

## Potential use of cod liver oil in a pig diet: effects on the chemical, physical and sensory parameters of a bologna sausage

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**Abstract:** The aim of the study was to substantially increase PUFA n-3 content in bologna sausage based on meat of pigs fed a diet fortified with 8% fish oil. The control group of pigs was fed a standard feed ration without any added oils. The addition of oil into the feed ration did not cause increasing the fat level in bologna sausage. Fatty acid profile was affected significantly. The amount of these acids: C16:0, C18:0 and C18:2n-6 was significantly lower ( $p < 0.05$ ) for the experimental group. On the other hand, the amount of following acids: C20:5n-3, C22:5n-3 and C22:6n-3 was higher ( $p < 0.05$ ). Overall, the content of SFA decreased and the PUFA n-6/PUFA n-3 ratio was significantly lower ( $p < 0.05$ ). The addition of fish oil into the diet did not affect the oxidative stability of the product ( $p > 0.05$ ). Results of an instrumental and sensory analysis have shown a significant difference ( $p < 0.05$ ) between both groups, when the experimental group was evaluated as harder and less juicy. Colour changes was recorded for redness ( $a^*$ ) parameter, the experimental group shown higher values ( $p < 0.05$ ). The addition of fish oil did not affect ( $p > 0.05$ ) the flavour, nor the odour of the product.

**Key Words:** meat products, fish oil, fatty acids, EPA, DHA, functional food

### INTRODUCTION

On the one hand, meat and meat products are an important source of proteins, some vitamins, and minerals, on the other hand, they are criticized as a significant source of fat with an unsuitable lipid profile. According to FAO (2010) the daily recommended fat intake should be between 30–35% of overall energy intake, 10% of it should be SFA, TFA up to 1%, PUFA 6–11% and the rest should consist of MUFA. The recommendations are also specified for the EPA and DHA intake, where AI (adequate intake) is 0.25 g/day. According to Simopoulos (2002), a diet with high content of PUFA n-6 supports the development of cancer and other cardiovascular, inflammatory, and autoimmune diseases. The n-6/ n-3 ratio in a western diet is 15.0–16.7 (Simopoulos 2002), however, Elvevoll and James (2000) recommend the ratio around 4.0–10.0. Wood et al. (2008) state that the n-6/ n-3 ratio is around 7.6 for pork meat with adipose tissue and 7.2 for lean meat. From this reason, there is an effort to improve the fatty acid profile in meat products. Pérez-Palacios et al. (2019) summarize improving options of the fatty acid profile for meat products as: enrichment of meat through a fodder, enrichment of meat products by a direct addition of PUFA, PUFA emulsification or microencapsulation. Vegetable oil (linseed oil, chia oil, perilla oil), fish oil or algae oil are most used as PUFA sources. However, according to USDA (2010), vegetable sources of PUFA n-3 are less significant than oils acquired from sea sources. Wood et al. (2008) summarize knowledge of meat quality with respect to the fatty acids composition that had been affected by a fodder. The fatty acid composition affects mainly the meat texture, its oxidative stability, taste, and colour. In most cases, according to Pérez-Palacios et al. (2019), when the diet of pigs was enriched by oils rich in PUFA, there was a problem with subsequent oxidation and sensory quality of meat products, mainly regarding dried meat products. The aim of the study was to substantially increase PUFA n-3 content in bologna sausage based on meat of pigs fed a diet fortified with 8% fish oil.; concurrently we presumed that the expected decrease of oxidative stability will not damagingly affect the sensory acceptance of the products.

## MATERIAL AND METHODS

### Material

12 hybrid-breed sows were used for the experiment: 50% Landrace x 50% Large White (Bioprodukt Knapovec, Ústí nad Orlicí, Czech Republic). In the age of 12 weeks, these sows were divided into two groups of 6 pieces. For the following 30 days, the C group (control group) was fed a standard compound feed (De Heus, Maref, Czech Republic), the F group (experimental group) was fed the same feed compound with 8% addition (w/w) of fish oil (pharmaceutical cod liver oil). The analysis of fatty acids of used fish oil is shown in the Table 1. The experiment was conducted in compliance with the Czech National Council Act No. 246/1992 Coll. to protect animals from cruelty (amendment to the Act 255/2017 Coll.) After fattening, slaughter was performed, and the meat was processed in pilot plants (CZ 22067) at Department of Food Technology within Mendel University in Brno.

