Alpine vegetation type affects composition of nutritionally important C18 fatty acids in tissues of lambs from different breeds

Willems H.[1], Kreuzer M.[1], Leiber F.[1,2*]

[1]ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland
[2]FiBL – Research Institute of Organic Agriculture, 5070 Frick, Switzerland
*E-mail: florian.leiber@fibl.org

Abstract

A total of 110 lambs of the breeds Engadine Sheep and Valaisian Black Nose Sheep were fattened on one lowland and three different alpine pasture types. The experiment was conducted in two consecutive years and lasted for 9 weeks of grazing in each year. Immediately afterwards, the lambs were slaughtered. Perirenal adipose tissue and the *Longissimus dorsi* muscle were analysed for fatty acid composition. The lambs on the lowland pasture had the lowest proportions of linoleic and α-linolenic acid in the lipid fraction of these tissues. Additionally, there was a clear differentiation in these proportions when lambs grazed different alpine vegetation types. This was related to the content of phenolic compounds rather than the fatty acid contents of the swards, and an increasing phenolic level probably resulted in a higher ruminal protection of the native plant fatty acids. Intermediates of biohydrogenation (vaccenic acid and conjugated linoleic acid) were highest in the lowland lambs. Animal breed effects were weak.

Introduction

The generally positive effect of grazing alpine pastures on the fat quality of ruminant-source foods is well established, especially for dairy foods (Leiber, 2011). There is evidence for an influence of plant secondary compounds from the alpine swards on ruminant lipid metabolism (Leiber, 2011), but there is only few data about actual phenolic concentrations in different vegetation types and the related fatty acid (FA) profiles in the foods produced (Falchero et al., 2010; Povolo et al., 2012). Further, there is only little knowledge about the lipid quality in lamb meat derived from mountain pastures (Ådnøy et al., 2005). Therefore, a study was designed to evaluate the FA composition in the lipids of adipose and functional tissues of lambs fattened on different lowland and alpine vegetation types. By fattening two different breeds on each pasture, it was also targeted to investigate the interaction of animal breed and vegetation type.

Animals, Pastures & Methods

In two summer seasons, 2010 and 2011, lambs of two indigenous Swiss mountain sheep breeds, Engadine Sheep (ES) and Valaisian Black Nose Sheep (VS), were fattened for respectively 9 weeks either on lowland vegetation or on one of three different alpine vegetation types (2000 m a.s.l.). In each year, 6-7 lambs per breed and pasture type were included in the experiment. At the start of the experimental periods the live weights of ES and VS were 32.7±3.5 kg and 30.8±2.9 kg (2010) and 35.1±4.3 kg and 37.5±5.0 kg (2011), respectively. The ages of ES and VS were 26±2, 18±7 (2010), and 26±2 and 27±3 weeks (2011), respectively.

The vegetation types were selected to clearly differ in forage quality and plant species composition. The lowland pasture ("lowland") contained 9 species (as found according to the method of Braun-Blanquet, 1964), while the alpine high quality pasture (nutrient-rich *Crepido-aurea Festucetum*; "alpine HQ") contained 30 species, the alpine moderate quality pasture (nutrient-poor *Crepido-aurea Festucetum*; "alpine MQ") contained 71 species and the alpine low quality pasture (*Geo montani-Nardetum*; "alpine LQ") contained 37 species (more details given in Willems et al., 2013). Neutral detergent fibre (NDF) was analysed according to Van Soest et al. (1991); phenolic compounds were determined as described by Jayanegara et al. (2010), and fatty acid proportions were measured gas chromatographically following the settings as presented by Leiber et al. (2011).

After 9 weeks, the lambs were slaughtered. Samples from the *Longissimus dorsi* muscle and the perirenal adipose tissue were taken from each animal. Lipids from these samples were extracted, transesterified and

analysed for fatty acid profiles by gas chromatography as described by Leiber et al., (2011). Data was subjected to analysis of variance considering pasture breed and year as fixed effects and animal as random effect using SAS (version 9.2, SAS Institute, Cary, NC, USA).

Results and Discussion

On the two high-quality pastures (lowland and alpine) the NDF contents were lower in 2011 than in 2010; on the other pastures the values did not differ between years. On average of both years, the neutral detergent fibre contents (NDF) were 51, 42, 44, and 67 g/100 g dry matter (DM) for lowland, alpine HQ, alpine MQ, and alpine LQ, respectively. The corresponding contents of total phenolic compounds (averages over both years) were 1.6, 2.9, 4.6, and 2.3 g/100 g DM, respectively. This means that the alpine MQ pasture had almost three times higher concentrations of phenolic compounds than the lowland pasture ($P<0.001$). This effect was also true for both individual years. Proportions (g/100 g total FA) of linoleic acid (LA; 18:2n-6;) and α-linolenic acid (ALA; 18:3n-3) in herbage were 16.9 and 49.7 for the lowland pasture, 16.2 and 48.6 for the alpine HQ, 16.1 and 38.3 for alpine MQ, and 14.0 and 32.2 for alpine LQ, respectively. Overall, proportions of these fatty acids were lower in the alpine pasture swards ($P<0.001$). For ALA, there was a year effect (2010 higher than 2011), but no interaction with pasture type; the pasture effect was the same in both years.

