PAPER

CHARACTERISTICS OF LIPIDS FROM IMMUNOCASTRATED MEDIUM-HEAVY PIGS FED EITHER A RESTRICTED DIET OR *AD LIBITUM*

G. MINELLI*[a,b], P. MACCHIONI[c], F.MEZZETTI[b], A.M. BELMONTE[b], L.A. VOLPELLI[a,b], V. FAETI[d] and D.P. LO FIEGO[a,b]

[a]Department of Life Sciences, University of Modena and Reggio Emilia, Padiglione Besta, Italy

[b]Interdepartimental Research Centre for Agri-Food Biological Resources Improvement and Valorisation, University of Modena and Reggio Emilia, Padiglione Besta, Italy

[c]Department of Agricultural and Food Sciences, University of Bologna, Italy

[d]Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA - Council for Agricultural Research and Economics), Research Centre for Animal Production and Aquaculture, Modena Unit, Italy

*Corresponding author: Tel.: +39 0522 522085; Fax: +39 0522 522027

E-mail address: gminelli@unimore.it

ABSTRACT

We studied the feeding level-related variations in lipid characteristics in the adipose tissues of pigs. The lipid content, fatty acid profile, oxidative stability, iodine value, thrombogenic and atherogenic indices were determined in individual samples from 24 immunocastrated males (Duroc x Large White), fed either restricted or *ad libitum*. In backfat, feed restriction increased the polyunsaturated fatty acid proportion and iodine value and lowered the thrombogenic and atherogenic indices. Intramuscular lipid content was reduced by restriction, which did not affect either the fatty acid composition or the oxidative stability in both raw and cooked muscle. Feed restriction improved the nutritional quality of lipids without impairing their technological attributes.

Keywords: adipose depots, fatty acid profile, feeding restriction, immunocastration, medium-heavy pigs

1. INTRODUCTION

The consumption of meat, a common constituent of western balanced diets, is associated with the development of serious cardiovascular diseases (KONTOGIANNI *et al.,* 2008; ABETE *et al.,* 2014; LARSSON and ORSINI, 2014) because of the amount and characteristics of the fat it provides (SIMOPOULOS, 1999; WILLIAMS, 2000).
In pig meat, several factors affect the deposition of lipids and their fatty acid (FA) composition. Of these, feeding strategies (WOOD *et al*., 1986; CAMERON *et al*., 2000; LO FIEGO *et al*., 2005a; LEBRET, 2008), genetic factors mainly related to the adipogenetic aptitude of breeds (CAMERON *et al*., 2000; PIEDRAFITA *et al*., 2001; WOOD *et al*., 2008), sex, age and live weight at slaughter (LEBRET and MOUROT, 1998; LO FIEGO *et al*., 2005b) have been demonstrated to play a role.
In Italy, pork for fresh consumption, obtained mainly from subjects slaughtered at 95-100 kg body weight (BW), is generally imported. National production is centered on the rearing of heavy pigs slaughtered when they reach 9/10 months of age and 160-170 kg BW, to be processed into typical, high quality, dry cured Protected Designation of Origin (PDO) products.
A production chain based on medium–heavy pigs, slaughtered at a final weight of approximately 140 kg at about 6-8 months of age and not subjected to the strict PDO rules, provides meat for both fresh consumption and processed products such as salami, sausages, seasoned and cooked hams. For this production, the same genetic types used for heavy pig production can be used. Compared to heavy pigs, medium–heavy pigs have a shorter fattening period and, thus, show a better feed conversion efficiency (LO FIEGO *et al*., 2010; DALLA BONA *et al*., 2016).
Male pigs destined to be slaughtered at such high BW and age are surgically castrated to limit aggressiveness and the development of boar taint, a potentially off-putting odor in the meat (DUNSHEA *et al*., 2001; XUE and DIAL, 1997). However, this technique is stressful for the animal, with negative effects on health and animal welfare (ZAMARATSKAIA *et al*., 2008). Hence, in view of possible EU legislative measures aimed at restricting this practice, the investigation of suitable alternatives has been encouraged.
One method of inhibiting sexual development and boar taint is immunization against gonadotropin releasing hormones (GnRHs) (DUNSHEA *et al*., 2001). The vaccine is administered in two doses. After the second vaccination, immunocastrated pigs perform similarly to surgically castrated subjects (FÀBREGA *et al*., 2010; GISPERT *et al*., 2010; PAULY *et al*., 2009). In fact, when fed *ad libitum*, immunocastrated subjects show a very high feed intake, which can lead to excessive fat deposition. This, in turn, can influence the fatty acid composition of lipid depots, which become more saturated. Conversely, decreasing energy intake brings about an increase in the degree of unsaturation in pig adipose tissue (LEBRET and MOUROT, 1998).
The effects of feed restriction in immunocastrated pigs after the second vaccination, aimed at improving feed efficiency and avoid excessive carcass fatness, have been poorly investigated, and studies have only been performed in lean light pigs. Moreover, according to a review by ČANDEK-POTOKAR *et al*. (2015), research has yielded conflicting results. Finally, the effect of this feeding practice on the fatty acid composition of subcutaneous adipose tissue and intramuscular fat has not been taken into account.
Thus, this research was carried out on finishing immunocastrated pigs slaughtered at about 140 kg BW, in order to verify the effects of feed restriction, on fatty acid composition of backfat and intramuscular fat.

