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Effect of condensed tannins in sainfoin on *in vitro* protein solubility of lucerne as affected by the proportion of sainfoin in the mixture and the preserving conditions

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SUMMARY – The present work was designed to assess the effects of condensed tannins (CT) of sainfoin (S) on the solubility of Lucerne (L) proteins when the 2 forages are mixed in different proportions in fresh or conserved forms (freeze dried or oven dried at 40°C or 60°C). The percentages of L:S were 100:0, 75:25, 50:50, 25:75, 0:100) for all preparations. Fresh forages were chopped and either mixed and homogenised in a Waring blender and incubated fresh, or dried and ground prior to incubation. Nitrogen solubility tests (SolN) in artificial saliva buffer (expressed as % of total N) and protein N (in SolN) after precipitation with TCA (expressed as % Nt) were performed on all preparations in the presence or absence of PEG (polyethylene glycol 4000) to assess the tannin effect. Nitrogen solubility was low for fresh and freeze dried sainfoin (10% of total N) and approximately doubled after oven drying ($p<0.05$). The main component of sainfoin SolN was non-protein N. In contrast, soluble nitrogen in fresh lucerne was high (57% Nt) and decreased slightly in dried samples. The SolN was mainly protein N (65% SolN) in fresh and freeze-dried samples and 40% SolN in oven-dried samples. The N solubility and percentage of protein in SolN decreased as the percentage of sainfoin increased. After PEG addition, which binds with tannins, SolN was not affected for lucerne but increased considerably for sainfoin ($p<0.05$). SolN in mixtures (without PEG) was lower than that calculated from N solubility of each forage of the mixture so that CT from sainfoin insolubilized some lucerne proteins. This effect was greater with fresh than with dried forages. The data support the hypothesis that the degree of binding between CT with plant protein is affected by the preservation method. Condensed tannins in sainfoin can reduce *in vitro* N solubility of other forages (e.g. lucerne) when intimately mixed. This effect is greater with fresh than with dried forages such as hays.

Keywords: Forage, tannin, protein solubility, sainfoin, lucerne.

RESUME *– "Effet des tanins condensés du sainfoin sur la solubilité* in vitro *des protéines de luzerne selon la proportion de sainfoin dans le mélange et le mode de conditionnement". Ce travail a pour but d'évaluer* in vitro *l'effet des tanins condensés du sainfoin sur la solubilité des protéines de luzerne selon le mode de conditionnement de ces fourrages. De la luzerne (L) et du sainfoin (S) récoltés au stade végétatif ont été mélangés à l'état frais, ou après lyophilisation ou après séchage à 40°C ou 60°C ont été mélangés dans de la salive artificielle dans les proportions respectives (L-S) : 100-0, 75-25, 25-75, 0-100). Les quantités de N total, de N soluble et de N protéique (après précipitation au TCA) ont été déterminées en présence ou non de PEG qui complexe les tanins et inhibe leur effet. Le N soluble de la luzerne à l'état frais représente 57% Nt, diminue de 4 à 7 points sous l'effet du séchage et de 11 points après lyophilisation. Dans le sainfoin, la part de N soluble est faible (10% Nt), elle n'est pas modifiée par la lyophilisation alors qu'elle s'accroît de 6 à 12 points sous l'effet du séchage à 40°C ou 60°C. Lorsque la proportion du sainfoin augmente dans le mélange, la teneur en N soluble mesurée diminue. Après ajout de PEG (qui se lie aux tanins), la teneur en N soluble de la luzerne seule n'est pas modifiée mais celle du sainfoin est fortement augmentée. Dans les mélanges, le PEG inhibe l'effet des CT et les teneurs en N soluble sont très proches de celles calculées, quel que soit le mode de conditionnement. Les teneurs en N protéique diminuent lorsque la proportion de sainfoin augmente dans le mélange. En présence de PEG, les teneurs sont proches et ne varient pas quelle que soit la proportion de sainfoin dans le mélange mais les valeurs sont plus faibles pour les échantillons séchés à 40°C et 60°C. Les tanins condensés du sainfoin peuvent réduire la solubilité de l'azote in vitro ainsi que l'azote protéique quand ils sont mélangés avec de la luzerne. Cet effet sera moins marqué sur les échantillons séchés et donc sur les foins.*

Mots-clés : *Fourrage, tannin, solubilité des protéines, sainfoin, luzerne.*

Introduction

Proteins of fresh forage legumes such as lucerne are highly degraded in the rumen leading to their inefficient use by the animal. The condensed tannins (CT) present in some forages can improve the

nutritional value of these forages and of associated feeds in the diet. Previous *in vitro* results (Waghorn and Shelton, 1997) have shown that CT from *Lotus corniculatus* are able to bind with and precipitate protein from a ryegrass/clover pasture; but when these forages were fed together to sheep, CT effects on digestion and animal performances were low. This highlighted the need of improved understanding of the mechanism of CT interaction between feeds. The present work was designed to assess the effects of CT of sainfoin on the solubility of lucerne proteins according to the mode of conditioning of the samples.

