

A laboratory assessment of various treatment conditions affecting the ammoniation of wheat straw by urea. 1. The effect of temperature, moisture level and treatment period

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The effect of temperature, moisture level and treatment period on the ammoniation of wheat straw by 75 g urea per kg wheat straw was investigated in a 4 × 2 × 6 factorial experiment. Independent variables included temperatures of 4; 14; 24 and 35 °C, moisture levels of 250 and 375 g/kg wheat straw and treatment periods of 0; 1; 2; 4; 6 and 8 weeks. Dependent variables investigated were total nitrogen content, urea content, free ammonia nitrogen content and *in vitro* organic matter digestibility (IVOMD).

Temperature, moisture level and treatment period significantly ($P \leq 0,01$) affected ammonia release from urea. The efficiency of ammoniation was accordingly affected. Slow ammonia release at 4 °C (at both moisture levels) and at 14 °C (only at the lower moisture level) resulted in lesser improvements in IVOMD after a treatment period of eight weeks. Ammoniation was accelerated at higher temperatures, particularly at the higher moisture level. Comparable IVOMD values were obtained after a treatment period of one to two weeks at 35 °C and approximately six weeks at 24 °C. Less ammonia was released from urea at 35 °C and the moisture level of 375 g/kg, possibly owing to a decrease in urease activity at this temperature. Significant ($P \leq 0,01$) temperature × treatment period and moisture level × treatment period interactions were obtained, indicating that the slow reaction at lower temperatures and at lower moisture levels can partly be compensated for by a longer treatment period.

S. Afr. J. Anim. Sci. 1984, 14: 55 – 58

Die invloed van temperatuur, vogpeil en behandelingsperiode op die ammonifisering van koringstrooi met 75 g ureum per kg strooi is in 'n 4 × 2 × 6-faktoriaal-eksperiment ondersoek. Onafhanklike veranderlikes het temperature van 4; 14; 24 en 35 °C, vogpeile van 250 en 375 g/kg strooi, en behandelingsperiodes van 0; 1; 2; 4; 6 en 8 weke ingesluit. Parameters wat ondersoek is, het totale stikstofinhoud, ureum-inhoud, vry-ammoniak-stikstof-inhoud en *in vitro*-organiese-materie-verteerbaarheid (IVOMV) behels.

Temperatuur, vogpeil en behandelingsperiode het ammoniakvrystelling vanaf ureum hoogsbetekenisvol ($P \leq 0,01$) beïnvloed. Die doeltreffendheid van ammonifisering is dienooreenkomstig beïnvloed. Stadige ammoniakvrystelling by 4 °C (by beide vogpeile) en by 14 °C (slegs by die laer vogpeil) het geringe stygings in die IVOMV veroorsaak na 'n behandelingsperiode van agt weke. Ammonifisering by hoër temperature, veral by die hoër vogpeil, is versnel. Vergelykbare IVOMV-waardes is verkry na 'n behandelingsperiode van een tot twee weke by 35 °C en ongeveer ses weke by 24 °C. By 35 °C en die vogpeil van 375 g/kg is minder ureum na ammoniak omgesit, moontlik as gevolg van 'n verlaagde urease-aktiwiteit by die temperatuur. Hoogsbetekenisvolle ($P \leq 0,01$) temperatuur × behandelingsperiode-en vogpeil × behandelingsperiode-interaksies is waargeneem. 'n Langer behandelingsperiode kan dus tot 'n mate vergoed vir die stadiger ammonifisering wat by lae temperature en lae vogpeile voorkom.

S.-Afr. Tydskr. Veek. 1984, 14: 55 – 58

Keywords: Urea ammoniation, temperature, moisture level, treatment period, *in vitro* organic matter digestibility, nitrogen content

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Received 16 November 1983

Introduction

The upgrading of low quality roughages by means of ammonia treatment was reviewed by Sundstøl, Coxworth & Mowat (1978). An indirect method of ammoniation of crop residues has recently been reported by Hadjipanayiotou (1982) and Jayasuriya & Perera (1982). This treatment is dependent on the enzymatic release of ammonia from urea added to the roughage in an aqueous medium.

