ABSTRACT

The aim of this study was to assess the influence of long-term fat supplementation on the fatty acid profile of heavy pig adipose tissue. Fifty-four Large White barrows, averaging 25 kg LW, were randomized (matched weights) to one of three isoenergetic diets supplemented with either tallow (TA), maize oil (MO), or rapeseed oil (RO). The fats were supplemented at 3% as fed from 25 to 110 kg LW, and at 2.5% from 110 kg to slaughtering. Following slaughter at about 160 kg LW, backfat samples were collected from ten animals per treatment and analyzed. Fatty acid composition of backfat closely reflected the fatty acid composition of the supplemented fats. The backfat of pigs fed TA had the highest saturated fatty acid content (SFA) (P<0.01); those fed MO had the highest content in polyunsaturated fatty acid (PUFA) and the lowest in monounsaturated fatty acid (MUFA); and those fed RO had the highest content of linolenic acid (C\(_{18:3}\)) and cis 11-ecosenoic acid (C\(_{20:1}\)). Only MO treatment had an effect on linoleic acid levels and the iodine value (IV) of backfat, resulting in levels higher than those (IV = 70; C\(_{18:2}\) = 15%) accepted by the Parma Consortium for dry-cured ham. The IV and unsaturation index in both layers of subcutaneous backfat tissue differed significantly between treatments. These results show that long-term dietary supplementation with different fats changes the fatty acid profile of heavy pig adipose tissue. Supplementation with rapeseed oil increases the proportion of “healthy” fatty acids in pig fat, thereby improving the nutritional quality, however the effects on the technological quality of the fat must be carefully assessed.

Key words: Heavy pigs, Adipose tissue, Fatty acid profile, Fat quality.

RIASSUNTO

EFFETTI DELL’ALIMENTAZIONE PROLUNGATA CON DIETE GRASSATE SULLA COMPOSIZIONE ACIDICA DEL TESSUTO ADIPOSO NEL SUINO PESANTE

Sono stati valutati gli effetti di un’alimentazione prolungata con diete grassate con diversi lipidi sul profilo acidico del tessuto adiposo nel suino pesante. Sono stati utilizzati 54 suini maschi castrati di razza Large White, suddivisi in tre gruppi e alimentati da 25 a 160 kg di peso vivo con diete sperimentali contenenti differenti tipi di grasso aggiunto: sego, olio di mais, olio di colza. I grassi sono stati inclusi nella dieta al 3% nell’intervallo di peso 25-110 kg, e al 2,5% da 110 kg alla macellazione. Alla macellazione sono stati effettuati campionamenti del grasso dorsale da dieci soggetti per trattamento. Il trattamento alimentare non ha influenzato umidità e tenore in grassi del tessuto adiposo, mentre si è osservata una riduzione del tenore in acqua ed un aumento dell’estratto etereo tra strato esterno e strato interno. La composizione acidica del tessuto adiposo riflette la composizione in acidi grassi dei grassi introdotti con la dieta. I suini alimentati con dieta contenente sego hanno presentato un più elevato contenuto di acidi grassi saturi: 35,5% contro il 33,9% ed il
**ROSSI - CORINO**

32,7\% rispettivamente per gli animali alimentati con olio di mais e l’olio di colza (P<0,01). Il grasso dorsale degli animali alimentati con olio di mais ha presentato un più elevato contenuto di acidi grassi polinsaturi (20,1\%) rispetto al gruppo olio di colza, che ne conteneva il 15,5 \% (P<0,01), e che a sua volta ha fatto rilevare un più elevato tenore in PUFA (P<0,01) rispetto al gruppo olio di colza, che ne conteneva il 13,1\% (P<0,01), ed il 45,7\% rispettivamente nel gruppo alimentato con sego e con olio di colza (P<0,01). Il tessuto adiposo degli animali alimentati con dieta addizionata di olio di colza mette in evidenza un elevato contenuto di acido linoleico e acido eicosenoico. Lo strato esterno del grasso dorsale risulta, indipendentemente dal trattamento, più ricco (P<0,05) in acidi grassi monoinsaturi (46,0\% contro il 44,6\% dello strato interno) e polinsaturi (16,5\% contro il 15,5 \% dello strato interno) a scapito degli acidi grassi saturi: il 32,7\% contro il 35,6\% (P<0,01). Nel tessuto adiposo sottocutaneo degli animali alimentati con dieta contenente olio di mais il numero di iodio è superiore a 70 e la percentuale di acido linoleico è superiore al 15\% in contrasto con il regolamento di produzione del prosciutto di Parma. Il numero di iodio e il coefficiente di insaturazione dei due strati del tessuto adiposo sottocutaneo presentano degli effetti significativi fra trattamenti alimentari. Solo il numero di iodio evidenzia una differenza significativa fra i due strati.

