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Original Research Paper

IMPACT OF FEEDING OF FLAXSEED AND PROBIOTICS ON MEAT QUALITY AND LIPID OXIDATION PROCESS IN PORK DURING STORAGE

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Summary: In our experiment effect of flaxseed, mixed in standard diet (alone and in combination with probiotic bacteria) during feeding period, on lipid oxidation and sensory properties of pork (thigh muscles) stored at freezing (-21 °C) and chilling (4 °C) conditions was studied. Oxidation processes expressed as changes in malondialdehyde (MDA) content in thigh muscles were monitored. Addition of flaxseed had impact on increasing of fat content in muscles. Results confirmed that feeding the flaxseed significantly ($P < 0.05$) increased oxidation processes during storage in comparison to control group. Sensory examination of pork from pigs fed with flaxseed showed significantly different properties (taste and odour) compared to control group. Therefore, if feedstuff contains plant oils with high proportion of polyunsaturated fatty acids (PUFAs), addition of adequate amount of antioxidants is recomended.

Key words: flaxseed; lipid oxidation; pork

Introduction

Lipids are inseparable part of human nutrition and they have no replacement in various chemical and biochemical processes. Mainly, they are important as an intake of energy into the organism, and partially, lipids are utilised as a source of substances necessary for synthesis of components important for homeostasis. Besides nutrition and dietetic properties, lipids have also impact on sensory perceptions, and they have important influence on food odour and taste (1).

The main contribution of fats in nutrition is presence of essential polyunsaturated fatty acids (PUFAs). It is confirmed, that consumption of n-3 PUFAs has positive impact on human organism. In general, it is recommended to decrease the consumption of saturated and *trans* fatty acids and to increase the intake of PUFAs. Various scientific studies were focused on increasing the PUFAs content in animal products by adding plant oils into the animal feedstuff. The amount and composition of dietary fatty acids influence the quality of fat tissue in pigs (1, 2). Flaxseed presents a rich source of linoleic acid (C18:3, n-3) and in poultry (3) and rabbits (4) addition of flaxseed into the diet significantly changed proportion between PUFA/SFA and concentration of n-3 acids in animal muscles was increased.

Probiotic bacteria are known for their action on the host body including its immune system. Despite the considerable body of evidence about probiotics, the mechanism of their effect has not been explained completely. One of the presumed mechanisms of the inhibitory effect of probiotics on pathogens of digestive tract is the competition for the intestinal mucosa receptors (5, 6, 7). The results of different studies suggest that the action of probiotics may be modulated by dietary PUFAs as polyunsaturated fatty acids increase the colonisation of small intestine with lactobacilli.

It is well known that lipid oxidation is one of the major causes of lipid-rich foods deterioration. Higher content of PUFAs in meat have negative influence on stability of lipids during storage. The

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oxidative deterioration of the polyunsaturated lipids of foods leads through formation of hydroperoxides to short-chain aldehydes, ketones and other oxygenated compounds, which are considered to be responsible for development of rancidity in stored foods (8, 9).

The aim of our experiment was observation of lipid oxidation changes in pork thigh muscles during storage at chilling (4 °C, 11 days) and freezing conditions (-21 °C, 9 months) after feeding flaxseed alone or in combination with probiotic bacteria. Oxidative changes of lipids were compared with changes in sensory properties of stored meat.

Material and methods

Thirty six piglets at the age of 14 days were involved in our experiment. Piglets were divided into three groups. Groups were fed from 10 days before weaning until 35 days after weaning according to following schemes: first group (K) was fed with standard diet OŠ-02 NORM TYP (Spišské Vlachy, SR), second group (MK) was fed with standard diet enriched with flaxseed (10 % in mixture; composition of fatty acids in flaxseed is presented in Table 1). Third group (LMK) was fed with standard diet in combination with flaxseed (10 % in mixture) and probiotic bacteria strains (L81 – *Lactobacillus plantarum* and 2I3 – *Lactobacillus fermentum*) in form of probiotic cheese (4g per pig per day).

On the 35th day of age pigs were slaughtered. Bleeding of pigs followed the stunning. Procedure was performed according to the rules established for slaughtering of animals and was performed by trained veterinary surgeon. Afterwards, all pig carcasses were weighed, deboned and had skin removed. Thigh muscles were packed under vacuum conditions. One part of samples was stored at chilling conditions (4 °C) for 11 days, and second part at freezing conditions (-21 °C) for 9 months.

Table 1: Fatty acid composition of flaxseed used in diet (percentage)

Fatty acid	Proportion (%)
16:0 Palmitic	5.1
18:0 Stearic	3.7
18:1 *n*-9 Oleic	18.4
18:2 *n*-6 Linoleic	16.1
18:3 *n*-3 α-Linolenic	56.8

Chemical composition of meat samples

Determination of water content, dry matter content and fat content in percentage was performed according to Veterinary laboratory methods (10).

