RELATIVE CONTRIBUTION OF PRODUCTION FACTORS ON THE FATTY ACID COMPOSITION OF DIFFERENTIATED PORK

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Abstract – The objective of this study was to investigate the relative contribution of breed (Duroc, Lacombe and Iberian boars crossed to Large White×Landrace dams), slaughter weight (115 and 135 kg), sex (barrows and gilts), diet (Control, Canola or Flax) and their interactions on the fatty acid composition of intramuscular and subcutaneous fat of pigs. In intramuscular fat, most of the variability observed in 18:3n-3 (0.76) was explained by dietary treatment (88.7%), followed by breed (5.26%) and the breed×diet interaction (4.4%). Furthermore, diet contributed more than 94% of the explained variability observed in n-6/n-3 (0.81). In subcutaneous fat, the same factors contributed to the explained variance in 18:3n-3 (0.84), and in n-6/n-3 (0.90), in a similar order. On the other hand, in the explained variance of 18:2n-6, for both intramuscular and subcutaneous fat (0.38 and 0.59, respectively) breed was the most influential factor (68.9 and 68.2%, respectively). Some individual fatty acids and indices were also affected by both sex and slaughter (P<0.05). Understanding the contribution of each factor and their interactions will help the pork industry in the production of consistent differentiated products.

Key Words – pork, breed, diet, sex, weight

I. INTRODUCTION

In Canada, pork differentiation at the retail level is minimal and mainly based on credence quality attributes. Improved eating quality and positive health effects are two of the main traits that can be enhanced in order to differentiate meat and meat products [1].

In order to increase efficiency, genetic selection has led to leaner pigs [2]. Thus, pork branding based on less selected "heritage breeds" focuses on differences in quality attributes [3]. Similarly, dietary manipulation can result in enhanced attributes, such as healthier fatty acid (FA) profiles [4]. The gradual increase observed in commercial slaughter weights [5] may also impact pork quality. All these factors interact with each other and may elicit different responses from males and females, due to their different fat deposition patterns [6].

The objective of this study was to investigate the effects of breed composition, slaughter weight, sex, diet and their interactions on pork FA composition, which could help the pork industry both develop, and optimize differentiated-value added products for a highly competitive market.

II. MATERIALS AND METHODS

A 3×2×3×2 experiment was designed to include genotype, sex, diet, and slaughter weight (578 animals). Sires from Duroc, Lacombe (Peak Swine Genetics, Leduc, AB, Canada) and Iberian (Semen Cardona, Cardona, Spain) breeds were crossed to commercial Large White × Landrace F1 dams (Hypor Inc., Regina, SK, Canada). All animals (females and castrated males) were fed a typical Canadian commercial finisher diet (Masterfeeds, Winnipeg, MB, Canada). Animals were penned (three pigs of the same sex per pen) at 70 ± 3.3 kg live weight and randomly allocated to one of two slaughter weights: 115 or 135 kg. Three weeks before slaughter, a third of the animals continued on the Control diet, one third were fed a Canola supplemented diet (10% ExtraPro; 50% full fat canola and 50% extruded field peas, O&T Farms, Ltd., Regina, SK, Canada), and one third were fed a Flaxseed supplemented diet (10% LinPro; 50% flaxseed and 50% extruded field peas, O&T Farms) diet. Diets were formulated in collaboration with Verus Animal Nutrition (Winnipeg, MB).

At slaughter, *longissimus thoracis et lumborum* (LTL) muscle was collected from the left side of the carcass. A sample of subcutaneous fat (inner layer)

from the shoulder and a chop from the LTL (between 8th and 9th ribs) were collected and stored at -80°C for FA analyses.

Lipids were extracted from intramuscular using 2:1 chloroform:methanol [7]. Intramuscular lipids, and freeze dried backfat were dissolved in toluene containing 19:0 as an internal standard. Samples were then methylated using 5% methanolic HCl [8]. Samples were then cooled and hexane and KCl (0.88%) added, dried and stored at -20 °C until analyzed using the equipment and procedures described by Turner et al. [9]. Statistical analyses for all the studied traits were conducted using the MIXED model Covtest procedure of SAS [10], including breed, slaughter weight, sex and diet, as well as their interactions as fixed effects. Individual live weight nested within treatment was used as a covariate. Slaughter date was included as a random factor. The adjusted multiple R^2 was calculated for the full model as previously described [11]. Individual factors were then removed from the model and the decrease in the R^2 value used to calculate the relative contribution of each on the variability observed. An F-test was then used to assess the significance of the relative contribution of each factor [12]. Treatment means were determined using the LSMEANS option and separated using an F-test protected LSD ($P \leq 0.05$). Only the significant interactions ($P > 0.05$) or those accounting for >5% of the variability for any trait are presented in the tables.

