Effect of legumes rich in bioactive compounds on fatty acid biohydrogenation and sensory quality of lamb meat

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Introduction

Meat from sheep finished on pasture may have a pastoral off-flavour, which is not appreciated by a part of the consumers (Young et al., 1997). Further descriptors of this off-flavour are 'sheepy', 'milky', 'grassy' and even sometimes 'rancid' and 'faecal'. Faecal flavour is, like in pork, associated with the presence of the lipophilic substances skatole and indole, and these compounds have been strongly related to pastoral off-flavour in sheep meat (Young et al., 2003). Their occurrence is caused by rapid ruminal protein degradation mainly as a result of the high inherent ruminal protein degradability and the unfavourable protein-to-carbohydrate ratio in the grass. Plants containing secondary metabolites such as condensed tannins (CT) or enzymes like polyphenol oxidase (PPO) have been shown to slow down ruminal protein degradation. A possible mechanistic explanation for these findings is that hydroxyl and phenolic groups of CT and *o*-quinones formed from *o*-diphenols, a process catalysed by the PPO, are very reactive molecules. They can bind nucleophilic sites of proteins and glycerol-based lipids in the rumen, thereby protecting them from microbial degradation by impairing indirectly (inaccessibility of the protein molecule) or directly microbial activities (Barry and McNabb, 1999; Igarashi and Yasui, 1985). It has been shown that these compounds also may impair lipolysis and biohydrogenation of dietary polyunsaturated fatty acids (PUFA). For instance, Lee et al. (2009) showed a greater PUFA to saturated fatty acid (SFA) ratio and a lower *n-6* to *n-3* fatty acid ratio in the meat of steers fed red clover compared to grass silage. Furthermore, Khiaosaard et al. (2009) described that CT may partially inhibit one step of ruminal biohydrogenation of *n*-3 fatty acids, thereby promoting the formation of *trans*-11 C18- isomers (including conjugated linoleic acids (CLA)). Thus, because of their effect on proteolysis and lipolysis, CT and PPO from forage legumes could be beneficial by increasing PUFA and CLA content of sheep meat.

The goal of the present experiment was to evaluate the potential of silages of legumes containing CT or displaying PPO activity to reduce the pastoral off-flavour of sheep meat and monitor changes in the fatty acid profile of lamb meat.

Material and methods

The feeding experiment was conducted with 48 ram lambs. At 56 ± 13 d of age and 21 ± 4 kg body weight (BW) they were assigned to four dietary groups. Each group had *ad libitum* access to either lucerne (LU; control), red clover (RC; containing PPO), or two CT-containing plants like sainfoin (SF) or birdsfoot trefoil (BT). Except for a mineral-salt mix, no other feed was offered and silage consumption of the group was recorded daily. Silage samples as well as refusals were collected weekly, then pooled monthly to obtain a sample for the nutrient compositional analysis. Individual BW was recorded weekly. At an average age of 182 ± 16 d, the lambs were slaughtered at the Agroscope research abattoir by percussion stunning using a captive bolt pistol. After exsanguination and removal of the organs, perirenal fat and gastrointestinal tract, the hot carcass was weighed. Perirenal fat was vacuum packaged and stored at -20°C for the determination of skatole and indole contents using the HPLC method described by Hansen-Moller (1994). Temperature and pH of the *longissimus dorsi* muscle (LM) was determined at 1, 3 and 24 h post-mortem (p.m.) using a pH meter (WTW pH197-S, WTW, Weilheim, Germany) equipped with an Eb4 electrode (WTW, Weilheim, Germany). One day post-mortem, the entire LM was excised from both carcass sides. The LM from the left side was immediately vacuum packaged and stored at -20°C. Then, it was used to assess the intramuscular fat content by methanolic HCl extraction and its fatty acid profile by a transmethylation followed by an extraction and purification of the methyl esters before gas chromatographic analysis. The LM from the right carcass side was aged for 7 d at 4°C, then vacuum packaged and stored for 3 months at -20°C. This part was then used for sensory evaluation by 10 trained panelists. The panel had to evaluate on a 9 point scale following descriptors: 'livery', 'sheepy', 'grassy', 'milky', 'sweet' and 'sour'.

