Reprod. Nutr. Dev. 42 (2002) 537–544

DOI: 10.1051/rnd:2002039

Original article

Rumen escape of methionine and lysine administered intraruminally to growing double-muscled Belgian Blue bulls

Eric FROIDMONT*, Pierre RONDIA, André THÉWIS, Yves BECKERS

Faculté Universitaire des Sciences Agronomiques, passage des Déportés 2, 5030 Gembloux, Belgium

(Received 16 April 2002; accepted 15 July 2002)

Abstract — In many dietary conditions, methionine (Met) and lysine (Lys) are the most limiting amino acids (AA) for ruminants. The AA protected from ruminal fermentation are not commercially available, with the exception of Met which is not always economical, especially for meat production. This study measured ruminal escape of free Met and Lys supplemented intraruminally to fast growing bulls. Six double-muscled Belgian Blue bulls, fed a high concentrate diet and fitted with a rumen cannula, received free Met (40 g·d^{-1}) and free Lys (60 g·d^{-1}), individually or simultaneously, in a duplicated Latin square design. The mean ruminal escape of Met and Lys reached 37 and 45% respectively, and did not differ if administered separately or together. Plasma Lys and Met concentrations were increased by 504 and 126%, respectively. Substantial proportions of free AA escaped ruminal fermentation and were available for absorption from the small intestine when they were administered at physiologically high levels.

ruminant / methionine / lysine / rumen escape / plasma

1. INTRODUCTION

Methionine (Met) and Lysine (Lys) are often the first and second limiting amino acids (AA) for milk [13, 15] and meat [18] production in cattle. For dairy cows, protected AA from rumen fermentation are sometimes incorporated into the diets in order to improve protein efficiency. These AA are less used for growing cattle because of a lower economical interest related to less intensive breeding systems.

* Correspondence and reprints
Current address: Centre de Recherches agronomiques de Gembloux, Département 'Productions et Nutrition animales', rue de Liroux 8, 5030 Gembloux, Belgium
E-mail: froidmont@cra.wallonie.be

Belgian Blue bulls (BBb) are widely used for meat production in Belgium [9]. These animals require a precise dietary protein nutrition in order to satisfy their requirements relative to their high growth potential of up to 2.1 kg·d^{-1} [8] and the high proportion of muscle (78.1%) in the carcass [12].

Dietary incorporation of free AA may be a practical way to improve the AA pattern of metabolisable protein in dairy cows [24]. The ruminal escape of free AA has been shown to vary with AA [22], dietary conditions [16], amount supplied [24], and whether the AA is administered individually or with other AA [23]. To our knowledge, only Campbell et al. [2] has investigated free AA supplementation for growing cattle, and observed no effect on animal performances although the amounts supplied were modest.

Our hypothesis is that large amounts of free AA could change the AA balance of intestinally metabolisable protein for fast-growing bulls fed high concentrate diets. This study determined the ruminal escape of free Met and/or Lys, administered directly into the rumen of double-muscled BBb.

2. MATERIAL AND METHODS

The animal care protocol was approved by the "Comité d'éthique en expérimentation animale", Gembloux University, Belgium (Registration number: FUSAGx00/01).

2.1. Animals and diet

Double-muscled BBb (n = 6; 257 ± 9 kg), fitted with a ruminal cannula (internal diameter: 67 mm) were individually penned (1.5 × 2.5 m) and received a diet (Tab. I) with a metabolisable protein/net energy ratio of 58 g·Mcal^{-1} according to Dutch

Table I. Ingredients and chemical composition of the diet.