I risultati della ricerca confermano che la composizione acidica dei grassi inclusi nella dieta influenzano la qualità del tessuto adiposo nel suino e permettono di quantificare l’influenza a lungo termine della grassatura delle diete sulle caratteristiche del tessuto adiposo. Si evidenzia inoltre come è possibile aumentare il contenuto in particolari di acidi grassi, come i polinsaturi della serie n-3, per migliorare la qualità nutrizionale del tessuto adiposo, restando nei limiti attualmente riconosciuti per garantire una buona qualità tecnologica del grasso.

**Parole chiave:** Suino pesante, Tessuto adiposo, Profilo acidico, Qualità del grasso.

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**Introduction**

The inclusion of fat in pig diets increases feed efficiency (Noblet et al., 1994). Furthermore it is established that the fatty acid composition of pig adipose tissue is influenced by the level and composition of the dietary fats (Busboom et al., 1991; Larick et al., 1992; Madsen et al., 1992). Specifically, it is possible to increase the adipose content of monounsaturated fatty acids (MUFA), such as oleic acid (C\(_{18:1}\)), and polyunsaturated fatty acids (PUFA) by increasing their levels in the diet (Romans et al., 1995; Fontanillas et al., 1998). Long chain fatty acids are deposited unmodified in adipose tissue while short and medium chain fatty acids are used preferentially as energy sources (Polidori et al., 1982; Mordenti et al., 1992).

Numerous studies have shown that the fatty acid composition of fats consumed by humans can have important effects on health. High dietary levels of saturated fatty acids (SFA) are a major cause of high plasma levels of LDL-cholesterol, which in turn correlate with increased risk of coronary heart disease (Kris-Etherton and Yu, 1997). Conversely, higher proportions of PUFA and MUFA in the diet decrease plasma LDL-cholesterol, and this is associated with reduced risk of cardiovascular disease (Caggiula and Mustad, 1997). Thus, the nutritional quality of meat products is improved by increased MUFA and PUFA levels in the fat. However high PUFA levels in pork fat are undesirable because they adversely affect fat consistency and oxidative stability (Mourot et al., 1991). Fat consistency and hence tissue firmness depend on degree of unsaturation as well as chain length. As the carbon chain lengthens melting point and viscosity increase, while rising numbers of double bonds progressively lower melting point and viscosity. Pig carcasses in which the fat has insufficient consistency, often called soft fat, have defects such as oily appearance, reduced shelf life of meat and meat products (Warnants et al., 1998) and lack of cohesion between muscle and adipose tissue on cutting (Bailey et al., 1973). The greater susceptibility of unsaturated fatty acids to oxidation can result in lowered nutritional value of the meat, with loss of vitamins A, E, and C, and the production of toxic molecules from cholesterol oxidation (Kubow, 1990; Paniangvait et al., 1995). It is for these reasons that interest in supplementing the diets of meat-producing animals with antioxidants has increased recently (Faustman et al., 1998; Corino et al., 1999).
There is a close correlation between linoleic acid (C18:2) levels in pig diet and the linoleic acid content in the fat, for which Mourot et al. (1991) reported a correlation coefficient of $r = 0.83$. Accordingly a linoleic acid limit of 2% DM in pig diet and 15% in pig backfat has been recommended by the Parma Ham Production Consortium in order to avoid fat quality problems in pig products (Wood, 1984; Mourot et al., 1991). Other studies emphasized the importance of the stearic acid (C18:0) content of fat which should be greater than 12% to ensure good stability (Girard et al., 1988).