Data were analyzed sstatistically by One-Way ANOVA using the MIXED procedure of SAS (version 9.2). The model included the effect of the diet (LU, RC, SF, BT), and individual animals were considered as the experimental unit. Least squares means were compared using the PDIFF statement with the Tukey adjustment. Probability levels of $P < 0.05$ were considered significant. Regarding the sensory evaluation, non-parametric tests of Conover-Iman were performed on the mid-ranks using the R package (version 3.0.2).

Results

Nutrient composition of diet and refusals

The nutrient composition of the silages and the refusals are presented in Table 1. Dry matter (DM) and organic matter contents were 30% greater in LU and the SF had up to 30% lower crude protein content compared to the other silages, respectively. The greatest crude lipid level was determined in the RC silage. Despite these differences, the proportions of SFA, monounsaturated fatty acid (MUFA) and PUFA were similar between the four silages and amounted on average to 23, 6 and 58%, respectively. The refusals contained more crude fibre than the silages offered to the lambs. The CT content was 5-fold greater in the SF than in the BT silage whereas the CT content of the SF refusals was as low as that of the BT silage and refusals.

Table 1. Nutrient composition (± *SD*) of the lucerne (LU), red clover (RC), birdsfoot trefoil (BT) and sainfoin (SF) silages and of the refusals (g/kg DM) in the experiment (n=4)

	Silages				Silage refusals			
	LU	RC	BT	SF	LU	RC	BT	SF
Dry matter (g/kg of wet weight) in g/kg DM	480	346	350	367	572	388	405	463
Organic matter	395	252	252	290	516	297	306	400
Crude protein	195	190	192	138	140	147	176	87
Crude lipid	23.2	30.0	28.1	21.5	9.1	17.5	21.8	10.0
Crude fibre	332	267	311	326	487	340	352	461
Condensed tannins			21	104			18	21

Growth performance, carcass characteristics and meat quality

The DM intake was greater in the LU and RC compared to the SF and BT group (1030, 935, 810 and 620 g/d for LU, RC, SF and BT, respectively). Lambs fed SF and BT silages grew twofold slower ($P<0.05$) and reached a lower ($P<0.05$) slaughter weight, carcass weight and dressing percentage compared to LU and RC lambs. Carcasses were warmer ($P<0.05$) at 1 and 3 h, but not 24 h, in LU and RC compared with SF and BT lambs. The skatole concentration in the perirenal fat was twofold lower ($P<0.05$) in the SF than in the LU group, with intermediate values for RC and BT silages (Figure 1). This was not the case with indole, where the large standard error of the mean indicated that a great intra-group variability existed.

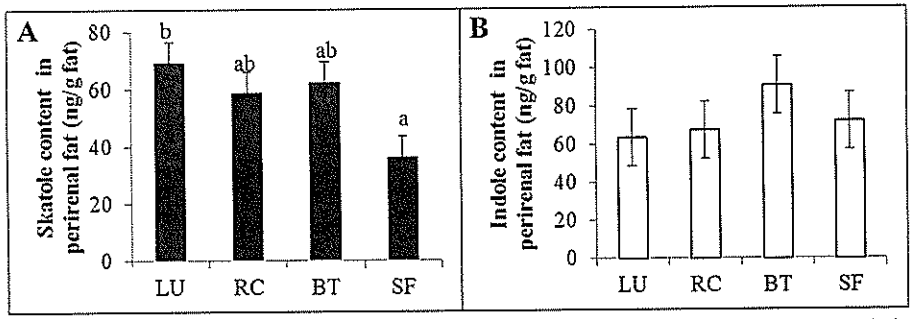

Figure 1. Skatole (A) and indole (B) levels in the perirenal fat of lambs fed lucerne (LU), red clover (RC), birdsfoot trefoil (BT) and sainfoin (SF) silages. [ab] Within a graph, means without a common superscript differ ($P < 0.05$).

The LM of RC lambs had a greater ($P<0.05$) intramuscular fat content than that of the BT and SF lambs, with intermediate values for the LU lambs. The intramuscular fat of the SF and BT lambs contained more ($P<0.05$) PUFA and less ($P<0.05$) SFA than that of the LU and RC lambs (Figure 2). These differences were mainly due to twofold greater levels (in percentage units; $P<0.05$) of arachidonic (AA), eicosapentaenoic (EPA) and docosapentaenoic acid (DPA). In addition, linoleic (LA), α-linolenic (ALA) and docosahexaenoic (DHA) acid proportions of the intramuscular fat were greater ($P<0.05$) in SF lambs than in the lambs of the three other experimental groups (Figure 3).

