J Sci Food Agric 1998, **78**, 95–101

Determination of Ammonia Nitrogen in the Urine of Small Ruminants

S López,* E Llamazares and J S González

Departamento de Producción Animal, Universidad de León, E-24007 León, Spain

(Received 5 June 1997; revised version received 17 October 1997; accepted 28 January 1998)

Abstract: The objective of the present work was to compare colorimetric and distillation–titration methods to determine the ammonia-nitrogen (NH_3-N) concentration in sheep and goat urine samples. Colorimetric methods used were based on the indophenol reaction, whereas titrimetric methods were based on the alkali distillation of ammonia from the urine and its titrimetric determination with a standard acid. Colorimetric methods were only reliable when urine samples were diluted at least 1 : 20. Both colorimetric and alkali-distillation methods gave quantitative recoveries with standard NH_3-N solutions, but when NH_3-N was determined in urine samples there was a significant discrepancy between analytical methods on the measured concentrations. These were between 1·3 and 10-fold (on average 2·6-fold) higher with alkali distillation than with the colorimetric method. The difference between concentrations measured by both analytical methods was significantly related ($R^2 = 0{\cdot}990$; $P < 0{\cdot}001$) to the concentration of urea in the urine samples. To study the effect of urea concentration, standard solutions containing variable concentrations of urea and NH_3-N were prepared, and NH_3-N concentration determined by both methods. Concentrations measured by colorimetry were similar to the actual concentrations irrespective of the urea concentration of the solutions. In urea-free solutions, alkali-distillation methods gave values similar to the actual concentrations, but when urea was present observed values were consistently higher than the expected concentrations. The overestimation increased with the urea concentration of the solutions, with a significant ($P < 0{\cdot}001$) relationship between both variables. Colorimetric methods were more reliable to measure NH_3-N concentrations in urine samples containing high urea concentrations. © 1998 Society of Chemical Industry.

J Sci Food Agric **78**, 95–101 (1998)

Key words: ammonia-nitrogen; analytical methods; urine; ruminants.

INTRODUCTION

In the ruminant ammonia is derived either from deamination of amino acids or from absorption from the digestive tract. This ammonia is highly toxic for the animal cells and has to be eliminated. In the liver, ammonia is rapidly converted into urea, which is excreted in the urine (Wright 1995) or recycled to the digestive tract. In ruminants, most of the urinary nitrogen (up to 70–90%) is excreted as urea (Bristow *et al* 1992), although that proportion can be much lower (up to 20%) when the N supply to the animal, relative to its requirements, is low. The proportion of nitrogen excreted as 'free ammonia' is very low (in most instances is less than 20 mg g^{-1} total N), and is usually derived from enzymatic degradation of glutamine (Huizenga *et al* 1994). However, in some physiological and clinical studies, the determination of urinary ammonia-nitrogen can be of special interest, because urea synthesis from ammonia and its degradation are important in the proton balance in the body (Atkinson 1992), and thus the concentration of ammonia in urine may serve as an index of the acid–base homeostasis of the body (Huizenga *et al* 1994).

In nutritional studies dealing with the partition of nitrogenous substances excreted in urine (Bristow *et al*

* To whom correspondence should be addressed.
Contract/grant sponsor: CICYT, Spain
Contract/grant number: AGF94–1357
Contract/grant sponsor: Junta de Castilla y León
Contract/grant number: LE 20/94

 J Sci Food Agric 0022–5142/98/$17.50. Printed in Great Britain

1992; Szanyiová *et al* 1995) or in tracer studies designed to quantify the body protein turnover (Reeds *et al* 1980) it can be important to measure the urinary excretion of both urea and ammonia. Information on the nitrogenous constituents in the urine and manure of ruminant livestock is also of interest due to their potential as organic fertilisers in grasslands (Bristow *et al* 1992) or when applied in the form of slurry (Sommer *et al* 1992).

