Intake, digestion and small intestinal protein availability in sheep in relation to ammoniation of wheat straw with or without protein supplementation

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The effects of ammoniation of wheat straw with or without supplementation of protein sources of either high (casein) or relatively low (potato protein) rumen degradability on intake and digestion were studied with four sheep in a 4 × 4 Latin square design. Rations offered were: (1) untreated wheat straw (UWS), (2) ammoniated wheat straw (AWS), (3) AWS supplemented with 3·2 g casein/kg live weight (W)$^{0.75}$ per d (AWSC) and (4) AWS supplemented with 3·9 g potato protein/kg W$^{0.75}$ per d (AWSP). Straw was offered *ad lib.* and all rations were supplemented with sugarbeet pulp and a mineral mixture. NH_3 treatment increased intake and digestion. Supplementation of AWS with potato protein increased total digestible organic matter intake (DOMI) compared with AWS whereas supplementation with casein did not affect total DOMI. Protein supplementation of AWS significantly reduced rumen digestion of cellulose, and when the supplementation was with casein it reduced rumen digestion of neutral-detergent fibre and hemicellulose also. This lower rumen digestion was compensated by a higher proportion of digestion occurring in the hindgut for hemicellulose ($P < 0.05$ for AWSC, $P > 0.05$ for AWSP), but not for cellulose. Across all rations, rumen fluid volume increased with increasing cell-wall intake. The efficiencies of microbial protein synthesis were (average of three different methods of estimation) 23·3, 26·2, 34·8 and 31·7 g N/kg apparently-rumen-degraded organic matter for UWS, AWS, AWSC and AWSP respectively. The difference between UWS and AWS was not significant, but values for AWSC and AWSP were significantly higher than that for AWS. The rumen digestion of feed amino acid-N (AA-N) was significantly higher for AWSC than for the other rations. The apparent small-intestinal digestion of AA-N and N was significantly higher for AWSP than for the other rations. The true small-intestinal digestion values were 0·86, 0·84 and 0·68 for AA-N, N and non-protein-N respectively. Ileal endogenous losses of AA-N were approximately 6 mg/g duodenal non-protein dry-matter flow. Linear relationships were observed between DOMI and N balance and truly absorbed AA-N, indicating that DOMI could have been limited by small-intestinal amino acid availability. Regression of N balance *v.* truly absorbed AA-N resulted in an estimate of net efficiency of utilization of truly absorbed AA-N of 0·54.

Protein availability: Ammoniated wheat straw: N balance: Microbial protein synthesis.

In ruminants, roughage intake may be limited by the intestinal supply of amino acids (AA; e.g. Doyle & McLaren, 1988; Doyle & Panday, 1990). The availability of AA for

absorption from the small intestine depends on the amounts of AA entering the duodenum, either from feed or of microbial origin, the amounts of endogenous protein added to the digesta, the true digestibility of the feed and microbial protein, and the proportion of endogenous protein that passes from the ileum to the caecum–colon (Van Bruchem *et al.* 1989). Limited rumen availability of nutrients such as peptides, branched-chain volatile fatty acids (VFA) and/or minerals in combination with a long retention time of microbes in the rumen may be reasons for the relatively low efficiency of microbial protein synthesis (Hespell & Bryant, 1979; Hoover, 1986), as found for untreated and ammoniated wheat straw (Oosting *et al.* 1993*b*). However, ileal endogenous AA losses were lower for rations based on untreated and ammoniated wheat straw (Oosting *et al.* 1993*b*) than for rations based on forages grown under temperate conditions (Van Bruchem *et al.* 1989). Hence, major constraints to AA availability in the small intestine for rations with straw as the major component are the low efficiency of microbial protein synthesis in the rumen in combination with a low flow of AA derived from straw, which probably have a low small-intestinal digestibility (Hvelplund, 1989).

The aim of the present experiment was to study the effects of ammoniation of wheat straw and of the supplementation of the ammoniated straw with proteins of low and high rumen degradability on intake, digestion and site of digestion, rumen fermentation and microbial protein synthesis, and small-intestinal AA availability.

MATERIALS AND METHODS

Animals, diets and experimental design

Four sheep (wethers, breed Swifter, a cross-breed of Texel and Flemish) with an average live weight (W) of 61 kg were fitted with a cannula in the dorsal rumen sac (25 mm i.d.) and with T-shaped cannulas (12 mm i.d.) in the proximal duodenum and terminal ileum. During the experiment the animals were kept in metabolism cages and received equal portions of their ration every 4 h. Water was freely available.

The experiment was set up as a 4 × 4 Latin square design. The four diets that were tested were: (1) untreated wheat straw (UWS), (2) ammoniated wheat straw (AWS), (3) AWS supplemented with casein (DMV-Campina, Veghel; AWSC) and (4) AWS supplemented with potato protein (Emsland-Stärke GmbH, Emlichheim, Germany; AWSP). Straw, chopped to a length of approximately 50 mm, was fed *ad lib*, by offering at least 25% excess. Ammoniation of the wheat straw was done as described by Oosting *et al.* (1993*a*).

All diets were supplemented with sugarbeet pulp, minerals and a commercially available mixture of vitamin A, cholecalciferol and trace elements (Mervit 318; Premervo, Utrecht, The Netherlands). The proportions of constituents in the sugarbeet pulp supplement without additional protein (SBP), with additional casein (SBPC) and with additional potato protein (SBPP) are given in Table 1. Sugarbeet pulp was offered at a level of 16·7 g DM/kg $W^{0.75}$ per d and the quantity of the mineral–vitamin mixture offered was 1·8 g DM/kg $W^{0.75}$ per d. Casein and potato protein supplements were given at levels of 3·2 and 3·9 g/kg $W^{0.75}$ per d, respectively. The supplementary casein and potato protein were approximately isonitrogenous. The chemical composition of each of the ration components, including the AA profile, is given in Table 2.

Each of the four experimental periods had a duration of 30 d. The adaptation period before the first experimental period and between experimental periods was 19 d.

Intake and digestion

Faeces and feed residue were collected for 10 d starting at day 3 and again at day 17 of each experimental period. No correction was made for withdrawal of digesta from the

Table 1. *Proportions of constituents (g/kg) in supplements of sugarbeet pulp + minerals and vitamins (SBP), SBP + casein (SBPC) and SBP + potato protein (SBPP)*

	SBP	SBPC	SBPP
Sugarbeet pulp	904	742	713
Mervit 318 (vitamin A, cholecalciferol and trace elements)	45	37	36
$NaH_2PO_4.2H_2O$	32	26	25
$FeSO_4.7H_2O$	0·5	0·4	0·4
$MgSO_4.7H_2O$	19	15·6	15
Additional protein	0	178	211

Table 2. *Chemical composition of wheat straw and protein supplements**

	Wheat straw		Protein supplement		
	Untreated	Ammoniated	SBP	SBPC	SBPP
DM (g/kg)	910	905	848	816	840
Ash (g/kg DM)	81	84	94	79	86
N (g/kg DM)	5·8	13·5	14·6	39·3	37·2
AA-N (g/kg DM)	3·4	3·4	10·5	32·0	27·6
NDF (g/kg DM)	781	758	395	342	361
Hemicellulose (g/kg DM)	289	263	171	161	176
Cellulose (g/kg DM)	425	439	204	161	164
Lignin (g/kg DM)	66	55	20	20	20
AA (mol/kg DM)	0·19	0·19	0·59	1·90	1·61
AA profile (mmol/mol)					
Cystine	10·4	11·3	11·5	4·7	8·9
Aspartic acid	104·9	102·1	86·2	70·9	115·6
Methionine	14·9	13·3	15·5	23·6	18·4
Threonine	58·3	57·1	59·4	49·0	58·1
Serine	74·3	72·1	76·9	73·6	68·9
Glutamic acid	122·1	121·6	110·9	162·6	102·7
Proline	57·4	60·6	59·9	104·0	54·1
Glycine	103·8	108·0	89·3	46·5	80·6
Alanine	96·9	97·6	80·2	52·3	69·8
Valine	73·1	71·5	83·0	75·9	78·0
Isoleucine	37·2	37·7	45·6	51·2	52·0
Leucine	58·7	58·8	70·1	85·3	85·2
Tyrosine	15·4	15·3	35·8	34·1	34·5
Phenylalanine	31·8	30·7	28·6	36·8	41·3
Lysine	53·4	58·4	46·1	30·2	27·7
Histidine	42·3	39·8	56·5	63·2	59·7
Arginine	36·0	33·1	37·0	28·9	35·1
Tryptophan	9·1	11·0	7·6	7·2	9·4

SBP, sugarbeet pulp + minerals and vitamins; SBPC, SBP + casein; SBPP, SBP + potato protein; AA, amino acids; NDF, neutral-detergent fibre.

