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Supplemental leucine and isoleucine affect expression of cationic amino acid transporters and myosin, serum concentration of amino acids, and growth performance of pigs

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ABSTRACT. Leucine (Leu) participates in the activity of cationic amino acid (aa) transporters. Also, branched-chain aa [Leu, isoleucine (Ile), and valine (Val)] share intestinal transporters for absorption. We conducted an experiment with 16 young pigs (body weight of about 16 kg) to determine whether Leu and Ile affect expression of aa transporters $b^{0,+}$ and CAT-1 in the jejunum and expression of myosin in muscle, as well as serum concentration of essential aa, and growth performance in pigs. Dietary treatments were: wheat-based diets fortified with Lys, Thr, and Met; basal diet plus 0.50% Leu; basal diet plus 0.50% Ile, and basal diet plus 0.50% Leu and 0.50% Ile. After 28 days, the pigs were sacrificed to collect blood, jejunum, and semitendinosus and longissimus muscle samples. The effects of single and combined addition of Leu and Ile were analyzed. Leu alone or combined with Ile significantly decreased daily weight gain and reduced feed conversion. Leu and Ile, alone or in combination, significantly decreased expression of $b^{0,+}$ and significantly increased CAT-1. Ile alone or combined with Leu significantly decreased myosin expression in semitendinosus and significantly decreased it in

longissimus muscle. Leu alone significantly decreased Lys, Ile and Thr serum concentrations; Ile significantly decreased Thr serum concentration; combined Leu and Ile significantly decreased Thr and significantly increased Val serum concentration. We conclude that dietary levels of Leu and Ile affect growth performance, expression of aa transporters and myosin, and aa serum concentrations in pigs.

Key words: Swine; Leucine; Amino acid transporter; mRNA expression; Isoleucine

INTRODUCTION

The essential amino acid (aa) Leu is recognized as a functional aa in mammals because it participates in the regulation of cell metabolism, especially protein synthesis (Dreyer et al., 2008) and energy metabolism (Yang et al., 2010). The activity of the mammalian target of rapamycin pathway, which plays a critical role regulating protein synthesis and cell hypertrophy in skeletal muscle, is in turn regulated by Leu as well as growth factors (Dreyer et al., 2008). Leu is also involved in the intestinal absorption and cellular uptake of cationic aa (Lys, Arg, and His), which are transported at high affinity by the Na-independent systems $b^{0,+}$ and CAT-1 (Majumder et al., 2009). The transporter $b^{0,+}$ is mainly expressed in epithelial cells, and exchanges Leu for Lys (Torrast-Llort et al., 2001); that is, the intestinal absorption of cationic aa by $b^{0,+}$ is coupled with the efflux of Leu (Pineda et al., 2004). Since Lys is the first limiting aa in most feed ingredients for pigs (NRC, 1998) and the intestinal absorption as well as the intracellular availability of Lys involve the presence of Leu, protein synthesis may be affected by the dietary contents of these two aa.

Diets for growing pigs are formulated to meet the requirement of Lys, but these diets contain excesses of several aa including Leu. The negative effects of Leu excess on the availability and metabolism of other aa such as Ile are well documented. Taylor et al. (1984) demonstrated an interaction between dietary Leu and Ile in growing pigs; the increase in the dietary Leu level resulted in a deficiency of Ile. Langer and Fuller (2000) found a reduced serum concentration (SC) of Ile that was caused by high dietary Leu levels; this response is associated with an increase in the activity of the enzyme branched-chain keto acid dehydrogenase (BCKA) in the liver (Wiltafsky et al., 2010). However, there is no available report showing the effect of dietary Leu at adequate or excess levels, on the absorption of cationic aa and activation of the protein synthesis pathway. Neither is it clear whether supplemental free Ile can counteract the negative effect resulting from excess dietary Leu. The reduction in dietary protein content, coupled with the supplementation of L-Lys, L-Thr, and DL-Met, eliminates excess Leu. A diet formulated only with wheat and supplemented with L-Lys, L-Thr, and DL-Met meets the requirements of these aa, supplying around 104% that of Leu, and becomes marginal in Ile (NRC, 1998). This diet formulation offers a good opportunity to study the interaction between Leu and Ile and its effect on the expression of the cationic aa transporters $b^{0,+}$ and CAT-1, the SC of aa, and the resulting performance of pigs.

