A laboratory assessment of various treatment conditions affecting the ammoniation of wheat straw by urea. 2. The effect of physical form, moisture level, and prolonged treatment period

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The effect of physical form, moisture level and prolonged treatment period on the ammoniation of wheat straw using 75 g urea per kg wheat straw was investigated in a 2 × 3 × 6 factorial experiment. Physical form was represented by ground (12 mm screen) and long wheat straw, moisture levels were 250, 375 and 500 g/kg air-dry wheat straw and treatment periods were 0, 4, 8, 12, 24 and 48 weeks. Total nitrogen content, urea content, free ammonia nitrogen, *in vitro* organic matter digestibility (IVOMD), and cell wall constituents (CWC) were determined. Physical form, moisture level and treatment period significantly ($P \leqslant 0{,}01$) affected ammonia release from urea. The efficiency of ammoniation was affected accordingly. Ammonia release was slower in long wheat straw, resulting in a slower and smaller increase in IVOMD and a slower and smaller decrease in CWC content with an increased treatment period, when compared to ground wheat straw. Ammoniation was accelerated at the two higher moisture levels compared to the lower moisture level. The IVOMD of wheat straw increased substantially up to a treatment period of 8 – 12 weeks, whereafter a slower increase was obtained up to a treatment period of 48 weeks. Wheat straw appeared to be adequately preserved by treatment with ammonia. No evident sign of deterioration was observed following prolonged treatment periods of up to 48 weeks.
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Die invloed van fisiese vorm, vogpeil en verlengde behandelingsperiode op die ammmonifisering van koringstrooi met 75 g ureum per kg strooi, is in 'n 2 × 3 × 6-faktoriaaleksperiment ondersoek. Fisiese vorm is verteenwoordig deur gemaalde (12 mm-sif) en ongemaalde koringstrooi, terwyl vir elke fisiese vorm vogpeile van 250, 375 en 500 g vog per kg lugdroë strooi vir behandelingsperiodes van 0, 4, 8, 12, 24 en 48 weke toegedien is. Afhanklike veranderlikes het totale stikstofinhoud, ureuminhoud, vry-ammoniakinhoud, *in vitro* organiesemateriaal-verteerbaarheid (IVOMV) en selwandinhoud behels. Fisiese vorm, vogpeil en behandelingsperiode het ammoniakvrystelling vanaf ureum hoogsbetekenisvol ($P \leqslant 0{,}01$) beïnvloed. Die doeltreffendheid van ammonifisering is dienooreenkomstig beïnvloed. Ammoniak is stadiger vrygestel in ongemaalde koringstrooi. Gevolglik is 'n stadiger en geringer toename in IVOMV en 'n stadiger en geringer afname in selwandinhoud in ongemaalde strooi waargeneem. Ammonifisering is versnel by die twee hoër vogpeile in vergelyking met die laer vogpeil van 250 g per kg strooi. Die IVOMV van koringstrooi is aansienlik verhoog tot 'n behandelingsperiode van 8 – 12 weke, waarna 'n stadiger verhoging tot 'n behandelingsperiode van 48 weke waargeneem is. Koringstrooi is oënskynlik doeltreffend bewaar deur ammonifisering. Geen opmerklike teken van agteruitgang is waargeneem tot en met 'n behandelingsperiode van 48 weke nie.
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Keywords: Urea ammoniation, physical form, moisture level, prolonged treatment period, *in vitro* organic matter digestibility, nitrogen content.

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Introduction

The upgrading of crop residues by means of ammoniation has received considerable attention over the past few years. More recently an alternative method of ammoniation, using urea as the source of ammonia, has been reported by several research workers (Dolberg, Saadullah, Haque & Ahmed, 1981; Kritzinger & Franck, 1981; Hadjipanayiotou, 1982; Jayasuriya & Perera, 1982; Ibrahim & Pearce, 1983; Cloete & Kritzinger, 1984). This treatment depends on urease activity in plant residues to release ammonia from urea in an aqueous medium.

