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RESEARCH ARTICLE

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Addition of arginine and leucine to low or normal protein diets: performance, carcass characteristics and intramuscular fat of finishing pigs*

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Abstract

The effect of dietary crude protein (CP) reduction, supplementation with arginine or leucine on intramuscular fat (IMF) content was evaluated in (Landrace × Duroc) × Pietrain pigs. One-hundred and eight barrows (67 ± 4 kg) were assigned to six diets (n=6 pens of 3 pigs each): four normal CP diets containing 16% CP from 60 to 90 kg and 13% CP from 90 to 115 kg live weight (normal protein; normal protein high Arg, normal protein high Leu or normal protein high Arg and Leu) and two low CP diets containing 14% CP from 60 to 90 kg and 11.8% CP from 90 to 115 kg live weight (with or without supplementation of both amino acids). The high Leu and Arg diets were supplemented to obtain ratios of standard ileal digestible Leu/Lys and Arg/Lys of 4 and 2, respectively. While feed to gain ratio tended to increase ($p<0.05$), final weight ($p<0.01$), average daily feed intake (ADFI) ($p<0.05$) and average daily gain (ADG) ($p<0.01$) were reduced in animals fed low-protein diets supplemented with Arg and Leu compared to the ones fed low-protein diet unsupplemented. Marbling and IMF content in loin were reduced when Arg was supplemented ($p<0.05$) in normal protein diets. Supplementing these diets with Arg also reduced belly weight ($p<0.01$) and increased lean meat percentage ($p<0.05$). Contrary to the initial hypothesis, reduction of CP or dietary supplementation with Leu had no effect on IMF content and supplementation with Arg reduced it.

Additional key words: amino acid; branched chain amino acids antagonism; meat quality; loin depth; marbling; subcutaneous fat.

Abbreviations used: ADFI (average daily feed intake); ADG (average daily gain); Arg (arginine); BCAA (branched chain amino acids); CP (crude protein); FGR (feed to gain ratio); IMF (intramuscular fat); Leu (leucine); LM (*Longissimus* muscle); LP (low protein); LPAL (low protein plus arginine and leucine); NIT (near infrared transmittance); NP (normal crude protein); NPA (normal crude protein plus arginine); NPAL (normal crude protein plus arginine and leucine); NPL (normal crude protein plus leucine); SM (*Semimembranosus* muscle).

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Introduction

One of the characteristics that influence the quality of meat as perceived by the consumer is the amount of intramuscular fat (IMF), which is located within the structure of muscle. In terms of taste, IMF is well correlated with pork acceptability. The threshold level of IMF needed for optimal eating quality of pork proposed is between 2.2 and 3.4% (Font-i-Furnols *et al*., 2012). In the last decades, the trend in pig production has been to increase the carcass lean content, reduce carcass fat, and the end result is that IMF content has decreased. Some studies indicate that for some modern genotypes

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IMF can be as low as 1% (Channon *et al*., 2001). One of the challenges for nutritionists is to increase the level of IMF without increasing backfat or impairing the animal growth.

It has been reported that supplementing pig diets with leucine (Leu) (Hyun *et al*., 2003, 2007) or arginine (Arg) could increase IMF (Tan *et al*., 2009; Ma *et al*., 2010). Leucine as a branched-chain amino acid (BCAA) is essential and therefore must be continuously available for protein synthesis. Although most of articles evaluating the excess dietary Leu in pigs have concentrated on the interaction of Leu with the other BCAA (valine and isoleucine) and its effects on growth performance (Morales *et al*., 2015), Leu may also promote global protein synthesis, insulin release and inhibit protein degradation (Harris *et al*., 2004). Leucine as a keto-acid may also further undergo oxidative decarboxylation to produce acyl-CoA derivatives and enter tricarboxylic acid cycle, promoting fatty acid synthesis. Therefore, the proper Leu concentration is critically important for maintaining body protein levels beyond simply the need of this essential amino acid for protein synthesis (Harris *et al*., 2004). Arginine is a semi-essential amino acid, some studies have demonstrated that dietary Arg may stimulate protein synthesis in young (Yao *et al*., 2008) and in adult pigs and at the same time reduce fat deposition in fattening pigs (He *et al*., 2009). These effects may be due to the role of Arg in regulating the metabolism of energy substrates (fatty acids, glucose, amino acids) partly through production of nitric oxide (NO). Nitric oxide increases fatty acid oxidation, decreases triacylglycerides synthesis and increases the basal lipolysis (Jobgen *et al*., 2006). However, Tan *et al*. (2011) suggested that dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle, favouring lipogenesis in muscle but lipolysis in adipose tissue. Therefore, it can be hypothesised that increasing the level of Arg and Leu in pig diets may increase the IMF content and consequently the acceptability of meat for consumers. However, data is not consistent throughout the literature. Hyun *et al*. (2007) only found an increase of IMF in the diets with a low level of Lys (0.5%) but no effect was observed when Lys was added at 0.7%. Madeira *et al*. (2014) did not find an alteration on IMF content in (Duroc × Pietrain) × (Large White × Landrace) fed reduced protein supplemented with Leu. Go *et al*. (2012) and Madeira *et al*. (2014, 2015) did not find effect of Arg on IMF.

