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Influence of continuous intravenous lysine and methionine infusion on N balance in growing sheep fed diets that differ in ruminal degradable protein*

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ABSTRACT

The effect of continuously infused lysine and methionine with glucose on nitrogen retention in young wethers (average liveweight 31.3 kg) fed diets with different protein degradation in the rumen in two 4 x 4 Latin squares design was investigated. The wethers of a particular Latin square were fed at a level of 1.2-times their maintenance energy requirement either with a diet based on ground barley and urea (B+U), with high degradable intake protein (HDIP) or a diet based on cracked maize and maize gluten meal (M+MGM), with high undegradable intake protein (HUIP). The wethers of each Latin square were continuously infused (jugular) with control solution (Control), or with L-lysine (Lys), DL-methionine (Met) or their combination (Lys+Met). Infusion rates for the above mentioned amino acids were 3.0, 1.5 and 3.0 g+1.5 g lamb^{-1} day^{-1}, respectively, estimated to be about 50% of maintenance. Glycine was added to the infusion solutions to equalise the total N infused. Glucose (12 g kg BW$^{0.75}$d^{-1}) was also supplemented in the infusion solution to minimise endogenous gluconeogenesis.

The average digestibility coefficients of DM and N were 73.6 and 60.2% for the B+U and 78.4 and 63.5% for the M+MGM diets, respectively, and were not affected by infusion treatments.

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The continuous infusions of Lys, Met and Lys+Met improved N retention, irrespective of the diet, by 30, 34 and 36%, respectively. The effect, however, depended on the diet. When the diet contained the HDIP (about 80%), based on barley and urea, N retention increased by 13, 20 and 29%, respectively with Lys, Met and Lys+Met infusions. Feeding a HUIP diet (about 60%), based on maize and maize gluten meal, increased N balance by 43, 44 and 40%, respectively with Lys, Met and Lys+Met infusions.

KEY WORDS: growing sheep, lysine, methionine, infusion, protein, rumen, degradation, N balance

INTRODUCTION

Lysine and methionine are most likely to be the first limiting amino acids for intensively growing and/or milk and wool producing ruminants (Storm and Ørskov, 1984; Fraser, 1988; Schwab et al., 1992). Cows fed these amino acids in the protected form or infused postruminally respond with increasing milk protein content (Piepenbrink et al., 1996; Pisulewski et al., 1996). Some investigations also show a positive increase in milk yield (Choung and Chamberlain, 1992; Robinson et al., 1995). A supply of intestinally available lysine and methionine can also improve N-balance and performance of growing sheep and cattle (Loerch and Oke, 1984; Urbaniak, 1984; Oke et al., 1986; Schmidt and Nguyen-Thanh, 1989).

It has been indicated that lysine is the limiting amino acid for intensive growth in lambs and methionine for wool production in adult sheep (Dvalishvili, 1995). How these amino acids limit protein synthesis in tissues of these animals depends also on the diet and directly upon the proportion of microbial protein (MP) and protein undegraded in the rumen. Storm and Ørskov (1984) showed that for growing sheep methionine is the first limiting amino acid if MP is the only protein source. When the diet contains a high proportion of rumen undegradable intake protein (UIP), its amino acid composition can have a great influence on total diet utilisation (Varvikko, 1986).

The aim of this experiment was to determine the effect of continuous lysine and methionine intravenous infusion in growing sheep fed diets containing protein of different degradability in the rumen. The intravenous infusion was chosen to avoid uncertainty over the absorbability of the supplementary amino acids (Choung and Chamberlain, 1995). Infusion of these amino acids was accompanied with glucose. According to Judson and Leng (1973), exogenous glucose diminishes endogenous gluconeogenesis from amino acids in ruminants and decreases urinary-N excretion by up to 50 to 60% (Matras and Preston, 1989), thus improving N balance. Under these conditions, it was expected that the infused amino acids would be utilised entirely for protein synthesis.

MATERIAL AND METHODS

Two experimental groups, 6 animals each of Rambouillet type wether lambs (31.3 kg), were placed in metabolism stalls after a two-week adjustment period. Each group was fed a different diet (Table 1). The diets were isoenergetic and isonitrogenous but differed in protein degradability in the rumen. One group was fed the diet based on barley and urea (B+U), containing high degradable intake protein (HDIP) and the second group received the diet containing maize and maize gluten meal (M+MGM), with high undegradable intake protein (HUIP).