A treatment period of 2 weeks (Jayasuriya & Perera, 1982) or up to 4 – 8 weeks (Hadjipanayiotou, 1982; Cloete & Kritzinger, 1984) is usually recommended for optimal ammoniation of crop residues by urea. The effect of prolonged treatment periods on the urea ammoniation of crop residues, however, has so far not been investigated. Furthermore, the effect of physical form of the roughage that is to be ammoniated is not known. Long roughages may react differently to ammoniation than roughages that have been ground through a hammermill. Furthermore, it is known that moisture level affects ammoniation markedly (Solaiman, Horn & Owens, 1979; Kritzinger & Franck, 1981; Sundstøl & Coxworth, 1984; Cloete & Kritzinger, 1984). In general, ammoniation is faster and more efficient at higher moisture levels. Urea ammoniation is also dependent on moisture for the conversion of urea to ammonia and carbon dioxide. Excessive moisture content of treated roughages may, however, be disadvantageous under certain circumstances. Results presented by Cloete & Kritzinger (1984) indicated a moisture level × treatment period interaction, suggesting that the slower reaction at low moisture levels can be partly compensated for by a longer treatment period.

A laboratory investigation of the effects of physical form, moisture level, prolonged treatment period and the relevant interactions was thus undertaken. Since ammonia serves as a preservative of moist roughages (Knapp, Holt & Lechtenberg, 1975; Ørskov, Reid, Holland, Tait & Lee, 1983; Tetlow, 1984), it is unlikely that roughages treated by this method will deteriorate.

Materials and methods

Ground (12 mm screen) and long wheat straw were mixed with urea solutions to provide a urea level of 75 g/kg wheat straw and moisture levels of 250, 375 and 500 g/kg air-dry wheat straw. Individual samples were sealed in 2000 ml airtight plastic containers. The urea included in these samples was subsequently allowed to react with the wheat straw for periods of 0, 4, 8, 12, 24 and 48 weeks at a constant temperature of 24°C. All samples included in the investigation were treated in duplicate, resulting in a 2 × 3 × 6 factorial trial with two replications.

Following treatment the samples were dried at 59°C in a forced draught oven prior to analysis. Total nitrogen content of the samples was determined by the Kjeldhal method (AOAC, 1970). Samples were also analysed for free urea (Technicon Auto Analyser, 1977) and free ammonia nitrogen content (Technicon Auto Analyser II, 1977). For these determinations urea and free ammonia (NH_3-N) were extracted from the respective samples using a 2,0 M potassium chloride solution, containing 5 mg phenyl mercury acetate per litre. *In vitro* organic matter digestibility (IVOMD) of the samples was determined according to the two-stage technique of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967). Cell wall constituents (CWC) of the respective samples were analysed according to the methods of Van Soest & Wine (1967). As IVOMD and CWC values were slightly affected by the presence of residual soluble urea in some samples, all these values were corrected for the urea content of the respective samples.

Standard procedures for the analysis of a factorial design were applied in the statistical analysis (Snedecor & Cochran, 1967). Results were presented as three-factor interactions where applicable. In cases where the three-factor interaction was not statistically significant, data were presented to depict significant two-factor interactions.

Results and discussion

Total nitrogen, urea, and NH_3-N content

Degrees of freedom, mean squares, and significance for the analyses carried out on total N, urea, and NH_3-N contents are presented in Table 1. Physical form, moisture level, and treatment period significantly ($P \leqslant 0,01$) affected all these dependent variables. All the relevant interactions were significant ($P \leqslant 0,05$) for total N and urea contents, whereas the physical form × treatment period interaction was significant ($P \leqslant 0,01$) for NH_3-N content. The results concerning total N and urea contents are presented as three-factor interactions in Figures 1 and 2 respectively. Results for NH_3-N content are presented as a two-factor interaction between physical form and treatment period (Figure 3).