III. RESULTS AND DISCUSSION

The statistical model used to evaluate FA composition in intramuscular fat explained a considerable percentage of variability (R^2) for only some individual n-3 FAs, especially 18:3n-3, total n-3 and the n-6/n-3 ratio (Table 1). For remaining FAs and indices, the model explained less than 40% of the observed variability. More than 88% of the variability observed in 18:3n-3 was explained by the dietary treatments, 5% by the breed and 4% by their interaction. In the case of total n-3, diet explained 59% of the variability, while breed was responsible for 25%, and their interaction explained 5%. Sex and slaughter weight also had a small influence (~4.5%). The n-6/n-3 ratio, the index with the highest R^2 from the model, was mainly explained by diet (94% of total explained variability).

The percentage of variability explained by the model for most saturated and mono-unsaturated individual FAs and indices was relatively low. This may be due to low variability in certain FAs, individual animal variation and/or large contributions of factors not included in the model. Low variance is typical of FAs which are intermediate metabolites of important metabolic routes [13], such as 16:0, 18:0 or 18:1.

Table 1. Relative contribution (%) of each factor to variance in intramuscular fat composition

	R^2	Breed (B)	Diet (D)	Sex (S)	Weight (W)	B×S	S×W
SFA	0.24	10.8	ns	62.4	5.91	13.3	ns
MUFA	0.33	81.9	8.67	4.32	1.65	ns	2.00
PUFA	0.40	62.9	5.12	23.1	4.27	1.44	1.37
n-3	0.61	25.3	59.5	5.17	4.32	0.16	0.26
18:3n-3	*0.76*	*5.26*	*88.7*	*0.14*	*0.79*	*ns*	*0.15*
22:5n-3	*0.45*	*57.6*	*12.9*	*18.7*	*4.16*	*1.85*	*0.67*
n-6	0.38	65.0	ns	26.3	3.56	1.61	1.46
18:2n-6	*0.38*	*68.9*	*0.97*	*22.0*	*3.40*	*1.09*	*2.32*
20:4n-6	*0.37*	*53.5*	*ns*	*36.0*	*3.45*	*2.93*	*ns*
PUFA/SFA	0.39	57.3	4.44	27.2	5.00	3.17	0.73
n-6/n-3	0.81	3.69	94.1	0.14	1.27	ns	0.02
Total fat	0.34	69.6	ns	16.9	ns	ns	5.79

SFA, MUFA, PUFA: saturated, mono-unsaturated and polyunsaturated fatty acids

Meat from Lacombe pigs had lower intramuscular fat content (1.5%) compared with the other two breeds (2.4%; Data not shown). Meat from females (1.9%) was always lower ($P<0.05$) in intramuscular fat than meat from castrated males, which increased from 115 (2.1%) to 135 kg (2.5%).

In backfat, 18:3n-3, total n-3 and the n-6/n-3 ratio were again well explained by the model ($R^2>0.80$). More than 50% of the variability observed in 18:2n-6, total PUFA and n-6, as well as the PUFA/SFA ratio, was also explained by factors included in the model (main factors and interactions; Table 2). Diet was the most influential factor for 18:3n-3, 22:5n-3, total n-3 and, especially, the n-6/n-3 ratio. The effect of breed was very important for 18:2n-6, total PUFA and n-6, and the PUFA/SFA ratio. Those same FAs and indices were also influenced by the diet during the last three weeks and sex of the animal. Slaughter weight did not explain more than 5% of the variability of any of the FAs and indices with high R^2. The interaction between breed and diet explained 6% of the variability in 18:3n-3 and total n-3, as was observed in intramuscular fat, as well as 22:5n-3. For 18:2n-6, 20:4n-6 and total n-6, the most

influential interactive effect was breed×sex, explaining 9-10% of the variability observed in these FAs.

