a protein-restricted diet can regulate muscle and serum free AA concentrations in pigs [34–36]. In the present study, weaned pigs fed the 20% CP diet exhibited a significant difference in the serum free AA concentrations of Arg ($P < 0.05$), His ($P < 0.05$), Leu ($P < 0.01$), Val ($P < 0.01$), and Tyr ($P < 0.05$) compared with the concentrations in those fed the 14% CP diet, while Ser ($P < 0.05$) was significantly different between the 14% CP and 17% CP groups (Table 2). The observed concentrations of His, Leu, and Val were not consistent with previous studies in starter pigs fed 19.2% CP and 28.1% CP diets [34]. A possible explanation for the discrepancies between this previous study and the present study could be that the variations in serum free AA concentrations depend on the investigated pig species, diet formulations and stages.

The variation in serum biochemical parameters depends on the external environment, dietary nutrition levels and endocrine status. Constantly altering and maintaining a dynamic balance in a relatively narrow range of characteristic concentrations is a sensitive indicator used for the assessment of nutritional and physiological status as well as the analysis of nutrient requirements [37]. Serum UN is commonly employed as an indicator of protein utilization efficiency, which reflects the status of the balance of AAs [35, 38]. When there is redundant CP, serum UN levels increase, as observed in the present work (Table 2); this excess CP cannot be stored in the body and is consequently degraded, with the production of urea, and excreted from the body [35, 39]. When weaned pigs were fed the protein-restricted diets (17% CP or 14% CP diets in our study), they exhibited a lower serum UN concentration ($P < 0.05$, Table 2), and these results were consistent with the results of previous studies [4, 7, 32, 35, 39]. These findings suggest that pigs in different stages exhibit either an increased N efficiency or decreased protein breakdown, which ultimately reduces N excretion and protects the surrounding environment [4, 6, 35]. Variations in serum cholesterol and triglyceride contents are a sensitive indicator of lipid metabolism and nutrition status in pigs and humans [35]. In the present study, the weaned pigs fed the 20% CP diet and those fed the14% CP diet exhibited a significant difference in the serum biochemical parameters of cholesterol ($P < 0.05$) and the level of triglycerides ($P < 0.05$) (Table 2). These results are consistent with a previous hypothesis proposed by other researchers that an excess low-protein diet or AA imbalance may cause high cholesterol and triglyceride concentrations in serum as well as increased fat content in the entire body [35]. This could be supported by the increasing trend in serum cholesterol and triglyceride levels observed in our study in pigs receiving the protein-restricted diets similar results have been reported elsewhere [35, 40-44]. Subsequent experiments also confirmed that an excessively CP-restricted diet and serious AA imbalance lead to a decrease in protein synthesis, with the remaining energy being transferred to fat deposition in broiler chickens and pigs [35, 45, 46].

A reduction of dietary CP by 6 points led to the highest ileal digestibility of CP and AAs in the present work (Table 3). These results were also consistent with previous findings [4, 47]. It is possible that the different responses to the dietary CP levels fed to the weaned pigs in terms of the ileal digestibility of CP and AAs is due to relative increases in the rate of protein digestion and the absorption of the resulting products in the GIT. However, when dietary CP was reduced by 6 points and the diet was supplemented with essential AAs, the growth performance and feed efficiency of the weaned pigs could not be maintained, even when the ileal digestibility of AAs was improved. Therefore, our findings support the possibility that the nutritional requirements of pigs can be met when dietary CP is reduced by 3 points if the diet is supplemented with adequate amounts of essential AAs.

The umami taste receptor, composed of TasR1 + TasR3, can recognize and respond to a diverse repertoire of chemical entities, including sugars, AAs, and other nutrients [48]. The released of gut hormones involved in the control of food intake is dependent on the acute

