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EFFECT OF N-3 PUFA ON REPRODUCTION PARAMETERS OF MODERN GENOTYPE SOWS

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1. Background and objectives of the research

Pig industry has changed significantly in recent decades as worldwide modern genotype, so-called "super prolific" breeds have spread. Beside larger litters, the piglets of these breeds have greater growth potential and fattening properties. The better reproductive performance caused by genetic selection is associated with higher stillbirth rate and a lower, inhomogeneous birth weight, which leads to high pre-weaning mortality due to the increased number of piglets with poor viability.

In case of these modern genotype sows, the satisfaction of energy and nutrient requirements significantly affects the efficiency of production and the longevity of sows. As fertility is one of the most important virtues of these high-performance, modern breeds, supporting this through feeding must be one of the main economic goals.

The energy content of pig feeds is traditionally increased by additional fat or oil sources, as their energy concentration is 2-2.5 times higher compared to other raw materials. However, some fat sources can be used not only to increase the energy content of feed, because due to their fatty acid content, they may have a positive effect on reproductive biology and other physiological processes as well. The effects of n-3 long-chain polyunsaturated fatty acids (LC PUFAs) on pig performance and health have been studied for years. In my research, the practical use of n-3 fatty acids in the feeding of sows was investigated, with particular regard to the reproduction biological aspects of n-3 fatty acids. During this research, I was looking for which production phases and what dose of n-3 fatty acids should be mixed into the feed of sows in order to improve their performance parameters.

The basic goal of my research was to develop a feeding method that can improve the reproduction biological parameters of modern genotype sows and thus make their production more effective. To earn this, the main objective of my work was to investigate the effects of n-3 fatty acids on the reproduction biological processes of sows using methods that have not been used before, and the acquired results could be used to further expand of knowledge related to swine reproduction and nutrition.

The objectives of the research were as follows:

1. My goal was to investigate the effect of fish oil fed at a dose of 6.3 g/kg on the nutrient content and fatty acid composition of the milk of the Danish large white × Danish landrace sows, in case of corn-soybean-based feeding.
2. I wanted to investigate the applicability of electronic nose based on ultrafast gas chromatography to detect the odour-modifying effect of fish oil and other feed

components, as well as whether the fish oil fed in different doses causes a change in the odour profile of sow milk, supplementary and mixed feeds.

3. In several experiments, I planned to investigate the effect of fish oil fed in different doses on the feed intake, reproduction biological parameters of sows, and the other performance parameters of sows and their piglets. I also aimed to assess whether the feeding of fish oil in a short-term, but higher dose (6.3 - 12.6 g/kg of feed) or in a long-term, but lower dose (3.15 g/kg) could be more beneficial for sows.
4. Among my goals was to investigate the effects of 6.3 g/kg fish oil feeding during lactation and the first trimester of pregnancy on the lipid peroxidation processes and on the antioxidant system of sows, by the changes in blood malondialdehyde (MDA) and glutathione (GSH) levels, and the activity of glutathione peroxidase (GPx).
5. I also planned to study the effect of 6.3 g/kg fish oil supplementation on the levels of reproductive hormones such as 17β-oestradiol, progesterone, prostaglandin F2α of sows in different production phases.

2. Materials and methods

2.1. Experiments

2.1.1. First experiment

The first experiment was conducted in two replications on a large pig farm with 2,500 DanBred sows. Sow feeds were based on corn, barley and soybean meal. Fish oil (FO) was mixed into the lactation feed of the experimental sows at a dose of 6.3 g/kg, while the control group received the same amount of sunflower oil (SO). The supplementary feeds were included in the lactation feed instead of the same amount of barley. It was not possible to mix the experimental and control supplementary feeds into the feed of pregnant sows, so animals received them "on top", in addition to daily feed dose. Due to the added SO and FO, the n-6/n-3 fatty acid ratio of the control and experimental lactation feeds changed significantly (15.08 vs. 7.38).

The first experiment started between the days 110-114 of pregnancy, by settling the animals to lactation buildings and ended by the pregnancy test (ultrasound diagnosis) 30 days after insemination. Only multiparous sows (2-6 parity) were included in the experiment. Milk samples were collected from 12 control and 12 experimental sows on the day 13-14 of lactation, whose nutrient content and fatty acid composition were examined. Blood samples were taken from 24 control and 24 experimental sows on the day 14 of lactation, on the day 5 after weaning, and on the day 30 after insemination (pregnancy test). Malondialdehyde (MDA) and reduced glutathione (GSH) levels, as well as glutathione peroxidase (GPx) activity, were determined from the blood samples. The levels of 17β-oestradiol (E2), progesterone (P4) and 6-keto-PGF1α were also determined from the blood serum. Animal experiment license number: PE/EA/872-8/2020.

2.1.2. Second experiment

The second experiment was conducted at the Herceghalom, on the experimental pig farm of the Institute of Physiology and Nutrition of the Hungarian University of Agriculture and Life Sciences. The number of sows were app. 100, F1 (large white × landrace) and Hypor genetics. The experiment was carried out in one replication, but in two subsequent reproduction cycles. SO rich in n-6 fatty acids was mixed into the feed of the control group's lactation feed, while FO rich in n-3 fatty acids was mixed into the experimental lactation feed at a dose of 12.6 g/kg. The supplementary feeds replaced the same amount of cornmeal in the feeds.