The hypothesis of this study was that excess Leu affects the absorption and availability of aa and the performance of pigs, and that free Ile supplementation may reverse the excess Leu effect. Therefore, this experiment was conducted to evaluate the effect of two levels (adequate and excess) of Leu and Ile on the expression of $b^{0,+}$ and CAT-1, SC of indispensable aa,

expression of myosin in muscle, and the performance of growing pigs fed wheat-based diets fortified with L-Lys, L-Thr, and DL-Met.

MATERIAL AND METHODS

Animals

The experiment was conducted with 24 crossbred (Landrace x Hampshire x Duroc) pigs with an average initial body weight (BW) of 15.9 ± 0.6 kg. Pigs were distributed into four groups based on initial BW, age, sex, and litter. Within each group, pigs were randomly assigned to one of four dietary treatments, according to a randomized complete block design (Steel and Torrie, 1980); there were six replicates (three barrows and three gilts) per treatment. All pigs were individually housed in raised-floor metabolism pens (1.2 m wide, 1.2 m long, and 1.0 m high) equipped with a stainless-steel self-feeder, a nipple water drinker, and iron mesh floor in a temperature-controlled room. Pigs were weighed every week to calculate the average daily gain (ADG). Average daily feed intake (ADFI) was measured on a weekly basis; the feed:gain ratio (FG) was also calculated. The pigs had unlimited access to feed and water. The average BW of the pigs at the end of the 20-day trial was 24.2 kg.

Diets

A basal diet was formulated with wheat as the sole source of protein (Table 1), and supplemented with 0.69% Lys, 0.28% Thr, 0.10% Met, vitamins, minerals, and cornstarch. This diet contained sufficient levels of the entire indispensable aa, and adequate levels of Leu and Ile (NRC, 1998). Dietary treatments (T) were: T1, basal diet; T2, basal plus additional 0.50% Leu; T3, basal plus 0.50% Ile, and T4, basal plus additional 0.50% Leu plus 0.50% Ile. Diets in T2 and T4 contained 50% excess Leu, and diets in T3 and T4 provided 50% excess Ile. Crystalline Leu and Ile were added to the diets at the expense of cornstarch. All diets contained a vitamin and mineral premix, sufficient amounts of Ca and available P to meet or exceed the requirements of the 10 to 20 kg pigs, and contained 2400 kcal NE/kg. A single batch of wheat grown by irrigation was used in all trials.

Table 1. Formulation of the experimental diets (%, as-fed basis).

Ingredients (%)	Diets[a]			
	Basal	+Leu	+Ile	+Leu+Ile
Wheat	95.35	95.35	95.35	95.35
L-Lysine-HCl, 78%	0.88	0.88	0.88	0.88
L-Threonine, 99%	0.28	0.28	0.28	0.28
DL-Methionine, 99%	0.10	0.10	0.10	0.10
L-Leucine, 99%	-	0.50	-	0.50
L-Isoleucine, 99%	-	-	0.50	0.50
Cornstarch	1.00	0.50	0.50	-
Calcium carbonate	1.35	1.35	1.35	1.35
Dicalcium phosphate	0.40	0.40	0.40	0.40
Iodized salt	0.35	0.35	0.35	0.35
Vitamin and mineral premix[b]	0.20	0.20	0.20	0.20

[a]Basal = wheat-based diet formulated to contain 1.05% Lys, 0.64% Thr, and 0.21% Met; +Leu = basal diet + 0.50% Leu; +Ile = basal diet + 0.50% Ile; +Leu+Ile = basal diet + 0.50% Leu + 0.50% Ile. [b]Supplied per kilogram of diet = 4800 IU vitamin A; 800 IU vitamin D3; 4.8 IU vitamin E; 1.6 mg vitamin K3; 4 mg riboflavin; 7.2 mg D-pantothenic acid; 16 mg niacin; 12.8 µg vitamin B12; 64 mg Zn; 64 mg Fe; 4 mg Cu; 4 mg Mn; 0.36 mg I, and 0.13 mg Se.

