

The potential of condensed tannin-rich feedstuff to affect the nutritional and sensory qualities of ruminant-based products

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Abstract. Over the last decade, interest has increased when it comes to using temperate forage legumes containing condensed tannins (CT) for ruminants. The reason for this is that CT has been shown to benefit animal health and performance, as CT reduce parasitic burden by gastro-intestinal nematodes, prevent bloat and reduce urinary nitrogen losses. Less is known about their impact on the quality of ruminant-based products. This short review discusses various quality issues which could, based on the current knowledge on the mode of action of CT, be positively affected by forages that are rich in CT. The main focus is the fatty acid composition, as well as the sensory traits of the meat, milk and cheese. The results presented here show the potential for 2 tanniferous forage legumes to positively affect the deposition of polyunsaturated fatty acids (PUFA), especially of the *n*-3 fatty acids in meat, milk and cheese, and to improve the organoleptic quality of these products. From the 2 plants tested (birdsfoot trefoil and sainfoin), it is evident that the CT effect in the digestive tract depends on various factors like the CT level, the chemical composition of the CT and whether the CT in the plant are available in a soluble form or bound to proteins or carbohydrates of the plants.

1. Issues with ruminant-based products

Worldwide, ruminant-based products are already an important part of the human diet, and the rate of consumption is expected to continue to rise in the years to come. However, especially in developed countries, their consumption has stagnated or even decreased. This is surprising from a nutritional point of view, as red meat contains high biological value proteins and important micronutrients, such as vitamin B, iron (both free iron and haem iron) and zinc. Similarly, milk and dairy products are rich sources of protein, calcium and vitamins A and D. The responsibility for the stagnation or decline in consumption can be attributed to, among other things, the increasing skepticism towards modern livestock husbandry practices, which affects consumption habits, and the bad reputation of livestock products with respect to health. This public perception is partly based on undifferentiated interpretations of scientific reports. For instance, it has been established that processing meat through curing, smoking, or cooking can result in the formation of known or suspected carcinogens, including N-nitroso-compounds, polycyclic aromatic hydrocarbons and heterocyclic aromatic amines [1]. The Working Group of the International Agency for Research on Cancer found that existing epidemiologic data is sufficient to classify processed meat as carcinogenic [1]. However, the same authors state that there is limited evidence for the carcinogenicity of unprocessed red meat, which is in line with the outcome of the systematic review of experimental results performed by Turner et al. [2]. These authors found no evidence of a mechanistic link between the intake of red meat as part of a healthy dietary pattern and the risk of colorectal cancer. Similarly, ruminant fat is portrayed as a negative component of ruminant-

based products, largely because it is a rich source of cholesterol and saturated fatty acids (SFA). The rationale behind the negative perception is that compared to dietary monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), dietary cholesterol and SFA raise risk factors for cardiovascular disease, such as the total serum cholesterol and the ratio of low-density lipoprotein to high-density lipoprotein cholesterol [3]. However, Kratz et al. [4] concluded that no evidence has been observed which supports the hypothesis that dairy fat contributes to any cardiometabolic risk.

The odor and flavor of food are crucial traits that strongly impacts consumers' acceptance and willingness to purchase. These qualities are affected by an animal's diet, as the degradation and oxidation of feed components can lead to the formation and deposition of lipophilic odorant molecules in the milk, the adipose tissue, the inter- and intra-muscular fat. Consequently, the presence of off-odor and off-flavor is dependent on the fat content of the products, and can thus be a greater issue in high-fat products like butter, cream or full-fat cheese. The deviant flavor which consumers primarily perceive if they have become accustomed to the flavor of meat from grain-finished animals is the pastoral or dairy flavor. This flavor can be found in the dairy products or meat from pasture-grazing ruminants. Pastoral flavor summarizes attributes like *grassy*, *milky*, *animal-like*, *barnyard* and *fecal*, and have been related to the presence of skatole and indole [5, 6], both of which originate from the ruminal tryptophan catabolism, which produces indole and indole acetic acid.