Table 1 Fatty acid profile of used cod liver oil (*Jecoris aselli oleum*)

Fatty acid	Content [wt %]	Fatty acid	Content [wt %]	Fatty acid	Content [wt %]	Fatty acid	Content [wt %]
14:0	4.6	18:2n-6	3.3	20:5n-3	10.4	MUFA	45.7
16:0	11.1	18:3n-6	0.8	22:4n-6	1.1	PUFA	34.7
16:1	10.4	18:3n-3	1.6	22:5n-3	1.9	n-6	7.2
17:0	1.0	20:1	15.8	22:6n-3	13.6	n-3	27.5
18:0	2.9	20:2n-6	1.0	22:6n-3	13.6	n-6/n-3	0.18
18:1n-9	19.5	20:4n-6	1.0	SFA	19.6		

6 sows were chosen to produce bologna sausage, three of each group. The other sows were used for the production of other meat products. Lean and fatty meat was used; no other meat (like beef) was added to the product. 10 kg of bologna sausage was produced from each individual piece. This amount was divided to three parts for the analysis and each part was analysed twice ( $n = 9$ ; except the sensory analysis). The bologna sausage were produced using 4.3 kg of the lean meat, 3.7 kg of the fatty meat, 0.194 kg of the nitrite salting mixture, 1.75 kg of water (in the form of ice), 0.126 kg of the spicing mixture (Combi-Bologna Sausage; Masoprofit, Czech Republic) consisting of phosphates, antioxidant (ascorbic acid), ground black pepper and white pepper. The whole mixture was ground up using a vacuum cutter (Seydelmann, Stuttgart, Germany) into the form of the meat emulsion, filled into the polyamide casing (diameter of 60 mm) by a filler (HTS 150; Germany) and heat-treated at 85 °C for 10 min in a smoker (Bastramat, Arnsberg, Germany). The bologna sausage were vacuum-packed immediately after production and stored at 2 °C.

### Chemical analysis and oxidative stability

The dry matter, protein, fat and salt content were specified. For total amount of protein was used the Kjeldahl method, the total fat content was analysed by Soxhlet extraction, the salt content was determined by Mohr method and the dry matter content was analysed gravimetrically (AOAC 2005).

Furthermore, the fatty acid profile was specified. The analysis was performed according to Komprda et al. (2017) with some modifications. The gas chromatograph Agilent 6890 with an autosampler (Agilent Technologies, Wilmington, USA) was used for the separation of fatty acids methyl esters (FAMES). A capillary chamber with dimensions of 20 m x 0.18 mm x 0.15  $\mu$ m, ZB FAST FAME brand (Phenomenex, Torrance, California, USA) was used. The temperature of an injector was 250 °C. A gas ionization detector (temperature of 260 °C) was used for detection. The temperature programme was as follows: the start temperature was 80 °C, held for two minutes; 10 °C/min until 160 °C; 3 °C/min until 185 °C; 30 °C/min until 240 °C, held for two minutes. The carrier gas flow (N<sub>2</sub>) was 0.5 ml/min a split ratio 1:100. The NU-CHECK 455 – 16 FAME (NU-CHECK Prep, Inc., Elysian, Minnesota, USA) was used as an external standard for FAME identification.

The oxidative stability of the product was specified according to Jezek et al. (2019). The extent of lipid oxidation was specified by the determination of thiobarbituric acid reactive substances

(TBARS). The TBARS values were obtained by multiplication of optical density by the factor of 7.8. Oxidative products were quantified as equivalents of malondialdehyde (MDA, mg/kg).

### Instrumental measurement

Sample colour was analysed pursuant to the CIELAB system (ISO/CIE 2019). Colour parameters were evaluated:  $L^*$  (axis from black to white),  $a^*$  (axis from green to red) and  $b^*$  (axis from blue to yellow). The specifications were obtained using a spectrophotometer Minolta® CM-3500d (Konica Minolta, Osaka, Japan) in the D65 lighting mode, a slit of 8 mm, SCE. Colour was measured on 2 cm slices that was taken from the central part of the product. Samples were measured within 5 minutes of their cutting.

The hardness of sausage was measured by TIRATEST 27025 (TIRA GmbH, Germany). The cylindrical slices of the bologna sausage (height 2 cm) were used for determination of hardness by the MORS knife with a crosshead speed of 10 mm/min and penetration to 10 mm. The hardness was also measured by the compression using 50% compression rate with a crosshead speed of 50 mm/min using the cylindrical samples with a diameter of 1 cm and height of 1 cm. In the both method the hardness of the bologna sausage samples was measured in the edge and the centre.

