

MODIFICATION OF DIET FATTY ACID COMPOSITION CHANGE THE FATTY ACID COMPOSITION OF RABBIT MEAT

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ABSTRACT

The objective of the study was to determine the effect of linseed oil supplementation on the performance and fatty acid composition of rabbit leg muscle and adipose tissue. Two experiments were done. First experiment: twelve male SIKA rabbits, divided in two groups, control (n = 4; commercial diet) and the linseed (n = 8; commercial diet with 9% of linseed oil sprayed onto the pellets). Second experiment: twenty-four (12 male and 12 female) SIKA rabbits, divided in two groups, palm fat (n = 12; 6% palm fat), linseed oil (n = 12; 6% linseed oil). Body mass gain, feed intake and feed efficiency were recorded. The fatty acid composition of leg intramuscular lipids and adipose tissue was determined by gas chromatography and malondialdehyde (MDA) concentration in plasma and leg muscle was measured. The results clearly show that the fatty acid composition of leg muscle and adipose tissue depends on the diet fatty acid composition. Linseed oil in both experiments reduced the proportion of saturated fatty acid (SFA) and increased the proportion of polyunsaturated fatty acids (PUFA) in both tissues. Linseed oil also reduced the n-6/n-3 PUFA ratio to more favorable (8.22 to 1.31 and 6.82 to 1.81 in first and second experiment, respectively). However, linseed oil addition leads to significantly higher MDA concentration in leg muscle.

Key words: rabbits / meat / animal nutrition / fatty acid composition / linseed oil / malondialdehyde

1 INTRODUCTION

The beneficial effects of polyunsaturated fatty acids (PUFA), especially n-3 PUFA on human health are well known and have been documented by numerous studies (review of Williams, 2000; review of Riediger *et al.*, 2009). The population in the developed world do not consumed enough n-3 PUFA, but relatively high amount of n-6 PUFA, which leads to a high n-6/n-3 PUFA ratio, ranging from 15/1 to 20/1 and increase the risk of developing various disease, especially cardiovascular disease. According to Simopoulos (2002) the optimal ratio between n-6/n-3 PUFA would be 4/1 or even lower. With the addition of n-3 PUFA (like linseed oil) in the rabbit's diet, the proportion of n-3 PUFA in the meat increase and leads to a lower n-6/n-3 ratio in the meat and consequently in

human nutrition if rabbits meat is included in the human nutrition. However, high content of PUFA could lead to a high susceptibility to lipid oxidation, which leads to the formation of aldehydes, like malondialdehyde (MDA).

The aim of this study was to investigate the effect of linseed oil supplementation into commercial diet (rise the fat content) and changing the saturated fatty acids (SFA) with PUFA on performance and fatty acid composition of rabbit's meat and adipose tissue.

2 MATERIAL AND METHODS

All procedures were performed according to current legislation on animal experimentation in Slovenia. Animals used in both experiments were reared and

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2.1 ANIMALS AND DIETS

There were two experiments. In first experiment twelve male SIKA rabbits (2284 g) were randomly (equally by mass) divided into two groups and assigned into two different dietary treatments, a control diet ($n = 4$) and a linseed diet ($n = 8$). The control diet was a commercial diet with normal addition of fat (soybean oil 2 %) and the linseed diet was commercial diet, sprayed 9% of linseed oil onto the pellets, which was prepared daily. In the second experiment twenty-four SIKA rabbits (12 male, 12 female), were randomly divided (equally by sex and mass) into two groups. In the palm fat diet was a large amount of SFA (6% of palm fat) and in linseed oil diet was a large amount of PUFA (6% of linseed oil). In both experiments animals had free access to drinking water (nipple drinkers) and diet. They were individually housed in standard cages. Samples of each diet were taken during the each experiment in the purpose of chemical and fatty acid analyses.

