

INFLUENCE OF BOTANICAL DIVERSITY IN THE NATURAL AND CULTIVATED PASTURES ON THE LIPID CONTENT AND FATTY ACID COMPOSITION OF GRASS IN THE REGION OF THE MIDDLE RHODOPES

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Abstract

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The aim of the study concerns an estimation of the lipids and their fatty acid composition of the grass associations occupying natural and cultivated pastures at 1200m altitude. Plant samples are taken from the region of the Middle Rhodopes from natural meadows containing mainly *Nardus stricta* and cultivated meadows predominantly with *Festuca fallax*, *Lolium perenne* L. and *Poa pratensis* during the period from 15 May to 15 July. The established differences in the molar concentration of SFA, MUFA and PUFA, as well as the changed proportions of the main fatty acids (palmitic, oleic, linoleic and linolenic) are supposed to have influence on the milk fat in the ruminant animals. The content of SFA increases from May to July and reaches from 39.90mol% to 45.31mol% in the natural and from 31.19mol% to 40.86mol% in the cultivated grass. It is mostly due to the molar concentration of the 16:0 and 22:0 ($p < 0.05$) which varies between 21.91mol% - 27.57 mol% and 0.88 mol% - 1.47 mol%, respectively in the cultivated grasses. The study shows that the total content of MUFA is increased ($p < 0.01$) during the experiment from 8.60mol% to 14.08 mol% in the cultivated meadows, while the differences are not significant in the grasses of the natural meadows. The concentrations of PUFA (60% - 45%) in the plant lipids at the examined altitude in the region of the Middle Rhodopes in May, June and July are connected with relatively higher levels of 18:3n-3 (24mol% - 42mol%) and 18:2 (13mol% - 21 mol%). It has to be noted that the high PUFA level during the whole vegetation period is favorable for providing an optimal supply of essential fatty acids to grazing animals, especially for productive ruminants, as well as for the increased synthesis and secretion of CLA in milk lipids, which prevent human beings from atherosclerosis, obesity, diabetes and other diseases and stimulate the immune system.

Key words: pasture, season, altitude, fat, fatty acid composition

Abbreviations: SFA-saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; CLA - conjugated linoleic acid, FAME - fatty acid methyl esters; CH₃OH - methyl alcohol; CHCl₃ – chloroform

Introduction

The botanic composition of the pastures as well as the relative part of every vegetation occupying the regions at 1000m and 1200m altitude (Collomb et al., 2002; Bau-

man and Griinari, 2003), have given to significant extent an impact on the quality of the produced milk and milk products from the ruminants: cows, sheep, goats, buffalo and others. The composition of plant species also shows that the phenological phase of the grasses plays

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an essential role in the modulation of their nutritional properties and characteristics (Dewhurst et al., 2001; Oprea and Cardasol, 2002). The biochemical characteristics and content of the main nutrients of herbage offered to dairy ruminants influence the milk quality, in particular the fat and fatty acid composition (Bauman and Griinari, 2003). Besides their quantitative contribution to dietary energy, the different fatty acids: saturated, mono- and polyunsaturated are potentially defined as positive or negative (in some cases) factors for the consumer health (Parodi, 1999; Williams, 2000). Kelly et al. (1998) show that the levels of conjugated linolenic acid (C18:2 cis-9, trans-11, CLA) from milk of lactating dairy cows varied significantly from 3 to 25 mg/g lipid depending on the dietary fatty acids. Animal breed can influence CLA in milk fat, feeding dietary fats and oils, season, altitude of the pasture, botanical composition of grasslands (Collomb et al., 2002) and other factors. Conjugated linoleic acid is the major cis-, trans- isomer of the milk fat. This acid in milk and dairy products plays very important role as anticarcinogenic, antiatherogenic, immunomodulatory, growth promoting and lean body mass enhancing properties (MacDonald, 2000; Parodi, 1999). The green grasses from meadows (Griinari and Bouman, 1999) contain considerable proportions of α -linolenic and linoleic acid and these fatty acids should increase the endogenous synthesis of CLA in the mammary gland as well as in other tissue of the ruminants. Elgersma et al. (2003) have found a high proportion of 18:3n-3 in the fresh herbage and this lead to increase in the contents of rumenic acid, which is a main precursor of CLA.

