



Effects of oilseed meal and grain-urea supplements fed infrequently on digestion in sheep

1. Low quality grass hay diets

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Abstract

An experiment examined intake, growth response and rumen digestion of young sheep fed ad libitum low quality grass hay alone or supplemented with approximately isonitrogenous amounts of barley grain and urea (Bar/N), safflower meal (SAF) or linseed meal (LIN) provided at 3 days intervals. Supplements comprised 13–20% of total DM intake. Sheep fed grass hay alone consumed 60.2 g DM/kg LW^{0.75}/day of hay and an estimated 6.09 MJ metabolizable energy (ME)/day, and were in liveweight (LW) maintenance. Hay intake was decreased ($P < 0.05$) by the Bar/N supplement with a substitution rate of 0.9, but was not changed by the oilseed meal supplements. Each of the supplements increased ($P < 0.05$) estimated ME intake to a similar extent, but LW gain and wool growth were lower ($P < 0.05$) in sheep supplemented with Bar/N than those supplemented with LIN. Rumen degradabilities of the SAF and LIN CP were estimated to be 0.72 and 0.62, respectively. Rumen ammonia concentrations in sheep fed hay alone (average 97 mg NH₃/l) were expected to be adequate for microbial activity. Fractional outflow rate (FOR) of liquid from the rumen measured with Co-EDTA (mean 0.109 h⁻¹) was greater than that of Cr-mordanted supplements (mean 0.056 h⁻¹), which was in turn greater than the FOR of Cr-mordanted hay (mean 0.031 h⁻¹). Diet did not affect these FOR. Supplemented sheep accommodated increased DM intake on Day 1 of the 3 day supplementation cycle by increasing rumen digesta load rather than by increasing rate of passage of digesta. Results show that the LW gain of young sheep fed low quality hay was

Abbreviations: ADF, acid detergent fibre; Bar/N, barley grain and urea; CP, crude protein; DM, dry matter; LIN, linseed meal; LW, liveweight; ME, metabolizable energy; N, nitrogen; MRT, mean retention time; NDF, neutral detergent fibre; Nil, no supplement; OM, organic matter; RDP, rumen degradable protein; RUP, ruminally undegraded protein; SAF, safflower meal

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increased more by either oilseed meal than by equivalent amounts of barley grain/urea supplement, apparently due to more efficient utilization of ME for LW gain.

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1. Introduction

In many grazing systems the only pastures available for part of the annual seasonal cycle, or during drought, are senesced forages of low nutritive value. Nutrient intake of animals consuming such pastures is often insufficient to support even maintenance, and thus productivity is often adversely affected. There has been extensive experimentation on the types and amounts of supplements required in various circumstances for optimal biological and economic efficiency. High nitrogen (N) supplements such as oilseed meals have often resulted in large increases in intake and animal productivity of sheep and cattle consuming low quality forage (Abidin and Kempton, 1981; Kellaway and Leibholz, 1983; Coombe, 1985; Coombe et al., 1987). However, there remains controversy about the roles of various supplements, in particular the relative importance of various forms of additional metabolizable energy (ME) or N to supply substrate for rumen microorganisms, or of digestible ruminally undegraded protein (RUP) to meet the needs of the animal for amino acid (Egan, 1984; Leng, 1990; Poppi and McLennan, 1995; Hennessy et al., 1996; Dixon and Egan, 2000).

The responses of ruminants fed low quality forages to supplements are determined by a number of factors. Supplements may decrease, have no effect, or increase both voluntary intake of forages and digestibility of the fibrous components of the forage, thus affecting ME intake (Leng, 1990; Dixon and Stockdale, 1999; Rafiq et al., 2002). In addition, the efficiency of utilization of ME for growth may be influenced by the balance of substrates available (MacRae and Lobley, 1986; SCA, 1990); supplements may change the efficiency of utilization of ME by changing this substrate balance. However, few experiments examining growth responses of ruminants to supplements have determined the extent to which responses could be attributed to a supplement increasing ME intake, or to changes in efficiency of utilization of ME. Furthermore although protein meal supplements have often given greater growth responses than those based on cereal grain plus inorganic N, protein meals are usually much higher in cost per tonne. Thus, even when the animal response per unit of supplement is lower, supplements based on cereal grains may be more cost-effective. A quantitative understanding of the animal response to various types of supplements is essential if supplements are to be used most cost-effectively to improve animal productivity. Furthermore, much of the information available has been obtained under conditions where supplements were fed daily or more frequently, whereas on-farm management often involves feeding supplements less frequently thereby creating large fluctuations in nutrient supply.

The present studies examined changes in voluntary forage intake, digestion in the rumen and across the entire gastrointestinal tract, estimated ME intake and liveweight (LW) change of young sheep fed a low quality hay in response to a single isonitrogenous level of three high N supplements offered at 3 days intervals. Two of the supplements consisted of linseed meal (LIN) (*Linum usitatissimum*), an oilseed of low fibre content expected to contain a substantial

proportion of the protein as RUP, and safflower meal (SAF) (*Carthamus tinctorius*), an oilseed meal of high fibre content expected to contain a low proportion of RUP. The third supplement consisted of barley grain mixed with sufficient urea to increase the N to a concentration similar to the LIN. The non-steady state conditions created by feeding the supplements at 3 day intervals were examined in order to evaluate the magnitude of the cyclic fluctuations and their effects on digestion and utilization of nutrients.

2. Materials and methods

2.1. Sheep and experimental design

Merino wethers (initially about 15 months of age, LW $32 \pm$ (S.D.) 3.1 kg and body condition score (Russel et al., 1969) 2.3–2.7) were, in two consecutive groups of 22 and 24 sheep, held in individual metabolism cages for 45 days. The 24 sheep in the second group were surgically prepared with rumen cannulae and allowed at least 4 weeks to recover from the surgery before the experiment commenced. All sheep were treated with anthelminthetics (Ranide providing 150 mg rafoxanide per sheep, MSD-Merck, Sydney, Australia; Nilvern providing 130 mg levamisol hydrochloride per sheep, ICI, Sydney, Australia). Sheep within each group were allocated by stratified randomization based on LW to four treatment diets. Following a feeding and metabolism trial, the sheep were euthanised and measurements made of the amounts of digesta present in various parts of the gastrointestinal tract.