After a 4-day adjustment period in metabolism stalls, during which the lambs were continuously infused with 750 ml d^{-1} of sterile saline, 4 of the most docile wethers from each of two groups fed different diets were chosen and used in the

TABLE 1

Composition and nutritive value of the diets

Item	Diet [a]	
	B+U	M+MGM
Diet composition, %		
ground barley	41.00	–
cracked maize	–	55.74
cottonseed hulls	30.00	30.00
molasses	6.00	6.00
urea	1.24	–
maize gluten meal	–	5.56
maize starch	18.86	–
dicalcium phosphate	0.60	0.30
$CaCO_3$	0.85	1.05
NaCl	0.50	0.50
K, $MgSO_4$ [b]	0.45	0.35
trace mineral premix [c]	0.25	0.25
vitamin A, D, E premix [d]	0.25	0.25
Content in the diet, %		
dry matter (DM)	87.1	87.3
organic matter	83.4	83.6
Nutritive value of 1 kg DM		
metabolisable energy, MJ	10.7	11.1
crude protein, g	121	127

[a] diets: B – U = barley – urea (Latin square I); M+MGM = maize – maize gluten meal (Latin square II)

[b] contained 18 % K, 11 % Mg and 22 % S

[c] added the following to the diet DM: Mn 6.6, Zn 18, Fe 9.9, Cu 1.95, I .45, Co .34 and Mg 30 mg/kg

[d] added the following to the diet DM: 1650 I U vit. A, 270 I U vit. D and 44 I U vit. E/kg

two separate 4 x 4 extra period Latin square design trials. This experimental design allows an estimate of direct and residual effects of treatment (Lucas, 1957). There were 4 treatments within each Latin square: Control treatment and treatments either with L-lysine (Lys), DL-methionine (Met) or combined L-lysine + DL-methionine (Lys+Met) intravenous infusion (Table 2). The amounts of infused lysine (3 g d^{-1}) and/or methionine (1.5 g d^{-1}) were equal to about half of their daily requirement for maintenance (Asplund, 1986). Glycine was infused to give isonitrogenous infusion solutions for all treatments. The amino acids were infused in saline solution (0.9 % NaCl). The solution also contained glucose, given in an amount of 160 g $head^{-1}$ day^{-1}, or about 12 g day^{-1} kg $BW^{0.75}$. Glucose infused at this rate in our previous experiment (Matras and Preston, 1989) maximally diminished urinary-N excretion and improved N balance. The saline-glucose solutions were sterilised in an autoclave and the amino acids were added just before infusion. The catheters were inserted into the jugular vein according to the method of Matras and Preston (1989). The solution was infused at the rate of 750 ml per day. Each infusion period lasted six days. Faeces and urine were collected during the last five days of each six-day infusion period to determine dry matter (DM), organic matter (OM) and nitrogen (N) digestibility coefficients, N and allantoin excreted in urine and N balance. Allantoin was determined according to Young and Conway (1942).

Each Latin square was statistically analysed separately (Lucas, 1957).

TABLE 2

L-lysine and DL-methionine infusion, g $head^{-1}$ day^{-1} in both Latin squares

Infusion treatment	Amino acid infused [a]	
	L-lysine	DL-methionine
Control	–	–
Lys	3.00	–
Met	–	1.50
Lys + Met	3.00	1.50

[a] glycine (3.84, 0.76 and 3.08 $head^{-1}$ day^{-1} in Control, Lys and Met treatments, respectively) was used in the infused solution to equalise the total infused N

RESULTS

All results and statistical comparisons are the direct effect of treatments, since there were no significant residual effects (P>0.20). The average daily DM intake by the lambs in the B+U and M+MGM Latin squares was 616 and 629 g, respectively. The energy consumed was about 1.2 times their maintenance energy requirement. Taking into account the energy contained in the infused glucose (160 g glucose head[-1]d[-1]), the total intake of energy was about 1.65 times their maintenance. The average daily total N intake (N in the diet + N in the infused amino acids) was 12.7 and 13.5 g, respectively, and there were no differences in N intake among treatments within both Latin squares (Table 5). The amount of N in the infused amino acids was the same in all treatments (0.7 g d^{-1}). The average OM digestibility coefficients of B+U and M+MGM diets were 73.6 and 78.4%, respectively, and digestibility coefficients of crude protein were 60.2 and 63.5%, respectively (Table 3) and they were not affected by the treatments.