2. MATERIALS AND METHODS

2.1. Animals, diet and sampling

A total of 24 crossbred (Italian Duroc x Italian Large White) pigs were involved in the trial, conducted at CREA (San Cesario S/P, Modena, Italy). All pigs were immunocastrated against GnRH using two doses of Improvac® vaccine (Zoetis, Belgium, S.A.), each dose consisting of 300 μg of GnRH-protein conjugate in 2 ml of aqueous solvent. Applications were performed by subcutaneous injection, at 90 and 162 days of age.
The animals were housed collectively until they reached 120 days of age (51.84±4.38 kg BW). Subsequently, the pigs were housed evenly in six adjacent pens, each with four subjects, balanced for weight, and were fed *ad libitum* until they were 162 days old (103.3±6.9 kg BW). Thereafter, until slaughtering, at 197 days of age (142.32±6.8 kg BW), three pens were fed restricted (R) at 7.5% $BW^{0.75}$ and the remaining three received the same farm concentrate *ad libitum* (AL). This dietary restriction level is commonly adopted in the finishing of traditional Italian heavy pigs. Feed composition is shown in Table 1. Water was always available through nipple drinkers.

Table 1. Determined and calculated diet analyses.

Determined analysis[(a)]	%
Crude protein	13.83
Crude fibre	5.05
Crude fat	2.50
Fatty acids (FAs)	(% total FAs)
C14:0 (myristic)	0.23
C16:0 (palmitic)	21.62
C16:1n-7 (palmitoleic)	0.33
C18:0 (stearic)	2.57
C18:1n-9 (oleic)	24.27
C18:2n-6 (linoleic)	48.27
C18:3n-3 (α-linolenic)	2.71
Σ SFA (saturated FAs)	24.42
Σ MUFA (monounsaturated FAs)	24.60
Σ PUFA (polyunsaturated FAs)	50.98
Calculated analysis[(b)]	
Starch, %	46.01
Digestible lysine, %	0.80
Net energy, Mcal/kg	2.29

The ingredients in the diet in % (as fed): corn meal 48.6, barley meal 21.0, beet pulp 6.0, soybean meal dehulled 13.5, wheat bran 7.5. Added amino acids 0.55 (l-lysine HCl 0.38; dl-methionine 0.05; l-threonine 0.07; l-tryptophan 0.05); added salts 2.45 (calcium carbonate 1.05; dicalcium phosphate 1.00; sodium chloride 0.40) vitamins and trace elements 0.40.

[(a)]According to the Association of Official Analytical Chemists (1995). [(b)]According to Sauvant *et al*. (2004).

All animals were slaughtered on the same day, following routine abattoir procedures. After carcass grading, 1 hour *post mortem*, individual samples of the *Longissimus lumborum* (LL) muscle and subcutaneous adipose tissue were collected at the last rib level. An

aliquot of the samples was vacuum-packed, in individual bags, and stored at -20°C, for subsequent laboratory analysis.

2.2. Analyses

2.2.1 Lipid content and oxidative stability

Each sample of LL muscle was analyzed in duplicate to determine:
- On fresh muscle (24 h *post mortem*) - lipid oxidation by the 2-thiobarbituric acid reactive substances (TBARS) measurement, according to SIU and DRAEPER (1978); results were expressed as mg of malondialdehyde (MDA)/kg muscle.
The same determination was carried out on vacuum-packed slices (approx. 100 g), put in a water-bath at 80°C, and left until the core temperature reached 70°C.
- After thawing - ether extract content using petroleum ether (Carlo Erba reagents, MI, Italy) and a Soxhlet apparatus (AOAC, 1995) with previous acid hydrolysis. Results were expressed as the percentage of wet matter.

