Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 261–265

Protein fractions (Cornell system) of lucerne dried or ensiled with different additives*

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ABSTRACT

Effects of formic acid, formaldehyde, tannic acid or mixture of each two of the additives on five protein fractions based on the Cornell Net Carbohydrate and Protein System (CNCPS) in hay and ensiled lucerne (*Medicago sativa*) were studied. After 35 d of ensiling, the additives significantly reduced fraction A in the ensiled forages. Fraction B_1 in all of the additive-treated silages was significantly higher than that in control silage. Large amounts of true protein in the formic acid/formaldehyde treated- and tannic acid/formaldehyde treated-silages were fractions B_3 and fraction B_2, respectively. No difference was observed on fraction C content between the control silage and silages treated with additives except for the formaldehyde or tannic acid treated-silages.

KEY WORD: protein fractions, lucerne, hay, silage, CNCPS

INTRODUCTON

It is well established that the nutritive value of ensiled legume forage is limited by extensive protein degradation that occurs in many grass species during ensiling (Albrecht and Muck, 1991). It may be concluded that the previous studies mainly focused on the inhibitory effects of the above additives on the formation of NPN in ensiled forage, and analysis of protein in silage is generally limited to determination of crude protein and NPN constituents. To our knowledge, however, the Cornell Net Carbohydrate and Protein System (CNCPS) of crude protein fractions has not been

* Supported by the Chinese Ministry of Agriculture, Project NYHYZX07-022
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used to improve characterization of the quality of ensiled lucerne and how silage additives may affect the fractions of proteins in ensiled lucerne. Our objectives were to determine the effect of formic acid, formaldehyde and tannins or mixture of each two of the additives on protein fractions based on the CNCPS.

MATERIAL AND METHODS

Lucerne (330 g DM per kg forage) was chopped to about 1.2 cm lengths using a paper-cutter and ensiled in laboratory silos and was either untreated (control) or treated with formic acid (5.4 g/kg fresh weight (FW)), formaldehyde (3 g/kg FW), tannic acid (15.2 g/kg FW) or mixture of formic acid (2.7 g/kg FW)/formaldehyde (1.5 g/kg FW), formic acid (2.7 g/kg FW)/tannic acid (7.6 g/kg FW) and formaldehyde (1.5 g/kg FW)/tannic acid (7.6 g/kg FW). Additives were prepared on the day of ensiling and applied, in solution, at a rate of 20 ml kg^{-1} of herbage. An equal volume of distilled water alone was added to the control. Mini-silos with triplicate were made for each treatment following the method described previously (Muck, 1987).

Triplicate silos from each treatment were opened at 35 d of ensiling and analysed for DM content, total nitrogen (TN) and non-protein nitrogen (NPN) (Muck, 1987). The crude protein in forage samples was partitioned into five fractions as proposed by the CNCPS (Sniffen et al., 1992; Chalupa and Sniffen, 1994). True protein nitrogen, buffer-insoluble protein nitrogen, neutral detergent-insoluble nitrogen (NDIN) and ADIN of forage samples were analysed as described by Licitra et al. (1996).

The data were subjected to analysis of variance using the one-way ANOVA procedure of the Statistical Package for the Social Science (SPSS 11.0, SPSS, Inc., Chicago, IL) and the differences among treatment means were tested with the LSD Multiple Range Test.

RESULTS AND DISCUSSION

Initial forage pH was 6.04 declining to 4.48 after 35 d of fermentation in the control silage (Table 1). All acid-treated silages had significant lower pH than the control silage, but in the silages treated with formaldehyde, tannic acid and combination of each two of the additives, the pH was significantly higher than in the control silage. Significant amounts of the NPN constituents of NH_3-N, free AA-N and peptide-N were observed in hay forage compared to the fresh forage (Table 1). All additives significantly reduced the NPN, NH_3-N and AA-N contents in the ensiled forage compared to the control silage. The most effective reduction of NPN and AA-N was observed in the formic acid/formaldehyde-treated silage, since when formic acid was

used with formaldehyde, the combined effect of rapid acidification and protein binding resulted in a much higher reduction in soluble nitrogen (Salawu et al., 1999).

Fraction A accounted for 15.3% of CP (DM basis) in the fresh forage (Table 2). In the hay forage, however, fraction A was significant higher than that in the fresh forage ($P<0.05$). More than half of CP in the fresh and wilted forage was the rumen rapidly degraded protein-fraction B_1; however, a small amount of fraction B_1 was observed in hay forage which was only 3.74% of CP (Table 2), and large amount of CP was consequently converted to fraction B_2.

