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A HIGHER PROPORTION OF PUFA IN DIET INCREASES THE PUFA CONTENT IN RABBIT MEAT, BUT REDUCES THE OXIDATIVE STABILITY OF MEAT

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SUMMARY

The aim of our study was to determine the changes in the fatty acid composition of rabbit meat, if palm fat (99% of saturated fatty acids (SFA)), as a source of fat in rabbit diet, was replaced with linseed oil (71% of polyunsaturated fatty acids (PUFA), containing 52% of α-linolenic acid (n-3 PUFA)). The Ganoderma lucidum or olive leaves were added in the diet as potential antioxidant in order to protect the PUFA against oxidation. 48 SIKA rabbits were randomly divided by mass and gender in four groups: CONT- 6% palm fat, CONT+ 6% linseed oil, REISHI 6% linseed oil and 1% Ganoderma lucidum, OLIVE 6% linseed oil and 1% olive leaves. After 22 days of the experimental procedure, the samples of back muscle were taken and divided in 7 portions. One was for fatty acid determination, other six for malondialdehyde (MDA) determination after different storage condition; fresh, 6 days at 4°C or 3 months at -20°C, raw or cooked (60 minutes, 85°C). Addition of linseed oil resulted in a significant higher proportion of PUFA (n-3 PUFA) and monounsaturated fatty acid (MUFA) and lower proportion of SFA in the back muscle, but the oxidative stability of meat was reduced, since the level of MDA was significantly higher. After cooking, the level of MDA increased in all the groups, but more in the groups with linseed oil in the diet, the addition of Ganoderma lucidum or olive leaves slightly decreased the level of MDA, but the difference was not significant.

Key-words: nutrition, rabbit meat, oxidative stability, PUFA, MDA

INTRODUCTION

Nowadays people are more and more aware of the importance of healthy diet, and healthy effect of polyunsaturated fatty acids (PUFA) is implied in this context. Western diets have excessive amounts of omega-6 polyunsaturated fatty acids (n-6 PUFA), compared to the omega 3 polyunsaturated fatty acids (n-3 PUFA), leading to a high n-6/n-3 PUFA ratio, more than 15/1, while the optimal ratio would be 4/1 (Simopoulos, 2002). Rabbit meat already contains high amount of n-3 PUFA compared to the other source of meat (Dalle Zotte, 2004), which is the result of alfalfa presence in the rabbit diet. Nevertheless, the fatty acid composition of rabbit meat can be improved by the addition of n-3 fatty acids in rabbit diet (Dalle Zotte, 2004) and in that way rabbit meat could be considered as a functional food (Dalle Zotte and Szendrő, 2011). However, PUFA can be easily oxidized, forming aldehydes like malondialdehyde (MDA) during storage or cooking of meat and consequently has impact on the nutritional value of meat (Gray et al., 1996; Tres et al., 2014). Incorporation of antioxidants in the diet can prevent lipid oxidation. Interest of using non-vitamin antioxidants of plant origin (polyphenols, flavonoids) has increased in the last years.

The objectives of the study were to evaluate the impact of adding PUFA in rabbit diet on the fatty acid composition of back muscle, the susceptibility of raw and cooked meat to oxidation under different storage conditions and the effectiveness of addition of potential antioxidants, *Ganoderma lucidum* or olive leaves on the oxidative stability of the meat.

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MATERIAL AND METHODS

Animals and diets

All procedures were performed by the current legislation on animal experimentation in Slovenia. Forthy-eight SIKA rabbits (80 days old, average initial body mass 2580±299 g) were randomly divided (by mass and gender) in four groups. The CONT- group received diet with 6% of palm fat (99% of saturated fatty acids - SFA), other three groups received diet with 6% of linseed oil (more than 70% of PUFA), CONT+ was unsupplemented, REISH was supplemented with 1% of *Ganoderma lucidum* and OLIVE was supplemented with 1% of olive leaves. All other ingredients of the diets were the same and at the same level, except the alfalfa level that was decreased in REISHI and OLIVE groups by 1%, as it was the supplementation level.