2.2. Diets and intake

The forage consisted of coarsely chopped low quality grass hay offered daily at about 20% in excess of anticipated intake. This forage was offered without additional supplement (Nil) or was supplemented each third day with barley grain mixed with urea and sodium sulphate (Bar/N; approximately 10 g DM/kg LW^{0.75}/day), SAF (approximately 16 g DM/kg LW^{0.75}/day) or LIN (approximately 10 g DM/kg LW^{0.75}/day). Bar/N was prepared by mixing whole barley grain with a solution of urea and sodium sulphate (64 g urea and 13 g sodium sulphate in 200 ml solution/kg of air dry grain) immediately before feeding, thereby adding 30 g urea N and 3 g sulphate S/kg air dry barley grain. Both oilseed meals had been prepared by solvent extraction. SAF was manufactured without prior removal of the hull from the seed and was high in fibre. Supplements were offered each third day at 0800–0900 h in a separate feeder. Minerals (15 g/day; Dixon et al., 1999) were provided mixed with the hay. Water was freely available. The amounts of hay and supplement offered and refused by each sheep were measured daily. Samples of hay and supplements offered and of refusals were bulked for each 3-day cycle and a subsample dried at 100 °C to determine DM content.

2.3. Synthetic fibre bag measurements

Synthetic fibre bags containing supplements were inserted into the rumen commencing at 0800 h on Day 16 and removed after 6, 24, 48 and 72 h. Bags containing hay were incubated

in the rumen commencing on Day 22 to measure rate of disappearance of DM. Bags were inserted at 0800 h on Day 1 of the supplementation cycle and duplicate bags removed after 24, 48 and 72 h of incubation. In addition, bags were inserted 24 and 48 h after provision of supplements and duplicate bags removed after 24 and 48, or 24 h, respectively, incubation. The synthetic fibre bags (110 mm × 45 mm) were made from monofilament nylon cloth (44 µm pore size, Nytal ASTM 325-44, Swiss Screens Australia PLC, Moorabbin, Vic.) and contained 2 g hay or 3 g supplement and a steel ball (8 g) as a weight. Hay and barley grain were ground through a 3 mm screen using a laboratory feed mill (Makla Mill, Crompton Parkinson, model 8302 A-P, Noyes Bros Ltd., Australia), but there was no additional processing of the SAF and LIN supplements. Following removal from the rumen the bags were washed briefly and stored at 5 °C until all bags had been removed. The bags were then washed thoroughly under running tap water with light squeezing until the washings were colourless, dried at 60 °C and re-weighed. Solubility of DM in each feedstuff was determined by soaking six bags in sodium chloride solution (9 g/kg) for 3 h before washing and drying.

2.4. Marker measurements and total collection

At 0800 h on Day 19 sheep were given, via the rumen cannula, a single dose of Co-EDTA (650 mg Co in 65 ml solution) as a liquid phase marker. The supplemented sheep were also given a single dose of Cr-mordanted (Uden et al., 1980) supplement (1.7–3.3 g Cr-mordant to 50 g supplement) as a marker of the respective supplement. Approximately 45 ml of rumen fluid was sampled 0.5 h before and 3, 6, 9, 12, 15, 24, 30, 36, 48, 60 and 72 h after offering supplements. Rumen fluid was obtained by gentle suction through a gauze-covered probe in the ventral sac. Rumen fluid pH was measured immediately (Model 701 pH meter, Orion Research Laboratories, Cambridge, MA) and samples were acidified to pH < 4 (0.6 ml 5 M sulphuric acid) before freezing (–20 °C). The faeces excreted were collected quantitatively each 8 h for the following 6 days, subsampled, dried at 100 °C and stored for subsequent analysis of Cr. Samples of rumen fluid obtained between 3 and 30 h after administration with Co-EDTA were subsequently analysed for Co content. Cr-mordanted hay (0.8–1.2 g Cr-mordant to 25 g hay DM) was dosed into the rumen of all sheep via the cannula on Day 28 at 0800 h and faeces were collected and subsampled at 8 h intervals for the next 6 days. All of the faeces and urine voided between Days 31 and 39 were collected, the urine into sufficient hydrochloric acid to reduce the pH to less than 4. A daily subsample of faeces was dried at 100 °C to determine DM content and a second subsample was bulked over the collection period and stored at –20 °C. The latter subsamples were subsequently freeze dried in preparation for laboratory analysis. The rate of intake of supplements on Day 1 of the 3-day supplementation cycle was measured on three occasions by weighing the amount of supplement remaining after 2, 4, 6, 9 and 24 h. Wool growth was measured by closely clipping midside patches (100 mm × 100 mm) on Days 20 and 35. The sheep were weighed at 0800 h each 6 or 9 days and on the third day of the 3-day feeding cycle.

2.5. Measurements at slaughter

Following the 45 days interval during which the above measurements were made, the amounts of digesta present in various parts of the gastrointestinal tract 6, 24 and 72 h

after provision of supplements were determined at slaughter. Four sheep given each diet were randomly allocated to each time of slaughter, except that no sheep fed the Nil diet were allocated to the 24 h slaughter time which for this diet was synonymous with the 72 h slaughter time. Sheep were shorn and then euthanised with sodium pentobarbitone given intravenously. As quickly as possible the abdominal cavity was then opened, the gastrointestinal tract was ligated at designated sites to prevent movement of digesta between anatomical sections of the tract, and the sections of the tract removed. The gastrointestinal tract was separated into the reticulo-rumen, omasum, abomasum, small intestine, and large intestine. Digesta was emptied from the sections of the tract, the sections washed and then the excess water removed with absorbent paper. Weights of wet digesta and of gastrointestinal tract tissue were determined and a subsample of digesta dried at 70 °C.

2.6. Laboratory analysis

Samples of feed offered, feed refused and faeces were ignited at 550 °C for 6 h to determine organic matter (OM) content. Total N was determined by Kjeldahl analysis (AOAC, 1975). Concentrations of ammonia in rumen fluid was determined by distillation and titration procedures. Concentrations of individual VFA were determined using a gas chromatograph (Hewlett Packard, Model 5890, Series 11). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed as described by Goering and van Soest (1970) without inclusion of sodium sulphate in the extraction solutions and, for the barley grain, use of α -amylase; values were expressed to include residual ash. Lignin was analysed by the method of van Soest (1963). Concentrations of Co in rumen fluid and Cr in faeces were measured by atomic absorption spectroscopy. Wool was washed with hexane and then dried (Chapman, 1960) to determine clean dry wool yield. Particle size distribution of the supplements was determined by dry sieving (Endecott Test Sieve Shaker, Model A, London, UK) 50 g samples for 20 min through 190 mm diameter sieves. The specific gravity of each of the separated fractions of the oilseed meals was measured by the displacement of paraffin by weighed amounts of the material.

