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Evaluation of the influence of dietary fat content and fatty acid composition in four diets based on different fat sources on loins (*M. Longissimus dorsi*) of newborn piglets

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1 Introduction

Litter size, piglet birth weight and survival have been important issues for the farming industry during a long time. Still mortality around 10% or more for piglets after birth has been accepted and is very common. This development in pig farming has been ascribed the selection for lean growth resulting in less physiologically mature status of piglets. The Swedish lean pig is no exception, generally suffering the same losses of piglets. So far sow diets have been composed with regard to protein, vitamins, minerals and energy demands, which have been more generally known as important, while the needs for essentially fatty acids in animal nutrition have not been fully recognized. Indeed, according to the recent BSAS standards (Whittemore et al. 2003), the minimum required level of essential fatty acids is unclear, and 1.0-2.5% linoleic acid in diets for all pigs is suggested as a guideline. Today, the most commonly used fat sources in pig diets are cereals, which contain mostly monounsaturated fatty acids and *n-6* polyunsaturated fatty acids. Attempts have not been made to design the composition of lipids to fulfil the physiological needs for polyunsaturates including a well balanced ratio of *n-6*/*n-3* fatty acids (Innis, 1999). The most obvious goal has been a cheap and not easily oxidised lipid source resulting in high ratio *n-6*/*n-3* fatty acids, often exceeding 10:1.

Studies on piglet performance as an effect of sow fatty acid nutrition have been performed by Rooke et al. (2001 a, b). The sources of fatty acids investigated in these experiments were mainly of fish origin. It is well established that the nervous system of newborns has a large demand for *n-3* polyunsaturated fatty acids. The retina and brain of mammals is in general very rich in docosahexaenoic fatty acid, 22:6*n-3* (DHA). The need of DHA from external sources in piglet retina and brain has been demonstrated by Leskanich and Noble (1999).

2 Objective

The aim of this study was to explore if a change in dietary fatty acid content from low fat, high fat-saturated, and two high fat-unsaturated diets (*n-6* rich and *n-3* rich, respectively) had impact on the tissue composition of newborn piglets.

3 Materials and methods

Animals, diets and experimental design

The study was performed on 39 litters. Sows were blocked with a randomised uneven (block) design, according to date of weaning of previous litter and parity/age group, to one of four experimental dietary treatments. There were no gilts included, and mean parity of sows was 3.9 (range 2-8). During service and pregnancy period sows were housed in large pens (52 m2) on deep straw bedding, with a maximum of 16 sows per group. One week before expected farrowing sows were moved to individual farrowing pens (8.5 m2), without crates, with partly drained (2.5 m2), and partly heated floors. Sows had free access to water.

The diets were fed to purebred Swedish Yorkshire sows at the Swedish University of Agricultural Sciences, Funbo-Lövsta experimental station, from before service until weaning. They consisted on four cereal based diets (with soy as protein enhancement):

- **Low Fat diet:** conventional low fat (3 %) sow diet (**Control diet**)
- Three high fat (6%) diets:
  - **High fat saturated Diet** (**Saturated diet**)
  - **High fat *n-6* Diet**: 40 % of high-fat oats was included (***n-6* diet**)
  - **High fat *n-3* Diet**: 20 % of high-fat oats and linseed oil as a source of *n-3* fatty acids (***n-3* diet**)

Sampling and lipid analysis

Immediately following birth, one piglet per litter, preferably the third born was stunned by CO_2, bled to death and dissected. The *longissimus* muscle was cut out with the subcutaneous fat layer included. Loins from newborn piglets (20 animals, 5 per treatment) were collected and stored in -80 ºC until analyses.

Lipids in loins were extracted according to Hara and Radin (1978) to analyze fat content and fatty acid composition in the main lipid fractions, triacylglycerols (TG) and phospholipids (PL) by using the thin-layer chromatography (TLC) methodology, which was performed in the solvent system hexane:diethylether:acetic acid (85:15:1, v/v/v). Methylation of the PL and TG muscle lipids was performed with dry methanol and BF_3 addition, described by Appelqvist (1968) and resulting fatty acid methyl esters (FAME) were stored in hexane at -80 ºC until further analysis. The analysis of FAME was performed by using gas chromatography (GC).

All samples were analysed in duplicate and ls-mean values are reported.

