

Rumen ammonia concentrations, and non-ammonia nitrogen passage to and apparent absorption from the small intestine of sheep ingesting subtropical, temperate, and tannin-containing forages

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Non-ammonia nitrogen (NAN) passage to the small intestine and disappearance of NAN in that organ have been studied in multi-cannulated sheep on pasture or fed indoors. Forages under investigation included a number of subtropical grasses, foggages and hay, temperate grasses, lucerne (*Medicago sativa*), triticale (*Triticale* × *Secale*), oven-dried ryegrass (*Lolium multiflorum*), and the tannin-containing forages sainfoin (*Onobrychis viciifolia*) and sheep's burnet (*Sanguisorba minor*). Digesta flow was measured with reference to Yb-acetate and Cr-EDTA as particulate and fluid markers, respectively. Rumen ammonia concentrations were exponentially associated with the nitrogen (N) content of the forage, with concentrations increasing progressively above 2.5% N in the forage dry matter. Ruminal fermentation of tannin-containing forages resulted in much lower ammonia concentrations than ruminal fermentation of forages without tannins. NAN passage to the small intestine as a proportion of N intake was significantly ($P \leq 0.05$) different between forage classes: 0.97 ± 0.09 for subtropical forages, 0.75 ± 0.09 for temperate grasses plus lucerne and triticale, and 1.12 ± 0.08 for tannin-containing forages and dried grass. NAN passage as a proportion of N intake declined with increasing N intake in all non-tannin forages, the decline being especially severe in forages with high total (above 3%) and/or high soluble N. NAN disappearance in the small intestine as a proportion of NAN entering that organ differed significantly ($P \leq 0.01$) between tannin-containing forages and subtropical foggages and hay on the one hand (0.64 ± 0.06), and all other forages on the other hand (0.76 ± 0.02). NAN disappearance as a proportion of N intake increased in the order: temperate grasses plus lucerne and triticale, subtropical foggages and hay, subtropical grasses, tannin-containing forages, and dried grass.

Die vloeï van nie-ammoniak stikstof (NAN) na, en die verdwyning daarvan in die dunderm is bestudeer in meervoudig-gefituleerde skape op weiding of binnenshuis gevoer. Ruvoere wat bestudeer is het ingesluit: 'n aantal subtropiese grasse, staande hooie, gematigde grasse, lusern (*Medicago sativa*), korog (*Triticale* × *Secale*), oondgedroogde raaigras (*Lolium multiflorum*), en die tannien-bevattende weidings sainfoin (*Onobrychis viciifolia*) en skaapburnet (*Sanguisorba minor*). Digestavloeï is gemeet deur gebruik te maak van Yb-asetaat en Cr-EDTA as vastestof- en vloeïstofmerkers, onderskeidelik. Rumenammoniakkonsentrasie was eksponensieel verwant aan stikstof(N)-inhoud van die ruvoer, veral bokant 2.5% N in die ruvoer droë materiaal. Rumenfermentasie van tannien-bevattende ruvoere het tot laer ammoniakkonsentrasies gelei as rumenfermentasie van ruvoere wat nie tannien bevat het nie. NAN-vloeï na die dunderm as 'n verhouding van N-inname het betekenisvol ($P \leq 0.05$) verskil tussen ruvoerkategorie: 0.97 ± 0.09 vir subtropiese ruvoere, 0.75 ± 0.09 vir gematigde grasse plus lusern en korog, en 1.12 ± 0.08 vir tannien-bevattende ruvoere en gedroogde gras. NAN-vloeï as 'n verhouding van N-inname het progressief afgeneem met 'n toename in N-inname by alle nie-tannien ruvoere, en die afname was veral merkbaar by ruvoere met hoë totale (meer as 3%) en/of hoë oplosbare N. NAN-verdwyning in die dunderm as 'n verhouding van NAN wat na die dunderm gevloeï het, het betekenisvol ($P \leq 0.01$) verskil tussen tannien-bevattende ruvoere en subtropiese staande en gesnyde hooie aan die een kant (0.64 ± 0.06), en alle ander ruvoere aan die ander kant (0.76 ± 0.02). NAN-verdwyning as 'n verhouding van N-inname het toegeneem, en wel in die volgorde: gematigde grasse plus lusern en korog, subtropiese staande en gesnyde hooie, subtropiese grasse, tannien-bevattende ruvoere, en gedroogde gras.

Keywords: Absorption, digestion, forage, non-ammonia nitrogen, sheep.