Materials and methods

Samples of lucerne (L) and sainfoin (S) were cropped in April 2004, at the vegetative stage. The forages were sown the year before in a field close to the INRA Research Center, Theix, at an altitude of 800 m on a deep silt loam soil. Measurements and subsequent analysis were done on fresh and dried forages either through freeze-drying or heating at 40° and 60°C for 48 h in a forced air oven. For each form of preparation, the following mixtures were studied (L:S): 100:0, 75:25, 50:50, 25:75, 0:100, giving a total of 20 preparations. Immediately after cutting fresh samples were finely chopped with scissors to allow representative sampling before measurement. Freeze-drying and oven drying simultaneously started. Dried samples were ground (1 mm screen size) before measurements. Five samples of each preparation (0.7 g of dry matter basis) were incubated with 50 ml of artificial saliva (Verité and Demarquilly, 1978) for one hour; in half of these samples PEG 4000 (polyethylene glycol) was added (600 mg for 0.7 g equivalent dry sample) to inhibit the effect of CT in sainfoin. 1.4 g (dry matter basis) samples of each preparation of fresh mixture were homogenised in 100 ml artificial saliva (pH 6.9) using a Waring blender (3×0.5 min) agitated for 60 min at 20°C and centrifuged (27000 g, 20 min 4°C). The same procedure was followed with dried mixture except that the amount of samples and buffer was half that used for fresh mixtures.

The supernatant of all mixtures with and without PEG was analysed for total N (Nt) before and after protein precipitation with TCA 10% (v/v). Soluble N (SolN) (%Nt) and protein N (%SolN) were calculated.

Results and discussion

Nitrogen content of the forages was high: on average 3.9% and 5.3% (dry matter basis) in sainfoin and lucerne respectively. Nitrogen solubility (SolN) was low in fresh and freeze dried sainfoin (10% of Nt) and nearly doubled in the heated forage ($p<0.05$, Table 1). The main component of sainfoin SolN was non-protein N (Fig. 1). In contrast SolN in fresh lucerne was higher (57% Nt). Preserving effects differed from those observed with sainfoin; freeze-drying reduced SolN (by 11 percentage units) more than heating (4-7 percentage units). Protein was the main component of fresh and freeze-dried lucerne (65-70% SolN) and was halved by oven drying.

Adding PEG which binds with tannins and releases protein (Makkar, 2003), had no effect on SolN and its protein fractions in lucerne whereas it increased SolN in sainfoin to roughly 40% Nt (and a little less in the freeze dried forage), which was 7 to 14 percentage units lower than corresponding lucerne samples. Tannins in sainfoin complexed and insolubilized 20 to 30% of Nt (Table 1), which are mainly proteins released by PEG addition, as indicated in Fig. 1 and Table 1.

After PEG addition, the protein fraction in sainfoin SolN was similar to that observed in lucerne (Fig. 1) and the mixture of the 2 forages. Hence mixing sainfoin with lucerne resulted in a decrease in SolN (Table 1) as well as soluble protein N (Fig. 1). It is noteworthy that the measured SolN was lower than the value calculated from SolN of each forage and its proportion in the mixture showing that the CT in sainfoin insolubilized some of the proteins in lucerne (Aufrère *et al.*, 2005). This interaction was more pronounced with fresh mixtures than dried preparations, perhaps because tannins and /or proteins are more reactive or because the mixing process enables a closer alignment of CT and protein from fresh forages.

Dried mixtures have been studied to simulate alterations during hay making and to evaluate a cheap method of sample conservation compared with freeze drying. However in our drying conditions losses of leaves (that contain the highest concentration of tannins compared to stems (Lees, 1993) were minimized and our results might overestimate the effect of CT in sainfoin fed as hay.