* For details of composition, see Table 1.

duodenum and ileum, which occurred on 4 d of the 10 d of faecal collection. Total DM withdrawn during duodenal and ileal sampling was approximately 15 g/d, which, averaged over the faecal collection period, would mean a maximal underestimation of faecal DM excretion of only 6 g/d.

Passage through the small intestine

From day 1 to day 10 and again from day 16 to day 24 of each experimental period, 10 g Cr-mordanted neutral-detergent fibre (NDF; Cr–NDF; average Cr concentration 53 g/kg)/d and 3 g CoEDTA (average Co concentration 148 g/kg)/d, both prepared according to Udén *et al.* (1980), were introduced into the rumen at intervals of 6 h (starting at 06.00 hours) to get steady-state concentrations of Cr and Co in the rumen.

Two-hourly samples of approximately 20 g duodenal and ileal digesta were collected from days 6 to 9 and again from days 20 to 23 from 08.30 hours until 18.30 hours. These samples were freeze-dried, ground and pooled for each sheep, week and cannula and subsequently analysed for DM, ash, Co, Cr, cell-wall composition, N and AA. As in the experiment described by Van Bruchem *et al.* (1993) with sheep fed on UWS and AWS, duodenal and ileal samples were representative in respect of fluid and particulate phases: the Cr: Co ratio introduced into the rumen was on average 1·19. In duodenal samples this ratio was 1·21 (SEM 0·018) and in ideal samples 1·24 (SEM 0·032), without significant differences between periods, animals and rations. Use of the double- or two-marker techniques to correct for non-representative sampling (Faichney, 1980), therefore, was not needed and daily duodenal or ileal flows of nutrients were calculated from the concentration of that nutrient: the concentration of Co or Cr in duodenal or ileal digesta multiplied by the daily Cr or Co flow. Flows presented or used for further calculations were averages of estimates based on Co and Cr. Correction of the ileal flow for withdrawal of digesta from the duodenum was not required because the concentration of a nutrient relative to the concentration of markers is not affected by duodenal sampling.

The duodenal flows of microbes were estimated by the following methods:

(1) Diaminopimelic acid (DAPA) method, based on the daily DAPA flow in the duodenum and the DAPA: N ratio in isolated rumen bacteria,

(2) AA profile method, based on the AA profiles of ingested protein, bovine pepsinogen (Siddons *et al.* 1982), microbial protein and duodenal digesta. Dietary, pepsinogen and microbial AA were mixed by an iterative procedure in such proportions that the computed AA profiles best matched the actual AA profiles of duodenal protein. This was tested by minimizing the objective function:

$$\sum_{AA=1}^{AA=16} (1 - AA_{computed}/AA_{actual})^2.$$

This procedure was done for sixteen AA, omitting cystine (because of the high analytical variation) and tryptophan (the tryptophan concentration in pepsinogen was unknown).

(3) Purine derivatives method, based on excretion of purine derivatives in the urine measured according to the method described by Chen *et al.* (1990*b*). From the daily urinary excretion of purine derivatives the corresponding amount of microbial purines absorbed by the animal was estimated based on the model described by Chen *et al.* (1990*a*). The duodenal flow of microbial N was then calculated from the absorbed quantity of microbial purines by assuming a digestibility of microbial purines of 0·83 and a purine-N: total microbial-N value of 0·116 (Chen *et al.* 1991).

Rumen fluid, rumen fermentation and rumen passage

The steady-state concentration of Co in rumen fluid was measured in samples of 50 ml taken from days 6 to 9 and again from days 20 to 23 at 08.00 and 10.00 hours. The rumen fluid volume was estimated from the Co concentration in the rumen fluid samples by the following equation, applying to a steady-state situation in the rumen:

$$\text{rumen fluid volume (litres)} = \frac{\text{daily dosage of Co (mg/d)}}{\text{concentration of Co (mg/l)} \times k_l\text{(/d)}},$$

where k_l is the fractional rate of passage of Co.

Rumen samples for determination of pH (immediately after sampling) and concentration of VFA, NH_3-N and Co were taken two-hourly on day 10 and again on day 24 starting at 08.00 hours, 2 h after the last dosing of Cr–NDF and CoEDTA, to 20.00 hours. One additional sample was taken on days 11 and 25. Co was also determined in rumen fluid samples taken at 10.00 and 12.00 hours on days 11 and 25. Faecal samples (total collection) for analysis of Cr concentration were collected over the following time intervals after the last dosing of Cr–NDF on days 10 and 24: 24–28, 28–32, 32–36, 36–40, 48–52, 52–56, 56–60, 60–64, 72–76, 76–80, 80–84, 84–88, 96–104, 104–112, 112–120, 120–128 and 128–136 h. The fractional rate of passage of rumen fluid from the rumen (k_l; based on rumen Co concentration) and the fractional rate of passage of the particulate-phase marker Cr–NDF (k_p; based on the descending part of the faecal excretion curve of Cr) were estimated according to Grovum & Williams (1973).

Rumen microbes

From rumen fluid samples of 50 ml, taken at the same moment as those for estimation of steady-state Co concentration in rumen fluid, rumen microbes were isolated as described by Oosting *et al.* (1993*b*). After freeze-drying, grinding through a 1 mm sieve and pooling for each animal and week, samples were stored pending N and AA analyses. In rumen-fluid samples taken for estimation of the steady-state Co concentration, the concentration of DAPA was also determined.

Dacron bag analysis

For the straw and supplements in the present experiment, the disappearance of N from small Dacron bags introduced into the duodenal cannula was measured in cattle. Feed samples were all ground through a 1 mm sieve and incubated in Dacron bags (size 70×120 mm, pore size 41 μm $\times$ 41 μm) for 12 h in the rumen of two steers fed on UWS. Straw was also incubated for 24 and 48 h. After collection and washing of the bags, the residue was dried at 70° for 48 h and pooled for each feed. A sub-sample was taken for DM and N analysis and eighteen subsamples of approximately 0·5 g of each feed were weighed into small Dacron bags (size 30×60 mm, pore size 41 μm $\times$ 41 μm). These samples were incubated in pepsin–HCl (1 g pepsin/l 1 M-HCl) at 39° for 1 h and subsequently introduced into the distal duodenum of six cows in triplicate per feed per cow. These cows were fed on a ration consisting of (g/kg): dried grass 400 and concentrates 600. After collection of the bags voided with the faeces, washing in tap-water and drying at 70° for 48 h, the residues were pooled for each feed and analysed for DM and N.

Chemical analysis

DM, ash, N, NH_3-N in rumen fluid, NDF, acid-detergent fibre (ADF), acid-detergent lignin (ADL), Co and Cr and VFA were all determined by the methods described by Oosting *et al.* (1994). Hemicellulose was calculated as NDF − ADF, and cellulose as ADF − ADL. AA including DAPA were determined as described by Oosting *et al.* (1993*b*). Tryptophan was analysed after alkaline-hydrolysis. A sample containing 25–50 mg protein was mixed in a test-tube with 16 ml 4 M-LiOH. After cooling in ice, the tubes were evacuated and subsequently put into an oven at 120°. After boiling for 16 h the pH was reduced to 4·5 by adding HCl (12 M) with continuous stirring. Then the sample was centrifuged at 550 ***g*** and the supernatant fraction quantitatively transferred into a 50 ml volumetric flask and filled to the mark. Of this solution, 5 ml was mixed with a phosphate

buffer (11·4 g $K_2HPO_4.3H_2O$ and 6·8 g KH_2PO_4/l water and an internal standard (5-methyl-DL-tryptophan; 1 mmol/l; Sigma). After centrifugation at 70000 *g*, the supernatant fraction was analysed by HPLC (isocratic system; eluent 13·6 g sodium-acetate.$3H_2O$ and 5·7 ml acetic acid in 2 litres water to which 353 ml methanol was added; 100 mm Lichiosorb RP-18 column; u.v. detection at 280 nm).