Table 1. The pH and composition of total nitrogen fractions

Treatment	pH	CP % of DM	Composition of total N, g kg^{-1} total N			
			NPN	NH_3-N	AA-N	peptide-N
Lucerne						
fresh	6.04^{b}	22.20^{c}	150.30^{g}	2.80^{h}	66.52^{i}	80.99^{bc}
wilted	6.18^{a}	22.70^{b}	170.80^{f}	3.43^{gh}	68.25^{i}	99.12^{b}
hay	6.29^{a}	23.65^{a}	286.81^{e}	5.67^{g}	120.09^{h}	161.04^{a}
Ensiled forage						
control	4.48^{e}	21.36^{d}	684.02^{a}	66.92^{a}	518.73^{a}	98.37^{b}
FA[1]	3.86^{g}	22.24^{c}	506.59^{c}	27.29f	316.94^{f}	162.37^{a}
FD	4.69^{d}	22.50^{bc}	571.71^{b}	31.56^{e}	434.71^{c}	105.44^{b}
TA	4.84^{c}	21.64^{d}	574.48^{b}	50.85^{b}	480.06^{b}	43.58^{d}
FA+FD	4.14^{f}	22.46^{bc}	433.75^{d}	34.70^{d}	242.01^{g}	157.03^{a}
FA+TA	3.96^{g}	21.26^{d}	437.40^{d}	36.28^{d}	335.26^{e}	65.87^{cd}
FD+TA	4.69^{d}	21.36^{d}	511.23^{c}	47.09^{c}	384.79^{d}	79.35^{bc}
SEM	0.14	0.13	28.93	3.62	27.59	7.40
P-value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

[1] non-protein nitrogen (NPN), ammonia-nitrogen (NH_3-N); free amino acid-nitrogen (AA-N); and peptide nitrogen (peptide-N) in fresh, wilted, hay and ensiled lucerne; FD - formaldehyde; FA - formic acid; TA - tannic acid

[abcdefghi] means within columns not sharing a common letter differ ($P<0.05$)

After 35 d of ensiling, most of forage true proteins were converted into fraction A (Table 2). All of the added additives significantly reduced fraction A content in the ensiled forages and the mixture of formic acid and formaldehyde was more effective in inhibiting formation of fraction A in ensiled forage than the remainder additives. In addition, large amounts of true protein in the formic-acid/formaldehyde treated-silages were fractions B_3. In the control silage, only 1.46% of CP of fraction B_1 was detected (Table 2). The content of fraction B_1 in all of the additive-treated silages was significant higher than that in control silage ($P<0.05$), especially in formaldehyde-treated silage. It is probably due to more effective protection with formaldehyde to forage proteins than that of formic acid or tannins to forage proteins (Salawu et al., 1999). Adding each of the three mixture additives at ensiling significantly increased the content of true protein

Table 2. Protein fractions of fresh, wilted, hay and ensiled lucerne

Treatment	Protein fractions, g/100 crude protein				
	Fraction				
	A[1]	B_1	B_2	B_3	C
Lucerne					
fresh	15.03^{g}	56.99^{a}	13.52^{cd}	1.95^{d}	12.47^{bc}
wilted	17.08^{f}	55.38^{a}	14.50^{c}	2.71^{d}	10.33^{e}
hay	28.68^{e}	3.738^{d}	41.14^{a}	15.37^{b}	11.07^{cd}
Ensiled forage					
control	68.40^{a}	1.46^{e}	14.56^{c}	3.26^{d}	12.33^{bc}
FA	50.66^{c}	11.11^{c}	11.04^{ef}	14.00^{b}	12.65^{b}
FD	57.17^{b}	14.89^{b}	10.22^{f}	3.41^{d}	14.31^{a}
TA	57.45^{b}	13.41^{b}	12.22^{de}	2.08^{d}	14.84^{a}
FA+FD	43.37^{d}	9.77^{c}	13.40^{cd}	21.63^{a}	11.83^{bc}
FA+TA	43.74^{d}	10.82^{c}	18.23^{b}	14.57^{b}	11.98^{bc}
FD+TA	51.12^{c}	10.36^{c}	18.73^{b}	8.00^{c}	11.79^{bc}
SEM	2.72	2.86	1.28	1.24	0.25
P-value	P-0.001	P-0.001	P-0.001	P-0.001	P-0.001

^{abcdefg} means within columns not sharing a common letter differ ($P<0.05$)

A - nonprotein nitrogen compounds instantaneously degraded in the rumen; B_1, B_2, B_3 - rapidly, intermediately, and slowly degraded protein, respectively; C - bound protein undegraded in the rumen and indigestible in the intestine; FD - formaldehyde; FA - formic acid; TA - tannic acid

fractions when compared to the control silage that was due to the combination of these additives to forage proteins, and large amounts of true protein were fraction B_2 in the silage treated with tannic acid/formaldehyde.

No difference was observed on fraction C content between the control silage and silages treated with additives except for the formaldehyde or tannic acid treated-silages. The significant amounts of fraction C observed in silages treated with formaldehyde or tannic acid alone probably resulted from the formation of formaldehyde- or tannic acid-protein complexes when each of the additives was added to the forage at ensiling with the concentrations applied in the present study. This implies that formaldehyde or tannic acid increased the content of proteins in ensiled forage that are unavailable to ruminants. The previous studies also proposed that high concentration of formaldehyde (Ashes et al., 1984) or tannin (Salawu et al., 1999) may irreversibly bind to the proteins and make them unavailable to the animal.

CONCLUSIONS

More than half of CP in the fresh and wilted forage was the rumen rapidly degraded protein-fraction B_1; however, a large amount of CP was converted to fraction B_2 and B_3 in hay forage showing that the rumen degradation of hay protein

is lower than of protein in fresh lucerne. Silage making increased the content of instantaneously degradable protein, but all the tested additives could to some extent reduce fraction A in the silage. Fraction C was increased by using formaldehyde and by using tannin showing that there is a risk of making the protein totally indigestible by using these additives.

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