The fixation of nitrogen in urea ammoniated wheat straw by means of different acids

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The fixation of nitrogen in urea ammoniated wheat straw by means of different acids was investigated in two laboratory trials and in a voluntary-intake trial with adult SA Mutton Merino wethers. Results from the laboratory trials showed hydrochloric and sulphuric acid to be superior to phosphoric and acetic acid as fixation agents, and indicated that the N content of urea ammoniated wheat straw could be effectively increased by as much as 60%, following treatment with the former two acids. However, the low and variable voluntary intakes obtained with ammoniated straw treated in this way limits practical use.
S. Afr. J. Anim. Sci. 1984, 14: 173–176

Die fiksering van stikstof in ureum-geammonifiseerde koringstrooi deur middel van verskillende sure is in twee laboratoriumproewe en in 'n vrywillige inname-studie met volwasse SA Vleismerinohamels ondersoek. Resultate uit die laboratoriumproewe toon dat fiksering van stikstof met soutsuur of swawelsuur meer doeltreffend is as met asynsuur of fosforsuur, en dui daarop dat die stikstofinhoud van ureum-geammonifiseerde koringstrooi met tot 60% verhoog kan word deur die fiksering van ammoniak met eersgenoemde twee sure. Die praktiese gebruikswaarde van hierdie behandeling word egter beperk deur die lae en wisselvallige vrywillige innames verkry op geammonifiseerde strooi daarvolgens behandel.
S.-Afr. Tydskr. Veek. 1984, 14: 173–176

Keywords: Ammoniated wheat straw, nitrogen fixation, acids, voluntary intake

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Received 14 February 1984

Introduction

Ammoniation is now a generally accepted method of improving the nutritive value of low-quality roughages. Prior to feeding ammoniated roughages to animals, it is usually thoroughly aerated to remove excessive amounts of ammonia. The loss of ammonia-nitrogen from roughages treated in this way is quite extensive and may amount to 66% (Sundstøl, Coxworth & Mowat, 1978) or from 60 to 65% (Solaiman, Horn & Owens, 1979) of the ammonia originally included. The retention of this nitrogen by means of fixation by different acids was investigated by Borhami, Sundstøl & Garmo (1982) and Jayasuriya & Pearce (1983). These results indicated that ammonia-nitrogen in treated roughages can effectively be fixed by treatment with acids. Fixation of ammonia by formic and acetic acid significantly improved the apparent digestibility of nitrogen, when measured with sheep (Borhami *et al.*, 1982). Published results furthermore indicate that treatment with sulphuric acid advantageously affected the *in sacco* degradability of barley straw (Fahmy & Ørskov, 1984).

The fixation of nitrogen in ammoniated roughages may also be of value under South African conditions. Treatment conditions for the fixation of nitrogen in urea ammoniated wheat straw were thus investigated in two laboratory trials and in a voluntary-intake trial.

Materials and Methods

In a preliminary laboratory trial, wheat straw was ammoniated with 75 g urea/kg straw at moisture levels of 250 and 375 g/kg straw in 26 glass containers of 450 ml each. After ammonia treatment for 6 weeks, 24 of the samples in these containers were treated with four different acids (acetic, phosphoric, hydrochloric and sulphuric acid) at three fixation levels (calculated to fix approximately 25, 50 and 100% of the nitrogen added to the straw), sealed airtight, and incubated for a further 2 weeks. After incubation all these containers were opened and the samples dried in a forced-draught oven at 59 °C before being analysed for total nitrogen (N) content (AOAC., 1970), urea (Technicon Auto analyser, 1977), free ammonium (Technicon Auto Analyser II, 1977), *in vitro* organic matter digestibility (Engels & Van der Merwe, 1967), and neutral detergent fibre (Van Soest & Wine, 1967). *In vitro* organic matter digestibility (IVOMD) and neutral detergent fibre (NDF) were corrected for the urea and free ammonium (NH_4^+) contents of the respective samples. The two containers (one at each moisture level) to which no acid was added served as controls and were not included in the statistical analysis. Standard procedures for the analysis of a 4 × 3 × 2

factorial design with one replication were applied, including four acids, three fixation levels and two moisture levels (the latter referring to the urea ammoniation pretreatment).