2.2.2 Fatty acid profile

Fatty acid (FA) composition of lipids of subcutaneous adipose tissue and LL muscle was determined using a TRACE™GC Ultra (Thermo Electron Corporation, Rodano, Milan, Italy) equipped with a Flame Ionization Detector, a PTV injector, and a TR-FAME Column (Thermo Scientific, Rodano, Milan), 30 m long, 0.25 mm i.d., 0.2 μm film thickness. Total lipids were extracted from the samples of subcutaneous adipose tissue, separately for the outer and inner layers (IUPAC, 1979) and from LL muscle (FOLCH *et al*., 1957).
An aliquot of 25 mg was then subjected to methylation by means of a methanolic solution of potassium hydroxide (KOH 2N) according to FICARRA *et al*. (2010), using tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Malmö, Sweden) as internal standard. The injection of the fatty acid methyl ester sample (1 μl) was performed in split mode with a split flow of 10 mL/min, operating at a constant flow of 1 mL/min of helium as a carrier gas. The temperature of the injector and detector was kept at 240°C. The temperature program used for the analysis started from 140°C, was maintained for 2 min, then increased to 250°C, at a rate of 4°C/min, and kept at this temperature for 5 min.
The peaks of the fatty acids were recorded and integrated using Chrom-Card software (vers. 2.3.3, Thermo Electron Corporation, Rodano, Milan, Italy) and identified by comparison with the retention times of standard solutions with known quantities of various methyl esters (Supelco® 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellafonte, PA, USA). For quantification purposes, the response factor was calculated, and an internal standard was used. The amount of each FA in the sample was expressed as FA relative percentage with respect to the total amount of FAs. The iodine value (IV) of the outer and inner layers of backfat was calculated adopting the equations proposed by LO FIEGO *et al*. (2016):

$$IV = 85.703 + [C14:0] \times 2.740 - [C16:0] \times 1.085 - [C18:0] \times 0.710 + [C18:2n\text{-}6] \times 0.986$$

In addition, atherogenic and thrombogenic indices (AI and TI) were calculated after ULBRICHT and SOUTHGATE (1991):

$$AI = \frac{(C12:0 + 4xC14:0 + C18:0)}{(MUFA + PUFA)}; \quad TI = \frac{(C12:0 + C16:0 + C18:0)}{0.5x(MUFA + n-6\ PUFA) + 3x(n-3\ PUFA) + (n-3\ PUFA/n-6 PUFA)}$$

2.3. Statistical analysis

The data were statistically analyzed by a MIXED model (SAS Institute Inc., Cary, NC, USA) including dietary treatment as the fixed effect and pen as the random effect.

3. RESULTS AND DISCUSSION

As expected, and in agreement with QUINIOU *et al*. (2012) and BATOREK *et al*. (2012), R pigs gained less final BW (135.4 *vs* 149.3 kg), yielded lighter carcasses (113.4 *vs* 125.1 kg) and thinner backfat (22.7 *vs* 28.1 mm) compared to the AL group (P<0.01) (*data not reported*). Table 2 shows the data regarding the intramuscular fat (IMF) content and lipid oxidation (TBARS values) in LL muscle.

Table 2. Intramuscular fat (IMF) and TBARS (thiobarbituric acid reactive substances) content of *Longissimus lumborum* muscle of immunocastrated male pigs submitted to different feeding regimes (least squares means).

Items	Treatment		SE[$]
	***Ad libitum* (n=12)**	**Restricted (n=12)**	
IMF (%)	3.97[a]	2.43[b]	0.617
TBARS (mg MDA/kg raw muscle)	0.057	0.052	0.008
TBARS (mg MDA/kg cooked muscle)	1.159	1.298	0.139

[$]Standard error of the differences; [a,b]=$P<0.05$.

The IMF content was lower than that found by MINELLI *et al*. (2013) in pigs slaughtered at a heavier weight and similar to those observed by ROSSI *et al*. (2014) and by DALLA BONA *et al*. (2016) in medium-heavy pigs slaughtered at 135-140 kg body weight. Feeding restriction significantly lowered ($P<0.05$) the IMF content. BATOREK *et al*. (2012), instead, found no difference in this trait between *ad libitum* or restricted fed immunocastrated pigs.
The feeding regimen did not affect the oxidative stability of fresh and cooked LL muscle. The TBARS values fell within the range observed by ROSSI *et al*. (2014) and indicated a very low level of lipid oxidation in both fresh and cooked muscle.
Table 3 shows the data on the effect of the dietary treatment on the fatty acid composition of the outer layer of backfat.
Total saturated fatty acids (SFAs) were lower in restricted pigs ($P<0.05$); the difference being mainly represented by the variations in the proportions of palmitic acid (C16:0; $P<0.05$) and stearic acid (C18:0), although the latter did not decrease significantly. The percentage of monounsaturated fatty acids (MUFA), except for heptadecenoic acid (C17:1) that increased in the R pigs ($P<0.05$), was not affected by the feeding regimen, whereas the percentage of total polyunsaturated fatty acids (PUFAs) was significantly higher in the R group ($P<0.01$). This outcome is mainly ascribable to the variation in the proportion of linoleic acid (C18:2n-6; $P<0.05$), the single most represented PUFA in backfat, although all the individual PUFA percentages, except for eicosadienoic (C20:2n-6), eicosapentaenoic (C20:5n-3) and docosadienoic (C22:2n-6) acids, were significantly higher in the R group.
Accordingly, restricted pigs showed higher PUFA/SFA ratio values ($P<0.01$), total n-6 ($P<0.05$) and n-3 FAs, IV ($P<0.01$), and lower values for TI ($P<0.01$) and AI ($P<0.05$).