A number of methods have been described for determination of ammonia in biological fluids (Lepo and Ferrenbach 1987; Huizenga *et al* 1994). For rumen fluid and urine from ruminants, the methods most commonly used are colorimetry or alkali-distillation of ammonia and titration with standard acid. In some preliminary tests conducted in our laboratory, it was observed that both methods were consistent when used for determinations in rumen fluid, but an important discrepancy was observed when urine samples were analysed. The objective of the present work was to study the suitability of colorimetric and alkali-distillation methods to determine the ammonia concentration in the urine of sheep and goats, investigating the reasons of the apparent discrepancy in the results obtained with both analytical methods.

MATERIALS AND METHODS

Samples

Two Merino sheep and two Alpine goats were used in this study. All animals were housed in individual cages and were fed alfalfa hay. Eleven spot samples (six from the sheep and five from the goats) were collected at different times of the day. Urine was taken at the time of excretion, collecting the total amount of urine produced during one micturition in polythene bottles. Approximately 30 ml of each sample were acidified by adding 0·2 M HCl in the proportion 1 : 1 (v/v). Another subsample (40 ml) of each urine was acidified by adding 10 ml of a solution containing 0·36 M H_2SO_4 and 0·01 M formaldehyde. pH was measured in all acidified samples and values were always below 3. Acidified samples were frozen at −20°C and stored until use.

Analytical techniques

The water used was purified by reverse osmosis and ion exchange (NH_3-free H_2O), and all reagents were of analytical grade. Urea concentration was determined by manual colorimetry using the reaction with diacetyl monoxime (Coulombe and Favreau 1963). All analyses were performed in duplicate for each urine sample and, in almost all instances, replicates did not differ more than 5% from the mean value.

Direct distillation and titrimetric determination of ammonia

Ammonia was separated by steam distillation using an automated distillation-titration unit for Kjeldahl nitrogen determination (KjelTec Auto 1030 Analyzer), collected in boric acid-indicator, and determined with standard acid. In this experiment, 10 ml aliquots of acidified urine were used, 35 ml of a saturated solution of $Na_2B_4O_7 \cdot 10H_2O$ (borax) were added to the urine sample for distillation of ammonia and titration was with 0·05 M HCl (method DD1). Alternatively, the procedure described by Bremmer and Keeney (1965) to eliminate interference with some alkali-labile organic nitrogen compounds was used. In this procedure (method DD2), 0·2 g of carbonate-free MgO were added to 10 ml of the sample for distillation of ammonia, and titration was with 0·005 M HCl.

Colorimetric determination of ammonia

The phenol-hypochlorite procedure described by Weatherburn (1967), based on the indophenol reaction, was used to determine NH_3-N concentration in urine (method C1). An aliquot of each diluted urine was mixed with a combination of the two reagents freshly prepared, phenol plus nitroprusside (catalyst) and alkali plus hypochlorite. The mixture was incubated at 39°C for 15 min and absorbance at 630 nm was measured. This procedure was compared with that described by Fawcett and Scott (1960), as automated procedures for ammonia determination are based on this last manual method. In this procedure (method C2) the following solutions were added immediately after each other to the aliquot of urine: sodium phenate, sodium nitroprusside (0·1 g litre^{-1}) and sodium hypochlorite (*c* 0·02 M), incubated at 27°C for 15 min and optical density at 630 nm was measured.

The optimum dilution rate of the urine samples for colour development during the indophenol reaction was investigated. Two specimens of urine (one from a sheep and one from a goat) were diluted 1 : 2, 1 : 4, 1 : 10, 1 : 20, 1 : 50 and 1 : 100 (v/v), and ammonia concentration in the diluted urines was determined by the colorimetric procedure C1.