2. Fate of dietary PUFA

Despite the fact that grass and hay, the typical dietary components of ruminant rations, contain high quantities of unsaturated fat (more than 70% PUFA, primarily of the *n*-3 family), fats of ruminant-based products are mainly saturated. The primary reason is that after lipolysis by plant and microbial lipases, up to 90% of free oleic (18:1*n*-9), linoleic (18:2*n*-6) and linolenic acids (18:3*n*-3) in the rumen undergo ruminal biohydrogenation [7], which results in the accumulation of stearic acids (18:0) and trans-fatty acids (e.g. vaccenic acid [*t*11-18:1]) [8] in the small intestine. After absorption, a part of these fatty acids are converted in the mammary gland, muscle and adipose tissue by Δ 9-desaturase to palmitoleic acid (16:1*n*-7), oleic acid or rumenic acid (*c*9,*t*11-18:2 [Conjugated Linoleic Acid], [8]). Since neither linoleic nor linolenic acids can be synthesized by mammals, linoleic and linolenic acids need to be ingested through the mammal's diet. Consequently, to naturally enrich ruminant-based products with PUFA, the extent of ruminal biohydrogenation needs to be reduced. To do this, approaches have been tested that have proven successful, such as coating the dietary fat source and/or using *n*-6 and/or *n*-3 PUFA rich ingredients [9]. These strategies targeted the dietary PUFA source whereas an alternative approach could be inhibiting the microbial population responsible for ruminal biohydrogenation (e.g. *Butyrivibrio* spp., *Propionibacterium acnes*, *Megasphaera elsdenii* [10]). The latter method would allow the exploitation of the natural PUFA sources, namely, grass and hay, without necessarily requesting additional sources of fat.

3. Bioactive compounds of plants

The aforementioned quality constraints of ruminant-based products requires remedying, and the bioactive compounds, also called secondary metabolites, found in forage legumes could provide such a solution. The secondary metabolites in plants are allelochemicals [11] and include compounds, which are not necessary for vital functions like plant reproduction or growth. There are 3 main families of bioactive compounds: *alkaloids*, *terpenoids* and *phenolic compounds*. Among the wide class of phenolic compounds rich in hydroxyl and in phenolic groups, condensed tannins (CT) are of particular interest for the quality of ruminant-based food.

3.1. Condensed tannins

Condensed tannins, also called proanthocyanidins, are oligomers (2 to 10 monomers) or polymers (> 10 monomers) of flavan-3-ol units; their name is based on how proanthocyanidins, when treated with acidic alcohol, will degrade to anthocyanidins, the pink-purple pigments responsible for the color of flowers. CT are not susceptible to anaerobic enzyme degradation [12], and it has been assumed until now that

their structure is only marginally altered in the digestive tract [13]. Based on the position of the –OH and –H groups, several flavan-3-ol units give rise to classes of polymers such as procyanidins, prodelphinidins, profisetinidins and prorobinetinidins [11]. When found in plants, CT can be present as a mixture of these classes. For instance, CT from quebracho (*Schinopsis lorentzii*) are polymers of profisetinidins and prorobinetinidins, while CT from temperate forage legumes are polymers of procyanidins and prodelphinidins. Furthermore, within species of forage legumes, procyanidins are found in greater abundance in birdsfoot trefoil, whereas prodelphinidins constitute the main polymer class in sainfoin [11].

3.2. Interaction of CT with other molecules

Owing to their hydroxyl and phenolic groups, CT can establish different types of interactions with proteins, lipids, carbohydrates and metal ions [14]. The interaction between CT and other molecules is based on weak linkages such as hydrogen bonding or hydrophobic interactions [15]. The weak linkages are reversible and can dissociate depending on physico-chemical conditions such as temperature and pH. In the context of this review, the ability of CT to precipitate protein and lipids under certain physico-chemical conditions is of interest. The most abundant water-soluble protein of fresh forages, dried herbages, silages or hay is RuBisCO (ribulose- 1,5-bisphosphate carboxylaseoxygenase). A pH between 3.5 and 7, like in the rumen, is generally favorable to generate the CT-RuBisCO complex, whereas at a lower, like in the abomasum, or higher pH, this complex becomes dissociated and RuBisCO is released [16, 17]. In addition, the affinity to form CT-protein complexes is affected by the size of the polymers and the procyanidin-to-prodelphinidin ratio. In general, as the polymer length increases and the concentration of prodelphinidin becomes greater, the protein precipitation properties of CT also increase (reviewed by Girard et al. [13]).