nutritional status of the body, suggesting that nutrient sensing mechanisms are involved in the control of their release [49]. The nutrient sensing of CaSR is expressed in a number of tissues and is involved in calcium homeostasis and the regulation of a variety of cellular processes, including proliferation, differentiation, expression, ion channel function and hormone secretion, as well as fluid absorption [23]. CCK can stimulate gallbladder contraction and pancreatic growth [50], and L-phenylalanine L-tryptophan can stimulate the release of CCK from isolated CCK cells [51]. The diets AAs with higher affinity for CaSR are the aromatic AAs L-tryptophan, L-phenylalanine, L-tyrosine and L-histidine, where basic and branch-chain AAs are the least effective [52]. Administration of L-phenylalanine elevated plasma levels of CCK and reduced food intake in human [53]. AAs also affected the release of INR via the CaR in isolated rat intestinal loop [54]. A previous study also showed dietary CP levels also affect GI hormones sensing and regulation of a variety of nutrient sensing gene expression in rat [55]. There was a significant difference between the 17% and 20% CP diet groups ($P < 0.05$) but not the 14% CP diet group ($P > 0.05$) in the mRNA levels of TasR1 (Figure 2) and a significant difference in the CaSR level (Figure 2) in the jejunum between the 14% and 20% CP diet groups ($P < 0.05$). However, the weaned pigs fed the 17% CP diet showed higher mRNA levels of CCK-1R than pigs fed the 14% CP diet ($P = 0.031$) (Figure 2). Studies have linked TasR1, CaSR, and CCK, demonstrating that knockdown of TasR1 in STC-1 cells (an immortalized intestinal enteroendocrine cell line) attenuates the stimulatory effects of L-phenylalanine, L-leucine and L-glutamate on CCK release [56] and that some AAs stimulate CCK release through the CaSR receptor [51]. Therefore, our results showed that the expression of nutrient-sensing genes in the jejunum can be regulated in response to a protein-restricted diet.

Several reports on pigs have shown that the levels of the main digestive enzymes are dependent on different dietary formulations and determined how effectively a given dietary formulation may enhance body growth [57, 58]. The digestion of dietary proteins and other nutrients in pigs is accomplished by many different parts of the GIT and results in a hydrolysated protein mixture of oligopeptides and free AAs as well as glucose [4, 23]. A previous study showed that low-protein diets can increase the digestibility of energy and AA [4, 59]. In the present study, weaned pigs fed the 17% CP diet displayed higher mRNA levels of pepsinogen than pigs fed the 14% CP diet ($P = 0.021$). There was no significant difference ($P > 0.05$) between the 17% and 20% CP diet groups (Figure 3). This result is consistent with previous results showing that a protein-restricted diet can affect the expression of chymotrypsin, aminopeptidase, amylase, and carboxypeptidase [50, 60]; however, it is not consistent with previous results indicating that a protein-restricted diet cannot affect the expression of pepsinogen and trypsinogen in pigs [4, 50, 60]. Therefore, in the present study, the 17% CP-restricted diet can increase the expression of genes encoding pepsinogen in the stomach but induced a trend toward higher mRNA levels of trypsinogen, chymotrypsin, CPA, and α-amylase than those in other groups, although there were no significant differences. A possible explanation for the different responses of digestive enzymes to the protein-restricted diet is the complex regulation at both the transcriptional and translational levels by a variety of factors, including the composition and balance of dietary AA and GIT health status. Future studies are needed to determine the protein abundances of digestive enzymes in pigs fed a protein-restricted diet or an adequate CP diet.

The transport of AAs in the intestines is critical for the supply of AAs to all tissues and the homeostasis of plasma AA levels, and the intracellular presence of available AAs is regulated by many different kinds of AA transporters [6, 9, 10, 61]. In different cell types, all types of AA transporters are ubiquitously localized in membranes. These transporters sense AA availability, relay nutrient signals (including AAs) to the cell interior, regulate the uptake and efflux of AAs and trigger a series of cascade responses, which ultimately provides dual transporter and receptor functions in response to nutrient stimulation. In the present study, pigs fed the 20% CP diet showed higher mRNA levels of EAAC1 than pigs fed the 14% CP and 17% CP diets; however, there was no significant difference ($P > 0.05$) between the 14% and 17% CP diet groups (Figure 4A). Likewise, pigs fed the 14% CP diet showed higher mRNA levels of ASCT2 than pigs fed the 20% CP diet ($P = 0.041$) (Figure 4A), but there was no significant difference ($P > 0.05$) between the 17% CP and 20% CP diet groups (Figure 4A). Notably, there was no significant difference in the mRNA levels of PepT1 ($P > 0.05$) between the 17% CP and 20% CP diet groups (Figure 4C). A previous report showed that reducing the dietary CP content increased the levels of AA transporters (EAAC1 and ASCT2) in weaned pigs [6] and demonstrated that the supplementation of a reduced-CP diet with branched-chain AAs increased the levels of the AA transporter PepT1 in growing pigs fed a 17.1% CP diet compared with those in pigs fed a 20.9% CP diet [62]. A possible explanation for the discrepancies between this previous study and the present study could be that the mRNA levels of transporters depend on the investigated pig species, diet formulations and stages.

