INFLUENCE OF THE SEASON ON THE TOTAL LIPIDS AND FATTY ACID COMPOSITION OF GRASSES AT THE DIFFERENT ALTITUDES IN THE REGION OF THE MIDDLE RHODOPES

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Abstract

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The investigation is related to a study of the lipid content and fatty acid profile of the grass associations containing mainly Gramineae species and Fabaceae species from the region of Middle Rhodopes. Plant samples are taken from the meadows at 800m, 1000m and 1200m altitudes and results show a low lipid content in the grasses during the spring period April - June.

The established differences in the concentrations of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids as well as the changed proportions of the main fatty acids (palmitic, oleic, linoleic and linolenic) are supposed to have influence on the milk fatty acid profile. The content of saturated fatty acids increases from April to June and reaches 22.5mol%, 27.5mol% and 26.3mol%, respectively at 800m, 1000m and 1200m, predominantly due to the molar concentration of the palmitic acid which varies between 16 and 20mol%, while the level of monounsaturated fatty acids doesn’t change during the experiments. The higher concentrations of polyunsaturated fatty acids (64-72 mol %) at three altitudes are due to 18:3 (48-61mol%; 48-56mol% and 50-57 mol %) and 18:2 (from 13 to 18 mol%). It has to be noted that the high polyunsaturated fatty acids levels during the whole vegetation period are favorable for providing an optimal supply of essential fatty acids to grazing animals as well as for the increased synthesis and secretion of conjugated linoleic acid in milk lipids, which prevent human beings from atherosclerosis, obesity, diabetes and stimulate the immune system.

Key words: grasses, fat, fatty acids, altitude, season
Abbreviations: SFA-saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; CLA - conjugated linoleic acid, FAME - fatty acid methyl esters

Introduction

The meadows from regions with different altitude – lowlands, mountains and high-lands are characterized with abundance of flora diversity. The botanic composition of the pastures as well as the relative part of every vegetation occupying the regions with 800m, 1000m and 1200m altitude (Michylova at., 2007; Bauman and Grinari, 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 pasture plays an essential role in the modulation of their nutritional properties and characteristics (Marques and Belo, 2005). The biochemical characteristics and content of the main nutrients of herbage offered to dairy ruminants influence the milk quality, in particular to the fat and fatty acid composition. Besides their quantitative contribution to dietary energy, the different fatty acids: short- and medium-chains, branched, cis- and trans- isomers, saturated, mono- and polyunsaturated are potentially defined as positive or negative (in some cases) factors for the consumer health (Williams, 2000).

Conjugated linoleic acid (C18:2 cis-9, trans-11, CLA) mainly is a precursor of the trans - vaccenic acid (C18:1 trans-11) in the rumen and a product of the “-9 desatur- ration of this fatty acid in the mammary gland. CLA from milk and milk products played very important role as anticarcinogenic, antiatherogenic, immunomodulatory and lean body mass enhancing properties (MacDonald, 2000). The green grasses from meadows (Elgersma et al., 2003; Michaylova et al., 2007) contain considerable proportions of 6-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.

The objective of the current experiments is to investigate the total lipids content and fatty acid composition of grass associations at different altitudes in the region of Middle Rhodopes during the period April - June.

Material and Methods

The vegetation materials are collected from 9 experimental plots at 800m, 1000m and 1200m altitude in the region of Middle Rhodopes during the period 30 April, 30 May and 30 June. The botanical composition of grass samples from pastures has been studied and total lipids are extracted according to the method of Bligh and Dyer (1959). The total lipid content is analyzed by gas chromatography using triarain (20:0) as an internal standard. The fatty acid methyl esters are prepared by the method of Lepage and Roy (1988) and separated by gas chromatography using a Pye Unicam 304 equipped with a Carbowax 20M capillary column (30m x 0.32mm i.d., 1 μm film thickness). The injector and the flame ionization detector are kept at constant temperature (T°) of 250 and 260°C, respectively. The 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. For peak identification, a standard mix of 37 FAME (Supelco Inc., Bellefonte, PA) is used. The carrier gas used is H₂. The results are subjected to Student’s test for determination of significance differences.