Evaluation of Thiobarbituric acid reactive substances (TBARS)

Decomposition of fats was assessed by TBARS assay. Evaluation of TBARS was performed according to Marcincak et al. (11) and measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, Thermo spectronic, Great Britain). Results were calculated on amount of malondialdehyde in 1 kg of sample. Individual analysis were carried out on 1, 3, and 11 day of storage at chilling conditions and 0, 3, 6, and 9 month of storage at freezing conditions.

Sensory evaluation

Thigh muscles were used for sensory evaluation. Samples were evaluated 24 hours after slaughter processing of pigs (fresh meat) and after 9 months of storage at freezing conditions (- 21 °C). Professional evaluation committee presented panel of 7 assessors and they worked according to Methods intended for sensory evaluation of meat (12). The samples were boiled and 5 point schema and triangle test were applied for evaluation (13).

Statistical analysis

Statistical processing of results was performed by Graph Pad Prism 3.0 (1999). Results are expressed as arithmetic mean (x) and standard deviation (± s.d.). Increase of malondialdehyde between different groups during storage was compared by one-way ANOVA test. Tukey comparison test was used to compare statistical differences between values and $P < 0.05$ was considered as statistically significant difference. Values of observed parameters, which are presented in tables, are mean values obtained by calculation from six samples of meat.

Results

Results of determination of chemical composition of thigh muscles are shown in Table 2. Feeding of flaxseed, as a source of PUFAs caused increase in fat content in samples from experimental groups (MK and LMK). Statistically significant differences

were recorded between control and experimental groups ($P < 0.05$). Comparison of protein content revealed higher values in samples from group LMK than in control samples ($P < 0.05$); however protein content in samples from group MK and in control group was similar.

Table 2: Chemical composition of thigh muscles (percentage)

	Water (%)	Fat (%)	Proteins (%)
K	76.75 ± 1.29^{a}	3.54 ± 1.08^{a}	16.83 ± 0.23^{a}
LMK	76.73 ± 0.69^{a}	6.29 ± 1.19^{b}	17.28 ± 0.14^{b}
MK	76.43 ± 0.32^{a}	5.26 ± 0.63^{b}	16.98 ± 0.15^{a}
^{a,b} – values with different labelling in column are statistically different			

In diagram 1 results of TBARS determination in samples of muscles stored at chilling conditions (4 °C, 11 days) are shown. Immediately after the slaughtering, amount of malondialdehyde (main product of lipid oxidation) in thigh muscles (MK and LMK) increased rapidly. Statistically significant difference was present between experimental and control groups ($P < 0.05$) in the amount of MDA. Storage of samples (3 and 11 days) caused increase in MDA in all groups, although the increase was higher in samples from experimental groups. Increase of MDA in control group was significantly lower ($P < 0.05$) in comparison to experimental groups, what reflects significantly lower oxidation stability of meat samples obtained from animals fed with flaxseed alone or in combination with probiotic bacteria (*Lactobacillus*).

The oxidative changes were increased also during the storage of meat samples at freezing conditions (Graph 2). In experimental groups MK and LMK level of lipid decomposition, expressed as amount of MDA present in muscles, was significantly higher in comparison to control group ($P < 0.05$) already 24 hours after slaughter, and thereafter during the whole storage period. However, there was no significant difference between groups MK and LMK. At the end of nine month of storage, amounts of MDA in groups MK and LMK were twice as high (2.28 and 2.99 $mg.kg^{-1}$) as in control group (1.08 $mg.kg^{-1}$).