Tissue collection

Four pigs from each treatment group were euthanized at the end of the 20-day trial by electrical stunning and exsanguination. The carcasses were immediately eviscerated and samples (approximately 0.5 g) of mucosa scraped from the middle jejunum were collected into 2-mL Eppendorf microtubes; the proximal jejunum is the major site of aa and peptide absorption (Silk et al., 1985; Broer, 2008). Also, samples (0.5 to 1.0 g) from the longissimus dorsi (LDM) and semitendinosus (STM) muscles were collected, and immediately stored in liquid nitrogen. The total collection process took no longer than 10 min to maximize the quality of the extracted RNA. Blood samples from the carotid artery (approximately 10 mL) were collected during the exsanguination of the animals to analyze the concentration of free aa in serum. All samples were transported to the molecular biology lab and stored at -82°C until analysis.

Total RNA extraction and purification

The samples from jejunal mucosa, LDM and STM were treated to extract total RNA by pulverization in liquid nitrogen and then following the instructions for the Trizol reagent (Invitrogen, Corp.). Purified RNA was then eluted with 30 µL nuclease-free distilled water and stored at -82°C. The concentration of total RNA was determined spectrophotometrically (Helios β, Thermo Electron Co.) at 260 nm, and purity of RNA was assessed by the A_{260}/A_{280} ratio, which ranged from 1.8 to 2.0 (Sambrook and Russell, 2001). The integrity of total RNA was evaluated by gel electrophoresis on 1% agarose gels. All RNA samples were of good quality with a 28S:18S rRNA ratio around 2.0:1 (Sambrook and Russell, 2001).

Reverse transcription

Approximately 2 µg total RNA was treated with 1 U (1 U/µL) DNase I (Invitrogen) and 6 µL 5X reverse transcription buffer in a 30-µL reaction mixture completed with DEPC-treated water; the reaction was carried out for 15 min at room temperature and another 15 min at 70°C. Reverse transcription was initiated with DNase-treated RNA samples, adding 1 µL 150 ng/µL random primers and 1 µL 10 µM of each dNTP solution. The reaction was incubated at room temperature and then chilled on ice for 1 min. DTT (3 µL, 0.1 M), 1 µL 40 U/µL RNase OUT (Invitrogen), and 2 µL 5X reverse transcription buffer were added to the reaction mixture, followed by incubation at 42°C for 2 min to stabilize the reaction before adding 1 µL 200 U/µL RT-Superscript III reverse transcriptase enzyme (Invitrogen). Reaction mixture was incubated at 42°C for 50 min. The mixture was further incubated at 70°C for 15 min and then chilled on ice to stop the reaction. cDNA samples were quantified spectrophotometrically and diluted to a final concentration of 50 ng/µL.

Quantitative PCR (qPCR)

Specific primers for each amino acid transporter mRNA and myosin were designed according to their published sequences in GenBank (Table 2). Also, a housekeeping 18S rRNA gene was used as an endogenous control to normalize variations in mRNA. Before starting, end point PCR was carried out to standardize the amplification conditions for each pair of

primers. To confirm the specificity of the PCR products related to its mRNA, samples of every PCR product were sequenced at the Davis Sequencing Facilities (Davis, CA, USA).