Table 3. Fatty acid (FA) composition (%) of the outer layer of backfat in immunocastrated male pigs submitted to different feeding regimes (least squares means).

Fatty acids	Treatment		SE[($)]
	***Ad libitum* (n=12)**	**Restricted (n=12)**	
C 10:0 (capric)	0.09	0.08	0.006
C 12:0 (lauric)	0.08	0.08	0.004
C 14:0 (myristic)	1.37	1.39	0.041
C 16:0 (palmitic)	25.48[a]	24.21[b]	0.471
C 17:0 (heptadecanoic)	0.37	0.41	0.050
C 18:0 (stearic)	12.91	12.03	0.504
C 20:0 (eicosanoic)	0.18	0.15	0.012
C 16:1n-7 (palmitoleic)	2.21	2.41	0.170
C 17:1n-7 (heptadecenoic)	0.32[b]	0.43[a]	0.041
C 18:1n-7 (vaccenic)	2.51	2.69	0.114
C 18:1n-9 (oleic)	39.31	38.47	0.612
C 20:1-n9 (eicosenoic)	0.79	0.75	0.052
C 18:2n-6 (linoleic)	12.50[b]	14.66[a]	0.776
C 18:3n-3 (α-linolenic)	0.65[B]	0.76[A]	0.034
C 18:3n-6 (γ-linolenic)	0.15[B]	0.19[A]	0.011
C 20:2n-6 (eicosadienoic)	0.53	0.60	0.043
C 20:3n-3 (eicosatrienoic)	0.10[b]	0.12[a]	0.007
C 20:4n-6 (arachidonic)	0.25[B]	0.32[A]	0.018
C 20:5n-3 (eicosapentaenoic)	0.01	0.01	0.001
C 22:2n-6 (docosadienoic)	0.01	0.01	0.001
C 22:4n-6 (docosatetraenoic)	0.11[B]	0.13[A]	0.006
C 22:5n-3 (docosapentaenoic)	0.06[B]	0.08[A]	0.005
C 22:6n-3 (docosaesaenoic)	0.03[B]	0.04[A]	0.003
Σ SFA (saturated FA)	40.47[a]	38.34[b]	0.755
Σ MUFA (monounsaturated FA)	45.15	44.74	0.226
Σ PUFA (polyunsaturated FA)	14.38[B]	16.91[A]	0.882
PUFA/SFA	0.36[B]	0.44[A]	0.026
Σ n-6 (ω-6 FA)	13.54[b]	15.91[a]	0.840
Σ n-3 (ω-3 FA)	0.85[B]	1.00[A]	0.044
n-6/n-3	15.98	15.85	0.313
IV (iodine value)	64.98[B]	69.17[A]	1.151
TI (thrombogenic index)	1.21[A]	1.09[B]	0.039
AI (atherogenic index)	0.52[a]	0.48[b]	0.014

[a,b]=$P<0.05$; [A,B]=$P<0.01$; [($)]standard error of differences;
IV=85.703 + [C14:0] x 2.740 - [C16:0] × 1.085 - [C18:0] × 0.710 + [C18:2n-6] × 0.986);

$$TI = \frac{(C12{:}0+C16{:}0+C18{:}0)}{0.5x(MUFA+n-6\ PUFA)+3x(n-3\ PUFA)+(n-3\ PUFA/n-6PUFA)}\ ;\quad AI = \frac{(C12{:}0+4xC14{:}0+C18{:}0)}{(MUFA+PUFA)}$$

Table 4 reports the data regarding the effects of feed restriction on the fatty acid composition of the backfat inner layer.

Table 4. Fatty acid (FA) composition (%) of the inner layer of backfat in immunocastrated male pigs submitted to different feeding regimes (least squares means).