Urea hydrolysis increased with prolonged treatment periods (Figures 1, 2 and 3). Total N and urea content correspondingly decreased, while NH_3-N content increased. Ammonia release

Table 1 Degrees of freedom, mean squares, and significance for the analyses of total N, urea, NH_3-N, IVOMD, and CWC content

		Mean squares				
Parameter	*df*	Total N	Urea	NH_3-N	IVOMD	CWC
Independent variables						
Physical form (P)	1	5,825^b	62,83^b	0,4604^b	240,75^b	53,99^b
Moisture level (M)	2	3,558^b	21,82^b	0,0728^b	41,43^b	9,42^{ns}
Treatment period (T)	5	3,149^b	40,10^b	0,5165^b	409,14^b	86,66^b
Interactions						
P × M	2	0,361^b	1,07^b	0,0035^{ns}	8,89^{ns}	1,21^{ns}
P × T	5	0,171^b	2,93^b	0,0621^b	34,05^b	11,34^b
M × T	10	0,433^b	1,89^b	0,0061^{ns}	12,15^b	3,94^{ns}
P × M × T	10	0,098^a	0,65^b	0,0023^{ns}	5,15^{ns}	3,41^{ns}
Error	36	0,032	0,18	0,0030	3,07	3,49

^{ns}Not significant; ^{a}Significant ($P \leqslant 0,05$); ^{b}Significant ($P \leqslant 0,01$).

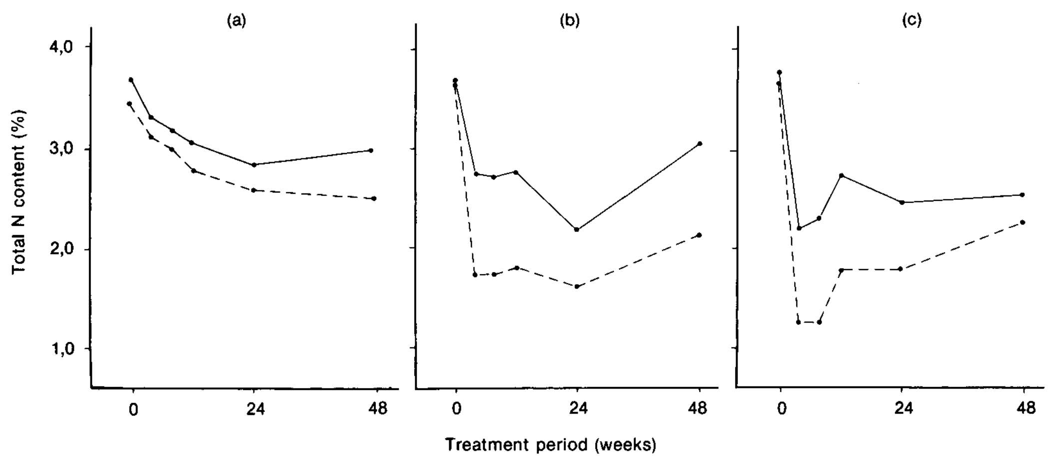

Figure 1 The total N content of long (______) and ground (----------) wheat straw ammoniated with urea for prolonged treatment periods at moisture levels of (a) 250 g/kg, (b) 375 g/kg, and (c) 500 g/kg

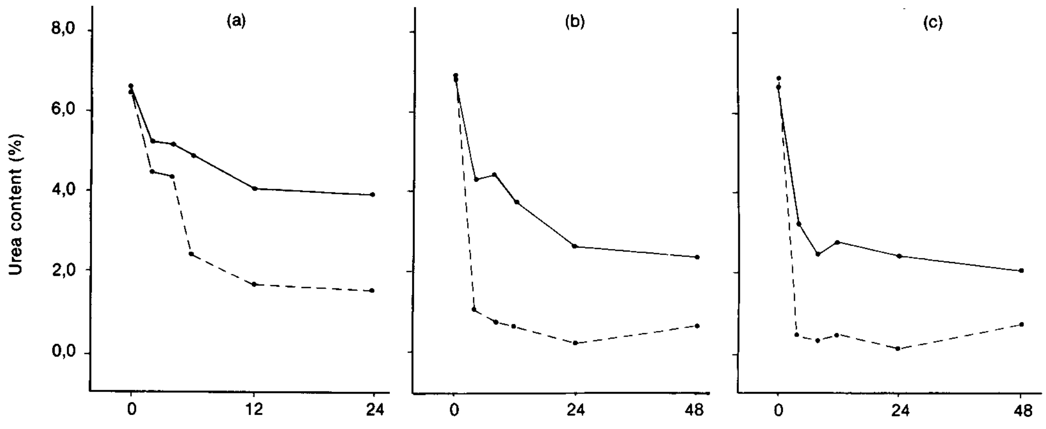

Figure 2 The urea content of long (______) and ground (-----------) wheat straw ammoniated with urea for prolonged treatment periods at moisture levels of (a) 250 g/kg, (b) 375 g/kg, and (c) 500 g/kg