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Introduction

The absorption of optimal quantities of amino acids from the small intestine is of paramount importance to the productive capabilities of the ruminant. On pasture, MacRae & Ulyatt (1974) found that daily gain correlated better with amino acids apparently absorbed from the small intestine than with the energy yielding parameters. John & Lancashire (1981) and Marten *et al.* (1987) showed higher rates of gain in sheep and cattle grazing Lotus and sainfoin than in those grazing lucerne and ryegrass, owing to a better absorption of amino acids on the former pastures (Thomson *et al.*, 1971). Sainfoin protein is

degraded less than most grass proteins (Barry, 1984; Acheampong-Boateng, 1991) and therefore supplements the amino acid flow to the small intestine. Extensive hydrolysis of highly soluble and degradable proteins often results in insufficient protein reaching the small intestine (Aii & Stobbs, 1980). The problem is of particular concern in temperate pastures high in nitrogen (N) content and is further aggravated by N fertilization.

It follows, therefore, that it would be of value to predict the amino acid uptake from different forage types. This is of particular importance in subtropical forages, which have received

comparatively less attention than temperate forages. Secondly, more quantitative information on amino acid-N passage to the small intestine is required to enable better prediction by protein evaluation systems and standards. Consequently, the present study aimed to investigate differences in apparent absorption between subtropical pastures, foggages and hay, temperate pastures, dried grass and forages containing tannins.

Methods

The forages investigated and their chemical composition in terms of N content, neutral detergent fibre (NDF) and *in vitro* digestibility of organic matter (IVDOM) are shown in Table 1. *In vitro* DOM was measured by the Tilley & Terry (1963) method, as adapted by Engels *et al.* (1981). Nitrogen content was determined by Kjeldahl (AOAC, 1980) and NDF by the method of Van Soest & Wine (1967).

Nitrogen intake and NAN flow and digestibility were measured in partial digestion trials either on pasture or when pasture material was fed to sheep in metabolic cages. On each pasture, at least four Merino type wethers were fitted with cannulae in the rumen, abomasum and terminal ileum. At least two more received oesophageal fistulae. Pasture intake was estimated from the ratio faeces (OM) voided in collection bags and the indigestibility of oesophageal samples. Faeces were collected over five days after an adaptation period of 14 days.

Oesophageal samples were obtained at the beginning and the end of the faeces collection period after the sheep had been fasted overnight. *In vivo* DOM was calculated from IVDOM before intake calculation. The following regression equation, developed at this laboratory, applied:

$$\begin{aligned} \text{In vivo DOM (\%)} &= (0.746 \pm 0.038) \text{IVDOM (\%)} + \\ &\quad (18.16 \pm 2.51) \\ r^2 &= 0.975 \\ \text{Sy. x} &= 1.80 \end{aligned}$$

Nitrogen intake was calculated by multiplying the N content of the pasture OM with the calculated OM intake.

Flow of NAN to the small intestine as well as apparent absorption of NAN in that organ was determined from digesta flow measurements, using ytterbium-acetate (Yb-acetate) and chromium-EDTA (Cr-EDTA) as particulate and fluid markers, respectively (Siddons *et al.*, 1985). Markers were infused by a peristaltic pump fitted to the back of the sheep (Corbett *et al.*, 1976). Infusion commenced on day 10 of the adaptation period with a primer dose and continued at a rate of about 100 mg Yb-acetate and 200 mg Cr-EDTA/d. Dry matter and fluid flows were reconstituted to resemble true digesta passage by the mathematical procedure of Faichney (1980). Digesta samples from the rumen, abomasum and ileum were collected at regular intervals for four days and pooled for analysis (Faichney, 1980).

Table 1 Forages used in the investigation and their chemical composition (DM basis, except digestibility^a)