3.3. Biological properties and presumed mode of action of CT

The mechanism of CT action in the digestive tract has not been fully unraveled. In view of the previously described interaction of CT with other molecules, in ruminant nutrition the following mechanisms have been proposed:

- 1) Binding and dissociation, based on the surrounding pH milieu of proteins, carbohydrates and lipids, and by that protecting the substrate (e.g. nutrients from the diet) from microbial degradation in some parts of the digestive tract (e.g. in the rumen but not the small intestine).
- 2) CT change the conformation and the microbial enzyme activities and by chelation the availability of vital ions of rumen microorganisms.

These effects in the digestive tract could ultimately be relevant for the final quality of ruminant-based products.

4. From theory to practice — to what extent do CT affect ruminant-based products?

The following 2 chapters will summarize the results of experiments performed during the European-funded Marie Curie Initial Training Network project named LegumePlus.¹ The detailed information of the studies is available online [18, 19]. Two major CT-containing forage legumes were used in the studies: sainfoin and birdsfoot trefoil.

4.1. Effects of tanniferous forage legumes on quality traits of lamb meat

As previously mentioned, low PUFA levels and the pastoral flavor can be determinant traits for consumer acceptance of ruminant-based products. To assess the potential of CT to alter these traits, an experiment was performed with growing lambs. The animals weighing 21 kg were offered either birdsfoot trefoil, sainfoin or alfalfa silage *ad libitum* for, on average, 127 d. The sainfoin contained 5 times more CT than the birdsfoot trefoil (10 vs 21 g/kg dry matter). Average daily gain, hot carcass weight and dressing percentage of lambs fed birdsfoot trefoil and sainfoin were lower compared to lambs

¹ <http://legumeplus.eu/>

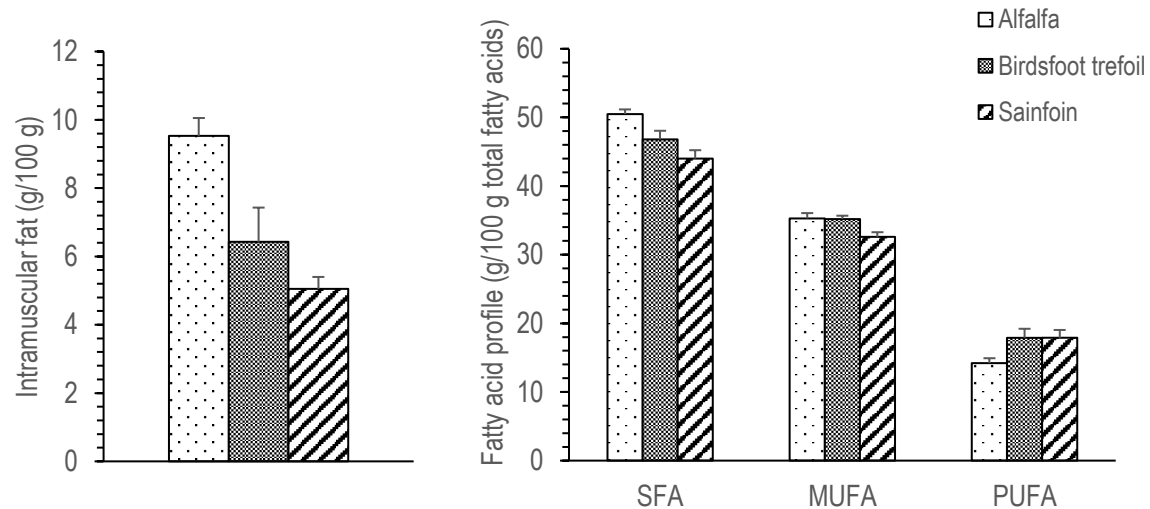


Figure 1: The intramuscular fat content and fatty acid profiles of the intramuscular fat of the longissimus thoracis muscle of lambs fed alfalfa, birdsfoot trefoil or sainfoin silages.