TABLE 3

Digestibility coefficients of organic matter and crude protein, %

Component	Latin square [1]	Infusion treatment Control	Lys	Met	Lys + Met	Average	Standard error
Organic matter	I	72.6	73.4	74.5	73.7	73.6	1.42
	II	78.3	77.8	78.5	78.9	78.4	0.95
Crude protein	I	61.6	60.5	59.9	58.9	60.2	1.96
	II	64.6	62.5	62.9	64.0	63.5	1.17

[1] square I, B+U diet; square II, M+MGM diet

Urinary-N expressed as a percentage of the total N intake (diet N + infused N) is presented in Table 4. The portion of N excreted in urine was similar in the control treatments for both Latin squares (43%). In both trials lysine and methionine infusion decreased urinary-N excretion considerably compared with the control. The results, however, were somewhat different between B+U and M+MGM diets. For the B+U diet a statistically significant ($P<0.05$) difference in comparison with the control treatment was noted only for the Lys+Met infusion, whereas for the M+MGM diet a significant ($P<0.01$) difference was noted both for the Lys+Met treatment and for the treatments, where these amino acids were infused individually.

Lysine and methionine infused both individually and in combination considerably increased N balance (Table 5). Irrespective of the diet, the N retention (g d^{-1}) for Lys, Met and Lys+Met infusions, when compared with Control was higher by

TABLE 4

Influence of lysine and methionine infusion on N excreted in urine, % of total N intake (N intake in the diet + N infused)

Square (Diet)	Infusion treatment Control	Lys	Met	Lys + Met	Average	Standard error
I (B+U)	43.1[a]	39.4[a b]	38.4[a b]	36.1[b]	39.2	1.34
II (M+MGM)	43.0[A]	33.0[B]	33.2[B]	35.1[B]	36.1	1.11

[a, b] – $P < 0.05$
[A, B] – $P < 0.01$

30, 34 and 36%, respectively. The results, however, were not the same for B+U and M+MGM diets. In the M+MGM one, lysine and methionine infused individually and in combination improved N retention ($P<0.05$) to a similar extent (40 to 45%). In contrast, the improvement in N retention for Lys, Met and Lys+Met infusions in the B+U diet was 13, 20 and 29%, respectively and was not statistically significant. Similar tendencies were observed when N balance was expressed as a per cent of N intake and as a per cent of N digested. The lambs on the B+U and M+MGM diets excreted 420 and 255 mg allantoin per day in the urine, respectively. The infusion treatments did not influence allantoin excretion.

TABLE 5

Influence of lysine and methionine infusion on N retention

Item	Square (Diet)	Infusion treatment				Average	Standard error
		Control	Lys	Met	Lys+Met		
N intake[1], g/day	I (B+U)	12.7	12.7	12.7	12.7	12.7	–
	II (M+MGM)	13.5	13.5	13.5	13.5	13.5	–
N retention, g/kg	I (B+U)	1.92	2.17	2.30	2.48	2.22	0.17
	II (M+MGM)	2.47[b]	3.53[a]	3.57[a]	3.46[a]	3.25	0.21
N retention, % of N intake	I (B+U)	15.1	17.1	18.1	19.5	17.5	1.34
	II (M+MGM)	18.3[b]	26.1[a]	26.4[a]	25.6[a]	24.1	1.56

[1] N intake = N intake in the diet (12.0 g N day^{-1} Latin square I, 12.8 g N day^{-1} Latin square II) + 0.7 g N in amino acids intravenous infusion

[a, b] – $P < 0.05$

[A,B] – $P < 0.01$

DISCUSSION

The applied investigations with lysine and methionine given in the protected form to the ruminant diets in many cases revealed a positive influence on N balance and production (Loerch and Oke, 1984; Potkański et al., 1985; Oke et al., 1986). Some studies, however, did not show any response (Wylie et al., 1986; Wright and Loerch, 1988). The proportion of DIP and UIP in the diet as well as the amino acid profile of the UIP had a major influence on the effectiveness of these amino acids (Rock et al., 1982; Varvikko, 1986). In this study, to avoid the digestive tract, lysine and methionine were given to wether lambs by continuous intravenous infusion. The infusion of amino acids was accompanied with glucose, which according to Judson and Leng (1977) and Matras and Preston (1988), would decrease the utilisation of amino acids for gluconeogenesis and/or oxidation processes and thus increase their availability for body protein synthesis.

Allantoin excretion in the urine is useful as an indicator of rumen microbial protein synthesis (Antoniewicz, 1988; Lindberg et al., 1989). According to Antoniewicz (1988), from 1 g of digested N of microbial origin, 50 mg N is excreted as urine allantoin. Assuming that microbial protein digestibility is about 85% (Lindberg et al., 1989), the contribution of microbial N entering the intestine is calculated to be 82% of N flow to the duodenum for the B+U diet and only 48% for the M+MGM diet. These values correspond fairly well with the values 80 and 44% DIP, respectively, when calculated from tables by Preston (1987).

