Effects of infused methionine, lysine and rumen-protected methionine derivatives on nitrogen retention and wool growth of Merino wethers

J. Coetzee*, P.J. de Wet and W.J. Burger

Division of Sheep and Wool Science, Department of Animal Sciences, University of Stellenbosch, Stellenbosch, 7600 Republic of South Africa

Nitrogen (N) retention and wool growth rate (WGR) responses to various amino acids and derivatives as well as protein sources were determined with six ruminally and six abomasally fistulated Merino wethers in two balanced cross-over design experiments. The six treatments of the first trial consisted of a control (RC), ruminally infused: methionine (RM), maleyl methionine (RMM) and 2-methyl-maleyl methionine (RMMM), as well as other essential amino acids supplied by a low (RFM: fish meal) and high degradable dietary protein source (RSOM: sunflower-oilcake meal), respectively. The latter two treatments (AFM and ASOM, respectively) and a control (AC) were included in the second trial as well as abomasally infusion of methionine (AM) and/or lysine (AL or AML). Six roughage-based diets were compiled to be iso-nutritious and complementary to the different treatments and were fed at a maintenance level. Live weight was not affected ($P > 0.10$) by any treatment whereas the apparent DM digestibility was enhanced ($P < 0.05$) with the inclusion of fish meal in the diets (RFM: 56.7%; AFM: 57.5%). The percentage N retained (15.6 and 35.8% vs. 26.1%) decreased ($P = 0.03$) by ruminally infused methionine (RM) and increased ($P = 0.04$) by inclusion of fish meal (RFM). Abomasally infusion of methionine (AM) resulted in an increased ($P = 0.02$) percentage N retained (39.0%) but the infusion of lysine (AL) or both (AML) gave no response (26.5 and 36.6% vs. 30.1%). WGR was enhanced by 0.21 ± 0.05 mg/cm^2/day ($P = 0.0002$) and 0.27 ± 0.05 mg/cm^2/day ($P = 0.0001$) by ruminally infusion of derivatives (RMM and RMMM) and the inclusion of natural protein sources (RSOM and RFM), respectively. Abomasally infusion of methionine (AM) and a mixture of amino acids (AML) as well as diets containing natural protein sources (ASOM and AFM) increased ($P < 0.0004$) WGR with 31, 35, 19 and 38%, respectively, whereas lysine infusion (AL) had no effect. MMM (2-methyl-maleyl methionine) and fish meal show great potential as a source of bypass methionine, especially to increase wool growth (30% and 36–38%), whereas MM (maleyl methionine) (12%) and sunflower-oilcake meal (17–19%) appears to be about half as efficient.

Stikstof (N)-retensie en wolgroeitempo (WGT)-respons tot verskeie aminosure en -derivate asook proteïenbronne is met ses ruminale en ses abomasale gefistuleerde Merinohamels in twee gebalanseerde oorskakelproefontwerp-eksperimente bepaal. Die ses behandelings van die eerste eksperiment het bestaan uit 'n kontrole (RC), ruminaal ingedrupte: metionien (RM), maleïelmetionien (RMM) en 2-metielmaleïelmetionien (RMMM) sowel as ander essensiële aminosure wat onderskeidelik deur 'n laags (RFM: vismeel) en hoogs degradeerbare dieetproteïenbron (RSOM: sonneblomoliekoekmeel) voorsien is. Laasgenoemde twee behandelings (AFM en ASOM, onderskeidelik) en 'n kontrole (AC) is in die tweede eksperiment ingesluit asook abomasale indruppeling van metionien (AM) en/of lisien (AL of AML). Ses ruvoergebaseerde diëte is met inagneming van die verskillende behandelings op 'n iso-voedingstofbasis saamgestel en teen onderhoudspeil gevoer. Liggaamsmassa is nie deur enige behandeling beïnvloed nie ($P > 0.10$) terwyl die skynbare verteerbaarheid van die DM deur die insluiting van vismeel (RFM: 56.7%; AFM: 57.5 %) in die diëte verhoog ($P < 0.05$) is. N-retensie, as persentasie van N ingeneem (15.6 en 35.8% vs. 26.1%), is verlaag ($P = 0.03$) deur ruminaal ingedrupte metionien (RM) en verhoog ($P = 0.04$) deur die insluiting van vismeel. Abomasale indruppeling van metionien (AM) het N-retensie, as persentasie van N ingeneem (39.0%), verhoog ($P = 0.02$), maar die indruppeling van lisien (AL) of albei aminosure (AML) het geen respons (26.5 en 36.6% vs. 30.1%) gehad nie. WGT is met onderskeidelik 0.21 ± 0.05 mg/cm^2/dag ($P = 0.0002$) en 0.27 ± 0.05 mg/cm^2/dag ($P = 0.0001$) deur ruminale indruppeling van die derivate (RMM en RMMM) en die insluiting van natuurlike proteïenbronne (RSOM en RFM) verhoog. Abomasale indruppeling van metionien (AM) en 'n mengsel van aminosure (AML) asook diëte wat natuurlike proteïenbronne (ASOM en AFM) bevat, het WGT met onderskeidelik 31, 35, 19 en 38% verhoog ($P < 0.0004$) terwyl lisien indruppeling (AL) geen invloed gehad het nie. MMM (2-metiel-maleïelmetionien) en vismeel toon groot potensiaal as bron van deurvloeimetionien, veral om wolgroei te verhoog (30% en 36–38%), terwyl maleïelmetionien (MM) (12%) en sonneblomoliekoekmeel (17–19%) ongeveer die helfte so doeltreffend was.