2.2 EXPERIMENTAL PROCEDURE AND SAMPLE COLLECTION

In both experiment each day animals received weighed daily meal and the residue from the day before was weighed and discarded. Body masses were recorded each week during the experiment. In the first experiment, which was longer, after 52 days of treatment, and in the second experiment, after 22 days of treatment, rabbits were slaughtered by electric stunning and exsanguinated. Blood samples, for the purpose of measuring MDA concentration in plasma, were collected into 6 ml evacuated tubes containing EDTAK₂ anticoagulant (367864, BD-Plymouth, UK). Plasma was separated by centrifugation (1000 g for 15 min at 4 °C), transferred into Eppendorf tubes and stored at -70 °C until analysis. The part of the leg musculature and adipose tissue were taken, weighted and stored at -70 °C until analyses were performed.

2.3 DETERMINATION OF MALONDIALDEHYDE (MDA) AND FATTY ACID COMPOSITION

The methodology of Wong *et al.* (1987) modified by Chirico (1994) and Fukunaga *et al.* (1995) was used to measure the concentration of MDA in blood plasma and samples of leg muscles by HPLC using reversed-phase

chromatography column (HyperClone 5u ODS (C18) 120A, 4.6 × 150 mm 5 mikron; Phenomenex Inc., USA). A Waters Alliance 2690 apparatus equipped with a Waters 474 scanning fluorescence detector was applied. The results of the analysis were evaluated by the Millenium32 Chromatography Manager program.

The fatty acid composition of intramuscular lipids and adipose tissues samples were analyzed using a gas chromatographic method after the in situ transesterification of lipids. For the fatty acid composition of the diet samples was used the same method. Each sample was analyzed in duplicate. Methyl esters of fatty acids were prepared according to the procedure of Park and Goins (1994) by gas chromatography using an Agilent 6890 series gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with an Agilent 7683 Automatic Liquid Sampler, a split injector, a flame ionization detector and a fused silica capillary column Omegawax 320 (Supelco, USA). The chromatograms were evaluated by the Agilent GC Chem Station software. Separated FAMES were identified by retention time. Results are expressed as percentage of the total fatty acids.

2.4 STATISTICAL ANALYSIS

The data were analyzed by the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA), taking into consideration the diet as the only main effect. Differences among groups were determined using Tukey's multiple comparison test. Significance was considered established at $P < 0.05$, if not stated otherwise. Results in the tables are presented as LS-means ± standard deviation with P-values.

3 RESULTS AND DISCUSSION

3.1 FATTY ACID COMPOSITION OF THE DIETS

The fatty acid composition of the diets from the first experiment (control diet and linseed diet) and second experiment (palm fat diet and linseed oil diet) are presented in the Table 1. As expected, the both diets with the addition of linseed oil had higher proportion of PUFA and lower proportion of SFA compared to the control diet. In the first experiment the linseed diet had higher proportion of n-3 PUFA and lower proportion of n-6 PUFA as compared to the control diet and with that more suitable ratio n-6/n-3 PUFA (0.63 and 6.30 in linseed diet and control diet, respectively). But, in the second experiment the proportion of both, n-3 and n-6 PUFA and also monounsaturated fatty acids (MUFA) were higher in lin-

Table 1: Fatty acid composition of the diets (% of the total fatty acids)

	1. experiment		2. experiment	
	Control diet	Linseed diet	Palm fat diet	Linseed oil diet
C12:0	0.10	0.06	0.20	0.04
C14:0	0.22	0.11	0.89	0.12
C16:0	14.31	7.85	35.81	8.00
C18:0	3.25	3.56	41.73	4.00
Σ C18:1	21.13	19.37	7.75	23.48
C18:2 n-6	50.26	25.85	9.01	22.01
C 18:3 n-3	7.93	40.74	2.78	40.23
Σ SFA	19.64	12.60	80.08	13.48
Σ MUFA	22.05	20.85	8.05	24.14
Σ PUFA	58.32	66.59	11.86	62.38
n-6/n-3 PUFA	6.30	0.63	3.16	0.55

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

seed oil group as compared to palm fat group. The ratio n-6/n-3 PUFA was again more suitable in the diet with linseed oil addition (0.55 and 3.16 in linseed oil group and palm fat group, respectively). Since in palm fat the content of SFA is almost 100%, the amount of PUFA and MUFA in palm fat diet was low (less than 20% together). Even that the ratio n-6/n-3 PUFA was favorable, the amount of PUFA in the palm fat diet was 6 times lower than in linseed oil diet.