### Sensory analysis

The evaluation was ongoing under ČSN ISO 6658 (560050) condition (ÚNMZ, 2010). Samples were assessed by a 12-member trained panel consisting of researchers. For sensory analysis was selected 100mm non-structured line scale with a description of anchored points, where 0 is the sign for minimum (not perceived) and 100 for maximum perception. For the descriptors “saltiness”, “intensity of colour” and “hardness”, 50 was considered as the optimum value. Each sample was coded with a randomly selected three-digit number. Unsalted rolls and room temperature water were served as neutralizers. Two slices (1.0–1.5 mm thick) of each samples of the bologna sausage were served at room temperature on the white porcelain plate. The slices were taken from the central part of the product. On the beginning of the evaluation, sausage was assessed as a whole piece (acceptability of the overall appearance). Other descriptors were assessed in the cut (intensity of colour, appearance, and odour). The intensity of strength on the bite, juiciness, saltiness and overall taste acceptability were also evaluated.

### Statistical analysis

Data were tested for normality by Shapiro–Wilk test. The differences between samples were analysed by either a Mann-Whitney U test or an analysis of variance (one-way ANOVA), including Tukey’s test ( $p < 0.05$ ). STATISTICA 12 (StatSoft, USA) was used for statistical evaluation.

## RESULTS AND DISCUSSION

Results of chemical analysis are stated in the Table 2. Even though the F group was fed the food with added oil, no significant differences ( $p > 0.05$ ) in fat content were observed between the F group and the C group. Comparing to the control, fish oil in the food increased the protein content in bologna sausage ( $p < 0.05$ ). Besides proteins, also NaCL content increased. Bryhni et al. (2002) also added fish oil to a compound feed but they did not state fundamental chemical parameters within their study. When adding fish oil into a meat product, the fat content in meat is obviously increasing in proportion to the dose of added oil (Cáceres et al. 2008); a consumer can perceive this as a negative factor.

The influence of fish oil in a pig fodder on the fatty acid content in bologna sausage was proven for some fatty acids (Table 3). Contents of myristic acid 16:0, stearic acid 18:0 and linoleic acid 18:2n-6 were reduced. On the contrary, the increase was recorded for EPA C20:5n-3 (twelve times higher), DPA C22:5n-3 (almost five times higher) and DHA C22:6n-3 (more than twelve times higher). The n-6/n-3 ratio decreased significantly ( $p < 0.05$ ) to 2.57, this value corresponds with the recommendations according to Elvevoll and James (2000). Overall content of SFA ( $p < 0.05$ ) also decreased, the content of MUFA was not affected by the addition of fish oil into a fodder ( $p > 0.05$ ). Bryhni et al. (2002) confirm the increasing amount of DPA in sausages, the DHA level was not increased because of the low dose of added fish oil (0.2%, 0.4%). The amount of EPA was not monitored. According to Cáceres et al. (2008), direct addition of fish oil into bologna sausage increased the amount of MUFA and PUFA, furthermore, the n-6/n-3 ratio decreased to 2.54 for 6% of batch.

Table 2 Basic chemical analysis of bologna sausage according to different diets

Parameter	C ( $\bar{x} \pm \text{SEM}$ ) [g/100g]	F ( $\bar{x} \pm \text{SEM}$ ) [g/100g]
Protein	13.02 $\pm$ 0.45 <sup>b</sup>	14.47 $\pm$ 0.50 <sup>a</sup>
Fat	9.30 $\pm$ 2.03	7.07 $\pm$ 3.01
Dry matter	27.70 $\pm$ 2.04	27.69 $\pm$ 3.03
NaCl	2.10 $\pm$ 0.03 <sup>b</sup>	2.25 $\pm$ 0.02 <sup>a</sup>

Legend: C – standard diet, F – diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

