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A moderate threonine deficiency differently affects protein metabolism in tissues of early-weaned piglets

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ABSTRACT

A moderate threonine deficiency may affect differently tissue protein metabolism. In this study, we compared protein metabolism in the small and large intestines, the liver, and the carcass of piglets (*Sus scrofa*) pair-fed either a control well-balanced diet (C: 9.3 g threonine/kg diet) or a low threonine diet (LT: 6.5 g threonine/kg diet) for 2 weeks. In the small intestine, the LT diet did not modify protein deposition, fractional protein synthesis rate (K_S) and AA protein composition. Ubiquitin mRNA level, a component of the ubiquitin-dependent proteolytic pathway, was significantly decreased in the jejunum of the LT piglets. Protein deposition measured in the carcass and the colon, and K_S measured in the *semitendinosus* muscle and the colon, did not differ between LT and C piglets. Nevertheless, in these compartments, threonine content was reduced indicating deposition of proteins less rich in threonine. In the liver, protein retention was reduced, K_S was increased and AA protein composition was modified in the LT compared to the C piglets. In conclusion compared to the other compartments, small intestinal protein metabolism seems to be less sensitive to a moderate dietary threonine deficiency. This indicates that dietary threonine extraction by the small intestine may reduce threonine availability for the other tissues when young piglets were fed a diet marginally deficient in threonine.

1. Introduction

In mammals, threonine is an indispensable amino acid (AA). This implies that functions involving threonine are dependent on an adequate dietary supply. Dietary threonine is absorbed in the small intestine and, then, used by the peripheral tissues mainly for protein deposition associated with growth and with protein mass maintenance. During its intestinal absorption, more than one half of orally supplied threonine is extracted in first-pass by the small intestine of young pigs (Stoll et al., 1998; Le Floc'h and Sève, 2005; Van der Schoor et al., 2002). The high threonine extraction by the small intestine is an important nutritional question, notably because it determines threonine availability for tissues depending on threonine supplied by the blood, namely the colon and the non digestive tissues. In this context, our questioning was to determine if threonine extraction by the small intestine may compromise protein metabolism in the other tissues when young pigs are fed a diet marginally deficient in threonine.

In the present study, the objective was to compare the response of protein metabolism to a moderate threonine deficiency in the small intestine and the colon, the liver, the carcass and one skeletal muscle. In a first experiment, we measured the protein synthesis rate in the ileal and jejunal mucosa, the colon, the *semitendinosus* muscle and the liver after piglets were injected with a flooding dose of ^{15}N valine. Proteolytic systems also were investigated in the ileum, jejunum, and large intestine by measuring the mRNA levels for components of the lysosomal, the Ca^{2+}-activated, and the ATP-ubiquitin-dependent pathways. In a second experiment, we analysed the AA composition of the whole small and whole large intestines, the liver and the carcass in order to determine the impact of a low threonine supply on the AA profile of protein deposited in the different tissues. Threonine and protein retention were evaluated by the comparative slaughter technique.

2. Materials and methods

2.1. Animals and feeding

These experiments were conducted under the guidelines of the French Ministry of Agriculture for animal care. Experiment 1 was conducted on twenty two and experiment 2 on thirty Piétrain x (Landrace × Large White) piglets (*Sus scrofa*) from the experimental herd of INRA (Saint-Gilles, France). The same experimental design was used in both experiments. Piglets were weaned at 7 days of age (day 0) and blocks of two (experiment 1) and three (experiment 2) piglets were selected within litter on a body weight (BW) basis (2.57 ± 0.05 kg

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Table 1
Ingredients and nutritional values of the control well-balanced (C) and the low-threonine diet (LT)