A simple measure of adipose tissue softness is given by the iodine value (mg iodine/100 g of lipids), which is in fact an estimate of the degree of fatty acid unsaturation that is usually calculated rather than determined. The maximum permitted IV for ham fat is 70 (Regulations for Parma Ham Production) (Baldini et al., 1993).

The effects of long-term dietary supplementation with fats on the adipose tissue quality of heavy pigs have not been extensively investigated. The aim of this study was therefore to assess changes in the fatty acid composition of heavy pig adipose tissue, and consequent effects on the nutritional and technological quality, following long-term supplementation of diets with one of three types of fat: tallow, high in saturated fatty acids; maize oil, high in unsaturated fatty acids (particularly linoleic acid); or rapeseed oil high in MUFA but also in linolenic acid.

**Material and methods**

*Animals and diets*

Fifty-four Large White barrows were assigned to one of the three dietary treatments according to a balanced block design. The treatment began at 25 kg live weight (LW) and continued up to about 160 kg LW. The diets, formulated to be isoenergetic, differed in terms of the added fat which was either tallow (TA), maize oil (MO) or rapeseed oil (RO). Fat supplementation levels were 3% as fed from 25 to 110 kg LW, and 2.5% as fed from 110 kg LW to slaughtering. The animals received different diet formulations according to weight: grower phase (25-50 kg LW), fattening phase (50-110 kg LW) and finisher phase (110-160 kg LW); these differed mainly

| Table 1. Composition of basal diets. |
|--------------------------------------|
| Ingredient (%) | 25 to 50 kg | 50 to 110 kg | 110 to 160 kg |
| Degermed maize | 51.5 | 56.5 | 60.0 |
| Soybean meal (44% CP) | 24.0 | 22.0 | 18.5 |
| Barley | 9.0 | 6.5 | 6.5 |
| Beet pulp | 4.0 | 3.5 | 3.5 |
| Wheat bran | 2.5 | 3.5 | 2.5 |
| Molasses, beet | 3.0 | 3.5 | 4.0 |
| Fat (1) | 3.0 | 3.0 | 2.5 |
| Mineral and vitamin premix | 3.0 | 2.5 | 2.5 |

**Calculated analysis:**

- Crude protein %: 16.3 15.5 14.2
- Lysine %: 0.83 0.78 0.69
- Energy (kcal NE/kg): 2308 2338 2323

(1) Fat: either tallow, maize oil, or rapeseed oil
in energy, crude protein and lysine content (table 1). The diets were fed at 9% LW0.75. The fatty acid composition of the added fats are shown in table 2. Immediately after slaughtering, at about 160 kg LW, samples of subcutaneous adipose tissue, which included both layers of backfat, were collected from ten pigs per treatment, vacuum-packed, frozen and stored at -20°C pending analysis.

Chemical analysis

To determine DM content, 50 g of minced sample was freeze-dried for 48 hours. The ether extract was determined by Soxhlet extraction with diethyl ether. For fatty acid analysis lipids were extracted from subcutaneous fat by chloroform/methanol (2:1) using the method of Folch et al. (1957) and the extract evaporated under nitrogen.

Methyl esters of the fatty acids were prepared with 20% boron trifluoride/methanol solution as described by Morrison et al. (1964). The methyl esters were separated on a Perkin Elmer autosystem gas chromatograph equipped with fused silica capillary column (0.25 mm i.d.x 25 m) containing cyanopropyl siloxane (CP-Sil 88) stationary phase (0.25 µm film thickness) (Chrompack, Middelburg, The Netherlands). The conditions used were as follows: temperature program, 11 min at 177°C, from 177 to 225°C at 7°C/min and then 11 min at the latter temperature; injector temperature 270°C; detector temperature 300°C. The injection volume was 0.2 to 0.5 µl. The various esters were identified by comparison of retention times with those of pure standards (Supelco 37 Component FAME mix).