Ingredient composition:	% of DM
Wheat straw	13.4
Rolled barley	29.1
Sugar beet pulp, pellets	28.8
Roasted soybeans	14.9
Soybean meal, 48% CP, solvent	3.9
Linseed meal, schilffers	3.8
Mineral and vitamin mixture[a]	3.3
Na-bicarbonate	1.8
Liquid beet molasses	1.0
Chemical composition:	g·kg^{-1} DM
Organic matter	885
Crude protein	142
Neutral detergent fibre	335
Acid detergent fibre	192
Cellulose	148
Ether extract	52
Metabolisable AA[b]	105
Net energy, kcal·kg^{-1} DM[b]	1.815

[a] Composition (%): 14 Ca, 7 P, 3.5 Na, 2.5 Mg; (mg·kg^{-1}): 40 I, 40 Co, 500 Cu, 2850 Fe, 2500 Mn, 3500 Zn, 12 Se, 500 vitamin E; (IU·kg^{-1}): 500000 vitamin A, 100000 vitamin D_3.
[b] Metabolisable amino acids and net energy: calculated according to the Dutch system [17, 20].

recommendations [17, 20]. This ratio has been found to be optimal for growing double-muscled BBb [6]. The intake level was fixed at 87 g DM·kg^{-1} LW$^{0.75}$ and fresh water was available at all times. Feed was offered in equal amounts at 8 a.m. and 8 p.m.

2.2. Experimental protocol and sample collection

After 21 d of diet adaptation, three 1 d treatments were administered in order to supply in the rumen, with the morning meal, 40 g of DL-Met ('Met'), 60 g of L-Lys as L-Lys-HCl ('Lys') or 40 g of DL-Met and 60 g of L-Lys ('Met + Lys') in a duplicated Latin square design (3 treatments × 6 animals). These AA were dissolved in 500 mL of water containing 1.4 g of Co as CoEDTA [19], used as a liquid phase tracer. Treatment days were separated by 2 d without AA supplementation. Ruminal fluid (1 L) was sampled by suction at 1, 2, 4, 6, 8, 10 and 12 h after AA administration. Ruminal fluid was immediately filtered (250 μm) after sampling and manually homogenised by blending for a few seconds. A 100 mL aliquot was retained for laboratory analyses and the remainder was returned to the rumen. Blood samples were collected by jugular venipuncture into heparinised tubes (250 IU·60 mL^{-1} of blood) 3 h after the morning feeding.

2.3. Laboratory analyses

Concentrate and straw were ground through a 1 mm screen (Pulverisette 14; Fritsch, Idar-Oberstein, Germany) prior to DM, ash, N, ether extract [1], neutral detergent fibre [21], acid detergent fibre and cellulose [1] analyses were conducted.

Ruminal fluid samples were centrifuged 10 min at 1200 × *g*. A part (50 mL) of the supernatant was frozen until Co determination by atomic absorption spectrophotometry (Spectrophotometer 1100B, Perkin-Elmer, Norwalk, CT). After cooling on ice, the proteins of the other part (10 mL) of the supernatant were precipitated by addition of 5 mL of TCA (15%). The supernatant from the 20 min 9900 × *g* centrifugation was frozen at –20 °C until free AA determination by high performance liquid chromatography [3].

Whole blood was centrifuged 15 min at 2600 × *g* immediately after collection. Plasma was removed and frozen at –20 °C. After thawing, plasma was deproteinised using 3000 Da ultrafiltration (Amicon, Micropartition method, kit MPS No. 4010; Amicon, Beverly, MA) before determination of free AA by high performance liquid chromatography [3].

2.4. Calculations

The concentration of a liquid phase tracer such as Co evolved with time according to the relation: $[Co]_t = [Co]_{t0}\exp^{-kt}$, where $[Co]_t$ is the Co concentration at time *t*, $[Co]_{t0}$ is the initial Co concentration, *k* is the liquid outflow rate and *t* is the time. Therefore, *k* and $[Co]_{t0}$ were calculated from the slope and Y-axis junction of the regression of the natural logarithm of rumen liquid Co concentration versus time, respectively. Liquid pool sizes were finally determined by dividing the amount of Co administered by $[Co]_{t0}$. The r^2 values of these regressions were higher than 0.89 for all animals. Calculations of ruminal AA escape and degradation were calculated as [4]: Rumen AA escape (g) = mean concentration of AA during the interval (g·L^{-1}) × liquid outflow rate (L·min^{-1}) × interval (min). Rumen AA degradation (g) = [initial concentration (g·L^{-1}) – final concentration (g·L^{-1})] × pool size (L) – rumen escape (g). Amino acid concentration (g·L^{-1}) at time zero (*t* = 0): = AA dosage (g)/pool size (L). Ruminal AA escape and degradation for the 12 h after AA administration were calculated by summing the values for escape and degradation for each sampling

interval. Due to the large AA doses, no corrections were made for basal free AA concentrations in the rumen fluid.