Calculations and statistics

The true digestion of individual and total AA (TAA), AA-N, N and non-protein-N (NPN) in the small intestine or over the whole digestive tract (only for N) was estimated by Lucas equations (Van Soest, 1982) of the following general form:

$$DX = a + bX,$$

where X and DX represent the concentrations of a nutrient and the disappeared nutrient respectively (in the duodenal flow of non-protein DM (NPDM) for estimation of the small-intestinal true digestion and in organic matter intake for estimation of the whole-tract true digestion), *a* is the endogenous loss of the nutrient per 100 g duodenal NPDM flow or organic matter intake and *b* is the true digestion as a proportion of intake (for whole-tract digestion of N) and of duodenal influx (for true small-intestinal digestion of nutrients).

The equation for estimation of the small-intestine true digestion as given previously was derived from the equation used by Van Bruchem *et al.* (1989). The latter equation to estimate endogenous AA output and true AA digestion in the small intestine had the following form:

$$\text{ileal AA flow} = a + b_1(\text{duodenal AA flow}) + b_2(\text{duodenal NPDM flow}).$$

However, in the Van Bruchem *et al.* (1989) results and in the present experiment, the intercept, *a*, in the equation given previously was never significantly different from zero, either for individual AA or for TAA. It, therefore, can be omitted. AA disappearance in the small intestine (D) is then given by:

$$\begin{aligned} D &= \text{duodenal AA flow} - \text{ileal AA flow}, \\ &= (1-b_1)(\text{duodenal AA flow}) - b_2(\text{duodenal NPDM flow}). \end{aligned}$$

DX, which is AA disappearance as a function of duodenal NPDM, is defined as

$$\begin{aligned} DX &= D/NPDM, \\ &= ((1-b_1)(\text{duodenal AA flow}) - b_2(\text{duodenal NPDM flow}))/\text{duodenal NPDM flow}, \\ &= a + bX, \end{aligned}$$

where X is duodenal AA concentration in duodenal NPDM, $a = -b_2$ and $b = 1-b_1$.

Means for each animal within period (average of two repeated measurements) were statistically analysed by the program DBSTAT (Brouwer, 1989) with the model:

$$Y_{ijkl} = \mu + \text{period}_i + \text{animal}_j + \text{ration}_k + \text{error}_{ijkl},$$

with *n* 16. Differences between ration means were compared by Student's *t* test (Snedecor & Cochran, 1967), only if the ration effect was significant.

RESULTS

Intake and digestion

NH_3 treatment increased intake and whole-tract and rumen digestion of all dietary constituents significantly (Table 3). Supplementation of AWS with potato protein increased total organic matter intake ($P < 0·05$), whereas supplementation with casein

Table 3. *Intake and digestion of organic matter and fibre fractions and average weight of sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) and with casein (AWSC) or potato-protein (AWSP) supplements**

Ration...	UWS	AWS	AWSC	AWSP	SEM
Organic matter					
Intake: g/kg $W^{0.75}$ per d					
Whole ration	41·2[a]	49·2[b]	49·4[b]	56·2[c]	1·32
Straw	24·4[a]	32·6[bc]	29·4[b]	35·7[c]	1·22
g/animal per d					
Whole ration	872[a]	1070[b]	1090[b]	1260[c]	33·5
Straw	516[a]	709[bc]	649[b]	800[c]	30·5
Digestion: g/kg ingested					
Whole tract	619[a]	629[ab]	628[ab]	643[b]	4·7
g/kg digested					
Rumen	775[b]	773[b]	703[ab]	663[a]	26·2
Small intestine	123	124	175	205	28·0
Large intestine	102	104	123	132	19·0
Digestible organic matter intake (g/kg $W^{0.75}$ per d)	25·5[a]	30·9[b]	31·0[b]	35·9[c]	0·90
Neutral-detergent fibre					
Intake (g/kg $W^{0.75}$ per d)	28·1[a]	34·4[bc]	32·2[b]	37·6[c]	1·02
Digestion: (g/kg ingested)					
Whole tract	597[a]	647[c]	622[b]	642[c]	6·0
Rumen	555[a]	602[b]	547[a]	564[ab]	12·3
g/kg digested					
Rumen	933	935	880	883	23·5
Hemicellulose					
Intake (g/kg $W^{0.75}$ per d)	10·9[a]	12·6[bc]	12·3[b]	14·2[c]	0·43
Digestion: g/kg ingested					
Whole tract	639[a]	699[b]	696[b]	712[b]	7·3
Rumen	564[a]	614[b]	565[a]	592[ab]	9·6
g/kg digested					
Rumen	883[b]	878[b]	813[a]	830[ab]	18·9
Cellulose					
Intake (g/kg $W^{0.75}$ per d)	15·0[a]	19·4[bc]	18·1[b]	20·8[c]	0·51
Digestion: g/kg ingested					
Whole tract	659[a]	699[b]	666[a]	674[a]	7·4
Rumen	604[a]	651[b]	600[a]	604[a]	10·3
g/kg digested					
Rumen	918	930	900	898	26·1
W (average; kg)	58·8[a]	60·4[ab]	61·3[ab]	63·0[b]	1·28

[a,b,c] Values in a row with different superscript letters were significantly different ($P < 0·05$).
W, live weight.
* For details of composition, see Tables 1 and 2.

did not affect organic matter intake. The differences between rations with regard to intake of cell-wall components reflect differences in straw intake, since intake of cell-wall components from the supplements was similar for all rations. Intakes of NDF, hemicellullose, cellulose and lignin from the supplements were 7·5, 3·4, 3·7 and 0·4 g/kg $W^{0.75}$ per d respectively.

Compared with AWS, AWSC and AWSP had significantly lower whole-tract and rumen digestion of cellulose. Rumen digestion of hemicellulose was, in contrast to its whole-tract digestion, significantly lower for AWSC than for AWS.

Table 4. *Rumen fluid volume, rumen passage rates and rumen fermentation characteristics of sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements*†*

Ration...	UWS	AWS	AWSC	AWSP	SEM
Rumen volume (litres)	6·0[a]	7·5[bc]	6·6[ab]	8·9[c]	0·43
k_l (% per h)	7·5	8·1	8·2	7·4	0·32
k_p (% per h)	3·5	3·8	4·1	3·7	0·17
VFA concentration (mmol/l)	101·4[a]	109·8[ab]	117·9[b]	120·6[b]	4·10
Molar proportions of VFA (mmol/mol)					
Acetate	710[a]	729[b]	700[a]	709[a]	3·2
Propionate	188[b]	177[a]	194[b]	188[b]	3·5
Butyrate	95	90	89	93	2·9
Isovalerate	3[a]	1[a]	8[b]	5[ab]	1·5
Valerate	3[a]	3[a]	11[c]	5[b]	0·6
pH	6·3	6·2	6·2	6·2	0·05
NH_3-N concentration (mg/l)	101·4[a]	109·8[ab]	117·9[b]	120·6[b]	4·10

[a, b, c] Values in a row with different superscript letters were significantly different ($P < 0·05$).

k_l, fractional rate of passage of Co; k_p, fractional rate of passage of particulate-phase marker; VFA, volatile fatty acids.

* For details of composition, see Tables 1 and 2.

† For details of procedures, see pp. 348–351.

The small-intestinal digestion of cell-wall components did not differ significantly from zero and is not given in Table 3. No differences in the contribution of the rumen to whole-tract digestion were observed between UWS and AWS. The proportion of digestion occurring in the rumen was lower for AWSC and AWSP than for AWS (significantly for hemicellulose in the case of AWSC and for organic matter in the case of AWSP).

Duodenal flows of NDF were 264, 295, 326 and 369 (SEM 18·4) g/d for UWS, AWS, AWSC and AWSP respectively, being significantly higher for AWSP than for UWS and AWS. The daily quantities of NDF digested in the rumen were 333, 451, 392 and 473 (SEM 12·6) g for UWS, AWS, AWSC and AWSP respectively, being lowest for UWS ($P < 0·05$) and significantly lower for AWSC than for the other two AWS-based rations.