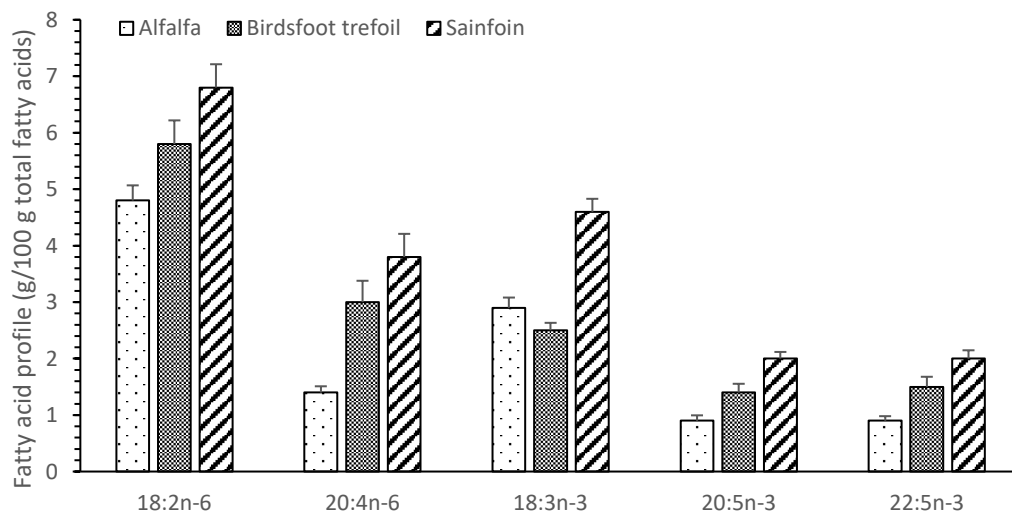


Figure 2: PUFA levels of the intramuscular fat of the longissimus thoracis muscle of lambs fed alfalfa, birdsfoot trefoil or sainfoin silages.

fed alfalfa (56 and 58 vs 102 g/d; 11.4 and 12.0 vs 15.6 kg; 39.9 and 41.1 vs 45.5%). The diets showed no effects with respect to meat quality traits such as water holding capacity, meat color and shear force. There are contradictory results regarding the impact of diets containing elevated CT levels on animal growth, feed intake and feed palatability [20, 21]. Lower palatability has been related to the interaction between CT and salivary proteins such as proline, which creates an impression of astringency, thereby reducing voluntary feed intake [22]. In addition, greater concentrations of CT impair the degradation of ruminal nutrients (desired in the case of the protein). This may explain why growth performance and consequently carcass weight of both, birdsfoot trefoil and sainfoin lambs, were inferior to alfalfa lambs.

In accordance with the lower growth rate, the intramuscular fat content was lower in lambs of the birdsfoot trefoil and sainfoin groups compared to the alfalfa group (Figure). The intramuscular fat of lambs fed birdsfoot trefoil and sainfoin contained less SFA and more PUFA than those fed alfalfa (Figure), resulting in a PUFA-to-SFA ratio of 0.38, 0.53 and 0.28, respectively. In addition, compared to alfalfa, the tanniferous plants increased the relative amounts of the long-chain fatty acid homologues of the *n*-3 and *n*-6 families by 117 and 196%, respectively (Figure 2). Interestingly, the level of long

chain PUFA, such as arachidonic acid (20:4 n -6), eicosapentaenoic acid (20:5 n -3) and docosapentaenoic acid (22:5 n -3), were up to 43% greater in the intramuscular fat of lambs fed sainfoin compared to those fed birdsfoot trefoil (Figure 2). This finding suggests that the relative levels of the elongation products of linoleic acid (18:3 n -3) in the intramuscular fat could be dose-dependent in terms of the CT intake (the CT level was greater in sainfoin than in birdsfoot trefoil and feed intake was similar) and/or chemical structure-dependent in terms of the CT molecules (sainfoin contains more prodelphinidins and fewer procyanidins compared to birdsfoot trefoil). As the linoleic acid intake per lamb was estimated to amount to only 3 g for lambs fed birdsfoot trefoil and sainfoin compared to 5 g for the lambs fed alfalfa, it can be assumed that the linoleic acid transfer rate was greater as a result of a reduced ruminal biohydrogenation rate in the lambs that were fed CT-rich legumes. The same is valid for linolenic acid.