Table 2. Relative contribution (%) of each factor to variance in backfat composition

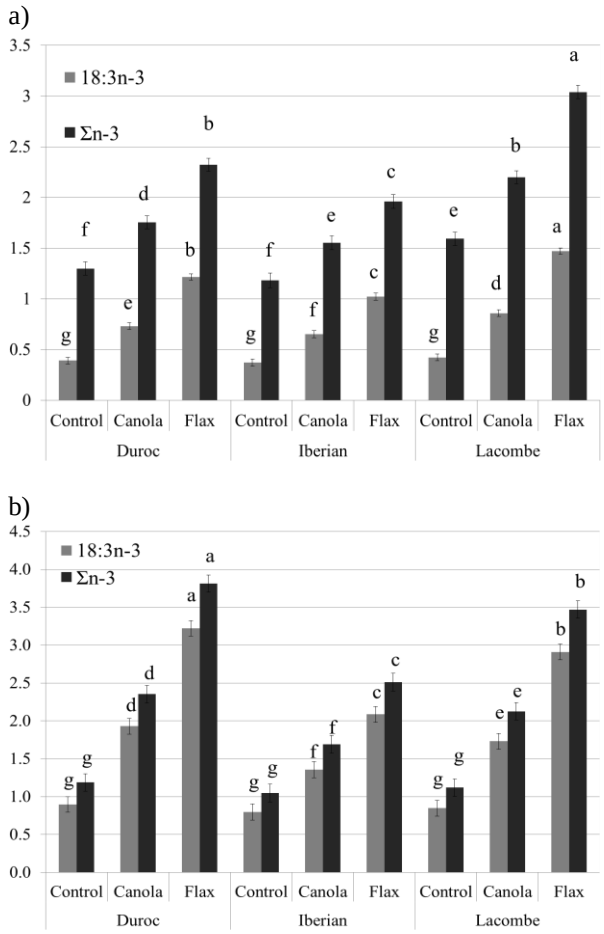
	R^2	Breed (B)	Diet (D)	Sex (S)	Weight (W)	B×D	B×S
SFA	0.24	42.7	13.6	12.8	11.2	4.81	14.0
MUFA	0.17	26.5	67.9	ns	ns	ns	ns
PUFA	0.70	46.0	38.6	6.54	1.56	3.25	3.08
n-3	0.84	6.95	83.9	0.55	0.20	6.26	ns
18:3n-3	*0.84*	*6.48*	*84.2*	*0.61*	*0.20*	*6.35*	*ns*
22:5n-3	*0.41*	*11.7*	*70.2*	*ns*	*4.07*	*6.87*	*1.07*
n-6	0.60	69.2	8.79	10.3	2.28	0.32	8.83
18:2n-6	*0.59*	*68.2*	*9.69*	*10.3*	*2.37*	*0.45*	*8.75*
20:4n-6	*0.21*	*51.6*	*3.93*	*16.5*	*18.1*	*ns*	*9.84*
PUFA/SFA	0.65	45.8	34.2	8.22	2.60	3.77	4.60
n-6/n-3	0.90	ns	97.4	ns	ns	1.25	0.20

Due to the interaction between breed and diet, the changes due to the diet were relatively higher in intramuscular fat from Lacombe and backfat from Duroc, followed by Lacombe (Figure 1). Differences in total fat content and fat deposition could be responsible for this interactive effect. Zhang et al. [14] published another aspect of this study and reported that the Lacombe breed had the lowest intramuscular fat content, while, carcasses from Duroc-crossed pigs had the lowest backfat content, followed by those from Lacombe-crossed animals. Therefore, similar absolute changes in FA composition would have a lower relative impact on breeds with higher fat content, due to smaller relative contribution of membrane phospolipids in intramuscular fat and a dilution effect on backfat. The significant effect of breed could also partly be explained by variations in one or more key lipogenic enzymes [15].

Changing the diet led to a huge impact on the content of 18:3n-3 and n-6/n-3 that was only partly modified by the other factors employed in this study. Differences in dietary fat composition are known to be reflected in the intramuscular and subcutaneous fat of pigs [16].

As expected, inclusion of flax led to higher 18:3n-3 and total n-3 in both intramuscular and subcutaneous fat [17]. Although to a lesser degree than for flax, feeding canola also led to significant increases in 18:3n-3 and total n-3 compared with the control diet, in both intramuscular and subcutaneous fat. A similar effect was reported by Bertol et al. [16]. The use of canola as a means to increase n-3 content in Canada may have several advantages, from its competitive price (lower than the control diet in this study; data not shown) to a credence attribute in the development of Canadian heritage pork.

Figure 1. Breed×diet interaction for 18:3n-3 and total n-3 (% total fat) in intramuscular (a) and backfat (b). Means separation within fatty acid.