The objective of the experiment is to investigate the total lipid content and fatty acid composition of grass associations in natural and cultivated pastures at 1200 m altitudes in a region of the Middle Rhodopes during the period from 15 May to 15 July.

Material and Methods

The vegetation materials are collected from experimental plots of a natural pasture (n-6) and of a cultivated pasture (n-6) at 1200 m altitude in the region of Middle Rhodopes during the periods 15 May, 15 June and 15 July. The botanical composition of fresh grass

samples from natural and cultivated pastures has been studied. The samples are finely cut (1-2cm) and are grinded in a mill equipped with chromed knives. Total lipids of plant samples are extracted according to the method of Bligh and Dyer (1959). The cold extraction is made with a mixture of CH_3OH and CHCl_3 (2:1 v/v). The organic solvents are evaporated in vacuum evaporator at 30°C and the lipids are dissolved in CHCl_3 and analyzed immediately. The fatty acid methyl esters (FAME) are prepared by the method of Lepage and Roy (1988) and separated by using a gas chromatography (Shimadzu GC, model 2010, Duisburg, Germany) equipped with a flame ionization detector, an automatic injection system (AOC-2010) and a SP-2560 capillary column (100mx0.25 mm i. d. with 0.20 μm film thickness; Sopolco Sigma-Aldrich Co., USA). The injector and the flame ionization detector are kept at constant temperature (T°) of 250°C and 260°C, respectively. The column oven T° is held at 165 °C for 2 min, increased at 8 °C/min to 235 °C and held until the analysis is finished. Hydrogen is used as the carrier and make up gas with a linear velocity set at 30 cm/s. For peak identification, a standard mix of 37 FAME (Supelco Inc., Bellefonte, PA) is used. The results are subjected to Student's test for determination of significance differences.

Results and Discussion

The botanic composition of herbage from the plots in natural and cultivated pastures at 1200m altitude is presented in the Table 1. The examined meadows in the region of the Middle Rhodopes from 15 May to 15 July occupy rich floristic diversity. The natural and cultivated pastures are composed of ten species and are dominated by the two families *Poaceae*, *Fabaceae* and others plant species. It must be noted that in natural grasslands the content of *Poaceae* (94.60%) is predominant, while in the cultivated meadows there is a growth of *Poaceae* (55.66%), *Fabaceae* (6.00%) and *Others plant species* (30.00%), mostly. The composition of two pastures consists as follows: permanent mainly of *Nardus stricta* and the grass mixtures of: *Festuca fallax*, *Lolium perenne*, *Poa pratensis*, *Nardus stricta*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Trifolium hybridum*, *Trifolium repens*, *Trifolium pratense*, *Bromus inermis* and others

Table 1
The botanical composition (as percent of total number of species) of natural and cultivated pastures in the region of the Middle Rhodopes

Botanical families	Cultivated pastures		Natural pastures	
Poaceae	Festuca fallax	29.63%	-	-
Poaceae	Poa pratensis	10.71%	-	-
Poaceae	Nardus stricta	1.39%	-	-
Poaceae	Anthoxanthum odoratum	1.05%	Nardus stricta	94.60%
Poaceae	Lolium perenne	15.32%	-	-
Poaceae	Dactylis glomerata	3.00%	-	-
Fabaceae	Trifolium hybridum	3.11%	-	-
Fabaceae	Trifolium repens	1.18%	-	-
Fabaceae	Trifolium pratense	1.71%	-	-
Other plant families	Bromus inermis	2.90%	-	-
	Other plant species	30.00%	Other plant species	5.40%

plant species. Michaylova et al. (2007) and Leiber et al. (2010) have found similar to these results in the region of the Eastern Rhodopes. Jeangros et al. (1999) found that the lowland meadows consist only some plant species and legumes, but their botanical diversity is less than that of the highland grasses.