* To whom correspondence should be addressed

Introduction

Amino acid supply at the duodenum may limit production in sheep. Increases in body growth (Storm & Ørskov, 1984), nitrogen retention (Loerch & Oke, 1984) and wool growth (Reis, 1979) have been observed following postruminal infusions of methionine or mixtures of amino acids. To increase the supply of methionine, regarded as the first limiting amino acid for sheep (Reis, 1979), at the duodenum it should be protected against microbial fermentation in the rumen (Ferguson, 1975) so that methionine in a biologically available form is released postruminally.

The method of blocking or protecting the amino groups of amino acids with maleic anhydride holds promising possibilities for supplying essential amino acids at the abomasum and the duodenum of animals receiving low protein diets and to enhancing the use of non-protein nitrogen (De Wet, 1975). Increased animal performance, especially wool growth, has been demonstrated with dietary supplementation of maleyl

methionine (MM) (De Wet, 1982), but, as far as could be established from the literature, 2-methyl-maleyl methionine (MMM) has not been tested as a methionine source for ruminants.

The objectives of this study were to determine indirectly the extent to which MMM is protected against microbial or chemical degradation in the rumen and the effectiveness in delivering methionine postruminally. This was monitored as N retention and wool growth rate (WGR). To evaluate the potential of this derivative, it was infused in the rumen and compared with a control (no infusion), ruminally infused methionine and MM, abomasally infusions of unprotected amino acids (methionine and/or lysine, the first- and second-limiting amino acids in microbial protein: Storm & Ørskov, 1984) as well as with other essential amino acids supplied by a low (fish meal) and high ruminally degradable dietary protein source (sunflower-oilcake meal).

Materials and methods

Animals

Sixteen six-year-old medium-woolled Merino wethers, approximately 62 kg in weight, were allocated at random to two groups of eight sheep each. Eight sheep were fitted with a permanent ruminal and the remainder with abomasal cannulae (Van Niekerk & Belonje, 1968). Animals were allowed a recovery period of six weeks after surgery before being used in experiments during which they were dosed against internal parasites and injected with a vitamin ADE preparation. The sheep were housed individually indoors in pens fitted with a slatted wooden floor. Water was freely available to animals throughout the experiment.

Diets

Six experimental diets (Table 1) in which urea was partly or completely substituted by infused amino acids (methionine and/or lysine), maize meal, sunflower-oilcake meal or fish meal were formulated (as-fed basis) to be approximately iso-nutritious. Diets 5 and 6, containing natural protein sources, were included in the experiment to compare the responses of

Table 1 Composition of experimental diets on an air-dry basis

	Diet					
Ingredients (%)	1	2	3	4	5	6
Oat straw	55.0	55.0	55.0	55.0	55.0	55.0
Lucerne hay	20.0	20.0	20.0	20.0	20.0	20.0
Maize meal	16.84	16.87	16.98	17.02	5.26	11.43
Sunflower-oilcake meal	–	–	–	–	13.22	–
Fish meal	–	–	–	–	–	7.95
Urea	1.5	1.47	1.36	1.32	–	–
Molasses meal	5.0	5.0	5.0	5.0	5.0	5.0
Limestone	1.16	1.16	1.16	1.16	1.02	0.12
Ammonium chloride	0.5	0.5	0.5	0.5	0.5	0.5

the animals with those receiving urea-containing diets (Diet 1–4) supplemented with amino acids. The sheep were gradually adapted, during the pretreatment period, to these pelleted diets and were fed daily at 06:00 on a maintenance feeding level (60 g $DM/W^{0.75}$). The chemical composition of the experimental diets is presented in Table 2.

