PE9.30 Fatty acid profile of Meat and Fermented Sausages influenced by including Linseed, Fish oil or Microalgae in Pig Feed 194.00

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***Abstract*—It is now well established that n-3 polyunsaturated fatty acids have a protective influence on several chronic diseases. The objective of the present study was to investigate the fatty acid profile of fresh meat and dry fermented sausages of pigs fed different n-3 fatty acid sources. Crossbred pigs were given an experimental diet supplemented with soybean oil (CON), linseed oil (LIN), fish oil (FO) or three different concentrations of microalgae (ALG LOW, ALG MEDIUM and ALG HIGH). The fatty acid composition of the fresh meat and dry fermented sausages was analyzed by gas chromatography. For the fresh meat significantly higher ALA proportions in the LIN group (1.38 g/100g FA) and higher proportions of EPA in the FO group (1.30 g/100g FA) were found compared to all other groups. The DHA proportions in the FO group (1.02 g/100g FA) and ALG groups were significantly higher compared to the CON (0.20 g/100g) and LIN (0.45 g/100g) group. The DHA content in the meat increased with increasing amounts of microalgae in the feed (0.78, 1.69 and 2.32 g/100g FA in the ALG LOW, MEDIUM and HIGH respectively). For the dry fermented sausages, the ALA proportion was higher in the LIN group (2.73 g/100 g FA), and an increased EPA and DHA was found in the FO group (0.35 and 0.56 g/100g FA resp.). Increased DHA proportions in ALG MEDIUM (0.88 g/100 g FA) and ALG HIGH (1.38 g/100g FA) were found compared to the other groups.**

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***Index Terms*—microalgae, fish oil, n-3 fatty acids, pork, sausage**

I. INTRODUCTION

It is generally accepted that the n-3 polyunsaturated fatty acids (PUFA) α-linolenic acid (ALA; C18:3n-3) and especially its metabolites eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) have a protective influence on several chronic diseases [1-3]. The superior Health Council of Belgium recommends for healthy humans that at least 0.3% of the total daily energy intake should originate from EPA and DHA. Based on these recommendations, several studies have been performed to increase the n-3 PUFA content of animal products.

Sources of n-3 fatty acids used for this purpose in the diet of farm animals are grass, rapeseed, linseed, linseed oil, fish oil or microalgae [4-8]. Although meat only partly contributes to the total fat intake of the diet, optimizing the fatty acid profile of meats still deserves attention due to the high meat intake in industrialized countries [9].

The aim of the present trial was to study the effect of linseed, fish oil and microalgae added to the diet of pigs on the fatty acid profile of fresh meat and dry fermented sausages.

II. MATERIALS AND METHODS

Experimental set-up

Six groups of five crossbred pigs each were fattened under commercial conditions on different diets from 75 kg until 110 kg live weight. Water and feed was offered *ad libitum*. Different amounts and sources of n-3 PUFA were fed, i.e. linseed oil (LIN group, rich in ALA), fish oil (FO group, rich in EPA and DHA) or dried microalgae (ALG group, rich in DHA) at three different levels: ALG LOW, ALG MEDIUM and ALG HIGH. In the control group (CON group) soybean oil was added to the diet (See Table 1 for the PUFA composition of the feed). The total PUFA content was kept constant and the total fat content was similar for all groups (between 4.3 and 4.8%). All diets were supplemented with 150 mg/kg α-tocopherylacetate.

Animals were slaughtered at a live weight of approximately 110 kg in a commercial slaughterhouse. After slaughtering and cooling for 24h, the *longissimus thoracis* was sampled and stored vacuum packed at -20°C until analysis (slices of 2.5 cm). Shoulder meat and subcutaneous fat of three pigs per group were sampled for the production of dry fermented sausages (a mixed sample was made per group). Dry fermented sausages were produced using a standard commercially recipe, with 70% shoulder meat and 30% subcutaneous fat. Ripening was carried out for 23 days according to a commercial ripening procedure. The sausages were stored vacuum packed at 4°C.

Chemical analysis

The lipids were extracted from the fresh meat and dry fermented sausages using chloroform/methanol (2/1; v/v) (modified after Folch, Lees, & Stanley [10]). Fatty acids were methylated and analysed by gas chromatography (HP6890, Brussels, Belgium) on a CP-Sil88 column for FAME (100 m × 0.25 mm × 0.25 µm; Chrompack, The Netherlands) [11]. Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., IL, USA; Sigma, Bornem, Belgium). Nonadecanoic acid (C19:0) was used as an internal standard to quantify the individual and total fatty acids. The fatty acid profiles are expressed in g/100g of total FAME (fatty acid methyl esters) and the total fatty acid (FA) content is expressed in g FA/100g meat or sausage.