Experimental procedure and sample collection

Throughout of the experiment, 22 days; animals had free access to the water (nipple) and pelleted diet and were individually housed in wired cages. During the experiment, the body mass of the animals were recorded each week and just before slaughtering. Diet intakes were recorded daily, animals received weighted daily meal and the residue from the day before was weighted and discarded. Rabbits were slaughtered at the 102 days of age whereas a sample of back muscle (*M. longisimus dorsi*) from each animal was taken and divided in 7 equal portions in order to determine the MDA concentration in raw and cooked samples, stored in three different ways: fresh (at -70°C until the analyses were performed), 6 days in refrigerator at 4°C and 3 months in freezer at -20°C. Cooking was performed at 85°C one hour, after the storage. In one sample of back muscle, the fatty acid composition was determined for each meat treatment. Before the analyzing, samples were homogenized in laboratory homogenizer by liquid nitrogen.

Determination of fatty acid composition and MDA concentration

The fatty acid composition of diets and back muscle were analyzed using a gas chromatographic method after the in situ transesterification of lipids. Methyl esters of fatty acids were prepared according to the procedure of Park and Goins (1994) whereas analysis of fatty acids methyl esters (FAMEs) was performed by gas chromatography using an Agilent 6890 series gas chromatograph. FAMEs are identified by retention time and results are expressed as a percentage of the total fatty acids content.

The MDA concentration was determined following the method of Vilà et al. (2002) with minor modifications by HPLC using reversed-phase chromatography column. A Waters Alliance 2690 equipped with Waters 474 scanning fluorescence detector was used to determine MDA concentration.

Statistical analysis

The data were analyzed using the General Linear Models procedure of the SAS/STA module (SAS 8e, 2000) considering the diet as the only main effect. Differences among the groups were determined using Tukey's multiple comparison test. Unless stated otherwise, a least significant difference of 0.05 was used to separate treatment means. Results in the tables are presented as least square means (LSM) ± SEM with *P*-values.

RESULTS AND DISCUSSION

Growth performance of rabbits were similar in the all groups and normal by the age (weight gain between 28.8 g/day and 34.8 g/day and feed conversion rate between 5.18 g/g and 6.09 g/g), with tendency of better values in groups with linseed oil addition and no additional effect of olive leaves or *Ganoderma lucidum* supplementation. This is in accordance with the notification of Meartens et al. (1986), that more saturated fatty acids are less digestible, although some other authors (Bianchi et al., 2009; Casado et al., 2013) detected lower growing performance, when linseed or linseed oil was added in the diet.

Since the differences in the composition of the diet were only in the source of fat (palm fat or linseed oil) and in the supplementation (1%) of olive leaves or *Ganoderma lucidum* or no supplementation, the composition (Table 1) of the diets was similar. On the contrary, the fatty acid composition of the diets differs. After the substitution of palm fat with linseed oil the proportion of SFA decreased and the proportion of monounsaturated fatty acids (MUFA) and especially PUFA increased, with no additional supplement effects (Table 2).

Table 1. Proximate composition (g/kg) and fatty acid composition (% of total fatty acids) of the diets

	CONT –	CONT +	REISHI	OLIVE
Chemical composition (g/kg DM)				
Dry matter (g/kg)	933	912	924	922
Crude protein	191	196	196	193
Crude fat	116	95	92	93
Crude fibre	244	250	247	246
Crude ash	74	76	77	76
Main fatty acids (% of total fatty acids)				
C12:0	0.20	0.04	0.04	0.04
C14:0	0.89	0.12	0.12	0.12
C16:0	35.81	8.00	8.06	8.07
C18:0	41.73	4.00	3.98	3.99
∑ C18:1	7.75	23.48	23.70	23.56
C18:2 n-6	9.01	22.01	22.20	21.92
C18:3 n-3	2.78	40.23	39.77	40.21
∑ SFA	80.08	13.48	13.53	13.56
∑ MUFA	8.05	24.14	24.36	24.22
∑ PUFA	11.86	62.38	62.11	62.22
∑ n-3 PUFA	2.85	40.33	39.86	40.31
∑ n-6 PUFA	9.01	22.05	22.25	21.92
n-6/n-3 PUFA	3.16	0.55	0.56	0.54

CONT- 6% palm fat in a diet; CONT+ 6% linseed oil in a diet; REISHI 6% linseed oil in a diet with addition of 1% *Ganoderma lucidum*; OLIVE 6% linseed oil in a diet with addition of 1% olive leaves; DM – dry matter; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Table 2. Fatty acid composition (% of total fatty acids) of back muscle (*M. longisimus dorsi*)