2.5. Statistics

Statistical analyses were conducted with the general linear model procedures of SAS software [14] for a duplicated Latin square design. Three effects (animal, period and treatment) were considered for volume, outflow rate, outflow volume, Met and Lys concentrations of ruminal fluid, total amounts of these AA escaping or being degraded in the rumen and plasma AA concentrations. The differences among treatment means were compared using a least significant difference test [5]. For both AA studied, differences between individual or simultaneous AA supply on ruminal concentrations, and on their escape and degradation rates were analysed for each sampling hour by a three-way ANOVA (animal, period and supplying mode).

3. RESULTS AND DISCUSSION

Intraruminal administration of Met, Lys or both had no effect on ruminal liquid pool size or outflow rate (Tab. II). However, outflow volume was lower with treatment 'Lys', due to a numerically lower liquid volume and outflow rate.

Concentrations of free Met and free Lys in the ruminal fluid decreased progressively with time after their administration (Fig. 1). Individual or simultaneous supply of these AA little influenced their concentrations in rumen liquid ($P > 0.05$). Ruminal concentrations of free Met and free Lys were very low when these AA were not supplied.

Total amounts of free Met leaving the rumen reached respectively, 15.7 ± 2.5 and 13.9 ± 2.6 g when Met was supplied alone or simultaneously with Lys (Fig. 2), and did not differ between treatments ($P = 0.45$). Total ruminal outflow of free Lys did not differ when it was supplied with or without Met ($P = 0.08$) and reached, respectively, 26.6 ± 5.5 and 27.5 ± 5.5 g. In this study, high ruminal escape of Met and Lys were observed despite dietary conditions favourable to AA degradation. High concentrate diets actually induce an efficient growth of microorganisms [16] and a selection of amylolytic bacteria that utilise high proportions of AA [24]. However, more than 65% of the total amounts of free Met and free Lys that escaped ruminal fermentation did so during the first 4 h.

According to Velle et al. [23], ruminal escape of free AA is increased when they are administered with larger amounts of other AA. Our results did not support this hypothesis since no variation in Met or Lys escape was observed with the simultaneous supply of these AA. Velle et al. [23] showed

Table II. Effect of intraruminally administered methionine (Met), lysine (Lys) or methionine plus lysine (Met + Lys) on liquid pool size, outflow rate and outflow volume of ruminal fluid in double-muscled Belgian Blue bulls.

	Treatment			
	Met	Lys	Met + Lys	SEM
Pool size (L)	28.6	27.7	31.1	0.61
Outflow rate (%·h^{-1})	10.5	9.3	9.3	0.44
Outflow volume (L·h^{-1})	2.8^{y}	2.5^{z}	2.8^{y}	0.04

^{y,z} Within a row, means with a different superscript differ ($P < 0.05$).

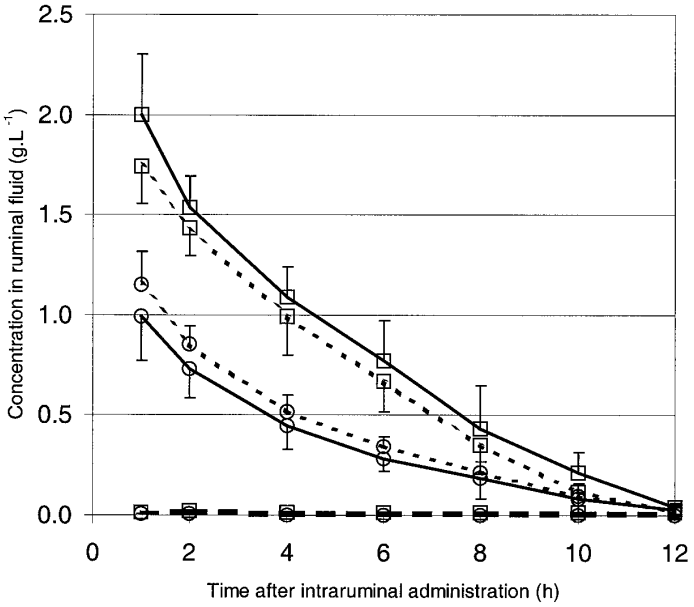

Figure 1. Concentration of Met (○) and Lys (□) in rumen liquid when these amino acids were administered individually, (——) simultaneously, (- - -), or not at all (▬▬) in the rumen of double-muscled Belgian Blue bulls.