The concentration of skatole and indole in the perirenal fat was determined using high-performance liquid chromatography [23]. The perirenal fat of the sainfoin lambs, but not of the birdsfoot trefoil lambs, contained almost double the skatole concentration than that of the lambs fed alfalfa (Figure 3). In contrast, neither the concentration of indole nor the sum of indolic compounds were affected by the dietary treatments. It is well established that decarboxylation of tryptophan in the rumen by *Lactobacillus* sp., *Clostridium scatologenes*, and *Clostridium drakei* [24] forms indole and indole acetic acid, which is further decarboxylated to indole-3-methyl (skatole) [25]. Indole and skatole pass the rumen wall, and, regulated by specific CYP450 isoenzymes, undergo hepatic clearance [26]. However, depending on the amount of skatole and indole produced in the rumen, degradation by specific cytochrome P450 isoenzymes can be incomplete, resulting in an accumulation of both substances in the adipose tissue. Studies performed *in vitro* found that the inhibitory effect of CT targeted the transformation of indole acetic acid to skatole more than it targeted the formation of indole itself [27], which is in line with our findings *in vivo*. The lack of birdsfoot trefoil's effect on skatole and indole was surprising, as studies in New Zealand have reported lower concentrations of skatole and indole in the body fat of lambs that graze birdsfoot trefoil swards [6]. As for the fatty acid metabolism, it appears that the quantity and/or the chemical structure of a plant's CT determines its effect on the production of skatole and indole.

To assess the impact of CT-containing legumes on the pastoral flavor of lamb meat, a sensory analysis was performed with a trained panel using known descriptors to judge the flavor of the lamb meat. The intensity of the descriptors *grassy*, *milky*, *sweet* and *sour* for the cooked lamb meat was judged to be similar among the 3 treatment groups. Of all 3 treatment groups, meat from the lambs of the sainfoin group had the weakest 'sheepy' odor and flavor. These findings are in line with the low skatole level, since skatole is believed to play a key role in the development of pastoral flavor [5].

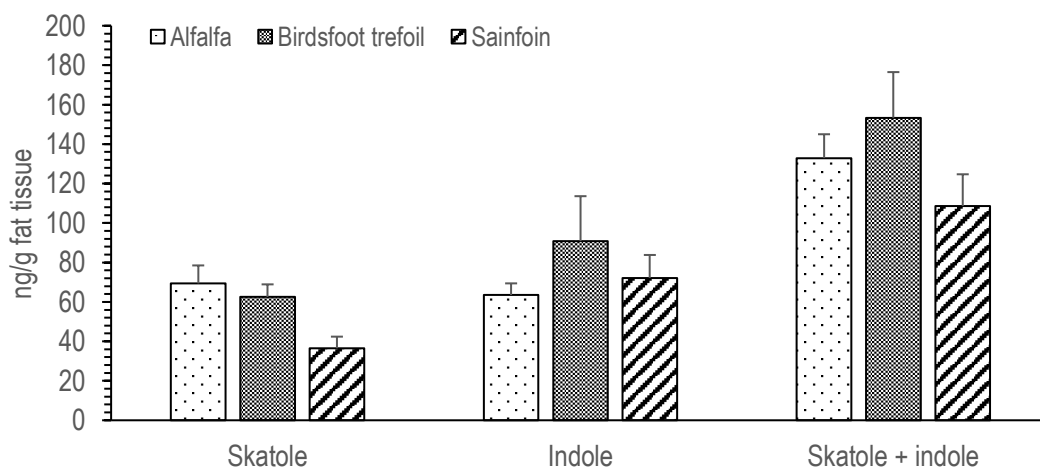


Figure 3: Skatole and indole levels determined in perirenal fat of lambs fed alfalfa, birdsfoot trefoil or sainfoin silages.

In conclusion, the present data has demonstrated the potential of ensiled legume species with elevated CT contents to modify the nutritional value and sensory properties of lamb meat. Feeding lambs solely these legumes increased the relative content of long chain *n*-3 fatty acids, but the growth performance and intramuscular fat deposition rate were impaired. Among the CT plants tested, sainfoin was more efficient than birdsfoot trefoil at reducing the ruminal biohydrogenation of dietary PUFA, reducing skatole concentrations, and, to some extent, reducing the pastoral off-flavor.