Unsaturation index and IV were calculated from the fatty acid composition.

Unsaturation index = 
\[
\frac{[(%C_{16:1}+ %C_{18:1}) \times 1 + (%C_{18:2}) \times 2 + (%C_{18:3}) \times 3]}{[%(C_{16:1} + C_{18:1} + C_{18:2} + C_{18:3})]}
\]

Table 2. Fatty acid composition of fats.

| Fatty acid, % | Tallow | Maize oil | Rapeseed oil |
|--------------|--------|-----------|--------------|
| C12:0        | 0.30   | -         | -            |
| C14:0        | 4.80   | Tr.       | Tr.          |
| C14:1        | 0.50   | -         | -            |
| C15:0        | 2.10   | -         | -            |
| C16:0        | 28.70  | 11.50     | 4.90         |
| C16:1        | 7.60   | 0.10      | 0.20         |
| C17:0        | 0.90   | 0.10      | -            |
| C18:0        | 9.50   | 1.70      | 1.50         |
| C18:1        | 41.60  | 27.70     | 62.40        |
| C18:2        | 1.70   | 57.10     | 19.80        |
| C18:3        | 0.40   | 0.80      | 8.30         |
| C20:0        | -      | 0.30      | 0.40         |
| C20:1        | -      | 0.20      | 1.10         |
| C20:2        | 0.40   | -         | -            |
| C22:0        | -      | 0.10      | 0.20         |
| C22:1        | -      | 0.10      | 0.20         |
| Total saturated (SFA) | 46.30  | 13.70     | 7.00         |
| Total monounsaturated (MUFA) | 49.70  | 28.10     | 63.90        |
| Total polyunsaturated (PUFA) | 2.50   | 57.90     | 28.10        |
| MUFA: SFA Ratio | 1.07   | 2.05      | 9.13         |
| PUFA: SFA | 0.05 | 4.23 | 4.01 |
The iodine value (Castaing et al., 1988) =

\[
(\%C_{16:1}) \times 0.95 + (\%C_{18:1}) \times 0.86 + (\%C_{18:2}) \times 1.732 + \\
(\%C_{18:3}) \times 2.616 + (\%C_{20:1}) \times 0.785 + \\
(\%C_{22:1}) \times 0.723
\]

Statistical analysis

One-way analysis of variance was used to test the effect of diet on the variables examined. Differences between means were tested by the Student-Newman-Keuls t test.

Results and discussion

Growth performance was not influenced by dietary fat supplementation (data not shown). There were also very limited effects on carcass characteristics (data not shown) as also reported by Pantaleo et al. (2000).

The diets had no influence on the moisture content or ether extract of the outer and inner layers of backfat (table 3), whereas there were significant differences (P<0.05) in the chemical composition of the outer and inner layer of subcutaneous adipose tissue. These differences are consistent with those reported by Girard et al. (1988), Kies et al. (1991) and Camara et al. (1994). Specifically, the backfat of pigs fed TA had the highest SFA content: 35.5% vs. 33.9% for MO-fed and 32.7% for RO-fed animals (SEM = 0.91; P<0.01) (figure 1). Pigs fed MO had the lowest MUFA content in backfat: 41.4% vs. 47.2% and 46.7% in TA- and RO-fed animals, respectively (SEM = 0.93; P<0.01) (figure 1). The backfat of MO-fed pigs also had the highest PUFA content: 20.1% vs. 13.1% and 15.5% in TA- and RO-fed animals respectively (SEM = 0.48; P<0.01) (figure 1).

The fatty acid composition of subcutaneous adipose tissue reflected the fatty acid composition of the added fat (tables 4 and 5). TA supplementation significantly increased the myristic acid (C_{14:0}) content of the outer layer but not of the inner layer of backfat; palmitoleic acid (C_{16:1}) and oleic acid (C_{18:1}) were significantly increased in both outer and inner layers, while stearic acid (C_{18:0}) was increased only in the inner layer. As expected, the backfat of animals fed MO had the highest linoleic acid (C_{18:2}) content and that of RO-fed animals had the highest linolenic acid (C_{18:3}) content. The lower oleic acid (C_{18:1}) content of the backfat of pigs fed MO compared to pigs fed TA is consistent with the results of Kouba and Mourot (1998) and is probably due to the low C_{18:1} content of diet and to reduced Δ−9 desaturase activity in pigs supplemented with MO.