In the second laboratory trial, a plastic bag of wheat straw was ammoniated with 50 g urea/kg straw at a moisture level of 400 g/kg straw. After 8 weeks of ammoniation approximately 90 g of the ammoniated straw was transferred to each of 62 plastic containers, acid-treated and sealed airtight. Treatments included two acids (HCl and H_2SO_4) at two fixation levels (50 and 100%) and three acid-dilution levels (the appropriate amount of acid diluted to 20, 30 and 40 ml with distilled water respectively) for five treatment periods (0; 2,5; 5; 10 and 20 days), while two samples were left untreated for control purposes. None of the parameters investigated were significantly affected by treatment period, and this effect was thus used as replications in the statistical analysis, resulting in a 2 × 2 × 3 factorial trial with five replications. Subsequent treatment and laboratory analyses were similar to that described for the first laboratory trial.

Based on the results of the previous laboratory trials, 128 bales of urea ammoniated wheat straw was acid treated with HCl and H_2SO_4 at a fixation level of 50%. After a treatment period of 14 days, the acid-treated straw was dried on a cement floor to approximately 90% dry matter and hammermilled to pass a 12 mm screen before being fed to 10 adult SA Mutton Merino wethers in a voluntary intake trial.

Results and Discussion

The effect of nitrogen fixation by different acids at different fixation levels on the NH_4^+ content of urea ammoniated wheat straw is presented in Figure 1. It is evident that nitrogen was effectively fixed by hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) at all fixation levels. The lower NH_4^+ content of wheat straw ammoniated at the lower moisture level and acid treated at the 100% fixation level may possibly be explained by partial digestion after acid treatment. Less urea is converted to ammonia after urea ammoniation at low moisture levels (Kritzinger & Franck, 1981). It is thus possible that insufficient amounts of ammonia were available for the neutralization of the acids, especially when higher acid levels were added to the straw. As a result, the remaining acid may have reacted with the organic matter and/or remaining urea in these samples. Both acetic acid and phosphoric acid appeared to be inferior to HCl and H_2SO_4 as fixation agents, especially at the higher moisture level. No apparent explanation exists for the high NH_4^+ content of wheat straw treated with phosphoric acid at the lower moisture level and at the 100% fixation level. According to results obtained by Borhami, *et al.* (1982), fixation of ammonia by acetic acid was more effective than by phosphoric acid. In the present study no significant differences in N and NH_4^+ content was obtained between these two acids.

Degrees of freedom and appropriate mean squares for the second laboratory trial are presented in Table 1. Means for the respective dependent variables are presented as third-order interactions in Table 2, and as overall means for the main effects in Table 3. According to these results the N content of samples treated with HCl was significantly ($P < 0,01$) higher than in samples treated with H_2SO_4. This difference appears to be caused by a higher level of urea conversion by the latter acid. The reaction whereby urea was converted by the respective acids is not clear. NH_4^+ content was not significantly affected by acids. Total N and NH_4^+ was significantly ($P < 0,01$) higher at the 100% fixation level, while urea content tended to be significantly lower, possibly owing to a higher level of acid conversion. The latter trend was only discernible in the case of HCl, resulting in a significant ($P < 0,01$) acid × fixation level interaction (Tables 1 and 2). Fixation of nitrogen in ammoniated wheat straw, by treatment with H_2SO_4 and HCl at the 100% fixation level, resulted in respective increases of 59,3 and 64,4% in the N content, when compared to the untreated control samples. Corresponding increases at the 50% fixation levels were 57,2 and 59,4% respectively. IVOMD of samples treated with H_2SO_4 was significantly ($P < 0,01$) higher than in samples treated with HCl. The higher fixation level also tended to reduce the IVOMD of samples significantly ($P < 0,01$) when compared to the 50% fixation level. Fixation of nitrogen in urea ammoniated wheat straw with HCl tended to reduce the IVOMD by 5,6%, when compared to the untreated control samples.

