The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein

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Abstract

The effects on silage composition of ensiling perennial ryegrass (PRG) with three commercial tannins (mimosa, myrabolam and quebracho tannins) were assessed (Experiment 1). The effects on silage composition and mobile bag disappearance of DM, nitrogen and true protein of the addition of these tannins or a combination of tannin plus formic acid, formaldehyde alone, formic acid alone, or a combination of formaldehyde and formic acid (Experiment 2) were also studied. In Experiment 1, the PRG was a third cut with a mean oven-dry DM content of 200 g/kg. All silages were prepared on a small laboratory scale (500 g), with the additives added in 20 ml aliquots/kg herbage fresh weight. The tannins were added at the rate of 5 or 50 g/kg herbage DM and in Experiment 1, samples were examined after 7 or 32 days of ensiling. In Experiment 2, a 1st cut PRG with a mean oven-dry DM content of 188 g/kg was used and the samples were taken on days 7, 14 and 49. In Experiment 1, treatment with tannins reduced the soluble nitrogen (SN) and ammonia content of the silages. In Experiment 2, the tannins used also reduced silage SN during ensiling, and were able to reduce degradation of silage nitrogen and true proteins in the rumen. Of the tannins used, quebracho tannin was also able to reduce SN and rumen degradation better than mimosa tannin. However, the...
tannins were not as good as formaldehyde at protecting silage proteins both during ensiling and in the rumen, neither were they better than formic acid in enhancing silage quality. For both the tannins and formaldehyde, formic acid addition further reduced the SN content as a result of the combined effect of rapid acidification and protein binding. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords**: Mimosa tannins; Mobile bags; Myrabolam tannins; Proteolysis; Quebracho tannins; Silage

1. Introduction

Throughout the world, grass is often ensiled and the resultant silage used as feed when fresh grass is unavailable. During ensilage, some grass proteins are degraded to non-protein nitrogen (NPN). Furthermore, residual silage protein is readily degraded in the rumen, with the liberation of ammonia. There is considerable evidence to suggest that ruminal undegradable protein is a desirable component of some feeds (ARC, 1984), as proteins that by-pass the rumen digestive process are a potential source of amino acids for absorption. Treatments of silage with chemicals like formaldehyde (Barry et al., 1978a, b; McDonald et al., 1991; Henderson, 1993) have been used to protect proteins from microbial and plant enzyme hydrolysis. High concentrations of formaldehyde may, however, irreversibly bind the proteins and make them unavailable to the animal (Ashes et al., 1984). Formic acid is also used to improve silage fermentation by lowering the pH (Henderson, 1993), and thus reducing the activities of plant proteases. Reducing the pH also prevents the growth of undesirable microbes (listeria, clostridia, enterobacteriaceae, moulds etc. McDonald et al., 1991). Both formaldehyde and formic acid are toxic chemicals which may cause severe skin, eye and respiratory irritation, and which may release toxic fumes. Formaldehyde is also potentially carcinogenic (Sigma-Aldrich, 1988). Although, improvement in ensiling technologies has made safe handling of chemical additives possible, bulk handling of these chemicals is hazardous and less toxic alternatives would be valuable. Tannins have been identified as showing potential value for the protection of proteins from degradation in the rumen (Waghorn et al., 1987; McNabb et al., 1993; Salawu, 1997). However, questions remain regarding the release of protein by the dissociation of tannin-protein complexes post-ruminally. Salawu et al. (1999), has for example reported that with the tannin-rich browse legume *Calliandra calothyrsus*, the utilization of by-pass protein in the intestine is poor. Separately, other tannin-protein complexes have been reported to disappear almost totally in the rumen (Muhammed, 1997). Notwithstanding the above, tannins appear to have potential as protein protection agents during the ensilage of grass and its subsequent utilization by ruminants. These studies were aimed at evaluating the potential of selected tannins as silage additives. Experiments were conducted to study the effect of treating silages with commercially available tannins on silage composition. The effect on silage composition and total tract disappearance of tannins alone or in combination with formic acid, were also compared with those of formic acid alone or formaldehyde alone or a combination of formic acid and formaldehyde.
2. Materials and methods