Infusions

Stock infusion solutions were prepared and stored in a refrigerator to prevent microbial growth. Daily allowances were removed prior to use and allowed to reach room temperature. Preparation of MMM and MM stock solutions (0.15 g methionine/ml) took place according to the method of Dixon & Perham (1968) and De Wet (1975), respectively. The unprotected methionine solution (M) contained all the chemicals as the protected forms (MM and MMM) except the blocking agents (maleic and 2-methyl-maleic anhydride). In the blank solutions methionine was also excluded. For the abomasal infusion, methionine (0.15 g/ml) and lysine (0.3 g/ml) were dissolved separately or together in 5 N HCl. A hydrochloric acid solution was used to assure that the infusion solution had approximately abomasal pH. Aqueous solutions were prepared daily from the stock solutions and infused into the rumen (1 000 ml) or abomasum (500 ml). Methionine and lysine were infused at a daily rate of 2.46 and 5 g per sheep, respectively. The infusates were administered by means of a peristaltic infusion pump, over an 8 h period, which was connected with a poly-ethylene pipe to the cannula. In the case of a rumen cannula, the pipe was inserted 20 cm into the rumen to assure thorough mixing with the digesta. The infusion commenced daily immediately after the sheep were fed and fastened. Thereafter the pipes were disconnected and the sheep could move freely.

Treatments and design

From the 16 animals, six best adapted ruminal and six best adapted abomasal fistulated sheep were used in a balanced cross-over design (no. 14) as described by Patterson & Lucas (1962). More than one replicate is needed if carry-over effects are to be estimated. In our experiments only one replicate was used, but a period of adaptation of two weeks was allowed between the administration of the six treatments, in the hope of eliminating carry-over effects. Two experiments (ruminal and abomasal infusion) were conducted to evaluate the six treatments (Table 3). The design made provision for six periods (consisting of a 14-day adaptation and a 21-day treatment

Table 2 Chemical composition of experimental diets (DM basis except DM)

Nutrients		Diet					
		1	2	3	4	5	6
DM	(%)	89.0	89.1	88.9	88.6	88.5	89.1
OM	(%)	94.5	94.6	93.6	93.3	94.4	95.0
CP	(%)	12.5	12.9	11.6	12.3	12.6	12.7
ME*	(MJ/kg)	7.2	7.2	7.1	7.0	7.0	7.4

* ME = 12 + (0.008 CP) + (0.023 EE) – (0.018 CF) – (0.012 ASH) (MAFF, 1984)

Table 3 Experimental treatments

Ruminal infusion experiment			Abomasal infusion experiment		
Treatment	Diet	Infusate	Treatment	Diet	Infusate
RC	1	Blank (Control)	AC	1	Blank (Control)
RM	2	Methionine	AM	2	Methionine
RMM	2	Maleyl methionine	AL	3	Lysine
RMMM	2	2-methyl-maleyl methionine	AML	4	Methionine & lysine
RSOM	5	Blank	ASOM	5	Blank
RFM	6	Blank	AFM	6	Blank

period), six treatments and six animals. Each of the six sheep in each of the two experiments received each of six treatments (consisting of a specific diet and an infusate) according to a specific order, one after the other.

Sampling and measurements

Feed intakes were measured daily; live mass (24 h fasting mass) was recorded on two occasions (beginning and end) during each period and the average mass was taken as the average mass of the period.

N balance was measured for all the sheep over a 7-day period commencing seven days before the end of each 35-day experimental period. Before the commencement of the N balance, the diets were weighed simultaneously into paper bags for the duration of the N balance; samples were taken at random and analysed. Each sheep was harnessed and fitted with a faeces collection bag and urine funnel. The faeces collection bags were emptied twice daily, weighed and two representative samples, each comprising one tenth of the total, were retained. One was dried at 60 °C in a forced-air oven and kept for dry-matter (DM) determinations while the other was preserved as a wet sample for determination of N. The urine was collected in plastic bottles containing 100 ml diluted hydrochloric acid as a preservative. The collected urine was weighed, sampled (10% of total weight) and stored at 4 °C until further analysis. The representative feed refusals, faeces and urine samples of the 7-day collection period of each animal were pooled for further analysis.

WGR (mg clean dry wool/cm^2/day) was measured by closely clipping a tattooed defined area, approximately 100 cm^2 on the midside of each sheep, with a small animal clipper (Oster, size 40) at the beginning and end of treatment periods. Each time the size of the defined area was measured accurately and a mean for the duration of the experiment was calculated for each sheep.

Analytical methods

All chemical analyses were done on a DM basis, in duplicate. Feed and faeces samples were analysed for DM, N, ash, ether extract (EE), crude fiber (CF), neutral (NDF) and acid detergent fibre (ADF) and the urine samples for N content according to standard AOAC (1984) procedures. Apparent digestibility coefficients were subsequently calculated for DM, OM, CP, EE, CF, NDF and ADF. N retention results were also obtained.