Furthermore, in a previous study, using Duroc × Landrace pigs, reduction of the level of dietary protein maintaining the levels of Lys as the ones in the control diet or reduction of dietary Lys maintaining the levels of protein as the ones in the control diet resulted in an increase of IMF, while when both parameters protein and Lys were reduced IMF was not affected (Tous *et al*., 2014). Wood *et al*. (2004) found that a low protein diet slowed growth and increased neutral lipid fatty acid concentrations in *Longissimus* (LM) and *Psoas* muscles in the two modern breeds compared with two traditional genotypes. Hence, there are genotypic differences regarding the effects of dietary protein level on IMF.

The first objective of the current study was to evaluate whether the addition of Arg or Leu to low or normal protein diets would increase the IMF content on LM of pigs. The second objective was to determine if the reduction of dietary crude protein (CP) would increase IMF without negatively affecting growth performance and other carcass traits in a leaner genotype ([Landrace × Duroc] × Pietrain) than the one used in our previous study (Duroc × Landrace crossbreed; Tous *et al*., 2014).

Material and methods

The experiment was conducted in compliance with the Spanish guidelines (BOE, 2007) for human care and use of animals in research and the protocol was approved by the Ethical Animal Committee of Institut de Recerca i Tecnologia Agroalimentàries (IRTA, Spain).

Animals and diets

One hundred and eight 67 ± 4 kg live weight (Landrace × Duroc) × Pietrain barrows chosen from a larger group were blocked by weight balancing the effect of litter within the block, and were housed in the same farm in adjacent pens with 3 pigs per pen (6 pens per treatment). Pigs within blocks were randomly assigned to one of the six dietary treatments up to slaughter at approximately 115 ± 7 kg live weight (56 or 70 days). The six treatments differed in the CP, Arg and Leu content: normal CP (NP), normal CP diet + Arg (NPA), normal CP diet + Leu (NPL), normal CP diet + Arg + Leu (NPAL), low CP diet (LP), low CP + Arg + Leu (LPAL). The high Leu and Arg diets were supplemented to obtain ratios of standardized ileal digestible Leu/Lys and Arg/Lys of 4 and 2, respectively. Glutamic acid, a non-essential amino acid, replaced the additions of the different amino acids to maintain constant levels of nitrogen and protein in the diets (Table 1). The level of dietary protein in the normal protein diets was in accordance to requirements (NRC, 1998). Low-protein diets were first formulated based

Table 1. Ingredient composition of experimental diets (as-fed basis).

Ingredient, %	1st period (60-90 kg)						2nd period (90-115 kg)					
	NP	NPA	NPL	NPAL	LP	LPAL	NP	NPA	NPL	NPAL	LP	LPAL
Maize	72.9	72.8	71.8	72.8	77.0	77.2	78.6	78.7	77.8	78.7	81.1	81.3
Soyabean meal 44%	18.7	18.7	18.7	18.7	11.3	11.3	11.3	11.4	11.3	11.4	6.97	7.04
Glutamic acid	3.20	1.57	1.75	-	4.13	-	2.70	1.14	1.60	-	3.20	-
Leucine	-	-	1.40	1.39	-	1.56	-	-	1.03	1.02	-	1.12
Arginine	-	0.60	-	0.60	-	0.86	-	0.53	-	0.53	-	0.68
Starch	2.55	3.67	3.62	3.84	4.31	5.81	4.80	5.61	5.58	5.74	5.69	6.77
Dicalcium phosphate	0.78	0.78	0.78	0.78	0.87	0.87	0.63	0.63	0.64	0.63	0.69	0.68
Calcium carbonate	0.55	0.55	0.55	0.55	0.55	0.55	0.57	0.57	0.57	0.57	0.57	0.57
Lard	0.50	0.50	0.50	0.51	0.50	0.51	0.50	0.50	0.50	0.50	0.50	0.50
Mineral-vitamin premix[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium chloride	0.33	0.33	0.32	0.33	0.05	0.05	0.02	0.02	0.02	0.02	-	-
L-lysine HCl	0.08	0.08	0.08	0.08	0.29	0.29	0.12	0.12	0.12	0.12	0.25	0.24
L- threonine	-	-	-	-	0.08	0.08	-	-	-	-	-	-
Sodium bicarbonate	0.03	0.03	0.04	0.03	0.43	0.43	0.35	0.34	0.35	0.34	0.58	0.58
DL- methionine	-	-	-	-	0.02	0.02	-	-	-	-	-	-
L-tryptophan	0.01	0.01	0.01	0.01	0.04	0.04	0.02	0.01	0.02	0.01	0.04	0.04

NP: normal protein; NPA: normal protein + arginine; NPL: normal protein + leucine; NPAL: normal protein + arginine + leucine; LP: low CP; LPAL: low CP + arginine + leucine. [1] One kilogram of feed contains: 5,000 IU vitamin A; 1,000 IU vitamin D_3; 15 IU vitamin E; 1.3 mg vitamin B_1; 3.5 mg vitamin B_2; 0.025 mg vitamin B_{12}; 1.5 mg vitamin B_6; 10 mg calcium pantothenate; 15 mg nicotinic acid; 0.1 mg biotin; 0.6 mg folic acid; 2 mg vitamin K; 80 mg Fe (from $FeSO_4 \cdot H_20$); 6 mg Cu (from $CuSO_4 \cdot 5H_20$); 0.75 mg Co (from $CoCO_3 \cdot H_20$); 60 mg Zn (from ZnO); 30 mg Mn (from MnO); 0.75 mg I (from $CaIO_3$); 0.10 mg Se (from Na_2SeO_3); 125 mg ethoxiquin.

on the normal protein diet (with the supplementation of both Arg and Leu) but reducing level of protein as much as possible maintaining Lys and the minimum requirements for the essential amino acid to Lys ratios although the supplementation with Arg and Leu in LPAL diet. In order to obtain isonitrogenous diets, the inclusion of amino acids (Arg, Leu and glutamic acid) in the diets was adjusted. Diets were based on maize and soybean meal 44%. Net energy was calculated according to Sauvant *et al.* (2004). All diets had a calculated net energy content of 10.0 MJ/kg (Table 2). L-glutamic acid (Ajinomoto, Mesnil-Saint-Nicaise, France), L-leucine (Ajinomoto, Limeira-SP, Brazil) and L-arginine (Ajinomoto, Morodomi-Cho Saga, Japan) had a purity greater than 98.5%. During the experimental period, pigs received the diets for *ad libitum* consumption and had free access to water.