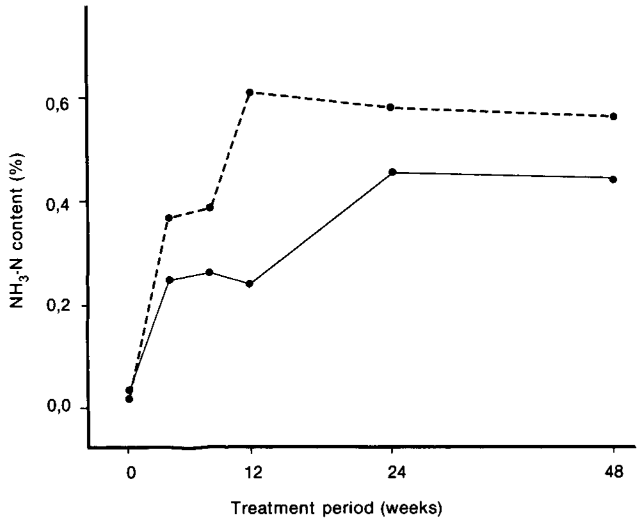

Figure 3 The NH_3-N content of long (____) and ground (--------) wheat straw ammoniated with urea for prolonged treatment periods

in long wheat straw was relatively slow, resulting in significantly ($P \leqslant 0{,}01$) higher total N and urea levels and a lower NH_3-N level than in ground wheat straw. This result may be related to an uneven distribution of the urea solution in the long wheat straw. This may also be related to the smaller surface area in long wheat straw that was in direct contact with the urea solution. It is interesting to note that total N content tended to increase slightly after approximately 24 and 48 weeks at moisture levels of 375 g/kg and 500 g/kg wheat straw respectively. This trend may be explained partially by unexpected higher urea levels in ground samples treated to provide the moisture levels and treatment periods mentioned. Another possible explanation is the possibility of more nitrogen bound to the straw as a result of prolonged treatment periods.

Increasingly more urea was hydrolysed at higher moisture levels (Figures 1 and 2). This finding is in agreement with results reported by Kritzinger & Franck (1981); Williams, Innes & Brewer (1984) and Cloete & Kritzinger (1984). No interactions involving the effect of moisture level on NH_3-N content of wheat straw samples were obtained. Overall means, pooled across treatment period and physical form, for samples treated at moisture levels of 250, 375 and 500 g/kg wheat straw were 0,30; 0,38 and 0,38% NH_3-N respectively. Differences between the lower and the two higher moisture levels were statistically significant ($P \leqslant 0{,}01$) while no significant difference was obtained between the latter two moisture levels.

The NH_3-N content of ground wheat straw samples levelled off at approximately 0,61 to 0,57% following 12 weeks of treatment. Long wheat straw samples, correspondingly, levelled off at 0,46 to 0,45% NH_3-N following 24 weeks of treatment. These results can be compared to results obtained by Hadjipanayiotou (1982) and Solaiman, *et al.* (1979). The former author reported a NH_3-N content of 0,48% in barley straw ammoniated by a 10% urea solution at a moisture level of 400 g/kg for a period of 90 days. In the present investigation the NH_3-N content of ground and long wheat straw, treated for a comparable treatment period (12 weeks) at a comparable moisture level (375 g/kg), was 0,59 and 0,25% respectively. Solaiman, *et al.* (1979) reported the NH_3-N content of wheat straw, ammoniated by ammonium hydroxide at a level of 3,3% and a moisture level of 540 g/kg for a period of 50 days, to be 0,56%. Corresponding results, obtained under comparable treatment conditions (viz. 500 g moisture/kg wheat straw and a treatment period of 8 weeks), in the present investigation were 0,38 and 0,31% NH_3-N for ground and long wheat straw respectively.