Table 2. Primers used for the qPCR analyses of cDNA derived from two amino acid transporters, myosin and 18S ribosomal RNA.

mRNA	Primer	Primer sequence (5'-3')	Location (bp) on the template	Product size (bp)
Cationic amino acid transporter-1, CAT-1 (GenBank: AY371320)	Sense	5'-GTCGGTTGCAAAGACCATTT-3'	4239-4258	329
	Antisense	5'-GAGCGGTGCTGACAACAGTA-3'	4548-4567	
Amino acid transporter $b^{0,+}$ (SLC7A9) (GenBank: EF127857)	Sense	5'-CGGAGAGAGGATGAGAAGT-3'	1-19	562
	Antisense	5'-GCCCGCTGATGATGATGA-3'	545-562	
Myosin, heavy-chain 4 (GenBank: NM_001123141)	Sense	5'-AGATTTCTGACCTGACTG-3'	4582-4599	340
	Antisense	5'-TCTCCCTCCATCTTCTTC-3'	4904-4921	
18S ribosomal RNA (GenBank: AY265350)	Sense	5'-GGCCTCACTAAACCATCCAA-3'	236-255	295
	Antisense	5'-TAGAGGGACAAGTGGCGTTC-3'	511-530	

Expression of aa transporters in jejunum and muscles was estimated by qPCR assays, using the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Corp.) in a Chromo 4-DNA Engine (Bio-Rad), with the MJ Opticon Monitor 3.1 software. The equipment was calibrated with a standard curve using 18S rRNA cloned into a TOPO vector 4.0 (Invitrogen), from which a calibrator's cDNA was produced. The standard curve was obtained using known concentrations of 100-fold serial dilutions of the 18S cDNA. Reactions for qPCR contained 50 ng cDNA, 0.5 µM of each specific primer, 12.5 µL 2X SYBR green/ROX qPCR Master Mix, and DNase-/RNase-free water to complete a final volume of 25 µL. 18S rRNA was also used as housekeeping gene to standardize the amount of amplified DNA. The PCR conditions used for the amplification and quantification were an initial denaturation step (95°C for 1 min), followed by 45 cycles of amplification (denaturation at 95°C for 30 s, annealing at 56°C for 15 s and extension at 72°C for 30 s), and a melting curve program (60° to 90°C). For DNA quantification, fluorescence was measured at the end of every cycle and every 0.2°C during the melting program.

Serum concentration of free amino acids

The serum concentration of free aa was determined according to Sunde et al. (2003). Clotted blood samples were centrifuged at 425 *g*, at 4°C for 10 min to separate serum from blood cells. The serum was then deproteinized with a Millipore Ultrafree-MC 10,000 NMWL filter unit (Millipore, Bedford, MA, USA), at 5000 *g*, 4°C, for 30 min. The filtrate of the supernatant was derivatized with Waters AccQ**.**Tag reagent. The analysis of aa was performed by HPLC (method 982.30E; AOAC, 2006).

Statistical analysis

Analyses of variance of the data for each variable were performed using the GM of SAS (SAS, 2000). Since the main objective of this study was to analyze the effect of adding Leu and Ile, alone or in combination, three contrasts were constructed to compare their effects with that of the basal diet: C_1, basal *vs* Leu; C_2, basal *vs* Ile, and C_3, basal *vs* Leu + Ile. In addition, the main effects of Leu and Ile, and their interaction were tested. Effects and differences were considered to be significant when $P < 0.05$.

RESULTS

The performance results of pigs fed wheat-based diets with excess levels of Leu or Ile or both, as compared to those of pigs fed the basal diet, are presented in Table 3. Excess Leu alone (P = 0.002) or combined with Ile excess (P = 0.006) reduced the ADG of pigs; excess of Ile alone also tended to reduce the ADG (P = 0.068). The ADFI was not affected (P > 0.10). Excess Leu (P = 0.007) or Ile (P = 0.040) alone or in combination (P = 0.017) worsened the FG ratio. The factorial analysis showed that excess Leu deteriorated the ADG and FG ratio (P < 0.05).

Table 3. Effect of the excess levels of Leu and Ile in low protein, wheat-based diets fortified with Lys, Thr, and Met, on the average daily weight gain (ADG), average daily feed intake (ADFI), and feed:gain (FG) ratio of growing pigs.