4 Data analyses

Analysis of variance was performed using PROC GLM procedure with the computer software Statistical Analysis System (SAS system for windows, v 8.01, SAS Institute Inc, Cary, NC).

5 Results and discussion

Diets

Fatty acid composition of the four diets used in the experiment is shown in Table 1. Fat content was 3 % by weight in the control feed compared to 6 % in the high fat feeds (Saturated, *n-6* and *n-3* feeds). The highest level of 18:3*n-3* was found in the linseed oil added feed (*n-3* feed), and it was nearly 6 times higher in comparison to saturated and *n-6* feeds. Accordingly, *n-3* feed has the highest level of PUFA *n-3* and therefore, the lowest value of *n-6*/*n-3* ratio (1.95). The highest level of SAFA was found in saturated feed (43 %), and the lowest one in *n-3* feed (17 %). The PUFA content in control feed was comparable, by percentage (53 % vs. 51 %) to the PUFA content in the *n-3* feed. No differences were found between *n-6* and *n-3* feeds when comparing PUFA *n-6* percentage (34.17 % vs. 33.88 %).

Piglet loin fatty acid content

Results of total fat content show no differences (P>0.05) among diets (mean= 4.05 ± 0.72 %) although three of them were designed as High Fat diets, containing twice the total amount of fatty acid. This suggests that the extra fat if stored it would probably be as an extra fat tissue was not found in muscle tissue as intramuscular fat.

Piglet loin fatty acid composition

TG fraction had higher saturated (SAFA) and monounsaturated (MUFA) fatty acid content whereas the long-chain PUFA of interest (arachidonic acid, AA; eicosapentaenoic acid, EPA; docosapentaenoic acid, DPA and DHA) were more prevalent in the PL fraction than in the TG. However, α-linolenic acid (18:3 *n-3*), which was the one used to enrich *n-3* diet, was higher in TG fraction. Despite these differences in fatty acid content, similar diet effects were observed in both fractions regarding to fatty acid (FA) composition on piglet loin muscle: they showed significant changes among treatments, specially the linseed group n-3 diet, which is a source of α-linolenic acid, (Table 2 and Table 3).

Saturated diet did not modify fatty acid composition in piglet loins in comparison with Control diet. However, some differences could be found when studying *n-3* and *n-6* diets.

In this study, *n-6* fatty acids were composed of 18:2 *n-6*, 18:3 *n-6*, 20:3 *n-6*; 20:4 *n-6*, 22:4 *n-6* and 22:5 *n-6*, and the *n-3* fatty acids were composed of 18:3 *n-3*, 20:3 *n-3*, 20:5 *n-3*, 22:5 *n-3* and 22:6 *n-3*. With respect to total *n-6* PUFA content, no differences were found among diets. However, linoleic acid (18:2*n-6*) content was higher when *n-6* and *n-3* diets were used in the TG ($P<0.01$) and the PL ($P<0.1$) fractions. On the other hand, arachidonic acid (20:4*n-6*) content was lower in *n-6* and *n-3* diets in comparison to control one in the TG fraction, whereas in PL no differences were found .

Both fractions (TG and PL) showed differences ($P<0.01$) in *n-3* fatty acid content among diets: α-linolenic acid content was greater ($P\leq0.001$) in *n-3* diet group than in other treatments, and same results were found in longer *n-3* fatty acids, such as EPA (20:5*n-3*; $P\leq0.001$), DPA (22:5*n-3*; $P\leq0.001$) and DHA (22:6*n-3*; $P\leq0.01$). Therefore, the *n-3* content in newborn piglets' loins was higher in *n-3* diet ($P\leq 0.001$) and, consequently, *n-6*/*n-3* ratio was lower ($P\leq 0.01$) in comparison with other diets. Same results were found in growing pigs by Kouba *et al* (2003) when using a high linseed diet (6%).

Non-essential fatty acids are readily obtained by the foetus either by synthesis from appropriate precursors or by absorption via the placenta, although the placental transfer of fatty acids in swine has been reported to be quite limited (Ramsay *et al*, 1991). In this study we have observed an elevation in *n-3* PUFA in pig loins born to sows fed *n-3* diet, which was enriched with linseed oil.