In vitro organic matter digestibility

Statistical information regarding the effect of physical form, moisture level, prolonged treatment period and the relevant interactions on the IVOMD is presented in Table 1. IVOMD was significantly ($P \leqslant 0{,}01$) affected by all the independent variables investigated, whereas the physical form × treatment period and the moisture level × treatment period interactions were statistically significant ($P \leqslant 0{,}01$), too. These two-factor interactions are presented in Figures 4 and 5 respectively.

Ammoniation appeared to be more effective and faster in ground wheat straw than in long straw (Figure 4). This finding is consistent with the previously mentioned results regarding the total N, urea and NH_3-N contents of the samples. It is interesting to note that a further improvement in IVOMD was obtained following a substantial increase during the first 8 – 12 weeks of treatment in the present investigation. The IVOMD of ground wheat straw, pooled across moisture levels, increased from 48,9% at 8 weeks to 54,4% at 48 weeks of treatment. The IVOMD of long wheat straw was correspondingly increased from 42,1% to 51,2% over the same period. Both Waiss, Guggolz, Kohler, Walker & Garret (1972) and Hadjipanayiotou (1982) investigated ammonia treatment for periods up to 90 days under comparable conditions. These authors reported little further improvement in digestibility after

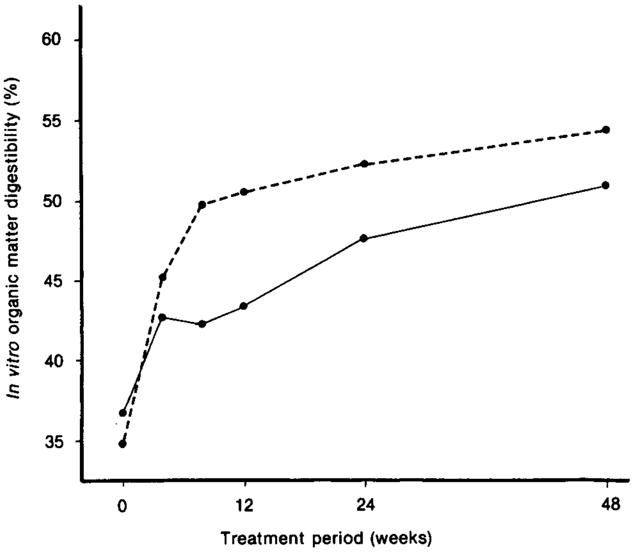

Figure 4 The *in vitro* organic matter digestibility (corrected for urea content) of long (____) and ground (--------) wheat straw ammoniated with urea for prolonged treatment periods

treatment periods of 30 days and approximately 30 to 45 days respectively. It should be noted that the present investigation was carried out over a period of 336 days, compared to the shorter treatment periods reported by the authors mentioned.

Following 48 weeks of treatment no evidence of deterioration was observed in the ammoniated wheat straw, which can apparently be preserved for indefinite periods of time under these conditions. The lack of deterioration of ammoniated wheat straw is in agreement with results obtained by Knapp, *et al.* (1975) and Ørskov, *et al.* (1983).

Ammoniation tended to be more effective at higher moisture levels (Figure 5). A significant ($P \leqslant 0,01$) difference was obtained between the moisture level of 250 g/kg wheat straw and the other two moisture levels when data were pooled across physical forms and treatment periods. The difference between the latter two moisture levels was relatively small and not statistically significant. No evident explanation exists for the drop in IVOMD at the moisture level of 250 g/kg and the treatment period of 8 weeks. The effect of moisture level

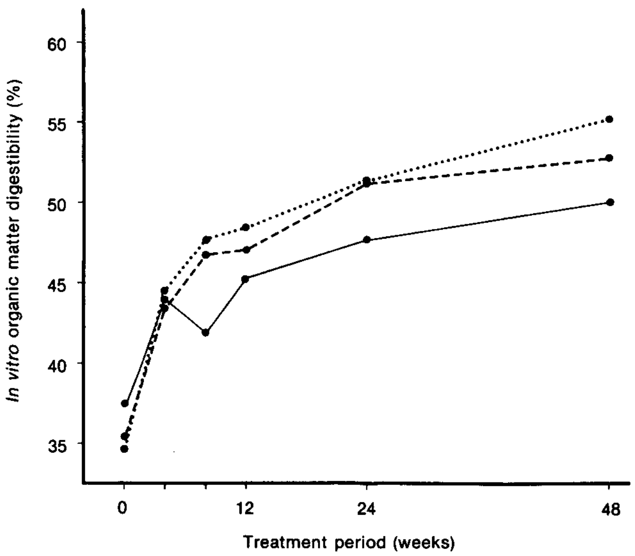

Figure 5 The *in vitro* organic matter digestibility (corrected for urea content) of wheat straw ammoniated with urea for prolonged treatment periods at moisture levels of 250 g/kg (____), 375 g/kg (--------), and 500 g/kg (........)