Previous work at this institute proved ammoniation by urea to be effective in improving the *in vitro* organic matter digestibility of wheat straw (Kritzinger & Franck, 1981). Encouraging results were also obtained in a voluntary intake and *in vivo* digestibility trial (Cloete, De Villiers & Kritzinger, 1983). This method of ammoniation appears to be relatively safe, uncomplicated and inexpensive when compared to some other chemical treatments. It does, however, require a treatment period of approximately six to eight weeks for optimum results (Kritzinger & Franck, 1981). Results obtained by Oji, Mowat & Winch (1979) and Waagepetersen & Vestergaard Thomsen (1977) indicate that anhydrous ammoniation can effectively be accelerated by treatment at elevated temperatures of up to 90°C. The effect of temperature on the ammoniation of crop residues by urea has so far not been investigated. Literature reviewed by Du Preez (1983), indicated the optimum temperature for urease activity in soil to be approximately 30°C. According to Ørskov, Reid, Holland, Tait & Lee (1983) and Du Preez (1983) urease activity tends to decrease at temperatures lower than 20°C. Soil urease activity, on the other hand, was only marginal at a temperature of 45°C (Wahhab, Khan & Ishaq, 1960).

The effect of moisture level on the ammoniation of low quality roughages was investigated by Sundstøl *et al.* (1978); Solaiman, Horn & Owens (1979) and Kritzinger & Franck (1981) with moisture levels ranging from 80 to 540 g/kg straw. In general, it can be concluded that ammoniation was consistently faster and more effective at higher moisture levels. Excessive moisture content of treated straw may however present some practical problems under certain circumstances. A laboratory investigation into the effect of temperature on urea ammoniation of wheat straw was thus undertaken. The effect of moisture level, treatment period and the relevant interactions were investigated simultaneously.

Materials and Methods

Ground wheat straw (12 mm screen) was thoroughly mixed with a urea solution to provide a urea level of 75 g urea/kg straw and moisture levels of 250 and 375 g/kg wheat straw.

Individual samples were sealed airtightly in 2 000 ml plastic containers. Subsequently the urea included in these samples was allowed to react for periods of nil, one, two, four, six and eight weeks at temperatures of 4, 14, 24 and 35°C respectively. Temperatures of 4, 14 and 24°C were obtained by incubation of samples in constant temperature rooms, while an incubator was used for the treatment of samples at 35°C. All samples included in the investigation were treated in duplicate, resulting in a 4 × 2 × 6 factorial design with two replications.

Following treatment the samples were dried at 59°C in a forced-draught oven prior to analyses. Total nitrogen content of the samples was determined by the Kjeldahl method (A.O.A.C., 1970). Samples were also analysed for urea remaining (Technicon Auto Analyser, 1977) and free ammonia nitrogen (Technicon Auto Analyser II, 1977). For these determinations urea and free ammonia nitrogen (NH₃-N) were extracted from the respective samples by a 2,0 molar 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). As IVOMD-values were slightly affected by the presence of residual soluble urea in some cases, all these values were corrected for the urea content of the respective samples. Standard procedures for the analyses of a factorial design were applied in the statistical analyses (Snedecor & Cochran, 1967).

Results and Discussion

Total nitrogen, urea and free ammonia nitrogen

Degrees of freedom, mean squares and significance for the independent variables, interactions and error in the statistical analyses for total nitrogen content (N content), urea and NH₃-N content are presented in Table 1. The effects of temperature, moisture level and treatment period on N content, urea and NH₃-N are graphically illustrated in Figures 1, 2 and 3 respectively.

Table 1 Degrees of freedom, mean squares and significance

	df	Mean squares			
		Total N	Urea	NH ₃ -N	IVOMD
Independent variables					
Temperature (T)	3	2,9328 ^a	24,757 ^a	0,35803 ^a	355,01 ^a
Moisture level (M)	1	5,3865 ^a	48,650 ^a	0,20336 ^a	608,08 ^a
Treatment period (P)	5	1,2511 ^a	14,926 ^a	0,19771 ^a	146,31 ^a
Interactions					
T × M	3	1,3683 ^a	8,055 ^a	0,02067 ^a	58,52 ^a
T × P	15	0,3389 ^a	2,335 ^a	0,02506 ^a	17,14 ^a
M × P	5	0,1829 ^a	1,931 ^a	0,00677 ^a	13,35 ^a
T × M × P	15	0,1277 ^a	0,885 ^a	0,00408 ^a	4,12 ^b
Error	48	0,0053	0,051	0,00052	1,67