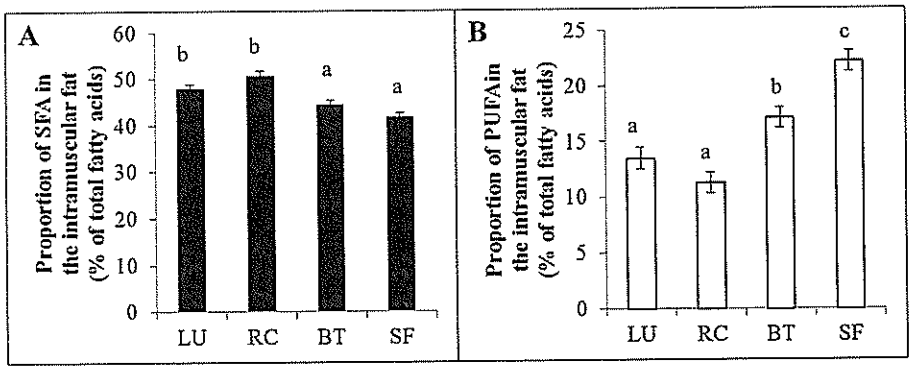

Figure 2. Proportions of saturated fatty acids (SFA) (A) and polyunsaturated fatty acids (PUFA) (B) in the intramuscular fat of lambs fed lucerne (LU), red clover (RC), birdsfoot trefoil (BT) and sainfoin (SF) silages. [abc] Within a graph means without a common superscript differ ($P < 0.05$).

Sensory assessment

In the cooked lamb, the intensity of the descriptors 'milky', 'sweet' and 'sour' was judged similar ($P>0.05$) for the four treatment groups. By contrast, the LM of SF lambs was less ($P<0.05$) frequently assessed 'livery' and 'sheepy' compared to BT and RC lambs, with intermediate values for the LM of LU lambs. However, the intensity of 'grassy' was stronger ($P<0.05$) in the LM of SF lambs compared to the LM of the three other treatment groups.

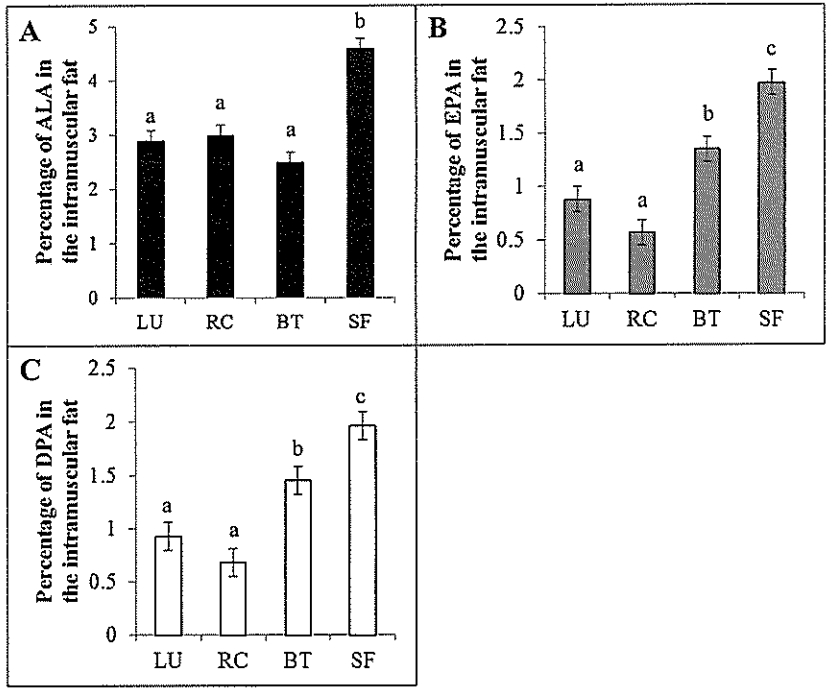

Figure 3. Percentage of α-linolenic acid (ALA) (A), eicosapentaenoic acid (EPA) (B) and docosapentaenoic acid (DPA) (C) in the intramuscular fat of lambs fed lucerne (LU), red clover (RC), birdsfoot trefoil (BT) and sainfoin (SF) silages. [abc] Within a graph means without a common superscript differ ($P < 0.05$).