The proportions of LA and ALA in the perirenal adipose tissue of the lambs were the lowest when grazing in the lowlands (2.28 and 1.19 g/100 g FA), followed by alpine LQ (2.73 and 1.43), alpine HQ (2.97 and 1.84) and alpine MQ (3.53 and 2.23) (vegetation effect, $P<0.001$). There was no year effect for any of both FA. The proportion of conjugated linoleic acid (CLA; 18:2*c*9*t*11) was highest when lambs grazed in the lowlands (0.88 g/100 g total FA) compared to the alpine pastures (0.72, 0.69 and 0.66 in alpine HQ, MQ, and LQ, respectively; vegetation effect, $P<0.001$). in 2011, the CLA concentrations in adipose tissue were higher than in 2010 ($P<0.05$). Vaccenic acid (VA; 18:1*t*11) proportion in adipose tissue was highest in the lowland group ($P<0.001$); here, no year effect occurred.

In the intramuscular lipids, the proportions of LA and ALA were more than twice as high compared to the adipose tissue. Again, the lambs that grazed the alpine MQ pasture showed the highest proportions of these FA. In detail, the respective proportions (g/100 g FA) of LA and ALA were 8.69 and 3.44 in alpine MQ lambs and 5.71 and 2.18 in lowland lambs ($P<0.001$). The ALA and LA concentrations were intermediate for the lambs from the other two alpine pastures. In 2011, the concentrations of LA and ALA in the muscle were lower than in 2010 ($P<0.001$). Only the proportion of CLA (but not of VA) was increased in the muscle lipids under lowland grazing conditions compared to the alpine grazing treatments.

Only few effects of the sheep breed occurred, like a higher proportion of polyunsaturated FA concentration in the adipose tissue of VS compared to the ES ($P<0.05$).

The results show that increasing total phenol concentrations in the forage were associated with an increase in ALA and LA proportions in the body tissue lipids, regardless of the proportions of these FA in the forage lipids. The same was indicated by the year effect on ALA and LA proportions in the intramuscular fat, which corresponded to the year effect on phenolic compounds in the swards. The phenolic compounds may have reduced the first steps of ruminal biohydrogenation, resulting in enhanced carry-over of ALA and LA, depending on the botanical and chemical composition of the swards. Also in agreement with data from other *in vivo* and *in vitro* studies (e.g. Cabiddu et al., 2005, 2010; Leiber et al., 2005; Jayanegara et al., 2011), it becomes clear that phenolic compounds from herbs may act as efficient inhibitors of the first biohydrogenation step and that this influences the lipid composition of meat and milk, also in mountain pasture grazing systems.

By contrast, the biohydrogenation intermediates VA and CLA are more accumulated under lowland grazing conditions, indicating a different effect of the same phytochemicals on these FA. A general promoting effect of fresh pasture on these two FA has been repeatedly shown for cattle (Leiber et al., 2005; Khiaosa-ard et al., 2011), but the differentiated effects of varying pasture types are not the same as for LA and ALA.

Conclusion

In conclusion, the positive effect of alpine grazing on the lipid composition of ruminant-source foods has also been shown for lambs, and furthermore, a clear phenolic-compound linked differentiation between

contrasting alpine pasture types with respect to their influence on the FA profile in animal tissues was demonstrated.

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References

Ådnøy T., Haug A., Sørheim O., Thomassen M.S., Varszegi Z., and Eik, L.O. (2005). Grazing on mountain pastures – does it affect meat quality in lambs? *Livest. Prod. Sci*., 94, 25–31.

Braun-Blanquet, J. (1964). Pflanzensoziologie. Grundzüge der Vegetationskunde. 3rd ed., Springer, Vienna, New York.

Cabiddu A., Decandia M., Addis M., Piredda G., Pirisi A., and Molle G. (2005). Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. *Small Rum. Res.* 59, 169–180.

Cabiddu A., Salis L., Tweed J.K.S., Molle G., Decandia M., and Lee M.R.F. (2010). The influence of plant polyphenols on lipolysis and biohydrogenation in dried forages at different phenological stages: *in vitro* study. *J Sci. Food Agric.* 90, 829-835.

Falchero L., Lombardi G., Gorlier A., Lonati M., Odoardi M., and Cavallero, A. (2010). Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. *Dairy Sci. Technol.* 90, 657–672.

Jayanegara A., Kreuzer M., Wina E., and Leiber F. (2011). Significance of phenolic compounds in tropical forages for the rumen bypass of polyunsaturated fatty acids and the appearance of biohydrogenation intermediates as examined *in vitro*. *Anim. Prod. Sci*. 51, 1127–1136.

Khiaosa-ard R., Soliva C.R., Kreuzer M., and Leiber F. (2011). Influence of alpine forage either employed as donor cow's feed or as incubation substrate on *in vitro* ruminal fatty acid biohydrogenation. *Livest. Sci*. 140, 80-87.

Leiber F., Kreuzer M., Nigg D., Wettstein H-R., and Scheeder, M.R.L. (2005). A study on the causes for the elevated omega-3 fatty acids in cow's milk of alpine origin. *Lipids* 40, 191–202.

Leiber F. (2011). The added nutritional value of mountain dairy products. In: *Proceedings of the 10th International Meeting on Mountain Cheese*, Dronero, Italy. pp 43-46.

Leiber F., Hochstrasser R., Wettstein H.-R., and Kreuzer M. (2011). Feeding transition cows with oilseeds: Effects on fatty acid composition of adipose tissue, colostrum and milk. *Livest. Sci.* 138, 1–12.

Povolo M., Pelizzola V., Lombardi G., Tava A., and Contarini G. (2012). Hydrocarbon and fatty acid composition of cheese as affected by the pasture vegetation type. *J. Agric. Food Chem.* 60, 299–308.

Van Soest P.J., Robertson J.B., and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.

Willems H., Kreuzer M., and Leiber F. (2013). Effect of vegetation type for performance and meat quality of growing Engadine and Valasian Black Nose sheep grazing alpine pastures. *Livest. Sci.* 151, 80–91.