Effect of breed and reduced protein diets on pork fat content and fatty acid composition

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Abstract — **Intramuscular fat (IMF) content and fatty acid composition have a foremost impact on eating quality and human health. A high level of IMF (marbling fat) in muscle has been associated with improved eating quality of meat. Some studies have reported that feeding reduced protein diets increase the IMF in pig muscle. Therefore, the objective of this study was to evaluate the combined effect of breed, dietary protein and lysine levels on fat deposition and fatty acid composition of the *longissimus dorsi* muscle of pig. Sixty intact male pigs (30 Alentejano purebred and 30 Large White x Landrace x Pietrain crossbred), finished from 60 ± 2 to 95 ± 5 kg of live weight and submitted to one of the three diets (normal protein equilibrated for lysine, reduced protein corrected for lysine and reduced protein not corrected for lysine) were used in this experiment. The IMF was extracted according to the Soxhlet method with previous acid hydrolysis. Fatty acids were extracted, methylated and analyzed by gas–liquid chromatography. Significant interactions between breed and diet were observed for IMF as well as for 10 fatty acids, whereas diet had a stronger effect on crossbred than in Alentejano pigs. Breed had a strong effect ($P<0.001$) on fatty acid composition of pork (affected 11 of the total 19 fatty acids identified as well as on SFA, n-3 PUFA and n-6/n-3). By contrary, small differences in fatty acid composition (16:0, 18:3*n*-3 and sums of SFA and *n*-3 PUFA) among diets were observed. These results contribute for a novel understanding on production options which exploit both meat quality and healthiness in meat production.**

Keywords — **Fatty acid composition, pork fat, diet**

I. INTRODUCTION

Fat content and fatty acid composition in meat producing animals has received considerable attention in view of their implications for meat quality and human health [1]. In general a high polyunsaturated fatty acid (PUFA) to saturated fatty acids (SFA) improves the nutritional value of meat. Many efforts have been made to improve the nutritional value and the sensory quality of meat by controlling intramuscular fat deposition and its fatty acid composition [2]. It is well established that intramuscular fat (IMF) content and composition is determined by genetic and environmental factors, with breed and diet being important underlying factors [3]. Previous studies have shown that the use of leaner crossbred pigs elsewhere reduced the IMF levels and marbling in pork, perhaps to the detriment of meat quality [4]. In addition, some authors observed that reduced protein or lysine diets increased the expression of muscle lipogenic enzymes, such as the stearoyl CoA desaturase, which catalyses the cellular biosynthesis of monounsaturated fatty acids, and hence increase de novo fatty acid synthesis [5,6,7]. Therefore, a major challenge in the pork industry is to produce meat lean pigs without compromising pork quality. In this study we investigated the combined effect of breed (Alentejano purebred *versus* crossbred pigs), dietary protein and lysine levels on fat deposition and fatty acid composition of the *longissimus dorsi* muscle.

II. MATERIAL AND METHODS

Sixty entire male pigs (30 Alentejano purebred and 30 Large White x Landrace x Pietrain crossbred) were used. The trial was conducted under the guidelines for the care and use of experimental animals in INRB (Instituto Nacional dos Recursos Biológicos). Live weight of the pigs at the beginning was 60 ± 2 kg. Animals were fed the same commercial concentrate diet until of the beginning of the experiment. Pigs were then separated into groups of 10 and randomly

assigned to one of the three diets in a 2 x 3 factorial arrangement. The experimental diets were NP, normal protein diet with 18% protein equilibrated for lysine, RP, reduced protein diet with 14% protein corrected for lysine (0.8%) and RL, reduced protein diet with 14% protein not corrected for lysine (0.5%). The caloric value of the diets was 14 MJ/kg of digestible energy. Animals were slaughtered at 93 ± 2 kg live body weight at the INRB experimental abattoir. *Longissinus dorsi* muscle was collected, trimmed of connective and adipose tissue before being blended in a food processor, vacuum packed and stored at −20 ºC until further analysis. Meat samples were liophylized (-60ºC and 2.0 hPa) to constant weight. Intramuscular fat was extracted according to the Soxhlet method with previous acid hydrolysis [8]. Fatty acids were extracted according to the Folch method [9] and converted to fatty acid methyl esters (FAME) as described by [10]. FAME was analyzed using a HP6890A chromatograph (Hewlett–Packard, Avondale, PA, USA), equipped with a flame-ionization detector (GC–FID) and fused silica capillary column (CP-Sil 88; 100 m × 0.25 mm i.d. × 0.20 µm of film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA). using nonadecanoic acid (19:0) as the internal standard. The MIXED procedure of SAS, version 9.1 [11] was used to perform a 2×3 factorial analysis, with a model that included the main effects and their interaction. The level of significance was set at $P<0.05$.