8 control and 8 experimental sows were included in the experiment, 3 multiparous, and 5 primiparous in each treatment. At the end of the experiment, on the day 5 and on the day 12 after weaning, 2-2 sows were culled, slaughtered and the ovaries were macroscopically examined.

2.1.3. Third experiment

The third experiment was carried out in pig farm with 2100 F1 (large white × landrace) and DanBred sows, in two replications, through a complete reproduction cycle. During the experiment, we evaluated the data of 161 control and 164 experimental sows. Experimental sows received FO at a dose of 3.15 g/kg of feed, mixed into the lactation, mating and pregnant sow feeds. The feeds of the control groups did not contain any supplements. At the same time, the feeds of the control groups (lactation and mating feeds) contained an important proportion of extruded linseed-based supplementary feed (45 g/kg feed). In all cases, the experimental supplementary feed was added to the diets instead of the same amount of extruded flaxseed-based supplementary feed.

2.2. Chemical and other analyses

The dry matter, fat and protein content of the milk samples were analysed, as well as the chemical composition (dry matter, crude protein, crude fat, crude fibre and ash) of the control and experimental sow feeds according to the regulations of the Association of Official Analytical Chemists (AOAC 2006). Analysis of the fatty acid composition of milk (Experiment 1) and feed samples (Experiments 1, 2, 3) was performed in all experiments using a gas chromatography method (GCMS-QP2010 SE; Shimadzu, S.A., Kyoto, Japan).

The blood samples were examined at the Department of Food Safety in the Institute of Physiology and Nutrition on the Hungarian University of Agriculture and Life Sciences. The GSH content of blood plasma and red blood cell hemolysates was determined based on the complex formation of non-protein sulfhydryl groups with 5,5-dithiobis-2-nitrobenzoic acid, while the MDA content by using the 2-thiobarbituric acid complex formation method. The GPx activity was determined using a direct end-point assay.

Hormone testing of blood serum samples was performed in the Endocrinology Laboratory of the Department of Obstetrics and Reproductive Biology on the University of Veterinary Medicine. A radioimmunoassay developed and validated in the laboratory was used to detect

progesterone and oestrogen in the samples. 6-keto-PGF1α was tested by enzyme-linked immunoassay (ELISA) method (Abcam, Cambridge, UK).

2.3. Electronic nose measurements

Aroma profile tests were performed with an Alpha MOS Heracles NEO 300 electronic nose device (Alpha MOS, Toulouse, France) in the laboratory of ADEXGO Kft. The equipment is a two-column ultrafast gas chromatograph with an automatic sample handling unit (CTC Analytics AG, Zwingen, Switzerland), two flame ionization detectors (FID) and a trap concentrating the volatile components. For the measurements, AlphaSoft ver. 16 (Alpha MOS, Toulouse, France) data analysis software was used. Kováts retention indexes were assigned to the chromatograms taken from the samples. The peaks of the chromatograms marked with the given retention index were interpreted as virtual sensors, while the area under the peak was interpreted as the intensity of the given sensor. Based on the retention indexes, the volatile components were identified using AroChemBase (Alpha MOS, Toulouse, France).

From the first experiment the aroma profile of 24 sow milk samples were examined. In case of the second experiment, we performed e-nose measurements on 16 (8 supplementary and 8 mixed feed samples), while in the third experiment on a total of 45 (10 supplementary and 35 mixed feed samples) feed samples.

2.4. Statistical analyses

The mathematical statistical evaluation of the experimental results was performed using the SPSS Statistics 26.0 for Windows program (IBM, Armonk, NY, USA). The Kolmogorov–Smirnov test, the Levene test, the independent sample t-test, and the Mann–Whitney U test were used to analyse the results of feed and milk samples, as well as hormone levels and reproductive biological parameters. The chosen significance level for all statistical analyses was $p=0.05$, in the case of $p\leq0.1$ a trend was established. The statistical analysis was started by filtering the experimental data. Values greater than 2.5 times the standard deviation, as well as values marked by the outlier test, were excluded from the evaluation. Significantly different results were marked with the letters a;b, where the two different markings indicate a difference, and the two identical letters indicate statistical identity. To analyse electronic nose data, AlphaSoft ver. 16 (Alpha MOS, Toulouse, France) data analysis software was used. The odour patterns were described using principal component analysis (PCA), and the separability of individual groups based on odour was examined using linear discriminant analysis (LDA).

3. Results and discussion

3.1. The performance parameters of sows and their piglets

The main production parameters of the sows and their piglets of the first experiment are presented in Table 1. The feeding of supplementary feeds with different n-6/n-3 ratios (SO, FO) had no significant effect on the performance of either the sows or their piglets. However, it should be noted that the time from wean to oestrus and the proportion of sows heated later than seven days were more favourable in the experimental group from a practical point of view. In the control group, there were 25 sows that heated only after the seventh day after weaning, while in the experimental one there were 10 (13.66% *vs*. 6.33%). The average time till oestrus in the control group was 5.72 days, while in the experimental group it was 4.94 days (non-significant difference).

