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FATTY ACID COMPOSITION AND OXIDATIVE STABILITY OF MUSCLES IN LAMBS FED COCONUT OIL SUPPLEMENTED DIET

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Abstract

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A study with 10 male lambs of Bulgarian dairy synthetic population was carried out to investigate the effect of dietary coconut oil supplementation on the lipid components, fatty acid composition and oxidative stability during storage in *m. Longissimus dorsi* and *m. Semimembranosus*. Dietary coconut oil supplementation did not induce significant changes in the amount of lipid components in both muscles. Triacylglycerols of *m. Longissimus dorsi* and *m. Semimembranosus* in the lambs from the group fed coconut oil supplemented diet had significantly higher content of C14:0 and C16:0 ($P<0.01$; $P<0.05$) and lower amount of C17:0 ($P<0.01$). The differences in the phospholipid fatty acid composition of the studied muscles were due to both diet and muscle type. Coconut oil supplementation increased significantly the amount of C16:1 ($P<0.05$) and lowered that of C17:0 and C22:5 ($P<0.05$; $P<0.01$). The content of C20:4 ($P<0.05$) and the total amount of PUFA was higher ($P<0.05$) in *m. Semimebranosus* in the control and coconut oil supplemented group of lambs, compared to *m. Longissimus dorsi*. The oil oxidative stability of both *m. Longissimus dorsi* and *m. Semimebranosus* was influenced by the time of storage whereas no significant effect of the dietary coconut oil supplementation was observed.

Key words: fatty acids, lambs, muscles, coconut oil, oxidation

Introduction

Saturated fatty acids are usually connected with increased risk of development of cardiovascular diseases. Since meat is considered to be the major source of saturated fatty acids in the human diet attempts are being made to alter the fatty acid composition of animals for meat producing towards more unsaturated fatty acids. This could be achieved by modifications of the animal diet and it is easier in monogastric animals. In ruminants it is known that considerable amount of the unsaturated fatty acids coming from the diet are hydrogenated by the rumen microorganisms and turned into saturated.

Although saturated fatty acids are generally considered unhealthy some of them have positive influence, such as lauric acid (C12:0), which possesses antibacterial (Hinton et al., 2006; Nakatsuji, 2009) antioxidative and antiviral (Hornung et al., 1994) properties.

Relatively limited research has been done to study the role of specific saturated fatty acids in the lipid metabolism of ruminants (Bozzolo et al.,

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1993; Castro et al., 2005). Hence it is interesting to examine whether feeding diets high in saturated fatty acids will i[1]ncrease significantly their deposition in muscles and influence their oxidation.

The objective of this study was to investigate the effect of coconut oil (rich in lauric acid) added to feed on the fatty acid composition and oxidative stability in two muscles in lambs.

Material and Methods

The study was carried out with 10 male lambs of Bulgarian dairy synthetic population, divided in 2 groups (5 animals each). The initial live weight of the animals was 18.04±2.6 kg and 18.12±2.74 kg, respectively for the control and experimental groups. The lambs from the two groups were fed concentrate diet (20 % corn, 30% barley, 50 % soy meal, vitamin premix 0.0013g per animal) and hay. For a period of 3 months each animal of the experimental group additionally received coconut oil in amount 20 g per day. Diet and coconut oil fatty acid composition are presented in Table 1.

The animals were slaughtered at mean live weight 37.07±4.62 kg for the control and 37.02±4.35kg for the experimental group. The carcasses were divided in two halves and 24 *h post mortem m. Longissimus dorsi* and *m. Semimembranosus* were carefully dissected form each left side.

Samples for analysis of lipid composition and fatty acids were taken from both muscles. Total lipids were extracted from the muscles according to the method of Bligh and Dyer (1959). Aliquots of the lipid extracts were subjected to phospholipid content assay, using the method of Bartlett (1959) and determination of the cholesterol content (Sperry and Webb, 1954).