Pigs were selected on two different days (50 animals day 56: NP=10; NPA=9; NPL=10; NPAL=7; LP=12; LPAL=2; and 58 animals day 70: NP=8; NPA=9; NPL=8; NPAL=11; LP=6; LPAL=16) in order that all animals had similar final body weight 48 h before slaughter (115 ± 7 kg live weight). It was not possible to select the same number of pigs per treatment for each day of slaughter because pigs fed the LPAL treatment grew more slowly. Individual body weights were determined at the beginning of the trial (67 ± 4 kg live weight), on day 21 (88 ± 3 kg live weight), and before the first shipment of animals to the slaughterhouse (day 56; 107 ± 7 kg live weight). The average daily feed intake, average daily gain and feed efficiency were calculated until the day before the first shipment of the pigs to the abattoir (day 56). However, pigs that did not reach the weight the first day were fed with the same previous diet until they reached slaughter weight.

Slaughter conditions

Pigs were transported to the IRTA experimental abattoir (2 h) on 2 different days (day 56 or 70) and they underwent approximately 16 h of fasting time before slaughter, with free access to water. Pigs were weighed and slaughtered minimizing the stress using standard *ante-mortem* procedures and stunned with 85% CO_2 for 120 s using CO_2 Dip Lift (Butina, Alps, Copenhagen, Denmark). Once carcasses were eviscerated, they were split longitudinally and weighed within 45 min (blooming time). Perirenal fat and liver were also separately weighed.

Carcass quality measurements

Fat and muscle thickness were measured with the Fat-O-Meat'er probe (Carometec A/S, Herlev, Denmark) between the third and the fourth last ribs at 6 cm off the midline. From these measurements, lean meat

Table 2. Nutritional composition of the experimental diets[1].

	1st period (60-90 kg)						2nd period (90-115 kg)					
	NP	**NPA**	**NPL**	**NPAL**	**LP**	**LPAL**	**NP**	**NPA**	**NPL**	**NPAL**	**LP**	**LPAL**
Net energy, MJ/kg	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
CP, %[2]	16.4	16.7	16.6	16.6	14.2	14.5	13.3	13.1	13.0	13.1	11.8	11.9
Standardized ileal digestible amino acids, g/kg												
Lys	6.50	6.50	6.50	6.50	6.50	6.50	5.20	5.20	5.20	5.20	5.20	5.20
Leu	12.2	12.2	26.0	26.0	10.5	26.0	10.6	10.7	20.8	20.8	9.64	20.8
Arg	8.15	13.0	8.12	13.0	6.08	13.0	6.11	10.4	6.10	10.4	4.89	10.4
Thr	4.55	4.55	4.52	4.55	4.36	4.36	3.60	3.63	3.59	3.62	3.46	3.46
Met	2.18	2.18	2.16	2.18	1.99	1.99	1.85	1.86	1.84	1.86	1.64	1.65
Trp	1.28	1.28	1.28	1.28	1.29	1.29	1.03	1.03	1.03	1.03	1.03	1.03
Ile	5.24	5.24	5.21	5.24	4.04	4.05	4.07	4.10	4.06	4.10	3.37	3.39
Val	6.02	6.02	5.98	6.02	4.82	4.82	4.86	4.89	4.84	4.89	4.16	4.18
Phe	6.31	6.30	6.27	6.30	5.01	5.02	5.06	5.09	5.04	5.09	4.30	4.33
Hys	3.51	3.51	3.49	3.51	2.83	2.83	2.85	2.87	2.84	2.87	2.45	2.47

For treatments, see Table 1. [1] Calculated values according to Sauvant *et al.* (2004). [2]Analysed.

percentage was calculated using the Spanish official equation (Lean meat (%) = 66.91 – 0.895 * backfat thickness + 0.144 * muscle depth; Font-i-Furnols & Gispert, 2009). In addition, backfat thickness at the last rib at 6 cm off the midline and between the third and the fourth lumbar vertebrae at 8 cm off the midline were determined with the same probe. The minimum fat thickness over the *Gluteus medius* muscle and fat thickness in the cranial position of the first lumbar vertebrae and in the shoulder at the level of first rib were measured with a ruler over the carcass midline.

