Genotype and dietary lysine deficiency affect carcass and muscle amino acid composition of pigs growing from 10 to 25 kg body-weight

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Abstract

Amino acid (AA) composition of body protein is considered constant although there are evidences that AA pattern in pigs may be altered by different factors. Pigs with different body composition and protein deposition rates -like fatty and lean pigs- may differ in AA composition, with possible consequences on their AA requirements. This work investigates effects of genotype and dietary lysine deficiency on AA composition of carcass and muscles of Iberian and Landrace × Large White pigs. Twenty-eight barrows (10 kg body weight (BW)), 14 from each breed, were used. They were randomly assigned to 2 experimental diets according to a factorial arrangement (2 breeds × 2 diets). Diets were isonitrogenous and isoenergetic (200±1 g CP/kg dry matter (DM); 14.7±0.1 MJ ME/kg DM) and with identical chemical composition except for lysine concentration (10.9 and 5.20 g lysine/kg DM, for lysine-adequate (AL) diet and lysine-deficient (DL) diet, respectively). Pigs were individually housed, and daily feed allowance was adjusted on a weekly basis according to BW. Pigs were slaughtered at 25 kg BW. Isoleucine, valine, and phenylalanine concentration were higher in carcass protein of Iberian pigs ($P<0.01$). In longissimus muscle, higher concentration of arginine, isoleucine, phenylalanine, lysine and valine ($P<0.001$ to $P<0.05$), and lower of methionine ($P<0.001$) were detected in Iberian pigs; whereas phenylalanine, leucine, lysine, threonine and methionine concentration decreased and arginine increased ($P<0.001$ to $P<0.05$) when pigs were fed DL diet. Genotype and lysine deficiency effects were moderate in the AA composition of protein of biceps femoris muscle. The results show that AA proportions in protein of carcass and longissimus muscle can be influenced by pig genotype and conditions of lysine shortage. The biceps femoris muscle, with different functional and metabolic properties, shows more constant AA composition than longissimus, which seem to prevail independent from genotype or nutritional challenges.

Keywords: amino acid composition; pig genotype; lysine deficiency; muscle protein; carcass protein

1. Introduction

Establishing optimal requirements for protein and amino acid (AA) allows designing balanced diets to optimize growth, development and body functions, minimize budget costs and reduce the environmental impact of pig production. For this purpose, knowledge of chemical carcass composition and its response to nutrient supply is a key tool. The Iberian pig is a fatty, non-selected pig-type (Nieto et al., 2019), which according to its lower body protein content requires less dietary protein than conventional lean pigs of similar body weight (BW) (Nieto et al., 2012). In previous studies, dietary protein was formulated following the optimum AA pattern -in terms of g AA/kg crude protein (CP)- established for conventional pigs (NRC,1998; BSAS, 2003). However, it remains questionable if using the dietary AA pattern established for conventional pigs is adequate for feeding these slow-growing, fatty pigs.

Although it is generally assumed that the AA composition of body protein is constant, there are some evidences in pigs that AA content of body proteins may be affected by different factors such as protein and energy intake (Bikker, Verstegen, & Bosch, 1994), body weight (Mahan & Shields, 1998) and essential AA supply (Conde-Aguilera, Cobo-Ortega, Mercier, Tesseraud, & van Milgen, 2014). Such changes in the body AA pattern have been related to alterations in the relative proportions of body proteins (actin, myosin, collagen, etc.) which have different AA proportions (Chung & Baker, 1992). In addition, deficiencies in indispensable AA supply have been associated to changes in muscle AA composition and metabolic properties (Katsumata, Matsumoto, Kobayashi, & Kaji, 2008; Conde-Aguilera et al., 2016). It is also possible that pigs with marked differences in body composition and protein deposition rates, like fatty and lean pig genotypes, could differ in AA composition of body proteins. One of the objectives of this work was to assess differences in AA composition of carcass and muscles between Iberian and conventional lean pigs in identical experimental conditions. These measurements could be considered as a starting point from which differences in AA requirements between pig genotypes -in terms of AA composition of dietary protein- could be envisaged, despite the influence of other factors such as tissue turnover rates and maintenance AA requirements (Mahan & Shields, 1998). In this sense, in a study designed with Iberian piglets to determine the proportion of lysine in dietary protein for optimal growth and carcass protein deposition, it was observed that higher rates of carcass protein retention were obtained with 64 g lysine/kg protein (Nieto, Barea, Lara, Palma-Granados, & Aguilera, 2015), a ratio approximately 14% lower than that recommended for conventional piglets (NRC, 2012). In addition, Rivera-Ferre, Aguilera, & Nieto (2006) found a greater decrease in N retention on a metabolic body size basis in Landrace than in Iberian gilts when fed a lysine deficient diet (35% of the recommended lysine content).