Fatty acid analysis was done in both triacylglycerols and phospholipids after their isolation by preparative thin layer chromatography and methylation as described by Christie (1973). Fatty acid composition was determined using Carlo Erba gas chromatograph with a capillary column. Fatty acids methyl esters were identified comparing the retention times of the standards. Fatty acids were expressed as percentages of the total fatty acid methyl esters (Christie, 1973).

Table 1
Fatty acid composition of diet and coconut oil

Fatty acids, %	Coconut oil	Concentrate	Hay
C10:0	7.59	0.49	2.60
C12:0	50.65	0.13	1.65
C14:0	17.9	0.58	4.18
C15:0			2.71
C16:0	10.47	15.59	26.24
C16:1		0.28	1.32
C17:0		0.16	0.99
C18:0	3.59	3.55	10.40
C18:1	8.33	22.4	23.97
C18:2	1.47	54.8	11.91
C18:3		2.01	14.04

Lipid oxidation was studied in both *m. Longissimus dorsi* and *m. Semimembranosus*. The samples of the two muscles were wrapped in oxygen permeable foil and stored at 4ºC. Oxidative stability of the samples was measured after storage of 24h, 48h, 4 and 6 days. The results were expressed as mg malondialdehyde/ kg meat or TBARS units.

Two ways ANOVA was applied to determine the influence of treatment and muscle type in the current study. Statistical evaluation was performed using JMP v.7 (2007).

Results and Discussion

Lipid components and fatty acid composition

Lipid composition of the two studied muscles is presented in Table 2. The total lipid content varied between 1.5-1.8 % and is relatively low when compared to other studies on the influence of saturated fatty acids in lamb diets (Solomon et al., 1992; Bhatt et al., 2011). Total lipid content

Table 2

Lipid components in *m. Longissimus dorsi* and *m. Semimembranosus* in lambs

Lipid components, mg/100g	Muscles				Significance			
	m. Longissimus dorsi		*m. Semimembranosus*		Diet	Muscle	Inter action	S.E.
	Diet							
	C[a]	CO[b]	C	CO				
Total lipids	1764.82	1510.20	1657.44	1849.98	NS	NS	NS	539.23
Triacylglycerols	1363.49	1096.65	1218.18	1416.07	NS	NS	NS	492.84
Phospholipids	351.46	355.24	386.49	387.34	NS	NS	NS	79.57
Total cholesterol	49.87	58.31	52.77	46.56	NS	NS	NS	14.82

[a]Control group

[b] Coconut suppelemented diet

NS-non significant

was lower in *m. Longissimus dorsi* and higher in *m. Semimembranosus* in the lambs fed coconut oil supplemented diet but the differences between groups were insignificant. Triacylglycerol content followed the same tendencies as total lipids in both muscles and together with phospholipids was not significantly influenced by the inclusion of the coconut oil in the diet of the lambs.

The widely accepted recommendation is to limit the level of cholesterol in the human diet not to exceed 300 mg/d (Regulation 23/2005). Cholesterol content of lamb meat is consistent with this recommendation and its values are comparable with those of fish, beef and poultry. Feeding coconut supplemented diet did not result in increased cholesterol content in both muscles in this study.

Coconut oil supplementation led to significant increase in the amount of myristic (C14:0) ($P<0.01$) and palmitic (C16:0) ($P<0.05$) fatty acids in the triacylglycerols of the studied muscles (Table 3). Lauric acid, though abundant in the coconut oil was not significantly changed. However its amount into both muscles of the animals from the experimental group was increased by 32 % in the triacylglycerol fraction. In the literature accessible there are limited researches on the effect of coconut oil on the fatty acid composition of muscles in ruminants. However our results are in accordance with those of Castro et al. (2005) and Manso et al. (2009) who studied the influence of palm oil, rich in stearic acid on the intramuscular fatty acid composition and did not find significant increase of this fatty acid in the muscle lipids. On the other hand Solomon et al., 1992 found significant increase of stearic acid in the muscle lipids in lambs fed palm oil supplemented diet. The lack of significant increase in the levels of the lauric acid in the lambs fed coconut oil could suggest that it passes through the rumen of the animals relatively unaltered and is deposited in the muscle tissue.