2.1. Silage preparation, sampling and treatments

2.1.1. Experiment 1

The silages were made from third cut perennial ryegrass (PRG; *Lolium perenne*) harvested in August 1995 and stored for 2 weeks at \(-20^\circ\)C. One day prior to silage making, the grasses were removed from the freezer and thawed overnight. The tannins (mimosa tannin, myrabolam tannin and quebracho tannin; Hodgson Chemicals, London, UK) were dissolved in water and dispensed in aliquots of 20 ml/kg fresh PRG. Two levels of tannins (5 and 50 g tannin/kg DM) were used. For the 500 g model ensiling, and an estimated DM of 200 g/kg, 5 or 0.5 g tannins were dissolved in 10 ml distilled water to achieve the desired level of 50 or 5 g/kg DM, respectively. An equal volume of distilled water was also added to the controls. Hand mixing of tannins with the grasses was carried out in bulk at the same time for each treatment, after which, the grass was stored in 500 g amounts in polythene bags simulating conditions in a silo. Four replicate treatments were set up and the grass was ensiled for 7 and 32 days. Opened silages were divided into two parts, of which one was used for the extraction of silage juice using a silage press. pH was determined in the silage juice. The other part was freeze-dried and milled to pass through a 1 mm aperture before chemical analyses. The treatments were thus:

1. Control
2. Mimosa tannin (5 g/kg DM)
3. Mimosa tannin (50 g/kg DM)
4. Myrabolam tannin (5 g/kg DM)
5. Myrabolam tannin (50 g/kg DM)
6. Quebracho tannin (5 g/kg DM)
7. Quebracho tannin (50 g/kg DM).

2.1.2. Experiment 2

Perennial ryegrass (1st cut) was mown with a precision chopper on 12th June, 1996. Grasses were chopped to about 5 cm in length using a silage chopper and ensiled on the same day. Silages were prepared as described in Experiment 1 above. The tannins (mimosa and quebracho) were added at a rate of 10 g/kg FW or 50 g/kg DM, and the formaldehyde and formic acid were added at a rate of 2.5 g/kg FW or 12.5 g/kg DM (w/w). The additions were based on an estimated DM content of 200 g/kg.

Three bags were opened from each treatment 7, 14 and 49 days after ensiling. The tie end of each bag was discarded, and samples taken from the mid-sections. Samples were divided into three portions, one of which was used to determine the DM and nitrogen. Silage juice was extracted from the second portion for the determination of VFAs, lactate, ethanol, ammonia, SN and WSC. The third portion was stored at \(-20^\circ\)C, and later freeze-dried. Freeze-dried samples were ground to pass through 3.2 mm or 1 mm apertures (for chemical analysis). Samples milled to pass through 3.2 mm screens were used for the mobile bag degradability study in the gastrointestinal tract (GIT) of dairy cows. The treatments were thus:
1. Control
2. Control +12.5 g formic acid/kg DM (FA)
3. Control +12.5 g formaldehyde/kg DM (F)
4. Control +12.5 g formaldehyde/kg DM +12.5 g formic acid/kg DM (F + FA)
5. Control +50 g mimosa tannins/kg DM (MT)
6. Control +50 g mimosa tannins/kg DM +12.5 g formic acid/kg DM (MT + FA)
7. Control +50 g quebracho tannins/kg DM +12.5 g formic acid/kg DM (QT)
8. Control +50 g quebracho tannins/kg DM +12.5 g formic acid/kg DM (QT + FA)

2.2. Chemical analysis

Nitrogen (Kjeldahl nitrogen) was determined in wet silage. Lactate, ethanol, volatile fatty acids (VFAs), ammonia, soluble nitrogen (Kjeldahl nitrogen) and water-soluble carbohydrates were determined in the silage juice. The silage oven DM and nitrogen were determined following the procedure of the official method of analysis (AOAC, 1990). The DM content of the silages used for the mobile bag experiment was determined before incubation, and on residual materials after incubation. The residual materials were first freeze-dried and the freeze-dried weights taken. Six bags from the freeze-dried residual material were randomly taken and further oven-dried at 50°C for 48 h. The calculated oven-dried DM was then used to correct the DM content of the freeze-dried residual materials. For the analysis of DM, N and amino acid disappearance in the mobile bags, dried residues were pooled into groups of two according to the cows in which they were incubated in the rumen. This gave a final total of three replicates per sample.

The pH of the silage was determined directly from the silage juice using a portable Corning 103 pH meter (Corning Labware and Equipment, Corning, NY). The ammonia contents of the silages were determined using the automated phenol-hypochlorite procedure (Technicon method 321-74A). The fermentation product concentration of silage juice was determined by high performance liquid chromatography (HPLC) using a modification of the method described by Salawu et al. (1997b). Silage juice (0.1 ml) was prepared for analysis by adding 0.1 ml of 50 mM H2SO4 plus 0.1 ml of internal standard (1 ml of 2-ethyl-n-butyric acid made to volume with deionised distilled water in 100 ml volumetric flask) plus 0.7 ml of deionised distilled water. The mixture was centrifuged at 9853 × g for 15 min., and the aliquot analysed immediately. The stock standard contained 5, 2.5, 2.5, 5, 2.5 and 2.5 mg/ml of acetate, propionate, butyrate, lactate, formic acid, and ethanol, respectively. The fermentation products were quantified by reference to an internal standard (2-ethyl-n-butyric acid), and standard chromatogram obtained from the stock standards.

