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# Effects on fatty acids profile of milk from transhumant small ruminants related to the floristic composition of mountainous rangelands

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**Abstract.** The available knowledge regarding the effects of grazed species composition on sheep and goat milk quality in Greece is limited. This study contributes to fill in this gap by presenting the results of a combined study of rangeland floristic composition and milk quality of transhumant flocks. The vegetation cover measured and the species composition and richness were calculated early in summer of 2013 and 2014. At the same period milk samples were taken from the refrigeration tanks where milk is collected before transportation to industries and dairies. Samples were analyzed for the profile of fatty acids, which were divided in six categories (SFA, MUFA, PUFA, CLA, omega-3 and omega-6). The results showed correlations between floristic composition and the concentration of specific fatty acids, some of which are potentially beneficiary for human health.

**Keywords.** Mountainous rangelands – Extensive production – Grazing – Milk quality.

## ***Effets sur le profil des acides gras du lait provenant de petits ruminants transhumants liés à la composition floristique des pâturages montagneuses: Résultats préliminaires de Grèce***

**Résumé.** Les effets de la composition des espèces de pâturages sur la qualité du lait de moutons et de chèvres en Grèce n'ont pas reçu attention. Cette étude présente les résultats d'une étude de la composition floristique des pâturages et de la qualité du lait des troupeaux transhumants. Des mesures de la couverture végétale et de la production fourragère ont été prises dans les premiers jours de pâturage pendant l'été de 2013 et 2014 et la composition et la richesse des espèces ont été calculées. À la même période, des échantillons de lait ont été prélevés par les réservoirs de réfrigération du lait et ont été analysés pour le profil des acides gras, qui ont été divisés en six catégories (SFA, MUFA, PUFA, CLA, oméga-3 et oméga-6). Les résultats ont révélé des relations entre la diversité floristique et la concentration d'acides gras spécifiques, dont certains sont potentiellement favorables à la santé.

**Mots-clés.** Pâturages montagneuses – Production extensive – Pâturage – Qualité de lait.

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## **I – Introduction**

Greece represents a Mediterranean setting of particular importance in terms of biodiversity. A significant part of the country's Usable Agricultural Areas is characterized as High Nature Value farmland (53%), especially in the mountainous areas of the country, which are the most abundant. Agropastoralism has played a crucial role in the formation and maintenance of unique landscapes with significant biodiversity in these areas, with transhumant sheep and goat flocks being the main grazers. These flocks remain in the highlands for at least four months (May/June – early/late October) and graze in rangelands with considerable ecological and floristic diversity. Recent experimental data have revealed that properly grazed areas are characterized by increased floristic diversity and moderate grazing provides important ecosystem services (Karatassiou *et al.* 2016a).

At the international level there is significant evidence that grazing is reflected in the production of milk of higher quality. Morand-Fehr *et al.* (2007) reported that pasture-based livestock produce milk rich in fat and in micro-components, which are beneficial to human health. Chilliard *et al.* (2003) reported increased Linoleic Conjugated Acid (LCA) concentration in cow milk when animals were fed off fresh grass. Galina *et al.* (2007) found that many of the properties of raw unpasteurized milk were passed to cheese produced in the artisanal way and pointed out differences between cheeses from milk from grazing and milk from indoors feeding. Economic and marketing literature shows that consumers are interested in the consumption of these products, because they require less forage and concentrates and increase animal welfare (Nicholas *et al.*, 2014), especially when they are produced from raw milk, which ameliorates their organoleptic characteristics (Colonna *et al.*, 2011).

In Greece, research has focused on milk quality of small ruminants fed indoors with various types of feedstuff, for instance by substituting soy meal by legumes (Manousidis *et al.*, 2015), or on the differences between various systems of ewe milk production (Tsiplakou *et al.*, 2010). Also there has been research on the uses of mountainous grasslands (Hadjigeorgiou *et al.*, 2003; Karatassiou *et al.* 2016b) and on their contribution to the economic performance of farms (Ragkos *et al.*, 2014), while Manousidis *et al.* (2016) examined the grazing behavior of goats in such settings. However, up to now the available knowledge regarding the effects of grazed species composition on milk quality is limited. This study contributes to fill in this gap by presenting preliminary results of a combined study of rangeland floristic composition and milk quality of transhumant flocks. In particular, the study focuses on grazing in semi-natural mountainous rangelands during summer.

## II – Material and methods

The study was undertaken in four different mountainous (summer) rangelands of Northern Greece. The common characteristic of the sampled farms was that they all spend the winter in the lowlands of Thessaly, Central Greece and move towards their summer domiciles in late May. The first area was Samarina-Dotsiko (Mountain Smolikas), which is situated in Grevena (North-Western Greece) and is one of the most important grazing areas for transhumant flocks during summer (52.183 sheep and goats, 128 flocks). The second area is Grammatiko (Mountain Vermio), where actually only 22 flocks rearing 9.161 sheep and goats graze, but used to be an important summer rangeland for transhumant flocks until the 1970s. The third area, Chaliki (Mountain Lakmos), is a small community between Thessaly and Macedonia; the village has a longstanding transhumance tradition and actually 19 farms rearing 9.058 small ruminants, mainly sheep, graze there during summer. The fourth area is Pyrra in Central Greece (Pindos), where the transhumant population has declined considerably (less than 1.900 animals spend the summer there).