With this background, the hypothesis tested in this work is if under similar nutritional and physiological conditions, two pig genotypes which show clear differences in potential for lean tissue deposition, as the Iberian and Landrace × Large-White (LDW) pigs, will have a different AA profile of body protein and will respond differently in terms of AA body pattern to cope with a restricted lysine supply. With this purpose, the AA composition of carcass and muscle protein of both pig genotypes was assessed in young pigs fed similar amounts of either a lysine-adequate (AL) or a lysine-deficient (DL) diet, both of similar total protein and ME contents. As plasma AA contents are the result of inputs from dietary intake and AA released by tissues (proteolysis and de novo synthesis, mainly by muscle), and outputs to AA oxidation and metabolism (synthesis of proteins and other molecules), plasma free AA contents were also tested.

2. Materials and methods

The experimental procedures and animal care were carried out according to current Spanish legislation (RD53/2013), and the authorization to experiment on living animals was approved by the Bioethical Committee of the Spanish National Research Council (CSIC, Spain) and the competent local authority (Junta de Andalucía, Spain).

2.1 Animals, experimental design, and treatments

Animals, diets and experimental design have been described in detail elsewhere (Palma-Granados et al., 2017). Briefly, 14 purebred Iberian (Silvela strain) barrows and 14 LDW barrows from a commercial lean cross were housed in individual pens of 2 m^2 in an environmentally controlled room (10.3±0.3 and 10.4±0.3 kg BW; 58 and 50 days of age, respectively). Pigs from both breeds were randomly assigned to each of two experimental diets. Diets were isonitrogenous and isoenergetic [200±1 g crude protein (CP) and 14.7±0.1 MJ metabolizable energy per kg dry matter (DM), Table 1]. One diet was adequate in AA and rest of dietary nutrients (AL, 10.9 g lysine/kg DM) (NRC, 1998) and the other was of identical composition except for the lysine content (DL, 5.20 g lysine/kg DM). In the DL diet, synthetic L-lysine·HCl was replaced by L-glutamic acid and glycine to maintain the same nitrogen concentration as in AL diet. The rest of AA remained constant and in sufficient amounts (NRC, 1998; BSAS, 2003). Daily feed allowance was adjusted weekly for each pig according to a BW function developed for the Iberian pig breed (Conde-Aguilera, Aguinaga, Aguilera, & Nieto, 2011). To ensure similar feed intake among genotypes, pigs were fed at 85% of ad libitum intake (according to individual BW) of Iberian pigs, as they have greater intake capacity (Morales, Pérez, Baucells, Mourot, & Gasa, 2002). Feed refusals were monitored daily. Water was freely available.

At 25 kg BW pigs were slaughtered by exsanguination after electrical stunning. Immediately after slaughter blood, carcass and organs were weighed separately. Blood samples (approximately 100 mL obtained in EDTA) were processed to obtain plasma that was stored at -80ºC until analysed. The eviscerated carcasses (without head and feet) were weighed, divided longitudinally, and kept at −20ºC until analysis. The longissimus and biceps femoris (b. femoris) muscles from the left side were rapidly dissected, weighed and stored at -20ºC until analysis. The right half of the carcass was processed as described previously (Nieto et al. 2015). Carcass was cut into small pieces and ground in a mincer (Talleres Cato, Sabadell, Spain), homogenized in a cutter (Talleres Cato, Sabadell, Spain) and subsamples (approximately 500 g) were taken for freeze-drying. The whole longissimus (300-500 g) and b. femoris (170-300 g) muscles were cut into small pieces, ground in a mincer (Moulinex, Barcelona, Spain) and freeze-dried. After freeze-drying carcass and muscles samples were ground with liquid nitrogen (Retsch Ultra Centrifugal Mill ZM 200; Restch GmbH, Haan, Germany) before analyses.

[Insert Table 1 near here]

2.2 Chemical Analysis

All analyses were performed in duplicate. The dry matter and ash content of feeds were analyzed by standard procedures (method 934.01 and 942.05, respectively, AOAC, 2005). The crude fat content of feeds was determined by ether extraction (method 920.39C: AOAC, 2005). Gross energy of feeds was determined in an isoperibolic bomb calorimeter (Parr Instrument Co., Moline, IL). Total nitrogen in feeds and in freeze-dried carcass and muscles was determined by Dumas method (AOAC method 990.03) in a Leco TruSpec CN equipment (St. Joseph, Michigan, USA). Crude protein content was calculated using the factor of 6.25.