After carcass refrigeration at 3 °C for 24 h, carcasses were weighed. Carcass length was measured as the distance between the recess of the first rib and the anterior edge of the symphysis pubic and loin length as the distance from the atlas bone to the first lumbar vertebrae. Then, the left side of each carcass was cut following a simplified European reference method (Walstra & Merkus, 1995). The primary cuts obtained were: ham (included hind shank and hind foot), loin, belly (included the ventral part of the belly and jowl), shoulder (with front shank, front foot and neck), tenderloin and head (with cheek). Each cut was weighed and their proportion with respect to cold carcass weight was calculated. Furthermore, the *Semimembranosus* muscle (SM) was separated from the ham and the LM and subcutaneous fat (with skin) from the loin, and their respective weights were recorded. The LM was stored at 4 °C for 24 h. Then, the LM samples were ground using a food processor (Robot-Coupe Blixer 3, Montceau les Mines, France), and a 90 g subsample was used to determine the IMF content using the Infratec 1265 Meat Analyzer (Foss Tecator AB, Hoganas, Sweden) equipment, based on near infrared transmittance (NIT) (Gispert *et al*., 1997, 2010).

Muscle pH was measured in the left carcass side using a Crison portable meter (Crison, Barcelona, Spain) equipped with a Xerolyte electrode in the LM at the last rib level and in SM muscles at 45 min (pH45) and at 24 h (pHu) *postmortem*. Electrical conductivity was measured in the carcass using a probe (Pork Quality Meter-Kombi, Aichach, Germany) also at the last rib level in the LM and SM muscles at 24 h *postmortem*.

Instrumental colour (CIE, 1976) was measured at 24 h *postmortem* at the level of the last rib on a cross section of the LM after 15 min of blooming time with a colorimeter (using D65 as illuminant and standard observer of 10°; CR-400 Minolta Co., Osaka, Japan), and luminosity (L*), redness (a*) and yellowness (b*) were recorded. Subjective marbling (NPPC, 2000) measurements in LM samples were obtained from the cut lean surface of each chop on a scale of 1 to 5 (1 being low and 5 being high). Drip loss was obtained from the LM according the methodology described by Rasmussen & Andersson (1996).

Statistical methods

A preliminary statistical evaluation was performed using GLM procedure of SAS (SAS, Inst. Inc., Cary, NC, USA) with initial weight as covariable for growth performance. Final weight was also included as covariable for some backfat measurements (backfat thickness on the last rib, minimum fat thickness over the *Gluteus medius* muscle and lean meat percentage). Pen was considered the experimental unit for all variables (6 replicates per treatment). The LPAL treatment presented a large standard deviation compared

to the other treatments for all variables measured, indicating that variance was not homogeneous for all treatments. Consequently, it was eliminated from the overall data set. A Student t-test for not homogeneous variances was conducted to compare the LP and LPAL treatments. For the five remaining dietary treatments (NP, NPA, NPL, NPAL, and LP), the different variables studied were analyzed by two way ANOVA in a 2 by 2 plus one design (comparison of low protein versus normal protein diet and the effect of supplementation of Arg and Leu in normal protein diets as a 2 × 2 factorial design) with 6 blocks corresponding to body weight and location within the house. Differences among treatment means were investigated with a set of contrasts in four different comparisons: NP + NPAL *vs*. NPA + NPL (evaluating interaction between Arg and Leu: Int), NPA + NPAL *vs*. NP + NPL (evaluating the effect of supplementing arginine: Arg), NPL + NPAL *vs*. NP + NPA (evaluating the effect of supplementing leucine: Leu) and NP *vs*. LP (evaluating the effect of reducing dietary CP: Prot). Differences were considered significant at $p<0.05$ and $p<0.1$ was considered as trend.

Results

Growth performance

A trend in the interaction between dietary Arg and Leu was observed in animals fed normal protein diets for final weight and average daily gain (ADG) (p=0.083 and p=0.072, respectively; Table 3). Animals fed normal protein diets supplemented with Arg or Leu tended to have a greater final weight and ADG compared to animals fed normal protein diet or normal protein diet supplemented with both amino acids Arg and Leu. When both Arg and Leu were added in excess in normal protein diet final body weight and ADG were similar to normal protein diet without amino acid supplementation.

The reduction of dietary protein did not affect growth performance compared to normal protein diets. However, the addition of both amino acids Arg and Leu in the low protein diet reduced final weight (p=0.006), average daily feed intake (ADFI) (p=0.020) and ADG (p=0.001) and reduced gain to feed ratio (p=0.040) compared to low protein diet.

Carcass characteristics

A trend in the interaction between Arg and Leu was observed in muscle depth between the third and the fourth last ribs (p=0.070; Table 4), as it tended to be increased in normal protein diets supplemented with Arg or Leu with respect to the normal protein diet but it was not changed in normal protein diet supplemented with both Arg and Leu. The other variables measured were not affected by the interaction between Arg and Leu. A trend to reduce backfat thickness (p=0.059) between the third and the fourth last ribs and an increase of lean meat percentage (p=0.046) was observed by effect of Arg supplementation compared to normal protein diet and the normal protein diet supplemented with Leu. The supplementation of diets with Leu (NP and NPA *vs*. NPL and NPAL) reduced the length of the loin (p=0.041). Reduction of dietary protein level (NP *vs*. LP) tended to increase carcass yield (p=0.089). Animals fed the low protein diets supplemented with Arg and Leu had a lower body weight at the end of the trial, carcass length (p=0.018 and p=0.006, respectively) and tended to reduce carcass yield (p=0.071) compared to the ones fed low protein diet (LP *vs*. LPAL). Considering the LSMEANS adjusted for the covariable body weight (data not shown in Table 4), backfat thickness tended to be increased on the last rib and on the fist lumbar vertebrae (14.3 *vs* 16.9 p=0.096 and 19.6 *vs* 22.7 p=0.061, respectively) and muscle depth was decreased (p=0.011) and the lean meat percentage tended to be reduced (60.2 *vs* 57.7 p=0.072) by effect of supplementing diets with a reduced level

Table 3. Effect of dietary arginine, leucine and crude protein level on growth performance of finishing pigs (n=6, 6 pens of 3 pigs per treatment)[1].

