FATTY ACID COMPOSITION AND SENSORY ACCEPTABILITY OF DRY CURED HAM INFLUENCED BY LINSEED OIL, FISH OIL OR MICROALGAE INCLUDED IN THE PIG FEED

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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, lipid oxidation and sensory acceptability of dry cured hams 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 dried microalgae (ALG LOW, ALG MEDIUM and ALG HIGH). Dry cured hams were manufactured and ripened for 19 months. The fatty acid composition of the dry cured hams was analyzed by gas chromatography. TBARS, as a measure of lipid oxidation, were assessed spectrophotometrically. The sensory acceptability was evaluated by a semi-monadic method. Significantly higher ALA proportions in the LIN group (1.92g/100g FA) and higher proportions of EPA in the FO group (0.91g/100g FA) were found compared to all other groups. The DHA proportions in the FO group (0.86g/100g FA) and ALG groups were significantly higher compared to the CON (0.10g/100g) and LIN (0.27g/100g FA) group. The DHA content in the dry cured ham increased with increasing amounts of microalgae in the feed (0.61, 1.03 and 1.70g/100g FA in the ALG LOW, MEDIUM and HIGH respectively). The TBARS values of the CON group (0.32 µg MDA/g ham) were significantly lower compared to all ALG groups (0.47, 0.49 and 0.48 µg MDA/g ham for ALG LOW, MEDIUM and HIGH respectively). The TBARS values of both the FO and LIN groups (respectively 0.44 and 0.38 µg MDA/g ham) reached intermediate levels, but did not differ significantly from the other groups. No negative effects on the sensory characteristics were noticed by the consumer panelists. These results demonstrate that it is possible to produce dry cured ham with improved nutritional properties without negatively affecting the oxidative and sensory properties.**

***Index Terms*—dry cured ham, fish oil, linseed oil, microalgae, n-3 fatty acids**

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 (Ruxton, 2004). Currently, people in the Western world consume an insufficient quantity of n-3 fatty acids. Despite attempts to provide education about healthier eating patterns, there are several barriers such as a lack of interest towards changing one's diet, or concerns about having to compromise on taste or enjoyment (Kearney & McElhone, 1999). A successful strategy to improve the n-3 fatty acid content of the overall diet would be to provide these fatty acids in food products that are already popular. Besides adding n-3 fatty acid sources during the processing of foods, incorporation of n-3 fatty acid rich products like grass, rapeseed, linseed and fish oil in livestock feeds, resulting in accumulation of these fatty acids in animal products, is receiving increasing interest (Raes, De Smet, Demeyer, 2004). Unfortunately, the increased degree of unsaturation of n-3 fatty acids makes these enriched products more susceptible to oxidative damage which can negatively affect the meat quality (Wood & Enser, 2008). Dry cured hams are particularly susceptible to oxidation due to their long ripening time. While a small amount of oxidation products is required to get the typical aroma of dry-cured ham, an excessive oxidation leads to off-flavours (Coutron-Gambotti & Gandemer, 1999). The aim of the present trial was to study the effect of linseed oil, fish oil and microalgae added to the diet of pigs on the fatty acid composition, oxidative stability and consumer acceptability of dry cured hams.

II. MATERIALS AND METHODS

A. Experimental set-up

Six groups of six 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-tocopheryl acetate and 0.4 mg/kg organic selenium.

Animals were slaughtered (carcass lean meat = 62±2%) in a commercial slaughterhouse. After slaughtering and cooling for 24h, hams of six pigs per group were sampled for the production of dry cured hams. The dry cured hams were manufactured using a standard commercial protocol. After 19 months of ripening, the hams were stored vacuum packed at 4°C until sensory analysis. For the chemical analysis subcutaneous and intermuscular fat was removed from the dry cured hams and the sample were stored at -21°C until analysis.

B. Chemical analysis

The lipids were extracted from the trimmed dry cured hams using chloroform/methanol (2/1; v/v) (modified after Folch, Lees, & Stanley, 1957). Fatty acids were methylated according to Raes, De Smet & Demeyer (2001) 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). 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 as g FA/100g ham. Lipid oxidation of the trimmed dry cured hams was assessed spectrophotometrically by the TBA reactive species method (TBARS) based on Tarladgis et al. (1960) and is expressed as µg malondialdehyde (MDA)/ g ham.