The wool was ether-extracted for four hours, alcohol extracted for three hours, rinsed in five changes of distilled water with one-hour intervals and dried at 60 °C for 24 hours. After removal of all foreign organic matter, it was dried to constant weight at 80 °C. The clean dry weight of wool was used to calculate wool growth rates.

Statistical analysis

The analysis of one replicate of design 14 in Patterson & Lucas (1962) corresponds to the analysis of a Latin square design. The data of the experiments were analysed separately, using PROC GLM (SAS, 1989).

Results and discussion

Live weight change

The live weight change did not differ ($P > 0.10$) between treatments in either experiments (Tables 4 & 5); DMI (56 g DM/W$^{0.75}$) was close to the maintenance feeding level and only a small number of animals was used over a very short experimental period. The mean gain during the ruminal infusion experiment (Table 4) matches that reported by Stephenson et al. (1990) of 7 g/day with an average DM intake of 55 g/W$^{0.75}$ and performance was not changed when sheep were fed 50 g DM/W$^{0.75}$ and supplemented with protected methionine products (Alimet and MHA). Live weight tended ($P = 0.09$) to be decreased (−38.9 g/day) only when lysine alone was infused abomasally (Table 5) but tended to ($P = 0.08$) increased (40.5 g/day) when both methionine and lysine were infused simultaneously, suggesting that methionine was deficient and lysine was the second limiting amino acid.

Dry-matter intake and apparent digestibility

Although the diets were offered at maintenance feeding level (60 g DM/W$^{0.75}$), only one group (AML) consumed all their feed while the other groups had different refusal rates indicating a treatment effect on feed intake. Dry-matter intake (DMI) was not influenced ($P > 0.10$) by ruminal infusion of methionine (55 g DM/W$^{0.75}$) and maleyl derivatives (56 g DM/W$^{0.75}$) (Table 4). This matches the conclusion of Kelly & Thomas (1975) that methionine alone had a limited influence on feed intake. However, DMI increased with 131 ± 37 g/day

Table 4 LS means for initial weight and live weight change, dry-matter intake and apparent dry-matter (DM) digestibility of sheep receiving different ruminal infusions

Parameter	Treatment*						
	RC	RM	RMM	RMMM	RSOM	RFM	SE$_m$
Initial weight (kg)	62.5	62.0	63.0	63.8	63.1	62.9	0.62
Live weight change (g/day)	5.6	7.1	15.1	15.9	19.0	23.8	10.86
Dry-matter intake (g/day)	1 217	1 220	1 251	1 261	1 249	1 243	23.13
Apparent DM digestibility (%)	52.4[a]	53.4[a]	55.3[a]	55.6[a]	54.8[a]	56.7[b]	1.06

*   Refer to Table 3 for treatment codes
SE$_m$ Standard error of the mean
[a,b] Means within the same row with different superscripts differ significantly ($P < 0.05$)

Table 5 LS means for initial weight and live weight change, dry-matter intake and apparent dry-matter (DM) digestibility of sheep receiving different abomasal infusions

Parameter	AC	AM	AL	AML	ASOM	AFM	SE_m
	Treatment*						
Initial weight (kg)	60.9	62.3	62.0	62.0	61.8	62.6	0.42
Live weight change (g/day)	4.0	4.8	-38.9	40.5	-35.7	22.2	21.70
Dry matter intake (g/day)	1 080[a]	1 123[a]	1 133[a]	1 376[b]	1 199[a]	1 163[a]	32.08
Apparent DM digestibility (%)	53.4[a]	53.2[a]	52.6[a]	54.2[a]	55.6[a]	57.5[b]	1.31

* Refer to Table 3 for treatment codes
[a,b] Means within the same row with different superscripts differ significantly ($P < 0.05$)

($P = 0.002$) and 101 ± 39 g/day ($P = 0.02$) when amino acids were infused abomasally (AL, AM and AML) and when natural protein sources (AFM and ASOM) were fed, respectively (Table 5). The latter may alter the flow rate of the digesta and therefore the net effect is one of increasing forage intake (Siebert & Hunter, 1982).

Simultaneous infusion of methionine and lysine abomasally resulted in the largest increase ($P = 0.0001$) in DMI (27%), which matches the results of Schelling & Hatfield (1968) who found that intake increased when other essential amino acids were supplied abomasally with methionine. This phenomenon suggests that lysine was limiting only when methionine was infused and thus creating an amino acid imbalance. One biochemical change that occurs rapidly in animals fed an imbalanced diet is a change in their amino acid patterns (Young & Fukagawa, 1988), whereas the most consistent physiological change is decreased feed intake (Qi et al., 1994). Infusing the combination of amino acid (AML) corrected the imbalance and thus increased feed intake.