	Treatments				SE	Basal *vs* P		
	Basal	+Leu	+Ile	+Leu+Ile		+Leu	+Ile	+Leu+Ile
ADG (kg/day)[a]	0.500	0.358	0.426	0.379	0.026	0.002	0.068	0.006
ADFI (kg/day)	0.817	0.832	0.902	0.832	0.076	0.891	0.442	0.894
FG[a]	1.61	2.35	2.15	2.24	0.166	0.007	0.040	0.017

[a]Leu effect (P < 0.05). SE = standard error. For diet formulations, see legend to Table 1.

The expression values of $b^{0,+}$ and CAT-1 in jejunum and of myosin in STM and LDM are presented in Table 4. Excess Leu (P = 0.002) or Ile (P = 0.012) alone or in combination (P = 0.004) decreased the expression of $b^{0,+}$. In contrast, CAT-1 expression was higher in pigs with excess Leu (P = 0.001) or Ile (P = 0.013) or both aa (P = 0.001). The orthogonal contrasts resulting from the factorial analysis showed an interaction between excess Leu and Ile for $b^{0,+}$ (P = 0.041) and CAT-1 (P = 0.001). Regarding myosin, excess Leu alone did not affect the expression of myosin in STM (P = 0.465) or LDM (P = 0.867). Excess Ile alone or combined with Leu reduced the expression of myosin in STM (P = 001), but increased it in LDM either alone (P = 0.022) or combined with excess Leu (P = 0.028).

Table 4. Effect of the excess levels of Leu and Ile in low protein, wheat-based diets on the relative expression of the amino acid transporter proteins $b^{0,+}$ and CAT-1 in jejunum, and myosin in the semitendinosus (STM) and longissimus (LDM) muscles of growing pigs (arbitrary units; ratio of mRNA:18S rRNA).

Item	Treatments				SE	Basal *vs* P		
	Basal	+Leu	+Ile	+Leu+Ile		+Leu	+Ile	+Leu+Ile
AA transporter								
$b^{0,+}$ [a]	1.692	0.207	0.551	0.357	0.30	0.002	0.012	0.004
CAT-1[a]	0.720	4.177	1.872	2.282	0.31	0.001	0.013	0.001
Myosin in muscle								
STM	15.96	17.27	2.18	4.04	1.24	0.465	0.001	0.001
LDM	1.801	1.285	6.886	6.657	1.47	0.807	0.022	0.028

[a]Leu x Ile interaction (P < 0.05). SE = standard error. For diet formulations, see legend to Table 1.

The SC values of the indispensable aa are presented in Table 5. Leu excess alone reduced the SC of Ile (P = 0.008), Lys (P = 0.050), and Thr (P < 0.001), but increased that of Leu (P < 0.001). Ile excess alone decreased the SC of Thr (P < 0.001), but increased that of Ile (P < 0.001). The excess of both Leu and Ile increased the SC of Phe (P = 0.013), Val (P < 0.015), Leu (P < 0.001), and Ile (P = 0.002), but decreased the SC of Thr (P = 0.013).

Table 5. Effect of Leu and Ile supplementation on the concentration of amino acids in serum of pigs.

	Treatments				SE	Basal *vs* P		
	Basal	+Leu	+Ile	+Leu+Ile		+Leu	+Ile	Leu+Ile
Indispensable								
Arginine	0.288	0.326	0.238	0.268	0.058	0.657	0.551	0.815
Isoleucine[a,b]	0.171	0.070	0.985	0.463	0.105	0.008	<0.001	0.002
Leucine[a]	0.175	0.399	0.130	0.466	0.039	<0.001	0.784	<0.001
Lysine[b]	0.838	0.512	0.633	0.604	0.110	0.050	0.211	0.158
Methionine	0.089	0.089	0.070	0.085	0.013	0.969	0.325	0.817
Phenylalanine[c]	0.159	0.174	0.138	0.242	0.020	0.626	0.460	0.013
Threonine[c]	1.177	0.764	0.568	0.910	0.065	<0.001	<0.001	0.013
Valine[c]	0.151	0.151	0.138	0.229	0.019	0.993	0.644	0.015

[a]Leu effect: $P < 0.05$. [b]Ile effect: $P < 0.05$. [c]Leu x Ile effect: $P < 0.05$. SE = standard error. For diet formulations, see legend to Table 1.