The AA content of feeds, carcass and muscles were determined after hydrolysis in 6 N HCl plus 1% phenol in sealed, evacuated tubes at 110°C for 24 h by high-performance liquid chromatography (HPLC), according to the Waters Pico Tag method (Cohen, Meys, & Tarvin, 1989) with pre-column derivatization with phenylisothiocianate using a Waters 2695 separation module (Waters Cromatografía, SA, Spain). The cysteine and methionine contents were determined as cysteic acid and methionine sulphone, respectively, after oxidation with performic acid before protein hydrolysis (Moore, 1963). Tryptophan was not determined.

Plasma free AA concentration [μmol/L] were analysed by HPLC according to the Waters Pico Tag method for free AA (Cohen et al., 1989). Before derivatisation, 500 μL of plasma were deproteinized by diluting 1:1 with 20% Trichloroacetic acid and 2mM Norleucine as an internal standard, and centrifuged at 11,000 g and 4ºC for 15 min. A Millenium 32 chromatography manager system was used for gradient control and data processing.

2.3 Statistical analysis

Statistical analyses were assessed by ANOVA using the GLM procedure of SAS. The effects of genotype (Iberian, LDW), lysine content, and their interactions were included in the statistical model. Pig was considered the statistical unit. When interaction between main factors was significant, means from each of the four treatment combinations were compared by one-way ANOVA. Results are expressed as least squares means. Statistical significance was assessed by Tukey´s t-test. The level of significance was set to 5%. Statistical tendencies were considered at $P < 0.10$.

3. Results

Piglets remained healthy during the experiment. Results on growth performance have been published elsewhere (Palma-Granados et al., 2017). Briefly, average DM intake (g/day) was higher in Iberian piglets (p<0.05). Growth rate [g/kg BW·day] and feed efficiency (gain:feed) were higher in the LDW breed and decreased in both pig types when fed the DL diet, particularly in LDW pigs (genotype × diet interaction, p<0.01). Carcass yield was reduced in pigs fed the DL diet (p< 0.01). Muscle weights, higher in the LDW pigs (P<0.01), were reduced in both pig genotypes when fed the DL diet (p< 0.01).

3.1 AA composition of carcass

Results on protein and AA concentration in the carcass are shown in Table 2.

Carcass protein concentration was similar in both pig types when fed the AL diet (151 g CP/kg carcass). Lysine deficiency reduced carcass protein concentration, although the decrease was sharper in Iberian pigs (genotype × diet interaction, p<0.01). Genotype differences were found for most indispensable AA [g AA/kg CP carcass]. Isoleucine, valine, and phenylalanine were 5 to 9% higher in Iberian pigs (p<0.01), whereas threonine and methionine tended to be lower (p=0.06). No difference in leucine carcass concentration was detected. Genotype × diet interactions were found for lysine and histidine (p<0.05). Dietary lysine restriction reduced histidine concentration only in the carcass of Iberian pigs (13%). Lysine in carcass was decreased (13%) in LDW pigs but not in Iberian pigs when both were fed the DL diet (p < 0.05). Leucine decreased (6 to 10%, p<0.001) and arginine tended to increase slightly (2 to 4%, p=0.09) in carcass protein of both pig types when fed the DL diet.

For dispensable AA, LDW pigs had higher carcass concentrations of aspartic acid, glutamic acid and serine (4 to 14%; p<0.05) and lower of alanine (9%; p<0.01). When pigs were fed the DL diet, alanine and glycine increased (4 to 14%, p<0.01) while tyrosine and cysteine were reduced (6 to 10%, p<0.01). Total indispensable AA concentration in carcass was higher in Iberian than in LDW pigs ($p<0.01$), and it decreased in both pig genotypes when the DL diet was offered ($p<0.001$), whereas total dispensable AA concentration showed opposite effects: it was higher in LDW pigs ($p<0.05$) and increased in both pig types when fed DL diet ($p<0.001$).

[Insert Table 2 near here]

3.2 AA composition of muscles

For longissimus muscle (Table 3), protein concentration [g/kg] was higher in Iberian than in LDW pigs, and decreased when pigs were fed the DL diet, particularly in Iberian pigs (genotype × diet interaction, $p<0.05$). Whereas in carcass the most abundant AA were glutamic acid, glycine, arginine followed by alanine and aspartic acid, in this muscle the prevailing AA were glutamic and aspartic acids, lysine, leucine and arginine. Higher concentration of arginine, isoleucine, phenylalanine, lysine and valine (4.6 to 7.5%; $p<0.001$ to $p<0.05$) and lower of methionine (7.2%; $p<0.001$) were observed in longissimus of Iberian pigs compared with LDW pigs. No genotype differences were detected for leucine and threonine. Differences in histidine were detected only for Iberian pigs fed the DL diet (genotype × diet interaction, $p<0.05$). *P*igs fed the DL diet had decreased concentration of phenylalanine, leucine, lysine, threonine and methionine, and higher of arginine ($p<0.001$ to $p<0.05$). Regarding dispensable AA, increased concentration of alanine, proline and cysteine were found in Iberian pigs but not in LDW when fed the DL diet (genotype × diet interaction, $p<0.05$). Glycine increased and tyrosine decreased in pigs fed the DL diet ($p<0.01$).