Results and Discussion

The botanic composition of grass associations of the plots at 1200m altitude (similar to the other plots at 800m and 1000m) is presented of Trifolium incarnatum (302 g.kg⁻¹); Lolium perenne L. (54.5 g.kg⁻¹); Bromus mollis L. (55.5 g.kg⁻¹); Festuca fallax Thuil. (6.0 g.kg⁻¹); Trifolium repens L. (32.6 g.kg⁻¹); Poa pratensis L. (97.3 g.kg⁻¹); Pteridium aquilinium (16.8 g.kg⁻¹); Vicia villosa Roth (22.8 g.kg⁻¹); Nardus stricta (131.8 g.kg⁻¹) and other sorts (280.5 g.kg⁻¹). The examined meadows at the different altitudes in the region of the Middle Rhodopes from 30 April to 30 June occupy rich floristic diversity. This could explain to some extent the level of protein, fiber and lipid supply in the grasses. Keeping the optimal ratio between these three nutrient components is the requirement for a high protein: energy value of the grass associations in the spring-summer season. The values of the pastures show almost the same percent for the Gramineae species (362.1 g.kg⁻¹) as well as for the Fabaceae species (357.5 g.kg⁻¹), followed by the various grasses (280.5 g.kg⁻¹). Michaylova at al. (2007) has found similar to these results in the region of Eastern Rhodopes. It must note that in lowland and mountain pastures the content of Gramineae species is higher, while this of Fabaceae species is relatively low from April to June in the examined re-
region. Jeangros et al., (1999) found that the lowland meadows are composed only of various grasses and legumes and their botanical diversity is less than that of the highland grass.

The content of the total lipids in grass vegetation from the plots during the grazing period (April, May and June) at the corresponding altitudes – 800, 1000, 1200m is shown in Figure 1.

The values of the total lipids are lowest in April at three altitudes, and slowly increase in June (from 17.7 to 29.3 g.kg⁻¹, respectively), but the differences are not significant. Thus, a detailed examination on the fatty acid profile of grass lipids, at different altitudes, depending on the season, is additionally required. Assessment of the seasonal dynamics and one of the fatty acid compositions of the lipid grasses at different altitudes – lowlands, mountains and highlands show highest values of the 18:3, 16:0 and 18:2 (Tables 1, 2 and 3).

The lipid composition shows the presence of fatty acids with considerable number of carbon atoms from 14 to 24, including the main saturated and unsaturated acids. With the advance of the vegetation from April to June an increase in the concentration of the SFA is found in a higher extent at 1000m and 1200m, respectively 27.5% and 26.3%. The tendency is in relation mainly with the increase in the molar concentration of the 16:0 (4%) and to the lower extent with these of 14:0, 16:0DMA, 18:0 and 22:0 in the grass vegetation of the pastures at 800m, 1000m and 1200m.
Table 2

Fatty acid composition (mol%) of grasses at 1000m altitude in the region of the Middle Rhodopes