4.2. Effects of tanniferous forage legumes on the quality of milk and Gruyère-type cheese

As previously reported, due to ruminal processes, the PUFA level in milk and dairy products does not reflect the dietary PUFA intake. As proven in the lamb study, PUFA biohydrogenation is limited by CT, resulting in elevated levels of long-chain PUFA. Therefore, the feeding experiment lasting 52 d was conducted with 18 Holstein cows to determine whether diets containing CT from birdsfoot trefoil and sainfoin increase the PUFA, especially the *n*-3 fatty acid content, in the milk and cheese, without negatively affecting their physico-chemical and sensorial properties. Cows were assigned to the 3 treatment groups. The basal diet was composed of hay, corn silage, Extrulin (a rich source of linolenic

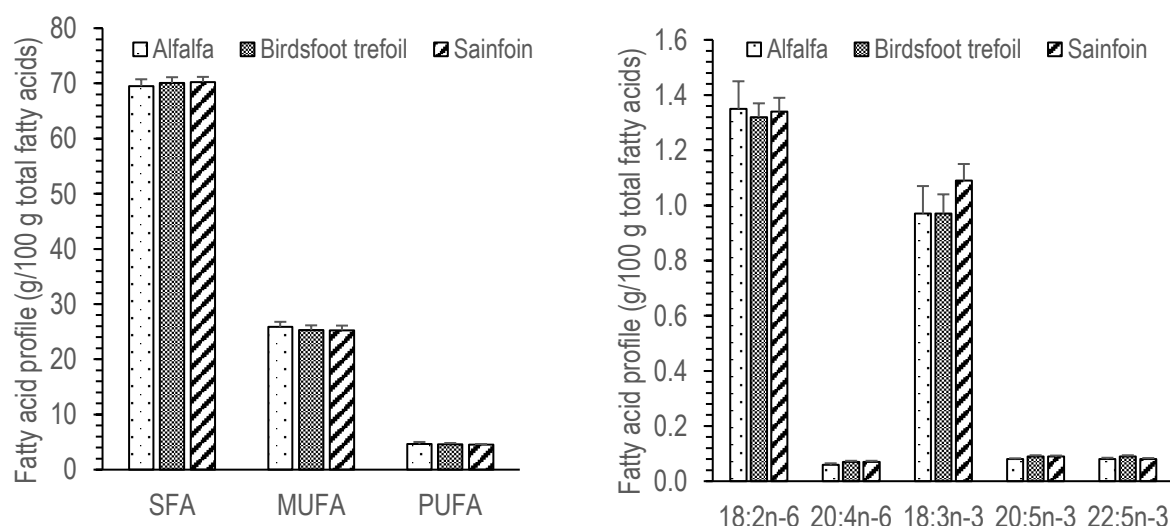


Figure 4: Fatty acid profiles of the milk from cows fed standard diets supplemented with either alfalfa, birdsfoot trefoil or sainfoin.