Recovery assays

Recovery experiments were carried out on two specimens of urine (one from a sheep and one from a goat) acidified with HCl (and thus already diluted 1 : 2). In the first assay, 100, 200, 400 and 600 µl of a solution containing 7·0 mg NH_3-N litre^{-1} were added to 400 µl of each urine, then made up to 1 ml with distilled water (acidified urines diluted 4 : 10) and analysed by colorimetry (C1). A second assay was conducted in which

25, 50, 100, 150, 200 and 400 µl of the 7·0 mg NH_3-N litre^{-1} solution were added to 100 µl of each urine, then made up to 1 ml with distilled water (acidified urines diluted 1 : 10) and analysed by colorimetry (C1). In another assay, different volumes of a solution containing 70 mg NH_3-N litre^{-1} were added to 5 ml of each urine sample to increase their NH_3-N concentration by 17·5, 35, 70 and 140 mg litre^{-1}, and analysed by direct distillation (DD1) to determine NH_3-N recovered. In all the assays, recovery was estimated as the slope when NH_3-N measured by each method was regressed against the amount of NH_3-N added in each case, the intercept being an estimate of the amount of NH_3-N in the volume of urine used.

Effect of urea on NH_3-N determinations

Aqueous solutions containing 0, 8, 16 and 32 g urea litre^{-1} were prepared. In each urea solution different amounts of ammonium sulfate were dissolved to obtain solutions containing 0, 11, 22, 44 and 88 mg NH_3-N litre^{-1}. Thus, a total of 20 aqueous solutions was prepared, resulting from all possible factorial combinations of four levels of urea concentration and five levels of NH_3-N concentration. All these solutions (pH 5·2–6·5) were analysed to determine their NH_3-N concentration using methods DD1, DD2 and C1.

Statistical analyses

The effects of analytical method and of the acid used to lower the pH were determined by performing analysis of variance using the GLM procedure of SAS (1989). In the experimental design used, the analytical method was the main source of variation, whereas the effect of the type of acid was nested within the analytical method. The variability among urine samples was excluded from the experimental error (Steel and Torrie 1980). Direct comparisons between two methods were carried out using Student's *t*-test for paired observations. Linear regression and correlation analyses were performed by the REG and CORR procedures of SAS (1989), respectively.

RESULTS AND DISCUSSION

Colorimetric method

The results presented herein describe the characteristics of the standard curve with the colorimetric method of Weatherburn (1967), the dilution required to analyse urine samples and the recovery of NH_3-N from sheep and goat urine samples when NH_3-N was added to increase its concentration by several levels.

The relationship between NH_3-N concentration of standard solutions and optical density at 630 nm was linear in the concentration range from 0·35 to 7 mg NH_3-N litre^{-1}. Residual standard deviations (RSD) of the standard curves were between 0·090 and 0·164 (mean value was 0·133). Although absorbances observed with undiluted urine samples were below maximum values suggested by other authors (Weatherburn 1967), urines had to be diluted in order to allow for full colour development. Ammonia nitrogen concentrations measured in urine samples diluted 1 : 2 with 0·2 M HCl were unexpectedly low because colour development and optical density were minimal. Measured NH_3-N concentration increased as urine samples were diluted further reaching a maximum for urines diluted 1 : 20. Although Fawcett and Scott (1960) suggested a dilution of 1 : 200 for urine samples, no apparent differences in estimated NH_3-N concentrations were observed when the sheep and goat urine samples were diluted 1 : 20, 1 : 50 or 1 : 100. It has been pointed out that the indophenol reaction may be inhibited by a number of non-protein nitrogen substances normally found in urine, including creatinine (Dilworth *et al* 1992).

Recovery was influenced by the dilution, so only the 1 : 20 dilution resulted in satisfactory recoveries of NH_3-N. Estimated recoveries for 1 : 20 diluted urines were 96·5% and 99·6% for the sheep and the goat urine samples respectively, whereas recoveries were 67·2% and 75·7% for the same urines when dilution was 1 : 5. These recovery assays confirm that samples need to be diluted to at least 1 : 20 to minimise the interference of other nitrogen compounds on colour development.

