The Content of Conjugated Linoleic Acid (CLA) Isomer Groups in Milk of two Polish Sheep Breeds Determined by Silver Ion Liquid Chromatography (Ag+-HPLC)

Agnieszka ROZBICKA-WIECZOREK, Aurelia RADZIK-RANT, Witold RANT, and Marian CZAUDERNA

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Sheep milk is rich in CLA isomers which are biologically active components influencing human health. There are four geometric CLA isomer pairs: cis,trans; trans,cis; trans,trans and cis,cis. The aim of the present study was the analysis of CLA isomer groups content by Ag+-HPLC in milk fat of Zelazneńska (ZS) and Wrozowska (WS) sheep breeds. The ewes of both breeds were kept under the same environmental and nutritional conditions. Milk samples were collected from 60-suckling ewes (30 from each breed), at the age of 3-4 years and in their 4th week of lactation. A higher total amount of all CLA isomer groups was obtained in milk of ZS ewes, however, this result was statistically insignificant. The percentage of the main 9,11 isomer in total CLA was higher in Wrozowska milk (68% vs. 74%). The content of the trans,trans isomer group in milk fat of the studied breeds was similar. The percentage of this group in total CLA in milk of WS and ZS constitutes 7.2% and 7.7%, respectively. The amount of cis,cis isomers in milk fat of ZS was higher than WS (P<0.01).

Key words: Sheep, milk, Ag+-HPLC, CLA isomers.

Among products of animal origin, sheep milk is rich in biologically active components positively affecting human health. Conjugated linoleic acid isomers undoubtedly belong to these components. The most recognizable include the isomer from cis,trans group 9,11 called rumenic acid (RA) and the 10,12 isomer from the trans,cis group. The 9,11 isomer comprises 75-90% of total CLA for which potential anticarcinogenic and antiatherogenic effects have been highlighted, whereas 10,12 CLA is a minor isomer and is probably responsible for effects on body composition, i.e. lowering of body weight and fat mass (COLLOMB et al. 2006; PARODI 2003). The other minor CLA isomers, of little or trace content, appear as geometric pairs cis,cis and trans,trans (PARK et al. 2007).

Most studies on ruminant milk fat quantified CLA isomers by gas chromatography (GC) and have shown that the GC peak can include more than one component and minor CLA isomers masked by the RA peak (LUNA et al. 2005). A more precise method, allowing quantification of isomers other than RA CLA or at least determining their group, is silver-ion high performance liquid chromatography (Ag+-HPLC). This method can provide separation of CLA not attainable by other means. Trans,trans isomers elute first, followed by cis,trans,cis and then cis,cis (DELMONTE et al. 2005).

It is not easy to obtain repeatable results using Ag+-HPLC. Potential sources of error include variation in silver loadings of the lipid columns, differences in instrument configuration, changes in elution volumes, solvent composition, and lack of internal standards as well as the number of analyzed samples (ADLOF 2003).
Attempts at determining CLA isomer content in milk of Polish sheep have been undertaken earlier, but in animals kept in different environmental and nutritional conditions, and using different analytical methods (BODKOWSKI et al. 2008; GABRYSZUK et al. 2007; RADZIK-RANT et al. 2010; SZUMACHER-STRABEL et al. 2008; SZUMACHER-STRABEL et al. 2011). The aim of the present study was to analyze CLA isomer group content including c9,t11 by Ag+HPLC in milk fat of the Żelazneńska strain of Polish Lowland Sheep and Wrzosówka breed, kept under the same environmental and nutritional conditions.

Material and Methods

The study was carried out according to the guidelines of the Third Local Ethics Committee in Warszawa (No. 54/2010 from the 14th July 2010).

Animals and treatment

Milk samples were collected from 60 suckling ewes of two breeds (30 ewes from each breed): Żelazneńska strain of Polish Lowland Sheep (ZS) and Wrzosówka sheep (WS), which were maintained in one flock belonging to the Experimental Farm of the Warsaw University of Life Sciences, SGGW. The diet of ewes of both breeds was based on local feeds: cereal meal, hay, straw and a mineral mixture. The chemical composition and nutritional value of fodder are presented in Table 1.

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Table 1

<table>
<thead>
<tr>
<th>Specification</th>
<th>Cereal meal</th>
<th>Hay</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>89.24</td>
<td>90.82</td>
<td>91.1</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>11.8</td>
<td>8.8</td>
<td>3.06</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>1.72</td>
<td>1.36</td>
<td>1.24</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>4.89</td>
<td>34.55</td>
<td>41.24</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.00</td>
<td>6.81</td>
<td>6.23</td>
</tr>
<tr>
<td>EN (MJ/kg s.m.)</td>
<td>7.54</td>
<td>4.27</td>
<td>–</td>
</tr>
<tr>
<td>UFL/1 kg s.m.</td>
<td>1.06</td>
<td>0.6</td>
<td>–</td>
</tr>
<tr>
<td>UFV/1 kg s.m.</td>
<td>1.02</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>PDI/1 kg s.m.</td>
<td>69</td>
<td>68</td>
<td>–</td>
</tr>
</tbody>
</table>

EN – net energy; UFL – unit energy for milk production; UFV – unit energy for meat production; PDI – protein digested in the small intestine.