CONCLUSIONS

In conclusion, our findings confirmed that weaned pigs chronically fed a moderately protein-restricted diet (17% CP) could catch up in terms of growth performance and feed efficiency. The provision of 20% dietary CP was beneficial to nutrient-sensing and nutrient transporter gene expression in the GIT, and the provision of 17% dietary CP was beneficial to lipid metabolism and the digestion of nutrient substances in the GIT. These findings may provide new insight for the application of nutritional strategies in the pig industry or even for human health. Further research is needed to confirm how a protein-restricted dietary regulates the complicated relationships from nutrient sensing, digestion, and absorption in pig nutrition.

MATERIALS AND METHODS

Ethics statement

All procedures involving animal subjects were approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture at the Chinese Academy of Sciences (Changsha, Hunan Province, China, No.: 20160711).

Experimental design and procedure

Twenty-four cross-bred pigs [Yorkshire × (Duroc × Landrace)] (Hunan Zhenghong Co., Ltd., Hunan Province, China) (initial body weight (BW), 9.57 ± 0.64 kg) were assigned randomly to one of three diet treatments (8 pigs/group), corresponding to a 14%, 17%, or 20% dietary intake of CP. All of the pigs were housed individually in metabolic cages and had free access to feedstuff and drinking water. The temperature in the metabolism room was maintained at approximately 24° C with constant lighting. Titanium dioxide (TiQ_2) (1 g/kg diet) was added to all of the experimental diets and served as a marker of indigestion to calculate total tract N digestibility [25]. The experimental design and procedure are shown in Figure 5. The dietary treatments of 14% CP and 17% CP diets were supplemented with L-Lys, L-Met, L-Thr, and L-Trp to meet the National Research Council (NRC) nutrient requirements for weaned pigs [26]. There was a 3-day acclimatization period prior to the commencement of each experiment. The experiments lasted 45 days. The ingredients and nutrient compositions of the experimental diets are shown in Table 4. Parameters related to BW and feed consumption, such as the ADG, average daily feed intake (ADFI), and ratio of feed to gain (F: G), were recorded at the beginning and end of the experimental period.