	NP	NPA	NPL	NPAL	LP	LPAL	RMSE	Int [2]	Arg [3]	Leu [4]	Prot [5]	LP *vs.* LPAL
Initial weight, kg	68.2	66.8	67.8	67.2	67.5	66.6	4.43	0.83	0.58	0.99	0.79	0.66
Final weight, kg	107.7	109.0	110.6	106.9	110.0	98.2	3.49	0.083	0.52	0.98	0.19	0.006
ADFI, kg/day	2.86	2.86	2.95	2.77	2.94	2.47	0.21	0.15	0.18	0.96	0.33	0.020
ADG, kg/day	0.91	0.95	0.97	0.90	0.97	0.70	0.08	0.072	0.54	0.96	0.18	0.001
G:F	0.32	0.33	0.33	0.32	0.33	0.28	0.02	0.31	0.60	0.97	0.37	0.040

For treatments, see Table 1. [1] Initial weight was used as covariable. [2] NP and NPAL *vs*. NPA and NPL. [3] NP and NPL *vs*. NPA and NPAL. [4] NP and NPA *vs*. NPL and NPAL. [5] NP *vs*. LP.

Table 4. Effect of dietary arginine, leucine and CP level on carcass characteristics (n=6, 6 pens of 3 pigs per treatment) [1].

Carcass characteristics	NP	NPA	NPL	NPAL	LP	LPAL	RMSE	Int [2]	Arg [3]	Leu [4]	Prot [5]	LP *vs.* LPAL
Live weight at slaughter, kg	116.3	116.8	118.7	116.1	116.6	107.6	2.76	0.19	0.37	0.47	0.85	0.018
Carcass yield, %	82.1	82.1	82.9	82.1	83.1	82.0	1.00	0.36	0.36	0.41	0.089	0.071
Chilling losses, %	2.21	2.15	2.18	2.16	2.20	2.19	0.08	0.51	0.20	0.94	1.00	0.69
Carcass length, cm	83.6	83.0	83.3	82.0	83.1	81.0	1.54	0.60	0.14	0.30	0.57	0.006
Loin length, cm	85.9	85.2	84.5	82.8	84.4	83.3	2.10	0.59	0.17	0.041	0.21	0.11
Backfat thickness, mm												
3rd-4th last ribs [6]	20.2	18.5	20.9	18.7	19.4	18.0	2.41	0.81	0.059	0.65	0.56	0.13
Last rib [6]	18.1	16.2	17.0	16.2	16.3	14.9	2.20	0.18	0.33	0.35	0.14	0.096
3rd-4th lumbar vertebrae [7]	23.3	20.8	22.5	22.1	21.6	19.5	2.73	0.35	0.20	0.87	0.28	0.25
Gluteus medius [8, 9]	19.1	18.7	20.0	18.6	18.5	16.0	1.83	0.99	0.46	0.86	0.52	0.22
1st lumbar vertebrae[8]	23.2	23.8	22.8	22.3	22.4	20.0	3.13	0.66	1.00	0.46	0.63	0.061
1st rib, mm [8]	35.5	36.8	36.5	36.9	36.4	34.7	2.53	0.65	0.44	0.61	0.57	0.55
Muscle depth, mm												
3rd-4th last ribs[6]	59.9	62.2	61.1	59.6	61.1	57.1	2.51	0.070	0.70	0.50	0.40	0.011
Lean meat % [10]	57.3	59.3	57.0	58.7	58.9	59.0	2.13	0.89	0.046	0.62	0.23	0.072

For treatments, see Table 1. RMSE: root means square error. [1] Final weight was included as covariable for backfat thickness on the last rib, minimum fat thickness over the *Gluteus medius* muscle and lean meat percentage. [2] NP and NPAL *vs*. NPA and NPL. [3] NP and NPL *vs*. NPA and NPAL. [4] NP and NPA *vs*. NPL and NPAL. [5] NP *vs*. LP. [6] Measurement taken at 6 cm to the carcass midline with Fat-O-Meat'er (Carometec A/S, Herlev, Denmark). [7] Measurement taken at 8 cm to the carcass midline with Fat-O-Meat'er (Carometec A/S, Herlev, Denmark). [8] Measurement taken on the carcass midline with a ruler. [9] Minimum fat thickness over the muscle. [10] Calculated from backfat thickness and loin depth between the third and the fourth last ribs using the Spanish official equation (Lean (%)=66.91 - 0.895 * Backfat thickness third and the fourth last ribs + 0.144 * Muscle depth third and the fourth last ribs; Font-i-Furnols & Gispert, 2009).

of protein with Arg and Leu compared to animals fed the low protein diet.