All sampled sheep were at the age of 3-4 years and in the 4th week of lactation. The length of lactation as well as litter size for both breeds was similar.

The lambs were separated from their dams two hours before milk collection, then ewes were hand milked and 100 ml samples for chemical analysis and CLA isomer determination were taken.

Chemical analysis

Chemical composition of fodder was analyzed according to procedure AOAC (1990). Each milk sample was analyzed for fat, protein, lactose, total solids (TS) and solids non-fat (SNF) with IR spectrometry using Milkoscan FT-120 (Poland).

Milk fat was extracted according to the Röse-Gottlieb method (AOAC 1990). CLA isomers were determined using a liquid chromatograph (Waters 625LC), a photodiode detector (DAD: model 996, Waters) and professional software Millennium 32 (ver.4.00). The isomers were separated by two silver-ion columns (Chrom. Sphere, 5μm Lipids: 250x4.6 mm; Chrompack), secured by the protective column (10x3 mm). The mobile phase consisted of hexane (98.39% v/v); acetic acid (1.60% v/v) and acetonitrile (0.013% v/v), at a flow rate of 1ml/min; the diode array detector was adjusted to 234 nm. Calibration of the HPLC system was conducted using individual standards of CLA isomers c9,t11 and t10,c12 and a mixture of CLA isomers (Sigma, USA; Larodon Fine Chemicals AB, Sweden) (CZAUDERNA et al. 2003).

Statistical analysis

Statistical treatment of the data of chemical composition of milk and CLA isomer groups of fat milk was performed using the SPSS 14.0 software (2003) based on one-way ANOVA, for P≤0.05.

Results and Discussion

The milk of Wrzosówka ewes was characterized by higher fat, lactose and TS content, but lower protein and SNF content than milk of Żelazneńska sheep, although differences were statistically insignificant (Table 2). Similar protein (4.68%) and lactose (5.12%) content, but lower fat portion (5.84%) in milk of the Wrzosówka breed was observed in earlier studies by NOWAK and NIŻNIKOWSKI (1996). RADZIK-RANT (2005) also reported lower fat content in milk of the Żelazneńska strain at the peak of lactation compared to the present study (6.22% vs. 8.21%), whereas the protein and lactose portions were similar. A much higher fat content reaching 12.6% was noted by WOHLT et al. (1981) for the
The chemical composition of milk of the studied ewes

<table>
<thead>
<tr>
<th>Analyzed traits</th>
<th>Wrzosówka n=30</th>
<th>Żelazneńska n=30</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>LSM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>8.91</td>
<td>8.21</td>
<td>0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>4.97</td>
<td>5.13</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>5.21</td>
<td>5.15</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>TS (%)</td>
<td>20.04</td>
<td>19.51</td>
<td>0.24</td>
<td>0.28</td>
</tr>
<tr>
<td>SNF (%)</td>
<td>11.13</td>
<td>11.3</td>
<td>0.11</td>
<td>0.52</td>
</tr>
</tbody>
</table>

TS – total solids; SNF – solids not fat; P>0.05 – not significant effect.

The differences in total content of trans,trans isomers in milk fat of the studied breeds were not statistically confirmed (Table 3). The proportion of this isomer group in milk of WS and ZS constitutes 7.2% and 7.7%, respectively. The differences in total content of cis,cis isomers in milk fat of the studied breeds were not statistically confirmed (Table 3). The proportion of this isomer group in milk of WS and ZS constitutes 7.2% and 7.7%, respectively. Similar results were reported by RADZIK-RANT et al. (2010) in milk of Polish Merino and Pomorska sheep and by PARTIDARIO et al. (2008) in milk of Portuguese breeds. However, in sheep cheese, the sum of the trans,trans isomers contributed 5-9% of total CLA.

The sum of cis,cis isomers in milk fat of ZS was higher (P<0.01) than in milk of WS (Table 3).
The proportions reached 18% of total CLA for Želazneńska sheep, but attained 11.6% for the Wrzosówka breed. The area of the cis,cis isomer elution is presented on an exemplary chromatogram (Fig. 1). The presence of this isomer group in milk fat of other Polish sheep breeds was also recorded in earlier research by RADZIK-RANT et al. (2010). Cis,cis isomers were undetected in milk and cheese derived from Portuguese sheep, even if the same Ag+HPLC method was applied (PARTIDARIO et al. 2008). In the present study, the high content of this group could be the result of the presence of other acids, masking isomers from trans,cis/cis,trans groups, which should be revealed earlier on this section of chromatogram (Fig. 1).

In all groups of CLA isomers, including c9,t11 C18:2 were negatively, but not significantly, correlated with milk fat content (Table 4). These results are consistent with those reported by TSIPPLAKOU et al. (2006) for the main c9,t11 isomer in milk of grazed sheep and goats. Also NALECZ-TARWACKA et al. (2008) obtained a negative relationship between RA and fat content in cow milk. A completely contrary relation between fat amount and CLA isomers was noted by RADZIK-RANT et al. (2010) in an earlier study.

In conclusion, it is possible to ascertain that the study of groups of CLA isomer content by Ag+HPLC in milk of sheep breeds kept under the same environmental conditions has confirmed preliminary research for sheep kept in different conditions. For greater repeatability of results, further research on the determination of CLA isomers by the Ag+HPLC method are required.

### References


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