EFFECT OF TANNINS ON GRASS SILAGE COMPOSITION

DJELOVANJE TANINA NA SASTAV SILAŽE TRAVE

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SUMMARY

Fresh meadow grass was ensiled with chestnut tannin extract (Farmatan 75®, Tanin Sevnica, Slovenia) at concentrations of 0 (control), 3, 15 and 30 g of extract/kg of ensiling material. Addition of tannins to the fresh grass material did not change substantially the dry matter (DM), ether extract and neutral detergent fibre (NDF) contents of silages. The crude fibre content decreased ($P < 0.05$) and nitrogen-free extracts increased ($P < 0.05$) with the increasing amounts of added chestnut extract. The crude protein (CP) content decreased from 133 in control silage to 116 and 117 g/kg DM ($P < 0.05$) in silages prepared with 15 and 30 g of chestnut extract/kg of ensiling material, respectively. Silages prepared with increasing amounts of chestnut tannin extract had greater amounts (from 11.2 to 13.6 g N in true protein/kg DM in control silage and silage prepared with 30 g of chestnut extract/kg of fresh grass, respectively) and proportion of N in true protein (TrueN) (from 52.3 to 72.5 % of N in control silage and silage prepared with 30 g of chestnut extract/kg of fresh grass, respectively). On the contrary, the amounts of soluble N (SolN) decreased significantly ($P < 0.05$) with increasing amounts of chestnut tannin extract from 8.9 to 3.0 and 2.9 g N/kg DM in control silage and silages prepared with 15 and 30 g of chestnut extract/kg of fresh grass, respectively. In accordance with the above mentioned trends, the proportions of SolN in total CP contents also decreased ($P < 0.05$) from 43.7 to 15.7 % in control silage and silage prepared with 15 g of chestnut extract/kg of ensiling material, respectively. Increasing the amounts of chestnut extract in silages also decreased the amounts of ammonia-N in silages. The addition of chestnut tannin extract did not influence pH values of silages. The highest amounts of added chestnut tannin extract (15 and 30 g/kg of fresh grass) significantly ($P < 0.05$) decreased lactic acid content (from 101 to 81 g/kg DM in control silage and silages with 15 and 30 g of chestnut extract/kg of fresh grass, respectively). On the contrary, the amounts of acetic, propionic and butyric acid did not change significantly. Obtained results suggest that tannins can be successfully used as silage additives, with favorable effects on protein solubility and true protein contents.

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INTRODUCTION

In earlier times hay making was the most common method of preserving forages. However, the weather conditions were often uncertain and cold and rainy weather often caused high losses of hay's nutrients (e.g. nutritive value) and the hay making method has been replaced by silage making. This shift in conservation methods was evident especially in Sweden, where 70% of forages were preserved as hay and 30% as silage in 1970 (Knicky, 2005). Nowadays this ratio is reversed. In Western Europe silages account for more than 60 % of conserved forages for winter feed. In some countries silages account for more than 85 % of conserved forages (Henderson, 1993). Although silages represent more than 60 % of the winter feed for ruminants, the silage fermentation process is largely left to chance. Silage additives have been developed to take some of the risk out of the ensilage process and to improve nutritive value of silages. Silage additives may be divided into two major groups: stimulants and inhibitors (Henderson, 1993; Knicky, 2005). The role of stimulants is to ensure the fermentation process by encouraging the lactic acid fermentation by promoting the growth of lactic acid bacteria through the provision of acids or fermentable substrate. Selected strains of lactic acid bacteria (LAB) are also members of this group. On the contrary, fermentation inhibitors partially or completely restrict microbial growth. The most used members of this group are mineral and organic acids such as sulphuric and formic acid.