3.2 BODY MASS GAIN AND DIET INTAKE

There were no significant differences in the initial and final body mass and also in the body mass gain, neither in first or second experiment. In the first experiment, the linseed diet had larger amount of fats in the diet (12.2% and 4.2% in linseed and control diet, respectively) and with that the energy content. The feed intake was significantly lower and consequently the feed efficiency was significantly higher in linseed diet (142.4 g/day and 22.7%) as compared to control diet (183.4 g/day and 17.3%). But in the second experiment, where the diets have similar amount of fat in the diet (10.8% and 8.6% of crude fat in palm fat and linseed oil, respectively), the feed intake (168.32 g/day and 178.51 g/day in palm fat and linseed oil, respectively) and feed efficiency (17.09% and 18.76% in palm fat and linseed oil, respectively) were similar. Since the level of SFA in palm fat diet was very high, the depression of body mass gain and feed efficiency were expected, as was detected by Voljč *et al.* (2011) on chicken and since Meartens *et al.* (1986) noticed a negative relationship between the degree of saturation and fat

digestibility (more saturated fats are less digestible than unsaturated).

3.3 FATTY ACID COMPOSITION OF TISSUES AND MDA CONCENTRATION

Diets supplemented with linseed oil in both experiment led to a higher proportion of total PUFA, due to reduction of SFA in leg muscles and in adipose tissue. As expected, the linseed oil addition significantly increased ($P > 0.0001$) proportion of the α -linolenic acid (C 18:3 n-3) and some other n-3 PUFA (C 20:5 n-3, C 22:5 n-3) in both experiments and in both tissues (Table 2 and 3). The differences among fatty acids composition inside the experiments were similar, except the amount of stearic acid (C 18:0) and linoleic acid (C 18:2 n-6). Since palm fat contain large amount of stearic acid, the amount was also increased in the leg muscles and in the adipose tissue, the difference wasn't notice in the first experiment. The amount of linoleic acid in the control diet of first experiment increased compared to linseed diet, but not in the second experiment. The reason is probably in some higher amount of linoleic acid in soybean oil in the pellets of first experiment (Table 1). Both those differences were more pronounced in adipose tissue (Table 3) than in leg muscles (Table 2). Our results are in general agreement with the other studies done on rabbits, such as addition of 16 g ground linseed per kg diet (Bernardini *et al.*, 1999) or 30 g extruded linseed per kg diet (Kouba *et al.*, 2008), or on chicken, where in kg diet was added 75 g of linseed oil (Voljč *et al.*, 2011).

Table 2: MDA concentration in plasma (nmol/ml) and in leg muscles (nmol/g) and fatty acid composition of leg muscle (% of the total fatty acids)

	1. experiment		2. experiment	
	Control diet (4)	Linseed diet (8)	Palm fat diet (11)	Linseed oil diet (12)
Plasma MDA	0.26 ^a	0.36 ^b	0.14	0.14
Muscle MDA	0.40 ^a	1.24 ^b	3.42 ^a	12.13 ^b
C12:0	0.10	0.08	0.20	0.16
C14:0	1.83 ^a	1.12 ^b	1.92 ^a	1.47 ^b
C16:0	21.86 ^a	15.04 ^b	22.69 ^a	16.52 ^b
C18:0	6.90	6.62	10.86 ^a	6.09 ^b
Σ C18:1	23.48	21.74	23.15	23.01
C18:2 n-6	28.98 ^a	25.77 ^b	27.83	28.06
C 18:3 n-3	3.30 ^a	20.17 ^b	3.99 ^a	16.26 ^b
C 20:4 n-6	3.49	2.45	1.85	1.60
C 20:5 n-3	0.08 ^a	0.30 ^b	0.06 ^a	0.18 ^b
C 22:5 n-3	0.57 ^a	1.37 ^b	0.33 ^a	0.59 ^b
Σ SFA	33.64 ^a	24.86 ^b	37.97 ^a	26.37 ^b
Σ MUFA	27.83 ^a	23.77 ^b	26.82	25.85
Σ PUFA	38.53 ^a	51.37 ^b	35.21 ^a	47.78 ^b
n-6/n-3 PUFA	8.22 ^a	1.31 ^b	6.82 ^a	1.81 ^b