Comparison between colorimetric procedures

There were no significant ($P > 0{\cdot}10$) differences between NH_3-N concentrations estimated by the colorimetric procedures described by Fawcett and Scott (1960) and by Weatherburn (1967). The linear relationship between values obtained with both methods was:

$$NH_3\text{-FS} = 1{\cdot}07(\text{SE } 0{\cdot}063)NH_3\text{-W} - 1{\cdot}45(\text{SE } 1{\cdot}097)$$
$$(R^2 = 0{\cdot}950;\ n = 11;\ P < 0{\cdot}001)$$

where NH_3-FS and NH_3-W are the NH_3-N concentrations (mg litre^{-1}) measured by the procedures of Fawcett and Scott (1960) and by Weatherburn (1967), respectively. The mean bias between values obtained by both methods was less than 1% and the slope of the regression line did not differ from unity ($P > 0{\cdot}10$). The slope of the relationship between NH_3-N concentration and optical density was lower with the Weatherburn procedure ($7{\cdot}63 \pm 0{\cdot}142$ vs $11{\cdot}80 \pm 0{\cdot}233$ mg NH_3-N per unit of absorbance), indicating that sensitivity could be slightly higher than with the other procedure. Concentration measured with the procedure described by

Weatherburn (1967) will be used for the comparison with distillation methods.

Direct distillation method

The relationship between NH_3-N concentration of standard solutions and the amount of 0·05 M HCl used in the titration was linear ($R^2 = 0{\cdot}998$, $P < 0{\cdot}001$). NH_3-N concentrations could be determined by distillation in undiluted urine samples providing the aliquots of urine contained up to 100 mg NH_3-N litre^{-1} (Bremmer and Keeney 1965). Quantitative recovery of NH_3-N was obtained by distillation, with recovery values of 95·6% and 97·6% for the sheep and goat urines used previously. However, the method cannot accurately detect low NH_3-N concentrations and requires greater amounts of sample than colorimetric methods. Sensitivity can be considerably increased by decreasing the molarity of the acid solution used for titration.

Comparison between colorimetric and distillation methods

Table 1 shows the source of each urine sample, with the concentration of urea and of NH_3-N measured by colorimetric (C1) and distillation methods (DD1). Values for the urea and ammonia concentrations were consistent with the partition of nitrogenous substances in the urine of sheep and goats reported by other authors (Bristow *et al* 1992; Szanyiová *et al* 1995). The concentration of NH_3-N was much lower than that of urea N, since this is the dominant form of urinary nitrogen, whereas NH_3-N represents only a small proportion of the total N in sheep and goat urine (Bristow *et al* 1992; Szanyiová *et al* 1995). Sommer *et al* (1992) found that total ammonium nitrogen (TAN) accounted for a high proportion of total nitrogen in pig and cattle slurry although, for these authors, TAN included both urea and ammonia N. The composition in urea and ammonia N of goat urine was similar to that of sheep urine.

Correlation coefficients between NH_3-N concentrations measured by colorimetric and distillation methods in urines acidified either with HCl or with H_2SO_4 are shown in Table 2.

Despite apparent differences for some samples, there were no statistically significant ($P > 0{\cdot}10$) effects of the type of acid used to lower the pH of the urines on the NH_3-N concentrations measured by both techniques. Linear and rank correlation coefficients between types of acidified urine within each analytical method (Table 2) were highly significant ($P < 0{\cdot}01$). Lepo and Ferrenbach (1987) have suggested that measurements of ammonia by colorimetry can be affected by small variations in H_2SO_4 added to the sample. Fawcett and Scott (1960) indicated that the phenate–hypochlorite reaction is influenced by pH, but concluded that the inclusion of buffers to acid solutions was unnecessary. Based on current results, it seems that the type of acid used does not have a significant effect on the determination of NH_3-N, providing the amounts of acid added are calculated for similar final pH values in the acidified urines.