The three fatty acids C12:0; C14:0 and C16:0 are known to be hyperlipidemic and increase the levels of serum cholesterol (Temme et al., 1997; Grundi, 2006). On the other hand, the increase of the amounts in C14:0 and C16:0 may indicate elongation of the lauric acid (Rioux et al., 2003).

In both muscles in the lambs fed coconut oil supplemented diet the contents of margaric acid (C17:0) was decreased significantly ($P<0.01$), which was observed also by Solomon et al, 1992 when feeding lambs with diet high in saturated fatty acids.

Table 3

Fatty acid composition of triacylglycerols in *m. Longissimus dorsi* and *m. Semimembranosus* in lambs

Fatty acids, %	Muscles				Significance			
	m. Longissimus dorsi		*m. Semimembranosus*		Diet	Muscle	Inter action	S.E.
	Diet							
	C[a]	CO[b]	C	CO				
C10:0	0.42	0.37	0.36	0.32	NS	NS	NS	0.1
C12:0	0.55	0.89	0.76	1.04	NS	NS	NS	0.48
C14:0	4.75	6.03	4.21	6.56	**	NS	NS	1.21
C15:0	0.47	0.43	0.47	0.51	NS	NS	NS	0.09
C16:0	27.68	29.20	26.40	29.33	*	NS	NS	1.99
C16:1	1.80	2.28	2.03	2.42	NS	NS	NS	0.55
C17:0	1.07	0.94	1.09	0.96	**	NS	NS	0.09
C18:0	16.99	15.55	16.25	14.07	NS	NS	NS	2.15
C18:1	41.79	40.30	43.88	40.54	NS	NS	NS	2.93
C18:2	3.50	3.13	3.58	3.19	NS	NS	NS	0.48
C18:3	0.37	0.29	0.35	0.35	NS	NS	NS	0.05
CLA[c]	0.61	0.57	0.61	0.71	NS	NS	NS	0.15
PUFA[d]	4.48	3.99	4.54	4.25	NS	NS	NS	0.54
MUFA[e]	43.58	42.58	45.91	42.96	NS	NS	NS	2.7
PUFA/SFA	0.09	0.08	0.09	0.08	NS	NS	NS	0.01
SFA[f]	51.94	53.43	49.54	52.79	NS	NS	NS	2.79
UFA[g]	48.06	46.57	50.46	47.21	NS	NS	NS	2.79

[a]Control group

[b] Coconut oil suppelemented diet

[c]Conjugated linoleic acids

[d]Total polyunsaturated fatty acids

[e]Total monounsaturated fatty acids

[f]Total saturated fatty acids

[g] Total unsaturated fatty acids

* P<0.05; ** P<0.01, NS-non significant

No differences in the fatty acid composition of the triacylglycerol fraction due to the muscle type were observed.

The changes in the content of some individual fatty acids in muscle phospholipids could be attributed to coconut oil presence in the diet as well as differences in the type of the studied muscles (table 4). There was significant increase (P<0.05) in the content of palmytoleic acid (C16:1) and decrease (P<0.05; P<0.01) of the amount of mar-

Table 4

Fatty acid composition of phospholipids in *m. Longissimus dorsi* and *m. Semimembranosus* in lambs