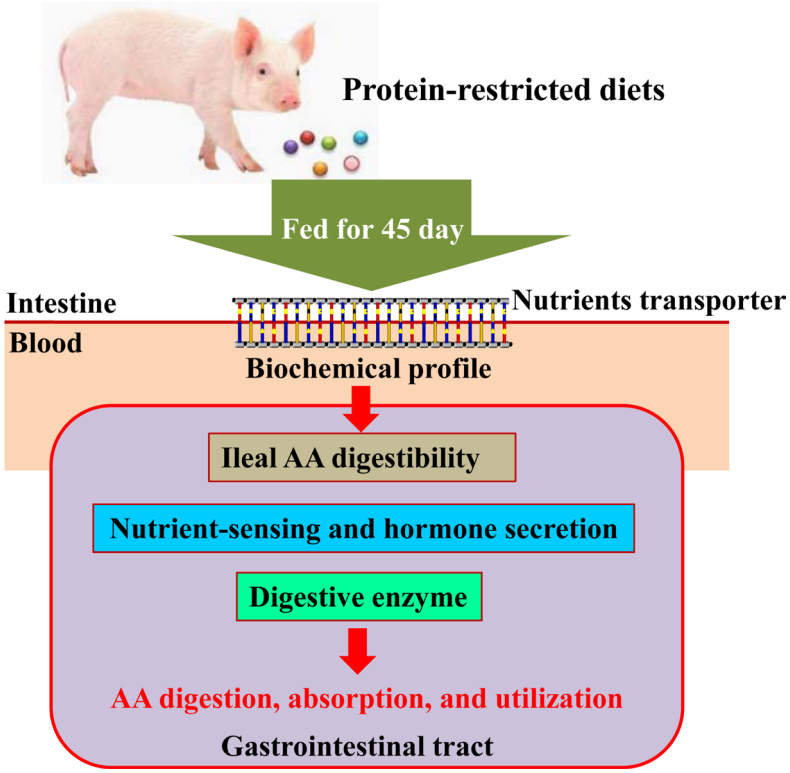

Figure 5: Experimental design. The experiments involved 24 [Yorkshire × (Duroc × Landrace)] young weaned pigs. Both the 17% CP diet and the 14% CP diet were supplemented with 4 essential amino acids (L-lysine, L-methionine, L-threonine, and L-tryptophan) to provide the same total concentrations as in the 20% CP diet. CP = crude protein.

Table 4: Feedstuff ingredients and nutrient composition in experimental diets for young weaned pigs (%).

Feed ingredient	CP levels[a]		
	14% CP	17% CP	20% CP
Corn (43% CP)	71.80	66.50	63.70
Soybean meal	13.40	18.80	19.80
Whey powder	4.40	4.30	4.30
Fish meal (64% CP)	1.50	4.00	9.00
Soybean oil	4.10	2.60	0.80
Lysine hydrochloride	0.88	0.62	0.38
Hydroxy methionine	0.27	0.19	0.10
L-threonine	0.33	0.21	0.09
L-tryptophan	0.08	0.04	0.01
$CaHPO_3$	1.15	0.74	0.00
Rock-powder	0.79	0.70	0.52
Salt	0.30	0.30	0.30
1% Premix[b]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated and analyzed nutrient composition[c]			
DE (MJ/kg)	14.60	14.60	14.60
CP	14.14	17.32	20.27
Total Ca	0.70	0.71	0.69
Total P	0.53	0.55	0.57
Starch	45.16	41.95	40.22
NDF	8.40	8.66	8.54
ADF	3.05	3.30	3.29
Lys	1.26	1.25	1.26
Met + Cys	0.63	0.65	0.62
Thr	0.76	0.75	0.76
Trp	0.20	0.20	0.20
Arg	0.71	0.93	1.09
His	0.30	0.37	0.44
Ile	0.46	0.60	0.71
Leu	1.11	1.32	1.52
Phe	0.56	0.70	0.81
Val	0.54	0.64	0.72

[a]Diet treatment: Crude protein (CP) levels contain 14 %, 17 % and 20 %, respectively and supplementation with appropriate crystalline AA. [b] Premix provided these amounts of vitamins and minerals per kilogram on an as-fed basis: vitamin A, 10,800 IU; vitamin D_3, 4,000 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 sulphate; 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. The values are expressed as percentage (%), except for digestible energy (DE; MJ/kg), essential amino acid (EAA)/nonessential amino acid (NEAA). [c]The DE was calculated according to NRC (2012). [c] All other values represent analyzed values.