There were significant differences ($P < 0{\cdot}001$) between analytical methods in the measured NH_3-N concentra-

TABLE 1

Source of each urine sample, with urea concentration and ammonia nitrogen concentration in urine samples acidified either with HCl or with H_2SO_4 measured either by colorimetric (method C1[a]) or alkali-distillation (method DD1[a]) methods

Urine number	*Source*	*Urea (g litre^{-1})*	*NH_3-N concentration (mg litre^{-1})*			
			Colorimetry		*Alkali-distillation*	
			HCl	*H_2SO_4*	*HCl*	*H_2SO_4*
1	Sheep	1·2	13·6	16·5	21·1	22·3
2	Sheep	18·9	101·7	131·3	189·2	217·9
3	Sheep	4·6	5·0	9·0	26·3	23·7
4	Sheep	3·2	4·8	5·3	15·2	19·0
5	Sheep	1·6	22·9	18·0	30·6	27·9
6	Sheep	7·1	21·6	16·0	54·2	28·4
7	Goat	12·2	25·6	28·1	89·0	75·5
8	Goat	13·2	16·4	21·7	81·1	90·7
9	Goat	6·8	3·3	3·1	35·1	—[b]
10	Goat	12·1	25·6	17·3	87·7	110·7
11	Goat	12·9	22·9	18·6	84·5	56·5

[a] See text for details.
[b] Not measured (not enough sample).

TABLE 2

Correlation matrix (Spearman rank correlation coefficients in the upper diagonal and Pearson linear correlation coefficients in the lower diagonal) between values of ammonia nitrogen concentration measured, either by colorimetric (method C1[a]) or alkali-distillation (method DD1[a]) methods, in urine samples acidified either with HCl or with H_2SO_4[b]

	Colorimetry		*Alkali-distillation*	
	HCl	*H_2SO_4*	*HCl*	*H_2SO_4*
Colorimetry				
HCl		0·827**	0·836**	0·854**
H_2SO_4	0·984***		0·763**	0·794**
Alkali-distillation				
HCl	0·910***	0·886***		0·939***
H_2SO_4	0·904***	0·894***	0·962***	

[a] See text for details.
[b] *** $P < 0{\cdot}001$; ** $P < 0{\cdot}01$.

tions. Values obtained by distillation and titration (DD1) were between 1·3 and 10-fold (on average 2·6-fold) higher than those measured by colorimetry (C1). However, both measurements were significantly correlated ($P < 0{\cdot}001$) and Spearman rank correlation coefficients were also significant ($P < 0{\cdot}01$), indicating that in general the ranking of samples according to their NH_3-N concentration was not affected by the analytical method. The difference between values obtained by each method did not seem to be associated to the NH_3-N concentration of the sample, but there was a significant ($P < 0{\cdot}001$) relationship between the difference in the values obtained by distillation methods relative to those obtained by colorimetry and the urea concentration of the urine samples. This relationship for the urines acidified with HCl is illustrated in Fig. 1.

Table 3 shows the measured NH_3-N concentrations (using methods C1, DD1 and DD2) in the aqueous solutions containing different concentration of NH_3-N and urea. In the urea-free solutions the NH_3-N concentrations measured by the three methods were similar to the actual concentrations of NH_3-N in the solutions. Agreement between observed and actual values was generally good (differences <15%) when NH_3-N concentrations were measured by colorimetry, irrespective of the quantity of urea added to the solutions. However, when urea was added to the solutions, the concentrations of NH_3-N measured by distillation methods were up to 8-fold greater than the actual concentrations.

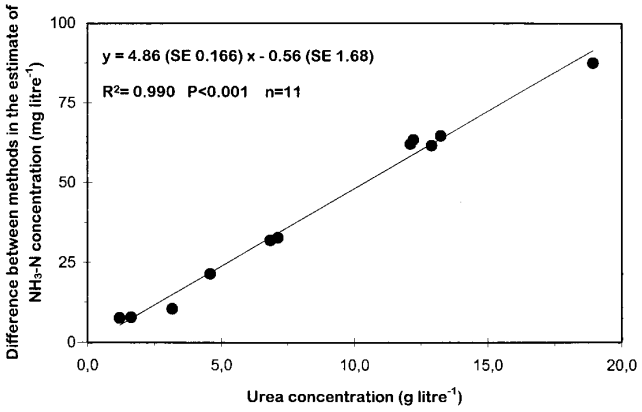

Fig 1. Relationship between urea concentration (g litre^{-1}) of urines preserved with HCl and the difference of the NH_3-N concentration measured by the alkali-distillation method, relative to that obtained by the colorimetric method.