The coefficient values of correlations between the dietary levels of Leu or Ile, the SC of Arg and Lys, the expression of aa transporters and myosin, and the performance of pigs are presented in Tables 6 to 8. The dietary level of Leu was negatively correlated ($P = 0.015$) with $b^{o,+}$ but positively ($P = 0.001$) with CAT-1 (Table 6); the level of Ile was not correlated with either $b^{o,+}$ or CAT-1 ($P > 0.05$). There was a positive correlation between the dietary Leu level and the SC of Arg ($P = 0.003$), but negative with the SC of Lys ($P = 0.035$); Ile was not correlated with the SC of either Arg or Lys ($P > 0.05$; Table 7). The SC of Leu was positively correlated with the expression of myosin in STM ($P = 0.001$; Table 8), but negatively correlated in LDM ($P = 0.050$). In contrast, Ile SC was negatively correlated with the expression of myosin in STM, but positively correlated in LDM ($P < 0.001$). The SC of Lys and Arg were not correlated with the expression of myosin either in STM or LDM ($P > 0.05$).

Table 6. Coefficients and P values of Pearson's correlations between the dietary content of Leu and Ile and the expression of $b^{o,+}$ and CAT-1 in jejunum.

mRNA	Dietary amino acids	
	Leucine	Isoleucine
$b^{o,+}$		
r	-0.425	-0.251
P	0.015	0.166
CAT-1		
r	0.65	-0.125
P	0.001	0.426

Table 7. Coefficients and P values of Pearson's correlations between the expression of $b^{o,+}$ and CAT-1 in jejunum or myosin in the semitendinosus and longissimus muscles, and the dietary content of Leu and Ile.

Cationic amino acids in serum	Amino acids in diet	
	Leucine	Isoleucine
Arginine		
r	0.503	-0.056
P	0.003	0.759
Lysine		
r	-0.374	-0.098
P	0.035	0.594

Table 8. Coefficients and P values of Pearson's correlations between the content of Leu and Ile in serum and the expression of myosin in the semitendinosus and longissimus muscles of growing pigs.

Muscle	Amino acids in serum			
	Leucine	Isoleucine	Lysine	Arginine
Semitendinosus				
r	0.645	-0.818	-0.023	0.156
P	0.001	0.001	0.901	0.394
Longissimus				
r	-0.339	0.549	0.079	-0.119
P	0.050	0.001	0.669	0.518

DISCUSSION

The basal diet used in this experiment contained only wheat as the major feed ingredient, and supplied 100% the requirements of Lys, Thr, and Met for growing pigs (10-20 kg; NRC, 1998) with their addition in free form. This diet supplied about 104% of the requirement of Leu and was marginal in Ile; if excess dietary Leu affected the availability of Ile, a negative effect on ADG and FG would be observed because of the marginal dietary Ile level. The supplementation of 0.50% Leu or Ile to the basal diet created a 58 and 116% excess of Leu and Ile, respectively. The ADG and FG ratio of pigs were negatively affected by excess Leu, but feed intake was not affected. Pigs fed the diet with excess Leu (1.6-fold the requirement; NRC, 1998) alone or in combination with Ile reduced the ADG by 28 and 24%, respectively. Gatnau et al. (1995) observed a 19% reduction in the ADG of pigs fed a corn-soybean meal-dried whey diet containing 2.24% Leu. Also, Wiltafsky et al. (2010) reported a linear reduction in ADG (from 6.2 up to 17.4%) and FG (from 5.2 up to 11.6%) as the dietary Leu content increased from 1.3 to 2.4%. In contrast, Edmonds and Baker (1987) found a decrease in ADG and feed conversion only when pigs were fed corn-soybean meal diets containing 6% Leu, but no effect was observed with 1, 2, or 4% dietary Leu. Henry et al. (1976) reported a tendency for ADG to decrease in 51-kg pigs fed diets based on fish meal and cornstarch, as the dietary Leu content was increased from 0.38 to 0.62%, although no significant change was observed in feed intake or feed efficiency. These response differences may be attributed to variations in the content and digestibility of the other indispensable aa in the diet, which may create specific interactions between some of them, affecting their availability for growth (Jackson et al., 2000; Matthews, 2005).