2.7. Calculations and statistical procedures

LW change of the lambs was calculated from the linear regressions of LW with time. The fractional outflow rate (FOR) from the rumen of Co-EDTA was calculated as the first order kinetic decline of Co in rumen fluid between 3 and 30 h after administration of Co-EDTA. The single exponential exponent explained >98% of the variance except in one case where it explained only 94%. The FOR from the rumen of Cr-mordanted supplements or hay was calculated as the slope of the exponential decline of the marker in faeces excreted between 40 and 80 h after administration of the Cr-mordant. In addition, the mean retention time (MRT) of the Cr-mordanted supplements or hay was calculated by numerical integration (Faichney, 1993) from the concentrations of Cr in faeces excreted at various times. The estimated ME content of diets was calculated as: $ME = 0.16 OMD - 1.8$, where ME was in MJ/kg feed DM and OMD was the percent OM digestibility measured by total collection (SCA, 1990). The ME contents of the supplements were assumed to be 12.2, 5.9 and 11.9 MJ ME/kg DM for barley grain, SAF and LIN, respectively (AFIC, 1987; SCA, 1990; AFRC, 1993).

The degradability of DM and CP of the SAF and LIN supplements incubated in the synthetic fibre bags in the rumen were calculated assuming a first order kinetic model of disappearance from the bags and the respective supplement FOR measured with the Cr-mordanted supplement (AFRC, 1993; Huntington and Givens, 1995). The predicted supply of metabolizable protein (MP; AFRC, 1993) was calculated assuming that the proportions of effective rumen degradable protein (RDP) and digestible undegraded protein (RUP), respectively, in CP were 0.65 and 0.19 for hay, 0.67 and 0.12 for the straw, 0.85 and 0.07 for barley grain, 0.72 and 0.18 for SAF, 0.62 and 0.37 for LIN. These values for hay, straw and barley grain were assumed from AFRC (1993). Values for the indigestible CP fraction of SAF and LIN were calculated from the disappearance of CP from synthetic fibre bags as $(1000 - (a + b))$, while the RDP and RUP were calculated by difference. The predicted supply of apparently digested CP leaving the stomach (ADPLS; SCA, 1990) was calculated with the same assumptions for the contributions of RDP and RUP, and that the standard reference LW of the sheep was 65 kg.

Data were analysed by a one-way ANOVA within randomized blocks, with the blocks consisting of the groups and LW sub-groups of sheep. Where the *F*-test was significant (i.e. $P < 0.05$) treatment means were compared using a least significant differences (LSD) test (Steel and Torrie, 1981). In addition, differences between treatments in wool growth were examined by an analysis of variance which included as a covariate growth of wool prior to the experiment. Data for the disappearance of hay DM from synthetic fibre bags at the various incubation times through the 3-day cycle were considered in a one-way ANOVA, while the effects of diets and days of the feeding cycle were considered in a two-way ANOVA.

3. Results

3.1. Intake, diet digestibility and productivity

Hay contained 16.1 g N/kg DM (Table 1). Bar/N supplement was calculated to contain 49.2 g N/kg DM and 64% of this N consisted of urea N. SAF contained less N and more NDF than the LIN, while 667 and 751 g/kg, respectively, of SAF and LIN N were sufficiently small to pass through a 1 mm screen were (Table 2). The specific gravity of the SAF and LIN fractions which passed through a 1 mm screen ranged from 1.15 to 1.32. The Bar/N, SAF and LIN supplements were calculated to provide 1.6, 1.1 and 1.4 MJ ME/day, respectively. The sheep were in good health throughout the experiment and generally consumed consistent amounts of hay.

Table 1
Chemical analyses of feedstuffs fed to the sheep (g/kg DM)

Feedstuff	OM	Nitrogen	NDF	ADF	Lignin
Grass hay	948	16.1	692	400	45
Barley grain	979	19.4	221	58	8
SAF	958	41.2	602	371	133
LIN	948	52.9	297	164	61

Table 2

Distributions of DM (g DM/kg DM) and nitrogen (mg N/g N) and the specific gravities of SAF and LIN retained on various screen sizes following dry sieving^a

Particle size fraction	Measurement					
	Dry matter		Nitrogen		Specific gravity	
	SAF	LIN	SAF	LIN	SAF	LIN
>2.0 mm screen	34	27	26	26	2.03	1.11
>1.0, <2.0 mm screen	442	235	307	223	1.13	1.19
>0.5, <1.0 mm screen	329	353	287	313	1.15	1.22
>0.25, <0.5 mm screen	119	228	216	247	1.27	1.19
<0.25 mm screen	76	157	164	191	1.32	1.26

^a Values for the DM fraction represent the mean of seven sieved samples. Fractions were bulked and analysed in duplicate for N and specific gravity.

With only occasional exceptions the sheep consumed their entire allocations of supplements within the 3-day supplementation cycle. At least 72% of the 3-day allocation of supplement was consumed within 4 h of offer (Table 3). Lower percentages of Bar/N and SAF than of LIN were consumed after 2, 4 or 6 h ($P < 0.001$, $P < 0.01$ and $P = 0.07$, respectively). The actual amounts of LIN and SAF consumed after 2, 4 or 6 h were similar; the lower percentage of SAF was associated with a larger amount of this supplement being offered.

On average, over the 3-day feeding cycle the Bar/N, SAF and LIN supplements constituted 15, 20 and 13%, respectively, of total DM intake and provided 6.2–7.7 g N/day (Table 4). Sheep offered Bar/N supplement consumed less hay ($P < 0.001$) than the sheep offered hay alone (51.9 and 60.2 g/kg LW^{0.75}/day, respectively; Table 4) and the substitution rate of Bar/N for hay was 0.9 g/g. The substitution rates for sheep offered SAF and LIN supplements were 0.3 and 0.5 g/g, respectively. Total DM intake was not changed by the

Table 3

Rate of consumption of a 3-day allocation of supplements during the 24 h after being offered ($n = 12$)^a

	Supplement			S.E.M.	Significance
	Bar/N	SAF	LIN		
Supplement offered (g air dry)	477	640	402	–	–
Percent consumed after:					
2 h	54.2 a	52.7 a	84.9 b	4.2	***
4 h	72.1 a	74.2 a	94.0 b	4.6	**
6 h	85.5	84.9	96.9	3.9	ns ^b
9 h	97.1	90.8	98.4	2.6	ns
24 h	100.0	96.8	100.0	1.2	ns

^a Diets consisted of grass hay offered ad libitum supplemented with barley grain, urea and sodium sulphate (Bar/N), SAF or LIN.

^b Not significant.