Forage class and species		Chemical composition		
Botanical name	Common name	N	NDF	Digestibility
Subtropical grasses				
<i>Anthephora pubescens</i>	Anthephora	3.3	54	64
<i>Chloris gayana</i>	Rhodes	2.6	69	64
<i>Digitaria eriantha</i> spp. <i>eriantha</i>	Smuts finger	1.8—2.7	62—64	51—61
<i>Panicum maximum</i>	Panicum	2.8—3.2	—	61
Subtropical foggages and hay				
	Anthephora foggage	1.5	65	51
	Rhodes foggage	0.9	72	46
	Panicum foggage	2.1	59	51
	Panicum hay: Early	2.2	54	59
	Late	1.8	59	55
<i>Pennisetum</i> hybrids	Bana foggage	1.7	66	60
	Green gold foggage	1.8	63	59
	Penaris foggage	1.9	65	54
Temperate grasses				
<i>Dactylis glomerata</i>	Cocksfoot	3.5	61	69
<i>Lolium multiflorum</i>	Italian ryegrass	3.0—4.4	46—48	71—78
	dried	2.9	49	79
<i>Lolium perenne</i>	Perennial ryegrass	2.5	60	70
<i>Medicago sativa</i>	Lucerne	3.4—4.5	35—48	65—69
<i>Triticale</i> × <i>Secale</i>	Triticale	2.8	56	69
Tannin forages				
<i>Onobrychis viciifolia</i>	Sainfoin	3.0—3.8	42—50	55—62
<i>Sanquisorba minor</i>	Sheeps' bumet	2.5—3.5	27—37	55—63

^a IVDOM.

Similar procedures were followed indoors, except that intake and digestibility figures were determined directly. Markers in this instance were infused via an autoanalyser pump.

Rumen, abomasal and ileal samples were analysed for N by Kjeldahl and for ammonia (NH₃) by means of an automated procedure (Technicon Auto Analyzer II. Industrial Method No. 334-74A, Jan. 1976). Non-ammonia N was calculated by difference.

The passage of NAN to the small intestine was related to N content of forages or N intake by linear regression. Similarly, NAN disappearance (apparent absorption) in the small intestine was related to NAN passage to the small intestine by linear regression. In contrast, the best fit for rumen ammonia levels against N content of forages was obtained by the power function $Y = ax^b$. Differences between forage categories in NAN flow as a proportion of N intake and NAN disappearance as a proportion of NAN flow, were tested by one-way analysis of variance and Tukey's *t* test. These methods are described by the manual of the GLM procedure of SAS (1985).

Results and Discussion

The relationship between the N content of the forage and rumen NH₃ concentrations is shown in Figure 1. Each data point in Figure 1 and subsequent figures represents the mean of at least four observations.

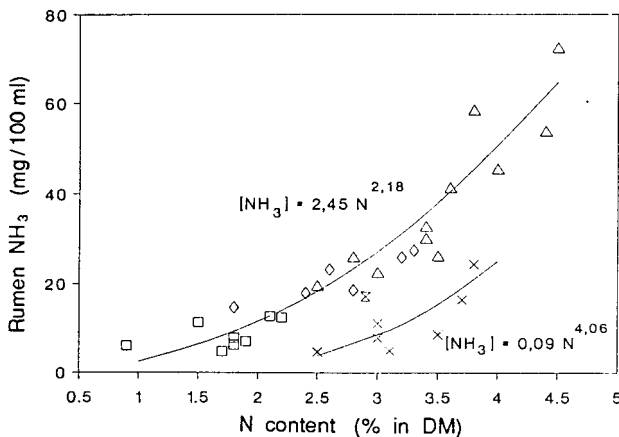


Figure 1 Relationship between N content of forage and rumen ammonia concentrations. \diamond Subtropical grasses; Δ temperate grasses, plus lucerne and triticale; \times dried grass; \square subtropical foggages and hays; \times forages containing tannins.

One power function fitted the data of subtropical grasses, foggages and hay, temperate grasses, plus lucerne and triticale, and dried grass, and another one the data of the tannin-containing forages (sainfoin and sheeps' burnet). Although NH₃ concentrations are not only a function of N content, but also of N solubility, energy content of the diet and level of intake (Van Soest, 1982), N content accounted for 81% and 70% of the variation in rumen NH₃ concentrations in the two relationships, respectively. The difference between tannin-containing forages and the other forage classes is explained by the lower N solubility and rumen degradation which result from tannin binding of protein (Barry, 1984; Acheampong-Boateng, 1991). The relationship for the other forages shows

that rumen NH₃ concentrations increase progressively above N contents of 2.5 to 3%. This observation corresponds with the *in vitro* study of Satter & Slyter (1974) which showed a response in microbial yield below about 2.5% but not above. Above 2.5% N content, rumen N is increasingly wasted as NH₃. The results of these authors, however, have been extrapolated to other conditions and consequently criticized. Most results, depending on the microflora (Mehrez *et al.*, 1977; Wallace, 1979), indicated optimal NH₃ concentrations between 2 and 20 mg NH₃/100 ml rumen fluid. Note that 2.5% N content (Figure 1) corresponds with a rumen NH₃ concentration of about 20 mg NH₃/100 ml in the present study.