Meat quality traits

Meat quality traits were not affected by the interaction between dietary Arg and Leu levels (Table 5). Addition of Arg (NPA and NPAL) produced a reduction of IMF content (p=0.033), marbling (p=0.041), meat lightness (L*, p=0.028) and yellowness (b*, p=0.045) with respect to the normal protein diet or the normal protein diet supplemented with Leu (NP and NPL). Electrical conductivity and drip loss in LM increased (p=0.035 and p=0.013, respectively) and redness tended to increase (a*, p=0.051) in animals fed normal protein diets supplemented with Leu (NP and NPA *vs*. NPL and NPAL). The IMF content or marbling was not affected by the supplementation of normal protein diet with Leu or the reduction of dietary protein. Lightness (L*, p=0.032) and yellowness (b*, p=0.045) were reduced in meat from animals fed the low protein diet (NP *vs*. LP). A reduction of pH at 45 min (p=0.044) in LM, a trend to reduce pH at 45 min in SM (p=0.058), a pH reduction at 24 h in SM (p=0.011), an increase of meat redness (a*, p=0.022) and a trend to increase conductivity in LM (p=0.097) was observed in low protein diets supplemented with both Arg and Leu compared to the unsupplemented low protein diets. Intramuscular fat content and marbling were not affected by supplementing low protein diets with Arg and Leu.

Weight of carcass cuts, liver and perirenal fat

A trend in the interaction between Leu and Arg (p=0.092; Table 6) was found for liver weight which tended to be reduced in normal protein diets supplemented with Arg or Leu (NPA or NPL) but no difference was observed when both amino acids Arg and Leu were added to normal protein diet (NPAL) compared to those of NP diet. Supplementation of normal protein diets with Arg (NPA and NPAL *vs*. NP and NPL) tended to increase shoulder weight (p=0.095) and significantly reduced (p=0.003) the belly weight (cut with a greater fat content). The weight of the different carcass cuts was not affected by the dietary supplementation of Leu. Reduction of dietary CP (LP *vs*. NP) tended to reduce belly weight (p=0.086) and tended to

Table 5. Effect of dietary arginine, leucine and CP level on meat quality traits in *Longissimus* and *Semimembranosus* muscles (as is) (n=6, 6 pens of 3 pigs per treatment).

Traits	NP	NPA	NPL	NPAL	LP	LPAL	RMSE	Int[1]	Arg[2]	Leu[3]	Prot[4]	LP *vs.* LPAL
Semimembranosus												
pH45	6.36	6.23	6.23	6.26	6.30	6.19	0.12	0.12	0.29	0.30	0.37	0.058
pHu	5.49	5.48	5.47	5.48	5.52	5.44	0.06	0.82	0.97	0.63	0.39	0.011
Electrical conductivity, mS	7.21	7.83	8.04	8.58	7.36	7.57	1.25	0.94	0.27	0.13	0.84	0.82
Longissimus												
pH45	6.27	6.13	6.11	6.12	6.23	6.04	0.12	0.14	0.22	0.11	0.61	0.044
pHu	5.48	5.48	5.46	5.47	5.48	5.42	0.06	0.75	0.86	0.46	0.88	0.12
Electrical conductivity, mS	4.66	5.44	6.36	5.98	5.13	6.60	1.23	0.26	0.70	0.035	0.52	0.097
Drip loss, %	4.02	4.36	5.75	4.99	3.70	4.84	1.08	0.22	0.64	0.013	0.62	0.19
Marbling	2.22	1.85	2.18	1.88	2.00	1.75	0.36	0.82	0.033	1.00	0.31	0.38
Intramuscular fat, %	2.23	1.88	2.27	2.17	2.00	2.25	0.25	0.24	0.041	0.14	0.13	0.21
Colour												
Lightness, L*	49.1	48.3	49.2	47.5	47.3	50.0	1.33	0.42	0.028	0.51	0.032	0.68
Redness, a*	6.96	6.92	7.25	7.78	7.18	7.91	0.69	0.33	0.39	0.051	0.58	0.022
Yellowness, b*	1.66	1.32	1.87	1.40	1.29	1.21	0.30	0.62	0.003	0.24	0.045	0.83

For treatments, see Table 1. RMSE: root means square error. [1] NP and NPAL *vs*. NPA and NPL. [2] NP and NPL *vs*. NPA and NPAL. [3] NP and NPA *vs*. NPL and NPAL. [4] NP *vs*. LP.

increase tenderloin weight (p=0.052). The weight of belly was also significantly reduced in animals fed low protein diets supplemented with Arg and Leu (p=0.039) compared to the ones fed unsupplemented diets.

Discussion

The current study was performed to evaluate if the reduction of dietary protein level below requirements (NRC, 1998) has the same effect on IMF than in the previous study (Tous *et al*., 2014) with a different crossbreed (Duroc × Landrace *vs*. Duroc × Landrace sows crossed with a Pietrain boar) in which IMF was expected to be lower. Therefore, the control diet had a protein level according to NRC's (1998) requirements (16% during the growing period and 13% during the fattening period) and the low protein diet had a level below these requirements (14% during the growing period and 11.8% during the fattening period). In order to avoid the protein × Lys interaction observed in a previous study (Tous *et al*., 2014) the level of Lys was kept constant between the different diets. In the current study, although diets were based on maize which has lower protein content than barley, the level of dietary protein could not be as low as in the previous study because diets were supplemented with two different amino acids (Arg and Leu). The levels of supplementation with Arg and Leu were selected based on studies in which supplementation of Arg and Leu increased IMF content (Hyun *et al*., 2003, 2007; Tan *et al*., 2009; Ma *et al*., 2010). In all studies, diets were also based on maize, and in the case of Hyun *et al.* (2003 and 2007) the basal Leu level was similar to that of our study. These levels were considered not toxic (Baker, 1989). In order to avoid the animal weight effect all animals were slaughtered at similar body weight, although animals fed the low protein diets supplemented with Arg and Leu had a lower body weight at the end of the trial. Therefore, the differences obtained for carcass characteristics, meat quality and carcass cuts between animals fed LP diet and the ones fed LPAL may be attributed to the difference in final body weight.

