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Role of branched-chain amino acid intravenous infusion in nitrogen utilization in growing ruminants

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ABSTRACT

Investigations were made of the effects on nitrogen retention of continuous intravenous infusions of branched-chain amino acids (BCAA) with glucose. Eight wether lambs (35 kg) fed a full feed diet (2.68 Mcal ME and 129 g CP kg^{-1}, DM basis) twice daily (50 g DM $W^{-0.75}$ d^{-1}) at a level of 1.2 times their maintenance energy requirement were continuously infused intravenously (jugular) with L-leucine (Leu), L-isoleucine (Ile) and L-valine (Val). Infusion rates were 2.1, 1.5 and 1.7 g $lamb^{-1}$ d^{-1}, respectively, estimated to be about 50 % of that absorbed from the gastrointestinal tract and / or required for muscle protein synthesis. Glycine was added to the infusion solutions to equalize the total N infused. Glucose (12 g W $kg^{-0.75}d^{-1}$) was also supplemented in the infusion solutions to maximise N-retention. Two 4 x 4 plus extra-period Latin squares were used. In square I, where the amino acids were infused individually, the treatments were: control, Leu, Ile and Val and in square II the treatments were: control, Leu, Leu + Ile and Leu + Ile + Val. Each period lasted 6 days. Digestibility coefficients of DM and OM were 75.8 and 77.1 %, respectively, and were not affected by the treatment. Average N-digestibility was 67.1%; the Ile + Leu treatment increased N-digestibility ($P<0.03$) compared to control. None of the individually infused amino acids significantly altered urinary-N (avg. 41% of total N-intake) or N-balance (avg. 22 % of total intake). In both squares, Leu alone failed to affect urinary-N or N-retention. Nitrogen balance was statistically ($P<0.05$) improved by 42%, when Leu was infused jointly with Ile. It is concluded that Leu infusion alone does not affect N-utilization in growing lambs, but when Leu is combined with Ile, N-utilisation is improved. The infusion of Val had little effect on N-utilization.

KEY WORDS: leucine, isoleucine, valine, intravenous feeding, growing sheep, nitrogen balance

INTRODUCTION

Branched-chain amino acids (BCAA), L-leucine (Leu), L-isoleucine (Ile) and L-valine (Val), comprise about 35 % of the essential amino acids in muscle protein and about 40 % of the amino acids required in the diet of mammals. Since BCAA make up almost 50 % of the essential amino acids in the food supply, unless the animals receive a low protein diet (Harper et al., 1984; Cronje et al., 1992), deficiencies do not generally occur. Investigations carried out *in vitro* with isolated tissues and perfused organs (Pösö et al., 1982; Tischler et al., 1982), have shown however that addition of Leu increased protein synthesis and decreased protein degradation, whereas Ile and Val had little effect on protein turnover. Furthermore, in a number of studies with postoperative patients infusion of BCAA improved nitrogen (N) balance (Freund et al., 1979, 1980). On the other hand, Harper et al. (1984) revealed that consumption of a high Leu diet by rats, ducks, pigs and turkeys resulted in retarded growth rates.

The purpose of this experiment was to study the influence of continuously infused BCAA on urinary-N and N-balance in growing sheep. Infusion of these amino acids was accompanied by glucose since amino acids play a significant role in gluconeogenesis in ruminants (Bergman, 1983). Exogenous glucose diminishes endogenous gluconeogenesis in ruminants by up to 50 to 60% (Judson and Leng, 1973) and glucose infusion (12 g of glucose $kg^{-0.75}$ d^{-1}) maximally depresses urinary-N excretion (Matras and Preston, 1989) thus improving N-balance. Under these conditions, it was expected that the infused BCAA would be utilized for protein synthesis rather than for gluconeogenesis and / or oxidation.