Water soluble carbohydrate (WSC) concentrations were determined using the anthrone reaction rate assay (Koehler, 1952). Extractable proanthocyanidin (PA) was determined from extracts of freeze-dried silages using the butanol-HCl method (Porter et al., 1986). The nitrogen content of the residues were determined according to the official method of analysis (AOAC, 1990), and amino acids were determined following the Altech modified procedure of AOAC (1984) as described elsewhere (Salawu et al., 1997b).
2.3. Total tract disappearance in mobile bags

The total tract disappearance of DM, nitrogen and true protein (sum of amino acids) of the silages were studied in Friesian dairy cows using the mobile bag technique described by Salawu et al. (1999). Mobile bags (18 in total i.e. six replicates each for rumen degradability, pepsin-HCl (200 mg pepsin in 2 l 0.004 M HCl pH 2.4) digestion and intestinal disappearance) were used for each silage sample. Silage samples were ground to pass through a 3.2 mm sieve and 1 (+0.2) g samples were weighed into each bag. The bags were made of 100% mononylon with pore size of 11 μm (Sefar AG, Mesh + Technology, 8803 Rüschlikon, Switzerland). The external dimensions of the bags were 6 × 6 cm and were closed by heat sealing (Elwis pack AS, Denmark).

Rumen degradability was measured in three fistulated non-lactating Friesian cows. The cows were about 3 years old and were maintained on a standard diet consisting of 675 g/kg grass hay and 325 g/kg concentrate. The concentrate was composed of 30 g/kg soya bean meal, 440 g/kg DM barley grain, 440 g/kg DM oat grain, 30 g/kg DM rape seed, 30 g/kg DM low ash fish meal and 30 g/kg DM sugar-beet molasses. The cows also received 200 g/day mineral mix and 21 g/day vitamin mix. Six cows fitted with T-shaped cannulae at the duodenum and the terminal ileum were used for the intestinal disappearance study. These cows were fed a complete diet made up of 550 g/kg grass silage, 245 g/kg grass hay, 133 g/kg concentrate, 68 g/kg barley, 2.7 g/kg mineral mix and 1 g/kg limestone. All the cows were fed twice daily at 0800 h and 1600 h in two equal portions. A maximum of 12 mobile bags was introduced per cow per day into the duodenum through T-shaped cannulae at the proximal duodenum. Bags were recovered from the faeces after 24 h by washing the faeces through a sieved bucket before washing in a front loading washing machine with cold water for 15 min without spinning, and then frozen.

2.4. Statistical analysis

All the data collected were analysed using the Minitab statistical package (Minitab Incorporation, 1995) as one way classified data. The means were separated using the least significant difference method of Steele and Torrie (1980).

3. Results

3.1. Silage pH

The pH of the silages used in experiments 1 and 2 are presented in Tables 1 and 2, respectively. In Experiment 1 the control silage had the lowest pH compared to those that were treated with the tannins. Between the tannin treated silages, those made with the lower level of the tannins (5 g/kg DM) had a higher (p < 0.05) pH after Day 32 than those made with higher tannins. In Experiment 2, the PRG had an average pH of 6.1 before ensiling. The addition of formic acid however, reduced (p < 0.05) the starting pH to 4.5 (Table 2). The pH of the silages dropped to between 4.0 and 4.5 after 7 days of ensiling,
with all the silages without added formic acid or formaldehyde having a lower pH. The silages FA and F‡ FA had a significantly higher pH than other silages after 49 days of ensiling.

### 3.2. Dry matter, WSC and PA content of the silages

The oven-dry DM, WSC and PA contents of the silages in Experiment 1 are presented in Table 3. Ensiling for 32 days generally resulted in a decrease in DM content of all the silages. After correcting for the added tannins, there was no significant difference in the DM of the silages. Silages treated with tannins generally had lower ($p < 0.05$) water...
soluble carbohydrate (WSC) content than the control silage. With the exception of myrabolam tannin-treated silages, treatment with high level of quebracho or mimosa tannins did not significantly affect the WSC content. The relationship between the WSC and PA contents of the silages was negative and strong ($r = 0.84$).