[Insert Table 3 near here]

The AA composition of b. femoris muscle is presented in Table 4. Protein concentration was higher in Iberian pigs and it was reduced in Iberian but not in LDW pigs when fed the DL diet (genotype × diet interaction, $p<0.05$). In general, less changes in AA concentration due to genotype or dietary lysine deficiency were detected in b. femoris in comparison to longissimus muscle. Arginine was higher and histidine lower in Iberian pigs (*p*<0.01). Also, less glycine and proline (*p*<0.001) and higher tyrosine and cysteine concentration (*p*<0.05) were observed for Iberian pig muscles. *P*igs fed the DL diet tended to have lower lysine concentration (*p*=0.08) and had higher glycine concentration (*p*<0.001). For alanine a genotype × diet interaction was detected (*p*<0.01). [Insert Table 4 near here]

3.3 Plasma free AA concentration

Plasma free AA concentrations [µmol/l] are shown in Table 5. Lower threonine and methionine in plasma were detected for Iberian pigs (*p*<0.001). Arginine, histidine and the branched chain AA remained similar among genotypes. Lysine concentration was higher in Iberian pigs, and its reduction was more pronounced in this genotype when fed the DL diet (genotype × diet interaction, *p*<0.001). Tryptophan was also higher in Iberian pigs, and decreased in this pig type when the DL diet was offered (genotype × diet interaction, *p*<0.05). Branched chain AA decreased (13 to 38%; *p*<0.05 to *p*<0.001) and phenylalanine increased (*p*<0.01) when pigs were fed the DL diet. Concentration of most dispensable AA was reduced in plasma of Iberian pigs in comparison with LDW pigs (except for hydroxyproline, taurine and tyrosine). The reduction of dietary lysine increased plasma contents of γ-amino-butyric acid, glycine, serine and tyrosine (*p*<0.05 to *p*<0.01) and reduced those of glutamic acid and hydroxyproline (*p*<0.001). Alanine decreased in LDW but not in Iberian pigs fed the DL diet (genotype × diet interaction, *p*<0.05). The sum of total AA in plasma was 34% lower in Iberian pigs (*p*<0.001). [Insert Table 5 near here]

4. Discussion

Comparing breeds of different growth potential implies some difficulties as developmental age of animals may differ, and it needs to be decided whether to use pigs of the same weight or age. To sort out this inconvenient, we used young pigs of the same weight at the start of the experiment, very close in age, and with similar nutritional requirements (Conde-Aguilera et al., 2011). An additional problem concerns their unequal capacity for food intake, considerably greater in Iberian pigs compared to leaner pig breeds (Morales et al., 2002). This was counteracted by the reduction practiced over the theoretical ad libitum consumption of the Iberian pig.

4.1 Genotype effects on AA composition of carcass and muscle

The AA composition of carcass protein found in this work is, in general, in the range of previously reported values for carcass of pigs of 20 to 45 kg BW (Bikker et al., 1994; Mahan & Shields, 1998; Hulshof, van der Poel, Hendriks, & Bikker, 2017). Regarding the present work, the most common limiting AA in pig diets (lysine, methionine and threonine) are in similar or slightly lower relative amounts in Iberian pig carcasses compared to LDW pigs. The differences in carcass concentration found for some indispensable AA (i.e., higher isoleucine, valine, and phenylalanine concentrations observed in carcass protein of Iberian pigs) deserve some attention. The isoleucine/lysine, valine/lysine and phenylalanine/lysine ratios were 0.51 and 0.49; 0.71 and 0.69; and 0.57 and 0.54 for carcass protein of Iberian and LDW pigs, respectively, which may indicate relatively higher requirements of these three AA for Iberian growing pigs. Most of the breed differences for AA composition noticed in carcass protein were also observed in longissimus muscle, in which additional differences for arginine, histidine and lysine were detected. The AA composition of b. femoris muscle was less affected by genotype, and restricted to arginine and histidine as indispensable AA. These differences in carcass and muscle indispensable AA composition were not reflected in circulating levels of plasma free AA with some exceptions (lysine, threonine and methionine), although the sum of indispensable, dispensable, and total AA was always higher in the LDW pigs, according to their higher protein pool. However, relations among these different AA pools are complex as plasma AA concentration are the result of inputs from dietary intake and AA released by tissues (proteolysis and de novo synthesis, mainly by muscle), and outputs to AA oxidation and metabolism (synthesis of proteins and other molecules) (see review by Liao et al. 2018).