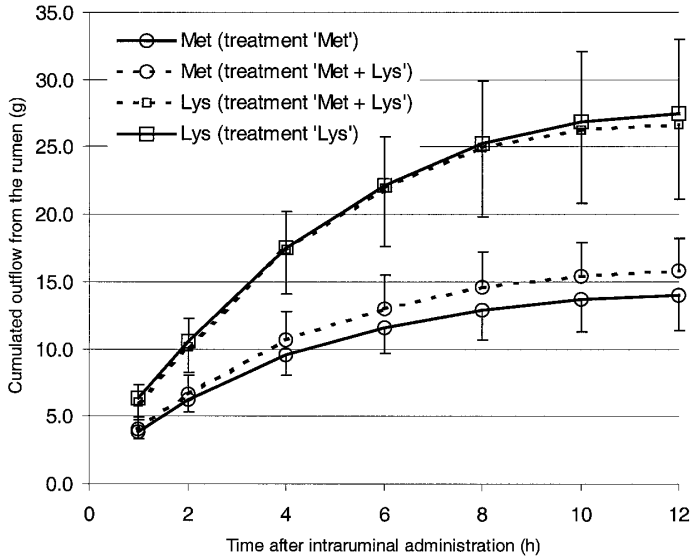

Figure 2. The effect of intraruminally administered methionine (Met), lysine (Lys) or methionine plus lysine (Met + Lys) on the cumulative outflow of free Met (○) and free Lys (□) from the rumen of double-muscled Belgian Blue bulls.

that such an effect was more pronounced with lower (< 150 mmol) versus higher AA dosages. The amounts of Met and Lys supplied in our experiment were high, reaching 270 and 410 mmol respectively, and the volume of ruminal fluid of BBb was widely smaller than in the dairy cows (28.7 vs. 61.4 L) used by Velle et al. [23].

The net sum of escaped and degraded Met and Lys 12 h after their administration differed slightly from the initial dosages. The differences, ranging from 1.2 to 6.2%, probably reflected some inaccuracy in the methods and the assumptions upon which they were based such as a constant ruminal fluid volume during the sampling time. Moreover, we observed that free Met and Lys concentrations in ruminal fluid were most variable 1 h after AA administration, suggesting that free AA was not perfectly mixed in the rumen. Nevertheless, the results suggest that no effect of free AA supplementation remained on the composition of intestinal content 12 h after AA administration. Velle et al. [22] showed that most free AA, except histidine, were gone from the rumen 8 h after ruminal administration, whatever the amounts supplied.

In our experiment, the proportions of administered free Met and Lys escaping ruminal degradation reached 37 and 45%, respectively. Feeding crystalline AA could be economically attractive when their ruminal escape rates exceed 20 to 25% [11]. Such rates, observed with dairy cows with high AA administration [22–24], have not previously been reported for fast-growing bulls. However, the main factor influencing the proportional ruminal escape of free AA is their concentration in the ruminal fluid, which depends on its volume and the dose of AA. High ruminal fluid AA concentrations could inhibit the activity of microbial deaminases in the rumen and, therefore, AA degradation [22]. Since it is during the growth phase that AA requirements in BBb are the most important [6, 7], when rumen volume is the lowest, the ruminal AA concentration is more influenced by AA administration and causes the highest proportional AA escape from the rumen. Thus, crystalline AA supplementation would appear to be well suited for growing BBb.