Table 5: Primers used for relative quantitative PCR analysis

Gene	5′-Primer (F)	3′-Primer (R)	Length (bp)	Accession No.
Nutrient-sensing gene				
TasR1[1]	TCCCTGGGCTTCATACTGG	TTCTCTGGCAAGTCCTTACCC	92	XM_003356140.1
TasR2[2]	TGTATCACGGTGCGCTCTTT	TGGTGTTGATCAGCAGTCCC	259	NM_001267894.1
TasR3[3]	GTAGGGTAGAGGCCCACTCA	ACTTTCAGAGGTTGGGGTGC	335	NM_001113288.1
GPRC6A[4]	CTTGAGAAAATCATAGCAGAAGCC	GGAATGGTAGTTATCTTGGTGGC	161	XM_003480266.1
CaSR[5]	GGGACCAGGAAAGGAATCAT	CACGGCAAAGAGGGTGAGT	219	XM_003132642.1
GPR40/FFAR1[6]	TGCTCTGACCTCCTGCTGG	CACACACCCCCCAGGAATAG	89	XM_003127043.1
GPR41/FFAR3[7]	GCTGCTGTTCCTGCCTTTC	TGAAGAAGATGAATCCAGAGAGTG	98	NM_005304.3
GPR43/FFAR2[8]	CCCATCCACATCCTCCTGC	GCTGCTGTAGAAGCCGAAAC	150	XM_003127046.1
Hormone secretion gene				
SST[9]	GCTGGGAAGCAGGTAAGGAG	GAACTGTGACCTACTGCGCT	238	AY596204.1
Ghrelin	GAAGGCACGGAGGACAAGC	GCTGGTCTCAGGGACAATCAC	398	NM_213807.1
Gastrin	AAAGAGCCACATGAGCTGGAT	GGTTCTAGGGACGCTGGTCT	120	NM_001004036
INR[10]	GGCATGGTGTACGAGGGAAA	AGGCCTCGTTGAGAAACTCG	124	XM_005654749.2
Leptin-R[11]	CAGTGACATTTGGCCCTCTT	AGGCCTGGGTTTCTATCTCC	396	NM_001024587.1
CCK-1R[12]	GTGGTCCACAGCCTTCTTAT	TCATTTTCGATCCCCAGTT	68	DQ496228
CCK-2R[13]	GCGGCGATCTTTCTGATGAG	GCAGGAAGGCGTTGGTGA	97	AY322551
GLP-1R[14]	TACTTCTGGCTGCTGGTGGAG	ACCCCAGCCTATGCTCAGGTA	105	NM_001256594.1
GLP-2R[15]	TGTCCTACGTGTCGGAGATGTC	TAATTGGCGCCCACGAA	76	NM_001246266.1
Gastrointestinal digestive enzyme gene				
Pepsinogen	CATGGACGGAGAGACCATCG	GCCCTGTCAAAGACGGTGTA	354	NM_213873.2
Trypsinogen	GTCTGCTGCTCACTGCTACA	GCCAGAGATGAGACACTCGG	253	LOC100302368
Chymotrypsin	GTACCTCAGCGGTGACACAT	AAGTCCGGGAGTTGCTGATG	101	NM_001244379.1
Carboxypeptidase A	TGGGGGCTGCTGATTTTTAGT	CAGATAGCCGGACGGTTGTT	515	NM_214244.1
Carboxypeptidase B	GGCTGCCGTGAAAGAACTTG	TGGCCAGCATTGTTTCCTCA	218	NM_214169.1
Amino peptidase A	ACACGGGGACAGTGAACATC	TCAGGTGGTAGAGACCCTCG	222	NM_214017.1
Amino peptidase N	GCTGTTGCCTGATTCCTACTTC	CTACCAGCTCAGTCCTGTCG	230	NM_214277.1
Pancreatic lipase	GGCTCCCGAACTGGATACAC	GATCCAGCCCTGTGATTCGT	205	NM_001177912.2
Sucrase	AAGTGGGAGGTGGGAACTCT	GCGTTAACTGCACAGGGTTG	166	XM_005657098.1
Maltase	AGAGTATCCTCACCGGGCAT	CAAATGACCGTCCAGCTCCT	154	XM_005657730.2
α-Amylase	ACTCCCGAGATAGAGAAAGTGTT	GACTGGGTTTGTGGGGCATA	241	XM_005663622.2
Nutrients transporter gene				
ASCT2[16]	GCTTCCGAGAGCCAAGAACT	TCCTTAACGCCTGGAAGCTG	152	XM_003127238.3
EAAC1[17]	GCTTCCTTCTTCCAGGGTCC	CTGGCCAATGTGGCTTGTTC	148	NM_001164649.1
$B^{0,+}AT$[18]	GAGAGGTTTGGTCTTACTGCG	GCTATGACCAAGACGGAGCG	96	XM_003353809.2
y^+LAT1[19]	TTTGTCTGACCGGCTCTTCC	GAGATCTCCTGCTGTCCTGG	286	XM_005666262.1
4F2hc[20]	CTCGAACCCACCAAGGAC	GAGGTGAGACGGCACAGAG	174	NM_001110171.1
PepT1[21]	CATCGCCATACCCTTCTG	TTCCCATCCATCGTGACATT	143	NM_214347.1
SGLT1[22]	TGTTATACCCCGAGGGCTGA	CGCAATCCATTGGGCATGAG	374	NM_001164021
GLUT2[23]	TCATGTCGGTGGGACTTGTG	GGCCTGGCCCAATTTCAAAG	105	NM_001164021
CAT1[24]	GCTGTCATGGCCTTCCTCTT	CTGGTACACCATGTTCGGCT	138	NM_001012613.1
Housekeeping gene				
β-actin	GGATGCAGAAGGAGATCACG	ATCTGCTGGAAGGTGGACAG	130	DQ845171