Genotype differences in AA composition of longissimus and b. femoris have been reported for Landrace and Bama mini-pigs at different growth stages (methionine, isoleucine and phenylalanine for longissimus; and threonine, methionine, leucine, lysine and histidine for b. femoris; mg AA/g tissue) (Liu et al., 2015). In contrast, in muscle homogenates from French Landrace, Belgian Landrace and Pietrain pigs, Duee, Calmes, & Desmoulin (1998) found an increase in concentration of some indispensable AA (g AA/100 g CP) with increasing slaughter weight (lysine, threonine, phenylalanine and methionine), but no difference according to genotype.

The reasons for the genotypes differences detected in our work are unknown, although it could be related to changes in the relative proportions of different carcass proteins, from muscle or other carcass tissues differing in AA composition. Although carcass AA composition is only one of the factors influencing pig AA requirements, it is worth to investigate further the requirements of the branched-chain AA valine and isoleucine, and phenylalanine of Iberian growing pigs. Improved growth performance could be achieved if diet composition can be optimally adjusted to animal needs. This can be particularly important in young Iberian growing pigs, a stage at which higher efficiency of protein utilization is observed compared to heavier animals (Nieto et al., 2012), similarly to leaner pig genotypes.

4.2 Effects of lysine deficiency on AA composition of carcass and muscle

Feeding a diet deficient in lysine affects the AA composition of carcass protein in young pigs of fatty and lean type. Lysine concentration decreased (by 13%) only in carcass protein of LDW pigs, probably because the lack of lysine was more severe for lean than for Iberian pigs. Actually, pigs from both types decreased their growth rate when fed the lysine deficient diet, but the decline was greater for LDW pigs (Palma-Granados et al., 2017). However, in longissimus muscle the reduction in lysine concentration was detected for both pig types. In contrast, Chang & Wei (2005) found no changes in AA concentration of several muscles (including longissimus) of pigs fed lysine deficient diets, although lysine restriction was shorter than in the present experiment.

Histidine concentration decreased only in protein of carcass and longissimus muscle of Iberian pigs. When muscle protein degradation occurs, part of histidine is unavailable for reutilization in muscle protein synthesis because of its post-transcriptional methylation to 3-methyl-histidine, which is excreted in urine, although in pigs partly is accumulated in muscle in the dipeptide balanine, in contrast to other mammalian species (Harris & Milne, 1981). Whether this lower concentration of histidine in the carcass and longissimus of Iberian pigs fed lysine deficient diets is related to higher muscle protein degradation remains uncertain.

Leucine concentration decreased in carcass and longissimus of both types of pigs. Decreased plasma free leucine, and also of the branched-chain isoleucine and valine, were observed in pigs fed the lysine deficient diet. Plasma free AA could resemble plasma free AA pools in tissues available for protein synthesis. In particular, the decrease in plasma free leucine concentration, along with the lower IGF-1 and insulin levels detected in both fatty and lean pigs fed lysine deficient diets (Palma-

Granados et al., 2017), agree with the decreased muscle protein synthesis rates reported in previous papers, as all these factors stimulate positively muscle protein synthesis via the mTOR signalling pathway (Miyazaki & Esser, 2009).

Contrary to longissimus, the AA composition of b. femoris muscle, particularly indispensable AA concentration, was not affected by lysine deficiency, except for a trend of lower lysine concentration. There are some differences in AA composition between the two muscles studied. Biceps femoris is richer in indispensable AA compared to longissimus. Variations in muscle composition and responses may relate to differences in their function and metabolic properties. Longissimus is a predominantly fast-twitch postural muscle, whereas b. femoris is a mixed type muscle (Andrés et al., 2001), involved in locomotion. In both types of pigs the response to lysine deficiency followed a similar pattern: both muscles have decreased growth, but only longissimus changed its protein content and amino acid composition significantly, whereas changes in composition of b. femoris were rather moderate. This finding agrees with previous observations in pigs and other species in which predominantly fast-twitch muscles seem more sensitive to nutrient restrictions or to other external factors than muscles with more oxidative metabolism (Kelly & Goldspink, 1982; Conde-Aguilera et al., 2016), and denotes that different proteins and body components may be affected differently by a shortage in lysine supply. It also has been suggested that muscles related to mobility, which develop earlier in life (based on their allometric growth coefficients) are less prone to be affected by external factors (as nutrient shortage) than muscles of higher allometric growth coefficients, like longissimus, considered a developmentally retarded muscle (Bee et al., 2007).