Table 3 Fatty acid profile of bologna sausage according to different diets

Fatty acid	C ( $\bar{x} \pm \text{SEM}$ ) [mg/100g]	F ( $\bar{x} \pm \text{SEM}$ ) [mg/100g]	Fatty acid	C ( $\bar{x} \pm \text{SEM}$ ) [mg/100g]	F ( $\bar{x} \pm \text{SEM}$ ) [mg/100g]
C14:0	100.1 $\pm$ 10.1	86.4 $\pm$ 12.0	C20:4n-6	30.9 $\pm$ 4.1	14.8 $\pm$ 2.9
C16:0	1837.2 $\pm$ 181.9 <sup>a</sup>	1144.5 $\pm$ 175.0 <sup>b</sup>	C20:5n-3	5.0 $\pm$ 0.1 <sup>b</sup>	60.4 $\pm$ 5.4 <sup>a</sup>
C16:1	187.0 $\pm$ 21.5	167.2 $\pm$ 20.7	C22:4n-6	8.9 $\pm$ 0.7	8.9 $\pm$ 0.9
C17:0	34.4 $\pm$ 2.8	28.4 $\pm$ 5.3	C22:5n-3	10.2 $\pm$ 0.9 <sup>b</sup>	46.2 $\pm$ 5.1 <sup>a</sup>
C18:0	986.1 $\pm$ 81.9 <sup>a</sup>	549.6 $\pm$ 85.9 <sup>b</sup>	C22:6n-3	5.3 $\pm$ 0.5 <sup>b</sup>	108.1 $\pm$ 13.7 <sup>a</sup>
C18:1n-9	3197.4 $\pm$ 336.6	2018.9 $\pm$ 319.8	SFA	2957.8 $\pm$ 276.1 <sup>a</sup>	1809.0 $\pm$ 271.5 <sup>b</sup>
C18:2n-6	992.0 $\pm$ 76.1 <sup>a</sup>	620.1 $\pm$ 90.2 <sup>b</sup>	MUFA	3366.3 $\pm$ 357.9	2186.1 $\pm$ 340.3
C18:3n-6	11.1 $\pm$ 1.4	10.6 $\pm$ 1.3	PUFA n-6	1100.2 $\pm$ 86.2 <sup>a</sup>	686.0 $\pm$ 97.8 <sup>b</sup>
C18:3n-3	65.9 $\pm$ 5.5	50.1 $\pm$ 7.6	PUFA n-3	82.2 $\pm$ 7.1 <sup>b</sup>	264.8 $\pm$ 35.0 <sup>a</sup>
C20:2n-6	48.4 $\pm$ 3.9 <sup>a</sup>	25.6 $\pm$ 3.8 <sup>b</sup>	n-6/n-3	13.45 $\pm$ 0.23 <sup>a</sup>	2.57 $\pm$ 0.07 <sup>b</sup>
C20:3n-6	9.0 $\pm$ 0.8	6.1 $\pm$ 0.7			

Legend: C – standard diet, F – diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

The results in the Table 4 shows that oxidative stability of bologna sausage was not significantly different when comparing with the control ( $p > 0.05$ ). The limitation of oxidative acceptability is 2.5 mg of MDA/kg (Zhang et al. 2019), the results of the oxidative stability did not affect the sensory analysis though. Bryhni et al. (2002) state that, in their case, results of the sensory analysis evaluating the oxidative stability was inconsistent.

Table 4 Oxidative stability of bologna sausage according to different diets

Parameter	C ( $\bar{x} \pm \text{SEM}$ ) [MDA, mg/kg]	F ( $\bar{x} \pm \text{SEM}$ ) [MDA, mg/kg]
Oxidative stability	2.18 $\pm$ 0.13	2.67 $\pm$ 0.23

Legend: C – standard diet, F – diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

When measuring the texture of bologna sausage (Table 5), it was observed that the addition of fish oil into a pig fodder increases hardness (in the both method - by MORS knife and by compression). A significant difference ( $p < 0.05$ ) between the C and the F group was observed both in the centre and in the edge part of the product. The texture may change for direct addition depending on other used raw materials, Cáceres et al. (2008) used caseinates that increased the hardness of the product.

Results of the colour measurement of bologna sausage are stated in the Table 6. A statistically significant difference was proven ( $p < 0.05$ ) in redness parameter ( $a^*$ ). In this case, the control group has a lower value of  $a^*$ . When directly adding fish oil, the lightness of the product increases proportionally to the amount of added oil;  $a^*$  value decreases (Cáceres et al. 2008). Vossen et al. (2017) did not observed any difference in colour parameters directly after slicing samples of dried hams made of pigs fed an algae oil.