The results of Richardson and Hatfield (1978) revealed that when the microbial protein was almost the only source of protein supply, methionine and lysine had the biggest positive impact on N retention in beef cattle. Our experiment was made with growing sheep, whose methionine allowance is presumably higher in comparison with young beef cattle because of a greater need for this amino acid for wool protein synthesis. This allowance is much higher than the methionine content in microbial or maize bypass protein and especially in maize gluten meal bypass protein. When the lambs were fed the diet containing high DIP (B+U diet), a fairly high (+20%) increase in N retention was noted when the lambs were infused with methionine. Lysine infusion had a somewhat smaller impact (+13%). Combining both of these amino acids elevated N retention by 29%.

The bypass methionine content in the protein of cracked maize and maize gluten meal is somewhat lower in comparison with MP, whereas the level of lysine in both of these feeds is very low. According to Ludden (1995), protein of maize grain and maize gluten meal contains only about 59 and 20% of lysine, respectively, in comparison with its level in microbial protein. The high need of growing sheep for methionine, which was accompanied by a very low lysine content in the diet based on maize grain and maize gluten meal, caused the significant ($P<0.05$) impact of infusion of these two amino acids on nitrogen utilisation, improving N balance by 40%.

CONCLUSIONS

Lysine and methionine infused intravenously with glucose to growing sheep considerably improved their N balance. The effect, however, depended on the diet. When the animals were fed a ground barley and urea diet (high DIP) in which the calculated contribution of microbial protein entering the small intestine was about 80%, N balance increased by 13, 20 and 29%, respectively, when lysine, methionine or lysine + methionine were infused. When the lambs received a diet based on maize grain and maize gluten meal (high UIP), containing protein which is about 55% undegradable in the rumen and contains a low level of both these amino acids, especially lysine, the influence of infusing these amino acids on N balance in lambs was larger, reaching 40%.

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STRESZCZENIE

Wpływ ciągłej dożylnej infuzji lizyny i metioniny na bilans azotu u rosnących owiec żywionych dawkami różniącymi się stopniem rozkładu białka w żwaczu

Badano wpływ ciągłej infuzji lizyny i metioniny wraz z glukozą na retencję azotu u młodych skopów (śr. masa ciała 31,3 kg), żywionych dawkami zawierającymi białko o różnym stopniu rozkładu w żwaczu, w układzie dwóch kwadratów łacińskich. W każdym doświadczeniu dawka odpowiadała 1,2-krotnemu zapotrzebowaniu bytowemu na energię i składała się ze śruty jęczmiennej i mocznika (B+U) z dodatkiem białka o wysokim stopniu rozkładalności (HDIP) lub z połupanego ziarna kukurydzy i mączki z glutenu kukurydzianego (M+MGM) z białkiem o niskim stopniu rozkładalności (HUIP). W obydwóch doświadczeniach były podawane ciągle dożylnie (*v. jugularis*): roztwór kontrolny (Control) lub z L-lizyną (Lys), DL-metioniną (Met) lub ich kombinacją (Lys+Met). Tempo infuzji tych aminokwasów wynosiło odpowiednio 3,0; 1,5 i 3,0 g + 1,5 g/jagnię/dzień, co stanowiło ok. 50% zapotrzebowania bytowego. Dla zrównoważenia ilości podawanego w infuzji azotu dodawano glicynę. W infundowanym roztworze podawano także glukozę (12 g kg $BW^{0.75}d^{-1}$) celem ograniczenia endogennej glukoneogenezy.

Średnie współczynniki strawności suchej masy i N wynosiły 73,6 i 60,2% przy skarmianiu dawki B+U oraz 78,4 i 63,5% przy podawaniu dawki M+MGM i nie zależały od rodzaju infuzji. Infuzja Lys, Met i Lys+Met poprawiała retencję N, odpowiednio o 30, 34 i 36%, niezależnie od rodzaju dawki. Jednakże gdy dawka jęczmienno-mocznikowa zawierała HDIP (około 80%) retencja N zwiększała się o 13, 20 i 29% przy infuzji odpowiednio Lys, Met lub Lys+Met, natomiast przy skarmianiu dawki „kukurydzianej" o wysokim udziale HUIP (ok. 60%) bilans N zwiększył się odpowiednio o 43, 44 i 40% przy infuzji wyżej wymienionych aminokwasów.