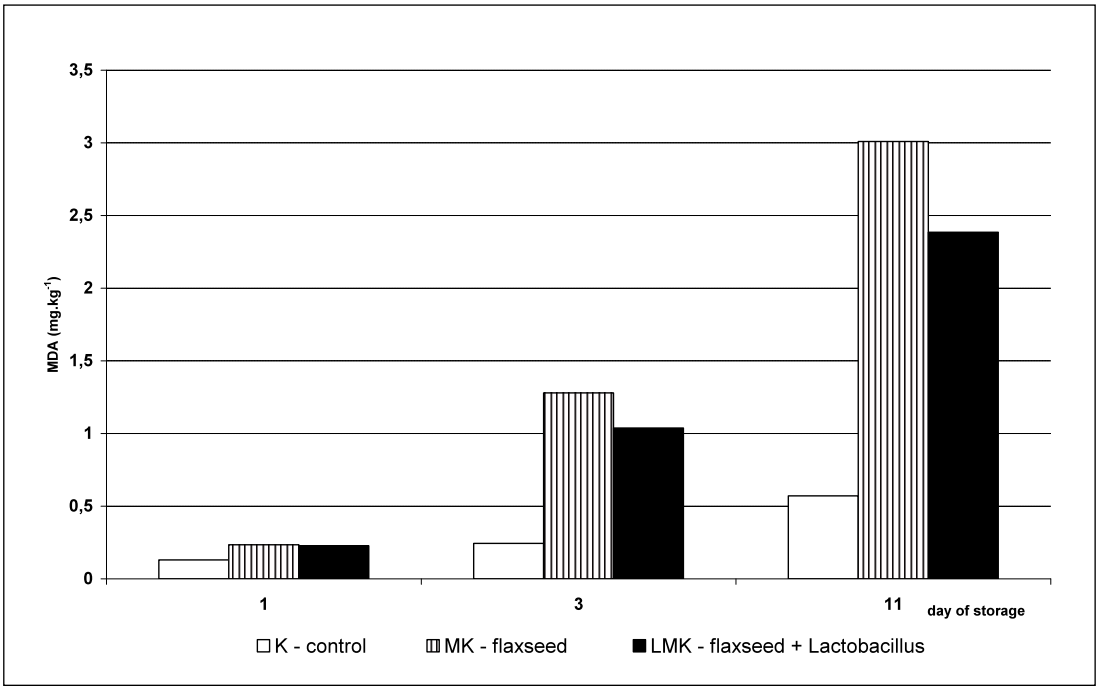

Diagram 1: Thiobarbituric acid reactive substances calculated as an amount of malondialdehyde in thigh muscles stored at chilling conditions (4 °C)

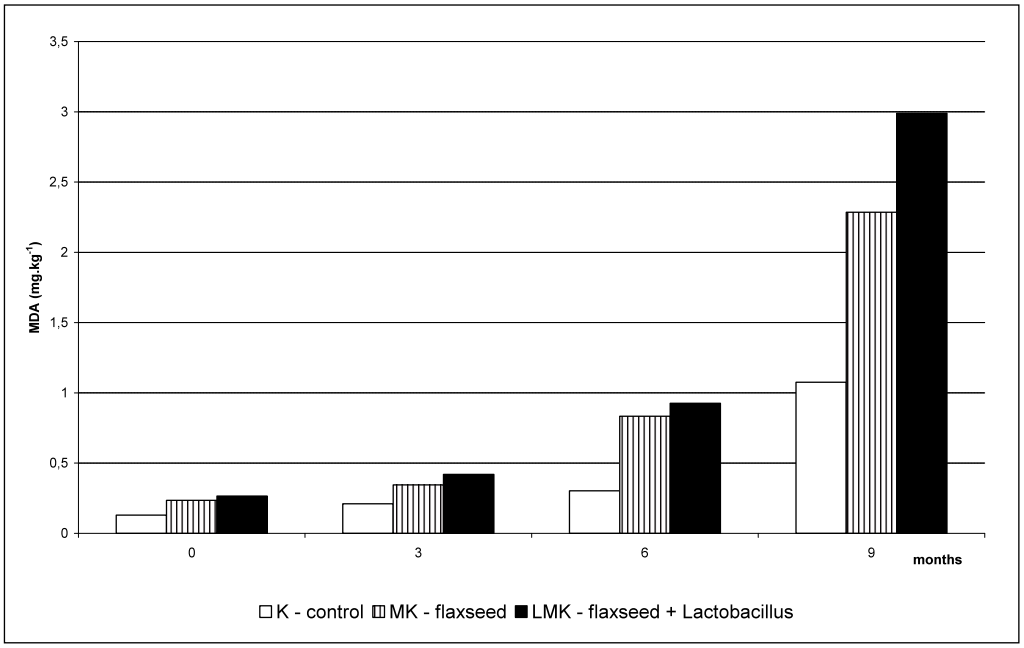

Diagram 2: Thiobarbituric acid reactive substances calculated as an amount of malondialdehyde in thigh muscles stored at freezing conditions (-21 °C)

Sensory evaluation of samples of thigh muscles was performed by professional evaluation committee. Taste, odour, succulence and texture of samples were evaluated. Immediately after slaughter, no statistically significant differences were recorded. However, control samples were evaluated as better in their sensory properties (table 3). Minimal differences were recorded between groups MK and LMK. In samples stored for 9 months at freezing conditions, higher final score, using 5 point schema, was given to samples from control group ($P < 0.05$). The most significant differences were in odour and taste of meat samples, which were considered negative properties in experimental groups.