Statistical analysis

The obtained experimental data were submitted to analysis of variance (GLM) with diet as main effect (SPSS 15.0). Mean differences between groups were tested using the Duncan post hoc test operating at a 5% level of significance.

III. RESULTS AND DISCUSSION

Table 1 shows the PUFA composition of the feed. As expected, the LIN diet had the highest ALA concentration and higher amounts of DHA were measured in the FO group and all three ALG groups. Only the FO diet contained EPA.

Table 2 shows the PUFA composition of the fresh meat samples. No significant differences for the total fatty acid content between the groups were found. The fatty acid composition of the experimental diets is clearly reflected in the fresh meat samples. The LIN diet resulted in a significantly higher ALA concentration in the meat compared to all other groups. The DHA proportions in the FO group and ALG groups were significantly higher compared to the CON and LIN group. The highest DHA proportion was found in the meat of the DHA HIGH group, which was 10 fold higher than in the CON group. The DHA content in the meat increased with increasing amounts of microalgae in the experimental feeds. As expected, the EPA proportion was the highest in the FO group, with a 7 fold higher proportion compared to the CON group. Less evident were the relatively high concentrations of EPA in the ALG groups, as no EPA was present in the ALG experimental feeds. Like for DHA, the EPA proportions increased with increasing amounts of microalgae in the experimental feeds. This could be due to retro-conversion of DHA into EPA [6]. The EPA proportion of the LIN group was 3 fold lower compared to the FO group and did not differ from ALG LOW and ALG MEDIUM. This suggests a rather poor conversion of ALA to EPA. Indeed, according to Brenna [12] only 25% of administrated ALA is available for the production of very long chain PUFA, whereas the largest part is used for β-oxidation.

DPA was found in the meat samples of all experimental groups, although it was not present in the experimental diets, as a result of elongation of EPA or retro-conversion of DHA to DPA. The DPA proportion in the fresh meat was the highest in the LIN and FO group.

The linoleic acid (LA; C18:2n-6) proportion was the highest in the LIN group. The metabolite of LA, arachidonic acid (AA, C20:4n-6) tended to be higher in the LIN, DHA MEDIUM and ALG HIGH group compared to the FO and ALG LOW group. This is surprising in view of the higher amount of LA in the CON diet compared to the other diets. ALA and LA compete for the use of the desaturase and elongase enzymes [13] during the conversion to very long chain PUFA. There is a preference for ALA by these enzymes. Consequently, lower amounts of AA in the LIN group were expected, as the highest proportion of ALA was found in this group. However, as shown in Table 2, this was not the case in this trial.

Table 3 shows the PUFA composition of the dry fermented sausages. No significant differences for the total fatty acid content between the groups were found. As for the fresh meat, the LIN diet resulted in a significantly higher ALA proportion in the dry fermented sausages compared to all other groups and a higher EPA proportion was found in the FO group. Also the effect of DHA was still present in the dry fermented sausages: higher concentrations of microalgae in the experimental diets resulted in higher proportions of DHA in the dry fermented sausages. However, the DHA proportion of ALG LOW was lower than the DHA proportion of the FO group. The ALG HIGH group had almost a 3 fold higher DHA proportion compared to the FO group. Although not measured, the subcutaneous fat must thus also have been affected by the microalgae supplementation.

Compared to the fresh meat lower proportions of long chain PUFA were found in all dry fermented sausages. This is probably due to relatively higher amounts of phospholipids in the intramuscular fat of the fresh meat. Phospholipids are characterised by a high PUFA content, mainly represented by long chain fatty acids [14]. The proportion of phospholipids in the fat fraction of the dry fermented sausages is lower compared to the fresh meat due to the high amount of subcutaneous fat, rich in triacylglycerols. This fat fraction could have weakened the effect of long chain n-3 PUFA enrichment since triacylglycerols contain less PUFA compared to phospholipids [15]. On a tissue weight basis, however, the content of total and individual PUFA is of course higher for the sausages than for the fresh meat as a result of the much higher total fatty acid content.

In general, the n-6/n-3 ratio of both the fresh meat and dry fermented sausages were positively affected by adding n-3 fatty acids to the feeds.

To verify the nutritional improvement of the fresh meat and dry fermented sausages, the increase of EPA+DHA in 100 g fresh meat and dry fermented sausages was calculated. For the fresh meat, a 2 to 3 fold higher intake of EPA+DHA was found for the LIN and ALG LOW group compared to the CON group. For ALG HIGH and the FO group even a 6 fold increase of EPA+DHA was achieved. For the dry fermented sausages all diets resulted in a significantly higher EPA+DHA content compared to the CON group, varying from a 2 fold increase for the LIN group to even an 18 fold increase for the ALG HIGH group. This means that, although for feed costs arguments low concentrations of n-3 PUFA were added to the feed, still a significant increase in the concentration of EPA and DHA could be achieved. According to Sioen et al. [16], the consumption of total meat, poultry and eggs contribute for 10.1% to the daily intake of n-3 PUFA and for 5.2% and 11.8% to the intake of EPA and DHA respectively for Belgian women. Likewise, Howe et al. [17] emphasized the importance of meat in the daily intake of long chain n-3 PUFA in Australia. They estimated that 43% of the consumed long chain n-3 PUFA originated from meat, poultry and game, because of the much larger consumption of meat and meat products compared with that of fish and seafood. Giving the fact that pork is the major meat source in most industrialized countries, supplementing feeds for pigs with n-3 PUFA might contribute to increase the intake of n-3 PUFA.