Rumen fluid volume, rumen passage and rumen fermentation

Rumen fluid volume was significantly higher for AWSP than for AWSC (Table 4). Ammoniation of wheat straw significantly increased rumen fluid volume. Regression of rumen fluid volume (litres) *v.* NDF intake (g/d) with inclusion of the animal effect as a factor in the regression model yielded the following equation (the intercept is the average over animals, with the SE of the estimate):

rumen fluid volume $= -1·2$ (SE 1·29) $+ 0·012$ (SE 0·0017)

$\times$ NDF intake (r^2 0·876, n 16, residual SD 0·73).

The data (uncorrected) are plotted in Fig. 1.

No significant differences were observed between rations with regard to k_l and k_p. The k_l and k_p were significantly correlated (r 0·854, n 16, $P < 0·001$). The pH and the VFA and NH_3-N concentrations in the rumen fluid were not significantly different between UWS and AWS and between AWS and AWSC or AWSP (Table 4). Molar proportions of individual VFA differed only slightly between rations. Only traces of isobutyrate were found (not included in Table 4).

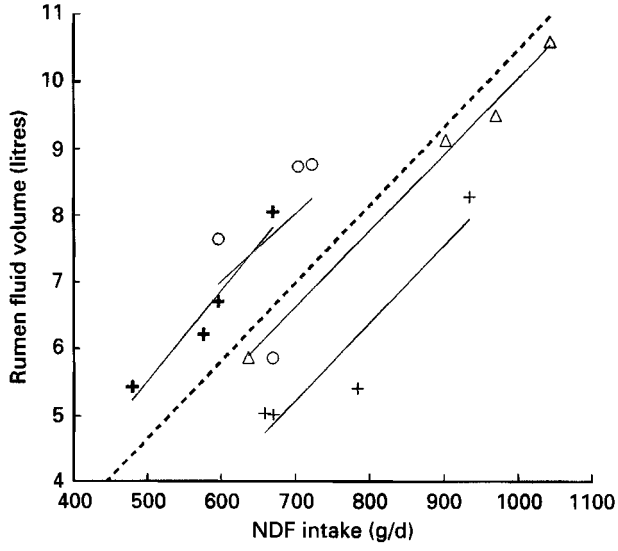

Fig. 1. Relationship between rumen fluid volume and neutral-detergent fibre (NDF) intake for four sheep given experimental rations containing untreated wheat straw or ammoniated wheat straw alone or with casein or potato-protein supplements. For details of rations and procedures, see Tables 1 and 2 and pp. 348–351. (+), Sheep 1; (△), sheep 2; (○), sheep 3; (✚), sheep 4. Values given for each sheep represent each of the experimental rations.

Table 5. *Disappearance (D) of N (g/kg) from Dacron bags in cattle for untreated wheat straw (UWS), ammoniated wheat straw (AWS) and sugarbeet pulp + minerals and vitamins (SBP), SBP + casein (SBPC) and SBP + potato protein (SBPP)*†*

(Values are from analysis in one pooled sample within feed and duration of rumen incubation)

	D_{Rumen}	$D_{Intestines}$‡	$D_{Whole\text{-}tract}$§
UWS			
12 h‖	221	652	729
24 h	147	715	756
48 h	86	774	794
AWS			
12 h	531	607	815
24 h	549	672	852
48 h	522	750	880
SBP			
12 h	452	904	947
SBPC			
12 h	856	966	995
SBPP			
12 h	447	974	985

* For details of composition, see Tables 1 and 2.

† For details of procedures, see p. 351–352.

‡ Incubation of residue after rumen incubation in pepsin (*EC* 3.4.23.1)–HCl followed by introduction into the duodenum and collection in faeces.

§ N disappearance from rumen and intestines ($= D_{Rumen} + (1000 - D_{Rumen}) \times D_{Intestines}/1000$).

‖ Duration of rumen incubation.

Table 6. *Molar proportions of amino acids (AA; mmol/mol total AA) of dietary intake, bovine pepsinogen, rumen microbes and duodenal digesta and percentage contribution of AA of microbial, feed and endogenous origin to total duodenal AA of sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements*†*

	Dietary intake						Duodenal digesta			
	UWS or AWS	AWSC	AWSP	SEM	Endogenous	Rumen microbes	UWS or AWS	AWSC	AWSP	SEM
Cys	11·2[c]	5·5[a]	9·2[b]	0·40	na	7·6	12·6[b]	12·7[b]	10·9[a]	0·29
Asp	92·2[b]	75·0[a]	113·4[c]	0·29	115·0	114·3	106·7[a]	105·6[a]	109·4[b]	0·72
Met	14·9[a]	22·3[c]	17·5[b]	0·35	10·8	19·4	15·9[a]	17·1[b]	16·8[b]	0·23
Thr	58·8[b]	50·1[a]	57·9[b]	0·23	76·3	62·6	61·7[b]	61·1[a]	61·7[b]	0·17
Ser	75·6[c]	73·5[b]	69·5[a]	0·35	143·2	59·1	63·8[b]	62·6[a]	65·1[c]	0·32
Glu	114·8[b]	157·2[c]	105·9[a]	0·66	92·2	104·2	106·3[b]	107·9[b]	100·1[a]	1·07
Pro	59·7[b]	98·4[c]	55·4[a]	1·21	44·3	34·9	43·6[a]	46·1[b]	48·5[c]	0·49
Gly	94·2[c]	54·6[a]	88·3[b]	0·46	99·4	92·4	93·3[b]	92·1[b]	90·3[a]	0·35
Ala	86·2[c]	58·2[a]	74·5[b]	0·61	46·0	103·1	96·8[b]	96·1[b]	87·4[a]	0·52
Val	79·3[b]	76·5[a]	76·9[a]	0·55	71·2	71·6	71·7	71·8	73·5	0·55
Ile	42·7[a]	49·4[b]	49·6[b]	0·17	90·1	57·5	55·0[a]	55·2[a]	58·6[b]	0·35
Leu	66·2[a]	81·8[c]	80·6[b]	0·32	72·0	70·6	73·4[a]	73·6[a]	80·5[b]	0·64
Tyr	28·7[a]	31·6[b]	31·2[b]	0·23	50·8	33·8	28·6[a]	28·6[a]	30·5[b]	0·20
Phe	29·4[a]	36·0[b]	39·5[c]	0·81	42·9	37·6	37·1[a]	37·1[a]	40·6[b]	0·38
His	49·5[b]	33·9[a]	32·9[a]	1·10	5·6	26·5	31·5[b]	31·5[b]	29·4[a]	0·52
Lys	51·2[a]	60·1[c]	56·3[b]	0·32	23·0	65·5	63·3[b]	63·0[b]	61·9[a]	0·26
Arg	36·1[b]	29·4[a]	34·7[b]	0·40	17·7	31·3	30·9	30·1	31·2	0·43
Try	8·5[b]	7·7[a]	9·7[c]	0·09	na	8·1	8·2	8·1	8·2	0·12
Composition of duodenal AA§										
Microbial							69·6[b]	82·1[c]	53·0[a]	1·80
Feed							28·1[b]	16·9[a]	44·6[c]	1·68
Endogenous							2·2	1·1	2·2	0·91

[a,b,c] Means in a row with different superscript letters were significantly different ($P < 0·05$).
na, Not available.
* For details of composition, see Tables 1 and 2.
† For details of procedures, see pp. 348–351.
‡ Composition of bovine pepsinogen adapted from Siddons *et al.* (1982).
§ Percentage of duodenal AA flow.

N disappearance from Dacron bags in cattle

Apparent N disappearance in the rumen of steers decreased with incubation time for UWS (Table 5), which should be attributed to contamination with microbial N. For UWS, values for DM disappearance from Dacron bags incubated in the rumen 227, 318 and 488 g/kg for incubation periods of 12, 24 and 48 h respectively. For AWS, also, contamination with microbial N was likely, since N disappearance from Dacron bags incubated in the rumen did not change with incubation period, while DM disappearance values were 190, 397 and 615 g/kg after rumen incubation for 12, 24 and 48 h respectively. For both UWS and AWS, post-rumen N disappearance from Dacron bags (pepsin (*EC* 3.4.23.1)–HCl incubation of residue after rumen incubation followed by introduction into the duodenum of cattle) and whole-tract N disappearance increased with increasing duration of pre-incubation in the rumen.