Discussion

The observation that the lipids content was highest in the RC silage might be due to the activity of the PPO because, during ensiling, PPO protects lipids from plant and microbial lipases (Lee et al., 2009). The greater fibre content of the refusals compared to the silages suggests that the lambs ate mainly the leaves. The observation regarding selective eating of leaves, known to be rich in CT, is

supported by the fivefold lower CT content in the SF refusals compared to the SF silages. For BT, CT remained the same in the silage and in the refusals. The unexpectedly low CT content could also have resulted from the 40% weeds present in the BT sward. Overall, this suggests that BT lambs were not selecting leaves over stems.

The low growth rate of the SF and BT lambs was closely related to the low DM intake recorded for the group. Diets containing elevated CT levels can have a reduced feed palatability. In this respect, the lower appetence may be caused by the interaction between CT and salivary proteins, which creates an impression of astringency, thereby reducing voluntary intake (Lamy et al., 2011). Lambs fed LU and RC were heavier, which also may explain the higher carcass temperatures during the first three hours post mortem.

Previous studies in New Zealand have shown that BT can reduce the skatole and indole content in the body fat of lambs (Schreurs et al., 2007a). However, in the present study only SF was effective. The markedly lower CT content in BT compared to SF (21 vs. 104 g/kg DM) could be the reason for the lack of effect. Moreover, the decarboxylation of tryptophan in the rumen forms indole and indole acetic acid, which then is converted to skatole. *In vitro*, Tavendale et al. (2005) found that the inhibitory effect of CT was more targeted on the transformation of indole acetic acid to skatole than on the indole formation itself. This could explain that indole levels were similar between experimental groups. Despite the decrease in skatole content in the perirenal fat of the SF lambs, it was difficult to relate skatole levels to the descriptors typically used to characterise pastoral off-flavour in the present study. On one hand, feeding forage with elevated CT content was effective in reducing the 'livery' and 'sheepy' flavour intensity but on the other hand it also produced a more 'grassy' flavour. These findings suggested that the skatole and indole content in the intramuscular fat was not elevated enough to cause a detectable sensory differentiation in the meat. From boar taint research it is known, that skatole levels >200 ng/g adipose tissue cause off-flavour in pork from entire males (Pauly et al., 2008). In the current study, the skatole levels in the perirenal fat were at least threefold lower. Furthermore, the influence of other factors and molecules such as antioxidant content, branched-chain fatty acids, phenols and aldehydes cannot be excluded (Priolo et al., 2009; Resconi et al., 2010; Schreurs et al., 2007b). For instance, as shown by Young et al. (1997), branched-chain fatty acids are related to species-specific odour in sheep.

As Lee et al. (2009) previously demonstrated in steers, the ram lambs fed RC in the present study had the lowest *n-6* to *n-3* fatty acid ratio compared to the three other treatment groups. Western diets are deficient in *n-3* fatty acids. Thus, one incentive for targeted ruminant nutrition is to increase their relative amount and decrease the overall proportion of SFA. Because of positive effects on cardiovascular diseases, brain development, immunological and inflammatory reactions

(Mozaffarian and Wu, 2012), EPA, DPA and DHA are, within the *n-3* fatty acids, of great interest. In the present study, the greatest PUFA to SFA ratio was found for lambs receiving CT-rich silages, which concurs with results of Vasta et al. (2009). In the current study, EPA, DPA and DHA levels in the intramuscular fat seemed to be CT dose-dependent because their proportions were greater in the intramuscular fat of the SF than BT group. Apart from CT level, also chemical properties play a role for the efficiency of tannins to prevent ruminal biohydrogenation of unsaturated fatty acids. Examples are the procyanidin to prodelphinidin ratio and the mean degree of polymerisation (Patra and Saxena, 2011).

Conclusion

The present study demonstrated the potential of some ensiled legume species to modify the nutritional value and sensory properties of lamb meat. On the one hand, red clover silage seemed promising to decrease the *n-6* to *n-3* fatty acid ratio in the intramuscular fat. On the other hand, legumes rich in CT have a greater potential to elevate the level of long chain *n-3* fatty acids compared to RC and LU. Among the tannin-containing plants, SF seemed more efficient than BT to reduce ruminal biohydrogenation of dietary PUFA. Of special interest was the finding that SF reduced skatole content and the pastoral off-flavour to some extent. Further studies have to show, if the different efficacy of BT compared to SF was the result of the lower CT content or was plant species specific.

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