C. Sensory analysis

The assessments were carried out in a sensory laboratory (VG Sensory, Deinze, Belgium) equipped according to ISO 8589-standards. Forty two male and forty eight female adults who regularly eat dry cured hams were recruited to participate. Each panelist received 4 whole slices (0.5 mm thick). The slices were taken from the refrigerator and distributed on plates 30 minutes before the start of each session so that the slices were at room temperature when consumed. The slices were randomly coded using a three-digit number. A semi-monadic method was used according to AFNOR (2000): the slices were assessed one after another, taking away the previous slice before the following one was served. The judgments were expressed on a 9-point hedonic scale ranging from disliked extremely (score 1) to excellent (score 9). The evaluated traits were: taste, visual perception, colour of the fat border, odour, general mouth feel and general perception. In the beginning of the product assessment, after tasting but before the detailed questions, panelists were also asked to write down spontaneous likes and dislikes.

D. Statistical analysis

The fatty acid composition and TBARS data were submitted to analysis of variance with diet as fixed effect (SPSS 15.0). Mean differences between groups were tested using the Tukey's post hoc test operating at a 5% level of significance. For the sensory analysis, a balanced, incomplete block design (BIBD) was used to evaluate the dry cured hams. Differences were considered significant if $P<0.05$.

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 a considerable concentration of EPA.

Table 2 shows the PUFA composition of the dry cured hams. No significant differences for the total fatty acid content between the groups were found. The fatty acid composition of the experimental diets was clearly reflected in the dry cured hams. The LIN diet resulted in a significantly higher ALA concentration compared to all other groups. 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 was the relatively high concentration of EPA in the ALG HIGH group, as almost no EPA was present in the ALG experimental feeds. This could be due to retro-conversion of DHA into EPA (Raes, De Smet, Demeyer, 2004). 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. However, the EPA proportion was still 2.5 fold higher compared to the CON group, which indicates a modest conversion of ALA to EPA. The highest DPA proportion was found in the FO group, but it was also high in the LIN group. The DPA proportion of all ALG groups did not differ from the CON group. The highest DHA proportion was found in the ALG HIGH group, which was 17 fold higher compared to the CON group. The DHA proportions of the FO and ALG MEDIUM group were similar and significantly higher than the CON and LIN group. For the ALG LOW group, the DHA proportion was 3 fold lower compared to the ALG HIGH group, but 2 fold higher compared to the LIN group. The DHA content in the dry cured hams increased with increasing amounts of microalgae in the experimental feeds. For the n-6 fatty acids, the highest proportions of linoleic acid (LA; C18:2n-6) and arachidonic acid (AA, C20:4n-6) were found in the LIN group. This was not expected as in other experimental diets higher amounts of LA were present.

Table 3 shows the TBARS values and sensory acceptability of the dry cured hams. The TBARS values of the CON group were 1.5 fold lower compared to all ALG groups. TBARS values of both the FO and LIN groups reached intermediate levels, but did not differ significantly from the other groups. Santos, Hoz, Cambero, Cabeza & Ordóñez (2008) found 3 times higher TBARS values in the *Biceps femoris* muscle of dry-cured hams from pigs fed a linseed based diet compared to a sunflower based control diet. In the present study no differences were found between the LIN and CON group. Different fatty acid composition, fat content and α-tocopheryl acetate supplementation could explain these differences. The overall low TBARS values in the present study are in agreement with the results of the sensory analysis: no significant differences between the diets for the sensory acceptability of the dry cured hams were found. The general perception was strongly correlated with general mouth feel and taste ($r^2>0.90$). No fishy odour and flavour were detected in any sample by the consumer panel. This was in contrast with other studies where adverse affects of n-3 fatty acids in dry cured hams were noticed by sensory panelists (rapeseed, Pastorelli et al., 2003; pure DHA, Sárraga, Guàrdia, Díaz, Guerrero, García Regueiro & Arnau, 2007; linseed oil, Santos et al., 2008; extruded linseed, Musella, Cannata, Rossi, Mourot, Baldini & Corino, 2009). The n-3 fatty acid percentages of the dry cured hams in the present study were similar or even higher compared to the above mentioned studies (except for Santos et al. 2008). Most likely, the n-3 fatty acid content expressed per 100 g of dry cured ham played a major role in the differences in acceptability. The fat content of the dry cured hams may greatly differ according to slaughter weight, pig genotype and ripening period of the dry cured hams. Musella et al. (2009) suggested that dietary addition of antioxidants can preserve the long chain fatty acids in products with a long shelf life such as dry cured ham. Possibly the high supplementation of α-tocopheryl acetate in the present study (150 mg/kg) delayed the oxidation processes and consequently influenced positively the sensory characteristics of the dry cured hams. Santos et al. (2008) reported no adverse effects on sensory traits of dry cured hams originating from pigs fed linseed oil with 220 mg/kg α-tocopheryl acetate, while dry cured hams with 20 mg/kg α-tocopheryl acetate were not accepted by trained experts. Also, the expertise of the sensory panelists (trained versus naive persons) could have affected the results of the sensory analysis.