Principal characteristic of tannins is their ability to form complexes with a variety of compounds such as proteins and carbohydrates. The formation of complexes non soluble and non degradable in the rumen could be of great importance in preserving forages as the rumen degradability of forage proteins normally exceeds 85 %, especially in immature forages and/or intensively fertilized forages or when unwilted grass silages are prepared (McNabb et al., 1996). Tannins can also react with microorganisms and their enzymes, thus inhibiting their activity (McSweeney et al., 2001; Chung et al., 1998). These characteristics of tannins would be beneficial also in ensiling forages to prevent excessive rumen degradation of plant proteins and to inhibit the appearance and growth of unwanted and harmful microorganisms.

The aim of this work is thus to evaluate the effect of hydrolysable tannins from sweet chestnut wood on chemical composition of silages prepared from fresh meadow grass. The present work differs from the experiment 1 of Lavrenčič and Levart (2005) where wilted grass was used as ensiling material.

MATERIAL AND METHODS

The silages were made from the first cut meadow grass. Harvested grass was stored for two weeks at - 20°C. One day prior to ensiling, the grass was removed from the freezer and thawed overnight. The chestnut extract (Farmatan 75®, Tanin Sevnica, Slovenia) was dissolved in distilled water and dispensed in aliquots of 125 ml/kg of grass. An equal volume of distilled water was also added for controls. Three levels of chestnut extract (3, 15 and 30 g/kg of grass) were used. Hand mixing of tannins with the grass was carried out in bulk for each treatment, after which the grass was packed in duplicate into five liter airtight containers and left for three months at room temperature.

When containers were opened, silages were divided into two parts, of which one part was used for the extraction of silage juice in which pH, ammonia-N (NH_3-N), lactic, acetic, propionic and butyric acids were determined. The other part was oven-dried and milled through a 1 mm sieve before chemical analyses. Weende proximate analyses (dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE)) as well as N content in true protein (TrueN) were performed according to Naumann and Basler (1999) while neutral detergent fibre (NDF) contents were determined with the Ankom apparatus according to Goering and Van Soest (1970). Soluble nitrogen (SolN) was determined as the difference between nitrogen content in dried material (total N content) and nitrogen content in the material extensively washed with cold tap water. Statistical evaluation of differences between silages within each experiment was carried out using the Tukey subroutine of GLM procedure (SAS/STAT, 1988)

RESULTS AND DISCUSSION

Chemical compositions of silages are presented in Table 1. The differences in DM contents between silages were small and they did not exceed 13 g/kg. This is in accordance with the results from experiment 1 of Lavrenčič and Levart (2005) who determined slightly greater difference (15 g/kg). On the contrary, Salawu et al. (1999) reported that the DM content decreases with increasing amount of tannins added to silages.

The CP, EE and CF contents decreased with increasing amounts of added chestnut tannin extract, while the NFE content increased. The CP content decreased from 133 g/kg DM in control silage to 116 g/kg DM in silage prepared with 15 g of chestnut tannin extract/kg of ensiling material. The EE and CF contents decreased from 39 and 341 g/kg DM in control silage, respectively to 28 and 306 g/kg DM in silages prepared with 30 g of chestnut extract/kg of fresh grass, respectively. The NFE contents increased from 435 g/kg DM to 469 g/kg DM in silages prepared with 30 g of chestnut tannin extract/kg of ensiling material. All these differences were not statistically significant ($P > 0.05$) when the lowest amount of chestnut tannin extract (3 g/kg of fresh grass) was applied. Differences became statistically significant ($P < 0.05$) when higher amounts of chestnut tannins (15 and 30 g/kg) were added to the ensiling material. These observations are in accordance with the results obtained in the experiment 1 of Lavrenčič and Levart (2005) but the differences in the CP, EE, CF and NFE contents in their experiment were much smaller, probably because they used wilted grass instead of fresh grass as ensiling material. The addition of chestnut extract did not affect the NDF content of silages, except in silage prepared with 3 g of tannin extract/kg grass which had a significantly lower NDF content. These results are in accordance with the observations obtained in the experiment 1 of Lavrenčič and Levart (2005).