Using triangle method, meat samples from experimental groups stored at freezing conditions were evaluated as significantly different in comparison control samples, and 28 samples from total 30 samples were correctly distinguished by a panel of 7 assessors (99.9 %). Once more, taste was the most significant resolution property in meat samples.

Table 3: Results of sensory evaluation using 5 point schema

	After slaughter	9 months freezing
K	18.4 ± 1.2^{a}	17.4 ± 2.1^{a}
MK	17.6 ± 0.9^{a}	15.1 ± 1.9^{b}
LMK	17.4 ± 1.4^{a}	14.3 ± 3.2^{b}
^{a,b} – values with different labelling in column are statistically different		

Discussion

Results of the present study showed that it is possible to raise a content of fat in pig muscle fairly quickly by adding flax into the diet of animals. It is generally known and confirmed by many studies, that feeding of flaxseed increased proportion of 18:3 n-3 and 20:5 n-3 PUFAs in fatty tissues and meat of animals (14, 15, 16). The fatty acid pattern in the diet substantially influenced the n-6/n-3 PUFAs ratio in meat (6). Kastel et al. (17) showed that elevated intake of n-3 PUFAs induced significantly higher levels of γ-linolenic, eicosapentaenoic and docosahexaenoic acids and reduction in arachidonic acid in blood serum of germ-free piglets.

Increased content of PUFAs in meat of pigs fed with flaxseed resulted in excessive production of lipid oxidation metabolites during storage of samples. Significant differences were recorded already 24 hours following slaughter processing. Storage of samples at chilling conditions (4 °C) for 11 days caused rapid growth of lipid decomposition processes. Rey et al. (16) showed that addition of 5 g of flaxseed oil per kg of feedstuff in pigs significantly increased lipid decomposition rate in muscles (*m. longissimus dorsi*) after 3, 6 and 9 days of storage when compared to control group without addition of flaxseed oil in the diet.

Time of storage in freezer influenced decomposition changes of fats in thigh muscles in all three

described positive effect of PUFAs on adhesion of probiotic strains in pig intestines. However, effect of probiotic bacteria on oxidative stability of PUFAs was not confirmed in our experiment. These results are in accordance with previously published data (2, 14, 16, 18), who stated that meat obtained from pigs fed diet fortified with flaxseed, had lower oxidative stability and higher concentration of TBARS during storage.

Sensory evaluation of pork, stored in a freezer, confirmed markedly worse final score of samples with higher content of PUFAs in the diet of pigs. These results are in agreement with Bryhni et al. (2) who stated that after 1 month of freezer storage meat and fat from pigs on the high PUFA diets showed more fishy and rancid odours and less meat odour than samples from pigs on the low PUFA diets.

In the present study, both sensory traits and TBA were affected by diets with PUFAs content. This is in agreement with Bryhni et al. (2) and Cameron and Enser (19), who showed that saturated and monounsaturated fatty acids were generally positively associated with the meat quality and stability, whereas PUFAs were correlated negatively. Although high PUFAs level might contribute to a healthier meat, it is important to be aware of reduced storage stability and problems connected with fat oxidation. Therefore, when plant oils with higher content of PUFAs are fed, it is advisable to use them together with appropriate amount of antioxidants.

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VPLIV HRANJENJA PRAŠIČEV Z LANENIMI SEMENI IN PROBIOTIKI NA KAKOVOST MESA IN OKSIDACIJO MAŠČOB MED SKLADIŠČENJEM MESA

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Povzetek: V poskusu smo proučevali oksidacijo maščob in senzorične lastnosti prašičjega mesa stegenskih mišic, ki je bilo skladiščeno bodisi v zamrznjenem stanju (-21 °C) ali ohlajeno (4 °C). Meso smo pridobili od prašičev, ki so bili hranjeni z lanenim semenom, mešano krmo, standardno krmo in standardno krmo z dodatkom probiotičnih bakterij. Oksidacijski proces smo spremljali s spremembami v vsebnosti malondialdehida (MDA) v stegenskih mišicah. Dodatek lanenega semena krmi je povečal vsebnost maščob v stegenskih mišicah in s tem tudi statistično značilno ($P < 0,05$) pospešil oksidacijske procese med hrambo mesa. Tudi senzorično ocenjevanje svinjine prašičev, ki so bili krmljeni z lanenim semenom, je pokazalo očitno razliko (v okusu in vonju) v primerjavi s kontrolno skupino. Ugotavljamo, da je pri krmljenju živali z rastlinsko hrano, ki vsebuje veliko večkrat nenasičenih maščobnih kislin (PUFA), smiselno dodajanje zadostne količine antioksidantov.

Ključne besede: laneno seme; oksidacija maščob; svinjina