Table 4 shows the oven-dry DM (corrected for added tannins) and WSC content of the silages in Experiment 2. The bags were filled immediately after harvesting, so the DM content of the silages was very low (188 g/kg), the control silage having a significantly lower ($p < 0.05$) DM content than the others. The DM (g/kg) content ranged between 151 for the control to 166 for the silage MT + FA after 49 days. All of the silages containing formic acid had significantly ($p < 0.05$) higher WSC concentrations than the corresponding silages without formic acid after 7 and 14 days of ensilage. After 49 days of ensilage, concentration of WSC was numerically higher ($p > 0.05$) in tannin alone silages in comparison with those containing a mixture of tannins and FA. Except for silage F + FA, the WSC content of the silages decreased with length of ensilage. The rate

### Table 3
Dry matter (DM; g/kg), water soluble carbohydrate (WSC; g/kg DM) and proanthocyanidin (PA; A550 nm/g) content of silage samples opened after 32 days of ensilage in Experiment 1a

<table>
<thead>
<tr>
<th>Silage</th>
<th>DM</th>
<th>WSC</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>183</td>
<td>20.3a</td>
<td>ND</td>
</tr>
<tr>
<td>Mimosa (5 g/kg)</td>
<td>182</td>
<td>7.2be</td>
<td>30.0a</td>
</tr>
<tr>
<td>Mimosa (50 g/kg)</td>
<td>186</td>
<td>8.2be</td>
<td>126.3b</td>
</tr>
<tr>
<td>Myrabolam (5 g/kg)</td>
<td>185</td>
<td>6.4b</td>
<td>30.3a</td>
</tr>
<tr>
<td>Myrabolam (50 g/kg)</td>
<td>193</td>
<td>14.2c</td>
<td>45.3c</td>
</tr>
<tr>
<td>Quebracho (5 g/kg)</td>
<td>179</td>
<td>11.3d</td>
<td>38.7ac</td>
</tr>
<tr>
<td>Quebracho (50 g/kg)</td>
<td>185</td>
<td>9.3de</td>
<td>172.3d</td>
</tr>
<tr>
<td>SEM</td>
<td>3.26</td>
<td>0.80</td>
<td>2.19</td>
</tr>
</tbody>
</table>

*a Means with common letters in columns are not significantly different ($p > 0.05$); SEM: standard error of means; ND: not determined.

### Table 4
Dry matter (DM; g/kg) and water soluble carbohydrate (WSC; g/kg DM) content of silage (Experiment 2)a

<table>
<thead>
<tr>
<th>Silage/days of ensilage</th>
<th>DM</th>
<th>WSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>166a</td>
<td>168a</td>
</tr>
<tr>
<td>FA</td>
<td>181b</td>
<td>177b</td>
</tr>
<tr>
<td>F</td>
<td>172c</td>
<td>165a</td>
</tr>
<tr>
<td>F + FA</td>
<td>180bd</td>
<td>175bc</td>
</tr>
<tr>
<td>MT</td>
<td>178de</td>
<td>173c</td>
</tr>
<tr>
<td>MT + FA</td>
<td>180bd</td>
<td>173c</td>
</tr>
<tr>
<td>QT</td>
<td>170c</td>
<td>176bc</td>
</tr>
<tr>
<td>QT + FA</td>
<td>176e</td>
<td>173c</td>
</tr>
<tr>
<td>SEM</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*a Means with common letters in columns are not significantly different ($p > 0.05$); SEM: standard error of means.
of decrease in WSC was, however, higher in silages made with a combination of tannins/formic acid or formaldehyde alone, in comparison with silages made with tannins alone, formic acid or the control silage.

3.3. Fermentation products

The fermentation products of the silages opened after 32 days of ensiling in Experiment 1 are presented in Table 5. The lactic acid concentration was generally lower \((p < 0.05)\) in silages treated with lower level of tannins in comparison with the controls or that of silages treated with higher level of tannins. All silages treated with tannins had higher \((p < 0.05)\) acetate concentration than the control. Treatment of the silages with higher levels of tannins, however, significantly reduced their acetate concentration when compared with silages made with the lower levels of tannins. The ethanol concentration was higher \((p < 0.05)\) in silages treated with mimosa tannins than the control or other tannin treated silage. Ethanol concentration was comparable \((p > 0.05)\) between the control silage and silages made with higher level of myrabolam or quebracho tannins. Butyrate concentration was lowest in silages treated with the higher level of the tannin, and was generally higher \((p < 0.05)\) in the control than in the tannin treated silage after 32 days of ensiling. The PA content of the silages was negatively related with the lactate \((r = -0.21)\) and butyrate \((r = -0.84)\), positively but weakly related to the ethanol \((r = 0.16)\) and showed no relationship with the acetate concentrations.

The fermentation products of the silages opened after 49 days of ensiling in Experiment 2 are presented in Table 6. The predominant products of fermentation in all the silages were lactate, acetate and ethanol. The lactate concentration was higher \((p < 0.05)\) in the control silage than all the other silages. Whereas a combination of formaldehyde and formic acid significantly reduced the lactate content than when formaldehyde was used alone, the effect of formic acid on lactate was not significant when used with tannins. Acetate concentrations was generally lower \((p < 0.05)\) in all the silages which received formic acid compared to the silages without formic acid. With the exception of silages treated with a combination of tannins and formic acid, the ethanol concentration of all the other silages was significantly lower than that of the control.