** $P < 0.01$.

*** $P < 0.001$.

Table 4

Intake, digestion and excretion of dietary components, LW change (g/day), feed conversion efficiency (FCE; g LW change/MJ ME intake) and wool production (mg clean wool/patch/day) in sheep

Measurement	Treatment				S.E.M.	Significance
	Nil	Bar/N	SAF	LIN		
<i>n</i>	10	12	12	12	–	–
DM intake						
Supplement (g/3 days)	0	382	568	355	–	–
Hay, Day 1 (g)	820 b	530 a	566 a	593 a	25	***
Hay, Day 2 (g)	867	782	842	871	29	ns ^a
Hay, Day 3 (g)	836	805	868	883	30	ns
Hay, mean (Days 1–3) (g/day)	841 c	706 a	759 ab	783 bc	26	**
Total (g/day)	842 a	833 a	948 b	901 ab	27	*
DM intake (per unit LW ^{0.75})						
Supplement (g/3 days)	0	28.1	41.9	25.6	–	–
Hay, Day 1 (g)	58.7 b	38.8 a	41.6 a	42.6 a	1.57	***
Hay, Day 2 (g)	62.1	57.7	62.2	62.7	1.79	ns
Hay, Day 3 (g)	59.9	59.3	64.1	63.6	1.76	ns
Hay, mean (Days 1–3) (g/day)	60.2 b	51.9 a	56.0 ab	56.3 ab	1.55	***
Total (g/day)	60.2 a	61.3 ab	69.9 c	64.9 b	1.53	***
Digestibility (g/kg)						
Dry matter	552 b	582 c	530 a	562 b	7	***
OM	566 b	596 c	543 a	575 b	7	***
NDF	600 b	586 b	543 a	581 b	8	***
ADF	597 c	584 bc	541 a	569 b	8	***
ME intake (MJ ME/day)	6.09	6.44	6.52	6.65	0.20	ns
ME intake (kJ ME/LW ^{0.75} /day)	436 a	474 b	481 b	479 b	11.7	*
LW change (g/day)	4 a	24 ab	50 bc	53 c	9.7	**
FCE (g/g)	0.6 a	3.6 ab	7.3 b	7.9 b	1.46	**
Wool growth (mg/patch/day)	81 a	81 a	90 ab	100 b	4.0	**
Nitrogen						
Hay N intake (g/day)	13.8 b	11.7 a	12.5 ab	13.1 b	0.48	*
Supplement N intake (g/day)	0	7.1	7.7	6.2	–	–
Total N intake (g/day)	13.8 a	18.8 b	20.1 b	19.3 b	0.52	***
Faecal N excretion (g/day)	7.3	7.5	8.5	8.4	0.37	ns
Digestibility (g/kg)	458 a	600 c	578 bc	563 b	11	***
Urine N excretion (g/day)	7.3 a	9.9 b	10.5 bc	11.2 c	0.41	***
N balance (g/day)	–0.8 a	1.4 b	1.1 b	–0.3 a	0.46	**

^a Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Bar/N supplement, but was increased by provision of SAF ($P < 0.001$) and LIN ($P < 0.05$) supplements. The decrease in voluntary hay intake by sheep fed Bar/N supplement was most pronounced on Day 1 of the feeding cycle when hay intake was decreased ($P < 0.001$) by 34%, while on Days 2 and 3 of the feeding cycle hay intakes were decreased by 7 and

1%, respectively. Intakes of total DM on Day 1 of the feeding cycle ranged from 66.9 to 83.5 g/kg $W^{0.75}$.

Provision of the Bar/N supplement increased ($P < 0.01$) OM digestibility across the entire gastrointestinal tract (Table 4). Provision of SAF decreased ($P < 0.05$ or $P < 0.001$) digestibility of OM, NDF and ADF, but LIN supplement did not change digestibility of any of the measured components. As a consequence of changes in both intake and OM digestibility the estimated ME intake, expressed per kg $LW^{0.75}$, was increased ($P < 0.05$) by 9–10% by each of the supplements. Total N intake was increased ($P < 0.001$) from 13.8 g N/day in sheep fed hay alone to 18.8–20.1 g N/day in supplemented sheep. N balance was increased ($P < 0.01$) by the Bar/N and SAF supplements, but not by the LIN supplement.

Sheep fed hay alone were in approximate LW maintenance (i.e. +4 g/day). Provision of SAF and LIN supplements increased ($P < 0.05$) LW gain to 50 and 53 g/day, respectively, while sheep fed LIN supplement had a higher ($P < 0.05$) LW gain than those fed Bar/N (24 g/day). Wool growth was increased ($P < 0.01$) by LIN supplement.

3.2. Rumen ammonia, pH and VFA

Rumen ammonia concentrations in sheep fed hay alone averaged 94 mg/l, and were increased ($P < 0.001$) during Day 1 of the feeding cycle by provision of each of the supplements (Table 5 and Fig. 1). The concentration increased to a higher maximum (553 mg/l),

Table 5

Concentrations in rumen fluid of ammonia and pH on each day of the supplementation cycle, and total VFA and the proportions of the major individual VFA in rumen fluid on Day 1 of the supplementation cycle following provision of supplements in sheep

Measurement	Treatment				S.E.M.	Significance
	Nil	Bar/N	SAF	LIN		
Rumen ammonia (mg NH_3 /l)						
Mean (Day 1)	92 a	290 c	230 b	222 b	12	***
Mean (Day 2)	97	109	118	113	9	ns ^a
Mean (Day 3)	93	85	74	91	8	ns
Rumen fluid pH						
Mean (Day 1)	6.3	6.3	6.4	6.3	0.05	ns
Mean (Day 2)	6.4	6.5	6.5	6.5	0.05	ns
Mean (Day 3)	6.4	6.5	6.5	6.5	0.07	ns
VFA, mean (Day 1)						
Total (mmol/l)	84.8	89.6	93.2	95.9	4.7	ns
Acetate (mmol/M)	728 c	691 a	719 bc	703 ab	6	**
Propionate (mmol/M)	168 a	209 c	173 ab	192 bc	7	**
Butyrate (mmol/M)	84	83	80	82	4	ns
<i>Iso</i> -butyrate (mmol/M)	6 b	3 a	9 c	7 b	0.7	***
Valerate (mmol/M)	6 a	9 b	9 b	9 b	0.4	***
<i>Iso</i> -valerate (mmol/M)	8 b	4 a	10 b	8 b	0.1	***

^a Not significant.