Table 1. *In vitro* nitrogen (N) solubility (SolN) in total N (%Nt) in mixtures (%) comprising different proportions of lucerne and sainfoin prepared by either fresh homogenising , freeze-drying or oven-drying at 40°C and 60°C. Measurements (mean ± SD) were performed without and with addition of PEG 4000. Calculated solubilities were derived from N solubility of individual forages and their proportion in the mixture

Preservation	Percentages of lucerne:sainfoin in mixtures (dry matter basis)				
	100/0	25/75	50/50	75/25	0/100
SolN (% Nt) and standard deviation					
Fresh					
Measured	57.1^{a} ± 2.5	42.1^{de} ±1.7	22.7^{f} ± 2.1	12.0^{e} ± 0.2	10.1^{f} ± 0.5
Calculated		47.5	37	24.7	
With PEG					
Measured	54.6^{ab} ± 2.5	52.8^{a} ± 1.7	48.3^{a} ± 3.1	46.9^{a} ± 0.8	40.6^{ab} ± 0.5
Calculated		51.8	48.9	44.9	
Freeze-drying					
Measured	45.6^{cd} ± 2.4	33.3^{f} ± 1.8	24.6^{e} ± 0.24	16.2^{de} ± 0.59	8.92^{f} ± 0.18
Calculated		35.7	29.9	20.1	
With PEG					
Measured	42.3^{d} ± 1.4	40.2^{de} ± 0.1	38.3^{c} ± 1.1	39.2^{b} ± 0.7	34.7^{c} ± 1.2
Calculated		40	39	38	
Drying 40°C					
Measured	53.2^{b} ± 0.7	42.5^{c} ± 0.1	33.4^{d} ± 0.4	24.6^{c} ± 0.2	22.3^{d} ± 0.2
Calculated		47.2	40.2	32.1	
With PEG					
Measured	49.5 ^{bc} ± 0.7	48.8^{b} ± 0.06	45.8^{ab} ± 0.4	47.0 ^{a} ± 0.2	42.6^{a} ± 0.2
Calculated		49.4	48.5	47	
Drying 60°C					
Measured	50.2^{b} ± 3.0	38.1^{e} ± 1.0	26.0^{e} ± 1.0	19.4^{d} ± 0.07	15.8^{e} ± 0.6
Calculated		42.7	34.7	26.2	
With PEG					
Measured	47.9 ^{bc} ± 3.0	47.2 ^{f}± 1.0	43.2^{b} ± 1.0	42.6^{b} ± 0.1	39.5^{b} ± 0.6
Calculated		46.8	45.4	42	

Comparisons between treatments are indicated by different superscripts within columns ($p < 0.05$). Calculated values are given for comparative purposes (see text for details).

Data from Palmer *et al.* (2000) support our hypothesis that the degree of complexing of CT with plant proteins is altered by heating temperature and oxidation. Oxidation is particularly effective at high drying temperatures. Stewart *et al.* (2000) found that CT contents in freeze dried leaves were closer to that in fresh leaves than in dried leaves. Their study suggests that careful air drying without excessive heating may not reduce the chemical parameters of nutritive value for *C. calothyrsus*.

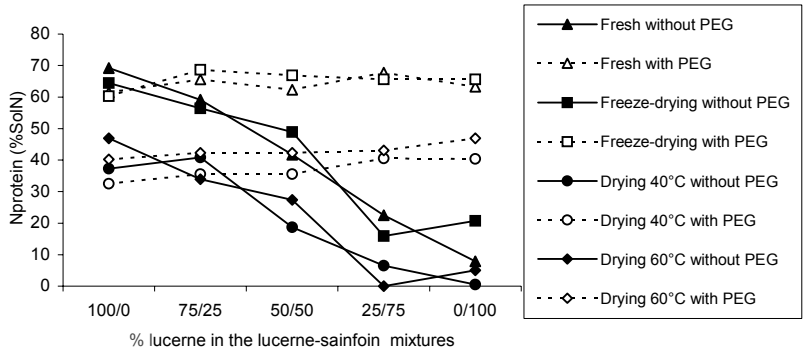

Fig. 1. Percentage of protein N in soluble N (protein N, % SolN) in fresh, freeze-dried or oven-dried (40°C and 60° C) when lucerne (L) and sainfoin (S) are mixed in different proportions. All measurements were done with and without PEG 4000 to assess the tannin effect on N solubility.

Conclusion

Excess CT in sainfoin can reduce N and protein solubility *in vitro* when mixed together fresh or after freeze drying. However these effects are likely to be reduced when samples have been dried at 40°C and 60°C, which resembles preparation for hays.

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