Fatty acids	Treatment		SE[($)]
	Ad libitum (n=12)	Restricted (n=12)	
C 10:0 (capric)	0.07	0.07	0.006
C 12:0 (lauric)	0.07	0.07	0.003
C 14:0 (myristic)	1.30	1.28	0.045
C 16:0 (palmitic)	26.62[a]	25.87[b]	0.347
C 17:0 (heptadecanoic)	0.34[b]	0.47[a]	0.049
C 18:0 (stearic)	16.05	15.94	0.691
C 20:0 (eicosanoic)	0.20[a]	0.18[b]	0.009
C 16:1n-7 (palmitoleic)	1.71	1.71	0.166
C 17:1n-7 (heptadecenoic)	0.24[b]	0.32[a]	0.037
C 18:1n-7 (vaccenic)	2.02	2.08	0.114
C 18:1n-9 (oleic)	37.70	36.62	0.740
C 20:1-n9 (eicosenoic)	0.82	0.79	0.057
C 18:2n-6 (linoleic)	11.22[B]	12.70[A]	0.452
C 18:3n-3 (α-linolenic)	0.55[B]	0.63[A]	0.022
C 18:3n-6 (γ-linolenic)	0.12[B]	0.16[A]	0.012
C 20:2n-6 (eicosadienoic)	0.50	0.55	0.032
C 20:3n-3 (eicosatrienoic)	0.09[b]	0.10[a]	0.005
C 20:4n-6 (arachidonic)	0.20[B]	0.24[A]	0.012
C 20:5n-3 (eicosapentaenoic)	0.00	0.01	0.001
C 22:2n-6 (docosadienoic)	0.01	0.01	0.001
C 22:4n-6 (docosatetraenoic)	0.09[b]	0.10[a]	0.005
C 22:5n-3 (docosapentaenoic)	0.05[B]	0.06[A]	0.003
C 22:6n-3 (docosaesaenoic)	0.02[B]	0.03[A]	0.002
Σ SFA (saturated FA)	44.66	43.88	0.780
Σ MUFA (monounsaturated FA)	42.50	41.53	0.947
Σ PUFA (polyunsaturated FA)	12.84[B]	14.59[A]	0.504
PUFA/SFA	0.29[B]	0.33[A]	0.013
Σ n-6 (ω-6 FA)	12.12[B]	13.77[A]	0.479
Σ n-3 (ω-3 FA)	0.72[B]	0.82[A]	0.026
n-6/n-3	16.88	16.70	0.225
IV (iodine value)	60.04[b]	62.35[a]	0.821
TI (thrombogenic index)	1.45	1.39	0.045
AI (atherogenic index)	0.58	0.55	0.015

[a,b]=$P<0.05$; [A,B]=$P<0.01$; [($)]standard error of differences;
IV=85.703 + [C14:0] x 2.740 - [C16:0] × 1.085 - [C18:0] × 0.710 + [C18:2n-6] × 0.986);

$$TI = \frac{(C12{:}0+C16{:}0+C18{:}0)}{0.5x(MUFA+n-6\ PUFA)+3x(n-3\ PUFA)+(n-3\ PUFA/n-6PUFA)}\ ;\quad AI = \frac{(C12{:}0+4xC14{:}0+C18{:}0)}{(MUFA+PUFA)}$$

In this layer the decrease in total SFAs was less marked than in the outer layer. Again, the most significant variation was shown by C16:0, which decreased significantly ($P<0.05$) in R pigs. In this group, eicosanoic acid (C20:0) decreased ($P<0.05$) and heptadecanoic acid (C17:0) increased ($P<0.05$). The feeding regimen did not affect total MUFA percentage and, as already observed in the outer layer for this class of FA, only the heptadecenoic acid (C17:1) proportion was higher in R pigs ($P<0.05$).
The total PUFA percentage was significantly higher ($P<0.01$) in R pigs. As found in the outer layer, all the PUFAs except C20:2n-6, C20:5-n3 and C22:2n-6 increased with feed restriction, however the difference was mainly related to the increase in linoleic acid proportion ($P<0.01$). As in the outer layer, also in the inner layer PUFA/SFA ratio, the total n-3 and n-6 fatty acid percentage ($P<0.01$) and IV ($P<0.05$) were higher in restricted fed subjects. The TI and AI values did not differ between treatments. The inner layer was thus characterized by a higher percentage of saturated fatty acids and a lower PUFA/SFA ratio than the outer layer, as also observed by DUNKER *et al*. (2007), DAZA *et al*. (2017) and BEE *et al*. (2002). According to MONZIOLS *et al*. (2007), this could be explained by the larger *de novo* synthesis of SFAs (especially C16:0) exhibited in the inner layer, which leads to a dilution of dietary PUFAs (especially C18:2n-6). Concerning the effect of feeding restriction in the finishing phase on subcutaneous fatty acid composition, our data show that decreasing the feed allowance and thus the availability of energy for the *de novo* synthesis, which mainly yields SFAs, leads to a higher unsaturation of backfat lipids connected to a relative increase in PUFAs, which are of strict feed origin (DAZA *et al*., 2007).
Table 5 shows the fatty acid composition of IMF in LL.
The feeding level only marginally affected the fatty acid composition of intramuscular fat. Palmitic acid decreased by 0.9 percentage points in restricted pigs ($P<0.05$) as well as C20:0 which decreased by 0.01 percentage points ($P<0.05$), whereas C20:2n-6 and C20:3n-3 increased by 0.03 ($P<0.01$) and 0.01 ($P<0.05$) percentage points, respectively. The non-significant variations exhibited by total SFAs and PUFAs resembled those observed by DALLA BONA *et al*. (2016). It is well known that diet affects fatty acid composition in muscle less than in subcutaneous adipose tissue (CORINO *et al*., 2002).
Our data indicate that the nutritional quality of adipose depots is in general improved by a dietary restriction. This is evident especially in backfat, where the PUFA/SFA ratio and total omega-3 PUFA percentage were higher ($P<0.01$) in both layers, and TI and AI were lower, however the differences were statistically significant only in the outer layer. These nutritional parameters showed the same trend in the IMF, where the differences were not statistically significant. However, in the present research, the n-6/n-3 ratio of both layers of backfat and of LL muscle was not favorably modified by the feed restriction which increased the proportions of both n-6 and n-3 PUFA. This confirms the findings of WIECEK *et al*. (2011) in *Longissimus thoracis* from light pigs slaughtered after 64 and 83 days of treatment.
Regarding the technological attributes of lipids, in both layers of backfat, the iodine value was significantly higher in restricted pigs but lower than 70, which is the threshold indicated by most authors (BARTON-GADE, 1987; MADSEN *et al*., 1992) as a guarantee of good preservation aptitude. GIRARD *et al*. (1988) suggest that in order to obtain an adequately firm fat that is not too susceptible to oxidation, its content in stearic and linoleic acid should be higher than 12% and lower than 15%, respectively. These requisites were met in both the *ad libitum* and restricted groups, in both layers. Thus, feeding restriction did not impair the technological quality of subcutaneous adipose tissue.