Table 1. Chemical composition of silages (g/kg DM) prepared with different amounts of chestnut tannin
Tablica 1. Kemijski sastav silaža (g/kg ST) pripremljenih s različitim količinama tanina kestena

	Control Kontrola	Amount of added chestnut tannin extract (g/kg) Količina dodanog ekstrakta tanina kestena (g/kg)		
		3	15	30
Dry matter (DM) - Suha tvar (ST)	212	217	206	219
Crude protein (CP) - Sirove bjelančevine (SB)	133[a]	132[a]	116[b]	117[b]
Ether extract - Eterski ekstrakt (EE)	39[a]	36[ab]	29[c]	28[c]
Crude fibre (CF) - Sirova vlaknina (SV)	341[a]	316[ab]	314[b]	306[b]
Nitrogen free extract (NFE) - Ekstrakt bez dušika (EBD)	435[c]	451[bc]	478[a]	469[ab]
Neutral detergent fibre (NDF) Neutralna deterdžentna vlakna (NDV)	578[a]	553[b]	577[a]	572[ab]

[abc] means with different letters in the same row are significantly different at $P < 0.05$

As reported above the CP (Table 1) and thus N (Table 2) content in silages decreased significantly ($P < 0.05$) with the increasing amounts of added chestnut tannin extract. In Table 2 are also presented various N fractions contents in silages. Increasing the amount of chestnut tannin extract increased significantly ($P < 0.05$) the TrueN amount from 11.1 in control silage to 13.6 g/kg DM in the silage prepared with 30 g of chestnut tannin extract/kg ensiling material (Table 2). Because of the decrease in the total N content and increase in TrueN content the portion of TrueN content in total N content increased from 52 to 73 % (Figure 1). These results are in agreement with the results obtained in experiment 1 of Lavrenčič and Levart (2005) when wilted grass was ensiled with different amounts of chestnut tannins. In addition, the SolN content decreased from 8.9 to 2.9 g/kg DM in control silage and silage prepared with the greatest amount of tannin extract, respectively. The SolN content was significantly different ($P < 0.05$) already between control silage and silage prepared with 3 g of tannin extract/kg of grass. These results are in accordance with the results of Salawu et al. (1999) and Lavrenčič and Levart (2005) who also found that increasing the amount of tannins decreased the SolN contents.

Table 2. Various nitrogen fractions contents (g/kg DM) in silages prepared with different amounts of chestnut tannins

Tablica 2. Sadržaj raznih frakcija dušika (g/kg ST) u silažama pripremljenim s raznim količinama tanina kestena

		Amount of added chestnut tannin extract (g/kg) Količina dodanog ekstrakta tanina kestena (g/kg)		
	Control - Kontrola	3	15	30
Total N	21.2^{a}	21.1^{a}	18.5^{b}	18.8^{b}
TrueN	11.1^{b}	12.2^{ab}	13.2^{a}	13.6^{a}
SolN	8.9^{a}	7.2^{b}	3.0^{c}	2.9^{c}
Ammonia-N	1.8^{a}	1.5^{b}	0.7^{c}	0.8^{c}

^{abc} means with different letters in the same row are significantly different at $P < 0.05$