	CONT -	CONT +	REISHI	OLIVE	SEM [1]	P-value
C12:0	0.15	0.09	0.14	0.11	0.02	0.057
C14:0	1.86 [a]	1.44 [b]	1.28 [b]	1.40 [b]	0.10	0.001
C16:0	23.21 [a]	17.31 [b]	17.66 [b]	17.62 [b]	0.34	<0.001
C16:1 n-7	3.48	2.64	2.41	2.55	0.45	0.319
C18:0	10.48 [a]	6.02 [b]	6.84 [b]	6.49 [b]	0.33	<0.001
∑ C18:1	23.38	23.50	22.76	23.12	0.30	0.286
C18:2 n-6	23.91	24.25	24.30	24.17	0.50	0.940
C18:3 n-3	3.15 [a]	14.68 [b]	12.50 [b]	13.48 [b]	0.74	<0.001
C20:4 n-6	4.12	3.43	4.44	3.91	0.39	0.309
C20:5 n-3	0.14 [a]	0.39 [b]	0.45 [b]	0.41 [b]	0.04	<0.001
C22:5 n-3	0.63 [a]	1.11 [b]	1.36 [b]	1.23 [b]	0.11	<0.001
C22:6 n-3	0.11 [a]	0.15 [ab]	0.19 [b]	0.17 [ab]	0.02	0.005
∑ SFA	38.52 [a]	27.50 [b]	28.98 [b]	28.43 [b]	0.53	<0.001
∑ MUFA	27.80	27.06	26.11	26.60	0.74	0.397
∑ PUFA	33.68 [a]	45.44 [b]	44.91 [b]	44.97 [b]	0.85	<0.001
∑ n-3 PUFA	4.07 [a]	16.52 [b]	14.68 [b]	15.50 [b]	0.68	<0.001
∑ n-6 PUFA	29.59	28.90	30.21	29.45	0.65	0.546
n-6/n-3 PUFA	7.28 [a]	1.81 [b]	2.11 [b]	1.96 [b]	0.13	<0.001

CONT- 6% palm fat in a diet; CONT+ 6% linseed oil in a diet; REISHI 6% linseed oil in a diet with addition of 1% *Ganoderma lucidum*; OLIVE 6% linseed oil in a diet with addition of 1% olive leaves; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; [a, b] values with different superscripts are significantly different ($P < 0.05$); [1] n = 46

In the fatty acid composition of the back muscle, the linseed oil addition resulted in a significant higher proportion of PUFA (substituting SFA), in particular n-3 PUFA, α-linolenic acid and also EPA and DHA (Table 2). The result was narrower n-6/n-3 PUFA ratio in all three groups with linseed oil in the diet, without any additional effect of *Ganoderma lucidum* or olive leaves, as compared to the group with palm fat in a diet. Some other authors (Gondret et al., 1998; Bernardini et al., 1998; Kouba et al., 2008; Gigand and Combes, 2008; Li et al., 2012; Du et al., 2013; Tres et al., 2014) also observed that fatty acid composition of the rabbit diet

have influence on the fatty acid composition of the meat. Two weeks of feeding of fortified diet with PUFA is enough to have changes in fatty acid composition in meat (Maertens et al., 2008).

However, higher amount of PUFA in meat (back muscle) leads to the lipid oxidation and degradation of n-3 PUFA into oxidative products, like MDA. MDA concentration in a back muscle was significantly higher in a groups supplemented with linseed oil, as compared to the group with palm fat (Figure 1). *Ganoderma lucidum* or olive leaves slightly reduce the level of MDA, but not to the level determined in the palm fat group, and the difference with CONT+ was not significant.

In the Figure 1 it could be seen that cooking increased the level of oxidation in the meat, but the highest oxidation degree was determined after 6 days of storage in the refrigerator in raw meat in the groups with linseed oil supplementation. The addition of *Ganoderma lucidum* was more effective in raw samples (except fresh row) and the olive leaves in cooked samples. Although for both those supplements *in vitro* antioxidative potential was already proven, it seems that in *in vivo* conditions they have no antioxidative potential, being in accordance with the results of Dal Bosco et al. (2014) on Spirulina or Thyme. If meat is cooked, the antioxidative protection was even less effective.