Table 1. Performance parameters of the first experiment's sows and their piglets

	Control	Experimental	P-value
Number of sows (pcs.)	209	176	-
Number of live born piglets (pcs.)	19.40±3.36	19.38±2.99	0.94
Number of weaned piglets/litter (pcs.)	13.42±1.24	13.15±1.51	-
Av. weight of weaned piglets (kg)	6.33	6.33	-
Number of inseminated sows (pcs.)	185 (86.05%)	162 (85.26%)	-
Number of pregnant sows (pcs.)	183 (98.92%)	158 (97.53%)	-
Sows with late oestrus (pcs.)[1]	25 (13.66%)	10 (6.33%)	-
Time from wean to oestrus (days)	5.72±5.14	4.94±3.89	0.12

Control = 6.3 g/kg sunflower oil; Experimental = 6.3 g/kg fish oil; Data in the rows without upper indexes are statistically non-significant; [1]More than 7 days from weaning to oestrus.

The most important production parameters of the control and experimental sows and piglets of the second experiment are presented in Table 2.

Table 2. Performance parameters of the second experiment's sows and their piglets

	Control	Experimental	P-value
Number of live born piglets (pcs.)	12.4±3.1	12.50±3.5	0.94
Av. litter weight (kg)	17.2±3.9	16.6±3.7	0.74
Av. piglet weight (kg/pcs.)	1.41±0.2	1.39±0.3	0.88
Feed intake of lactating sows (kg/sow)	200.1 ^{a} ±2.2	189.6 ^{b} ±8.9	0.01
Weight loss during lactation (kg/sow)	35.6±19.6	45.60±20.00	0.35
Number of weaned piglet (pcs.)	10.3±2.0	9.88±2.5	0.74
Weaning mortality (%)	11.50	15.10	-
Weight of weaned piglets (kg/pcs.)	8.41±1.3	8.42±1.2	-

	Control	Experimental	P-value
Rate of sows with oestrus within 7 days (%)	87.5	85.7	-
Time from wean to oestrus (days)	5.57±0.54	5.50±0.55	0.82
Pregnancy rate (%)	87.5	66.7	-
Subsequent farrow			
Number of live born piglets (pcs.)	9.43 [b] ±2.76	13.33 [a] ±2.8	0.03
Number of all born piglets (pcs.)	10.71 [b] ±2.50	14.17 [a] ±2.71	0.04
Av. litter weight (kg)	16.74±3.02	19.37±4.98	0.27
Av. piglet weight (kg/pcs.)	1.84 [c] ±0.29	1.49 [d] ±0.35	0.07
Number of weaned piglet (pcs.)	8.29±2.36	9.50±2.65	0.45
Weaning mortality (%)	12.12	25.00	-
Weight of weaned piglets (kg/pcs.)	7.71±0.99	7.50±1.71	0.80

[a,b] Different superscripts in the same row indicate a significant difference at a significance level of $P < 0.05$; [c,d] Different superscripts in the same row indicate a trend difference at a significance level of $0.05 < P < 0.1$. Data in rows without superscripts are not statistically different.

During the first lactation, experimental sows consumed significantly less feed than controls (189.6 *vs*. 200.1 kg), which affected their weight loss during lactation, as well as the performance of their piglets. Although the differences are not significant, the preweaning mortality and the weaned litter weight were also lower in the experimental group. We did not find differences between the reproduction parameters of the sows after weaning, but the pregnancy rate of the groups had great difference. Based on the pregnancy test results of the control group, 87.5% of the animals became pregnant, while this data was only 66.7% for the experimental sows.

During the next farrowing, compared to the previous one, the number of live born piglets in the control group decreased (12.40 → 9.43), while in the experimental group it was increased 9.43 → 13.33). The weight of the control litters was similar (17.20 → 16.74 kg) to the previous farrowing, but in case of the experimental ones it increased importantly (16.6 kg → 19.37 kg), caused by the larger number of piglets.

Table 3. The effect of fish oil feeding on the performance of sows and their piglets in the third experimental

	1st replication			2nd replication		
	Control	Experimental	P-value	Control	Experimental	P-value
Feed intake of sow during lact. (kg/day)	4.70	5.76		5.76	5.54	
Av. weaning weight of piglets (kg)	6.97	7.44		7.35	7.06	
Preweaning mortality (%)	12.58 [a]	8.74 [b]	0.00	12.64 [a]	9.53 [b]	0.04

[a,b] Different superscripts in the same row indicate a significant difference at a significance level of $P < 0.05$; Data in rows without superscripts are not statistically different.

Table 3 shows the average daily feed intake of the sows of the third experiment, the average weaning weight of the piglets and preweaning mortality. In the first replication

experimental sows, while in the second, the control ones ingested more feed per day, which was clearly reflected in the weaning weight of their piglets. Preweaning mortality in both replications was lower in the experimental group (1st rep.: 12.58% *vs*. 8.74%, p≤0.001; 2nd rep.: 12.64% *vs*. 9.53%, p≤0 ,04).