** $P < 0.01$.

*** $P < 0.001$.

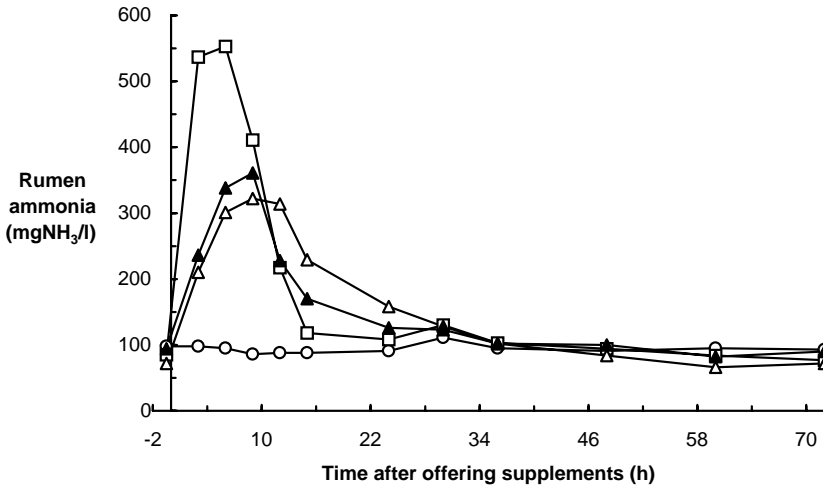


Fig. 1. The concentrations of ammonia in rumen fluid before and at intervals after offering supplements. The diets consisted of hay fed alone (○) or supplemented with Bar/N (□), SAF (△) or LIN (▲). Values are the means of six sheep and the S.E.M. ranged from 7 to 30 at the various times.

and more rapidly, with the Bar/N than with the oilseed meal supplements, but also declined more rapidly such that by 15 h after provision of the supplement the concentration in the Bar/N diet did not differ ($P > 0.05$) from the Nil diet. By 30 h after provision of SAF and LIN supplements, rumen ammonia concentrations were similar ($P > 0.05$) to the Nil diet. In sheep fed hay alone the pH of rumen fluid was in the range pH 6.3–6.5 (Fig. 2). Provision

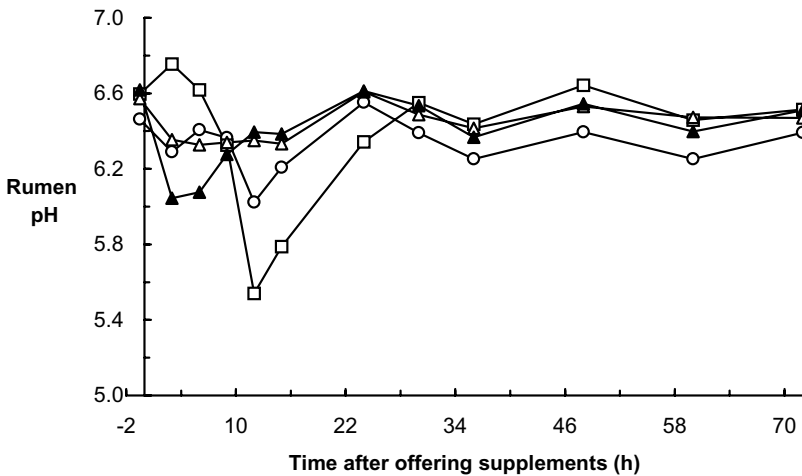


Fig. 2. The pH of rumen fluid before and at intervals after offering supplements. The diets consisted of hay fed alone (○) or supplemented with Bar/N (□), SAF (△) or LIN (▲). Values are the means of six sheep and the S.E.M. ranged from 0.05 to 0.11 at the various times.

of Bar/N supplement decreased ($P < 0.01$) this pH to 5.5–5.8 at 12 and 15 h after providing supplements, while provision of LIN supplement decreased ($P < 0.01$) this pH to 6.1 after both 3 and 6 h. Rumen pH was not affected by feeding SAF supplement. On Day 1 of the feeding cycle the proportion of acetate in rumen fluid was decreased ($P < 0.01$) from 728 mmol/M VFA in sheep fed hay alone to 691 and 703 mmol/M VFA in sheep fed Bar/N and LIN supplement, respectively (Table 5). Conversely, the proportion of propionate was increased by the Bar/N ($P < 0.01$) and LIN ($P < 0.05$) supplements. Butyrate proportion was not affected by the provision of supplements.

3.3. Synthetic fibre bag measurements

Degradability in the rumen of the SAF and LIN CP were predicted to be 0.72 and 0.62, respectively (Table 6). Disappearances of hay DM over 24 h on Day 1 were greater ($P < 0.05$) when the oilseed meal supplements than when the Bar/N supplement was fed. However, on Days 2 and 3 of the feeding cycle the supplements had no effect on the disappearance of hay DM from synthetic fibre bags. There was a tendency ($P = 0.07$) for 24 h hay DM disappearance in sheep fed SAF and LIN supplements to decrease from 543 g/kg on Day 1 to 494 g/kg on Day 3 of the feeding cycle.

3.4. Passage of digesta

The FOR of Co-EDTA, Cr-mordanted supplements and of Cr-mordanted hay averaged 0.109, 0.056 and 0.031 h⁻¹, respectively, and did not differ ($P > 0.05$) among diets (Table 6). On average the rumen volume measured from the dilution of Co-EDTA was 4.6 l. Outflow of liquid measured with Co-EDTA marker was on average 11.6 l/day, and tended ($P = 0.06$) to be greater for the SAF and LIN diets than for the Bar/N diet. FOR of Cr-mordanted supplement (FOR_{supp}/h) was related to the FOR of Co-EDTA (FOR_{liquid}/h) as

$$\text{FOR}_{\text{supp}} = 3.04 \text{ (S.E. 1.02)} + 0.229 \text{ (S.E. 0.0889)} \text{ FOR}_{\text{liquid}}$$

($n = 18$; $P < 0.05$; $r = 0.50$; R.S.D. = 0.836). In addition the FOR of Cr-mordanted hay (FOR_{hay}/h) was related to the FOR of Co-EDTA as

$$\text{FOR}_{\text{hay}} = 0.81 \text{ (S.E. 1.04)} + 0.201 \text{ (S.E. 0.0889)} \text{ FOR}_{\text{liquid}}$$

($n = 18$; $P < 0.05$; $r = 0.44$; R.S.D. = 0.847), and the FOR of Cr-mordanted supplements was related to the FOR of Cr-mordanted hay as

$$\text{FOR}_{\text{supp}} = 3.25 \text{ (S.E. 0.546)} + 0.769 \text{ (S.E. 0.169)} \text{ FOR}_{\text{hay}}$$

($n = 18$; $P < 0.001$; $r = 0.73$; R.S.D. = 0.658). FOR of Co-EDTA (FOR_{liquid}) was related to the total DM intake (DMI, g/day) as

$$\text{FOR}_{\text{liquid}} = -3.13 \text{ (S.E. 1.92)} + 0.0168 \text{ (S.E. 0.00216)} \text{ DMI}$$

($n = 18$; $P < 0.001$; $r = 0.88$; R.S.D. = 1.159). However, there was no relationship between the FOR of Cr-mordanted supplements or hay and the DMI.