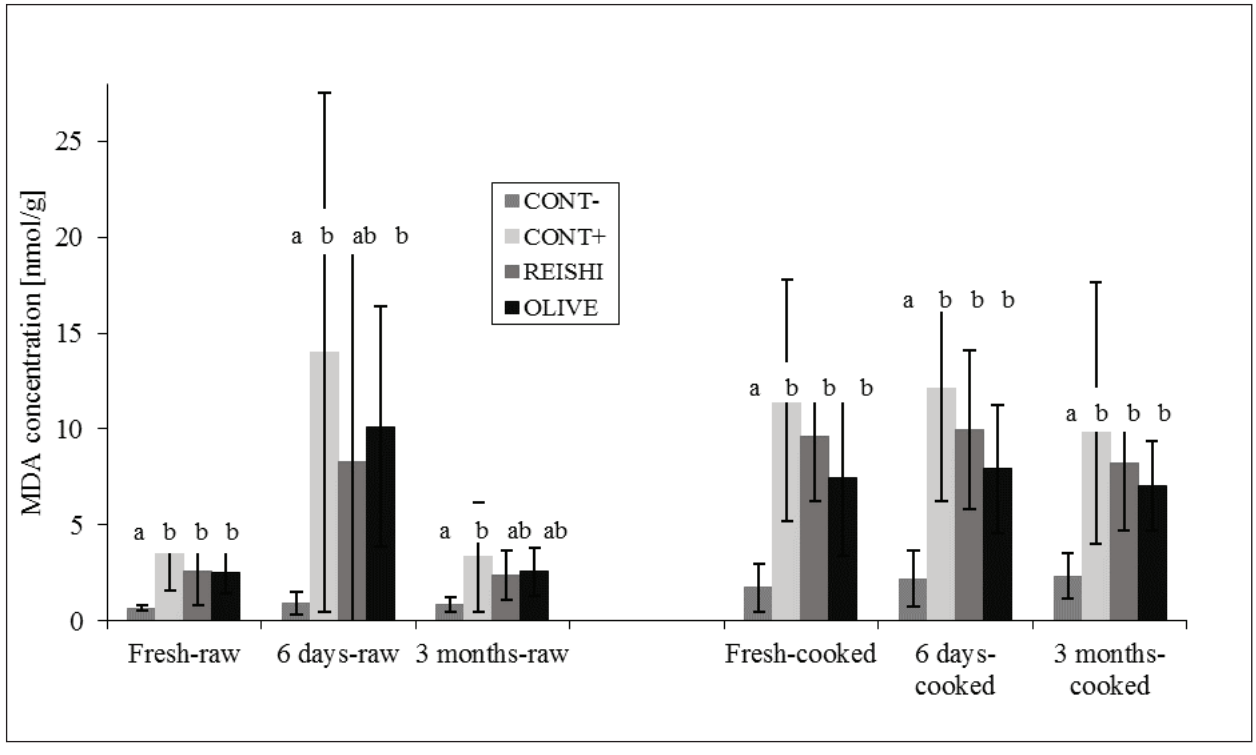

Figure 1. MDA concentration in raw and cooked (85°C, 60 min) back muscle (nmol/g) after different storage conditions, fresh, refrigerator stored (4°C, 6 days) or frozen stored (-20°C, 3 months) (CONT- 6% palm fat in a diet; CONT+ 6% linseed oil in a diet; REISHI 6% linseed oil in a diet with addition of 1% *Ganoderma lucidum*; OLIVE 6% linseed oil in a diet with addition of 1% olive leaves)

CONCLUSION

The meat oxidative stability was worse when PUFA content of meat increases. The addition of potential antioxidants (*Ganoderma lucidum* or olive leaves) does not protect meat from oxidation. Addition of *Ganoderma lucidum* decreased the concentration of MDA in both ways of stored raw samples to the level not statistical different from the palm fat group, but olive leaves only in the meat stored in freezer at -20°C. Cooking (heat processing) forwards the process of oxidation. The addition of *Ganoderma lucidum* negligible reduces the MDA concentration as well as olive leaves (slightly higher), but none of them did not completely prevent oxidation. Better way of using those (potential) antioxidants could be in the form of extracts, that could be worthy of future investigation.

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