^a - Significant ($P \leq 0,01$)

^b - Significant ($P \leq 0,05$)

Results obtained indicate that temperature, moisture level and treatment period significantly ($P \leq 0,01$) affected all dependent variables investigated. All interactions were statis-

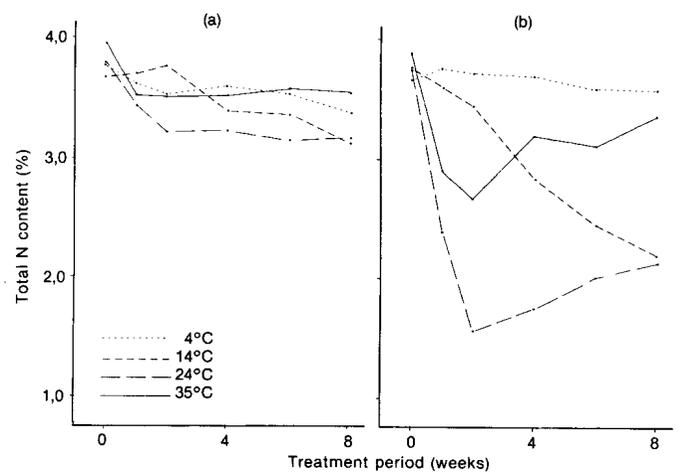


Figure 1 The total N content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

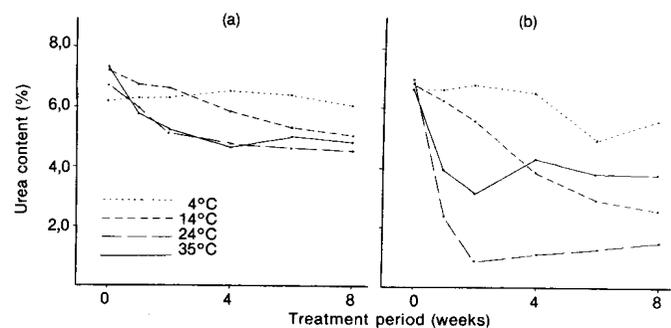


Figure 2 The urea content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

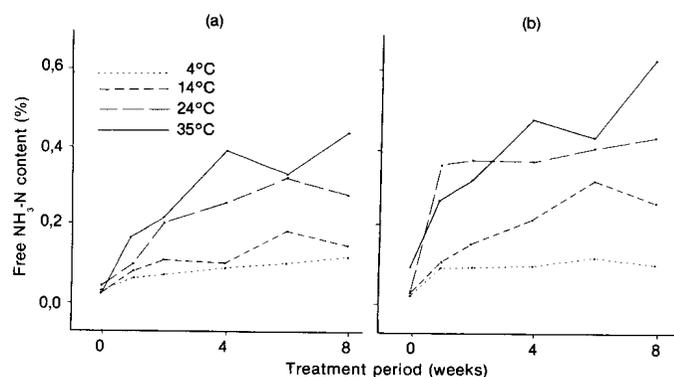


Figure 3 The free NH₃-N content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

tically significant ($P \leq 0,01$) too. According to Figures 1 to 3 little urea was converted to ammonia at 4°C at both moisture levels. Higher temperatures and the higher moisture level resulted in accelerated ammonia release, causing corresponding decreases in total N and urea contents (Figures 1 and 2 respectively). These observations resulted in the obtained significant ($P \leq 0,01$) temperature × treatment period and moisture level × treatment period interactions. At 4°C the effect of moisture level was small, resulting in almost no differences between moisture levels. At higher temperatures, con-

sistently more urea was converted to ammonia at the higher moisture level, causing a significant ($P \leq 0,01$) temperature \times moisture level interaction. At 35°C, however, total nitrogen loss was relatively small compared to that at 24°C. One factor contributing to this result was a lower conversion rate of urea to ammonia at 35°C (Figure 2). This trend may possibly be caused by a decline in urease activity at this temperature. Secondly, the $\text{NH}_3\text{-N}$ content of samples ammoniated at 35°C also tended to be higher than that of samples treated at 24°C (Figure 3). It thus appeared that the higher temperature catalysed the binding of extractable ammonia to the straw in some manner, resulting in higher $\text{NH}_3\text{-N}$ levels despite less ammonia released from urea at a temperature of 35°C. At 14°C total N content and urea decreased throughout the entire treatment period. At 24°C and 35°C both these dependent variables tended to reach a minimum level after a treatment period of two to four weeks. Total N content of samples ammoniated at 24°C and 35°C at the higher moisture level, appeared to increase after a treatment period of four weeks. Ammonia bound to the straw as $\text{NH}_3\text{-N}$ (Figure 3) and other nitrogenous compounds possibly contributed to this increase of total N content.