<table>
<thead>
<tr>
<th>Silage</th>
<th>Lactate</th>
<th>Acetate</th>
<th>Ethanol</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.5a</td>
<td>11.2a</td>
<td>26.0a</td>
<td>34.9a</td>
</tr>
<tr>
<td>Mimosa (5 g/kg)</td>
<td>2.1b</td>
<td>43.8b</td>
<td>40.9b</td>
<td>18.5b</td>
</tr>
<tr>
<td>Mimosa (50 g/kg)</td>
<td>17.3c</td>
<td>17.9c</td>
<td>45.8c</td>
<td>1.3c</td>
</tr>
<tr>
<td>Myrabolam (5 g/kg)</td>
<td>8.2d</td>
<td>33.4d</td>
<td>33.4d</td>
<td>20.5d</td>
</tr>
<tr>
<td>Myrabolam (50 g/kg)</td>
<td>28.0a</td>
<td>16.9c</td>
<td>23.8a</td>
<td>9.0e</td>
</tr>
<tr>
<td>Quebracho (5 g/kg)</td>
<td>1.5b</td>
<td>46.9e</td>
<td>31.9de</td>
<td>17.2b</td>
</tr>
<tr>
<td>Quebracho (50 g/kg)</td>
<td>5.9d</td>
<td>30.6f</td>
<td>26.7ae</td>
<td>4.2f</td>
</tr>
<tr>
<td>SEM</td>
<td>0.5</td>
<td>0.6</td>
<td>1.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5
Fermentation products (g/kg DM) of silages opened after 32 days of ensiling in Experiment 1

\(^a\) Means with common letters in columns are not significantly different \((p > 0.05)\); SEM: standard error of means.
A combination of formic acid and tannins appeared to increase \((p < 0.05)\) the ethanol concentration than when the tannins were used alone. Propionate was not detected in silages MT, QT and QT\(\,\,^\text{‡}\)FA, and only traces \((p > 0.05)\) was detected in the control silage and MT\(\,\,^\text{‡}\)FA. Propionate concentration of silage F\(\,\,^\text{‡}\)FA was significantly higher than that of other silages. The butyrate concentration was highest \((p < 0.05)\) in silage FA, and was significantly higher in all silages containing formic acid in comparison with silages that did not receive formic acid.

### 3.4. Total nitrogen (TN), ammonia nitrogen \((\text{NH}_3\text{-N})\) and soluble nitrogen \((\text{SN})\) content of silage

The nitrogen contents of the silages are presented in Tables 7 and 8, respectively, for experiments 1 and 2. In Experiment 1, significant difference was observed in the TN content of some of the silages after 7 days of ensilage. However, after 32 days of ensilage,
the difference in the TN content of all the silages was not significant. As with silages opened after 7 days in Experiment 1, significant difference was also observed in the TN content of the silages opened after days 7, 14 and 49 in Experiment 2. In both experiments 1 and 2, there was an increase in TN content of the silages as the length of ensiling increased.

At both sampling times in Experiment 1, the ammonia nitrogen (g/kg TN) content of silages with the lower level of tannins was higher \((p < 0.05)\) than those of the control silage or those receiving more tannins. In Experiment 2, the NH\(_3\)-N content was significantly higher \((p < 0.05)\) in the control silage than other silages after 7 days of ensiling. Treatment with a combination of formaldehyde/formic acid or mimosa tannin/formic acid significantly \((p < 0.05)\) reduced the NH\(_3\)-N content of the silages in comparison with treatment with formaldehyde or mimosa tannin alone after 7 days of ensilage. The NH\(_3\)-N content increased with increasing time of ensiling. However, the increase was less in the control silage and silages FA, F or F + FA than in silages containing tannins after 14 days of ensiling. Between Day 14 and 49 of ensiling, the rate of increase in NH\(_3\)-N content of the silages was higher in silages FA, F and F + FA than in the silages treated with tannins.

Silages made with the higher level of the tannins in Experiment 1 (Table 7) had a significantly lower \((p < 0.05)\) SN content than the control or silage made with lower tannins. In Experiment 2 (Table 8), treatment of PRG with all of the additives used here significantly \((p < 0.05)\) reduced the SN content of the silage. The reduction in SN was, however, greater \((p < 0.05)\) when the silages were made with a combination of formaldehyde/formic acid or tannins/formic acid. Thus, reductions in SN of 32, 23, 23, and 38\%, respectively, were obtained in silages FA, F, MT and QT in comparison with reductions in SN of 49, 39 and 46\% recorded in silages F + FA, MT + FA and QT + FA, respectively after 7 days of ensiling. After 49 days of ensiling, the reductions in SN content of silages F + FA, MT + FA and QT + FA were 61, 41 and 41\%, respectively. Between the tannins, quebracho tannins alone significantly lowered \((p < 0.05)\) the SN