In conclusion the breed differences found in concentration of some indispensable AA in carcass and muscle protein may indicate higher requirements of these amino acids relative to lysine in Iberian compared to lean pigs. This fact, that deserves deeper research, would question the application of the dietary AA pattern established for conventional pigs for feeding slow-growing, fatty pigs. Both fatty and lean pigs responded in general terms in a similar way to a dietary lysine deficiency, modifying the rate of body and muscle growth and modulating the protein composition of specific carcass components, a fact possibly related with their different body functions.

Disclosure statement

The authors declare no conflict of interest

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Table 1. Ingredients and nutrient composition of experimental diets fed to Iberian and Landrace × Large-White pigs.

	Experimental diets[†]	
	AL	DL
Ingredients [g/kg as fed]		
Corn	420	420
Barley grain, 2-row	340	340
Soybean protein concentrate, 65% CP	35	35
Corn gluten meal, 65% CP	112	112
Dibasic calcium phosphate	17	17
Calcium carbonate	6.6	6.6
NaCl	5	5
Vitamin/mineral pre-mix[‡]	3	3
L-lysine·HCl, 78.8%	8.16	-
L-threonine, 98%	2.65	2.65
DL-methionine, 99%	0.4	0.4
L-tryptophan, 98%	1.15	1.15
L-valine, 96.5%	1.8	1.8
L-glutamic acid, 99%	-	4.08
Glycine, 99%	-	4.08
Corn starch	47.14	47.14
Analysed nutrient composition [g/kg DM]		
Crude protein	201	199
Lysine	10.9	5.20
Methionine	3.77	3.70
Methionine+Cysteine	6.03	5.92
Threonine	9.61	9.58
Tryptophan[§]	2.40	2.40
Isoleucine	6.73	6.58
Leucine	22.4	22.5
Histidine	5.17	5.00
Phenylalanine and tyrosine	10.2	10.0
Valine	10.3	10.3
Lipids	25.5	25.8
Total ash	49.5	51.0
Gross energy [MJ/kg]	18.66	18.53

[†]AL, lysine-adequate content diet; DL, lysine-deficient-content diet.

[‡]Provided (per kg of diet) 3.38 mg retinol as retinyl acetate, 56.3 µg cholecalciferol, 25.2 mg dl-α-tocopherol as dl-α-tocopheryl acetate, 1.5 mg menadione as menadione sodium bisulfite, 0.15 mg thiamine, 3 mg riboflavin, 0.15 mg pyridoxine, 15 µg cyanocobalamin, 15 µg folic acid, 22.5 mg nicotinic acid, 15 mg d-pantothenic acid as calcium pantothenate, 15 mg Mn as $MnSO_4.4H_2O$, 75 mg Fe as $FeSO_4.7H_2O$, 120 mg Zn as ZnO, 450 µg I as KI, 60 mg Cu as $CuSO_4.5H_2O$, and 300 µg Co as $CoSO_4.7H_2O$.

[§]Calculated (de Blas, Mateos, & García Rebollar, 2010).

Table 2. Effects of genotype and dietary lysine content on crude protein and amino acid (AA) content of carcass of piglets growing from 10 to 25 kg BW[†]

Pig genotype	Iberian		LDW		SEM	*p*-value[‡]		
Diet	AL	DL	AL	DL		Genotype	Diet	Genotype × diet
Final BW [kg]	26.6	24.2	26.2	22.1	0.8	NS	***	NS
Carcass [g/kg	659	647	669	655	4	0.058	**	NS
CP content [g/kg]	152^{a}	126^{c}	150^{a}	132^{b}	1	NS	***	**
Indispensable AA [g/kg crude protein]								
Arginine	82.2	85.4	84.3	86.0	1.4	NS	0.091	NS
Histidine	38.0^{b}	32.9^{a}	38.9^{b}	37.4^{b}	0.8	**	***	*
Isoleucine	33.6	33.8	31.9	30.4	0.8	**	NS	NS
Phenylalanine	37.9	37.7	34.9	32.2	0.9	***	NS	NS
Leucine	67.6	63.5	66.8	60.2	1.3	NS	***	NS
Lysine	66.3^{b}	65.4^{b}	64.9^{b}	56.7^{a}	1.5	**	**	*
Threonine	39.0	39.8	41.1	40.1	0.6	0.060	NS	NS
Valine	47.2	48.0	44.6	44.3	0.7	***	NS	NS
Methionine	19.5	17.7	20.2	19.8	0.7	0.062	NS	NS
Σ indispensable AA	431	424	427	407	4	**	***	NS
Dispensable AA [g/kg crude protein]								
Alanine	72.0	75.2	66.0	72.3	1.5	**	**	NS
Aspartic acid	71.3	71.9	82.7	78.7	2.4	***	NS	NS
Glutamic acid	132	134	138	139	2	*	NS	NS
Glycine	102	112	96.1	110	2.7	NS	***	NS
Proline	67.7	68.6	67.1	69.7	1.6	NS	NS	NS
Serine	40.5	41.2	42.8	43.0	0.5	***	NS	NS
Tyrosine	30.8	28.2	30.2	28.3	0.6	NS	**	NS
Cysteine	10.2	9.4	10.5	9.5	0.3	NS	**	NS
Σ dispensable AA	526	540	533	551	4	*	***	NS