Performance of the animals in the current study could be considered adequate, according to the standard of the farm and the crossbreed used. The reduction of ADFI, ADG, final weight and the trend to increase feed to gain ratio (FGR) observed in animals fed low protein diets supplemented with Arg and Leu may be explained by an antagonism between Leu and other branched chain amino acids (BCAA). When Leu is in excess, isoleucine and valine may become limiting and depress performance, as Leu increases activity of enzymes involved in BCAA catabolism (Oestmer & Handson, 1973). The growth depression may occur despite the fact that the levels of isoleucine and valine were in excess of the ratios proposed by Chung & Baker (1992). Hyun *et al*. (2003) reported a reduction of ADG in Duroc × Yorkshire pigs when Leu (2%) was supplemented in the diet, as in the low protein diet supplemented with Arg and Leu in the current experiment. In the experiment of Hyun *et al*. (2003) the level of Lys in the control and experimental diet was the same (0.79%) but, contrary to our study, the Leu supplemented diet had a higher CP concentration (15.4%) compared with the control (14.1%). The same authors

Table 6. Effect of dietary arginine, leucine and CP level on the weight of some carcass cuts, liver and perirenal fat (n=6, 6 pens of 3 pigs per treatment).

	NP	NPA	NPL	NPAL	LP	LPAL	RMSE	Int [1]	Arg [2]	Leu [3]	Prot [4]	LP *vs.* LPAL
g/kg BW												
Liver	14.3	13.8	13.5	14.4	16.2	13.9	0.89	0.092	0.60	0.80	0.85	0.56
Perirenal fat	8.81	8.77	9.11	8.14	8.98	8.40	1.10	0.31	0.26	0.72	0.80	0.33
g/kg carcass[5]												
Head	75.1	74.0	73.1	75.5	76.0	78.0	2.96	0.16	0.57	0.83	0.59	0.19
Shoulder	276.1	277.1	273.2	279.4	281.0	281.5	5.03	0.22	0.095	0.87	0.11	0.87
Belly	147.4	138.7	142.6	139.3	142.8	137.3	4.46	0.14	0.003	0.25	0.086	0.039
Tenderloin	13.2	13.7	14.1	13.6	14.3	13.9	0.92	0.17	1.00	0.28	0.052	0.28
Ham	312.2	319.0	314.4	315.1	312.3	313.7	6.64	0.27	0.19	0.75	0.98	0.59
SM	29.6	31.0	30.4	30.7	30.0	30.0	1.47	0.35	0.15	0.70	0.61	1.00
Loin	172.5	173.0	178.0	173.0	173.8	170.1	5.10	0.19	0.28	0.19	0.67	0.26
LM	62.3	64.2	62.9	64.2	63.9	62.9	2.81	0.78	0.18	0.81	0.35	0.68
Backfat + skin	48.7	50.6	50.6	46.2	46.9	43.9	7.03	0.27	0.67	0.66	0.67	0.44

For treatments, see Table 1; SM: *Semimembranosus* muscle; LM: *Longissimus* muscle; RMSE: root means square error. [1] NP and NPAL *vs*. NPA and NPL. [2] NP and NPL *vs*. NPA and NPAL. [3] NP and NPA *vs*. NPL and NPAL. [4] NP *vs*. LP. [5] Carcass cuts following the European reference method (Walstra & Merkus, 1995) avoiding some of the cuts: ham (included hind shank and hind foot), loin, belly (included the ventral part of the belly and jowl), shoulder (with front shank, front foot and neck), tenderloin and head (with cheek).

did not find significant modifications of the growth performance in PIC line 327 × C22 pigs fed finishing diets supplemented diets with 1, 2, 3% Leu (Hyun *et al*., 2007) at two different levels of Lys (0.5% or 0.7%) but the level of dietary CP was not constant between diets. Tan *et al*. (2009) found an increase of ADG and a trend to reduce FGR of pigs when 1% Arg was added to the diet. Ma *et al*. (2010) and Go *et al*. (2012) did not find differences in performance of pigs fed diets supplemented with 1 % Arg compared to their respective control diets containing the same Lys and CP levels. The study of Madeira *et al*. (2014) reported that supplementation with Leu reduced the ADFI and that animals fed reduced dietary protein diets had a lower ADG and a higher FGR which may be caused by a deficiency in Lys, as Lys content was lower compared with the control diet. Therefore, the differences observed between studies could be explained because dietary protein, Lys and the genotype of the pigs were different.