### Table 8

<table>
<thead>
<tr>
<th>Silage</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 49s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN</td>
<td>NH(_3)-N</td>
<td>SN</td>
</tr>
<tr>
<td>Control</td>
<td>26.4ac</td>
<td>28.8a</td>
<td>619a</td>
</tr>
<tr>
<td>FA</td>
<td>27.9a</td>
<td>10.3b</td>
<td>421b</td>
</tr>
<tr>
<td>F</td>
<td>24.0bc</td>
<td>19.9c</td>
<td>476c</td>
</tr>
<tr>
<td>F + FA</td>
<td>26.4ac</td>
<td>10.6b</td>
<td>314d</td>
</tr>
<tr>
<td>MT</td>
<td>26.5ac</td>
<td>19.4c</td>
<td>474e</td>
</tr>
<tr>
<td>MT + FA</td>
<td>25.3c</td>
<td>13.0d</td>
<td>375f</td>
</tr>
<tr>
<td>QT</td>
<td>27.5a</td>
<td>13.7d</td>
<td>384f</td>
</tr>
<tr>
<td>QT + FA</td>
<td>25.4c</td>
<td>15.2e</td>
<td>337g</td>
</tr>
<tr>
<td>SEM</td>
<td>0.6</td>
<td>0.4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

a Means with common letters in columns are not significantly different \((p > 0.05)\); SEM: standard error of means.
Table 9
Cumulative disappearance of dry matter, nitrogen and true protein from grass silage in mobile bags in the rumen, pepsin-HCl and intestine of cows

<table>
<thead>
<tr>
<th>Silage</th>
<th>Dry matter (g/kg)</th>
<th>Nitrogen (g/kg TN)</th>
<th>True protein (g/kg total amino acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rumen</td>
<td>Pepsin-HCl</td>
<td>Intestine</td>
</tr>
<tr>
<td>Control</td>
<td>560a</td>
<td>592a</td>
<td>616ac</td>
</tr>
<tr>
<td>FA</td>
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<td>644b</td>
<td>678b</td>
</tr>
<tr>
<td>F</td>
<td>506cd</td>
<td>542ce</td>
<td>597a</td>
</tr>
<tr>
<td>F + FA</td>
<td>486cef</td>
<td>515d</td>
<td>594a</td>
</tr>
<tr>
<td>MT</td>
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<td>605ac</td>
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<tr>
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<td>520de</td>
<td>599ac</td>
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<tr>
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<td>474ef</td>
<td>522cd</td>
<td>606ac</td>
</tr>
<tr>
<td>QT + FA</td>
<td>469f</td>
<td>515d</td>
<td>622c</td>
</tr>
<tr>
<td>SEM</td>
<td>10.7</td>
<td>11.6</td>
<td>10.1</td>
</tr>
</tbody>
</table>

* Means with common letters in columns are not significantly different (p > 0.05); cumulative disappearance: incremental disappearance across the gastrointestinal tract; SEM: standard error of means.
content of the silage more than the mimosa tannins alone after 49 days of ensilage. Formaldehyde alone only reduced \((p < 0.05)\) the SN content more than MT alone. A combination of formaldehyde/formic acid also significantly \((p < 0.05)\) reduced the SN content of the silage compared with the tannin/formic acid combinations after 49 days of ensilage. The PA content of the silages was positively related with the TN \(r = 0.56\) and negatively related with the ammonia nitrogen \(r = -0.37\) and SN \(r = -0.90\) content of the silages.

3.5. Disappearance of dry matter (DM), nitrogen and total amino acid from mobile bags

The apparent cumulative disappearance of DM, nitrogen and true proteins from mobile bags in the rumen, pepsin-HCl solution and the intestine are presented in Table 9 as g/kg of the original material. All the silages containing tannins had lower \((p < 0.05)\) DM disappearance in the rumen than the control silage. Similarly the proportion of rumen undegradable DM which was lost in the lower tract was higher \((p < 0.05)\) in silages containing tannins (19.6%) and in silage F + FA (18%) than in the control (11.2%) or silage FA (9.4%) or F (15.6%). The total cumulative DM disappearance was, therefore, essentially the same \((p > 0.05)\) between the control silages and all the silages containing tannins. Dry matter disappearance was highest in silage FA and lowest in silage F + FA.