Overestimation between ammonia concentrations measured by alkali-distillation methods and actual concentrations was similar in all the solutions containing the same concentration of urea, irrespective of the NH_3-N concentration. However, the overestimation was consistently affected by the urea concentration, and the ensuing significant relationships were obtained:

$$\text{Diff (O-E)} = 2.30(\text{SE } 0{\cdot}043)\text{urea} + 0{\cdot}24(\text{SE } 0{\cdot}784$$

$$R^2 = 0{\cdot}998 \quad P < 0{\cdot}001 \quad n = 4$$

when borax was used for the distillation of ammonia, and

$$\text{Diff (O-E)} = 1{\cdot}76(\text{SE } 0{\cdot}007)\text{urea} - 3{\cdot}09(\text{SE } 0{\cdot}136)$$

$$R^2 = 0{\cdot}999 \quad P < 0{\cdot}001 \quad n = 4$$

when MgO was used in the distillation; where Dif. (O-E) is the difference between observed and expected NH_3-N concentrations (mg litre^{-1}) and urea is the concentration of urea (g litre^{-1}) in the aqueous solutions.

During the distillation of ammonia, high temperatures and alkaline pH values give rise to the hydrolysis of alkali-labile organic nitrogen compounds. This effect depends on the alkali strength, and thus it has been reported that more ammonia nitrogen is distilled

TABLE 3
Ammoniacal nitrogen and urea concentrations in prepared aqueous solutions and NH_3-N concentrations measured by colorimetry (method C1) or by alkali-distillation using either borax (method DD1) or MgO (method DD2)

Actual NH_3-N concentration (mg litre^{-1})	*Urea concentration (g litre^{-1})*	*NH_3-N concentration (mg litre^{-1})*		
		Method C1	*Method DD1*	*Method DD2*
0	0	0·1	0·8	0·0
0	8	0·0	20·3	12·2
0	16	0·0	40·4	23·4
0	32	0·1	77·0	50·8
11·4	0	11·1	11·6	9·5
11·6	8	12·6	38·6	21·3
11·3	16	10·6	56·8	34·8
11·4	32	11·2	95·5	61·9
22·8	0	25·0	27·3	19·9
23·3	8	24·6	49·8	34·6
22·7	16	22·2	70·1	51·6
22·7	32	22·3	105·1	79·2
45·7	0	48·7	51·0	39·1
46·5	8	50·6	83·0	52·6
45·3	16	47·4	95·8	66·3
45·5	32	47·2	130·8	95·7
86·7	0	89·3	93·7	86·2
88·3	8	98·6	123·6	100·2
86·0	16	93·9	137·3	111·1
86·2	32	91·4	171·8	138·3

with NaOH than with MgO in slurry (Sommer *et al* 1992) and in soil samples (Bremmer and Keeney 1965). The interference of some alkali-labile nitrogen compounds in alkali-distillation methods of determining ammonium ion can be eliminated by performing the distillation using a small amount of MgO and a very short period of incubation (Bremmer and Keeney 1965). In this experiment, an extensive hydrolysis of urea has been caused by both borax and MgO. Bremmer and Keeney (1965) found that glucosamine and galactosamine yielded ammonia under alkali-distillation, whereas the presence of urea and other nitrogen compounds did not interfere in determination of ammonia. However, it must be stressed that tests carried out by these authors were using solutions containing up to 10 mg urea-N litre^{-1}, whereas concentrations in sheep and goats urine are in the range of 3–20 g urea-N litre^{-1} (Bristow *et al* 1992).