No significant differences in the apparent digestibility of nutrient fractions (DM, OM, CP, EE, CF, NDF and ADF — only DM presented in Tables 4 & 5) were observed with ruminally or abomasally infused amino acids (protected or unprotected). This corresponds with the findings of Cottle (1988a), who supplemented sheep with one of two methionine derivatives (Mepron and Ketionin), as well as with the results of Oke et al. (1986) who supplemented diets with rumen-protected methionine and lysine. According to Bull & Vandersall (1973) sulphur-containing amino acids can have a positive influence on ruminal metabolism since supplementation of urea-containing diets with methionine increases nutrient digestion rate because of an improved ruminal fermentation rate (Clark & Petersen, 1988). Presumably, the level of methionine supplementation (2.46 g/day) in our experiment was insufficient. Doyle & Bird (1975) reported a linear increase in DM digestibility of a roughage-based diet containing 0.12% sulphur (S), with a N and S ratio of 13:1, as the level of methionine supplementation increased with a significant difference ($P < 0.05$) only at a daily supplementation of 15.4 g. Most research, demonstrating enchanced fermentation with methionine or S supplementation, has been with diets devoid of sulphur or with severely restricted methionine content (Judkins et al., 1994). Our study did not have such

severe conditions since the S content of the diets (0.15–0.20%) and the N:S ratio (10–13:1) were within the limits recommended by the NRC (1985).

DM digestibility in both our experiments was enhanced ($P < 0.05$) with the inclusion of fish meal in the diets (RFM: 56.7% and AFM: 57.5%) and might have been due to its excellent amino acid composition which has a favourable influence on rumen metabolism (Whitelaw et al., 1963), which may also be an indication of a shortage of other essential amino acids in the diets where fish meal was excluded.

Nitrogen (N) balance and retention

N retention differed ($P < 0.05$) between some treatments in both experiments (Tables 6 & 7), mainly owing to differences ($P < 0.05$) in urinary excretion of N although some differences in N intake ($P < 0.05$) also may have contributed, especially in the case of the abomasal infusions. Faecal nitrogen excretion was relatively constant and did not vary ($P > 0.05$) with treatment in either experiment. This agrees with the findings of Schelling et al. (1973) that abomasal infusion of amino acids influenced urinary N excretion and not faecal N excretion.

Ruminally infused methionine (RM) decreased ($P = 0.03$) and maleyl methionine (RMM) tended ($P = 0.10$) to decrease the percentage N retained (15.6 and 19.4 vs. 26.1%). This may be attributed to the degradation of methionine in the rumen and the unavailability of the methionine in the maleyl derivative. Bonifacino (1979) also found no improvement in N retention with dietary supplementation of M and MM. Although RMMM tended ($P = 0.07$) to perform better than the other derivative (RMM) in terms of percentage N retained (27.9 vs. 19.4%), it did not increase N retention to the same extent as abomasally infused methionine (39.0%) or the fish meal containing diets (RFM: 35.8%; AFM: 32.9%). A signif-

Table 6 LS means for nitrogen (N) balance and retention of sheep receiving different ruminal infusions

Parameter	RC	RM	RMM	RMMM	RSOM	RFM	SE_m
	Treatment*						
N balance							
N intake (g/day)	24.5	25.5	25.6	27.1	25.1	25.5	0.59
N excretion							
Faeces (g/day)	8.3	8.9	8.2	8.6	7.8	7.6	0.33
Urine							
g/day	9.9[a,b]	12.5[c]	12.5[c]	10.9[b,c]	10.5[a,b,c]	8.7[a]	0.68
g/100 g N[1]$_i$ (%)	39.9[a]	49.3[c]	48.6[c]	40.3[a,b]	42.0[b,c]	34.4[a]	2.63
N retention**							
g/day	6.4[a,b,c]	4.1[b]	5.0[a,b]	7.6[c,d]	6.8[a,c,d]	9.3[d]	0.88
g/100 g Ni (%)	26.1[a]	15.6[b]	19.4[a,b]	27.9[a,c]	26.8[a,c]	35.8[c]	3.11

* Refer to Table 3 for treatment codes
** [N intake – (faecal N + urinary N)]
1 Nitrogen intake
[a,b,c,d] Means within the same row with different superscripts differ significantly ($P < 0.05$)

Table 7 LS means for nitrogen (N) balance and retention of sheep receiving different abomasal infusions