Table 6

Disappearance of hay DM, and supplement DM and CP, from synthetic fibre bags incubated in the rumen, and ruminal measurements made with digesta markers in sheep offered four diets ($n = 6$)^a

	Treatment				S.E.M.	Significance
	Nil	Bar/N	SAF	LIN		
Supplement DM disappearance (g/kg)						
0 h	–	329	200	307	–	–
6 h	–	843 c	294 a	452 b	14	***
24 h	–	897 c	466 a	700 b	8	***
48 h	–	927 c	521 a	801 b	5	***
72 h	–	937 c	544 a	829 b	4	***
<i>a</i>	–	329 a	197 a	302 b	3	***
<i>b</i>	–	592 c	354 a	529 b	6	***
<i>c</i> (h^{-1})	–	0.342 b	0.058 a	0.060 a	0.009	***
Supplement CP disappearance (g/kg)						
0 h	–	–	290	380	–	–
6 h	–	–	631 b	436 a	29	***
24 h	–	–	826 b	762 a	17	*
48 h	–	–	908	910	6	ns ^b
72 h	–	–	921	932	4	ns
<i>a</i>	–	–	297 a	349 b	9	**
<i>b</i>	–	–	606	639	15	ns
<i>c</i> (h^{-1})	–	–	0.126 b	0.041 a	0.013	***
Degradability in rumen	–	–	0.72	0.62	–	–
Hay DM disappearance (g/kg)						
Day 1						
24 h	514 ab	491 a	541 b	544 b	14	*
48 h	674	677	690	677	7	ns
72 h	732	732	738	734	6	ns
<i>a</i>	107	106	108	108	1	ns
<i>b</i>	670	694	658	649	15	ns
<i>c</i> (h^{-1})	0.040	0.036	0.046	0.047	0.003	ns
Day 2						
24 h	505	508	500	530	15	ns
48 h	648	654	648	654	7	ns
Day 3						
24 h	497	509	494	494	13	ns
Marker measurements						
Co-EDTA						
FOR from rumen (h^{-1})	0.095	0.113	0.118	0.109	0.009	ns
Volume of rumen (l)	4.98	4.06	4.52	4.70	0.34	ns
Flow from rumen (l/day)	11.39	9.9	12.62	12.21	0.68	ns
Cr-supplements						
FOR from rumen (h^{-1})	–	0.058	0.055	0.056	0.004	ns
MRT of entire g.i.t. ^c (h)	–	37.3	34.1	34.5	1.1	ns
Cr-hay						
FOR from rumen (h^{-1})	0.033	0.032	0.028	0.033	0.004	ns
MRT of entire g.i.t. (h)	46.0	46.9	46.9	48.0	1.6	ns

^a Solubility of hay DM at 0 h was 108 g/kg.

^b Not significant.

^c Gastrointestinal tract.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 7
 Digesta present in various parts of the gastrointestinal tract at slaughter in sheep at 6, 24 or 72 h after feeding hay and supplements ($n = 4$)^{a,b}

Measurement	6 h after offering supplements				24 h after offering supplements				72 h after offering supplements				S.E.M.	Significance		
	Nil	Bar/N	SAF	LIN	Nil	Bar/N	SAF	LIN	Nil	Bar/N	SAF	LIN		D	T	D × T
Rumen wet digesta (g)	6361 bc	5983 abc	6229 bc	6842 c	–	4842 a	5516 ab	5200 ab	5039 ab	5133 ab	5531 abc	5087 ab	462	ns ^c	**	ns
Rumen DM content (g/kg)	110 ab	136 d	159 e	133 cd	–	112 ab	121 bcd	112 ab	114 abc	97 a	112 ab	101 ab	6.9	**	***	*
Rumen DM pool (g)	695 e	817 f	975 g	914 g	–	550 ab	664 de	583 bc	579 bc	496 a	621 cd	518 ab	71	ns	**	ns
Rumen OM pool (g)	634 d	759 e	901 f	839 f	–	505 abc	610 d	529 bc	430 a	448 a	566 cd	467 ab	76	*	**	ns
Rumen N pool (g)	16.7 ab	20.7 b	29.3 c	29.9 c	–	15.2 a	15.9 ab	15.8 ab	15.0 a	12.4 a	14.8 a	13.8 a	1.9	**	**	**
Rumen NDF pool (g)	543 ab	519 ab	524 ab	649 b	–	403 a	447 ab	428 ab	438 ab	358 a	458 ab	376 a	81	ns	*	ns
Rumen ADF pool (g)	265 bc	278 c	383 d	296 c	–	181 a	277 c	223 abc	197 ab	184 a	241 abc	189 a	26	**	***	ns
Post-rumen wet digesta (g)	2081 cd	1522 a	1746 abc	1863 abcd	–	2056 cd	1888 abcd	2234 d	1975 bcd	1770 abc	1660 ab	1942 bcd	135	**	**	ns
Rumen wet digesta (g/LW ^{0.75})	547 bcde	563 de	550 cde	603 e	–	411 a	493 abcd	436 a	456 abc	446 a	502 abcd	447 ab	35	ns	***	ns
Rumen DM pool (g/LW ^{0.75})	59.9 b	76.9 c	86.2 c	80.4 c	–	46.5 ab	59.4 ab	48.9 ab	52.1 ab	43.2 a	56.4 ab	45.5 ab	5.6	*	***	ns
Rumen OM pool (g/LW ^{0.75})	54.6 ab	71.5 bc	79.6 c	73.9 c	–	42.6 a	54.6 ab	44.3 a	36.9 a	39.0 a	51.4 a	41.0 a	6.3	**	***	ns
Rumen N pool (g/LW ^{0.75})	1.44 a	1.95 b	2.60 c	2.63 c	–	1.30 a	1.43 a	1.33 a	1.36 a	1.08 a	1.35 a	1.21 a	0.15	***	***	**
Rumen NDF pool (g/LW ^{0.75})	46.7 ab	48.9 ab	46.8 ab	57.1 b	–	34.1 a	39.9 ab	35.9 a	39.5 ab	31.1 a	41.6 ab	33.0 a	6.8	ns	*	ns
Rumen ADF pool (g/LW ^{0.75})	22.8 cde	26.3 e	33.9 f	26.1 e	–	15.3 a	24.6 de	18.7 abcd	17.7 abc	16.0 ab	21.9 bcde	16.6 ab	2.1	***	***	ns
Post-rumen wet digesta (g/LW ^{0.75})	178 bc	144 a	154 ab	165 abc	–	175 abc	169 abc	188 c	178 bc	154 ab	151 ab	170 abc	11.2	*	*	ns

^a No measurements were made for the sheep fed no supplement (Nil) at 24 h since for this treatment amounts and composition of digesta were expected to be the same at 24 and 72 h.