Results in the literature were generally obtained at ambient temperature or temperatures near to 24°C. Results obtained at 24°C and at the higher moisture level (375 g/kg straw) are in general agreement with results obtained by Solaiman *et al.* (1979) with ammonium hydroxide, and Hadjipanayiotou (1982) and Ibrahim & Pearce (1983) with urea. Solaiman *et al.* (1979) reported the total N and $\text{NH}_3\text{-N}$ content of wheat straw ammoniated by ammonium hydroxide to be respectively 1,9 and 0,56% after 50 days of treatment. Corresponding values for the present investigation after a treatment period of eight weeks (56 days) were 2,14 and 0,44% respectively. Hadjipanayiotou (1982) investigated the ammoniation of barley straw with 40 g urea/kg straw at a moisture level of 400 g/kg straw. Total N content in his investigation declined from 2,3% after one day to reach a minimum level of 1,15% after 75 days of treatment, while $\text{NH}_3\text{-N}$ increased from 0,1% after one day to 0,47% after 45 days, before levelling off at approximately 0,47%. In the present study, the total N content of wheat straw decreased from 3,77 to a minimum level of 1,56% after two weeks (14 days) of treatment. Subsequently, total N content tended to increase to 2,14% after eight weeks (56 days) of treatment. $\text{NH}_3\text{-N}$ content increased sharply from 0,03% to 0,37% after one week of treatment. A steady increase of $\text{NH}_3\text{-N}$ was observed afterwards, $\text{NH}_3\text{-N}$ reaching a level of 0,44 after eight weeks (56 days) of treatment. The total N content of 1,75% obtained after four weeks (28 days) of treatment in the present investigation agrees fairly well with 1,6% N after 28 days of treatment of barley straw ammoniated by 80 g urea/kg straw and at a moisture level of 1 000 g/kg straw, reported by Ibrahim & Pearce (1983).

In vitro organic matter digestibility

Statistical information regarding the effect of temperature, moisture level, treatment period and the relevant interactions on the *in vitro* organic matter digestibility (IVOMD) is presented in Table 1, and graphically illustrated in Figure 4. According to Table 1, IVOMD was significantly ($P \leq 0,01$) affected by temperature, moisture level and treatment period. All the relevant first-order interactions were significant ($P \leq 0,01$) too, while the temperature \times moisture level \times treatment period interaction was significant at $P \leq 0,05$. Ammoniation for two and one weeks at 35°C and at moisture levels of 250 and 375 g/kg straw respectively, resulted in IVOMD

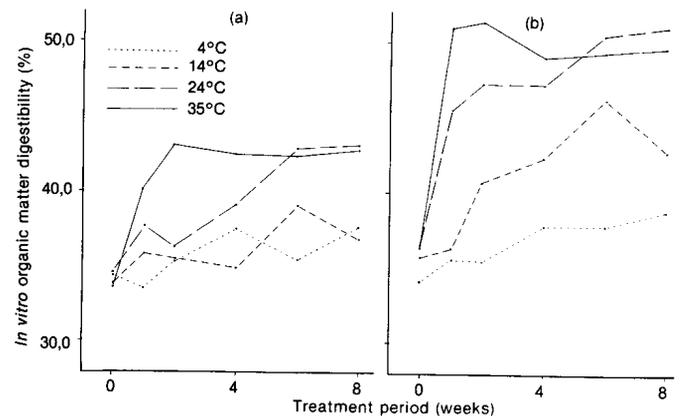


Figure 4 The *in vitro* organic matter digestibility (corrected for urea content) of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg (b).

values of approximately 43 and 50%. Corresponding IVOMD values were obtained after a treatment period of six weeks at 24°C for the respective moisture levels. Ammoniation thus appeared to be faster and more effective at 35°C when compared to 24°C, even though less urea was converted to ammonia at the former temperature (Figure 2). Furthermore, wheat straw ammoniated at 35°C also tended to include more $\text{NH}_3\text{-N}$ than that treated at 24°C (Figure 3). These results seem to indicate that the ammoniation reaction was somehow enhanced by the higher temperature. At 4°C the reaction was comparatively slow, resulting in a lesser improvement of IVOMD irrespective of moisture level or treatment period. A corresponding lesser increase was obtained at 14°C and the lower moisture level. At the higher moisture level, on the other hand, the increase of IVOMD appeared to be somewhat more pronounced. No evident explanation exists for the decrease in IVOMD after a treatment period of six weeks at 14°C, and this observation must thus be related to experimental and sampling errors.