The content of the total lipids in grass vegetation from the experimental plots during the grazing period (May, June and July) at the natural and cultivated pastures is shown in Figure 1. The values of the total lipids are lowest in May (0.96%) and significantly increase in June to July ($p < 0.01$) in the natural grasses, but their content insignificantly decrease in the cultivated pasture. Lieber et al. (2010) have founded a similar result

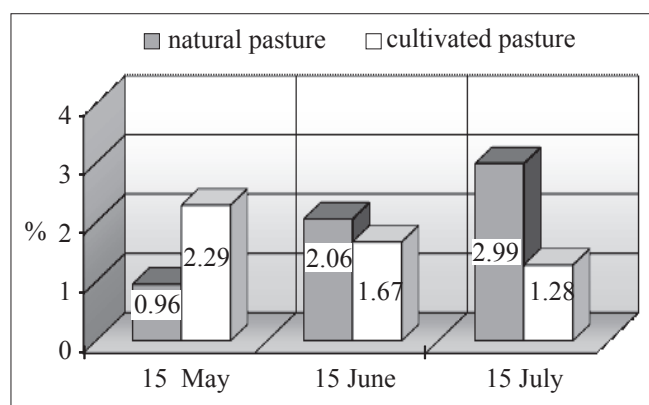


Fig. 1. Seasonal changes in the total lipid content in the grasses from natural and cultivated pastures in the region of the Middle Rhodopes

for the fat content, which continuously increased in the vegetations from April to June at 800m, 1000m and 1200m altitudes in the Eastern of Rhodopes.

Thus, a detailed examination on the fatty acid profile of grass lipids in two pastures depending on the botanical composition and season, is additionally required. There are very limited literatures (Oprea and Cardasol, 2002; Collomb et al., 2002; Elgesma et al., 2003) for fatty acid profile of grass from mountain regions. Changes in fatty acid composition at the end of vegetation period (May- July) at 1200m altitude, usually lead to decreasing of milk production of grazing animals, as well as, changing in fatty acid profile in milk lipids (Marques and Belo, 2005). The lipid composition of the grassland vegetations shows the presence of fatty acids with considerable number of carbon atoms, from 14 to 24, (Tables 2 and 3), including the main saturated mono-unsaturated and unsaturated acids. With the advance of the vegetation from May to July, an increase in the concentration of the saturated fatty acids (SFA) is found in a higher extent in cultivated meadows (31.19% to 40.86%) in comparison with natural meadows (39.90% to 45.31%). The tendency is in relation mainly with the increase in the molar concentration of the 16:0, 17:0 and 22:0 ($p < 0.05$) in the cultivated grasses, and to the lower extent with these of 14:0, 16:0, 17:0, 21:0 and 24:0 in the natural grasses.

The total amount of the monounsaturated fatty acids (MUFA) have not undergone substantial changes in

Table 2
Fatty acid composition (mol%) in a grass from natural pastures in the region of the Middle Rhodopes