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; a, b values with different superscript are significantly different ($P < 0.05$)

The addition of PUFA in the diet increase the lipid oxidation, the result was increased MDA concentration in the leg muscles (Table 2). The MDA concentration in leg muscles differed among the first and second experiment, but the enlargement between the groups inside the experiment was similar, around 3 times. The level of MDA in plasma also increased in the first experiment, but not in the second. The fact is that first experiment lasted 52 days and it is possible that the store of antioxidants run out and the animals were not able anymore to protect the body. The second experiment lasted only 22 days, before that the animals received the commercial diet with normal addition of antioxidants (vitamin C, vitamin E, Se), which were higher than in experimental diets. It is possible that the store of antioxidants lasted far enough, that the lipid oxidation in plasma was protected. Those results were some different from the results of the studies done on pigs (Rezar *et al.*, 2003) and chickens (Eder *et al.*, 2005; Voljč *et al.*, 2011), where higher consumption of PUFA cause lipid oxidation in plasma and tissues. Another possible explanation of the lower and equal level of plasma MDA could be the accumulation of MDA in the tissues, since the level in hind leg musculature MDA (also liver MDA 0.47 and 1.09 nmol/g in first experiment and 1.05 and 2.20 nmol/g in second experiment, with

higher value after the addition of linseed oil) was higher in second experiment as compared to the first one.

4 CONCLUSIONS

The linseed oil in both experiments caused a lower content of total SFA and a higher content of PUFA and also reduce n-6/n-3 PUFA ration in meat and adipose tissues. The content of MUFA decreased in the linseed diet of first experiment, compared to control, as direct result of the content in the diet. So the fatty acid composition of meat and adipose tissues depends on the fatty acid composition of the consumed diet. However, the higher content of PUFA in the tissues leads to a higher susceptibility to a lipid oxidation, which might reduce the shelf-life of meat and meat products, nevertheless the negative effect on animals wasn't detected.

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Table 3: Fatty acid composition of adipose tissue (% of the total fatty acids)

	1. experiment		2. experiment	
	Control diet (4)	Linseed diet (8)	Palm fat diet (11)	Linseed oil diet (12)
C12:0	0.14 ^a	0.11 ^b	0.19	0.15
C14:0	1.87 ^a	0.92 ^b	1.75 ^a	1.24 ^b
C16:0	21.96 ^a	12.31 ^b	24.81 ^a	15.86 ^b
C18:0	5.43 ^a	4.79 ^b	14.06 ^a	5.43 ^b
Σ C18:1	24.98 ^a	23.23 ^b	23.66 ^a	24.71 ^b
C18:2 n-6	34.47 ^a	27.41 ^b	25.72 ^a	27.23 ^b
C 18:3 n-3	4.72 ^a	28.02 ^b	4.21 ^a	20.31 ^b
C 20:4 n-6	0.15 ^a	0.09 ^b	0.14	0.14
C 20:5 n-3	0.01 ^a	0.03 ^b	0.001 ^a	0.03 ^b
C 22:5 n-3	0.05 ^a	0.11 ^b	0.06 ^a	0.11 ^b
Σ SFA	31.35 ^a	19.34 ^b	42.72 ^a	24.41 ^b
Σ MUFA	28.65 ^a	24.72 ^b	26.58	27.13
Σ PUFA	40.00 ^a	55.93 ^b	30.70 ^a	48.47 ^b
n-6/n-3 PUFA	7.31 ^a	0.98 ^b	6.02 ^a	1.40 ^b

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; a, b values with different superscript are significantly different ($P < 0.05$)

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