Parameter	AC	AM	AL	AML	ASOM	AFM	SEm
N balance							
N intake (g/day)	22.3[a]	23.7[a]	24.1[a]	27.7[b]	25.2[a,b]	24.1[a]	0.81
N excretion							
Faeces (g/day)	7.5	8.4	7.6	8.4	7.7	8.7	0.37
Urine							
g/day	8.0[a,b]	5.9[d]	10.1[c]	9.2[b,c]	10.4[c]	7.5[a]	0.49
g/100 g N^1_i (%)	35.9[a]	25.2[b]	42.0[c]	33.0[a]	41.8[c]	31.1[a]	1.83
*N retention***							
g/day	6.7[a]	9.3[b,c]	6.4[a]	10.2[b]	7.0[a]	7.9[a,c]	0.68
g/100 g N_i (%)	30.1[a,c]	39.0[b]	26.5[c]	36.6[a,b]	27.5[c]	32.9[a,b,c]	2.37

* Refer to Table 3 for treatment codes
** [N intake - (faecal N + urinary N)]
1 Nitrogen intake
a,b,c,d Means within the same row with different superscripts differ significantly ($P < 0.05$)

icant increase in N retention by lambs fed a diet supplemented with rumen-protected methionine was reported by Loerch & Oke (1984).

Sheep receiving the fish meal containing diet (RFM) retained a higher percentage N (35.8%) than those ruminally infused with M ($P = 0.0002$: 15.6%) and MM ($P = 0.001$: 19.4%). This agrees with the results of Lynch *et al.* (1991) who found that protein, but not rumen-protected methionine and lysine, increased N retention. Reasons for this phenomenon may be because other essential amino acids were limiting (Nimrick *et al.*, 1970) or because protein may improve hormonal balance. At lease part of the improved N balances reported for growing ruminants in response to abomasal casein may be explained by the increased insulin release and peripheral concentrations suggesting a changed endocrine status. Many hormones, including those produced by the pancreas, regulate how animals use nutrients. The increases in insulin secretion by the pancreas caused by increased protein intake are consistent with insulin's ability to promote nutrient storage by the animal tissue (Guerino *et al.*, 1991).

The infusion of methionine in the abomasum (AM) increased ($P = 0.01$) N retention with 2.6 ± 0.97 g/day as well as the percentage of N retained ($P = 0.02$: 39.0 vs. 30.1%) owing to a decrease ($P = 0.0005$) in the percentage of N excreted in the urine (25.2 vs. 35.9%), presumably the result of providing an amino acid which was physiologically limiting (Schelling *et al.*, 1973). The improvement in N retention resulted from less N excreted in the urine, suggesting absorbed N was used more efficiently at the tissue level (Meissner & Todtenhöfer, 1989) when methionine was abomasally infused. Since AM and AML infusion resulted in the highest (39.0 vs. 36.6%) and AL the lowest (26.5%) percentage N retained, this indicates that methionine and not lysine was the first limiting amino acid for N retention on these

Table 8 LS means for clean wool growth rate and efficiency of wool production of sheep receiving different ruminal infusions

Parameter	RC	RM	RMM	RMMM	RSOM	RFM	SEm
Clean wool growth rate							
mg/cm²/day	1.03[d]	1.06[c,d]	1.15[b,c]	1.34[a]	1.21[b]	1.40[a]	0.04
Relative value (%)	100	103	112	130	117	136	–
Efficiency of wool production ** (%)	43.0[d]	42.5[d]	46.0[c,d]	51.3[b]	49.9[b,c]	57.0[a]	1.73

* Refer to Table 3 for treatment codes
** Clean wool production/N intake (g/100 g)
a,b,c,d Means within the same row with different superscripts differ significantly ($P < 0.05$)

diets; however, lysine might have been co-limiting. Schingoethe (1991) suggested that minimal or no response, as in the case of AL supplementation, may occur because the second limiting amino acid is also close to being limiting or because the amino acid provided is not the most limiting amino acid. This might also imply that the infusion of lysine alone may have caused an imbalance with another amino acid (Sahlu & Fernandez, 1992).

Wool growth rate

Ruminal administration of methionine (Table 8) did not increase ($P > 0.10$) clean WGR, even though WGR was increased by 3%. This demonstrates the relatively poor utilization of unprotected methionine owing to extensive degradation by rumen micro-organisms (Reis *et al.*, 1978). In contrast, with the abomasally infusion of methionine an increase ($P = 0.0001$) of 31% in WGR was observed (Table 9). This agrees with several experiments where methionine was administered postruminally to bypass rumen degradation. Wool growth was stimulated by 30 to 60% with sheep offered maintenance diets under pen conditions (Reis, 1982) whereas typical responses have been 20 to 30% (Staples *et al.*, 1993).