[†]n = 7 individually housed piglets per each genotype × diet combination; LDW = Landrace × Large White pigs; AL = lysine-adequate diet (10.9 g lysine/kg DM); DL = lysine-deficient diet (5.20 g lysine/kg DM).

[‡]NS = not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Within a row means with different superscripts differ ($p < 0.05$).

SEM =standard error of the mean.

Table 3. Effects of genotype and dietary lysine content on protein [g/kg muscle] and amino acid (AA) content [g/kg crude protein] in longissimus muscle of piglets growing from 10 to 25 kg BW†

Pig genotype	Iberian		LDW		SEM	*p*-value‡		
Diet	AL	DL	AL	DL		Genotype	Diet	Genotype × diet
Muscle weight [g]	401	299	505	328	28	**	**	NS
Protein	204^{c}	158^{a}	180^{b}	157^{a}	6	*	***	*
Indispensable AA								
Arginine	73.1	79.0	69.5	72.8	1.1	***	***	NS
Histidine	50.1^{bc}	45.4^{c}	54.5^{ab}	57.4^{a}	1.6	***	NS	*
Isoleucine	43.1	41.3	40.7	39.7	0.9	*	NS	NS
Phenylalanine	36.5	35.6	34.9	33.8	0.5	***	*	NS
Leucine	76.9	76.4	77.1	74.0	0.9	NS	*	NS
Lysine	83.6	80.2	79.6	78.0	1.1	**	*	NS
Threonine	43.3	42.8	43.8	42.3	0.3	NS	**	NS
Valine	48.6	47.1	45.2	44.4	0.8	***	NS	NS
Methionine	19.2	14.3	20.7	17.9	0.7	***	***	NS
Σ indispensable AA	475	462	466	460	3	NS	*	NS
Dispensable AA								
Alanine	55.7^{b}	59.1^{a}	57.2^{ab}	57.4^{ab}	0.7	NS	*	*
Aspartic acid	99.6	95.9	96.1	97.5	1.6	NS	NS	NS
Glutamic acid	162	164	161	163	1	NS	0.054	NS
Glycine	45.6	54.5	51.7	57.0	2.1	*	**	NS
Proline	39.6^{b}	43.6^{a}	46.4^{a}	44.9^{a}	1.2	**	NS	*
Serine	37.6	38.7	39.1	38.5	0.6	NS	NS	NS
Tyrosine	31.9	28.8	29.4	28.3	0.6	*	**	NS
Cysteine	5.10^{b}	6.21^{a}	5.10^{b}	5.25^{b}	0.23	*	**	*
Σ dispensable AA	477	491	486	492	3	NS	**	NS

†n = 7 individually housed piglets per each genotype × diet combination; LDW = Landrace × Large White pigs; AL = lysine-adequate diet (10.9 g lysine/kg DM); DL = lysine-deficient diet (5.20 g lysine/kg DM).

‡NS = not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Within a row means with different superscripts differ ($p < 0.05$).

SEM =standard error of the mean.