As expected (Santoro et al., 1985; Girard et al., 1988) the inner layer of backfat was higher in SFA than the outer layer (35.6% vs. 32.7%, SEM = 0.91; P<0.01). Similarly, both the MUFA content (44.6% vs. 46.0%, SEM = 0.93; P<0.05) and the PUFA content (15.5 vs. 16.5 %, SEM = 0.91;
Numerous studies have reported that in pigs slaughtered at 100 kg LW the levels of particular fatty acids or fatty acid classes (e.g. MUFA or PUFA) in pig fat can be increased by adding these to the diet (Morgan et al., 1992; Mourot et al., 1994; Romans et al., 1995; Lebret and Mourot, 1998). We have found that similar effects are obtained in heavy pigs (those slaughtered at about 160 kg LW mainly for dry-cured pork products) in good agreement with the findings of Piva et al. (1996) and Della Casa et al. (1999). The latter authors found that use of hydrogenated fat and partially hydrogenated lard in heavy pig diets modifies the fatty acid composition of backfat and improves its consistency.

Bosi et al. (2000) reported high IV (>70) in the backfat of heavy pigs supplemented with high oleic acid sunflower oil. An expected, additional finding was that IV differed significantly between the pooled inner and outer layers of backfat of all animals (69.1 outer layer vs. 66.9 inner layer, SEM = 1.25; P<0.05). The subcutaneous fat of pigs fed RO had a fatty acid composition within the limit of acceptability for the production of Parma hams (tables 4 and 5). The maximum allowed level of linoleic acid is 15% of total fats, and the maximum permitted IV is 70: these limits were imposed to avoid problems with the technical quality of hams. Davenel et al. (1999) reported that the solidity of pig fat correlated strongly (R² = 0.94) with the proportion of saturated fatty acids present (palmitic and stearic acid content); correlations between pig fat solidity and iodine value (R² = 0.80) and linoleic acid content (R² = 0.48) were less strong. According to the authors, the weaker relationship between linoleic acid content and adipose tissue consistency could be connected with the

| Fatty acid, % | Tallow | Maize oil | Rapeseed oil | SEM |
|--------------|--------|-----------|--------------|-----|
| C14:0        | 1.39   | 1.31      | 1.32         | 0.048 |
| C16:0        | 21.44  | 20.79     | 20.58        | 0.488 |
| C16:1        | 2.02*  | 1.62*     | 1.75**       | 0.104 |
| C18:0        | 14.75* | 13.45*    | 11.82*       | 0.470 |
| C18:1        | 44.15* | 39.17*    | 44.30*       | 0.708 |
| C18:2        | 11.57* | 18.51*    | 13.82*       | 0.438 |
| C18:3        | 0.84*  | 0.91*     | 2.00*        | 0.122 |
| C20:0        | 0.15   | 0.27      | 0.17         | 0.077 |
| C20:1        | 0.70*  | 0.74*     | 1.11*        | 0.045 |
| C22:0        | 0.52*  | 0.74*     | 0.59*        | 0.032 |
| Total saturated (SFA) | 37.58* | 35.56* | 33.72* | 0.868 |
| Total monounsaturated (MUFA) | 46.16* | 40.79* | 46.06* | 0.753 |
| Total polyunsaturated (PUFA) | 12.42* | 19.23* | 15.72* | 0.429 |

* A,B,C: P<0.01; a,b: P<0.05.
Figure 1. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid content (as proportion of total fat) of heavy pigs fed diets supplemented with tallow (TA), maize oil (MO) or rapeseed oil (RO). The fatty acid content of the inner (IL) and outer (OL) layers of backfat pooled from all groups is also shown.