The expression of $b^{0,+}$ in jejunum was substantially reduced down to 12.4% in response to Leu excess and 21.3% in response to the combined excess of Leu and Ile, as compared to the basal diet. In general, the expression of $b^{0,+}$ was negatively correlated with the level of Leu in the diet (r = 0.43), but not with Ile. Similar results were found in a previous study conducted in this laboratory (Cervantes M, García H and Morales A, unpublished data), in pigs fed wheat- or wheat-soybean meal diets with excess Leu. There are no reports regarding the effect of branched-chain aa (BCaa) excess on the expression of $b^{0,+}$ in pigs, although Liao et al. (2009) reported that the ruminal infusion of hydrolyzed starch in steers, which increased the microbial protein and aa supply, reduced the abundance of the cationic aa transport systems y^+ and y^+L. These results indicate that Leu, but not Ile, is involved in the regulation of the absorption of cationic aa.

In contrast to the response observed with $b^{0,+}$, the expression of CAT-1 in jejunum

increased about 4.8- and 1.6-fold with dietary excess Leu and Ile alone, respectively; the combined excesses of Leu and Ile increased the expression of CAT-1 only 2.2-fold because of the Leu x Ile interaction. Expression of CAT-1 appears to be confined to the basolateral membrane (Kakoki et al., 2004), whereas $b^{0,+}$ is mainly expressed in the apical membrane. The reduced expression of $b^{0,+}$ caused by Leu excess could have provoked a reduction in the concentration of Lys in the enterocyte. In this regard, Hatzoglou et al. (2004) proposed the adaptive regulation theory, which implies that deficient aa supply increases the activity of the transporter. This may explain the increase in the expression of CAT-1 in jejunum observed in this study. However, since the SC of Lys was reduced in pigs fed the diet with Leu excess, it appears that the increase in the expression of CAT-1 was not sufficient to compensate for the reduction in the expression of $b^{0,+}$, which is in agreement with Closs et al. (2004), who speculated that CAT-1 cannot replace the function of other cationic aa transporters.

The SC of BCaa was selectively and differently affected by the dietary excess Leu or Ile. Excess dietary Leu increased the SC of Leu, decreased that of Ile, but had no effect on Val. Excess dietary Ile increased the SC of Ile, but did not affect the SC of Leu or Val. Similar results were previously reported by Henry et al. (1976) and Edmonds and Baker (1987), who found a decrease in the SC of Ile and Val in pigs fed dietary excess Leu. Wiltafsky et al. (2010) also observed a linear increase in plasma Leu levels and a decrease in Ile, as the dietary Leu content was increased. According to these authors, that response was accompanied by increases in the activity of basal BCKA in liver, and they concluded that excess dietary Leu increased the catabolism of Ile and Val. Likewise, Matthews (2005) indicated that BCaa share common enzymes for the transamination of BCaa and subsequent decarboxylation of the BCKAs. In addition, a competitive inhibition for absorption among the BCaa is well documented (e.g., Hagihira et al., 1961; Broer, 2008). Therefore, an excess of Leu could be expected to increase the catabolism or inhibit the absorption of Ile, which explains the reduction in the SC of Ile when excess dietary Leu was provided.