^b D: diet; T: time; D × T: diet × time interaction.

^c Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

3.5. Measurements at slaughter

The amount of wet digesta present in the rumen, expressed per kg LW^{0.75}, was greater ($P < 0.001$) at 6 h than at 24 or 72 h after providing the supplements but was not affected by the diet (Table 7). With each of the supplemented diets, the DM content of rumen digesta at 6 h (mean 142 g/kg) was higher ($P < 0.05$) than at 24 or 72 h (mean 109 g/kg), while DM content with the SAF diet was higher ($P < 0.05$) than with the Bar/N or LIN diets. Thus the DM pool in the reticulo-rumen was 54% greater ($P < 0.05$) at 6 h than at 24 or 72 h after providing supplements. Also, pools of OM, total N, NDF and ADF in the reticulo-rumen were greater ($P < 0.05$ or $P < 0.001$). Diet affected the size of the pools of DM, OM, N and ADF in the rumen, the largest pools generally being when the LIN or SAF diets were fed. Weights of wet tissue comprising the parts of the gastrointestinal tract were not affected by diet. The reticulo-rumen tissues weighed 68, 72, 67 and 69 g/kg LW^{0.75} (5% LSD 5.1), while the remainder of the gastrointestinal tract weighed 111, 119, 116 and 113 g/kg LW^{0.75} (5% LSD 10.3) in sheep fed the Nil, Bar/N, SAF and LIN diets, respectively.

3.6. Predictions of nutrient supply and sheep growth

Calculations based on AFRC (1993) of the estimated supply of ERDP indicated that the Nil sheep ingested about the amount of ERDP expected to be required to support fermentation of the diet (Table 8). The predicted MP:ME ratio for the Nil diet of 8.4 g/MJ ME was reduced by about 6% with the Bar/N supplement. However, the MP:ME ratio was predicted to be 18 and 29% higher for the SAF and LIN diets, respectively, than for the Bar/N diet. Predictions of the ADPLS:ME ratio (SCA, 1990) changed similarly between

Table 8

Intakes of CP and predictions from AFRC (1993) or SCA (1990) of the supply of ERDP, MP or apparently digested protein leaving the stomach (ADPLS), the ratios of these to metabolizable energy (ME) intake and of expected LW gain predicted from estimates of ME intake

Parameter	Treatment			
	Nil	Bar/N	SAF	LIN
Intake				
CP (g/day)	86	118	126	121
AFRC (1993) predictions				
ERDP in diet/ERDP required	1.00	1.44	1.44	1.26
MP (g/day)	51	51	61	68
MP (supply/required)	1.2	1.1	1.1	1.2
Ratio (MP:ME) (g/MJ)	8.4	7.9	9.3	10.2
Expected LW gain (g/day)	7	20	17	20
SCA (1990) predictions				
ADPLS (g/day)	40	40	47	52
ADPLS (supply/required)	1.1	1.0	1.0	1.1
Ratio (ADPLS:ME) (g/MJ)	6.6	6.2	7.2	7.8
Expected LW gain (g/day)	2	12	10	12

diets. Predictions of LW change were similar to the observed values for the Nil and Bar/N diets, but for both oilseed meal diets the actual LW changes was substantially higher than those predicted by SCA (1990) and AFRC (1993).

4. Discussion

4.1. *Effects of diet on ME intake and rumen digestion*

The measurements of disappearance of CP from synthetic fibre bags and the FOR of Cr-mordanted supplement indicated that both the SAF and LIN supplements were extensively degraded in the rumen. The estimate for LIN of 0.62 was similar to previous reports of 0.60–0.65 using similar techniques (Hosking et al., 1987; AFRC, 1993; Khorasani et al., 1994), while the estimate for SAF of 0.72 was similar to that reported by Hosking et al. (1987). The specific gravity of the particulate matter of these oilseed meals was in the optimal range for rapid escape from the rumen (Kennedy and Murphy, 1988). The observation that 33 and 25% of the CP of the SAF and LIN, respectively, was ingested in particle sizes >1 mm screen, which would not be expected to pass readily from the rumen (Dixon and Mora, 1983; Faichney, 1986), suggests that these measurements may have underestimated the actual rumen degradability. Nevertheless, the greater wool growth by sheep fed the oilseed meal diets than those fed the Bar/N diets indicates that at least some of the oilseed meal proteins escaped rumen digestion and were digested in the small intestine (Reis, 1979).

The volumes of rumen fluid measured by dilution of Co-EDTA were almost identical to the volumes of water measured at slaughter 72 h after provision of the supplements, giving confidence that the markers gave reliable estimates of digesta movement despite the non-steady state conditions associated with feeding the supplements at 3-day intervals. The measured FOR of Cr-mordanted supplements of 0.056 h^{-1} was higher than values previously reported for sheep on maintenance and sub-maintenance levels of feeding; for example AFRC (1993) suggests a typical FOR of 0.02 h under these circumstances. However, the intakes of DM on Day 1 of the feeding cycle when the markers were dosed ranged up to $83.5 \text{ g DM/kg LW}^{0.75}$ and were much higher than would usually occur for diets of this nature. Since FOR is generally positively related to DM intake (Eliman and Orskov, 1984; AFRC, 1993), these high intakes on Day 1 of the supplementation cycle provide a likely explanation for the high estimates of FOR for all of the markers in the present study. A much higher FOR for the liquid marker than for the Cr-mordanted supplement, and of the Cr-mordanted supplement than of the Cr-mordanted hay, is in agreement with the differential passage from the rumen of liquids and particles of various sizes (Dixon and Milligan, 1985; Kennedy and Murphy, 1988).