Figure 2. Adipose tissue iodine values of pigs supplemented with tallow, maize oil or rapeseed oil. The pooled iodine values of the inner and outer layers of backfat of all groups are also shown.

Figure 3. Adipose tissue unsaturation indices of pigs supplemented with tallow, maize oil, or rapeseed oil. The unsaturation indices of the inner and outer layers of backfat pooled from all groups are also shown.
The data presented in this study show that long-term dietary supplementation with different fats changes the fatty acid profile of heavy pig adipose tissue. Supplementation with rapeseed oil increases the proportion of "healthy" fatty acids in pig fat, thereby improving the nutritional quality, while the iodine value and linoleic acid level remain below the limits set for Parma ham production (Baldini et al., 1993). However, additional studies on the fatty acid profile and sensory qualities of Parma hams following long-term fat supplementation are required to confirm the potential benefit of rapeseed oil.

Table 5. Fatty acid composition of outer layer of subcutaneous adipose tissue of pigs fed diets added with tallow, maize oil, and rapeseed oil.

| Fatty acid, %       | Tallow   | Maize oil | Rapeseed oil | SEM     |
|---------------------|----------|-----------|--------------|---------|
| C14:0               | 1.49a    | 1.29ab    | 1.29ab       | 0.039   |
| C16:0               | 20.42    | 20.06     | 18.77        | 0.584   |
| C16:1               | 2.15a    | 1.74a     | 1.71b        | 0.100   |
| C18:0               | 12.16    | 11.01     | 11.51        | 0.511   |
| C18:1               | 45.38a   | 39.41a    | 44.27b       | 0.937   |
| C18:2               | 12.76a   | 20.04b    | 14.19b       | 0.533   |
| C18:3               | 0.91ab   | 0.80a     | 1.16a        | 0.067   |
| C20:0               | 0.20     | 0.18      | 0.13         | 0.023   |
| C20:1               | 0.72b    | 0.83a     | 1.37a        | 0.101   |
| C22:0               | 0.58a    | 0.83a     | 0.71ab       | 0.040   |
| Total saturated (SFA)| 34.08    | 32.36     | 31.61        | 0.941   |
| Total monounsaturated (MUFA) | 48.26a   | 41.99a    | 47.36a       | 1.067   |
| Total polyunsaturated (PUFA) | 13.67a   | 20.85a    | 15.36a       | 0.530   |

A,B: P<0.01; a,b: P<0.05.

Conclusions

The data presented in this study show that long-term dietary supplementation with different fats changes the fatty acid profile of heavy pig adipose tissue. Supplementation with rapeseed oil increases the proportion of "healthy" fatty acids in pig fat, thereby improving the nutritional quality, while the iodine value and linoleic acid level remain below the limits set for Parma ham production (Baldini et al., 1993). However, additional studies on the fatty acid profile and sensory qualities of Parma hams following long-term fat supplementation are required to confirm the potential benefit of rapeseed oil.

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fact that linoleic acid is mainly present as palmitoyl-stearoyl-linoleoyl-glycerol (POL) a triacylglycerol less correlated with solid fat content than palmitoyl-stearoyl-oleoyl-glycerol (PSO). Forward multiple regression relating solid fat content at 20°C (SFC20) to the proportion of individual triacylglycerols, indicated that the SFC20 could be predicted from the proportion of PSO, which explained 92% of SFC20 variability (Davenel et al. 1999).

Our results show that an MO-supplemented diet is not suitable for pigs destined for dry-cured ham production, as the linoleic acid content of the backfat exceeded 15% (20.04% in outer layer and 18.51% in inner layer) and IV was greater than 70. However, supplementation with RO seems to improve the nutritional quality of pig fat, and may therefore be useful from the point of view of human nutrition, while at the same time it does not increase the linoleic acid content and iodine value beyond the limits set for Parma ham. Finally, the linoleic acid contents of the finisher phase diets were 1.5%, 1.98% and 2.4% of DM in the TA, RO, and MO groups, respectively, and the value for the MO group was above the limit established by the Parma Ham Production Consortium (2% DM).
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