	Diet	
	Low threonine (LT)	Control (C)
Ingredients (g kg^{-1} diet)		
Skimmed milk powder	250	250
Soluble fish protein concentrate	74.3	74.3
Free amino acids mix†	54.9	54.9
Maltodextrins	430.15	430.44
Sunflower oil	62.37	62.37
Ammonium citrate tribasic	30	30
Dicalcium phosphate	49	49
Trace element and vitamin premix‡	10	10
L-aspartic acid	39.28	36.48
L-threonine	–	2.51
Composition		
Dry matter (%)	92.9	92.8
Crude Protein (*N*×6.25), %	24.4	25
Lysine, g kg^{-1} diet	13.9	13.9
Threonine, g kg^{-1} diet	6.5	9.3
Net energy (MJ kg^{-1} diet)	10.9	10.9

† Providing the following amount of free AA (g per kg diet): L–lysine HCl, 3.53; L–tryptophane, 0.85; L–leucine, 1.86; L–isoleucine, 1.35; L–valine, 1.39; L–phenylalanine, 1.42; monoNa L–glutamate /glutamic acid (50/50), 35.3; glycine, 9.2.
‡ Providing the following amount of vitamins and minerals (per kg diet): Ca, 1.82 g; Fe, 200 mg; Cu, 40 mg; Zn, 200 mg; Mn, 80 mg; Co, 4 mg; Se 0.6 mg; I, 2 mg; vitamin A, 30 000 UI; vitamin D3, 6000 UI; vitamin E, 80 UI; vitamin B1, 4 mg; vitamin B2, 20 mg; panthotenic acid, 30 mg; vitamin B6, 20 mg; vitamin B12, 0.1 mg; vitamin PP, 60 mg; folic acid, 4 mg; vitamin K3, 4 mg; biotin, 0.4 mg; choline, 1600 mg; vitamin C, 200 mg.

and 2.50±0.05 kg, respectively in the first and the second study). In experiment 1, piglets were fitted, at weaning, with a jugular catheter under general anaesthesia and aseptic conditions (Melchior et al., 2004). In experiment 2, one piglet per block was slaughtered at weaning (day 0) for initial body composition determination, whereas the other two piglets were slaughtered 2 weeks later.

In both experiments, piglets were transferred from weaning in individual stainless-steel cage in a room maintained at 30 °C. Within block, one piglet received a control well-balanced diet (C diet) and the second a low threonine diet (LT diet). The composition of the experimental diets is presented in Table 1. Protein was supplied by skimmed-milk powder and a soluble fish protein concentrate. Those raw materials are highly digestible and set the basal low threonine content in both diets. Free AA were added to match the ideal protein pattern as proposed by Chung and Baker (1992) for weaning piglets. Free threonine, 2.51 g/kg of diet, was added in the C diet. Threonine content was 9.3 g/kg in the C diet and 6.5 g/kg in the LT diet. Diets provided 250 g of protein (*N*×6.25) and 15 MJ of digestible energy (DE) per kg, that corresponds to 10.9 MJ of net energy (NE) per kg. The meals were delivered four times daily (7:00, 11:00, 16:00 and 21:00) in liquid form by mixing the air-dry dietary mixture with warm water (1:2). In a previous study, we noticed that LT piglets ate more than C piglets probably in order to compensate their lower threonine supply (Hamard et al., 2007). Therefore, in the present study, LT piglets were pair-fed the intake of C piglets from day 3. Piglets were weighed at days 1, 4, 6, 8, 11 and 13 and were slaughtered 2 weeks after weaning (day 14) for different measurements.

2.2. Experimental procedures and measurements

2.2.1. Blood sampling for plasma AA concentration measurement (experiments 1 and 2)

In experiment 1, blood was collected, from the jugular catheter, before the injection of the valine solution. In experiment 2, blood was collected at slaughter. In both experiment, blood was collected into heparinised ice-cooled tubes and immediately centrifuged at 3000 g for 15 min at 4 °C. Plasma, 1 mL, was deproteinised in a sulfosalicylic acid solution (60 g/L) containing norvaline as an internal standard and stored at −20 °C until analysis. Analyses of AA were performed by ion exchange liquid chromatography Biotronik LC 5001 analyser, Germany) according to the method of Moore and Stein (1954).