The above-mentioned results are in general agreement with those obtained by Waagepetersen & Vestergaard Thomsen (1977), Sundstøl *et al.* (1978), and Oji *et al.* (1979). Sundstøl *et al.* (1978), however, reported a substantial increase in IVOMD of oat straw ammoniated at 4°C. The contradictory results obtained in the present investigation can possibly be explained by the indirect nature of urea ammoniation which is, compared to direct ammoniation, largely dependent on the enzymatic release of ammonia from urea. Owing to the relative inactivity of the urease enzyme at low temperatures (Ørskov *et al.*, 1983; Du Preez, 1983) ammonia release at 4°C is slow, resulting in a less efficient ammoniation. The significant ($P \leq 0,01$) interaction between temperature and treatment period, indicating that lower temperatures can partly be compensated for by a longer treatment period, also agrees with results obtained by Sundstøl *et al.* (1978) and Oji *et al.* (1979).

Treatment was faster and more effective at the higher moisture level, except at a temperature of 4°C. This result is in agreement with results reported by Sundstøl *et al.* (1978), Oji *et al.* (1979), Solaiman *et al.* (1979), Kiangi, Kategile & Sundstøl (1981) and Kritzinger & Franck (1981). It is also in agreement with results published by Borhami & Sundstøl (1982) on the ammoniation of oat straw. It should, however, be mentioned that Borhami & Sundstøl (1982) investigated comparatively low moisture levels, ranging from 25 to 100 g/kg straw. The interaction between moisture level and treatment period is in agreement with results obtained by Oji *et al.* (1979). This

result indicates that the slow reaction at low moisture levels can partly be compensated for by a longer treatment period. At 4°C no difference in IVOMD was observed between moisture levels. At higher temperatures ammoniation became increasingly more effective at the higher moisture level, resulting in the observed interaction between temperature and moisture level.

In the present investigation the optimum treatment period at 24°C and at a moisture level of 375 g moisture/kg wheat straw was six to eight weeks. This finding agrees fairly well with results published by Kritzing & Franck (1981) and Hadjipanayiotou (1982). The former authors reported an optimum treatment period of six to eight weeks for wheat straw ammoniated by urea. Hadjipanayiotou (1982) obtained an improvement in the *in vitro* dry matter digestibility up to a treatment period of 45 days. Solaiman *et al.* (1979) and Jayasuriya & Perera (1982), on the other hand, reported little further improvement in the *in vitro* digestibility of wheat and rice straw, ammoniated by ammonium hydroxide and urea respectively, after respective treatment periods of 10 and 14 days (two weeks).

Conclusions

Temperature markedly affected ammonia release from urea, and accordingly the efficiency of ammoniation. Slow ammonia release at 4°C (at both moisture levels) and at 14°C (only at the lower moisture level) resulted in lesser improvements of IVOMD after a treatment period of eight weeks. At higher temperatures ammoniation was accelerated, particularly at the higher moisture level (375 g/kg straw). Treatment for one to two weeks at 35°C resulted in IVOMD-values comparable to that obtained after a treatment period of six weeks at 24°C, clearly demonstrating the beneficial effect of a rise in temperature on ammoniation as such. On the other hand, slightly less ammonia was released from urea at 35°C and the higher moisture level, indicating that urease activity may be reduced at this temperature. Significant temperature × treatment period and moisture level × treatment period interactions were obtained, indicating that the slow reaction at lower temperatures and at lower moisture levels can partly be compensated for by a longer treatment period.

The obtained results indicate that ammoniation by urea as a method of improving the nutritive value of low quality roughages may not be practical in extremely cold regions, as urease activity tends to be reduced by low temperatures. Extremely high temperatures, on the other hand, may also adversely affect ammonia release from urea, despite having a beneficial effect on ammoniation as such.