Administration of 40 $g \cdot d^{-1}$ of Met, alone or with Lys, increased its plasma concentration by 484 and 524% respectively as compared to treatment 'Lys' (Tab. III). In the same way, ruminal administration of Lys (60 $g \cdot d^{-1}$), alone or with Met, increased plasma Lys concentration by 117 and 135% respectively compared to treatment 'Met'. Since AA absorption by the rumen wall is negligible [10], these results confirmed that a substantial proportion of the administered AA escaped the rumen and was absorbed from the small intestine. The changes in plasma AA profile 3 h after AA administration confirmed that this mechanism was rapid. Except for Met and Lys, the treatments did not modify plasma concentrations of other essential AA. This suggests these AA were not better utilised with a particular treatment, following an improvement of N utilisation by animals for instance. However, the short length of treatments probably explains the absence of a metabolic response. In the same way, plasma concentrations of Arg, Cit and Pro, all being implicated in the urea cycle, were not influenced by the amount of AA administered, suggesting that ureogenesis was not modified when blood samples were realised. The stability of plasma Tau concentration among the treatments, which is an end-product of Met metabolism, confirmed that some metabolic pathways were probably not completely active 3 h after the treatments. After 4 d of adaptation to the treatments, Cottle and Velle [4] mentioned a reduction in plasma Gly level with Met administration reflecting the involvement of Gly in several reactions in which Met also participates. Without adaptation to the treatments, our results suggested that plasma Gly concentration is not influenced by Met supply. Except for Met and Lys, the stability of plasma AA concentrations observed in this study suggested that the effects of intraruminal AA administration on

Table III. Effect of intraruminally administered methionine (Met), lysine (Lys) or methionine plus lysine (Met + Lys) on plasma amino acid (AA) concentrations (μM) in double-muscled Belgian Blue bulls.

	Treatment			
	Met	Lys	Met + Lys	SEM
Essential AA (EAA)				
Arg	84.2	82.3	78.2	3.5
His	46.4	44.7	51.2	3.3
Ile	115.1	111.6	106.3	4.6
Leu	93.3	93.0	89.6	4.8
Lys	95.2^{y}	206.6^{z}	224.0^{z}	7.7
Met	121.4^{y}	20.8^{z}	129.8^{y}	4.2
Phe	47.9	44.5	45.7	2.2
Thr	63.7	61.3	66.1	3.8
Val	198.8	191.6	196.8	8.8
Non essential AA (NEAA)				
Ala	193.0	191.8	196.7	8.4
Asn	30.7	26.3	35.2	2.4
Asp	25.8	26.4	32.9	2.8
Cit	57.9	48.6	61.3	2.7
Glu	83.7	76.7	72.6	4.2
Gly	269.2	266.4	260.2	12.3
Pro	63.1	58.0	63.6	2.5
Ser	125.2	136.1	142.3	11.9
Tau	22.5	16.3	21.0	1.7
Tyr	90.9	83.7	87.7	4.3
EAA	865.9	856.0	987.6	35.6
NEAA	881.7	865.4	891.1	38.8
TAA[a]	1,748	1,721	1,996	70.3

[a] TAA: total amino acids.
[y,z] Within a row, means with a different superscript differ ($P < 0.05$).

metabolic responses and possible growth improvements of BBb should be determined in further studies with longer experimental periods.

4. CONCLUSIONS

For growing double-muscled BBb, a substantial proportion of crystalline AA administered intraruminally escaped from ruminal degradation to become available in the small intestine. The proportional rumen escape rates of free Met and free Lys reached 37 and 45%, respectively. The optimal amounts of crystalline AA for main practical dietary conditions need to be further investigated.

REFERENCES

[1] AOAC, Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Airlington, VA, 1990.

[2] Campbell C.G., Titgemeyer E.C., Cochran R.C., Nagaraja T.G., Brandt R.T. Jr., Free amino acid supplementation to steers: Effects on ruminal fermentation and performance, J. Anim. Sci. 75 (1997) 1167–1178.

[3] Cohen S.A., Strydom D.J., Amino acid analysis utilizing phenylisothiocyanate derivatives, Anal. Biochem. 174 (1988) 1–16.

[4] Cottle D.J., Velle W., Degradation and outflow of amino acids from the rumen of sheep, Br. J. Nutr. 61 (1989) 397–408.