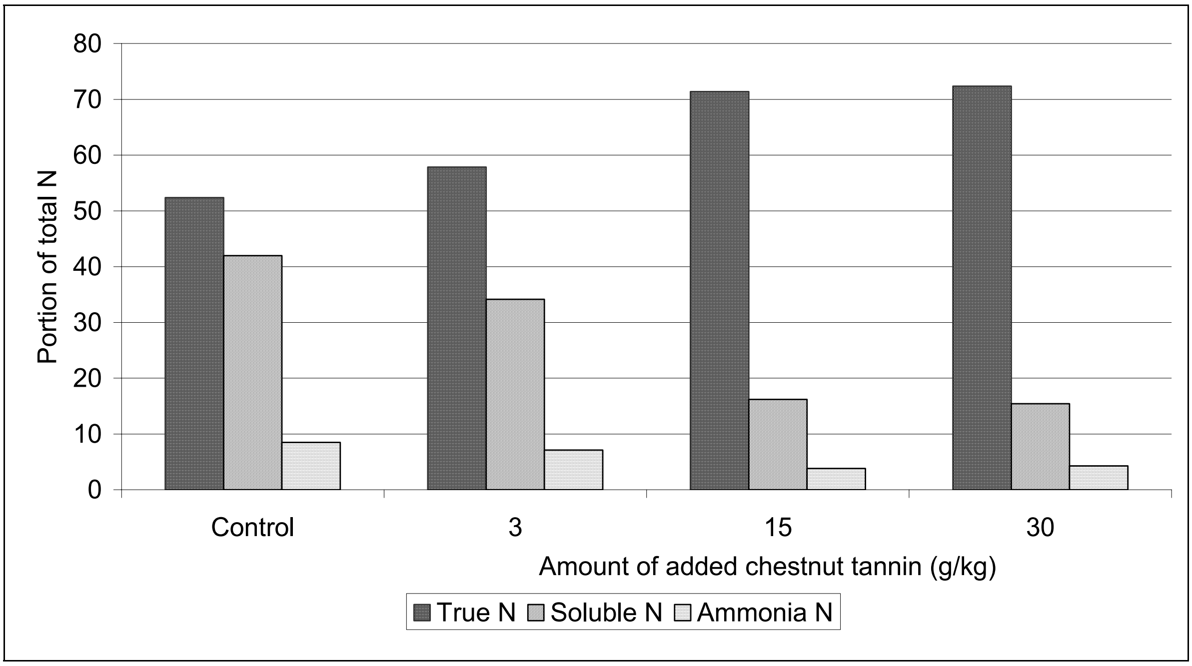

Figure 1. Portion of true and soluble N and ammonia N (% of total N) in grass silages prepared with different amounts of chestnut tannin extract

Slika 1. Udio pravog i topivog N i amonijaka N (% ukupnog N) u silaži trave pripremljenoj s različitim količinama ekstrakta tanina kestena

Silages prepared with chestnut tannins had significantly lower (P <0.05) amounts of ammonia-N contents (Table 2 and Figure 1) than control silage. In addition, with the increasing amounts of added chestnut tannins the reduction in ammonia-N increased. Neither control silage nor silages prepared with chestnut extract had ammonia-N content greater than 80 g/kg N (8 %), which is in accordance with the results of Lavrenčič and Levart (2005) who ensiled wilted grass. Such low levels of ammonia-N indicated that all procedures in preparation of silages were optimal. On the contrary, Salawu et al. (1999) observed much higher ammonia-N the contents in silages and they noted that only the highest concentrations of tannin extracts (50 g/kg of ensiling material DM) significantly reduced the ammonia-N contents. Lower amounts of ammonia-N (5 g/kg of ensiling material DM) and lower solubility of protein in silages prepared with tannins could be the result of the ability of tannins to form insoluble complexes with proteins (Hagerman and Butler, 1981; Makkar et al., 1990), which are resistant to proteolytic activity of microorganisms in silages and their enzymes (Gasparič et al., 1996).

Table 3. Composition of low molecular mass acids (g/kg DM) and pH of silages prepared with different amounts of chestnut tannin extract

Tablica 3. Sastav kiselina niske molekularne mase (g/kg ST) i pH silaža pripremljenih s različitim količinama ekstrakta tanina kestena

		Amount of added chestnut tannin extract (g/kg) Količina dodanog ekstrakta tanina kestena (g/kg)		
	Control - Kontrola	3	15	30
Lactic acid - Mliječna kiselina	101.1[a]	95.8[a]	80.6[b]	80.9[b]
Acetic acid - Octena kiselina	17.9	15.8	11.6	16.8
Propionic acid - Propionska kiselina	0.09	0.08	0.09	0.08
Butyric acid - Maslačna kiselina	1.31	1.21	0.16	0.09
pH	3.85[ab]	3.78[b]	3.84[ab]	3.92[a]

[ab] means with different letters in the same row are significantly different at P < 0.05

The concentration of low molecular mass acids and pH values of silages are presented in Table 3. Increasing levels of chestnut tannins in the ensiling material decreased significantly (P < 0.05) the lactic acid content of silages, especially when 15 and 30 g of chestnut tannin extract were used for ensiling the grass. This is in accordance with the results of Salawu et al. (1999) and the experiment 1 of Lavrenčič and Levart (2005). However, Salawu et al. (1999) observed that silages prepared with greater amounts of tannins also had greater amounts of lactic acid contents than those prepared with lower amounts of tannins.