In the first replication experimental sows had an oestrus earlier after weaning than control (1st rep.: 6.53 *vs*. 4.23 days, p≤0.019). In the second replication however, there was no difference in wean to oestrus interval between the groups (2nd rep.: 5.53 *vs*. 5.43 days, p≤0.915). The rate of animals heated after seven days was higher in the control group in both replications (1st rep.: 11.0% *vs*. 2.4%; 2nd rep.: 7.6% *vs*. 4.8%) (Table 4). As a result of the first inseminations, 10.1% and 8.5% more sows became pregnant in the replications in the experimental group than in the control (1st rep.: 80.3% *vs*. 90.4%; 2nd rep.: 72.1% *vs*. 80.6%). In the background of results of the second replication are probably the negative effects of summer heat stress. According to the farrowing results experimental animals maintained their advantage over the control sows during both repetitions (1st rep.: 75.0% *vs*. 87.7%; 2nd rep.: 67.6% *vs*. 73.6%).

Table 4. The reproduction performance of sows in the third experiment

	1st replication			2nd replication		
	Control	Experimental	P-value	Control	Experimental	P-value
Number of sows (pcs.)	82	82	-	79	82	-
Time to oestrus (days)	6.53 [b]	4.23 [a]	0.019	5.53	5.43	0.915
Sows in late oestrus (%)	11.0%	2.4%	-	7.6%	4.8%	-
Inseminated sows (pcs.)	76	73	-	68	72	-
Pregnancy rate (%)	80.3%	90.4%	-	72.1%	80.6%	-
Farrowing rate (%)	75.0%	87.7%	-	67.6%	73.6%	-
Non-pregnant/aborted sows (%)	3.9%	4.1%	-	7.4%	9.7%	-
Culled pregnant (%)	0.0%	9.6%	-	2.0%	5.6%	-
Later concepted (%)	13.2%	N.D.	-	20.6%	N.D.	-

[a,b] Different superscripts in the same row indicate a significant difference at a significance level of $P < 0.05$; Data in rows without superscripts are not statistically different.

In the first replication, during subsequent farrows, the number of live born piglets was significantly higher in the experimental group (first farrow: 14.20 *vs*. 13.01, p≤0.036; second farrow: 14.79 *vs*. 13.17, p≤0.007), while in the second replication there was no difference between the treatments. In the first replication, the number of live born piglets in the second farrow increased in both treatments compared to the first farrow, but while the control sows had 0.16 additional piglets, the experimental ones had 0.59 (control: 13.01 → 13.17; experimental: 14.20 → 14.79). In the second replication, the number of pigs in the control group decreased

by 1.12, while that of the experimental increased by 0.29 (control: 13.57 → 12.45; experimental: 13.34 → 13.63).

3.3.2. The composition and chemical analysis of sow milk

The dry matter, protein and fat content of sow's milk was not affected by supplementing the sow's feed with fish oil or sunflower oil during the first experiment.

3.3.3. Fatty acid composition and ratio of n-6/n-3 fatty acids in the sow milk

Sunflower and fish oil supplementation changed the fatty acid composition of the control and the experimental sows' milk (Table 5). Supplementation with sunflower oil increased LA (C18:2) (8.43 mg/ml *vs*. 6.63 mg/ml milk) and significantly ($p<0.02$) increased the amount of PUFA (9.92 mg/ml *vs*. 8.61 mg/ml milk) in the milk of control sows. In contrast, the content of EPA (C20:5), DPA (C22:5) and DHA (C22:6) was significantly higher in the milk of experimental sows ($p<0.001$). The amount of n-3 PUFA in the control milk samples was 0.69 mg/ml, while in the experimental it was 1.17 mg/ml ($p<0.001$).

Table 5. Fatty acid composition of the sow milk in the first experiment

Fatty acids (mg fatty acid/ml milk)	Control	Experimental	P-value
ΣSFAs	35.50 ± 4.43	33.22 ± 4.29	0.23
ΣMUFAs	27.29 ± 6.27	26.03 ± 6.61	0.67
C18:2 (n-6c)	8.43 ^{a} ± 1.05	6.63 ^{b} ± 1.05	0.001
C18:3 (n-3)	0.36 ± 0.05	0.36 ± 0.05	0.97
C20:5 (n-3)	0.04 ^{b} ± 0.01	0.17 ^{a} ± 0.03	0.001
C22:5 (n-3)	0.17 ^{b} ± 0.03	0.28 ^{a} ± 0.06	0.001
C22:6 (n-3)	0.09 ^{b} ± 0.02	0.33 ^{a} ± 0.06	0.001
ΣPUFAs	9.92 ^{a} ± 1.26	8.61 ^{b} ± 1.30	0.02
Σn-6	9.24 ^{a}	7.44 ^{b}	0.001
Σn-3	0.69 ^{b}	1.17 ^{a}	0.001
n-6/n-3	13.42	6.35	-

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; ^{a,b} Different superscripts in the same row indicate a significant difference at a significance level of $P < 0.05$; Data in rows without superscripts are not statistically different

The ratio of n-6/n-3 fatty acids in the control lactation feed was 15.08, which resulted in a ratio of 13.42 in the milk of the control animals. In the experimental lactation feed, this ratio was 7.38, that caused an n-6/n-3 ratio of 6.35 in the milk of the experimental sows.

3.3.4. Examination of odour profile of sow milk by electronic nose

The sow milk samples of the first experiment were examined in triplicate. Principal component analysis (PCA) was used to identify the outliers and the data of a total of 57 measurements were analysed.