Rumen N disappearance from Dacron bags was higher for SBPC than for SBP and

Table 7. *Composition of rumen microbes, the size of the rumen microbial pool associated with the rumen fluid and the efficiency of microbial protein synthesis for sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements*†*

Ration...	UWS	AWS	AWSC	AWSP	SEM
DAPA (mmol/kg DM)	17·2	16·5	16·5	17·4	0·49
AA (mol/kg DM)	2·87	2·68	2·81	2·99	0·072
N (g/kg DM)	70·2	66·7	71·0	73·6	1·57
AA-N (g/kg DM)	48·1	44·9	47·1	50·1	1·22
AA-N:N (g/kg)	685^{b}	675^{ab}	663^{a}	683^{b}	4·0
DAPA-N:N (g/kg)	6·88^{b}	6·88^{b}	6·50^{a}	6·59^{ab}	0·095
Microbial N pool associated with rumen fluid (g)	2·72^{a}	3·44^{ab}	3·02^{a}	4·54^{b}	0·373
Efficiency of microbial protein synthesis (g N/kg ARDOM) determined from‡:					
AA profiles	23·8^{a}	24·5^{a}	37·0^{c}	30·4^{b}	1·70
DAPA	22·7^{a}	23·4^{a}	31·6^{b}	31·7^{b}	2·35
Purine derivatives	23·3^{a}	30·6^{b}	35·8^{c}	33·2^{bc}	1·08

^{a,b,c} Means in a row with different superscript letters were significantly different ($P < 0·05$).
DAPA, diaminopimelic acid; AA, amino acids; ARDOM, apparently-rumen-degradable organic matter.
* For details of composition, see Tables 1 and 2.
† For details of procedures, see pp. 348–351.
‡ For details of methods, see p. 350.

SBPP. Rumen DM disappearance values were 638, 772 and 599 g/kg for SBP, SBPC and SBPP respectively. Post-rumen and whole-tract N disappearance of all supplements was high, although higher for SBPC and SBPP than for SBP alone.

Rumen microbes

The AA profiles of ingested protein, endogenous protein (bovine pepsinogen), rumen microbes and duodenal digesta are given in Table 6. The microbial AA profile did not differ significantly between rations and no significant differences were observed between UWS and AWS with regard to the AA profiles of dietary intake and duodenal digesta; for presentation in Table 6 these AA profiles were combined. The computed proportions of microbial AA as a percentage of duodenal AA flow were significantly higher for AWSC and significantly lower for AWSP than for UWS and AWS. The proportion of endogenous AA in the duodenal protein flow did not differ between rations.

The composition of rumen microbes, the rumen microbial N pool calculated from the rumen fluid pool size and the DAPA concentration in rumen fluid, and the efficiency of microbial protein synthesis (g microbial N/kg apparently-rumen-degraded organic matter) are given in Table 7. No significant ration effects were observed for DAPA, AA, N or AA-N concentrations in microbial DM. A significant ration effect was found for AA-N:N and DAPA-N:N, but differences were only small. AWSP had a significantly higher microbial N pool associated with rumen fluid than the other rations, which was mainly caused by differences in the rumen-fluid pool size. The DAPA concentration in the rumen fluid did not differ significantly between rations. Over rations the average DAPA concentration in rumen fluid was 0·113 (SEM 0·0152) mmol/l, which corresponded to an average concentration of microbial N in rumen fluid of 470 (SEM 10·7) mg/l.

Only the purine derivatives method showed a significantly higher efficiency of microbial protein synthesis for AWS than for UWS. Protein supplementation of AWS increased the

Table 8. *Intake and flow at the duodenum and ileum and faecal and urinary excretion of nitrogen of sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) and with casein (AWSC) and potato-protein (AWSP) supplements**†*

Ration...	UWS	AWS	AWSC	AWSP	SEM
Intake (g/d)					
N: Total	9·0[a]	16·2[b]	28·2[c]	30·4[c]	0·86
Straw	3·3[a]	10·4[bc]	9·6[b]	11·8[c]	0·56
AA-N: Total	6·0[a]	6·7[a]	17·6[b]	16·8[b]	0·68
Straw	2·0[a]	2·7[bc]	2·5[b]	3·1[c]	0·15
Duodenal flow (g/d)					
N	15·5[a]	21·3[b]	26·0[c]	34·7[d]	1·23
AA-N‡	10·0[a]	11·8[ab]	14·3[b]	20·8[c]	0·83
Microbial	6·8[a]	8·4[a]	11·7[b]	11·0[b]	0·50
Feed	3·0[a]	3·2[a]	2·4[a]	9·4[b]	0·43
Endogenous	0·2[a]	0·2[a]	0·2[a]	0·4[b]	0·02
AA-N:N	647[c]	560[a]	551[a]	599[c]	5·0
Ileal flow (g/d)					
N	7·7[a]	11·5[b]	13·1[bc]	15·1[c]	0·66
AA-N	4·3[a]	5·1[ab]	5·6[b]	6·9[c]	0·38
AA-N:N	553[b]	446[a]	432[a]	454[a]	6·7
Faecal excretion (g/d)					
N	6·7[a]	9·7[b]	10·4[bc]	11·5[c]	0·37
Urinary N excretion (g/d)	3·6[a]	7·1[b]	17·4[d]	15·9[c]	0·30
N balance					
mg/kg $W^{0·75}$ per d	−61·0[a]	−24·9[b]	10·2[c]	131·0[d]	5·18
g/animal per d	−1·3[a]	−0·6[b]	0·3[c]	3·0[d]	0·13

[a,b,c,d] Means in a row with different superscript letters were significantly different ($P < 0·05$).
AA, amino acids.
* For details of composition, see Tables 1 and 2.
† For details of procedures, see pp. 348–352.
‡ Based on AA profiles method; for details, see p. 350.

efficiency of microbial protein synthesis significantly, although the difference was not significant for AWSP when measured by the purine derivatives method. Estimates of the efficiency of microbial protein synthesis for AWSC and AWSP only differed significantly when based on AA profiles. The purine derivatives method gave significantly higher estimates for efficiency of microbial protein synthesis than DAPA and AA profiles for AWS, while for AWSC the efficiency of microbial protein synthesis was significantly lower for DAPA than for AA profiles. The correlation coefficient between DAPA and AA profiles was 0·84 (n 16, $P < 0·001$), between DAPA and purine derivatives 0·62 (n 16, $P < 0·01$) and between AA profiles and purine derivatives 0·63 (n 16, $P < 0·01$).

The difference in N intake between UWS and AWS was 7·2 g/d (Table 8) of which 85% was a result of N added through NH_3 treatment. The duodenal N and AA-N flows were higher than the intakes of these nutrients for all rations except for AWSC.

The AA profiles method, unlike the purine derivatives and DAPA methods, allowed division of the duodenal AA flow into subfractions of microbial, feed and endogenous origin. The duodenal AA flow of these fractions as given in Table 8 were based, therefore, on the AA profile method. Significantly higher estimates of microbial flows were observed

Table 9. *Apparent small-intestinal digestibility (g/kg) of rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements fed to sheep**†*

Ration...	UWS	AWS	AWSC	AWSP	SEM
N	501[a]	462[a]	494[a]	566[b]	18·1
AA-N	573[a]	571[a]	604[a]	671[b]	13·0
NPN	366	322	359	408	28·6

[a,b] Means in a row with different superscript letters were significantly different ($P < 0.05$).
AA, amino acids; NPN, non-protein-N.
* For details of composition of rations, see Tables 1 and 2.
† For details of procedures, see pp. 348–352.