Fatty acids, %	Muscles				Significance			
	m. Longissimus dorsi		*m. Semimembranosus*		Diet	Muscle	Inter action	S.E.
	Diet							
	C[a]	CO[b]	C	CO				
C14:0	0.43	0.35	0.25	0.40	NS	NS	NS	0.18
C16:0	21.67	22.15	19.70	21.16	NS	NS	NS	1.39
C16:1	0.93	1.17	0.83	1.03	*	NS	NS	0.16
C17:0	2.21	1.94	3.15	2.10	*	NS	NS	0.54
C18:0	17.31	18.38	17.58	18.15	NS	NS	NS	1.12
C18:1	27.23	27.02	24.03	25.64	NS	NS	NS	2.53
C18:2	18.18	17.89	20.20	18.51	NS	NS	NS	1.76
C18:3	0.56	0.56	0.61	0.55	NS	NS	NS	0.11
C20:2	0.92	0.98	1.01	1.01	NS	NS	NS	0.17
C20:3	0.75	0.77	0.70	0.86	NS	NS	NS	0.25
C20:4	8.73	7.82	10.50	9.45	NS	*	NS	1.01
C20:5	0.23	0.29	0.35	0.40	NS	NS	NS	0.11
C22:5	0.86	0.69	1.08	0.74	**	NS	NS	0.14
PUFA[c]	30.22	28.99	34.45	31.52	NS	*	NS	2.39
MUFA[d]	28.16	28.19	24.86	26.68	NS	NS	NS	2.65
PUFA/SFA	0.73	0.68	0.85	0.75	*	**	NS	0.06
SFA[e]	41.61	42.83	40.69	41.81	NS	NS	NS	1.37
UFA[f]	58.39	57.17	59.31	58.19	NS	NS	NS	1.37
n-6/n-3	18.09	18.18	15.92	17.88	NS	NS	NS	3.04

[a]Control group

[b] Coconut oil suppelemented diet

[c]Total polyunsaturated fatty acids

[d]Total monounsaturated fatty acids

[e]Total saturated fatty acids

[f]Total unsaturated fatty acids

* P<0.05; ** P<0.01, NS-non significant

garic (C17:0) and docosapentenoic (C22:5) acids in response to coconut oil supplementation. No significant difference in the total amount of saturated and polyunsaturated fatty acids was found in response to coconut oil supplementation. In an experiment with high content of saturated fatty acids in the diet of lambs Manso et al. (2007) did not observe significant difference in the total amount of saturated, mono- and polyunsaturated fatty acids in muscle fatty acid composition.

In our study the animals fed coconut oil supplemented diet showed significantly lower ($P<0.05$) values of the ratio between the polyunsaturated and saturated fatty acids.

The content of arahidonic acid (C20:4) was significantly higher in *m .Semimebranosus* compared to *m. Longissimus dorsi* and this corresponded to the significantly higher total amount of polyunsaturated fatty acids and the values of PUFA/SFA ratio. The higher total content of polyunsaturated fatty acids could be due to the metabolic differences between the two muscles.

As a whole the observed changes in the fatty acid composition, especially the increase of the amount of the hiperlipidemic fatty acids in *m. Longissimus dorsi* and *m. Semimembranosus* indicate certain negative effect of the coconut oil supplementation (in amounts of 20 g per animal per day) for the meat consumers. However more experiments with different doses and durations are needed to fully understand its influence especially in concerns to human healthy diet.

Oxidative stability of muscle lipids

The formation and content of specific compounds, mainly aldehydes such as malondialdehyde and hexanal is employed to measure the degree of oxidation in meat (McKee, 2006). In this study the oxidative stability of the muscles during storage was evaluated by determination of the content of malondialdehyde (MDA).

Oxidative stability in both *m. Longissimus dorsi* and *m. Semimembranosus* was not influenced by the dietary coconut oil supplementation. This corresponded to the changes in the fatty acid composition in response to the diet and the increase of the saturated fatty acids. They are more stable to oxidation than unsaturated and hence the lack of significant difference in the oxidative stability between the groups.

In the course of storage significant differences were observed among the intervals of oxidation measurement in the two groups of lambs in both muscles.

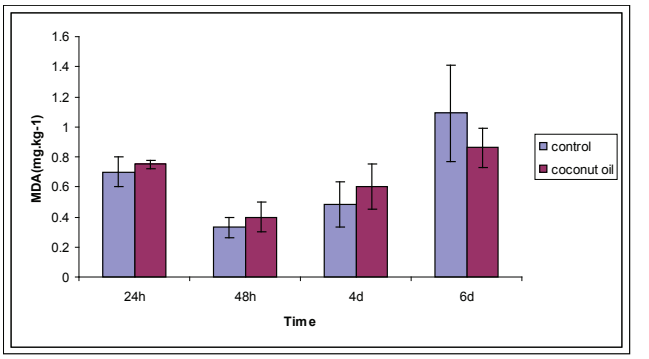

Fig. 1. Oxidative stability in *m.Longissimus dorsi* in lambs during storage at 4°C