The milk samples collected from differently fed animals can be clearly separated according to the feeding groups based on the aroma profile described by the electronic nose (Figure 1). As there is a clear difference between the experimental and control groups even with the unsupervised classification method (PCA) indicates a clear effect of feeding on milk odour. The selected sensors, which played a dominant role in distinguishing the groups, were more characteristic in the experimental group, which means that the volatile substances described by the relevant retention indexes were present in larger quantities in the experimental samples.

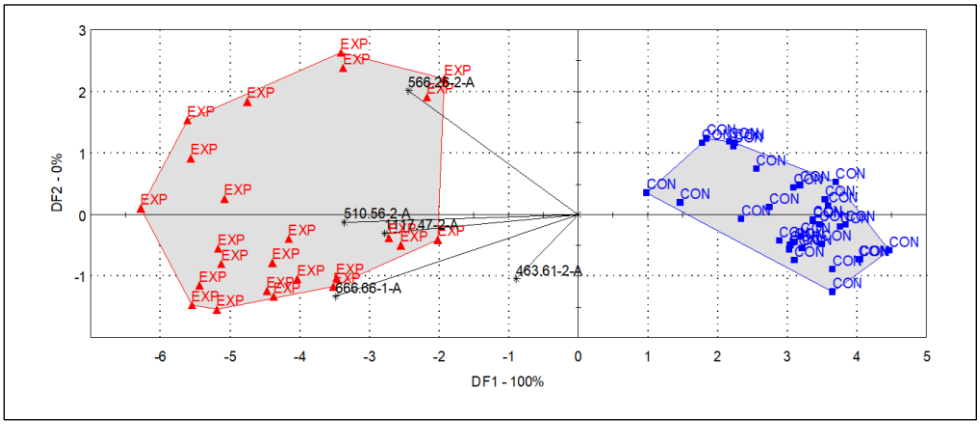


Figure 1. Bi-plot showing the LDA results for the discrimination of the control (CON, blue square) and experimental (EXP, red triangle) milk samples based on the odor profiles described by five selected sensors, and the loading vectors representing the dominance of the sensors.

3.3.5. Examination of different feed samples by electronic nose

The odour profiles of the supplementary feeds of the second experiment were described by principal component analysis. We examined the separability of the control and experimental supplementary feeds by their odour components. Based on the PCA analysis (Figure 2), the samples of fish oil and sunflower oil-based supplementary feeds showed a clear separation based on their odour profile.

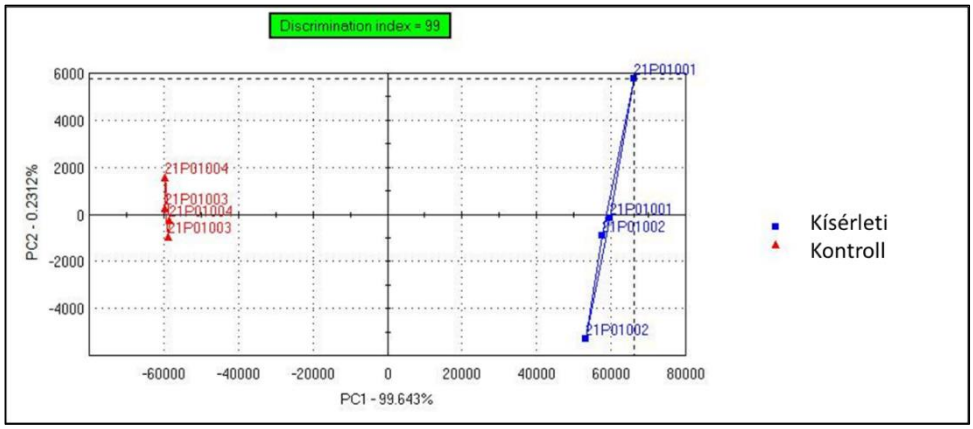


Figure 2. PCA of the control and experimental supplementary feeds in the second experiment

Based on the results, mixed sow feeds made with the addition of supplementary feeds will also be distinguishable based on their odour pattern. Figure 3 shows a comparison of the odour profile of the control and experimental mixed sow feeds of the second experiment using principal component analysis, where the control and experimental feed samples can be clearly separated from each other.

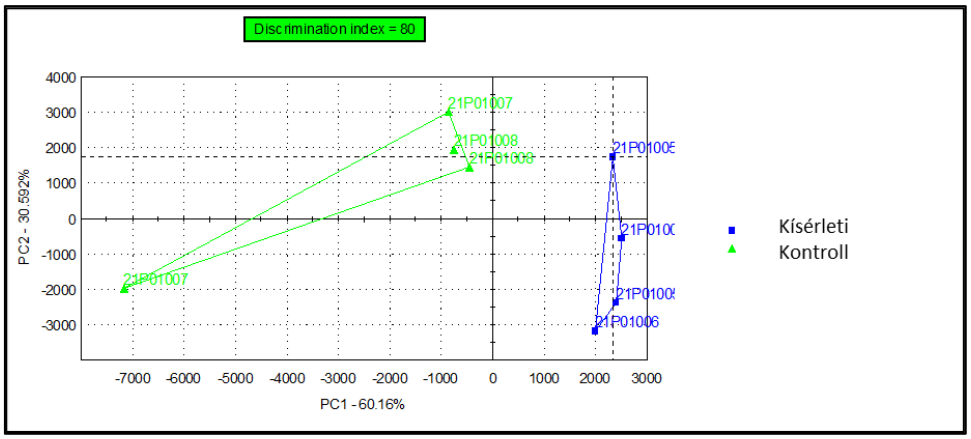


Figure 3. PCA of the control and experimental mixed sow feeds in the second experiment