Table 5. Fatty acid (FA) composition (%) of *Longissimus lumborum* muscle in immunocastrated male pigs submitted to different feeding regimes (least squares means).

Fatty acids	Treatment		
	***Ad libitum* (n=12)**	**Restricted (n=12)**	**SE[($)]**
C 10:0 (capric)	0.12	0.12	0.001
C 12:0 (lauric)	0.09	0.08	0.005
C 14:0 (myristic)	1.40	1.38	0.066
C 16:0 (palmitic)	27.99[a]	27.10[b]	0.413
C 17:0 (heptadecanoic)	0.23	0.27	0.028
C 18:0 (stearic)	14.43	14.63	0.745
C 20:0 (eicosanoic)	0.17[a]	0.16[b]	0.008
C 16:1n-7 (palmitoleic)	3.08	3.03	0.249
C 17:1n-7 (heptadecenoic)	0.24	0.25	0.026
C 18:1n-7 (vaccenic)	3.83	3.89	0.208
C 18:1n-9 (oleic)	42.53	41.95	0.731
C 20:1-n9 (eicosenoic)	0.80	0.80	0.040
C 18:2n-6 (linoleic)	4.18	5.11	0.743
C 18:3n-3 (α-linolenic)	0.19	0.20	0.025
C 18:3n-6 (γ-linolenic)	0.12	0.13	0.017
C 20:2n-6 (eicosadienoic)	0.15[B]	0.18[A]	0.011
C 20:3n-3 (eicosatrienoic)	0.02[b]	0.03[a]	0.003
C 20:4n-6 (arachidonic)	0.31	0.51	0.144
C 20:5n-3 (eicosapentaenoic)	0.00	0.01	0.002
C 22:2n-6 (docosadienoic)	0.00	0.00	0.000
C 22:4n-6 (docosatetraenoic)	0.07	0.11	0.026
C 22:5n-3 (docosapentaenoic)	0.03	0.04	0.010
C 22:6n-3 (docosaesaenoic)	0.01	0.01	0.002
Σ SFA (saturated FA)	44.44	43.75	0.990
Σ MUFA (monounsaturated FA)	50.48	49.91	0.880
Σ PUFA (polyunsaturated FA)	5.08	6.34	0.969
PUFA/SFA	0.11	0.15	0.026
Σ n-6 (ω-6 FA)	4.84	6.05	0.934
Σ n-3 (ω-3 FA)	0.25	0.29	0.038
n-6/n-3	19.52	20.62	2.020
TI (thrombogenic index)	1.50	1.45	0.061
AI (atherogenic index)	0.61	0.58	0.019

[a,b]=$P<0.05$; [A,B]=$P<0.01$; [($)]standard error of differences.

$$TI = \frac{(C12{:}0+C16{:}0+C18{:}0)}{0.5x(MUFA+n-6\ PUFA)+3x(n-3\ PUFA)+(n-3\ PUFA/n-6PUFA)}\ ;\quad AI = \frac{(C12{:}0+4xC14{:}0+C18{:}0)}{(MUFA+PUFA)}$$

The fatty acid composition of lipids is strongly influenced by the carcass fatness (LO FIEGO, 1996), therefore any factor that modifies carcass fat content, such as age or BW at slaughter (LEBRET and MOUROT, 1998; LO FIEGO *et al*., 2010), feeding strategies (CAMERON *et al*., 2000; BEE *et al*., 2002) and genetic type (PIEDRAFITA *et al*., 2001; LO FIEGO *et al*., 2005b), can also modify lipid composition, especially the PUFA content in backfat depots. In the present research, final BW and carcass backfat thickness were found to be significantly lower in R pigs, which also showed a markedly higher degree of lipid unsaturation in both layers of backfat. This thus confirms the effect of adiposity underlined in the quoted studies on light pigs and on Italian heavy pigs.