Table 2 shows estimates of the parameters of the simple linear regression of NAN passage to the small intestine (*y*) on the N content of all non-tannin forages (*x*). Tannin-containing forages were excluded because of poor fit.

Table 2 Parameters of the simple linear regression of non-ammonia nitrogen (NAN) flow to the small intestine (g/d) on N content of the forage^a (% of DM)

Slope	Intercept	r ²	Error of estimate (Sy . x)
8.41 ± 1.20	0.45 ± 3.28	0.71	4.76
8.58 ^b	-	-	4.89

^a All forages with the exclusion of tannin forages.

^b Adjusted slope of line with intercept forced to be zero.

On physiological grounds the intercept should be zero. The test confirmed that it did not differ significantly from zero. The variation between forage classes was reflected in a relatively large error of estimate (coefficient of variation of about 20%). Nevertheless, N content in all non-tannin forages *per se* accounted for 70% of the variation in NAN flow. The variation in NAN passage can also be seen in Figure 2 which depicts the relationship between N intake and NAN passage to the small intestine.

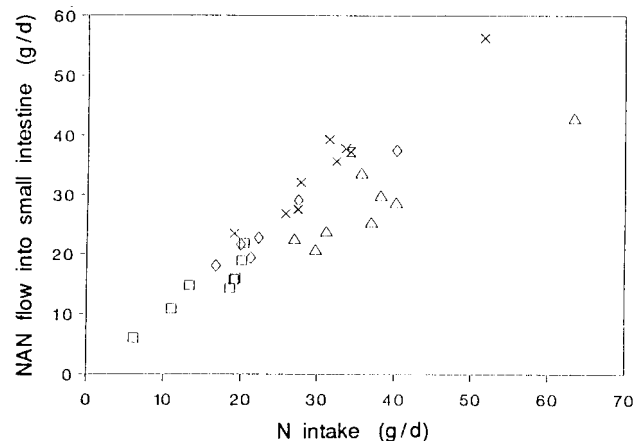


Figure 2 Relationship between N intake and NAN flow to the small intestine. \diamond Subtropical grasses; Δ temperate grasses, plus lucerne and triticale; \times dried grass; \square subtropical foggages and hays; \times forages containing tannins.

Table 3 Parameters of the simple linear regression of NAN passage to the small intestine (g/d) on N intake (g/d) and the difference between forage classes

Forage category	Slope	Intercept	r ²	Mean proportion ¹
Subtropical grasses ²	0.85 ± 0.09	3.62 ± 2.26	0.96	1.01 ± 0.07 ^{bc}
Subtropical foggages and hay	0.83 ± 0.16	1.52 ± 2.64	0.82	0.94 ± 0.11 ^b
Temperate grasses plus lucerne and triticale	0.56 ± 0.11	7.09 ± 4.27	0.81	0.75 ± 0.09 ^a
Tannin forages plus dried grass	1.09 ± 0.14	0.74 ± 3.39	0.96	1.12 ± 0.08 ^c

¹ NAN flow as a proportion of N intake.

² The parameters of the regression equations for subtropical grasses and subtropical foggages and hay do not differ significantly.

The combined equation is: slope = 0.84; intercept = 2.45.

^{a-c} Differences significant at the 5% level.

Parameters of the simple linear regression equations for different forage classes of NAN flow to the small intestine (y) on N intake (x) are shown in Table 3. The data of lucerne, triticale and the temperate grasses were grouped as one class, while the data of the dried grass which corresponded with the data of the tannin-containing forages were accordingly grouped as one class (Figure 2). Although the slopes and intercepts of the classes subtropical grasses and subtropical foggages and hay (Table 3) did not differ significantly, the regression parameters are shown separately for illustrative purposes. Table 3 also shows the differences between forage classes in NAN passage as a proportion of N intake.