To conclude from our data in the present study, an effect of diet could be recognised on the piglet loin fatty acid composition: the inclusion of linseed oil (18:3*n-3*) in the sow diet increased *n-3* fatty acid content in newborn piglets' tissues. Therefore, it may alter immune function and incidence of inflammatory disease in these piglets.

6 Conclusion

Sow diets is of great importance to the newborns. The FA composition of the diet is well reflected in the tissue of piglets, here the muscle. It is known that *n-3* PUFA have implications on the nervous development in mammals. We can also recognise that the dominant fatty acid in linseed, 18:3n-3 gave a higher DHA level in piglet tissue. This effect was found both in the TG and the PL fractions. This study indicates that the difference of DHA proportion and the low *n-6*/*n-3* ratio of the investigated muscle in the linseed group can be ascribed to the sow diets. DHA is known to contribute to the development of the nervous functions, which is also recognised by a behaviour study performed on the same piglet groups (Gunnarsson et al. 2003).

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Table 1 Fatty acid composition (% of identified) of the four diets used in the experiment

	Control feed	Saturated feed	*n-6* feed	*n-3* feed
Fat content, %	3	6	6	6
12:0	0.22	1.88	0.26	0.44
14:0	0.30	1.38	0.27	0.30
16:0	17.1	34.5	14.7	13.0
16:1	0.21	0.22	0.19	0.13
18:0	2.44	4.69	3.42	2.56
18:1 n-9	23.7	27.3	40.1	29.9
18:1 n-7	1.17	0.81	1.57	0.82
18:2 n-6	48.2	24.9	34.2	33.9
18:3 n-3	4.37	2.34	3.13	17.35
20:0	0.23	0.27	0.32	0.22
20:1 n-9	0.74	0.34	0.76	0.73
22:0	0.17	0.13	0.16	0.06
SAFA	20.6	43.0	19.2	16.7
MUFA	26.0	28.7	42.7	31.8
PUFA	52.6	27.26	37.30	51.2
PUFA n-6	48.2	24.92	34.17	33.9
PUFA n-3	4.37	2.34	3.13	17.4
n-6/n-3	11.0	10.7	10.9	1.95

Table 2 Fatty acid composition (% of identified) of loin triacylglycerols

Fatty acid	Control diet	Saturated diet	*n-6* diet	*n-3* diet	S.E.	p-value
Fat Content	4.05	3.99	3.30	4.32	0.51	ns
14:0	4.63	4.71	4.14	4.63	0.34	ns
14:1	0.12	0.11	0.05	0.16	0.04	ns
16:0	33.4	35.4	34.6	31.8	1.05	ns
16:1tr	3.80	3.80	3.69	3.90	0.17	ns
16:1 n-7	8.21	7.97	7.79	8.35	0.68	ns
17:0	1.12	0.96	1.01	0.99	0.07	ns
18:0	6.15	5.38	5.54	4.86	0.46	ns
18:1 n-9	23.4	22.0	20.2	21.9	1.77	ns
18:1 n-7	6.29	6.01	7.98	5.68	1.11	ns
18:2 *n-6*	5.23[c]	5.68[bc]	7.15[ab]	8.03[a]	0.51	**
18:3 *n-6*	0.57	0.54	0.57	0.66	0.04	ns
18:3 *n-3*	0.13[b]	0.09[b]	0.12[b]	0.61[a]	0.03	***
20:0	0.18	0.15	0.16	0.14	0.03	ns
20:1	0.40	0.37	0.39	0.35	0.04	ns
20:3	0.26	0.26	0.24	0.22	0.04	ns
20:3 *n-6*	0.44	0.46	0.55	0.63	0.06	ns
20:4 *n-6*	1.11	1.45	1.26	1.31	0.26	ns
20:3 *n-3*	0.11[a]	0.09[a]	0.02[b]	0.13[a]	0.02	*
20:5 *n-3*	0.02[b]	0.02[b]	0.00[b]	0.09[a]	0.01	***
22:4 *n-6*	1.10	1.13	1.20	1.03	0.15	ns
24:0	0.27	0.17	0.26	0.18	0.20	ns
22:5 *n-6*	0.57	0.89	0.57	0.63	0.26	ns
24:1	0.08	0.07	0.03	0.07	0.03	ns
22:5 *n-3*	0.37[b]	0.43[b]	0.36[b]	0.90[a]	0.06	***
22:6 *n-3*	0.33[b]	0.45[b]	0.34[b]	0.80[a]	0.07	**
% ID FA	95.5	95.7	96.6	95.8	0.33	ns
SAFA	45.8	46.8	45.7	42.6	1.14	ns
MUFA	42.4	40.4	40.1	40.6	1.07	ns
PUFA	9.98	11.23	12.2	14.8	1.26	†
PUFA *n-3*	0.96[b]	1.08[b]	0.85[b]	2.54[a]	0.14	***
PUFA *n-6*	9.02	10.2	11.3	12.3	1.13	ns
n-6*/*n-3	9.46[b]	9.68[b]	13.6[a]	4.83[c]	0.70	***