Table 10. *True small-intestinal digestibility (TD) and endogenous ileal losses of individual amino acids (AA), total AA (TAA), AA-N, N and non-protein-N (NPN) for sheep given rations containing untreated wheat straw (UWS) and ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements**†

(Mean values with their standard errors)

	TD		Endogenous ileal loss‡		
	Mean	SE	Mean	SE	
Cys	0·67	0·073	6·4	1·30	
Asp	0·84	0·016	32·5	2·67	
Met	0·90	0·032	4·1	0·79	
Thr	0·84	0·022	22·9	2·05	
Ser	0·82	0·022	25·0	2·07	
Glu	0·86	0·025	37·4	3·80	
Pro	0·85	0·020	18·7	1·33	
Gly	0·81	0·021	35·3	2·93	
Ala	0·84	0·026	33·7	3·56	
Val	0·88	0·018	27·0	1·89	
Ile	0·89	0·015	15·4	1·20	
Leu	0·90	0·018	23·2	1·99	
Tyr	0·88	0·022	7·9	0·97	
Phe	0·88	0·023	11·2	1·29	
His	0·78	0·049	18·8	2·23	
Lys	0·86	0·014	13·3	1·33	
Arg	0·91	0·018	8·6	0·83	
Try	0·76	0·047	2·4	0·57	
TAA	0·86	0·018	350	26·1	
AA-N	0·86	0·017	6·0	0·44	
N	0·84	0·032	11·1	0·69	UWS
			14·5	0·83	AWS, AWSC, AWSP
NPN	0·68	0·074	3·4	0·73	UWS
			5·8	1·10	AWS, AWSC, AWSP

* For details of composition, see Tables 1 and 2.
† For details of procedures, see pp. 348–352.
‡ mmol/kg non-protein DM (NPDM) for individual AA and TAA, g/kg NPDM for AA-N, N and NPN.

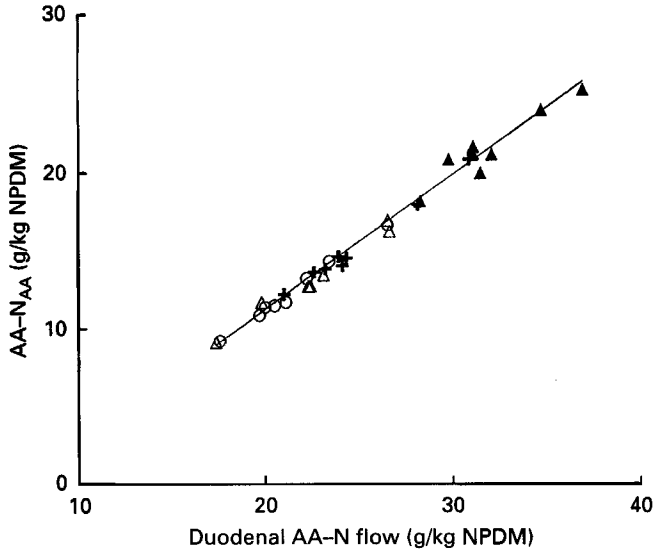

Fig. 2. True small-intestinal digestion of amino acid (AA)-N for rations containing untreated wheat straw (UWS) or ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements fed to sheep. (△), UWS; (○), AWS; (+), AWSC; (▲), AWSP. Relationship between apparently small-intestinally absorbed AA-N (AA-N_{AA}) and duodenal AA-N flow, both scaled to non-protein DM (NPDM). For details of rations and procedures, see Tables 1 and 2 and pp. 348–352.

for AWSC and AWSP than for AWS and UWS. AWSP had a significantly higher AA-N flow from feed origin and from endogenous origin than the other rations.

The AA-N:N in duodenal digesta was significantly higher for UWS than for AWS and was significantly higher for AWSP than for AWS and AWSC. Estimates of the rumen digestion of AA-N from feed origin were 499, 524, 862 and 444 g/kg for UWS, AWS, AWSC and AWSP respectively (SEM 20·4 g/kg), when calculated from intake and duodenal flow of feed AA-N. The AA-N:N ratio in ileal digesta was significantly higher for UWS than for the other rations. Some disappearance of N occurred between the terminal ileum and faeces. The average N disappearance in the large intestine was 183 (SEM 20·6) g/kg ileal N flow, without any significant ration effect.

Regression of digestible N intake:organic matter intake *v.* N intake:organic matter intake gave estimates for metabolic faecal N excretion of 7·2 (SE 0·59) g/kg organic matter intake and true whole-tract N digestion of 0·92 (SE 0·032). The residuals (digestible N intake:organic matter intake predicted minus digestible N intake:organic matter intake observed) were not significantly different from zero for any ration.

Urinary N excretion and N balances were significantly higher for AWS than for UWS. Protein supplementation to AWS increased urinary N excretion significantly, the increase being more for AWSC than for AWSP. Protein supplementation also increased N balance, although in this case the increase was significantly more for AWSP than for AWSC.

The apparent small-intestinal digestibilities of AA-N and N were significantly higher for AWSP than for the other rations, while no significant ration effect was found for apparent small-intestinal digestibility of NPN (Table 9). Ration differences as found for apparent small-intestinal digestion of N and AA-N were not found for true small-intestinal digestion (Table 10), indicating that ration differences with regard to apparent small-intestinal digestion were related to different duodenal NPDM and duodenal AA-N or N flows. The regression of apparent small-intestinal disappearance of individual AA, TAA, AA-N, N or

NPN *v.* duodenal flow of these nutrients (Y and X scaled to NPDM) included all thirty-two observations. By inclusion of ration as a factor in the regression model the existence of significant differences between rations with regard to endogenous ileal losses could be examined.

The NPDM flows were 463, 560, 586 and 659 (SEM 26·3) g/d for UWS, AWS, AWSC and AWSP respectively. Duodenal NPDM flows were significantly higher for AWS and AWSC than for UWS and significantly lower for AWS than for AWSP. For AA-N, TAA and individual AA no significant deviations from the general regression were observed, as illustrated in Fig. 2 for AA-N, while for N and NPN a significant ration effect was found (Table 10). No significant differences were observed between true small-intestinal digestibilities of N and AA-N, although NPN had a lower true digestibility than AA-N. Cystine, histidine and tryptophan had true digestibilities lower than 0·80 (Table 10). In addition, molar proportions in ileal endogenous protein losses of cystine and histidine were higher than those in duodenal protein resulting in even lower apparent small-intestinal digestibilities for these AA.

DISCUSSION

Intake and digestion

NH_3 treatment of wheat straw increased straw intake significantly. The increased intake was associated with a significantly higher cell-wall digestion in the rumen and a higher rumen fluid volume and, because of the relatively constant rumen DM concentration (Owens & Goetsch, 1986), also with a higher rumen DM fill; duodenal flows of undegraded cell walls and k_p were unaffected.

The relative contribution of the rumen to whole-tract digestion of organic matter and cell-wall components of UWS and AWS were consistent with results for rations based on UWS and AWS reported by Van Bruchem *et al.* (1993). However, the results of the present experiment do not support the suggestion by Demeyer (1991) that the importance of hindgut fermentation increases with decreasing digestibility of the ration.

The proportion of organic matter digestion occurring in the rumen was higher for UWS and AWS (average 774 g/kg) in the present experiment than the value of 650 g/kg observed by Zorilla-Rios *et al.* (1991) for AWS and UWS fed to cattle.

Protein supplementation of AWS reduced rumen cell-wall digestion; the reason for this is unknown. Rumen pH, rumen VFA concentration and retention times in the rumen were approximately similar for AWS, AWSC and AWSP. Also, it is unlikely that the relatively high NH_3-N concentration in the rumen fluid as found for AWSC and AWSP affected rumen digestion. Satter & Slyter (1974) did not observe toxic effects of NH_3-N concentrations up to 800 mg/l on microbial protein synthesis *in vitro*, and in the present experiment no negative effects of protein supplementation on efficiency of microbial protein synthesis were observed.

The lower rumen digestion of cell-wall components for protein-supplemented AWS was compensated by a higher large-intestinal digestion for hemicellulose, but not for cellulose.

Since the k_p did not differ between rations AWS, AWSC and AWSP, the higher duodenal passage of NDF observed for AWSP is indicative of a higher rumen NDF fill and consequently a higher rumen DM fill. This was supported by the higher rumen fluid volume for sheep fed on AWSP compared with those fed on AWS or AWSC.

Intake and protein status

The fact that NDF intake was highly correlated with rumen fluid volume indicates that factors other than rumen fill were limiting intake of UWS, AWS and AWSC and possibly AWSP. Intake of roughages could be limited by the amount of protein available for

absorption from the small intestine as suggested by Egan (1965, 1977), Doyle & McLaren (1988) and Doyle & Panday (1990), although no effects of increased small-intestinal protein availability were found in several other experiments (Kellaway & Leibholz, 1983; Ketelaars & Tolkamp, 1991). In the present experiment, digestible organic matter intake (DOMI) was linearly related to availability of truly absorbed AA-N (truly absorbed AA-N = duodenal AA-N flow × 0·86). Regression of truly absorbed AA-N *v.* DOMI with inclusion of the period effect as a factor yielded the following relationship (the intercept is the mean over periods, with the SE of the estimate):

truly absorbed AA-N (mg/kg $W^{0.75}$ per d) = −459 (SE 163·9) + 33·0 (SE 5·27) × DOMI (g/kg $W^{0.75}$ per d), (r^2 0·800, RSD 89·6, *n* 16).