After 24 hours of storage the amount of malondialdehyde in *m. Longissimus dorsi* (Figure 1) of the lambs from the control group decreased until the 6th day as the difference was significant between 24th and the 48th hour ($P<0.01$). Similar decrease in the content of malondialdehyde during storage was also observed in other experiments (Karwowska et al., 2008; Wattanachant et al., 2008). Malondialdehyde is a secondary product of oxidation, but according Melton (1983) TBARS values do not necessarily increase during storage. The observed decrease of the malondialdehyde content was probably due to reactions with proteins (Wattanachant et al., 2008).

The values of TBARS were highest on the 6th day of storage and we observed significant difference between this and the rest three points of measurement ($P<0.01$; $P<0.001$).

The changes in the oxidative stability in *m. Longissumis dorsi* of the animals fed coconut

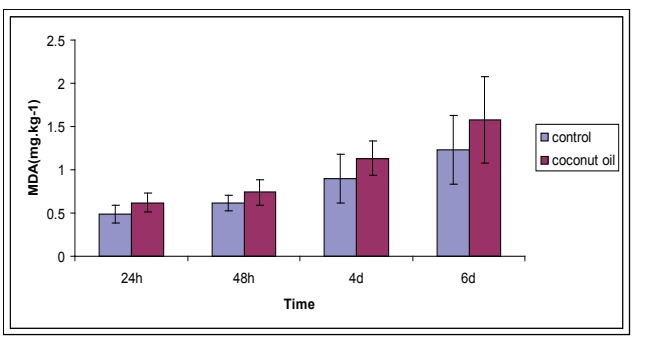

Fig. 2. Oxidative stability in *m.Semimembranosus* in lambs during storage at 4°C

supplemented diet followed the same trend as those in the control group. Significant differences were observed between the 24th and 48th hour of storage ($P<0.001$) as well as 48th h and 4th day ($P<0.05$), 48th h and 6th day ($P<0.001$) and 4th and 6th day ($P<0.01$).

The dynamic of oxidation in *m.Semimbranosus* was different than that observed in *m. Longissimus dorsi* (Figure 2). In the course of storage the amounts of TBARS gradually increased. The lowest values were observed on the 24th hour and the highest on the 6th day in both control and experimental group. In the muscles of lambs fed the control diet significant differences were observed between 24th h and 4th day ($P<0.05$) as well as 24th h and 6th and 48th with 6th day of storage ($P<0.001$). The experimental group showed significant differences ($P<0.05$; $P<0.001$) between all the intervals except between 24th and 48th hours of storage.

Malondialdehyde leads to off flavor when its amount in food products exceeds certain levels. For meat this critical value is 3mg.kg^{-1} (Wong et al., 1995). In the two muscles of both control and coconut oil supplemented group the content of malondialdehyde was lower than 3 mg.kg^{-1}

Conclusions

Coconut oil supplementation did not influence significantly the amount of lipid components in *m. Longissimus dorsi* and *m. Semimembranosus.*

Triacylglycerol fraction of both muscles in the lambs fed coconut supplemented diet had significantly higher content of C14:0 and C16:0 and lower amount of C17:0.

The differences observed in the fatty acid composition of the phospholipids of the studied muscles were due to both diet and muscle type. Coconut oil supplementation led to higher amounts of C16:1 and lower of C17:0 and C22:5.

The content of C20:4 and the total amount of PUFA were higher in *m. Semimembranosus*.

The observed changes in the fatty acid composition of the intramuscular lipids in lambs in response to coconut oil supplementation indicate certain negative effect of the latter in concerns to consumers' health.

The oxidative stability of both *m. Longissimus dorsi* and *m.Semimebranosus* was influenced by the time of storage whereas no significant differences due to dietary coconut oil supplementation were observed.

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