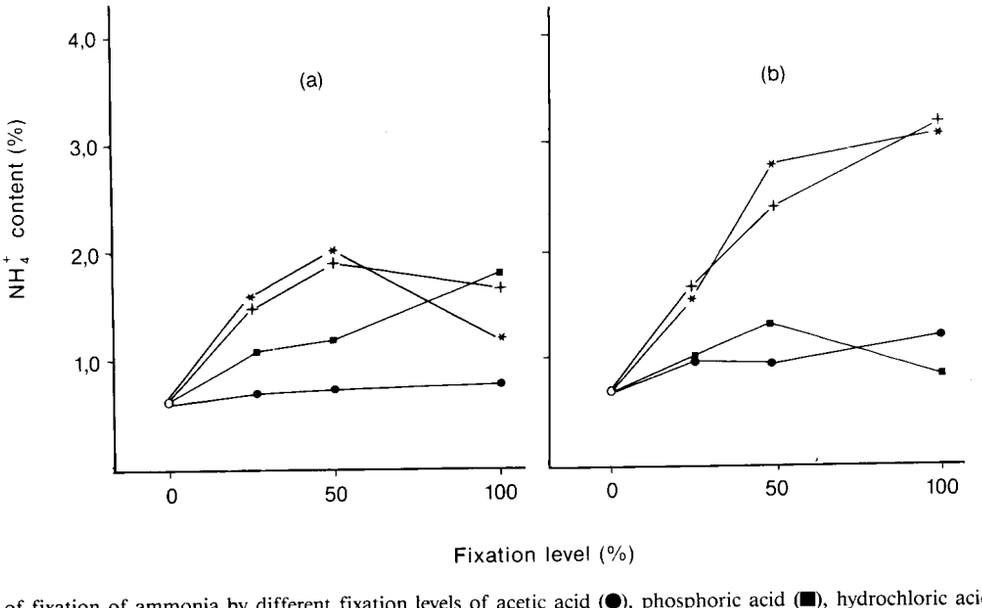

Figure 1 The effect of fixation of ammonia by different fixation levels of acetic acid (●), phosphoric acid (■), hydrochloric acid (★) and sulphuric acid (+) on the NH_4^+ content of wheat straw ammoniated by urea at moisture levels of 250 g/kg (a) and 375 g/kg (b), where (○) represents an untreated control value.

Table 1 Degrees of freedom and mean squares for variables investigated in the second laboratory trial where wheat straw was treated with two acids (HCl and H_2SO_4) at two fixation levels (50 and 100%) and three dilution levels (20, 30 and 40 ml)

Item	df	Mean square of dependent variables				
		Total N	Urea	NH_4^+	IVOMD	NDF
Independent variables						
Acids (A)	1	0,0770[b]	0,1135[b]	0,0120[ns]	190,46[b]	9,20[ns]
Levels of fixation (L)	1	0,0728[b]	0,0244[b]	1,4947[b]	29,32[b]	1780,41[b]
Dilution of acid (D)	2	0,0018[ns]	0,0012[ns]	0,0209[ns]	7,61[ns]	8,56[a]
Interactions						
A × L	1	0,0120[ns]	0,0213[b]	0,0212[ns]	19,43[a]	76,57[b]
A × D	2	0,0004[ns]	0,0003[ns]	0,0120[ns]	7,03[ns]	0,78[ns]
L × D	2	0,0069[ns]	0,0003[ns]	0,0145[ns]	9,48[a]	1,76[ns]
A × L × D	2	0,0120[ns]	0,0011[ns]	0,0025[ns]	4,99[ns]	1,62[ns]
Error	48	0,0046	0,0017	0,0136	2,71	2,49

[ns]Not significant; [a]Significant ($P<0,05$); [b]Significant ($P<0,01$).