The data, corrected for the period effect, are plotted in Fig. 3.

The basis of the hypothesis that the amount of protein available for absorption from the small intestine determines the intake of roughage could be that a balance is required between net energy and net protein availability to the tissues. DOMI could represent the net energy availability to the tissues. The conversion of DOMI to net energy probably occurs at a rather constant efficiency for *ad lib.*-fed rations, because (1) digestible energy intake is closely related to DOMI, (2) the metabolizable energy content in digestible energy is approximately 0·80 without much variation between different feeds (Agricultural Research Council, 1980; Oosting *et al.* 1993*a*) and (3) the efficiency of conversion of metabolizable energy to net energy for *ad lib.* rations fed to growing animals is also fairly constant (0·6) as postulated by Tolkamp & Ketelaars (1993), and confirmed by Oosting *et al.* (1993*a*) for UWS and AWS in sheep and cattle. In the present experiment, DOMI and N balance were linearly related. The N balance *v.* DOMI regression was done with inclusion of the period effect as a factor in the regression model. The following equation (the intercept is the mean over periods, with the SE of estimate) was found:

N balance (mg/kg $W^{0.75}$ per d) = −460 (SE 81·0) + 15·4 (SE 2·60) × DOMI (g/kg $W^{0.75}$ per d), r^2 0·773, RSD 44·3, *n* 16.

The data corrected for the period effect are plotted in Fig. 4.

The equation predicts a zero N balance for a DOMI of 29·7 g/kg $W^{0.75}$ per d, higher than the maintenance requirements of 26 g DOMI/kg $W^{0.75}$ per d (Agricultural Research Council, 1980). Although the regression coefficient has a high standard error, it is remarkably close to values observed by others for small ruminants fed on roughage-based diets. Ketelaars & Tolkamp (1991) observed a value of 14·4 mg N balance/g DOMI for West African Dwarf goats. Recalculation of results of Elliott & Topps (1964), who measured total digestible nutrient (TDN) intake and N balance in Blackhead Persian sheep, and Egan (1965), who measured digestible energy intake in Merino sheep receiving dietary N supplements, resulted in estimates of 14·6 and 15·3 mg N balance/g DOMI respectively. TDN values given by Elliott & Topps (1964) were converted to digestible organic matter by assuming that 1 g TDN contains 0·95 g digestible organic matter, and the regression coefficient derived from the regression of digestible energy intake *v.* N balance as given by Egan (1965) was converted to the regression coefficient of N balance *v.* DOMI by assuming 18·8 kJ digestible energy intake/g DOMI and that $b_{x.y}b_{y.x} = r^2$. Larger regression coefficients (up to 20·9 mg N balance/g DOMI) were found by Grenet & Demarquilly (1977) for Texel sheep. The linearity of these relationships between DOMI and N balance suggests that net protein and net energy availabilities to the tissues are balanced in *ad lib.* rations, hence that voluntary DOMI cannot increase without a concomitant increase in net protein availability. However, Ørskov (1982) proposed that energy intake may increase without increased N balance, if N availability becomes limiting.

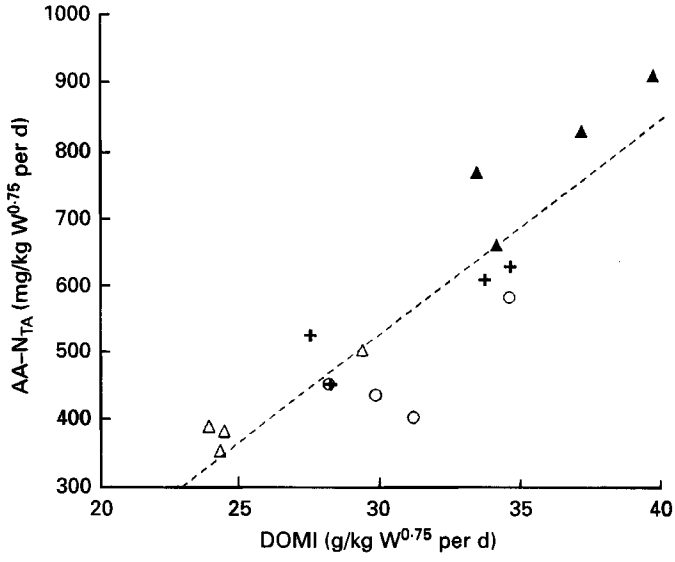

Fig. 3. Relationship between truly small-intestinally absorbed amino acid (AA)-N (AA-N_{TA}) and digestible organic matter intake (DOMI; data corrected for period effect) for rations containing untreated wheat straw (UWS) or ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements fed to sheep. (△), UWS; (○), AWS; (+), AWSC; (▲), AWSP. For details of rations and procedures, see Tables 1 and 2 and pp. 348–352. W, live weight.

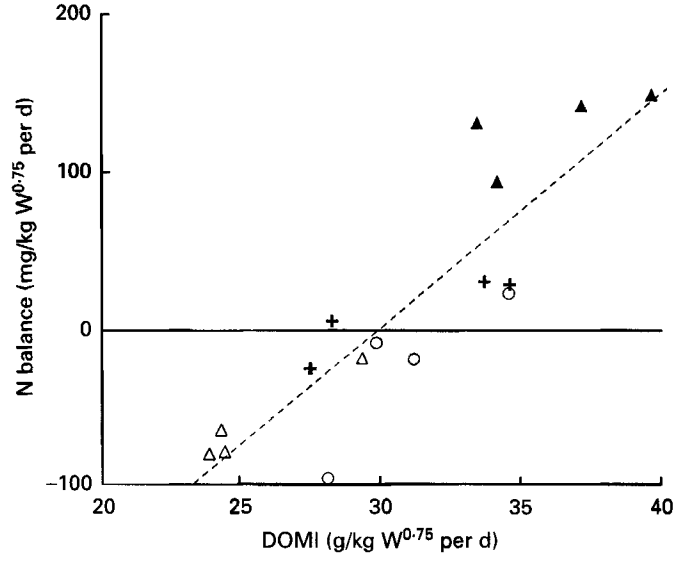

Fig. 4. Relationship between N balance and digestible organic matter intake (DOMI; data corrected for period effect) for rations containing untreated wheat straw (UWS) or ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements fed to sheep. (△), UWS; (○), AWS; (+), AWSC; (▲), AWSP. For details of rations and procedures, see Tables 1 and 2 and pp. 348–352. W, live weight.

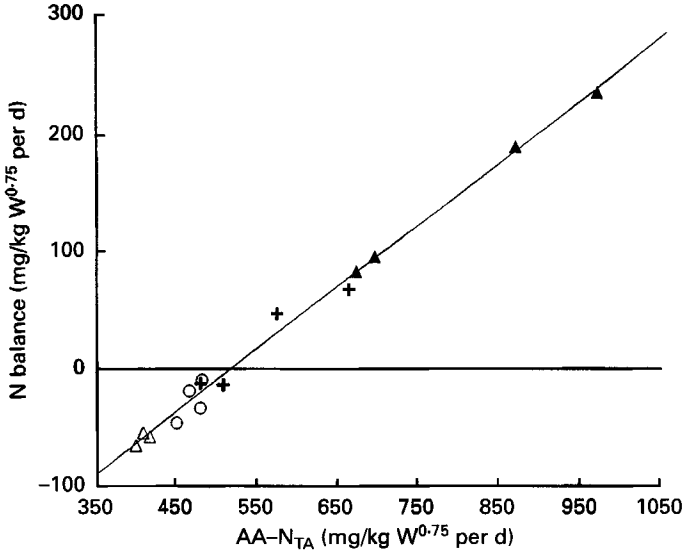

Fig. 5. Relationship between N balance and truly small-intestinally absorbed amino acid (AA)-N (AA-N_{TA}; data corrected for animal and period effect) for rations containing untreated wheat straw (UWS) or ammoniated wheat straw alone (AWS) or with casein (AWSC) or potato-protein (AWSP) supplements fed to sheep. (△), UWS; (○), AWS; (+), AWSC; (▲), AWSP. For details of rations and procedures, see Tables 1 and 2 and pp. 348–352. W, live weight.