The 2-methyl-maleyl methionine derivative (RMMM) showed great potential to resist microbial degradation and

Table 9 LS means for clean wool growth rate and efficiency of wool production of sheep receiving different abomasal infusions

Parameter	AC	AM	AL	AML	ASOM	AFM	SEm
Clean wool growth rate							
mg/cm²/day	0.95[d]	1.24[b]	0.95[d]	1.28[a,b]	1.13[c]	1.31[a]	0.03
Relative value (%)	100	131	100	135	119	138	–
Efficiency of wool production** (%)	43.7[d,e]	53.8[a,b]	40.0[e]	47.4[b,c,d]	45.6[c,d,e]	56.0[a]	2.33

* Refer to Table 3 for treatment codes
** Clean wool production/N intake (g/100 g)
a,b,c,d,e Means within the same row with different superscripts differ significantly ($P < 0.05$)

supply methionine postruminally. WGR was increased ($P = 0.0001$) (1.34 vs. 1.03 mg/cm²/day) when it was infused ruminally, a response (30%) of the same magnitude (31%) to abomasally infused methionine. In this regard, RMMM enhanced ($P = 0.002$) WGR with 0.19 ± 0.05 mg/cm²/day compared to ruminally infused maleyl methionine (RMM). This may be because RMMM is more efficiently protected against microbial degradation in the rumen and/or more of its methionine is being released postruminally and absorbed. In a chick growth assay (Kriel *et al.*, 1989) no significant difference was found between the effects of methionine, MMM and methionine-hydroxy analogue on growth rate and efficiency of feed conversion. However, MM did not produce growth rates equal to that from other products tested. Perhaps the rate of food passage was too rapid to allow hydrolysis in the proventriculus and gizzard so that methionine liberation was limited (Kriel *et al.*, 1989). The biological availability of MM for sheep could be limited by its hydrolysis rate [half-life of maleyl product is 11 to 12 h at pH 3.5 (Butler *et al.*, 1969) vs. approximately 2 h for the 2-methyl-maleyl derivative (Gibbons, 1970 cited by Butler & Hartley, 1972)]. If intestinal residence time is four hours a difference would be expected. The wool growth responses (12 and 30%, respectively) obtained with the two maleyl derivatives (RMM and RMMM) compared well with orally or dietary administration of several chemical protected derivatives: 1 to 8% with maleyl methionine (several authors, compiled by Coetzee, 1988); 9 to 23% with Mepron (Cottle, 1988a; 1988b; Stephenson *et al.*, 1990); 7% with MHA (Cottle, 1988c); 11 to 23% with Ketionin (Cottle, 1988a); 16 to 35% with Alimet (Stephenson *et al.*, 1990) and 6 to 27% with Smartamine (Staples *et al.*, 1993).

Although abomasally infused methionine (AM) increased ($P = 0.0001$) WGR (31%), lysine infusion (AL) had no effect ($P > 0.10$) which agrees with the results of Sahlu & Fernandez (1992). In this regard, Reis (1979) reported that, apart from the sulphur-amino acids, the administration of no other single amino acid has been shown to stimulate wool growth. This suggests that methionine and not lysine was the first limiting amino acid for wool growth under these conditions. With the simultaneously infusion of both methionine and lysine (AML) into the abomasum, WGR was stimulated ($P = 0.0001$: 35%), suggesting that methionine may be the first (Doyle & Bird, 1975) and lysine the second limiting amino acid (Storm & Ørskov, 1984). However, AM did not differ ($P > 0.10$) from AML, which agrees with the findings of Reis (1979). Based on the literature, wool growth can be limited by a deficiency of methionine (Reis & Schinckel, 1963) and lysine (Nimrick *et al.*, 1970), especially in sheep fed diets containing low quality roughage and non-protein nitrogen. As the level of sulphur-containing amino acid increases [normally 1 to 2 g/sheep supplemented (Reis *et al.*, 1973) vs. 2.46 g in our study], other amino acids become limiting for wool growth (Reis & Schinckel, 1964). According to Schingoethe *et al.* (1991) supplementation of the most limiting amino acid (e.g. methionine) makes the previously second limiting amino acid (e.g. lysine) the limitation. The level of the amino acid supplement is important since it has been indicated that optimal fiber production requires an optimal methionine level; too little results in a defiency, too much causes an imbalance. Both an optimal lysine level and an

ideal ratio of lysine to methionine are required for optimal fibre growth (Qi *et al.*, 1994).

The importance of lysine for wool growth was established in experiments with protein zein (which lacks lysine) and by omitting lysine from a mixture of essential amino acids (Reis, 1989). Because wool proteins are not rich in lysine, Reis (1989) suggested that its importance for wool growth may be partly related to the high content of lysine in histone proteins, which are important for cell division, and inner root sheath proteins. Unpublished data of J.L. Black and W.F. Colebrook (cited by Reis, 1991) also indicate a large effect of lysine intake on the rate of wool growth of pre-ruminant lambs receiving all their nutrients via the abomasum. The fact that AML infusion resulted in the highest WGR response of the amino acids infused agrees with Reis (1991) who identified cyst(e)ine, methionine and lysine as the amino acids having the greatest influence on fibre growth in non-breeding adult sheep. However, Sahlu & Fernandez (1992) found no synergistic effect in mohair yield with the intraperitoneal administration of both methionine and lysine in Angora goats. Presumably, these amino acids were more deficient in our basal diet which had a higher roughage content (75 vs. 48%) and a lower quality protein source (urea vs. soybean meal).