CONCLUSIONS

This study has confirmed the overestimation of NH_3-N in the urine of small ruminants by the alkali-distillation methods when compared with a colorimetric determination. The extent of overestimation is strongly related to the urea concentration of the urine and, given the high urea content of urine samples, alkali-distillation methods are inappropriate to determine their ammonia concentrations. Colorimetry gave quantitative determination of ammonia-N in aqueous solutions containing variable amounts of urea, and seems to be a more accurate technique to determine the urinary ammonia concentration. The NH_3-N concentration in urine samples amounts to a low proportion of total urinary nitrogen, and can be affected by a number of factors (pH and temperature during collection, interval between excretion and transfer to the cold store) which determine the hydrolysis of urea to ammonia immediately after urination (Bristow *et al* 1992). In nutritional studies, where urine is usually collected over 24 h periods, it seems that there will always be some urea breakdown, and could be appropriate to measure total urea + ammonia nitrogen, by enzymatic hydrolysis of all the urea and then determine ammonium ion either by colorimetry (Fawcett and Scott 1960) or by alkali distillation. The last alternative has been proposed by AOAC (1995) for the determination of urea and ammoniacal nitrogen in animal feeds. However, in some physiological and clinical studies it can be important to distinguish between urea and ammonia in the urine. In this case, ammonia N in urine can be reliably measured by the colorimetric method (and not by distillation), although it is important that no urea is degraded to ammonia during the interval from collection to analytical determination (samples have to be stored at low pH and temperature, and during very short periods of time).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support received from the Comisión Interministerial de Ciencia y Technología of Spain (Project AGF94-1357) and from Junta de Castilla y León (Project LE 20/94).

REFERENCES

AOAC 1995 *Official Methods of Analysis of AOAC International* (16th edition). AOAC International, Arlington, VA, USA.

Atkinson D E 1992 Functional roles of urea synthesis in vertebrates. *Physiol Zool* **65** 243–267.

Bremmer J M, Keeney D R 1965 Steam distillation methods for determination of ammonium, nitrate and nitrite. *Anal Chim Acta* **32** 485–495.

Bristow A W, Whitehead D C, Cockburn J E 1992 Nitrogenous constituents in the urine of cattle, sheep and goats. *J Sci Food Agric* **59** 387–394.

Coulombe J J, Favreau L 1963 A new simple semimicro method for colorimetric determination of urea. *Clin Chem* **9** 102–108.

Dilworth M J, Eldridge M E, Eady R R 1992 Correction for creatinine interference with the direct indophenol measurement of NH_3 in steady-state nitrogenase assays. *Anal Biochem* **207** 6–10.

Fawcett J K, Scott J E 1960 A rapid and precise method for the determination of urea. *J Clin Path* **13** 156–159.

Huizenga J R, Tangerman A, Gips C H 1994 Determination of ammonia in biological fluids. *Ann Clin* **31** 529–543.

Lepo J E, Ferrenbach S M 1987 Measurement of nitrogen fixation by direct means. In: *Symbiotic Nitrogen Fixation Technology*, ed Elkan G H. Marcel Dekker Inc, New York, USA, pp 221–255.

Reeds P J, Cadenhead A, Fuller M F, Lobley G E, McDonald J D 1990 Protein turnover in growing pigs. Effect of age and food intake. *Br J Nutr* **43** 445–455.

SAS 1989 *SAS*/STAT® *User's Guide*, *Version 6* (4th edition). SAS Institute Inc, Cary, NC, USA.

Sommer V K, Kjellerup V, Kristjansen O 1992 Determination of total ammonium nitrogen in pig and cattle slurry: sample preparation and analysis. *Acta Agric Scand*, *Sect B* (*Soil and Plant Sci*) **42** 146–151.

Steel R G D, Torrie J H 1980 *Principles and Procedures of Statistics* (2nd edn). McGraw-Hill, London, UK.

Szanyiová M, Leng L, Faix Š 1995 Partition of nitrogenous substances in the urine of sheep on different dietary protein intakes. *Vet Res* **26** 27–31.

Weatherburn M W 1967 Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* **39** 971–974.

Wright P A 1995 Nitrogen excretion: three end products, many physiological roles. *J Exper Biol* **198** 273–281.