Apart from the tannin forage-dried grass class, the slopes of all other classes were less than one, suggesting decreasing proportional NAN flow with increasing N intake. The decrease was particularly severe in the class temperate grasses plus lucerne and triticale. This class contained forages with high soluble N and/or high total N and it appeared that, with increasing N intake, the loss of NH₃ to the detriment of microbial protein production (Aii & Stobbs, 1980) was aggravated. The means of NAN flow as a proportion of N intake increased from 0.75 for temperate grasses plus lucerne and triticale, to 0.94–1.01 for subtropical grasses, foggages and hay (0.97 together), to 1.12 for tannin forages plus dried grass ($P \leq 0.05$). The figure of approximately 1.0 for subtropical forages corresponds with the suggested values of 1.00 to 1.035 of the NRC (1984; 1989) for diets where NAN flow consists almost entirely of microbial protein. The figure of 1.135 of the NRC (1989) which applies when NAN contains a high proportion of undegraded N, corresponds with the value of 1.12 obtained for tannin-containing forages and dried grass. These forages contained almost no soluble N and their predicted degradation was between 50 and 70% (Acheampong-Boateng, 1991; Du Preez & Meissner, 1992). In contrast, the soluble N content in immature lucerne (Acheampong-Boateng, 1991), Italian ryegrass (Erasmus *et al.*, 1990), and ryegrass and clover pastures (MacRae & Ulyatt, 1974), which would fall in the temperate forage class, was between 30 and 50% and their predicted degradation in excess of 80%.

NAN flow was also calculated for different forage N contents. When a N content was specified, the second equation of Table 2 was used to calculate NAN flow. This value was then used with the appropriate equation of Table 3 to solve for N intake, allowing the calculation of the ratio of NAN flow to

Table 4 NAN flow to the small intestine as a proportion of N intake, estimated at different forage^a N content

Forage category	N content (% of DM)					
	1.5	2.0	2.5	3.0	3.5	4.5
Subtropical grasses, foggages and hay	1.04	0.98	0.95	0.93	0.91	–
Temperate grasses, plus lucerne and triticale	–	0.96	0.84	0.77	0.73	0.69

^a Excluding tannin forages, plus dried grass.

N intake for a specified N content for the various forages listed in Table 3. The results of subtropical forages and temperate grasses plus lucerne and triticale are shown in Table 4.

The results showed that NAN flow as a proportion of N intake decreased with N content in both forage classes. However, the proportion at 2% N content was about equal in both classes and just below a value of 1.0. This confirmed the value of ≥ 1.0 for dietary N contents between 2 and 3% of DM reported by Hogan and Weston (1970) and Armstrong (1973), as cited by Van Soest (1982). From this data, and bearing in mind the suggestions from the rumen NH₃ results (Figure 1), it would appear that NAN flow as a proportion of N intake should be optimal when the N content of non-tannin forages is between 2 and 3%.

The plots of NAN disappearance in the small intestine against NAN passage to that organ (Figure 3) illustrate relatively small variation between forage classes. In fact, covariance analyses, distinguishing between all possible groups, indicated only two distinct groups: subtropical grasses, temperate grasses, lucerne, triticale and dried grass on the one hand, and subtropical foggages and hay, and tannin-containing forages on the other (Table 5).

Non-ammonia N disappearance as a proportion of NAN flow indicated a significant ($P \leq 0.01$) difference between the two groups (Table 5), with mean values of 0.76 and 0.64, respectively. Both figures correspond with the theoretical values of between 0.64 to 0.80 suggested by the NRC (1989) and the summary of literature values of between 0.65 and 0.70 as reviewed by the ARC (1980) and NRC (1985).

The lower disappearance of NAN of foggages and hay presumably resulted because of higher proportions of cell

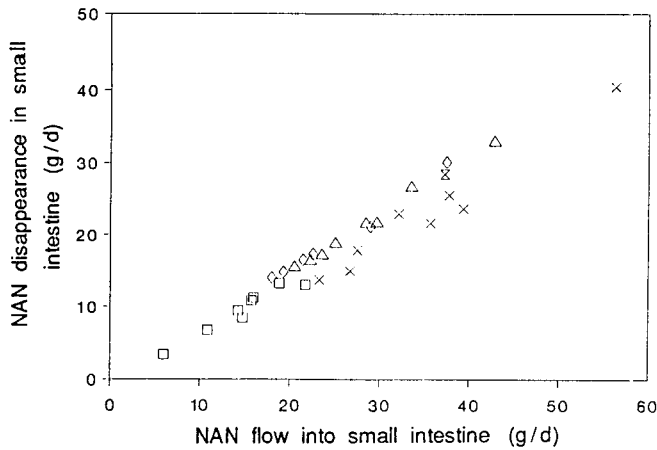


Figure 3 Relationship between NAN flow to the small intestine and its digestibility in that organ. \diamond Subtropical grasses; Δ temperate grasses, plus lucerne and triticale; \times dried grass; \square subtropical foggages and hays; \times forages containing tannins.