Acknowledgement

The authors wish to thank the laboratory staff and, in particular, Messrs. F. Franck and M.J. Herselman for technical assistance.

References

- A.O.A.C., 1970. Official methods of analysis. (11th Ed.). Association of Official Analytical Chemists, Washington D.C.
- BORHAMI, B.E.A. & SUNDSTØL, F., 1982. Studies on ammonia-treated straw. 1. The effects of type and level of ammonia, moisture content and treatment time on the digestibility *in vitro* and enzyme soluble organic matter of oat straw. *Anim. Fd Sci. Techn.* 7, 45.
- CLOETE, S.W.P., DE VILLIERS, T.T. & KRITZINGER, N.M., 1983. The effect of ammoniation by urea on the nutritive value of wheat straw for sheep. *S. Afr. J. Anim. Sci.* 13, 143.
- DU PREEZ, M., 1983. Hidrolise, nitrifikasie en vervlugtigting van ureum onder gekontroleerde toestande in dominante grondtipes van die Wes-Kaap. M.Sc.-verhandeling, Universiteit van Stellenbosch.
- ENGELS, E.A.N. & VAN DER MERWE, F.J., 1967. Application of an *in vitro* technique to South African forages, with special reference to the effect of certain factors to the results. *S. Afr. J. Agric. Res.* 10, 983.
- HADJIPANAYIOTOU, M., 1982. The effect of ammoniation using urea on the intake and nutritive value of chopped barley straw. *Grass Forage Sci.* 37, 89.
- IBRAHIM, M.N.M. & PEARCE, G.R., 1983. Effects of chemical pretreatments on the composition and *in vitro* digestibility of crop byproducts. *Agr. Wastes* 5, 135.
- JAYASURIYA, M.C.N. & PERERA, H.D.G., 1982. Urea-ammonia treatment of rice straw to improve its nutritive value for ruminants. *Agric. Wastes* 4, 143.
- KRITZINGER, N.M. & FRANCK, F., 1981. Die effek van ureuminkuiling op die *in vitro* verteerbaarheid van koringstrooi. *Els. J.* 5(1), 15.
- KIANGI, E.M.I., KATEGILE, J.A. & SUNDSTØL, F., 1981. Different sources of ammonia for improving the nutritive value of low quality roughages. *Anim. Fd Sci. Techn.* 6, 377.
- OJI, U.I., MOWAT, D.N. & WINCH, J.E., 1979. *In vitro* evaluation of processing conditions for thermo-ammoniation of corn stover. *Can. J. Anim. Sci.* 59, 813.
- ØRSKOV, E.R., REID, G.W., HOLLAND, S.M., TAIT, C.A.G. & LEE, N.H., 1983. The feeding value of straw and whole-crop barley and oats treated with anhydrous or aqueous ammonia or urea. *Anim. Fd Sci. Techn.* 8, 247.
- SNEDECOR, G.W. & COCHRAN, W.G., 1967. Statistical methods. Sixth edition. Iowa State University Press. Ames, Iowa, U.S.A.
- SOLAIMAN, S.G., HORN, G.W. & OWENS, F.N., 1979. Ammonium hydroxide treatment on wheat straw. *J. Anim. Sci.* 49, 802.
- SUNDSTØL, F., COXWORTH, E. & MOWAT, D.N., 1978. Improving the nutritive value of straw and other low-quality roughages by treatment with ammonia. *World Anim. Rev.* 26, 13.
- TECHNICON AUTO ANALYSER., 1977. Urea-nitrogen. Technicon Auto Analyser method no 4001 FD4, Domont France.
- TECHNICON AUTO ANALYSER II., 1977. Individual/simultaneous determination of nitrogen and/or phosphorus in BD acid digests. *Industrial Method* No. 334-74 W/B⁺.
- TILLEY, J.M.A. & TERRY, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassl. Soc.* 18, 104.
- WAAGEPETERSEN, J. & VESTERGAARD THOMSEN, K., 1977. Effect on digestibility and nitrogen content of barley straw of different ammonia treatments. *Anim. Fd Sci. Techn.* 2, 131.
- WAHHAB, A., KHAN, M. & ISHAQ, M., 1960. Nitrification of urea and its loss through volatilization of ammonia under different soil conditions. *J. Agric. Sci.* 55, 47.