The efficiency of utilization of truly absorbed AA-N was estimated by regression of N balance *v.* truly absorbed AA-N with inclusion of the (significant) animal and period effects as factors in the regression model. The regression equation obtained was (the intercept is averaged over animals and periods, with the SE of the estimate):

$$\text{N balance (mg/kg } W^{0.75} \text{ per d)} = -281 \text{ (SE } 15{\cdot}9) + 0{\cdot}54 \text{ (SE } 0{\cdot}029) \times \text{truly absorbed AA-N (mg/kg } W^{0.75} \text{ per d)}, r^2\ 0{\cdot}989, \text{RSD } 14{\cdot}3, n\ 16.$$

The data, corrected for the animal and period effect are plotted in Fig. 5.

The intercept can be interpreted as the obligatory N loss in the form of endogenous urinary N and metabolic faecal N. This value of 281 mg/kg $W^{0.75}$ per d is in the range of values varying from 201 to 427 mg/kg $W^{0.75}$ per d given by Ørskov (1982) for total urinary and faecal N excreted by fasting sheep or sheep maintained on N-free diets. Estimates of the intercept for individual animals ranged from −242 to −323 mg/kg $W^{0.75}$ per d and for periods from −221 to −344 mg/kg $W^{0.75}$ per d. With increasing period number the value for the intercept increased, indicating that the maintenance requirements decreased with increasing duration of the experiment. The regression coefficient implies an efficiency of utilization of truly-absorbed AA-N of 0·54 for all rations.

Hence, because (1) literature and the results of the present experiment suggest that a balance is required between net protein and net energy availability in growing animals and (2) the efficiency of utilization of truly absorbed AA-N was similar for all rations in the present experiments, it seems justified to conclude that a balance was required between truly absorbed AA-N and DOMI in the present experiment, i.e. that DOMI was limited by the availability of truly absorbed AA-N.

However, truly absorbed AA-N and N balance are in part a function of DOMI. Truly absorbed AA-N of microbial origin and, to a lesser extent originating from undegraded straw protein, increase with increasing straw DOMI. However, from microbial AA-N production per kg DOMI apparently degraded in the rumen, the proportion of DOMI that is apparently degraded in the rumen and a true small-intestinal digestion of microbial AA-N of 0·86, it can be calculated that for ration AWSC (the ration with the highest efficiency of microbial protein synthesis) only 14·0 g microbial truly absorbed AA-N could be produced per kg DOMI. This quantity is insufficient to achieve the required retention of 15 g N/kg DOMI (Fig. 4). Hence, additional straw consumption in the case of ration AWSC would result in an unbalanced net protein:net energy ratio, unless the quantity of feed truly absorbed AA-N increased considerably, which is unlikely in the case of AWS.

Why limited protein availability to the tissues restricts voluntary intake is unknown. MacRae & Lobley (1982) and MacRae *et al.* (1985) reported that the efficiency of metabolizable energy utilization increased, and consequently the O_2 consumption per unit metabolizable or net energy ingested decreased, with increasing protein availability. Tolkamp & Ketelaars (1992) postulated, as a control mechanism for intake regulation, that ruminants minimize O_2 consumption per unit net energy intake. These authors reported that the net energy intake level where the O_2 consumption per unit net energy ingested is minimal, increases with increasing efficiency of utilization of metabolizable energy. Hence, increased protein availability to the tissues could increase the efficiency of metabolizable energy utilization and consequently increase the voluntary intake.

Rumen microbes and small-intestinal protein digestion

In an earlier experiment (Oosting *et al.* 1993*b*) the efficiency of microbial protein synthesis was (average of DAPA and AA profiles methods) 24·6 and 19·0 g N/kg digestible organic matter apparently degraded in the rumen for UWS and AWS respectively. This value for AWS was lower than that from the present experiment, possibly because additional minerals including S were supplied in the present experiment.

The concentration of rumen microbes in rumen fluid did not differ significantly between rations. Ration differences in fluid-associated microbial pools could be attributed to differences in rumen fluid pools. The proportion of the total microbial pool associated with the fluid phase may vary from 20 to 47% (Owens & Goetsch, 1986). Oosting *et al.* (1993*b*) observed for UWS as well as for AWS, that 36% of the microbial pool was associated with the fluid phase.

As discussed by Oosting *et al.* (1993*b*), the DAPA method may give higher, lower or similar estimates of the efficiency of microbial protein synthesis compared with the AA profiles method. In the present experiment no significant differences were found between these two methods, except for ration AWSC. The purine derivatives method differed significantly from the other two methods only for ration AWS. Despite the variation between methods it could be concluded that the casein and potato-protein supplements supplied nutrients, probably peptides and/or branched-chain AA, that were limiting microbial growth with AWS and UWS.

From the true rumen degradability of feed AA-N, it was calculated that rumen NH_3-N contributed 63, 58, 0 and 33% to net microbial AA-N production for UWS, AWS, AWSC and AWSP respectively. Oosting *et al.* (1993*b*) reported values of 59 and 67% for UWS and AWS respectively. These results indicate that in rations with a limited rumen true protein availability the maximum contribution of NH_3-N to microbial protein synthesis may approximate 60–70%. The remaining contribution has to be provided by dietary true protein. The possible difference in the efficiency of microbial protein synthesis between AWSC and AWSP even indicates that the efficiency of microbial protein synthesis is higher

when all microbial AA-N can be synthesized from dietary protein as with AWSC, compared with AWSP where 33% of microbial AA-N originated from rumen NH_3.

The true small-intestinal protein digestion of 0·86 observed in the present experiment is consistent with the values reported by Van Bruchem *et al.* (1989; 0·85 for roughage-based diets in sheep) and by Storm *et al.* (1983) for AA of microbial origin. The major part of duodenal protein flow consists of microbial protein, which explains the similarity between the results from various experiments, but it could be concluded from the present experiment that dietary AA added through potato-protein supplementation were digested to the same extent as microbial protein. Although measured in cattle, the high small-intestinal digestion of N from Dacron bags introduced into the duodenum and collected in faeces also indicated that N from the supplements was highly digestible in the lower gut. The small-intestinal disappearances of N from Dacron bags observed in the present experiment for UWS (652–774 g/kg) as well as for AWS (607–750 g/kg) were considerably higher than those for untreated (317 g/kg) and ammoniated barley straw (357 g/kg) reported by Hvelplund (1989).

Sheep fed on UWS had lower NPN losses per 100 g NPDM from the ileum than sheep fed on rations based on AWS. This could probably be attributed to the fact that part of the N added through NH_3 treatment was not available for digestion. This is supported by the lower AA-N:N value in duodenal digesta for AWS and AWSC compared with UWS. The results of Oosting *et al.* (1993*b*) also suggested that part of the N added through NH_3 treatment could not be digested. Barnes (1988) observed by near i.r. diffuse reflectance spectrometry that NH_3 treatment leads to the formation of amide groups with both cell-wall and non-cell-wall components of wheat straw. It is likely that part of these amide groups is not digestible. However, as indicated by disappearance of N from Dacron bags over the whole digestive tract, potentially 800–900 g/kg total N in AWS could be digested, although it is possible that this could be attributed partly to solubilization.

Recalculation of the data from the experiment by Van Bruchem *et al.* (1989) by a model without intercept yielded as an estimate of ileal endogenous AA-N losses 7·9 mg/g NPDM for sheep fed on roughage-based diets of a relatively high quality. Oosting *et al.* (1993*b*) estimated ileal endogenous AA-N losses at 5·0 mg/g NPDM for sheep fed on UWS or AWS supplemented with sugarbeet pulp, while in the present experiment ileal endogenous AA-N losses were estimated at 6·0 mg/g NPDM. This indicates that sheep fed on straw-based diets have lower ileal endogenous protein losses than sheep fed on diets of higher quality with higher protein contents. Whether this is a result of a lower endogenous secretion in the distal part of the ileum or to higher re-absorption of secreted endogenous AA remains to be investigated.

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