IV. CONCLUSION

The EPA and DHA proportions of dry cured hams were markedly affected by feeding different marine n-3 fatty acid sources. Feeding linseed oil significantly increased the ALA content of the dry cured hams, while the EPA and DHA content was little affected. TBARS values were low and no adverse sensory effects of the dry cured hams were found by the consumer panelists. These results demonstrate that it is possible to produce long ripened dry cured ham with improved nutritional properties without negatively affecting the sensory properties.

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

	CON	LIN	FO	ALG LOW	ALG MEDIUM	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	0.02	0.06	2.19	0.12	0.13	0.25
DHA	0.02	0.19	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 oil 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 FAME) of the dry cured hams (n=6)

	CON	LIN	FO	ALG LOW	ALG MEDIUM	ALG HIGH	SEM
ALA	0.91^{bc}	1.92^{a}	1.00^{b}	1.01^{b}	0.87^{bc}	0.78^{c}	0.07
EPA	0.13^{d}	0.32^{c}	0.91^{a}	0.20^{cd}	0.29^{cd}	0.51^{b}	0.05
DPA	0.38^{c}	0.57^{b}	0.86^{a}	0.33^{c}	0.32^{c}	0.41^{c}	0.03
DHA	0.10^{d}	0.27^{d}	0.84^{b}	0.61^{c}	1.03^{b}	1.70^{a}	0.09
n-3	1.52^{d}	3.08^{ab}	3.64^{a}	2.15^{c}	2.52^{bc}	3.39^{a}	0.13
LA	13.3^{ab}	15.7^{a}	13.5^{ab}	11.0^{b}	11.9^{b}	12.5^{b}	0.4
AA	2.35^{ab}	2.88^{a}	2.01^{b}	1.92^{b}	2.14^{ab}	2.51^{ab}	0.09
n-6	16.4^{ab}	19.5^{a}	16.1^{ab}	13.7^{b}	14.9^{b}	16.0^{ab}	0.4
Total FA content (g/100g ham)	4.98	3.96	4.43	4.70	4.53	4.00	0.13

CON=control group; LIN=linseed oil 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=36); n-3 = C18:3n-3+C18:4n-3+ C20:5n-3+C22:5n-3+C22:6n-3; n-6 = C18:2n-6+C18:3n-6+C20:3n-6+C20:4n-6+C22:4n-6+C22:5n-6;
^{a,b,c,d} means with different letters in the same row indicate significant differences (Tukey, P<0.05)

Table 3 Effect of diet on the sensory acceptability and TBARS values of dry cured ham (mean±st.dev)[1]

Descriptor	CON	LIN	FO	ALG LOW	ALG MEDIUM	ALG HIGH	P-value
Overall opinion	6.86±1.22	6.40±1.26	6.78±1.30	6.26±1.66	6.62±1.37	6.43±1.20	NS
Aspect	6.62±1.26	6.60±1.13	6.67±1.13	6.28±1.35	6.56±1.32	6.62±1.31	NS
Colour fat border	6.44±1.10	6.07±1.22	6.43±1.05	6.26±1.20	6.08±1.39	6.27±1.23	NS
Odour	6.44±1.41	6.08±1.26	6.20±1.23	6.29±1.34	6.36±1.29	6.22±1.22	NS
General mouth feel	6.74±1.45	6.42±1.33	6.78±1.36	6.29±1.43	6.73±1.26	6.47±1.21	NS
Taste	6.73±1.41	6.34±1.33	6.61±1.33	6.22±1.54	6.62±1.45	6.34±1.15	NS
TBARS	$0.32±0.02^{b}$	$0.38±0.07^{ab}$	$0.44±0.09^{ab}$	$0.47±0.05^{a}$	$0.49±0.08^{a}$	$0.48±0.09^{a}$	0.002

CON=control group; LIN=linseed fed group; FO=fish oil fed group; ALG= microalgae fed group with different concentrations of microalgae (low, medium, high);
[1]The results judgements were expressed on a 9-point hedonic scale ranging from disliked extremely (score 1) to excellent (score 9); TBARS as μg malondialdehyde/g ham
^{a,b}means with different letters in the same row indicate significant differences (Tukey, P<0.05)