To optimize the response to protected methionine, Cottle (1988a) suggested that it is necessary to provide a diet which results in a balance of amino acids higher in the secondary limiting amino acids at the duodenum. Perhaps these amino acids are threonine, tyrosine, lysine, histidine and arginine (Buttery & Foulds, 1985). This is clearly demonstrated by the sheep receiving the fish meal containing diets (RFM and AFM) which produced a higher increase in WGR (36 and 38% respectively) and efficiency of wool production (57 and 56% respectively) than any other treatment. The WGR of sheep fed the sunflower-oilcake diets (RSOM and ASOM) was less than for sheep receiving RMMM and fish meal diets, mainly because of the high degradability of the sunflower-oilcake. The greater wool growth response with fish meal may be due to the presence of other essential amino acids, which limited wool growth, as well as the low ruminal degradation of fish meal. Reis & Schinckel (1963) reported that if casein was abomasally infused, a much higher increase in WGR was produced than when a similar amount of sulphur-containing amino acid was infused due specifically to the essential amino acids supplied by casein. Presumably, the extra energy available from the casein was far too little to account for the increases in wool growth obtained. Furthermore, the energy requirement for fibre growth represents less than 10% of the basal metabolic rate in sheep and generally is not a limiting factor (Black & Reis, 1979; Black, 1987). Reis & Sahlu (1994) concluded that maximum rates of wool growth can be sustained on maintenance intakes of energy if the supply of essential amino acids is adequate. Schingoethe (1991) indicated that providing two to five protected limiting amino acids has a greater probability to cause a response than providing only a single protected amino acid. According to Reis *et al.* (1990) a mixture of all essential amino acids stimulates wool growth more than a mixture of only five amino acids, even though the latter contains methionine, lysine, leucine and isoleucine. All are identified as the most important amino acids for wool growth.

Conclusions

The present results confirm those in the literature which indicate that derivatives of limiting amino acids resistant to microbial degradation and yet available for absorption in the lower-gut can improve production. MM seems to be inefficient as a source of bypass methionine to increase N retention and wool growth. MMM showed great potential in this regard suggesting that it may be a practical and efficient method of protecting amino acids against rumen degradation that could be applied in ruminant nutrition.

The poor results (decreased live weight, N retained and wool growth) with abomasally infused lysine (AL) can probably be attributed to the higher lysine infusion level in our study (5 g/day) than that required for optimal fiber growth, which induced an amino acid imbalance. The latest amino acid requirements for maintenance and fiber growth were calculated as 2.72 g S-amino acids and 1 g lysine daily for a 40 kg sheep growing 10 g clean, dry wool per day (Qi et al., 1994). Parsons (1990) suggested that excess lysine can elevate kidney arginase and decrease arginine synthesis which may depress growth. Keratin contains about 10% arginine, thus a lysine-induced arginine deficiency may depress fibre production as in the study of Sahlu & Fernandez (1992) with Angora goats.

Supply of both methionine and lysine appeared to limit wool growth of sheep limit fed high roughage diets containing non-protein nitrogen as the main protein source. Further enhancement was seen when other limiting essential amino acids also were postruminally provided in the form of fish meal. Substantial potential exists for improving the efficiency of utilization of non-protein supplements for roughage-based diets by supplementation with specific limiting amino acids protected from degradation in the rumen, particularly in woolled sheep. Practical application of these findings may include replacing fish meal with non-protein nitrogen and rumen-protected amino acids without depressing production.

MMM apparently resisted microbial degradation in the rumen while still allowing complete, or nearly complete, hydrolysis and absorption in the lower gut. If the 2-methyl-maleyl derivatives are to be of nutritional value, the nutrition of the host animal must be, according to Wallace (1992), limited by the amino acids being protected; this always is difficult to predict. Several other factors, like level of amino acid supplementation (Qi et al., 1994), quality and composition of basal diet (Cottle, 1988a; Qi et al., 1994), feeding level and genetic potential for wool growth (Stephenson et al., 1990), increases in fiber diameter (Reis, 1982; Sahlu & Fernandez, 1992) and physiological and environmental factors (Reis, 1982), must be considered in further research with MMM since all these factors can influence the magnitude of fibre growth responses.

Additional experiments with 2-methyl-maleyl methionine are needed to ascertain its potential value in ruminant nutrition, especially under field conditions. To maximize animal performance, further knowledge about limiting amino acids is needed.

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