In the third experiment, six types of mixed sow feeds were examined using an electronic nose. The experimental lactation and mating sow feeds contained aromatised supplementary feed, while the experimental pregnant sow feed contained non-aromatised one. From the principal component analysis of the experimental and control feed samples, we found that the lactation feed can be clearly distinguished from the pregnant and mating feed for both treatments (control, experimental), but the two latter groups are not separated from each other. It means, that the difference in the natural smell of the feeds hides the differences resulting from the aromatization of the supplementary feeds.

Finally, we examined whether the electronic nose could describe a clear difference between the smell of experimental and control sow feeds. The experimental and control samples can be separated from each other based on the PCA result of the mixed sow feeds in the first replication. However, in the case of the second replication, we could no longer clearly separate the experimental and control sow feeds from each other, because the PCA diagram of the experimental mating feed and the control lactation feed overlapped.

3.3.6. Lipidperoxidation and antixodiant system

Fish oil feeding induced statistically significant changes in GPx activity during lactation both in blood plasma and in red blood cell hemolysates (p=0.009 and p=0.003) and in GSH levels in red blood cell hemolysate on day 5 after weaing (p=0.038) compared to sunflower oil (Table 6). The treatment × production period had a significant effect on the GPx activity of the blood plasma of the experimental animals. In the case of red blood cell hemolysates, this interaction had an effect on the GSH content of the control group, and on all three parameters (GSH, GPx and MDA) in the experimental group, indicating that the different production periods significantly influenced (p<0.001) these parameters.

Table 6. Lipidperoxidation and antioxidant markers in the blood of sows in the first experiment (n=24/treatment)

	Day 14 of lactation			Day 5 after weaning			Day 30 after insemination			P-value, interaction (period × treatment)	
	CON	EXP	*p*	CON	EXP	*p*	CON	EXP	*p*	CON	EXP
Blood plasma											
GSH (µmol/g pr.)	2.74	2.82	0.99	3.01	2.97	1.00	2.61	2.82	0.80	0.080	0.461
GPx (E/g protein)	2.87^{b}	3.28^{aA}	0.01	3.00	2.83^{B}	0.70	3.11	3.01^{B}	0.91	0.082	0.005
MDA (µmol/ml)	9.67	10.94	0.42	10.50	9.70	0.84	10.71	11.05	0.10	0.220	0.139
Red blood cell hemolisates											
GSH (µmol/g pr.)	5.93^{B}	5.04^{B}	0.08	7.38^{bA}	8.38^{aA}	0.04	5.26^{B}	5.30^{B}	1.00	<0.001	<0.001
GPx (E/g protein)	3.75^{b}	4.74^{aA}	0.00	3.89	3.75^{B}	0.10	4.08	3.79^{B}	0.91	0.505	<0.001
MDA (µmol/ml)	12.18	12.81^{AB}	0.95	13.17	14.17^{A}	0.72	13.09	11.21^{B}	0.13	0.344	<0.001

CON=control treatment (sunflower oil); EXP=experimental treatment (fish oil); GSH=reduced glutation; GPx=glutation-peroxidase; MDA= malondialdehid

a, b: statistically significant difference between the control and the experimental group during a production period, at a significance level of P < 0.05; A, B: statistically significant difference between the control and the experimental group, in different production periods, at a significance level of P < 0.01

3.3.7. Hormone examinations

During the statistical analysis of the hormone levels, no difference was found between the data of replications (treatment × repetition), so the data were combined. During lactation, we found significantly lower levels of both E2 and 6-keto-PGF1α in the experimental group (p=0.035 and p=0.001, respectively), but there was no difference between P4 levels (Table 7). On day 5 after weaning, plasma P4 levels were significantly higher (p=0.036) in the experimental group, while 6-keto-PGF1α levels were lower (p=0.056), but there was no

difference in E2 levels (p=0.110). During early pregnancy (day 30 after insemination), there was no significant difference in P4 levels, but E2 was significantly lower (p=0.012) in the experimental group, similar to lactation. In addition, the level of 6-keto-PGF1α was also lower in the experimental group (p=0.077, trend).

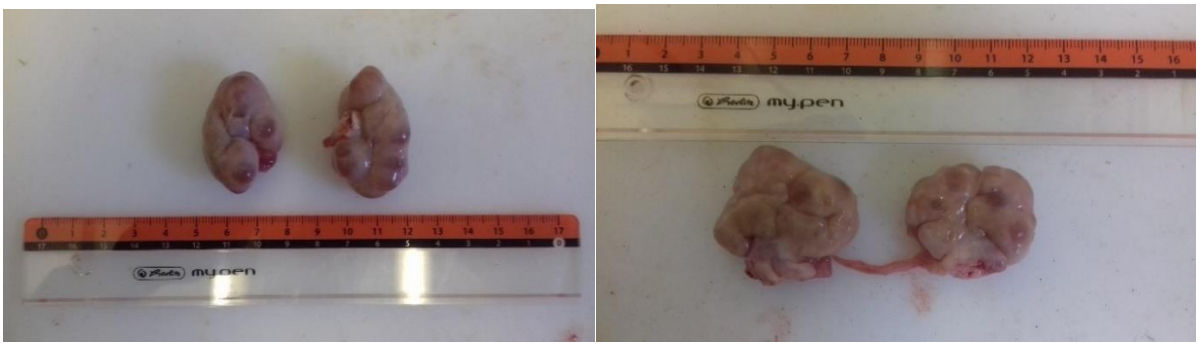
Table 7. Hormone levels of sow blood in different production period of the first examination (n=24/treatment)