Table 4. Effects of genotype and dietary lysine content on protein [g/kg muscle] and amino acid (AA) content [g/kg crude protein] in biceps femoris muscle of piglets growing from 10 to 25 kg BW†

Pig genotype	Iberian		LDW		SEM	*p*-value‡		
Diet	AL	DL	AL	DL		Genotype	Diet	Genotype × diet
Muscle weight[g]	252	170	305	176	13	*	***	NS
Protein	193^{c}	170^{b}	168^{ab}	161^{a}	3	***	***	*
Indispensable AA								
Arginine	84.0	85.0	80.4	80.3	1.2	**	NS	NS
Histidine	49.6	48.6	51.7	53.4	1.1	**	NS	NS
Isoleucine	43.1	43.8	44.2	43.4	1.0	NS	NS	NS
Phenylalanine	38.4	39.2	39.8	39.7	0.7	NS	NS	NS
Leucine	70.8	71.1	74.1	72.5	1.2	0.057	NS	NS
Lysine	87.3	84.1	86.1	85.4	1.1	NS	0.084	NS
Threonine	44.5	44.4	44.5	44.5	0.4	NS	NS	NS
Valine	46.9	47.1	47.4	46.3	0.0	NS	NS	NS
Methionine	24.9	23.6	23.8	24.4	0.9	NS	NS	NS
Σ indispensable AA	490	487	492	490	3	NS	NS	NS
Dispensable AA								
Alanine	53.6^{b}	55.2^{a}	54.9^{a}	54.8^{a}	0.3	0.069	**	**
Aspartic acid	94.2	92.2	90.6	90.7	1.4	0.083	NS	NS
Glutamic acid	146	144	143	142	2	NS	NS	NS
Glycine	44.4	47.9	46.9	49.7	0.4	***	***	NS
Proline	36.7	38.6	40.1	40.8	0.7	***	0.081	NS
Serine	38.6	38.6	38.1	38.6	0.6	NS	NS	NS
Tyrosine	38.8	37.7	37.3	37.2	0.3	***	*	NS
Cysteine	8.00	8.25	6.89	5.89	0.73	*	NS	NS
Σ dispensable AA	460	463	458	460	3	NS	NS	NS

†n = 7 individually housed piglets per each genotype × diet combination; LDW = Landrace × Large White pigs; AL = lysine-adequate diet (10.9 g lysine/kg DM); DL = lysine-deficient diet (5.20 g lysine/kg DM).

‡NS = not significant; * $p< 0.05$, ** $p< 0.01$, *** $p < 0.001$. Within a row means with different superscripts differ ($p <0.05$).

SEM =standard error of the mean.

Table 5. Effects of genotype and dietary lysine content on amino acid concentration in the plasma [μmol/l] of piglets growing from 10 to 25 kg BW†

Pig genotype	Iberian		LDW		SEM	p-value‡		
Diet	AL	DL	AL	DL		Genotype	Diet	Genotype × diet
Indispensable AA								
Arginine	117	80	97	107	12	NS	NS	NS
Histidine	88	72	75	99	10	NS	NS	NS
Isoleucine	175	141	189	135	10	NS	***	NS
Phenylalanine	55	79	64	91	7	NS	**	NS
Leucine	284	205	333	206	25	NS	***	NS
Lysine	126^{a}	48^{bc}	68^{b}	37^{c}	7	***	***	**
Threonine	182	286	558	708	103	***	NS	NS
Valine	401	347	399	303	29	NS	*	NS
Methionine	33	37	61	53	5	***	NS	NS
Tryptophan	49^{a}	32^{b}	39^{b}	33^{b}	2	0.064	***	*
Σ indispensable AA	1510	1320	1880	1770	180	*	NS	NS
Dispensable AA								
Alanine	449^{b}	503^{b}	1064^{a}	719^{b}	91	***	NS	*
Anserine	104^{b}	111^{b}	174^{a}	135^{b}	9	***	0.096	*
Aspartic acid	50^{a}	10^{b}	31^{ab}	37^{ab}	9	NS	0.087	*
γ-amino-butyric acid	30	41	47	67	6	***	*	NS
Glutamine	269	285	422	343	27	***	NS	NS
Glutamic acid	392	228	601	325	55	*	***	NS
Glycine	850^{a}	1116^{a}	1077^{a}	2165^{b}	187	**	**	*
Hydroxyproline	73	46	76	44	4	NS	***	NS
Ornithine	76	76	101	120	13	*	NS	NS
Proline	316	345	537	628	64	***	NS	NS
Phosphoserine	16	14	35	30	3	***	NS	NS
Serine	239	375	396	536	42	***	**	NS
Taurine	168	189	197	197	17	NS	NS	NS
Tyrosine	148	157	146	218	18	NS	*	NS
Σ dispensable AA	3870	4050	6240	6480	43	***	NS	NS
Σ Total AA	5380	5380	8120	8250	58	***	NS	NS

†n = 7 individually housed piglets per each genotype × diet combination; LDW = Landrace × Large White pigs; AL = lysine-adequate diet (10.9 g lysine/kg DM); DL = lysine-deficient diet (5.20 g lysine/kg DM).

‡NS = not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Within a row means with different superscripts differ ($p<0.05$).

SEM =standard error of the mean.