In these four areas, plots of 9 - 16 m<sup>2</sup> were fenced in selected rangelands in order to be protected from grazing. Measurements of the vegetation cover measured and the species composition and richness were calculated in early summer of 2013 and 2014 (Magurran, 2004). At the same period milk samples were taken from the refrigeration tanks where milk is collected before transportation to industries and dairies. Samples were taken in 2013 and 2014 from 23 farms on average 2-3 weeks after the flocks started grazing in summer rangelands. Collected samples were analyzed for the profile of fatty acids, which were divided in six categories: Saturated Fatty Acids (SFA, C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0); Monosaturated Fatty Acids (MUFA, C14:1, C16:1, C18:1, C20:1); Polysaturated Fatty Acids (PUFA, C18:2, C18:3, C20:4, EPA, DHA), Conjugated Linoleic Acid (CLA),  $\omega$ 3 Fatty Acids (C18:3, EPA, DHA);  $\omega$ 6 Fatty Acids (C18:2, C20:4). The fatty acid methyl esters were prepared by trans-esterification with potassium hydroxide according to ISO5509:2000L. The prepared fatty acid methyl esters were analyzed using a HP 5890 (Hewlett –Packard) gas chromatograph equipped with flame ionisation detector and a DB-23 (60 m×0.25 mm × 0.25  $\mu$ m) column (J & W Scientific, Inc., Folsom, California, USA). A 37 component mixture (Supelco, Bellefonte, Pennsylvania, USA) of fatty acids methyl esters (FAME)

was used as a reference standard. Fatty acids methyl esters were identified by comparing their retention times with the FAME mixture. The results are expressed as percent (%) of the total fatty acids present in the sample.

### III – Results and discussion

Table 1 reports the combined results of the species composition and milk quality experiments. The profile of the four areas differs in terms of floristic composition. Rangelands in Grevena exhibited the highest percentage of grasses, followed by other forbs (46% and 38,6% respectively). In Chaliki, no significant differences were found compared to Grevena, except the fact that legumes coverage was 4,5% more. However, different profiles were found in Vermio and Pyrra. In the former area, shrubs covered a significant part (14,4%), other other forbs were the most abundant type of vegetation (43,2%), while grasses were considerably less than in the two previous areas (30,5%). In Pyrra, on the other hand, grasses were the most scarce type of vegetation (18,4%), while legumes accounted for the highest acreage compared to the three other areas (29,4%), and forbs were, for once more, the most abundant (52,1%). The highest species richness was found in Pyrra (22,5 species on average) and Chaliki (20,6 species) followed by Grevena (18,1) and Vermio (16,6). Shrublands exhibited the lowest diversity (1-2 species) and other forbs the highest.

**Table 1. Species composition (%), number of species and milk quality components (fatty acids) in the four study areas**

	Grevena		Vermio		Chaliki		Pyrra	
	%	No. of species	%	No. of species	%	No. of species	%	No. of species
<i>Functional groups</i>								
Grasses	46,0	6,8	30,5	5,9	44,3	6,0	18,4	5,3
Legumes	14,6	2,9	11,8	2,6	19,1	4,4	29,4	5,7
Other forbs	38,6	8,1	43,2	7,6	36,7	10,1	52,1	11,3
Shrubs	0,8	0,3	14,4	0,5	0,0	0,0	0,0	0,0
Total	100,0	18,1	100,0	16,6	100,0	20,6	100,0	22,5
<i>Fatty acids</i>								
CLA	1,85		1,04		1,95		1,16	
SFA	63,09		65,97		59,67		58,33	
MUFA	29,56		28,54		31,73		35,17	
PUFA	5,49		4,45		6,65		5,34	
Total	100,00		100,00		100,00		100,00	
ω3	1,81		1,45		2,71		1,40	
ω6	3,68		3,00		3,94		3,94	

In the second part of Table 1 the average of the two measurements of fatty acids in the milk samples is reported. The highest CLA concentration was found in Chaliki and Grevena (1,85% and 1,95% respectively), which shows that grasses consumption can be related to higher CLA, which may be beneficial for human health (Whale *et al.*, 2004). PUFA are generally higher in cases where animals graze in mountain pastures (Morand-Fehr *et al.*, 2006). In this experiment the milk samples in Chaliki were the richest in PUFA followed by the ones in Grevena – i.e. the two areas with highest contribution of grasses – while the lowest was detected for Vermio, where shrublands occupied an important percentage of floristic composition. On the other hand, MUFA were considerably higher in Pyrra, indicating correlations with diets rich in legumes and/or other forbs. SFA were the most abundant types of fatty acids in all cases. The lowest percentage was found in Pyrra and Chaliki, where legumes were more abundant.

The establishment of relationships between grazing material and the quality of raw products has important economic prospects, as mentioned above. Indeed, this would support the standardization of traditional dairy products with high added value, which would improve their competitiveness in markets. Under such a scenario, farmers would probably choose to graze their animals more in order to increase and maintain high quality, even if this would entail less milk yield. Then, integrated management plans would be necessary for mountainous rangelands in order to support more grazing animals without threatening their ecological quality and floristic diversity. Nevertheless, the most important issue behind the economic use of these results is the production of cheese from raw milk. This traditional technique generally allows for the organoleptic characteristics of raw milk to be transmitted to the manufactured product. However, for Greece this is actually under examination, as the legislative framework is very strict regarding cheese-making from unpasteurized milk.

## IV – Conclusions

The preliminary results indicate a significant relationship between floristic diversity and the concentration of specific fatty acids, some of which are potentially beneficiary for human health. Differences are found among areas grazed by transhumant flocks during summer. These findings should be carefully analyzed also taking into account the breed of the animals, as it has been reported that Greek autochthonous breeds perform better than imported ones in terms of milk quality, especially in their area of origin.

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