Note: [1] TasR1, taste receptor type 1 member 1-like; [2] TasR2, taste 1 receptor member 2; [3] TasR3, taste 1 receptor member 3; [4] GPRC6A, G protein-coupled receptor, class C, group 6, member A; [5] CaSR, Calcium-sensing receptor, transcript variant 1; [6] GPR40, long-chain fatty acid receptors G-protein-coupled receptor 40 (also known as free fatty acid receptor 1, FFAR1); [7] GPR41, short-chain fatty acid receptors G-protein-coupled receptor 41 (also known as free fatty acid receptor 3, FFAR3); [8] GPR43, short-chain fatty acid receptors G-protein-coupled receptor 43 (also known as free fatty acid receptor 2, FFAR2); [9] SST, somatostatin; [10] INR, insulin receptor; [11] Leptin-R, leptin receptor; [12] CCK-1R, cholecystokinin type A receptor; [13] CCK-2R, cholecystokinin B receptor; [14] GLP-1R, glucagon like peptide 1 receptor; [15] GLP-2R, glucagon like peptide 2 receptor; [16] ASCT2, Na^+-dependent neutral amino acid exchanger 2; [17] EAAC1, solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 (SLC1A1); [18] $B^{0,+}AT$, $b^{0,+}$ amino acid transporter; [19] y^+LAT1, y^+ L-type amino acid transporter 1; [20] 4F2hc, solute carrier family 7 member 9 (SLCA9); [21] PepT1, dipeptide transporter 1; [22] SGLT1, sodium-glucose transporter 1; [23] GLUT2, glucose transporter 2; [24] CAT1, cationic amino acid transporter 1.

Samples of blood, tissue section, and digesta collection

All pigs were fasted overnight (for approximately 12 h), and the pigs were euthanized through intravenous injection of 50 mg/kg sodium pentobarbital and then sacrificed. The blood (5 mL) from the overnight fasting pigs was collected by vein puncture, centrifuged at 3,000 g for 15 min at 4° C, and immediately stored at –80° C for further analysis. The collection period was divided into different intervals to conduct experiments on apparent digestibility and N balance (days 3–7).

The 3 g tissue samples were collected from the midpoint of each section (i.e., the intestine (duodenum and jejunum), stomach, and pancreas) and immediately frozen in liquid nitrogen at –80° C for subsequent analysis of gene expression levels as previously described [9, 10].

Samples of digesta were collected from the ileum to analyze the digestibility of energy (DE), dry matter (DM), CP, and AAs as previously described [4, 27].

Analysis of serum biochemical parameters and the free AA profile

Serum biochemical parameters, including total protein, urea nitrogen (UN), glucose, cholesterol, and triglycerides, were measured using commercial kits according to the manufacturer's instructions (Sino-German Beijing Leadman Biotech Ltd., Beijing China) on a Biochemical Analytical Instrument (Beckman Chemistry Analyzer; Beckman Coulter, Inc., Brea, CA).

The contents of 18 free AAs in serum were determined via LC–MS/MS (HPLC Ultimate 3000 and 3200 QTRAP LC–MS/MS) as described previously [9, 10, 27, 28].

Chemical analysis

All samples of ileal digesta were pooled and homogenized in a blender, sub-sampled, freeze-dried, and finely ground in a grinder and thoroughly mixed for analysis as previously described [4]. All of the ileal digesta samples and the three diets were analyzed for conventional parameters, such as DM, DE, and N and the concentrations of AAs. DM was analyzed using the AOAC protocol (1990; method 925.09) [29], and gross energy (GE) was analyzed using an oxygen bomb calorimeter as previously described. N was analyzed via a previously described method [4, 29]. Samples for ileal AA determination were prepared via acid hydrolysis using a previously described protocol [4, 29]. Tryptophan was not analyzed because it was destroyed during preparation via acid hydrolysis [30].