on the ammoniation of roughages is in general agreement with results presented by Oji, Mowat & Winch (1979); Solaiman, *et al.* (1979); Kritzinger & Franck (1981); Sundstøl & Coxworth (1984) and Cloete & Kritzinger (1984). In this respect, Kiangi, Kategile & Sundstøl (1981) also reported a more efficient ammonia treatment at a higher moisture level (400 g/kg compared to 200 g/kg) for rice straw and wheat straw, but not for maize stover. The results are also in agreement with results reported by Borhami & Sundstøl (1982). The moisture levels reported by these authors were, however, very low compared to those in the present investigation.

Cell wall constituents (CWC)

Statistical information regarding the effect of physical form, moisture level, prolonged treatment period and the relevant interactions on the CWC content of wheat straw is presented in Table 1. CWC content was significantly ($P \leqslant 0,01$) affected by physical form and treatment period, while the physical form $\times$ treatment period interaction was statistically significant ($P \leqslant 0,01$). This two-factor interaction is presented in Figure 6.

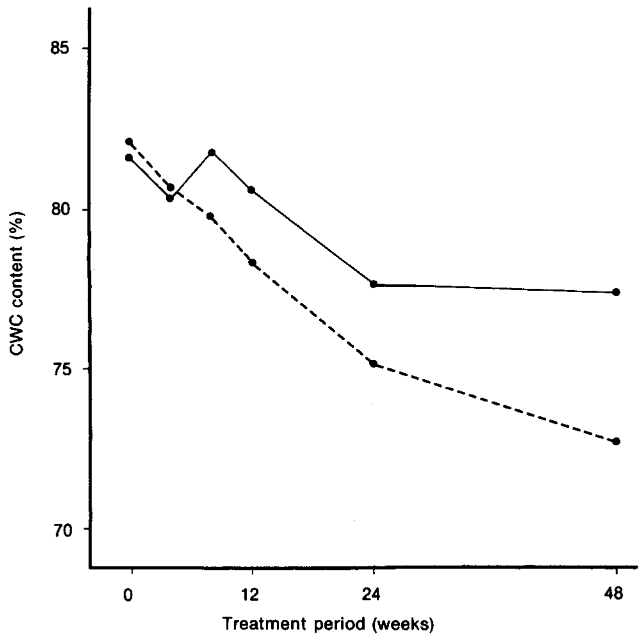

Figure 6 The cell wall constituents (CWC), corrected for urea content, of long (____) and ground (-------) wheat straw ammoniated with urea for prolonged treatment periods

CWC content of the respective samples decreased with an increased treatment period (Figure 6). This trend was faster in ground wheat straw, which is consistent with the results regarding total N, urea, NH_3-N and IVOMD reported earlier. This finding is in general agreement with results reported by Solaiman, *et al.* (1979) and Horton (1981).

Conclusions

Results from the present investigation indicate a definite advantage in the urea ammoniation of ground wheat straw when compared to long wheat straw. Possible explanations for this difference may either be problems with an even distribution of urea solution in long straw, or a reduction in the straw surface area in direct contact with the urea solution. Practical considerations, however, favour the treatment of long wheat straw, especially when large amounts of straw are to be treated.

Higher moisture levels resulted in a faster and more efficient ammoniation. Ammoniation at relatively low moisture levels thus requires a longer treatment period to obtain acceptable results. The digestibility of the wheat straw samples included in the present investigation tended to increase up to a treatment period of 48 weeks, without evident signs of deterioration. It therefore seems possible to preserve wheat straw almost indefinitely under ammoniation conditions. This result may be of practical significance, especially for the preservation of hay, an aspect which necessitates further investigation.

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