The SC of Lys and Thr were substantially reduced because of excess Leu. These results are similar to those published by Nair et al. (1992) in humans. The intestinal absorption of Lys is performed mainly by the cationic aa transporter $b^{0,+}$ (Broer, 2008), which exchanges Leu for Lys (Torrast-Llort et al., 2001); that is, the intestinal absorption of Lys by $b^{0,+}$ is coupled with the efflux of Leu (Pineda et al., 2004). The decrease in the SC of Lys was directly associated with the reduction in the expression of $b^{0,+}$ ($r = 0.98$). Furthermore, very high positive correlations between the expression of $b^{0,+}$ and ADG ($r = 0.98$), or the SC of Lys and ADG ($r = 0.98$) were observed. In contrast, the dietary Leu level ($r = -0.87$) and the Leu SC ($r = -0.77$) were negatively correlated with the ADG. Therefore, this response suggests that dietary excess Leu negatively affects the availability of Lys for growth by reducing the abundance of $b^{0,+}$ in jejunum. Although excess Ile reduced the expression of $b^{0,+}$, the decrease in the SC of Lys was not significant, suggesting that Leu is the main aa affecting the availability of Lys for growth.

Excess Ile (2.2-fold the requirement; NRC, 1998) also reduced the SC of Thr. The transport of Thr across the plasma membrane is mediated by the Na-independent transporter asc-1 (Fukasawa et al., 2000). Maenz and Patience (1992) reported that Thr uptake by preparations of pig jejunal brush border membrane vesicles was inhibited by several aa, especially Ile, Leu, and Tyr. This information may explain the reduction in the SC of Thr in pigs fed a diet with excess Ile and Leu. The basal diet was supplemented with free L-Thr to meet, without excess, its requirement for growing pigs. Thus, the reduction in the SC of Thr resulting from

the apparent absorption inhibition caused by excess Ile and Leu may indicate that Thr became deficient in the diet. In addition, the SC of Lys was not significantly reduced by excess Ile. Therefore, the decreased SC of Thr might have been responsible for the tendency of excess Ile to reduce ADG.

Myosin is the most abundant of all muscle proteins, and it is the major component of thick filaments (Czerwinski and Martin, 1994); type IIB fibers account for about 80% of the total fibers in some pig muscles. According to Lefaucheur et al. (2002), this isoform of fast contraction is extensively expressed in STM and LDM of pigs. However, the available reports regarding myosin IIB expression in pigs are scarce and show conflicting results; furthermore, no report related to the effect of excess Leu or Ile was found. It seems that the stimulation of protein synthesis by Ile is dependent on the type and physiology of muscle fiber because the expression of myosin isoform IIB in this study was increased in STM but decreased in LDM with dietary excess Ile. Hemmings et al. (2009) found a high expression of myosin IIB isoform in STM of sheep, but not in LDM. In contrast, Gunawan et al. (2007) reported that myosin IIB expression was higher in LDM of pigs, as compared to STM. Brodsky et al. (2004) concluded that protein deprivation in humans reduces the expression of fast twitch myosin heavy chain and that dietary amino acid scarcity produces a change in myosin isoform distribution via posttranscriptional mechanisms. The lack of effect of dietary excess Leu on the expression of myosin in this study is consistent with Verhoeven et al. (2009), who mentioned that Leu does not affect the muscle mass in adult human males. On the contrary, Rieu et al. (2007) demonstrated that muscle protein synthesis in adult males is stimulated with the ingestion of a high protein-high Leu diet. Thus, further studies need to be conducted to elucidate the differential effect of dietary excess Leu or Ile.

In general, dietary excess Leu negatively affected the performance of growing pigs, which was directly associated with a reduction in the expression of the cationic aa transporter $b^{0,+}$ in jejunum and SC of Lys and Thr. Ile did not prevent the negative effect of excess Leu. Excess Ile tended to reduce the growth rate and feed efficiency of pigs, and this response was also associated with a decrease in the expression of $b^{0,+}$ and SC of Thr. Expression of myosin isoform IIB was differently affected by excess Ile, depending on the muscle type, but there was no effect of excess Leu, although Ile and Leu increased the expression of CAT-1. In conclusion, dietary excess Leu and Ile affect performance, expression of aa transporters, and SC of aa. Myosin was affected only by excess dietary Ile.

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