Fatty acids	15 May	15 June	15 July
14:0	2.13 ± 0.14	2.33 ± 0.41	2.87 ± 0.33
15:0	0.42 ± 0.18	0.67 ± 0.53	0.42 ± 0.09
15:1	0.24 ± 0.11	0.28 ± 0.04	0.31 ± 0.09
16:0 DMA	1.03 ± 0.29	0.63 ± 0.29	0.81 ± 0.12
16:0	25.68 ± 3.29	26.71 ± 3.44	26.14 ± 2.79
16:1n-9	0.44 ± 0.21	0.38 ± 0.04	0.48 ± 0.08
16:1n-7	4.38 ± 0.68	4.19 ± 0.48	3.86 ± 0.36
17:0	0.30 ± 0.08	0.36 ± 0.03	0.41 ± 0.06
17:1	0.20 ± 0.04	0.27 ± 0.02	0.35 ± 0.08
18:0	1.98 ± 0.83	1.46 ± 0.20	1.56 ± 0.22
18:1n-7	3.02 ± 0.84	2.32 ± 0.23	4.36 ± 0.67c*
18:1n-9	0.36 ± 0.09	0.25 ± 0.01	0.48 ± 0.07
18:2	12.62 ± 2.52	13.74 ± 1.55	13.42 ± 1.00
18:3n-6	0.18 ± 0.06	0.14 ± 0.04	0.21 ± 0.01
18:3n-3	38.66 ± 4.26	36.09 ± 3.05	31.22 ± 5.65
20:0	3.44 ± 0.94	2.51 ± 0.66	2.48 ± 0.48
21:0	0.14 ± 0.03	0.31 ± 0.07	0.26 ± 0.12
22:0	0.93 ± 0.30	1.05 ± 0.55	0.93 ± 0.18
24:0	3.86 ± 1.32	6.31 ± 1.22	9.43 ± 1.81
SFA	39.90 ± 12.97	42.35 ± 8.92	45.31 ± 5.46
MUFA	8.65 ± 1.89	7.69 ± 0.71	9.84 ± 1.16
PUFA	51.46 ± 14.70	49.97 ± 9.63	44.85 ± 6.61

*p<0.05; **p<0.01; a –15 May/15 June; b –15 May/ July; c –15 June/15 July

Table 3
Fatty acid composition (mol%) in a grass from cultivated pastures in the region of the Middle Rhodopes

Fatty acids	15 May	15 June	15 July
14:0	1.20 ± 0.28	1.24 ± 0.27	1.91 ± 0.33
15:0	0.45 ± 0.24	0.48 ± 0.04	0.66 ± 0.15
15:1	0.20 ± 0.16	0.20 ± 0.42	0.22 ± 0.04
16:0 DMA	0.87 ± 0.08	0.96 ± 0.21	1.22 ± 0.43
16:0	21.91 ± 1.30	22.24 ± 0.68	27.57 ± 0.54 b*
16:1n-9	0.30 ± 0.04	0.38 ± 0.08	0.44 ± 0.04
16:1n-7	3.54 ± 0.36	3.37 ± 0.09	3.50 ± 0.13
17:0	0.24 ± 0.02	0.31 ± 0.05	0.50 ± 0.08 b*
17:1	0.48 ± 0.53	0.67 ± 0.45	0.26 ± 0.04 b***
18:0	1.89 ± 0.84	1.64 ± 0.39	2.91 ± 0.23
18:1n-7	3.69 ± 1.68	3.20 ± 0.26	8.75 ± 0.69
18:1n-9	0.39 ± 0.19	0.51 ± 0.06	0.92 ± 0.54
18:2	20.15 ± 3.19	17.19 ± 2.42	21.35 ± 2.56
18:3n-6	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.02
18:3n-3	39.99 ± 3.44	42.47 ± 1.02	23.56 ± 2.10b**
20:0	1.13 ± 0.81	1.06 ± 0.66	1.51 ± 0.53
21:0	0.13 ± 0.08	0.16 ± 0.02	0.14 ± 0.01
22:0	0.88 ± 0.09	0.90 ± 0.05	1.47 ± 0.16 b*
24:0	2.41 ± 0.98	2.86 ± 0.94	2.98 ± 0.95
SFA	31.19 ± 1.36	31.84 ± 3.53	40.86 ± 1.74
MUFA	8.60 ± 1.99	8.33 ± 0.56	14.08 ± 1.20 c**
PUFA	60.30 ± 1.85	59.81 ± 3.20	45.06 ± 0.56 b***c**

*p<0.05; **p<0.01; a – 5May /15June; b –15May/15July; c–15June/15July

June and July comparing to May. The content of the oleic acid (18:1n-7 isomer) is increased ($p < 0.05$) in natural flora, while in grass mixture they have appreciable changes at the end of the grazing period.