III. RESULTS AND DISCUSSION

The effect of breed (Alentejano purebred *versus* crossbred) and diet (level of dietary protein with or without correction for lysine) on IMF levels (g/100 g muscle) and fatty acid composition (weight %) of *longissimus dorsi* muscle of pigs are shown in Table 1. Regarding IMF content, a significant breed and diet interaction ($P<0.05$) was observed (2.16-5.79 g/100 g muscle), in which no dietary effect was observed for Alentejano purebred, whereas in crossbred pigs the RL diet increased the intramuscular fat. This could be outcome from genetic specificities of fat deposition [3] since the energy intake by pigs was similar.

The major fatty acids in IMF were 18:1*c*9 (33-38%), 16:0 (23-26%), 18:0 (12-14%) and 18:2*n*-6 (7-12%). Similar fatty acids profile was reported for pork [12]. Breed had a strong effect on fatty acid composition of pork (affected 11 of the total 19 fatty acids identified). The individual fatty acids, namely 16:0 ($P<0.001$), 18:0 ($P<0.001$), 20:0 ($P<0.01$) ($P<0.001$) and SFA ($P<0.001$) showed higher percentages in Alentejano breed than in crossbred line (except for 17:0, 18:3*n*-3, 20:4*n*-6 and *n*-3 PUFA). These results agree with previous reports that find that the contents of saturated and monounsaturated fatty acids increase with increasing fatness [3]. In contrast, dietary protein and lysine diets had a little influence in fatty acid composition (16:0, 18:3*n*-3 and sums of SFA and *n*-3 PUFA) of *longissimus dorsi* muscle. The fatty acid ratios, which are related to human health, are also presented in Table 1. In crossbred pigs, the PUFA/SFA remained near to 0.4 which is the limit recommended by [13]. Regarding the *n*-6/*n*-3 ratio, which a maximum value of 4.0 is recommended [13] because it is a risk factor for coronary heart diseases, the values were significantly higher, especially in crossbred pigs ($P<0.001$). The values for the ratio of n-6/n-3 fatty acids are difficult to reduce due to the high content of 18:2*n*-6 in the cereal-based diets [7]. In summary, significant interactions between breed and diet were observed for most of the individual and partial sums of fatty acids as well as for PUFA/SFA ratio.

IV. CONCLUSION

The results suggest that the reduced protein diets without lysine correction increase IMF in crossbred pigs but not in Alentejano pigs. In addition, lysine correction in diets reverts the effect suggesting its involvement on the increase of IMF. The fatty acid composition seems to be more affected by the breed than by the diets under analysis.

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Table 1 – Intramuscular fat (g/100 g muscle), fatty acid composition (g/100 g of total fatty acids), partial sums of fatty acids (g/100 g of total fatty acids) and nutritional ratios fatty acids of *longissimus dorsi* muscle in Alentejano purebred and Large White x Pietran x Landrace crossbred pigs.