In comparison with the other silages, the control silage and silage F + FA, respectively, had the highest \((p < 0.05)\) and lowest \((p < 0.05)\) nitrogen disappearance in the rumen. The cumulative disappearance of nitrogen after the passage of the mobile bags through the intestine was comparably higher \((p < 0.05)\) in the control and silages FA, MT + FA, QT, and QT + FA than in other silages. The actual disappearance of rumen undegradable nitrogen, in the intestines was highest in silage QT + FA (18.6%) and lowest in the control (5.9%) silage.

The pattern of true protein (defined as the sum of the amino acids measured) disappearance in the silages was similar to that of the nitrogen disappearance. The control silage lost a significantly higher \((p < 0.05)\) proportion of its true protein in the rumen, and only about 10% of the silage true protein was digested post ruminally. In contrast with the control silage, a significantly lower percentage of the silage true protein disappeared in the rumen with all the other silages that were treated with additives. A combination of formic acid with formaldehyde or tannins significantly reduced \((p < 0.05)\) the disappearance of true protein in the rumen, and increased the actual disappearance in the intestine of the rumen undegradable true protein, than formic acid, formaldehyde or tannins alone. The silage F + FA had the lowest \((p < 0.05)\) disappearance of true protein in the rumen and also in the pepsin-HCl solution, and the highest actual disappearance (33.4%) of true protein in the intestine.

4. Discussion

One of the main reason for using tannins as silage additives was to see if they would be able to protect grass/herbage proteins from rapid hydrolysis during ensiling, whilst being less hazardous than existing alternative treatments. Hydrolysis of proteins is mainly due
to plant peptidase activities (Carpintero et al., 1979; McKersie, 1981; Heron et al., 1988). It is optimal between pH 5–7, and continues at much reduced rate at a value below 4 (Henderson, 1993). Not surprisingly, acidifying the PRG with formic acid reduced the initial pH to below the optimum pH level for plant peptidase activities, and thus reduced the silage proteolysis (as indicated by reduced SN). Reduction in proteolysis by formic acid has been documented by Barry et al. (1978a,b), Carpintero et al. (1979), Nsereko (1996). The lowest pH of 4.6 attained by the control silage after 7 days of ensiling in Experiment 1 was above the pH (4) below which the activities of plant proteases were thought to be greatly reduced (Heron et al., 1988). Degradation of plant true proteins to amino acids by plant proteases was, therefore, thought to be considerable in all the silages. High levels of proteolysis may increase the buffering capacity of the silages and this may further delay the attainment of low stable pH (Ohshima and McDonald, 1978; Papadopoulos and McKersie, 1983; Henderson, 1993). The low final pH achieved in the control silage in Experiment 2 and all the other silages that have no formic acid treatment after 49 days of ensilage suggest that good fermentation occurred.

The high WSC content of control silage may have been due to an increased production of WSC from cell wall carbohydrates (Carpintero et al., 1979; McDonald et al., 1991). In contrast, the interaction of tannins with and their possible inhibition of cell wall degradation (Barry and Manley, 1984; Terrill et al., 1989; Reed, 1995) may contribute to the general reduction in WSC content of the tannin treated silage. However, the higher WSC of silages treated with a combination of either tannins or formaldehyde and formic acid on days 7 and 14 may be due to the effect of the rapid reduction in pH associated with formic acid on the rate of interaction of the tannins or formaldehyde. Low pH has been reported to reduce the interaction of the tannins with nutrients (Oh and Hoff, 1987; Field and Lettinga, 1992). The oven DM reported in this study was only corrected for the added tannins and not for volatile losses. However, lesser DM loss in silages treated with tannins, formaldehyde or formic acid may be due to reduction in natural fermentation in these silages. Tannins have been reported to inhibit the digestibility of carbohydrates and proteins in plants (Barry and Manley, 1984; Terrill et al., 1989; Reed, 1995; Perez-Maldonado and Norton, 1996). The higher level of tannins used here has also been reported to reduce the activities of silage bacteria and moulds (Salawu, 1997), and could have reduced the conversion of lactate to acetate, ethanol or butyrate. Since, clostridia are considered to be mainly responsible for the production of butyric acid (Woolford, 1984), the lower butyrate concentration in silages made with 50 g/kg DM tannins in Experiment 1, may be due to inhibition of clostridia by the tannins. Formic acid alone or in combination with formaldehyde appears to reduce the activities of lactic acid bacteria. However, the high concentration of ethanol and butyrate in relation to lactate and acetate may suggest secondary fermentation in these silages. When formic acid was used with tannins, lactate fermentation was evident. However, as mentioned earlier, a reduction in pH by formic acid when used with the tannins was thought to reduce the rate of fermentation.