Regarding sex, Geri et al. [6] observed a preferential deposition of 18:2n-6 in females, which could partly explain the sex effect, as well as the breed×sex interaction, for this FA. Sex and breed differences in total fat content could also be responsible for the effects of these two factors on 20:4n-6, especially in intramuscular fat, as this FA is mainly found in cell membranes.

The impact of slaughter weight on most of the FAs was very small compared to the other factors. This indicates that an increase in slaughter weight will not affect the FA profile of pork as much as the other factors.

IV. CONCLUSIONS

Although all the factors had a significant effect on several of the individual FAs and indices, further data analysis revealed that diet, followed by breed and sex, had the highest impact on those traits well explained by the model. The interaction effect of diet and breed should be considered when attempting to manipulate the n-3 content in pork.

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REFERENCES

1. Grunert, K.G., Bredahl, L. & Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sector - A review. Meat Science 66: 259-272.
2. Lonergan, S.M., Huff-Lonergan, E., Rowe, L.J., Kuhlers, D.L. & Jungst, S.B. (2001). Selection for lean growth efficiency in Duroc pigs influences pork quality. Journal of Animal Science 79: 2075-2085.
3. Carrapiso, A.I., Bonilla, F. & García, C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. Meat Science 65: 623-629.
4. Juárez, M., Dugan, M.E.R., Aldai, N., Aalhus, J.L., Patience, J.F., Zijlstra, R.T. & Beaulieu, A.D. (2010). Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower-finisher pigs. Meat Science 84: 578-584.
5. Brewer, M.S., Jensen, J., Sosnicki, A.A., Fields, B., Wilson, E. & McKeith, F.K. (2002). The effect of pig genetics on palatability, color and physical characteristics of fresh pork loin chops. Meat Science 61: 249-256.
6. Geri, G., Poli, B.M., Zappa, A., Campodoni, G. & Franci, O. (1990). Relationships between adipose tissue characteristics of newborn pigs and subsequent performance: III. Histological and chemical characteristics of backfat. Journal of Animal Science 68.
7. Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497.
8. Kramer, J.K.G., Sehat, N., Dugan, M.E.R., Mossoba, M.M., Yurawecz, M.P., Roach, J.A.G., Eulitz, K., Aalhus, J.L., Schaefer, A.L. & Ku, Y. (1998). Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography. Lipids 33: 549-558.
9. Turner, T.D., Mapiye, C., Aalhus, J.L., Beaulieu, A.D., Patience, J.F., Zijlstra, R.T. & Dugan, M.E.R. (2014). Flaxseed fed pork: n−3 fatty acid enrichment and contribution to dietary recommendations. Meat Science 96: 541-547
10. SAS (2003). SAS® user's guide: Statistics. SAS for Windows, version 9.1. SAS Institute, Inc., Cary, NC.
11. Edwards, L.J., Muller, K.E., Wolfinger, R.D., Qaqish, B.F. & Schabenberger, O. (2008). An R^2 statistic for fixed effects in the linear mixed model. Statistics in medicine 27: 6137-6157.
12. Juárez, M., Basarab, J.A., Baron, V.S., Valera, M., Larsen, I.L. & Aalhus, J.L. (2012). Quantifying the relative contribution of ante- and post-mortem factors to the variability in beef texture. Animal 6(11): 1878-87.
13. Juárez, M., Horcada, A., Alcalde, M.J., Valera, M., Mullen, A.M. & Molina, A. (2008). Estimation of factors influencing fatty acid profiles in light lambs. Meat Science 79: 203-210.
14. Zhang, H., Aalhus, J.L., Gariépy, C., Uttaro, B., López, C., O., Prieto, N., Dugan, M.E.R., Jin, Y. & Juárez, M. (2016). Effects of pork differentiation strategies in Canada on pig performance and carcass characteristics. Canadian Journal of Animal Science (In Press).
15. Marriott, D.T., Chevillon, P., Spencer-Phillips, P.T.N. & Doran, O. (2013). Effect of breed on fatty acid composition and lipogenic enzyme abundance in the subcutaneous adipose tissue of pigs. Journal of Food Science 78: C1130-C1135.
16. Bertol, T.M., de Campos, R.M.L.D., Ludke, J.V., Terra, N.N., de Figueiredo, E.A.P., Coldebella, A., dos Santos Filho, J.I., Kawski, V.L. & Lehr, N.M. (2013). Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. Meat Science 93: 507-516.
17. Juárez, M., Dugan, M.E.R., Aldai, N., Aalhus, J.L., Patience, J.F., Zijlstra, R.T. & Beaulieu, A.D. (2011). Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. Food Chemistry 126: 1716-1723.