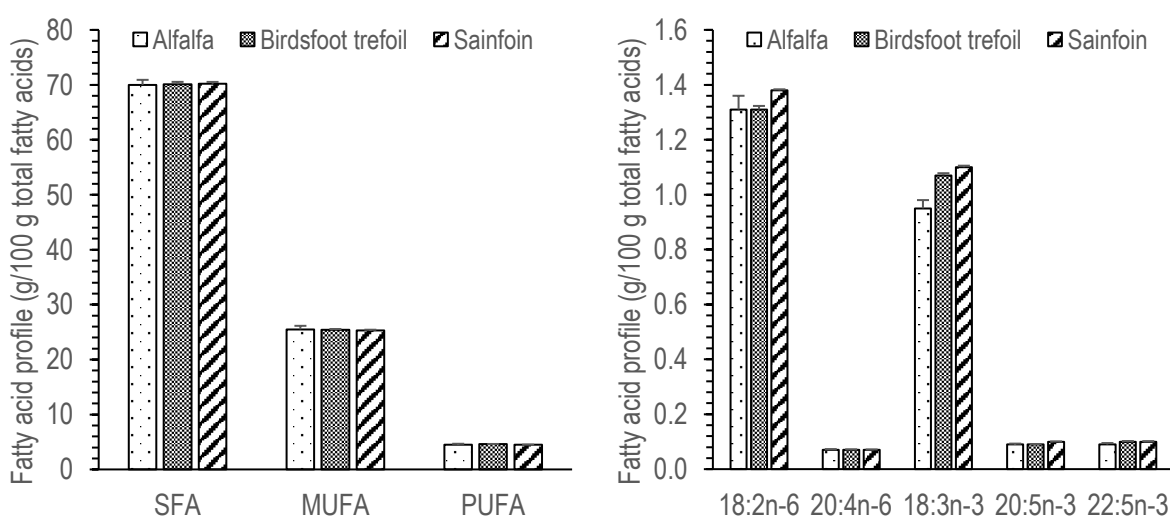


Figure 5: Fatty acid profiles of cheeses produced from the milk of cows fed standard diets supplemented with either alfalfa, birdsfoot trefoil or sainfoin.

acid), concentrate and alfalfa in a ratio of 45:25:5:7:18. For the CT groups, the portion of alfalfa pellets was replaced by either birdsfoot trefoil pellets (CT = 3%) or sainfoin pellets (CT = 19%). The milk was collected on 3 consecutive days. The milk from each individual cow from each day was analyzed for milk gross composition and fatty acid profile. The milk of all cows from each day of treatment was processed into Gruyère-type cheese. A trained panel assessed the sensory quality of the raw milk and cheese using discriminative and descriptive tests [28].

The dry matter intake, milk yield and milk gross chemical composition did not differ among treatment groups. Except for small changes in the proportions of some individual fatty acids, the proportions of total SFA, MUFA, PUFA, *n*-3 and *n*-6 fatty acids were not affected by the dietary treatments (Figure). Similar to the milk fat composition, the relative proportion of the main fatty acid groups in the cheese were not significantly affected by the diet (Figure). However, compared to the alfalfa group, the sainfoin pellets slightly but significantly elevated the linoleic and linolenic acid levels as well as the proportions of 2 of its elongation products, eicosapentaenoic and docosapentaenoic acid, in the cheese. The treatment effects observed in the cheese but not in the milk can be explained by the greater variability in the fatty acid profile of the milk from the 6 individual cows per treatment compared to the fatty acid profile of the cheeses produced from a mix of the milk from those individual cows. For instance, after feeding the sainfoin pellets, the linolenic acid level increased by 17% in both the milk and the cheese. The increase is statistically significant only in the cheese for which the variability expressed as standard error of the mean was 12 times lower than in the milk. As with the sheep, there were several indications to suggest that dairy cows also experience ruminal biohydrogenation inhibition via CT-rich forage legumes (by sainfoin greater than by birdsfoot trefoil): first, the increase in the level of the *n*-3 fatty acid family; second, the elevated levels of oleic acid; third, the lower proportion of stearic acid (the terminal product of ruminal biohydrogenation). As the effects of birdsfoot trefoil were not as great as the effects of sainfoin, and as birdsfoot trefoil had markedly lower CT content than sainfoin, it is implied that a certain CT level is needed for the inhibition of PUFA biohydrogenation. However, the present data does not allow the establishment of a distinct threshold value.

Regarding sensory evaluation, panelists did not detect any differences in neither the milk odor, nor the intensity of flavor in the cheese between the birdsfoot trefoil and sainfoin groups compared to the alfalfa group. These findings corroborate with the lack of difference in the gross chemical composition of the milk and the cheese, and the changes occurring in the fatty acid profile of the milk and cheese were apparently too small to affect the sensory assessment. With respect to texture, cheeses from the