Table 5 Parameters of the simple linear regression of NAN disappearance (g/d) in the small intestine on NAN passage (g/d) to that organ, and the difference between forage classes

Forage category	Slope	Intercept	r ²	Mean proportion ¹
Subtropical and temperate grasses, lucerne, triticale, and dried grass	0.81 ± 0.03	-1.34 ± 0.71	0.99	0.76 ± 0.02 ^b
Subtropical foggages and hay, and tannin forages	0.69 ± 0.03	-0.85 ± 0.71	0.98	0.64 ± 0.06 ^a

¹ NAN disappearance as a proportion of NAN flow.

^{a,b} Differences significant at the 1% level.

wall-captured unavailable N (ADIN) in mature forages (Van Soest, 1982; Erasmus *et al.*, 1990). In the case of tannin forages the tannin-protein complexes were either not totally released in the abomasum and consequently interfered with protein digestion in the small intestine (Reid *et al.*, 1973), or the tannin concentrations were too high (Barry & Duncan, 1984). The latter possibility appears remote since the tannin levels analysed by Acheampong-Boateng (1991) in sainfoin and sheeps' burnet were much less than the 4% of DM considered as the limit above which tannins may become detrimental (Barry & Duncan, 1984). The type of tannin may have an additional role. Sainfoin contains condensed tannins and sheeps' burnet apparently contains hydrolysable tannins (Acheampong-Boateng, 1991). It was noticed that NAN disappearance as a proportion of NAN flow was 0.60 to 0.74 in sainfoin and only 0.47 to 0.64 in sheeps' burnet.

The lower disappearance of tannin forage partially negates the advantage of a high NAN flow to the small intestine. This can be seen in Table 6 where NAN disappearance as a proportion of N intake is indicated. Table 6, furthermore, shows proposed values of NAN flow and disappearance for the

Table 6 Proposed values for NAN flow to and disappearance in the small intestine for different forage classes

Forage category	NAN	
	flow ^a	disappearance ^b
Subtropical grasses	0.95	0.75 (0.70)
Subtropical foggages and hay	0.95	0.65 (0.60)
Temperate grasses, plus lucerne and triticale	0.75	0.75 (0.55)
Dried grass	1.10	0.75 (0.80)
Tannin forages	1.10	0.65 (0.70)
Error of estimate	0.09	0.04

^a NAN flow as a proportion of N intake.

^b NAN disappearance as a proportion of NAN flow.

In brackets: NAN disappearance as a proportion of N intake (calculated by substitution).

forage classes under investigation. The relatively large error of estimate suggests that in particular studies values may deviate substantially from those predicted. Nevertheless, the 0.55 proposed value for temperate grasses plus lucerne and triticale corresponds with the 0.58 of fresh temperate pasture N which was apparently absorbed in the study of MacRae & Ulyatt (1974), and the 0.70 to 0.80 proposed value for dried grass and tannin forages with the 0.75 to 0.80 of freeze-dried pasture N of Beever *et al.* (1969; 1971).

Conclusions

The present results confirm those in the literature which indicate that amino acid uptake in the small intestine may be limiting on temperate forages containing high levels of total and soluble N. On subtropical forages which normally under dryland conditions are not heavily N-fertilized, the problem is less severe. Indications are that N is wasted as NH₃ in the rumen if forage N content exceeds 3%.

Tannin-containing forages are advantageous from the point of view of supplementing microbial protein flow to the small intestine. However, part of the advantage may be negated by lower digestibility of N in the small intestine, depending on level and type of tannin. Lower digestibility may also realize on subtropical foggages and hay owing to high levels of ADIN. As low N content *per se* is another limiting factor, N supplementation of subtropical foggages and hay should increase microbial and small intestine digestion to rectify both problems.

Non-ammonia N passage to the small intestine as a proportion of N intake in general appears to be lower on forages than predicted (NRC, 1984; 1985; 1989). Principle reasons may be inadequate energy supply in comparison to mixed diets and an imbalance between N and soluble carbohydrate (Aii & Stobbs, 1980), mostly on temperate pastures. Oven-dried grass and forages containing tannins on the other hand, do behave according to expectation.

Expressed as a proportion of NAN supply to the small intestine, NAN disappearance in most forages should not deviate

appreciably from a value of 0.75. For tannin-containing forages and forages high in ADIN, a value of 0.65 may be more appropriate, although the actual levels of tannins and ADIN will be decisive, as suggested by the standard deviation of 0.06 (Table 5).

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