[5] Dagnelie, P., Théories et méthodes statistiques, Vol. 2, Presses agronomiques de Gembloux, Belgium, 1986.

[6] Froidmont E., Beckers Y., Théwis A., Determination of the methionine requirement of growing double-muscled Belgian Blue bulls with a three-step method, J. Anim. Sci. 78 (2000) 233–241.

[7] Froidmont E., Beckers Y., Théwis A., Determination of the methionine requirement of finishing double-muscled Belgian Blue bulls with a three-step method, Can. J. Anim. Sci. 82 (2002) 95–102.

[8] Gengler N., Seutin C., Boonen F., Van Vleck L.D., Estimation of genetic parameters for growth, feed consumption, and conformation traits for double-muscled Belgian blue bulls performance-tested in Belgium, J. Anim. Sci. 73 (1995) 3269–3273.

[9] INS, Institut National de Statistique, Recensement agricole et horticole au 15 mai 1999, Ministère des Affaires économiques, Bruxelles, 1999.

[10] Leng R.A., Nolan J.V., Nitrogen metabolism in the rumen, J. Dairy Sci. 67 (1984) 1072–1089.

[11] Mbanzamihigo L., Vandyke E., Demeyer D.I., Degradation of methionine by rumen contents in vitro and efficiency of its protection, Anim. Feed Sci. Technol. 67 (1997) 339–347.

[12] Michaux C., Stasse A., Sonnet R., Leroy P., Hanset R., La composition de la carcasse de taureaux culards Blanc-Bleu Belge, Ann. Med. Vet. 127 (1983) 349–375.

[13] Rulquin H., Détermination de certains acides aminés limitants chez la vache laitière par la méthode des administrations post-ruminales, Reprod. Nutr. Dev. 27 (1987) 299–300.

[14] S.A.S., SAS user's guide: Statistics (Version 5 Ed.), SAS Inst. Inc., Cary, NC, 1985.

[15] Schwab C.G., Bozak C.K., Whitehouse N.L., Mesbah M.M.A., Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation, J. Dairy Sci. 75 (1992) 3486–3502.

[16] Sulu N., Bjørnstad K., Grønseth D., Velle W., Ruminal degradation and outflow of amino acids in cows, J. Vet. Med. A 36 (1989) 55–63.

[17] Tamminga S., Van Straalen W.N., Subnel A.P.J., Meijer R.G.M., Steg A., Wener C.J.G., Block M.C., The Dutch protein evaluation system: The DVE/OEB system, Livest. Prod. Sci. 40 (1994) 139–155.

[18] Titgemeyer E.C., Merchen N.R., Berger L.L., Deetz L.E., Estimation of lysine and methionine requirements of growing steers fed corn silage-based or corn-based diets, J. Dairy Sci. 71 (1988) 421–434.

[19] Udén P., Colucci P.E., Van Soest P.J., Investigation of chromium, cerium and cobalt as digesta flow markers in rate of passage studies, J. Sci. Food Agric. 31 (1980) 625–632.

[20] Van Es A.J.H., Van der Honing Y., Het nieuwe energetishe voederwaarderingssysteem voor herkauwers: Wijze van afleiding en uiteindelijk voorstel, Report IVVO 92 (1977) 1–48.

[21] Van Soest P.J., Robertson J.B., Lewis B.A., Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597.

[22] Velle W., Sjaastad Ø.V., Aulie A., Grønset D., Feigenwinter K., Framstad T., Rumen escape and apparent degradation of amino acids after individual intraruminal administration to cows, J. Dairy Sci. 80 (1997) 3325–3332.

[23] Velle W., Kanui T.I., Aulie A., Sjaastad Ø.V., Ruminal escape and apparent degradation of amino acids administrated intraruminally in mixtures to cows, J. Dairy Sci. 81 (1998) 32331–3238.

[24] Volden H, Velle W., Harstad O.M., Aulie A., Sjaastad Ø.V., Apparent ruminal degradation and rumen escape of lysine, methionine, and threonine administrated in mixtures to high yielding cows, J. Anim. Sci. 76 (1998) 1232–1240.