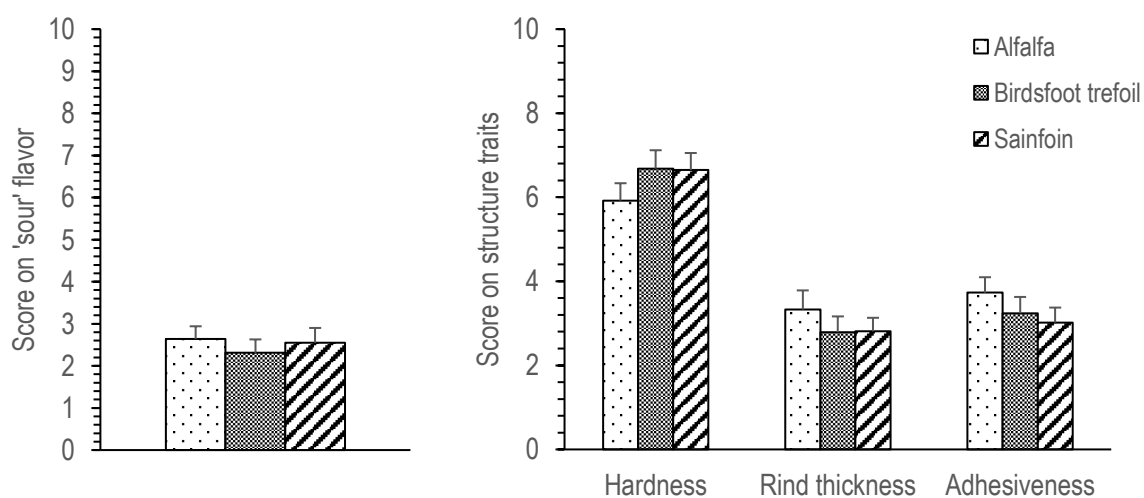


Figure 6: Sensory evaluation grades for the flavor and structure traits of the cheeses aged for 8 months, produced using the milk from cows fed standard diets supplemented with either alfalfa, birdsfoot trefoil or sainfoin. The scale ranges from 0 (low intensity/thin) to 10 (high intensity/thick).

birdsfoot trefoil and sainfoin groups were judged harder and tended to be less adhesive to the palate compared to those cheeses from the alfalfa group (Figure 6). In addition, birdsfoot trefoil and sainfoin cheeses had less rind. The firmness of cheese depends on the palmitic acid (16:0) and oleic acid content in the milk fat, with palmitic and oleic acid levels correlating to the hardness and the softness of cheese, respectively [29, 30]. An increase in the moisture content of the cheese has been correlated to an increase in cheese adhesiveness [31]. However, neither the levels of the palmitic acid and the levels of the oleic acid nor the moisture content were responsible for the observed structural changes, as these traits were similar among the cheeses produced from the 3 experimental treatments.

In conclusion, the results of the present study show that with a CT-rich forage legume like sainfoin, resulting in a diet containing 3% total CT, the level of linolenic acid and of long-chain *n*-3 fatty acids can be elevated in dairy products. This confirms that elevated dietary CT intake can increase the proportions of some beneficial PUFA in both milk and cheese. However, the effects of birdsfoot trefoil were not comparable to the effects of sainfoin, which supports the hypothesis that the effects depend on the plant species and in this case most likely to the difference in the CT content. The present study has also demonstrated that the inclusion of 18% of sainfoin makes it possible to produce cheese with only a few distinguishable sensory changes, and these descriptors concerned structure more than flavor. This largely disproves that CT negatively affects the odor and flavor in the tested dietary situations. Another important finding which promotes the use of sainfoin in the diet of dairy cows was that sainfoin can replace a high quality legume like alfalfa without adverse side effects on feed intake and milk yield.

5. Overall conclusion

The objective for using CT-rich plants in ruminants was to determine the efficacy of CT to increase the content of PUFA, especially of *n*-3 fatty acids, and to decrease the content of SFA in the ruminant-based products by reducing the ruminal biohydrogenation of dietary PUFA. From the 2 CT-rich forage legumes tested, sainfoin was always the most effective, but the effect was greater in meat than in milk. Several reasons could determine these differences, from species (lamb vs cow), to forage form (silage used with lambs vs dehydrated pellets used with dairy cows), to CT levels and feeding duration. As we have shown, the type of forage form can affect the structure of CT [32]. In the silage, the main proportion of CT is bound to plant proteins, while in the pellets, CTs are in a soluble form. Until now, it has been unclear to what extent the form of binding impacts the efficacy of the CT action. Furthermore, where the lambs were fed solely CT-rich silages for over 100 d, the dairy cows consumed the CT-rich plants as only a portion (18%) of the whole diet, and it was offered for a much shorter period (30 d). This obviously raises the question of the level of CT to include in rations to show a significantly positive effect, as well as the duration for which CT should be offered to the animals before a maximal effect can be seen.

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