The reduction in SN observed with the formaldehyde treated silage is presumably the result of binding of the proteins by the formaldehyde (Barry et al., 1978a,b). However, not all of the proteins were bound to the formaldehyde as indicated by the SN content when formaldehyde was used alone. Also, some of the proteins would have been
hydrolysed before the reaction occurred. Rapid proteolysis has been noted to start immediately after harvesting (Henderson, 1976; McKersie, 1981), and most of the hydrolysis occurs during the first week of ensiling (Kemble, 1956). When formaldehyde was used with formic acid, the combined effect of rapid acidification and protein binding resulted in a much higher reduction in SN concentration (McDonald et al., 1991). Reduction in SN with formaldehyde or formaldehyde/formic acid means a reduction in soluble precursors for ammonia production, and hence a reduction in ammonia concentration of the silage.

Treatment of herbage with tannins also reduced the SN content of the silage. Tannins protect proteins from enzyme hydrolysis in a similar way to formaldehyde, by binding to the proteins or free amino acid and rendering them inaccessible for enzyme hydrolysis (Hagerman and Butler, 1981; Barry, 1989; Wang et al., 1996). Tannins also bind to enzymes (Kumar and Singh, 1984; Horigome et al., 1988; Makkar et al., 1990; Salawu et al., 1998). The observation of reduced SN made here with tannins is in agreement with the findings of Albrecht and Muck (1991), who also showed a strong negative relationship between tannin concentrations and SN concentrations in legume silages. The differences in the tannins in the concentration of their active materials, molecular weight and degree of polymerisation (Field and Lettinga, 1992; Reed, 1995), and pH of the silages, may account for the differences observed in the ability of the different tannins to cross link the protein/proteases and thus reduced proteolysis. As with formaldehyde, when tannin was used in combination with formic acid, there was a combined effect of the acid (rapid reduction in pH and thus activities of plant proteases) and the tannins (complexing with the proteins). This is perhaps a little surprising since the reduced pH may reduce the ability of tannins to complex with the various nutrients (Oh and Hoff, 1987; Field and Lettinga, 1992; Salawu et al., 1997a). The tannins alone were not as effective at reducing proteolysis as formaldehyde alone. Similarly, a combination of tannin/formic acid was not as effective as formaldehyde/formic acid. The reason for the stronger effect of formaldehyde is not known, but the lower mass of this compound may enable it to gain access to binding sites unavailable to tannins. It is also possible that the chemical structure, molecular weight and degree of polymerisation of the tannins used here does not permit effective cross linking with the silage proteins and enzymes. Quebracho tannins for example have a branched chain structure which is compact and not very accessible for binding to macromolecules (Hagerman and Robbins, 1993). It should also be noted that the tannins used here are crude extracts that contain mixture of compounds. The reason for the differences in TN between silages and with length of ensilage is not known. It may, however, be partly due to the differences in DM content of the silages.

In addition to reducing proteolysis during ensiling, both formaldehyde and the tannins also reduced the disappearance of DM, nitrogen and amino acids in the rumen, and shifted the point of disappeararance of undegraded nitrogen and amino acids to the lower digestive tract. Reduced intestinal disappearance was not expected with the level of formaldehyde used here, however, higher rates of inclusion have been reported to be capable of binding to proteins irreversibly, rendering them unavailable to the animal (Ashes et al., 1984). Similarly, because of crude nature and the likely changes during ensilage to the tannins used here, the higher rate of addition (50 g/kg DM) may not
adversely affect microbial metabolism in the rumen. This will, however, require verification in a feeding trial. Thus, in both the formaldehyde and tannin treated silages, the reductions in rumen degradation were compensated for by increased disappearance in the intestine. It should however, be noted that contrary to our expectations, most of the nitrogen in the tannins and formaldehyde treated silages disappeared in the rumen. This suggests that both the tannins and formaldehyde were able to protect herbage proteins from plant/microbial enzyme hydrolysis during ensiling, but were unable to sufficiently protect the protein from hydrolysis in the rumen. As mentioned earlier, the low final pH of the silage may weaken the strength of tannin-protein or formaldehyde-protein complexes thereby making them more susceptible to loss in the rumen. This characteristic has also been found with tannin-casein and tannins-bovine serum albumin complexes (Muhammed, 1997; Salawu et al., 1997a).

5. Conclusions

The fermentation and quality of silages obtained in Experiment 1 with or without tannins were generally poor. However, the results obtained in Experiment 2 suggest that tannins might be used as silage additives. The most obvious effect of tannins in both experiments is in the reduction in SN and as such proteolysis during ensilage. The tannins were also able to reduce degradation of silage true proteins in the rumen, and protected proteins were digested in the intestine without any adverse effect on the overall DM and nitrogen digestion. The tannins used here were not as good as formaldehyde in protecting silage proteins, and were unable to enhance silage quality like formic acid. However, the wide variety of tannins available, and the extremely hazardous properties of formaldehyde, justify further work to establish the potential for the use of a variety of tannins at differing concentrations and conditions of use as silage additives.

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References