It can be noted (Tables 2 and 3) that the level of polyunsaturated fatty acids (PUFA) from 15 May to 15 July have taken considerable part of the total lipid content, in the permanent grasses (51.46%-44.85%) as well as in the cultivated pastures (60.30%-45.06%), distinctly expressed in May. Assessment of the seasonal dynamics of the fatty acid composition in the grasses of the two pastures show highest values for the 18:3n-3 and 18:2n-6. The differences in the natural pasture (predominant over *Nardus stricta*) are not statistically reliable, because of the significant individual varies between the samples. The investigations of longchain fatty acids show a decrease in the percent of the linolenic acid (18:3 ω -3) with 2.48mol% in June and 16.43mol% ($p < 0.01$) in July compared to May in the grass of cultivated meadows. The molar concentration of 18:2 ω -6 in the flora of the two estimated pastures is not altered considerably, but their level is relatively higher in the cultivated vs. the natural pastures and it may be connected with the botanical diversity in the grass mixture. There is a higher level of PUFA in cultivated grasses (60 mol%), but at the end of vegetation period the level is the same like in natural (mainly *Nardus stricta*, 45 mol%) pasture..

Investigations show that the changes in the molar concentrations of fatty acids from grass mixtures and permanent pastures at 1200 m altitude have a relation with the different stages in the growth of grass species from May to July in the region of the Middle of Rhodopes.

It has to be noted that the high PUFA levels during the whole vegetation are favorable for providing an optimal supply of essential fatty acids to grazing animals, especially for ruminants (Leiber et al., 2008), as well as for the increased synthesis and secretion of CLA in milk lipids. Cabiddu et al. (2005) have also studied the effect of botanical composition and the content of different legumes from the meadows, with particular attention on CLA and related metabolites on milk fatty acids.

Nevertheless, the grass associations with their floristic diversity at the two studied pastures gave a good possibility for syntheses and secretion of high levels of CLA. The established differences in the molar concentration

of SFA, MUFA and PUFA, as well as, the changed proportions of the main fatty acids are supposed to have their influence on the milk fatty acid profile. Collomb et al. (2002) correlated the botanical families and individual plant species with fatty acids in milk fat of sheep and cows grazing in the Alps.

The health aspects of grass vegetations that are due to the basic ω -3 fatty acid: α -linolenic acid has been studied (Razminowicz et al., 2004). This acid and longchain ω -3 fatty acids as well as the conjugated linoleic acids are desirable for human nutrition and health, and could be enhanced in the grazing ruminants.

Conclusion

The investigations have shown that the natural and cultivated meadows at 1200m altitude in the region of the Middle Rhodopes are occupied by rich floristic diversity. The highland grass is composed mainly of Poaceae, Fabaceae and other plant families. A much greater number of plant species than those one from the natural pasture characterizes the pastures from the cultivated grasses.

The total lipid content is low in herbage during the investigation periods. The concentration increases in natural grasses from May to June and July ($p < 0.01$), but in the cultivated pasture the levels insignificantly decrease.

The concentration of the SFA is higher in June and July compared to May in the two pastures and the tendency is mostly related to growth of the molar concentration of the 16:0, 17:0 and 22:0 ($p < 0.05$) in cultivated grasses.

The total amount of the monounsaturated fatty acids is lower ($p < 0.01$) in July compared to May in grass mixture.

The established high concentrations of PUFA – over 60 mol% in the cultivated and 51 mol% in the natural grasses from the starting and kept appreciable high during the grazing periods (45 mol%) have created favorable circumstances to the ruminant animals for syntheses and secretion of high levels of the conjugated fatty acids during the lactation period. These fatty acids have high biological activity and possess preventive and healthy effects on the human beings.

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