	Alentejano			Crossbred			Significance levels		
	NP	RP	RL	NP	RP	RL	Breed	Diet	B × D
IMF	4.16±0.36^{b}	5.79±0.92^{b}	4.47±0.39^{b}	2.68±0.28^{a}	2.16±0.16^{a}	3.74±0.35^{b}	***	ns	*
Fatty acid composition									
12:0	0.09±0.01^{ab}	0.07±0.00^{a}	0.08±0.01^{ab}	0.08±0.01^{ab}	0.09±0.00^{b}	0.08±0.00^{a}	ns	ns	**
14:0	1.32±0.02	1.40±0.03	1.44±0.03	1.49±0.07	1.38±0.05	1.45±0.04	ns	ns	ns
16:0	25.13±0.27	26.12±0.12	26.06±0.18	23.44±0.31	23.33±0.47	24.34±0.26	***	**	ns
16:1c7	0.25±0.01^{bc}	0.22±0.01^{ab}	0.22±0.01^{a}	0.24±0.01^{abc}	0.27±0.01^{c}	0.22±0.01^{a}	ns	**	*
16:1c9	2.74±0.09	3.05±0.06	3.15±0.06	3.11±0.15	2.84±0.18	3.08±0.11	ns	ns	ns
17:0	0.24±0.02	0.19±0.01	0.19±0.01	0.30±0.02	0.31±0.02	0.27±0.03	***	ns	ns
18:0	13.33±0.24	13.55±0.13	13.32±0.16	11.66±0.19	12.34±0.25	12.45±0.27	***	ns	ns
18:1t	0.13±0.01	0.15±0.01	0.15±0.01	0.16±0.01	0.15±0.01	0.15±0.00	ns	ns	ns
18:1c9	36.99±0.67^{cd}	38.36±0.63^{d}	37.42±0.62^{cd}	34.26±0.88^{ab}	33.48±0.88^{a}	36.45±0.61^{bc}	***	ns	*
18:1c11	5.39±0.18	5.72±0.21	5.70±0.20	6.13±0.48	5.27±0.19	5.91±0.18	ns	ns	ns
18:2*n-6*	8.47±0.49^{bc}	6.67±0.37^{a}	7.19±0.44^{ab}	11.06±0.75^{d}	11.78±0.69^{d}	8.93±0.42^{c}	***	**	**
18:3*n-3*	0.37±0.02	0.34±0.01	0.34±0.02	0.45±0.02	0.44±0.02	0.37±0.01	***	**	ns
20:0	0.17±0.01	0.16±0.01	0.16±0.01	0.14±0.01	0.13±0.01	0.14±0.01	**	ns	ns
20:1c11	0.65±0.02^{b}	0.68±0.03^{b}	0.63±0.04^{ab}	0.65±0.03^{b}	0.56±0.01^{a}	0.65±0.02^{b}	ns	ns	*
20:2*n-6*	0.24±0.01^{b}	0.21±0.01^{a}	0.20±0.01^{a}	0.34±0.02^{c}	0.33±0.01^{c}	0.27±0.01^{b}	***	***	*
20:3*n-6*	0.22±0.02^{b}	0.14±0.01^{a}	0.17±0.01^{a}	0.35±0.04^{c}	0.36±0.03^{c}	0.25±0.02^{b}	***	*	*
20:3*n-3*	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.05±0.01^{a}	0.09±0.01^{b}	0.08±0.01^{b}	***	*	*
20:4*n-6*	1.39±0.12	0.88±0.10	1.16±0.12	2.29±0.25	2.52±0.31	1.80±0.17	***	ns	ns
22:4*n-6*	0.24±0.02^{b}	0.16±0.01^{a}	0.20±0.02^{ab}	0.42±0.04^{cd}	0.46±0.05^{d}	0.32±0.02^{c}	***	*	*
Others	2.63±0.22^{bc}	1.89±0.18^{a}	2.20±0.19^{ab}	3.39±0.28^{de}	3.87±0.40^{e}	2.82±0.22^{cd}	***	ns	*
Partial sums									
SFA	40.27±0.47	41.49±0.24	41.24±0.33	37.12±0.40	37.58±0.65	38.72±0.41	***	*	ns
MUFA	46.14±0.70^{b}	48.18±0.63^{cd}	47.26±0.63^{bd}	44.55±1.19^{ab}	42.57±1.08^{a}	46.45±0.75^{bc}	***	ns	*
PUFA	10.96±0.65^{b}	8.45±0.50^{a}	9.29±0.61^{a}	14.95±1.10^{c}	15.99±1.11^{c}	12.01±0.63^{b}	***	*	*
n-6 PUFA	10.54±0.65^{bc}	8.06±0.50^{a}	8.91±0.59^{ab}	14.45±1.09^{d}	15.46±1.09^{d}	11.56±0.62^{c}	***	*	*
n-3 PUFA	0.41±0.02	0.38±0.01	0.38±0.02	0.50±0.02	0.53±0.02	0.45±0.14	***	*	ns
Fatty acid ratios									
PUFA/SFA	0.27±0.02^{bc}	0.20±0.01^{a}	0.23±0.02^{ab}	0.41±0.03^{d}	0.43±0.04^{d}	0.31±0.02^{c}	***	**	*
n-6/n-3	25.80±1.42	21.03±1.39	23.63±1.15	29.32±2.34	29.03±1.39	25.93±1.56	***	ns	ns

Significance: ns, $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$; means in the same row with different letters are significantly different ($P<0.05$); SEM, standard error of mean. Other: include unidentified peaks, SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; NP -normal protein diet with 18% protein equilibrated for lysine, RP – reduced diet with 14% protein corrected for lysine (0.8%) and RL- reduced diet with 14% protein and not corrected for lysine (0.5%).