ns: non significant; *. p<0.05; **. p<0.01; ***. p<0.001
[a.b.c]**:** least squares means within a row with different superscript differ (p<0.05)
Control diet: conventional low fat (3%); **Saturated Diet**: high saturated FA (6%); ***n-6* Diet**: High *n-6* FA (6%); ***n-3*diet**: High *n-3* FA (6%).
IDFA: Identified Fatty Acid; **SAFA**: Saturated Fatty Acid; **MUFA**: Monounsaturated Fatty Acids; **PUFA**: Polyunsaturated Fatty Acids; ***n-3***: *n-3* Fatty Acids; ***n-6***: *n-6* Fatty Acids.

Table 3 Fatty acid composition (% of identified) of loin phospholipids

Fatty acid	Control diet	Saturated diet	*n-6* diet	*n-3* diet	S.E.	p-value
14:0	0.26	0.25	0.26	0.38	0.08	ns
16:0	16.0	16.8	16.3	16.2	0.93	ns
16:1tr	0.38	0.34	0.34	0.43	0.09	ns
16:1 n-7	1.90	1.75	1.82	1.79	0.10	ns
17:0	1.47	1.22	1.32	1.38	0.11	ns
18:0	13.7	13.6	13.8	14.2	0.73	ns
Unknown	2.85	2.42	2.48	2.25	0.61	ns
18:1 n-9	16.0	17.0	16.8	17.2	0.61	ns
18:1 n-7	7.38	7.94	7.28	7.31	0.37	ns
Unknown	2.12	1.42	1.80	1.68	0.29	ns
18:1 n-5	1.75	1.58	1.49	1.36	0.14	ns
18:2 *n-6*	10.8	11.3	13.2	13.3	0.73	†
18:3 *n-6*	0.39	0.30	0.31	0.36	0.05	ns
18:3 *n-3*	0.01 [b]	0.02 [b]	0.04 [b]	0.36 [a]	0.03	***
20:3	0.57	0.48	0.37	0.33	0.07	ns
20:3 *n-6*	1.18	1.03	1.19	1.31	0.07	ns
20:4 *n-6*	16.9 [a]	16.4 [ab]	15.2 [b]	13.4 [c]	0.52	**
20:5 *n-3*	0.29 [b]	0.28 [b]	0.23 [b]	0.88 [a]	0.06	***
22:4 *n-6*	1.77 [a]	1.73 [a]	1.76 [a]	1.15 [b]	0.07	***
24:0	0.23	0.19	0.16	0.00	0.17	ns
22:5 *n-6*	1.73 [a]	1.84 [a]	1.69 [a]	1.04 [b]	0.18	*
22:5 *n-3*	0.84 [b]	0.80 [b]	0.85 [b]	1.60 [a]	0.07	***
22:6 *n-3*	1.05 [b]	1.07 [b]	1.06 [b]	1.61 [a]	0.08	***
% ID FA	97.8	96.9	98.4	96.2	0.97	ns
SAFA	31.7	32.1	31.8	32.1	0.67	ns
MUFA	27.47	28.6	27.8	28.2	0.67	ns
PUFA	35.0	34.8	35.6	35.1	0.71	ns
PUFA *n-3*	2.20 [b]	2.17 [b]	2.18 [b]	4.45 [a]	0.17	***
PUFA *n-6*	32.8	32.6	33.4	30.6	0.77	ns
n-6*/*n-3	15.0 [a]	16.0 [a]	15.5 [a]	6.90 [b]	1.47	**

Abbreviations: see Table 1; †. p<0.1.