		CON	EXP	SEM	P-value
Day 14 of lactation	P4 (nmol/l)	4.99	4.85	0.12	0.561
	E2 (pg/ml)	4.60[a]	3.94[b]	0.15	0.035
	6-keto-PGF1α (pg/ml)	723[a]	201[b]	76.8	<0.001
Day 5 after weaning (insemination)	P4 (nmol/l)	4.94[b]	5.76[a]	0.19	0.036
	E2 (pg/ml)	5.72	5.31	0.12	0.110
	6-keto-PGF1α (pg/ml)	207	166[+]	12.6	0.056
Day 30 after insemination (pregnancy test)	P4 (nmol/l)	52.58	49.95	1.12	0.301
	E2 (pg/ml)	5.84[a]	4.99[b]	0.17	0.012
	6-keto-PGF1α (pg/ml)	242	184[+]	17.0	0.077

CON=control treatment (sunflower oil); EXP=experimental treatment (fish oil); SEM=standard error of mean
P4= progesterone; E2=17β-oestradiol; 6-keto-PGF1α=6-Keto prostaglandin F1α
a, b: statistically significant difference between the control and the experimental group during a production period, at a significance level of $p<0.05$; +: statistically significant difference between the control and the experimental group during a production period, $0.05<p<0.1$

3.3.8. Ovarian examinations

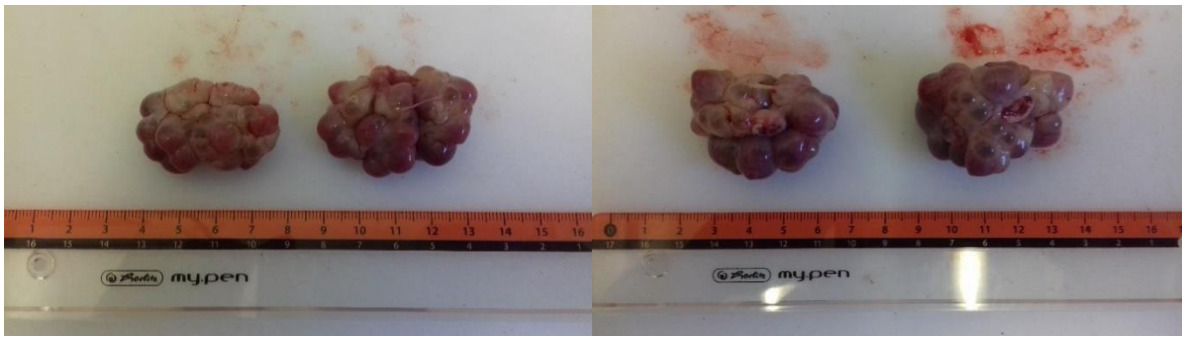
Sows number 2790 (control) and 1022 (experimental) were slaughtered 5 days after weaning, so they showed the symptoms of oestrus. We found 10 tertiary follicles on the left, and 13 on the right ovary of the control sow (Fig. 4a). On the left ovary of the experimental one we found 16 tertiary follicles, hence there were 13 on the right. Some corpus albicans could also be identified on the ovaries indicating previous sexual activity (Fig. 4b).



Figures 4a-4b Ovaries of the control (2790) and experimental sows (1022)

Sows number 1035 (control) and 1005 (experimental) were slaughtered on the day 12 after the weaning. We found 13 corpus haemorrhagicum (CH) and 12-13 inferior follicles on the left and 12 on the right ovary of the control sow (Fig. 5a). On the right and left ovaries of

the experimental sow there were 13 CH and 15-14 inferior follicles (Fig. 5b). Corpus albicans were also found on the ovaries.



Figures 5a-5b Ovaries of the control (1035) and experimental sows (1005)

Based on the ovarian examinations, fish oil feeding in a dose of 12.6 g/kg during lactation had a positive effect on the quantity and quality of follicles on the ovaries of sows, as well as other ovarian structures (CA, CH), compared with the same amount of sunflower oil containing n-6 fatty acids.

4. Conclusions and suggestions

Feeding of fish oil in a practically low dose (6.3 g/kg) significantly increased the amount of the highest biological valued n-3 fatty acids (EPA, DPA, DHA) in sow milk, which also improved the fatty acid supply of piglets. Fish oil feeding compared to sunflower oil caused a distinct change in the odour profile of sow's milk recorded with electronic nose, for which the compounds formed during the oxidation of the unsaturated fatty acids in the fish oil can be at least partly responsible. Even at low doses, fish oil has a detectable effect on the smell of feed and milk, therefore it is also important to study the effects on the feed intake of animals. In the case of higher dose of fish oil (e.g. 12.6 g/kg), the possible reduction in feed intake must be taken into account, which can negatively affect the performance of the sows and their piglets.