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>30 April</th>
<th>30 May</th>
<th>30 June</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.57±0.06</td>
<td>0.67±0.05</td>
<td>1.03±0.12</td>
</tr>
<tr>
<td>15:0</td>
<td>0.34±0.02</td>
<td>0.34±0.03</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>16:0 DMA</td>
<td>0.41±0.03</td>
<td>0.91±0.06a*</td>
<td>0.72±0.03b**</td>
</tr>
<tr>
<td>16:0</td>
<td>18.26±0.28</td>
<td>20.52±0.43</td>
<td>20.07±0.25</td>
</tr>
<tr>
<td>16:1n-9</td>
<td>0.39±0.04</td>
<td>0.31±0.03</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.03±0.21</td>
<td>2.09±0.19</td>
<td>2.75±0.06</td>
</tr>
<tr>
<td>17:0</td>
<td>0.18±0.08</td>
<td>0.21±0.02</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>17:1</td>
<td>1.09±0.03</td>
<td>1.45±0.63</td>
<td>0.38±0.01b**</td>
</tr>
<tr>
<td>18:0</td>
<td>1.80±0.04</td>
<td>2.40±0.11a*</td>
<td>2.38±0.13</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>1.76±0.08</td>
<td>2.51±0.07a*</td>
<td>2.96±0.04b*</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>0.30±0.03</td>
<td>0.47±0.06</td>
<td>0.43±0.01</td>
</tr>
<tr>
<td>18:2</td>
<td>14.13±0.91</td>
<td>17.90±0.83</td>
<td>16.97±0.70</td>
</tr>
<tr>
<td>18:3</td>
<td>56.29±1.21</td>
<td>47.77±1.14a*</td>
<td>48.69±0.85b*</td>
</tr>
<tr>
<td>20:0</td>
<td>0.40±0.05</td>
<td>0.73±0.06</td>
<td>0.72±0.04b*</td>
</tr>
<tr>
<td>22:0</td>
<td>0.61±0.07</td>
<td>0.91±0.06</td>
<td>0.88±0.06</td>
</tr>
<tr>
<td>24:0</td>
<td>0.44±0.09</td>
<td>0.81±0.10</td>
<td>1.05±0.02b*</td>
</tr>
<tr>
<td>SFA</td>
<td>23.01</td>
<td>27.5</td>
<td>27.48</td>
</tr>
<tr>
<td>MUFA</td>
<td>6.57</td>
<td>6.83</td>
<td>6.86</td>
</tr>
<tr>
<td>PUFA</td>
<td>70.42</td>
<td>65.67</td>
<td>65.66</td>
</tr>
</tbody>
</table>

DMA - Dimethylacetal; *P<0.05; **P<0.01; ***P<0.001; a-April/May; b-April /June; c-May /June

Fig. 1. Seasonal dynamics in the lipid contents at the different altitudes in the region of the Middle Rhodopes
altitudes. At each altitude the total amount of the MUFA did not undergo substantial changes in May and June comparing to April. However, the researches have shown significant increase (P<0.01) of the content of the oleic acid (18:1n-7 and n-9 isomers) at 800m altitude, whilst at 1000m and 1200m (P<0.05) altitudes this is found only for 18:1n-7 isomers.

It can be noted (Table 1, 2 and 3) that the level of PUFA from April to May have taken considerable part (73.5%-64.4%) of the total lipid content in the grass at experimental plots and this is distinctly expressed in April. The examined long chain fatty acids show a decrease in the percent of the linolenic acid (18:3 n-3) from 7% to 13% (P<0.01) in May and from 4% to 7% (P<0.05) in June compared to April in the grass at each altitude. Parallel with this, the concentration of linoleic acid (18:2 n-6) at 800m (P<0.01) and 1000m altitude has increased with 2-3% in May and June, in comparison to April.

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 and 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 particularly attention on CLA and related metabolites on milk fatty acids.

Nevertheless, the grass associations at different studied altitudes gave a good possibility for syntheses and secretion of high levels of conjugated linoleic acids. The established differences in the molar concen-
tration 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 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 omega-3 fatty acid - α-linolenic acid have been studied (Razminowicz et al., 2004). This acid and longchain n-3 fatty acids as well as the CLA are desirable for human nutrition and health, and could be enhanced in the grazing ruminants.

Conclusions

The investigations have shown that the meadows at 800m, 1000m and 1200m altitude in the region of Middle Rhodopes occupied by rich floristic diversity. The highland grass is composed of Gramineae species, Fabaceae species and various grasses in relatively equal ratios. The lowland and mountain pastures are composed predominantly of Gramineae species. The total lipid content is relatively low in the pastures at different altitudes in the Middle Rhodopes during the spring. The concentration of the saturated fatty acic is higher in June compared to April mainly at 1000 and 1200m altitude and the tendency is related to the increase of the molar concentration of the palmitic acid and in low extent of 14:0, 16:0DMA, 18:0, 22:0 and 24:0. The total amount of the monounsaturated fatty acids has not undergone substantial changes in May and June compared to April. The established high concentrations of PUFA - above 65% in the lipids of lowland, mountain and highland plots in the region of Middle Rhodopes, have created favorable circumstance 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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References


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