IV. CONCLUSION

The addition of different n-3 fatty acid sources to the diets of pigs alters correspondingly the fatty acid composition of fresh meat. Administration of microalgae to the pigs resulted also in higher levels of DHA in dry fermented sausages.

This outcome could be of interest in terms of improving the nutritional value of pork. Further research is needed to evaluate the impact of these PUFA sources on other meat products.

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Table 1 Fatty acid composition of the experimental diets (g FA/100 FA)

	CON	LIN	FO	ALG LOW	ALG MED	ALG HIGH
LA	32.5	24.63	24.4	29.9	28.8	26.7
ALA	3.50	6.70	2.73	3.45	3.40	2.63
EPA	/	/	2.19	/	/	/
DHA	/	/	1.87	1.80	3.62	7.12
PUFA	36.3	31.9	32.2	35.4	36.1	37.0

CON=control group; LIN=linseed fed group; FO=fish oil fed group; ALG= microalgae fed group with different concentrations of microalgae (low, medium, high); PUFA = C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6 + C20:2n-6 + C18:3n-3 + C20:5n-3 +C22:5n-3 + C22:6n-3

Table 2 Mean fatty acid composition (g/100g FA) of the fresh meat (n=5)

	CON	LIN	FO	ALG LOW	ALG MEDIUM	ALG HIGH	SEM
ALA	0.535[b]	1.38[a]	0.622[b]	0.601[b]	0.506[b]	0.524[b]	0.060
EPA	0.168[d]	0.488[bc]	1.30[a]	0.312[cd]	0.704[b]	1.06[a]	0.084
DPA	0.557[b]	0.918[a]	0.988[a]	0.395[b]	0.422[b]	0.493[b]	0.049
DHA	0.198[e]	0.452[de]	1.02[c]	0.784[cd]	1.69[b]	2.32[a]	0.148
LA	11.9[b]	15.4[a]	11.9[b]	9.69[b]	12.0[b]	12.9[b]	0.500
AA	2.96	3.57	2.55	2.39	3.52	3.95	0.182
n-6/n-3	10.5[a]	6.03[b]	3.80[d]	6.14[b]	5.03[c]	4.09[d]	0.427
Total FA content (g/100g meat)	1.44	1.18	1.42	1.49	1.28	1.07	0.057

CON=control group; LIN=linseed fed group; FO=fish oil fed group; ALG= microalgae fed group with different concentrations of microalgae (low, medium, high); SEM= standard error of the mean calculated from all groups (n=25); n-6/n-3 ratio was calculated as (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)

[a,b,c,d,e] Values with different letters in the same row indicate significant differences (Duncan-test; P<0.05)

Table 3 Mean fatty acid composition (g/100g FA) of the dry fermented sausages (n=2)

	CON	LIN	FO	ALG LOW	ALG MEDIUM	ALG HIGH	SEM
ALA	1.36[c]	2.73[a]	1.29[c]	1.53[b]	1.40[c]	1.11[d]	0.161
EPA	0.043[e]	0.072[d]	0.349[a]	0.067[d]	0.178[b]	0.138[c]	0.031
DPA	0.217[c]	0.361[b]	0.686[a]	0.240[c]	0.317[b]	0.356[b]	0.047
DHA	0.049[f]	0.106[e]	0.562[c]	0.412[d]	0.880[b]	1.38[a]	0.139
LA	14.7[a]	14.2[b]	13.3[c]	12.8[d]	14.3[b]	11.5[e]	0.324
AA	0.447[a]	0.400[b]	0.349[c]	0.393[b]	0.461[a]	0.466[a]	0.013
n-6/n-3	9.21[a]	4.54[d]	4.80[c]	5.43[b]	5.43[b]	4.54[d]	0.491
Total FA content (g/100g sausage)	26.2	24.3	26.6	27.1	24.6	29.1	0.603

CON=control group; LIN=linseed fed group; FO=fish oil fed group; ALG= microalgae fed group with different concentrations (low, medium, high); SEM= standard error of the mean calculated from all groups (n=12); n-6/n-3 ratio was calculated as (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)

[a,b,c,d,e,f] Values with different letters in the same row indicate significant differences (Duncan-test; P<0.05)