Table 5 Instrumental measurement of texture of bologna sausage according to different diets

Parameter		C ( $\bar{x} \pm \text{SEM}$ ) [N]	F ( $\bar{x} \pm \text{SEM}$ ) [N]
Edge	Hardness: MORS knife	2.30 $\pm$ 0.16 <sup>b</sup>	3.87 $\pm$ 0.21 <sup>a</sup>
	Hardness: compression	23.60 $\pm$ 1.18 <sup>b</sup>	31.75 $\pm$ 1.98 <sup>a</sup>
Centre	Hardness: MORS knife	2.23 $\pm$ 0.15 <sup>b</sup>	3.93 $\pm$ 0.28 <sup>a</sup>
	Hardness: compression	26.16 $\pm$ 1.32 <sup>b</sup>	36.10 $\pm$ 1.40 <sup>a</sup>

Legend: C – standard diet, F – diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

Table 6 Instrumental measurement of bologna sausage colour according to different diets

Parameter	C ( $\bar{x} \pm \text{SEM}$ )	F ( $\bar{x} \pm \text{SEM}$ )
L*	67.7 $\pm$ 0.59	67.2 $\pm$ 0.71
a*	7.6 $\pm$ 0.28 <sup>a</sup>	7.9 $\pm$ 0.16 <sup>b</sup>
b*	9.7 $\pm$ 0.18	9.5 $\pm$ 0.17

Legend: C – standard diet, F – diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

Results of the sensory analysis of the bologna sausage are stated in the Table 7. A statistically significant difference was proven ( $p < 0.05$ ) in the descriptors: strength on the bite (hardness), juiciness and saltiness. The results of the strength on the bite correspond to the instrumental measurement of hardness. The F group was evaluated worse than the control group in the descriptor: juiciness and hardness ( $p < 0.05$ ). The flavour and odour of the F group and the C group were practically the same ( $p > 0.05$ ). The evaluators didn't notice any off-flavours or oxidative changes. Vossen et al. (2017) reported neither fishy odour nor fishy flavour in dry-cured hams produced from pork of pigs fed a diet enriched with algae. However, LC-PUFA n-3 content was lower in the diet (1.2 g of algae/100 g feed). Bryhni et al. (2002) report that a small amount (0.4%) of fish oil in the feed did not affect the flavour or odour, but interacted with other PUFAs to produce a stale odour. The bologna sausages with direct addition of fish oil was generally acceptable for evaluators in all doses of 1–6% (Cáceres et al. 2008).

Table 7 Sensory analysis of bologna sausage according to different diets

Descriptor	C ( $\bar{x} \pm \text{SEM}$ )	F ( $\bar{x} \pm \text{SEM}$ )
Overall appearance (hedonic)	84.1 $\pm$ 4.2	89.3 $\pm$ 3.1
Cross-section colour (intensity, from dark to light)	74.6 $\pm$ 5.6	70.7 $\pm$ 7.3
Cross-section appearance (hedonic)	72.1 $\pm$ 6.2	73.5 $\pm$ 5.5
Odour (hedonic)	81.7 $\pm$ 4.2	78.1 $\pm$ 4.6
Strength on the bite (intensity, from soft to hard)	52.0 $\pm$ 6.8 <sup>b</sup>	64.3 $\pm$ 6.3 <sup>a</sup>
Juiciness (intensity, from dry to juicy)	73.2 $\pm$ 4.9 <sup>a</sup>	63.1 $\pm$ 6.6 <sup>b</sup>
Saltiness (intensity, from unsalted to over-salted)	51.3 $\pm$ 3.2 <sup>b</sup>	55.9 $\pm$ 2.9 <sup>a</sup>
Flavour (hedonic)	75.8 $\pm$ 6.2	75.6 $\pm$ 6.7

Legend: C – sausage produced from pork meat of pigs fed a standard diet, F – sausage produced from pork meat of pigs fed a standard diet with 8% of fish oil; Values with different superscripts in the same rows means there are significant differences ( $p < 0.05$ ).

## CONCLUSIONS

Generally, it can be said that bologna sausage made of meat of pigs fed the food enriched by fish oil (8%) was very positively perceived by evaluators within the sensory analysis. Despite the higher level of malondialdehyde, no significant differences in the odour or flavour between the control and experimental group were recorded. The texture of the product was rated less positively but the improvement in nutritional value of bologna sausage, in which the SFA content decreased significantly, should be noted. The n-6/n-3 ratio decreased as well, while the content of EPA and DHA



increased. In conclusion, it should be noted that the DHA content was so high that a health claim can be made: DHA contributes to maintain normal brain, vision and heart function (European Union, 2010).

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