One of the variables most related to meat acceptability for consumers is the IMF content (Font-i-Furnols *et al*., 2012). In our previous study, when protein was reduced and the dietary Lys was the same as the control, or when dietary Lys level was reduced but the percentage of dietary protein was the same as the control, the IMF content in LM and SM increased (Tous *et al*., 2014). In the present study, the reduction of dietary protein did not affect IMF content in LM. Supplementation of normal protein diet with Leu did not affect IMF and Arg supplementation reduced IMF and marbling in LM contrary to our hypothesis. These results are in contrast with previous studies which had shown an increase of IMF when Leu (Hyun *et al*., 2003, 2007) and Arg (Tan *et al*., 2009; Ma *et al*., 2010) were supplemented to pig diets, but in agreement with others (Go *et al*., 2012; Madeira *et al*., 2014, 2015) that found no effect of Arg supplementation on IMF. Hyun *et al*. (2007) found that the increase of IMF content was dependent of the level of dietary Lys, as Leu enhanced IMF content in LM when was combined with dietary low Lys but not with high dietary Lys. In a similar study performed with (Duroc × Pietrain) × (Large White × Landrace) boars, diet supplementation with 1% of Arg or 2% of Leu had no effect on the IMF content in LM, while the reduction of dietary protein increased it (Madeira *et al*., 2014). The difference between the studies of Madeira *et al*. (2014) and Tous *et al*. (2014) is that in the study of Madeira *et al*. (2014) the level of Lys in the low protein diet was reduced compared with the normal protein diet but it was not in the study of Tous *et al*. (2014). This difference between the low protein diets in the experiments suggests that the level of Lys instead of the level of protein may be responsible for the increase of IMF as previously suggested by Madeira *et al*. (2013) in (Large White × Landrace) × (Large White × Pietrain) boars. Some studies evidenced the increase of IMF by dietary protein restriction is mediated through Lys deficiency (Madeira *et al*., 2013, 2014, 2015; Katsumata, 2011) as Tous *et al*. (2014) found an interaction between these two parameters. Go *et al*. (2012) did not observe an increase of IMF in pigs fed diets supplemented with 1% Arg when the level of Lys in

the control and the experimental diet was similar (0.76 and 0.75 g / 100 g of diet, respectively), although they attributed the lack of response to the duration of the treatment or high BW at the beginning of the trial (from 80 to 110 kg live weight). Taking into account these considerations the supplementation of Arg and Leu may be only effective increasing IMF when the level of Lys in the diet is below the dietary requirement for pigs, or because low Lys and not Arg or Leu supplementation is the responsible of the increase on IMF. The difference observed between the current and the previous experiment with the Duroc x Landrace crossbreed (Tous *et al*., 2014), suggests that the genetic origin may also influence the changes on IMF and fat deposition as affected by dietary factors. In the study of Tous *et al*. (2014), the lean meat percentage of pigs fed the control diet was 50% while in the current study it was 57.3%, and in the current study the muscle depth was 10 mm higher and backfat thickness was 5 to 7 mm lower in all measurements compared with the previous study. Additionally, in the previous trial, animals fed the control diet presented an IMF of 2.38% and the animals fed the low-protein diet presented an IMF of 2.68%. In the current trial, although not significant, the animals fed the low-protein diet presented a lower IMF content in LM than the control diet (2.23 for NP *vs* 1.99% for LP). The differences in lean meat percentage between crossbreeds may explain, at least in part, the differences observed between these two studies. Wood *et al*. (2013) reported that feeding a regime for pigs with a lean genotype (Large White × Landrace) which provided a low protein diet but with the same intake of essential amino acids as pigs fed a control diet produced carcasses similar in fat content but higher content of IMF in LM, while feeding a reduced protein diet without maintaining the levels of essential amino acids, produced fatter pigs and increased IMF in LM and SM. In the studies of Wood *et al*. (2013) and Tous *et al*. (2014) the genotype did not include Pietrain sires, while in the study of Maderia *et al*. (2014) and in the current study, the genotype included Pietrain sires. Therefore, it could be hypothesized that some genes associated with the Pietrain breed may inhibit the response of IMF to certain nutrients in the diet. Further studies are required to confirm the hypothesis that Pietrain sires are less prone to increase IMF due to dietary modification.

Dietary supplementation with Arg increased lean meat percentage and reduced belly weight in relation carcass weight (cut with a high content of fat). These results together with the trend to reduce fatness in the 3rd-4th last ribs and the reduction of IMF and marbling, suggests a reduction of the whole animal fatness in animals fed diets supplemented with Arg. He *et al*. (2009) also found that dietary Arg supplementation decreased fat deposition and increased protein accretion in pigs. These results are opposite to those of Tan *et al*. (2009, 2011), showing higher lipogenesis and high fat deposition in muscle, but higher lipolisis and a reduction of total carcass fat in pigs fed diets supplemented with Arg, due to differential regulation of lipid-metabolic genes in adipose tissue and skeletal muscle. Ma *et al*. (2010) did not find differences due to Arg supplementation in carcass fat depth or meat percentage. Supplementation with Leu did not affect carcass backfat thickness or carcass lean meat percentage in agreement with Hyun *et al*. (2007), although Hyun *et al*. (2003) found a reduction of backfat on the last lumbar vertebrae and a trend to increase LM area when Leu was supplemented. Reduction of dietary protein did not affect carcass backfat thickness or carcass lean meat percentage in contrast to the results obtained in the previous study (Tous *et al*., 2014). The same reasons (Lys, genotype and cereal source in the diet) as stated previously for IMF might explain the differences observed between studies.

Although some differences were observed for conductivity and colour measurements by the effect of the dietary treatments, these differences were too small in magnitude to change the classification of meat as pale soft and exudative (PSE) or dark firm and dry (DFD). In the studies of Hyun *et al*. (2003, 2007), Madeira *et al*. (2014) and Ma *et al*. (2015) these variables were not significantly affected either.

In conclusion, in the conditions of the current study, and contrary to the initial hypothesis, reduction of crude protein or dietary supplementation with leucine had no effect on intramuscular fat content while supplementation with arginine reduced it. Furthermore, arginine seems to reduce whole animal fatness and increase the lean content. Attention must be paid when leucine and arginine are added in low crude protein diets as they impaired performance, possibly due to an antagonism between leucine and the other branched chain fatty acids.

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