2.2.2. Measurement of protein synthesis (experiment 1)

Fractional protein synthesis rate was measured *in vivo* according to the procedures described by Sève et al. (1993). Briefly, a solution of L-[^{15}N] valine was prepared by mixing 20% of L-[^{15}N] valine (99% mol% excess; Tracer Technologies, Sommerville, MA) with 80% of unlabelled L-valine to get a final enrichment of 19.8 mol% excess. Valine was diluted with water to reach a final concentration of 150 mmol.L^{-1}. All the piglets were fed 3 h before receiving 1.05 mmol.kg^{-1} BW of valine solution through the jugular catheter. Two mL of blood were taken just before the injection, then 7 and 14 min after the injection. Fifteen minutes after the injection, piglets were sacrificed with a lethal dose of pentobarbital and exsanguinated. Samples of *semitendinosus* muscle and liver were immediately collected and frozen in liquid nitrogen. The gastrointestinal tract was quickly removed. The small intestine, from the Treitz ligament to the ileo-caecal junction, was weighed empty of contents and its length was measured. It was divided in three parts of equal length, the proximal jejunum, the distal jejunum and the ileum. In the middle of the proximal jejunum and the ileum, 10 cm-segments were collected; mucosa was scraped on an ice-bed and frozen in liquid nitrogen. The large intestine was emptied, rinsed with water and weighed. A 10 cm-segment was collected; mucosa was scraped on an ice-bed and frozen in liquid nitrogen. All tissues were sampled and frozen 20–30 min after the injection. For all pigs, the tissues were collected and frozen following the same sequence, i.e. the liver, the muscle, the small intestine, and then the large intestine. This implies that, for each tissue, the time before freezing was similar between pigs.

Plasma samples (1 mL) were deproteinized in a trichloroacetic acid solution (10%) and centrifuged at 1800 g for 20 min at 4 °C. This step was repeated twice and the two supernatants were mixed. Plasma free AA contained in the supernatant were purified on Dowex cation exchange resin (50×8, 100–200 mesh, Sigma), eluted with NH_4OH 4M, dried and recovered in water. Tissue samples (1 g) were ground in a perchloric acid solution (0.2 mol/L). The protein precipitate was collected after centrifugation at 1800 g for 20 min at 4 °C and was washed three more times with the perchloric acid solution. Then, it was homogenized with 10 mL of NaOH (0.3 mol/L) and shaken for 1 h at 37 °C. One mL of the solution was removed for protein concentration measurement. Proteins were precipitated one more time with perchloric acid (2 mol/L) and centrifuged at 1800 *g* for 20 min at 4 °C. Total RNA concentration was determined in the supernatant. After two more washings with perchloric acid (0.2 mol/L), protein precipitate was hydrolysed in HCl (12 mol/L) for 48 h at 110 °C, filtered, dried, and suspended in water.

Plasma free valine enrichments (S_A) were measured by GC-MS (HP 5890 series II gas chromatograph coupled to a HP series 5971 mass spectrometer, Agilent technologies, USA) after derivatisation with N-tert-butyldimethylsilyl N-methyl-trifluoroacetamide (Mawhinney et al., 1986). Protein-bound valine enrichments (S_B) in the tissues were determined by GC-C-IRMS (HP 6890N gas chromatograph coupled to an IsoPrime mass spectrometer, GV Instruments, Manchester, UK) after derivatisation with ethyl chloroformate (Husek, 1991). The fractional synthesis rate, corresponding to the percentage of protein mass synthesised per day, was calculated as follows: K_S (%/day)=(S_B×100)/(S_A *x t*), where *t* is the L-[^{15}N] valine incorporation time (time elapsed between the injection of ^{15}N-Val and the sacrifice of the animals). Mucosal protein and RNA concentrations were measured as reported by Sève et al. (1993). Ribosomal capacity for protein synthesis (C_S) is used as an indicator of cellular potential for protein synthesis and calculated as the ratio of RNA to protein (mg/g). Finally the efficiency of protein synthesis was evaluated as the amount of protein