4. CONCLUSIONS

Our results indicate that, from a nutritional point of view, the fatty acid profile of the lipids of immunocastrated medium-heavy pigs can be improved by feeding restriction in the finishing period, without significantly impairing their technological attributes.
Rearing medium–heavy pigs is becoming increasingly common in Italy. This production chain uses the same genetic types as for PDO productions and, by adopting suitable feeding plans, yields pigs for either fresh pork or seasoned non-PDO salami. The optimal feeding strategy thus depends on carcass destination. *Ad libitum* feeding, which favors carcass fat covering and the deposition of less unsaturated intramuscular fat and, thus, is more appropriate for processing, is suitable for seasoned products. If the production goal is pork for fresh consumption, some degree of feeding restriction is advisable since it elicits leaner carcasses and meats with healthier lipids.
However, further studies are needed to define the most appropriate level of restriction to prevent excessively lengthening the rearing period.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. G. Della Casa (CREA-Research Centre for Animal Production and Aquaculture - Modena Unit – San Cesario sul Panaro, Modena) for taking care of the animals, and for formulating and supplying the feeds. The research was carried out with private funding.

REFERENCES

Abete I., Romaguera D., Vieira A.R., Lopez de Munain A. and Norat T. 2014. Association between total, processed, red and white meat consumption and all-cause, CVD and IHD mortality: A meta-analysis of cohort studies. Brit. J. Nutr. 112:762-775.

AOAC. 1995. Official methods of analysis, 16th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Barton-Gade P.A. 1987. Meat and fat quality in boars, castrates and gilts. Livest. Prod. Sci. 16:187-196.

Batorek N., Čandek-Potokar M., Bonneau M. and Van Milgen J. 2012. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6:1330-1338.

Bee G., Gebert S. and Messikommer R. 2002. Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. J. Anim. Sci. 80:1564-1574.

Cameron N.D., Enser M., Nute G.R., Whittington F.M., Penman J.C., Fisken A.C., Perry A.M. and Wood J.D. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci. 55:187-195.

Čandek-Potokar M., Batorek Lukač N. and Labussière E. 2015. Immunocastration in pigs. Proceedings of the 4th International Congress, New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, Serbia, October 7–9:324-335.

Corino C., Magni S., Pagliarini E., Rossi R., Pastorelli G. and Chiesa L.M. 2002. Effects of dietary fats on meat quality and sensory characteristics of heavy pigs loins. Meat Sci. 60:1-8.

Dalla Bona M., Schiavon S., Carraro L. and Gallo L. 2016. Growth performance, carcass traits and meat quality of growing pigs on different feeding regimes slaughtered at 145 kg BW. It. J. Anim. Sci. 15:419-427.

Daza A., Rey A.I,. Menoyo D., Bautista J.M., Olivares A. and López-Bote C.J. 2007. Effect of level of feed restriction during growth and/or fattening on fatty acid composition and lipogenic enzyme activity in heavy pigs. Anim. Feed Sci. Technol. 138:61-74.

Daza A., Olivares A., Latorre M.A., Rey A.I., Callejo A. and López-Bote C.J. 2017. Fatty acid composition of different adipose tissues in heavy pigs. It. J. Food Sci. 29:657-666.

Dunker A., Rey A.I., López-Bote C.J. and Daza A. 2007. Effect of the feeding level during the fattening phase on the productive parameters, carcass characteristics and quality of fat in heavy pigs. J. Anim. Feed Sci. 16:621-635.

Dunshea F.R., Colantoni C., Howard K. McCauley I., Jackson P., Long K.A., Lopaticki S., Nugent E.A., Simons J.A., Walker J. and Hennessy D.P. 2001. Vaccination of entire boars with Improvac® eliminates boar taint and increases growth performance. J. Anim. Sci. 79:2524-2535.

Fàbrega E., Velarde A., Cros J., Gispert M., Suárez P., Tibau J. and Soler J. 2010. Effect of vaccination against gonadotropin-releasing hormone, using Improvac®, on growth performance, body composition, behaviour and acute phase proteins. Livest. Prod. Sci. 132:53-59.

Ficarra A., Lo Fiego D.P., Minelli G. and Antonelli A. 2010. Ultrafast analysis of subcutaneous pork fat. Food Chem. 121:809-814.

Folch J., Lees M. and Sloane Stanley G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497-509.

Girard J.P., Bout J. and Salort D. 1988. Lipides et qualités des tissus adipeux et musculaires de porc: Facteur de variation. I: Lipides et qualités du tissu adipeux – Facteurs de variation. II: Lipides e qualités du tissu musculaire – Facteurs de variation. Journées Rech. Porcine en France 20:255-278.

Gispert M., Àngels Oliver M., Velarde A., Suarez P., Perez J. and Font-i-Furnols M. 2010. Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. Meat Sci. 85:664-670.