Relative quantification of the mRNA expression levels of nutrient-sensing and hormone secretion genes, gastrointestinal digestive enzymes, and nutrient transporters

The software program Primer 5.0 (Primer-E Ltd., Plymouth, UK) was used to design the primers, which are listed in Table 5. The *β-actin housekeeping gene* was employed as an internal control to normalize the expression of target gene transcripts.

Total tissue RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as previously described [9, 10]. cDNA was then reverse transcribed and amplified via quantitative real-time PCR using an ABI 7900 PCR system (ABI Biotechnology, Eldersburg, MD, USA) as previously described [9, 10]. Each sample had a total volume of a 10 μL, including 1 μL of 4x-diluted cDNA, 5 μL of SYBR Green mix, 0.2 μL of ROX reference dye (50 times), and 0.2 μL of each of forward and reverse primer. We used the following protocol: (i) pre-denaturation (10 s at 95° C); (ii) amplification and quantification for 40 cycles (5s at 95° C, 20s at 60° C); (iii) melting curve analysis (60–99° C, at a heating rate of 0.1° C s^{-1}, with fluorescence measurements). The mRNA expression levels of target genes, in arbitrary units, were determined from the real-time PCR threshold cycle (Ct) in relation to *β-actin*, using the comparative Ct, via the $2^{-\Delta\Delta Ct}$ method [9–11].

Statistical analysis

The data were tested via ANOVA using the SAS 8.2 software program (Version 8.2; SAS Inst. Inc., Cary, NC), followed by Duncan's multiple comparison test with the pen as the experimental unit. The data obtained from mRNA measurements were also analyzed through multifactor ANOVA and the GLM procedure was used to assess the effects of different diets. The results were presented as the mean ± standard error of the mean (SEM). Differences between significant means were considered significantly different at $P < 0.05$. The results of statistical analyses were introduced and mapped with GraphPad Prism 6.0 software (GraphPad Software, Inc.).

Abbreviations

GIT: gastrointestinal tract; AA: amino acid; CP: crude protein; ADG: average daily gain; ADFI: average daily feed intake; F: G: ratio of feed to gain; TiQ_2: titanium dioxide; BW: body weight; DM: dry matter; DE: digestibility of energy; N: nitrogen; GE: gross energy; SEM: standard error of the mean; GPRC6A: G protein-coupled receptor class C group 6 member A; CCK: cholecystokinin; UN: urea nitrogen; TasR1: taste receptor type 1 member 1; TasR2: taste 1 receptor member 2; TasR3: taste 1 receptor member 3; GPRC6A: G protein-coupled receptor, class C, group 6, member A; CaSR: calcium-sensing receptor, transcript variant 1; GPR40: long-chain fatty acid receptor G-protein-coupled receptor 40; GPR41: short-chain fatty acid receptor G-protein-coupled receptor 41; GPR43: short-chain fatty acid receptor G-protein-coupled receptor 43; SST: somatostatin; GLP-1R: glucagon-like peptide 1 receptor; GLP-2R: glucagon-like peptide 2 receptor; INR: insulin receptor; Leptin-R, leptin receptor; CCK-2R: cholecystokinin B receptor; CCK-1R: cholecystokinin type A receptor; CPA: carboxypeptidase A; CPB: carboxypeptidase B; APA: amino peptidase A; APB: amino peptidase B; P-lipase: pancreatic lipase; EAAC1: solute carrier family 1; ASCT2: Na^+-dependent neutral amino acid exchanger 2; PepT1: dipeptide transporter 1; CAT1: cationic amino acid transporter 1; $B^{0+}AT$: $b^{0,+}$ amino acid transporter; y^+ LAT1: y^+ L-type amino acid transporter 1; 4F2hc: solute carrier family 7 member 9; SGLT1: sodium-glucose transporter 1; GLUT2: glucose transporter 2.

CONFLICTS OF INTEREST

No potential conflicts of interest was reported by the authors.

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