The measurements of rumen digesta loads at slaughter indicated that the increased intakes of DM on Day 1 of the supplementation cycle were accommodated by the sheep by an increased rumen digesta load on this day. This provides an explanation for the similar FOR of hay from the rumen for each of the diets despite the differences in total DM intake. There is considerable evidence that sheep do have the capacity to substantially increase the rumen digesta load following meals during the daily cycle, even when rumen digesta load

remains constant for a specific animal and dietary circumstance (Aitchison et al., 1986; Forbes, 1995).

The large increases in voluntary intake of roughage, estimated ME intake and LW status in response to provision of supplement in the present study are consistent with numerous reports where low quality forages have been supplemented with high N concentrates, including oilseed meals (Abidin and Kempton, 1981; Kellaway and Leibholz, 1983; Egan et al., 1987; Dixon and Egan, 2000). Although Bar/N caused a larger decrease in forage intake, because OM digestibility was higher with the Bar/N than the oilseed meal supplemented diets the overall effect of the three supplements on total ME intake were similar.

Since the supplements were provided only each third day the sheep were not in the steady state conditions of digesta flow through the gastrointestinal tract that is assumed for calculations of rumen degradability of protein meals described by Huntington and Givens (1995), and used in the present study. Since the MRT calculated by numerical integration, which provided an estimate of the retention time in the entire gastrointestinal tract, does not depend on an assumption of steady state it may be considered a more reliable estimate of passage of supplements from the rumen of sheep fed supplements at 3-day intervals. However, the time for movement of digesta from the omasum to the rectum is included in this MRT. In the present experiment the Cr-labelled supplement did not appear in faeces until at least 8 h, and the Cr-labelled hay until at least 16 h, after administration of the marker into the rumen. Thus the 11–20 h greater MRT of the Cr-mordanted supplements or hay than the respective reciprocals of the FOR (which provided an estimate of retention time in the rumen pool) was expected and provides evidence that the estimates of rumen degradability of the protein meals were reliable despite the non-steady state conditions.

The adverse effects of the starch in Bar/N supplement to reduce rumen pH and fibre digestion were small; rumen pH was depressed to the critical range of pH 6.0–6.2 for only a small proportion of the 3-day feeding cycle even though the Bar/N comprised 42% of the total intake on Day 1 of the cycle. This contrasts with prolonged intervals of depression in rumen pH in other experiments when comparable amounts of cereal grain or lupin grain supplements have been fed each 3 days (Egan et al., 1987) or 2 days (Chase and Hibberd, 1989). The small effect in the present studies was probably due to the slow ingestion of the Bar/N supplement (only 72% was ingested during the 4 h after being provided) and because the barlev was fed in the whole grain form which would have reduced the rate of fermentation of the grain in the rumen (Orskov and Fraser, 1975; Dixon and Stockdale, 1999). Possibly the slow consumption of Bar/N was associated with an adverse flavour of, or a conditioned feed aversion (Provenza, 1985) to, the urea and sodium sulphate solution mixed with the grain. The lower intake of forage, particularly on Day 1 of the feeding cycle, with the Bar/N than the SAF or LIN supplements may have been associated with reduction in the rate of rumen fibre digestion by the Bar/N supplement even in the absence of a severe depression in rumen pH (Dixon and Stockdale, 1999); the 24 h hay disappearance from synthetic fibre bags tended to be slightly lower for the Bar/N diet. Alternatively when the Bar/N supplement was fed, voluntary intake of the hay may have been limited by metabolic constraints associated with the balance of absorbed nutrients, despite the low ME intake. There was evidence that metabolic constraints limited the voluntary intake of barley straw leaf in the experiment of Rafiq et al. (2002). LIN supplement also reduced rumen pH, but

not as much as the Bar/N supplement and not to the critical range of pH < 6.1 where fibre digestion is severely reduced. The lower digestion across the entire gastrointestinal tract of OM and fibre components when the SAF supplement was fed was presumably due to its high fibre content and the indigestible nature of this fibre.

The 3-day cycle of provision of supplements led to large fluctuations in rumen ammonia concentration. High concentrations of rumen ammonia followed by rapid disappearance by absorption following ingestion of cereal grain and urea mixtures have been reported previously (Bartley and Deyoe, 1977; Fishwick et al., 1978), while grain legume and protein meal supplements appear to increase rumen ammonia concentrations for longer intervals of 24–48 h (Egan et al., 1987). In sheep fed hay alone the rumen ammonia concentrations averaged 94 mg/l and thus the availability of ammonia as a substrate probably at least approached the needs of the microbes (Dixon, 1987). This is consistent with the prediction from AFRC (1993) calculations that, with this diet, the ERDP supply was sufficient to balance the fermentable dietary components available. The tendency for the SAF and LIN supplements to increase digestion of hay in the rumen compared to the unsupplemented diet was likely due either to the additional supply of peptides and amino acids or the higher ammonia concentration as microbial substrates (Dixon, 1987; McAllan, 1991; Morrison et al., 1988; Stritzler et al., 1992).

4.2. Utilization of ME for LW gain

Since the sheep fed hay alone were at maintenance and the supplements led to LW gain, it appeared that the ME obtained from the oilseed meal diets was used with greater efficiency than that from the Bar/N diet. Similarly, some experiments with sheep (Hassan and Bryant, 1986; Dixon and Egan, 2000; Rafiq et al., 2002) or cattle (Ortigue et al., 1989, 1990; Hennessy and Williamson, 1990) fed diets based on low quality roughage have indicated greater efficiency of utilization of ingested ME for LW gain with supplements high in RUP and which would thus be expected to increase the MP:ME ratio. An increase in efficiency of utilization of ME for growth and LW gain with increased MP:ME is consistent with the hypothesis that this efficiency depends on the balance of absorbed nutrients (MacRae and Loble, 1986; Black et al., 1987a,b). Nevertheless in numerous experiments the increased ME intake from a range of supplements, including those low and high in RUP, appears to have been used with similar efficiency for LW gain (Kempton and Leng, 1979; Abidin and Kempton, 1981; van Houtert and Leng, 1993; Dixon et al., 1999). Thus supplements providing RUP for low quality forage diets have not always been reported to increase the efficiency of utilization of ME for LW gain.

5. Conclusions

The present study supports the hypothesis that, in growing animals, protein meal supplements may give greater LW gain responses than supplements providing fermentable carbohydrate and inorganic N. In addition, protein meals often have other advantages such as reduced risk of urea toxicity or of acidosis. These advantages may, in some circumstances, justify the generally higher cost of protein meals rather than alternatives such as

grain legumes or fermentable carbohydrate and urea in supplements for ruminants fed low quality forage.

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