IUPAC. 1979. Standard methods for the analysis of oils, fats and derivatives. 6th ed. International Union of Pure and Applied Chemistry, Pergamon Press, New York, NY, USA.

Kontogianni M.D., Panagiotakos D.B., Pitsavos C., Chrysohoou C. and Stefanadis C. 2008. Relationship between meat intake and the development of acute coronary syndromes: the CARDIO2000 case-control study. Europ. J. Clin. Nutr. 62:171-177.

Larsson S.C. and Orsini N. 2014. Red meat and processed meat consumption and all-cause mortality: A Meta-Analysis. Am. J. Epidemiol. 179:282-289.

Lebret B. and Mourot J. 1998. Lipides et qualités des tissus adipeux chez le porc. Facteurs de variation non génétiques. INRA Prod. Anim. 11:131-143.

Lebret B. 2008. Effects of feeding and rearing systems on growth, carcass composition and meat quality. Animal 2(10):1548-1558.

Lo Fiego D.P. 1996. Carcass fatness and lipid quality in the heavy pig. Meat Focus Int. 5:261-263.

Lo Fiego D.P., Macchioni P., Santoro P., Pastorelli G. and Corino C. 2005a. Effect of dietary conjugated linoleic acid (CLA) supplementation on CLA isomers content and fatty acid composition of dry-cured Parma ham. Meat Sci. 70:285-291.

Lo Fiego D.P., Santoro P., Macchioni P. and De Leonibus E. 2005b. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. Meat Sci. 69:107-114.

Lo Fiego D.P., Macchioni P., Minelli G. and Santoro P. 2010. Lipid composition of covering and intramuscular fat in pigs at different slaughter age. It. J. Anim. Sci. 9:200-205.

Lo Fiego D.P., Minelli G., Volpelli L.A., Ulrici A. and Macchioni P. 2016. Calculating the iodine value for Italian heavy pig subcutaneous adipose tissue from fatty acid methyl ester profiles. Meat Sci. 122:132-138.

Madsen A., Jakobsen K. and Mortensen H.P. 1992. Influence of dietary fat on carcass fat quality in pigs. A review. Acta Agric. Scand. 42: 220-225.

Minelli G., Macchioni P., Ielo M.C., Santoro P. and Lo Fiego D.P. 2013. Effects of dietary level of pantothenic acid and sex on carcass, meat quality traits and fatty acid composition of thigh subcutaneous adipose tissue in Italian heavy pigs. It. J. Anim. Sci. 12:329-336.

Monziols M., Bonneau M., Davenel A. and Kouba M. 2007. Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. Meat Sci. 76:54-60.

Pauly C., Spring P., O'Doherty J.V., Ampuero Kragten S. and Bee G. 2009. Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac®) and entire male pigs and individually penned entire male pigs. Animal 3:1057-1066.

Piedrafita J., Christian L.L. and Lonergan S.M. 2001. Fatty acid profiles in three stress genotypes of swine and relationship with performance, carcass and meat quality traits. Meat Sci. 57:71-77.

Quiniou N., Monziols M., Colin F., Goues T. and Courboulay V. 2012. Effect of feed restriction on the performance and behaviour of pigs immunologically castrated with Improvac®. Animal 6:1420-1426.

Rossi R., Ratti S., Pastorelli G., Crotti A. and Corino C. 2014. The dietary of vitamin E and verbascoside on meat quality and oxidative stability of *Longissimus dorsi* muscle in medium-heavy pigs. Food Res. Int. 65:88-94.

Sauvant D., Perez J.M. and Tran G. 2004. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses, fish. Wageningen Academic Publishers, Wageningen and INRA Editions, Versailles.
Simopoulos A.P. 1999. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 70:560-569.

Siu G.M. and Draeper H.H. 1978. A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43:1147-1149.

Ulbricht T.L.V. and Southgate D.A.T. 1991. Coronary heart disease: seven dietary factors. Lancet 338:985-992.

Wiecek J., Rekiel A., Batorska M. and Skomiał J. 2011. Effect of restricted feeding and realimentation periods on pork quality and fatty acid profile of M. longissimus thoracis. Meat Sci. 87:244-249.

Williams C.M. 2000. Dietary fatty acids and human health. Ann. Zootech. 49:165-180.

Wood J.D., Buxton P.J., Whittington F.M. and Enser M. 1986. The chemical composition of fat tissues in the pig: effects of castration and feeding treatment. Livest. Prod. Sci. 15:73-82.

Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I. and Whittington F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343-358.

Xue J.L. and Dial G.D. 1997. Raising intact male pigs for meat: Detecting and preventing boar taint. Swine Health Prod. 5(4):151-158.

Zamaratskaia G., Rydhmer L., Andersson H.K., Chen G., Lowagie S., Andersson K. and Lundström K. 2008. Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac™, on hormonal profile and behaviour of male pigs. Anim. Reprod. Sci. 108:37-48.

Paper Received February 27, 2018 Accepted May 23, 2018