The acetic and propionic acid contents did not change significantly with increasing amounts of added chestnut tannin extract, which is in accordance with the results of experiment 1 performed by Lavrenčič and Levart (2005). On the contrary, Salawu et al. (1999) found that the addition of tannins to ensiling material increased the acetic acid content of silages and that silages prepared with greater amounts of tannins (50 g/kg of forage DM) contained smaller amounts of acetic acid. The butyric acid contents decreased with increasing amounts of added chestnut tannins, which is in accordance with the observations of Salawu et al. (1999), who suggested that tannins decreased the conversion of lactic acid into butyric and acetic acid. Small butyric acid contents are probably the consequence of lower activity of clostridia, which are main producers of this acid in silages (Henderson, 1993).

The highest pH value (3.92) was observed in silage prepared with the greatest amount of added chestnut tannin (30 g of chestnut tannin extract/kg grass). This value was not significantly different from the pH value in control silage and silage prepared with 15 g of chestnut tannin extract/kg grass (Table 3). The lowest pH value (3.78) was obtained when silage was prepared with 3 g of chestnut tannin extract/kg of ensiling material. Salawu et al. (1999) reported that control silage had lower pH value than silages prepared with tannins. They noted that silages prepared with small amounts of tannins (5 g/kg ensiling material DM) had higher pH value than silages prepared with greater amounts of tannins (50 g/kg ensiling material). These observations are in contrast with our results. However, pH values of all silages were low enough to ensure their stability (Demarquilly, 1973; Knicky, 2005).

CONCLUSIONS

Tannins can be used as silage additives. The estimated qualities of silages, if low molecular mass acid contents, ammonia-N and pH value were considered (Knicky, 2005), were very good. However the quality of silages depended greatly on the condition at ensiling (good compression of material into containers), which were optimal in our experiment. Decreased soluble N content ratio and increased ratio and N content in true proteins suggest that the proteolysis in silages was inhibited probably also because of the formation of complexes between proteins and tannins. The extent of degradation of these complexes in the rumen is presumably lower, which allows better amino acid supply to ruminant animals and thus their better performance.

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SAŽETAK

Svježa livadna trava silirana je s ekstraktom tanina kestena (Farmatan®, Tanin Sevnica, Slovenija) u koncetracijama od 0 (kontrola), 3, 15 i 30 g ekstrakta/kg siliranog materijala. Dodatak tanina u svježu travu nije znatno promjenio suhu tvar (DM), eterski ekstrakt i sadržaj neutralnih deterdžentnih vlakna (NDF) silaže. Sadržaj sirove vlaknine se smanjio ($P < 0,05$) a ekstrakti bez dušika su porasli ($P < 0,05$) s povećanim kolličinama dodanog ekstrakta kestena. Sadržaj sirovih bjelančevina (CP) se smanjio od133 u kontrolnoj silaži do 116 i 117 g/kg DM ($P < 0,05$) u silažama pripremljenim s 15 odnosno 30 g ekstrakta kestena/ kg siliranog materijala. Silaže pripremljene s povećanim količinama ekstrakata tanina kestena imale su veće količine od 11,2 do 13,6 g N u pravoj bjelančevini/kg DM u kontrolnoj silaži i silaži pripremljenoj s 30 g ekstrakta kestena/kg svježe trave. Nasuprot tome količine octene, propionske i maslačne kiseline nisu se značajno promijenile. Dobiveni rezultati navode na zaključak da se tanini mogu uspješno upotrijebiti kao dodaci silaži s povoljnim djelovanjem na topivost bjelančevina i sadržaj pravih bjelančevina.