Long-term, moderate dose of n-3 fatty acids (fish oil) feeding can be more beneficial than short-term, but in a higher dose. Fish oil fed at 6.3 g/kg dose compared to sunflower oil supplementation had a significant effect on GSH levels and GPx activity in sows. In this dose, n-3 fatty acids beneficially affected the antioxidant system, but did not cause an over activation of the system compared to the control group, so the fed amount should be considered as a safe dose.

Our findings regarding the levels of the examined hormones (E2, P4, 6-keto-PGF1α) can be explained by the fact that n-6 fatty acids help the formation of PGF2α, while n-3 fatty acids inhibit it, that could be confirmed in all, three examined production phases. Feeding of n-3 fatty acids during lactation increased the number and size of ovarian follicles, which may contribute to increased embryo number and viability.

Feeding of n-3 fatty acids to sows can be a significant economic advantage for pig farms. By shortening the time till oestrus reduces the so-called number of "empty days", while the increasing rate of animals heating in time does the number of unproductive sows. Better pregnancy and farrowing results reduce sow culling and the number of gilts needed. The positive effects on the ovaries and on the vitality of embryos increase the number of piglets and fattening pigs that can be sold per sow through better utilization of the genetic potential of the sows. Supplying piglets with n-3 fatty acids through mother's milk can improve piglet viability and growth, which increases the number of animals that can be sold, and also reduces the expenses required to produce one kilogram of pork.

5. New scientific results

1. I found that, in case of corn-soybean-based feeding, fish oil fed at a dose of 6.3 g/kg during lactation significantly increased the amount of physiologically important n-3 fatty acids (EPA, DPA, DHA) in the milk of Danish large white × Danish landrace genotype sows, while the ratio of n-6/n-3 fatty acids decreased from 13.42 to 6.35.

2. In feeding experiments sunflower oil and fish oil applied in a dose of 6.3 g/kg caused a distinct change in the odour profile of sow's milk, that could be detected by electronic nose (e-nose) based ultrafast gas chromatography. This odour change can be originated from the oxidation compounds of unsaturated fatty acids found in fish oil (3-methylbutanol, lactic acid, acetyl pyrazine, dimethyl sulfide, acetaldehyde).

3. Based on the electronic nose analysis of the supplementary and mixed sow feed samples, I found that the supplementary feeds containing sunflower or fish oil and the mixed sow feeds containing these supplementary feeds in a dose of 12.6 g/kg can be separated based on their odour profile.

4. The long-term use of n-3 fatty acids (fish oil) in a low dose (3.15 g/kg) in sow feeds significantly reduced the preweaning mortality of piglets, improved the reproductive performance of sows and the number of piglets born during the subsequent farrowing, so it is more beneficial than the shorter-term, but higher dose (12.6 g/kg) feeding.

5. Fish oil fed at a dose of 6.3 g/kg increased the GSH level in the blood of sows and increased the activity of GPx, which means an improvement in the antioxidant status of the animals.

6. I found that n-3 fatty acid rich fish oil supplementation in a dose of 6.3 g/kg reduced the production of PGF2α in the blood of Danish large white × Danish landrace genotype sows.

6. Scientific articles published in the topic of the dissertation

6.1. Publications in foreign scientific journals

Roszkos, R., Tóth, T., Bazar, G., Fébel, H., Mézes, M., 2022. Effect of omega-3 polyunsaturated fatty acid supplementation on oxidative stress parameters and sex hormone levels of modern genotype sows. Veterinary Medicine and Science, 1–12. https://doi.org/10.1002/vms3.1026

Roszkos, R., Bazar, G., Tóth, T., Kovacs, Z., Febel, H., Mezes, M., 2021. Effect of n-3 polyunsaturated fatty acid feeding on the fatty acid profile and odor of milk in danbred sows, Journal of Applied Animal Research, 49:1, 447-459, https://doi.org/10.1080/09712119.2021.2005071

Roszkos R, Tóth T, Mézes M. 2020a. Review: practical use of n-3 fatty acids to improve reproduction parameters in the context of modern sow nutrition. Animals (Basel). 10(7):1141. https://doi.org/doi:10.3390/ani10071141

6.2. Publications in Hungarian scientific journals

Roszkos, R., 2023. Az n-3 zsírsavak hatása nagy teljesítményű tenyészkocák fontosabb termelési és szaporodásbiológiai paramétereire. Scientia et Securitas. (4):1-10. https://doi.org/10.1556/112.2022.00108

Roszkos, R., 2022. Az elektromiográfia fejlődése és alkalmazásának lehetőségei a tenyészkocák szaporodásbiológiai folyamatainak vizsgálatában (szemlecikk). Állattenyésztés és Takarmányozás. 71(2):88-104.

Roszkos R, Tóth T, Fébel H, Mézes M. 2020b. Effect of different n-6/n-3 fatty acid proportion oil sources on reproduction performance and fatty acid profile of milk in modern genotype sows - preliminary results. Acta Agrar Debr. 1:121–128. https://doi.org/10.34101/actaagrar/1/3742