Table 2 Treatment means for variables investigated in the second laboratory trial (DM basis) where wheat straw was treated with two acids at two levels of fixation and three dilution levels

Acid	Level of fixation (%)	Dilution (ml)	Treatment means (%) of dependent variables				
			Total N	Urea	NH_4^+	IVOMD	NDF
Sulphuric	50	20	3,07	0,162	2,13	54,00	65,13
		30	3,13	0,126	2,22	54,49	63,21
		40	3,09	0,140	2,21	53,59	62,81
	100	20	3,14	0,140	2,52	50,97	55,23
		30	3,09	0,144	2,49	53,00	55,09
		40	3,18	0,136	2,59	50,50	54,92
Hydrochloric	50	20	3,11	0,274	2,19	49,68	67,34
		30	3,13	0,276	2,31	49,00	66,77
		40	3,18	0,252	2,25	49,31	66,16
	100	20	3,26	0,198	2,51	46,85	54,11
		30	3,24	0,184	2,55	49,95	54,01
		40	3,21	0,186	2,52	50,40	52,69
Control (not included in the statistical analysis)			1,97	0,901	0,54	52,14	78,93

Table 3 Main-effect means for the variables investigated in the second laboratory trial (DM basis) where wheat straw was treated with two acids at two fixation levels and three dilution levels

Main effect	Main-effect means (%) of dependent variables				
	Total N	Urea	NH_4^+	IVOMD	NDF
Acids					
Sulphuric acid	3,12[1]	0,14[1]	2,36	52,76[1]	59,40
Hydrochloric acid	3,19[2]	0,21[2]	2,39	49,20[2]	60,18
Fixation levels					
50%	3,12[1]	0,21[1]	2,22[1]	51,68[1]	65,24[1]
100%	3,19[2]	0,16[2]	2,53[2]	50,28[2]	54,34[2]
Dilution levels					
20 ml	3,15	0,19	2,34	50,38	60,46[a]
30 ml	3,15	0,18	2,39	51,61	59,77[a,b]
40 ml	3,16	0,18	2,39	50,95	59,15[b]

[a,b]denotes significance ($P<0,05$) in columns
[1,2]denotes significance ($P<0,01$) in columns

Jayasuriya & Pearce (1983) reported a comparable reduction in the IVOMD of barley straw after treatment with acetic acid. Fixation by H_2SO_4, on the other hand, resulted in a slight increase of 1,2% when compared to the untreated control. Fahmy & Ørskov (1984) also reported an improvement in the *in sacco* degradability of ammoniated and untreated barley straw subsequently treated with sulphuric acid. In the present experiments, NDF content was significantly ($P < 0,01$) reduced at the fixation level of 100% for both acids. The dilution level of acids also tended to reduce the NDF content of samples significantly ($P < 0,05$). Acid treatment furthermore resulted in a marked decrease of NDF in comparison with the untreated control samples, especially at the higher fixation level.

A marked decrease in the consumption of urea ammoniated wheat straw treated with both acids was recorded over the first 4 days of the voluntary-intake trial. The mean voluntary intakes on the first day of the trial were 1 000 ± 135 g and 500 ± 282 g for straw treated with HCl and H_2SO_4 respectively (as fed basis). Observed individual intakes of treated straw ranged between 0 and 25 g per sheep per day for both acids over the next 2 days. It was decided to terminate the trial on the fourth day when it became evident that the experimental animals still refused to eat the acid-treated straw presented to them.

In conclusion, despite extremely encouraging preliminary findings, it appears that the use of HCl and H_2SO_4 as fixation agents for ammonia in ammoniated low-quality roughages is limited, at least at a fixation level of 50%. The low voluntary intake may be related to comparatively large amounts of unpalatable ammonium salts formed by the acid treatments. Lower acid-fixation levels may render acceptable voluntary-intake results, an aspect which requires further investigation.

Acknowledgement

The authors wish to thank Mr F. Franck and the laboratory staff for technical assistance.

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