MATERIAL AND METHODS

The experiment was carried out at Texas Tech. University, Lubbock, USA. Eight Rambouillet-cross wether lambs (35.2 kg, SD = 4.2) placed in metabolism stalls were fed twice a day a full-feed mixture, containing chopped lucerne hay, cottonseed hulls, cracked maize, cane molasses, soyabean meal and mineral-vitamin additives. The diet contained 2.68 Mcal metabolizable energy (ME) and 129 g of crude protein (CP) kg^{-1} DM. The animals received the mixture in an amount of 50 g DM W $kg^{-0.75}$ day^{-1}, which was an average equivalent of 1.5 times their maintenance energy requirement. Lambs were infused intravenously (jugular) according to the method described by Matras and Preston (1989). The experimental design was two 4 x 4 plus extra period Latin squares which estimate direct and residual effects of treatments (Lucas, 1957). The BCAA infused were L-leucine (Leu), L-isoleucine[1] (Ile) and L-valine[1] (Val). In square I the amino acids were infused

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TABLE 1

Experimental treatments and amino acid infusion rates, g $lamb^{-1}d^{-1}$

Square	Treatment	
	BCAA[a]	amount[b]
I	Control	–
	Leu	2.1
	Ile	1.5
	Val	1.7
II	Control	-
	Leu	2.1
	Leu + Ile	2.1 + 1.5
	Leu + Ile + Val	2.1 + 1.5 + 1.7

[a] branched-chain amino acid; Leu = L-leucine, Ile = L-isoleucine, Val = L-valine

[b] glycine, 3.2, 2.0, 2.3 and 1.9 g $lamb^{-1}$ day^{-1} in control, Leu, Ile and Val treatments, respectively (Square I) and 3.2, 2.0 and 1.1 g $lamb^{-1}$ day^{-1} in Control, Leu and Leu + Ile treatments, respectively (Square II) was added to the infused solution, to equalize the total N infused

individually whereas in square II they were infused in combination (Table 1). The amounts of the infused amino acids were calculated to be equal to approximately 50 % of that absorbed from the gastrointestinal tract and /or required for muscle protein synthesis. To equalize the total N infused, glycine was added to the infusion solutions. The amino acids were dissolved in sterilized saline and glucose solution. Glucose was infused at a rate of about 12 g glucose W $kg^{-0.75}$ d^{-1} (Matras and Preston, 1989).

Each infusion period lasted 6 d; faeces and urine were collected during the last 5 d of each period to determine DM, organic matter (OM) and N-digestibility coefficients, N excreted in the urine and N-balance.

Each square was statistically analysed, using procedures outlined by Lucas (1957).

RESULTS AND DISCUSSION

All results and statistical comparisons are the direct effect of treatments since there were no significant residual effects.

Feed intake was constant for each lamb during the entire experiment. The intake in square I and II was: DM – 718 and 713 g; OM – 672 and 667 g; N – 14.8 and 14.7 g $lamb^{-1}$ d^{-1} respectively. Average digestibility coefficients of DM and OM were 75.8 and 77.1%, respectively. They were similar in both Latin squares and were not affected by the treatment. The average N-digestibility was 67.1% and was not affected by individually infused amino acids; however, when Leu and

TABLE 2

Influence of Leu, Ile and Val infused individually on urinary-N excretion and N-retention in growing lambs – Square I

Item	Treatment				SE
	control	Leu	Ile	Val	
N excreted in urine, g day^{-1}	6.39	6.79	6.24	5.93	0.22
% of N intake[1]	41.0	43.5	40.0	38.0	1.40
N retention, g day^{-1}	3.51	3.08	3.55	3.73	0.21
N retention: N intake, %	22.5	19.7	22.8	23.9	1.36

[1] daily N intake in the diet + N in the infused amino acids (14.8 g + 0.6 g = 15.6 g)

Ile were infused together N-digestibility increased by about 4% ($P<0.03$) compared to control.

Leucine infused alone did not change ($P>0.05$) the amount of N excreted in urine or N retention in growing wethers (Table 2 and 3). Isoleucine and Val diminished the quantity of urinary N excreted, primarily when added to Leu (square II), but the differences were not significant.

Neither Leu nor Ile or Val infused individually significantly changed N retention in lambs (Table 2). Jensen and Asplund (1979), using wether lambs (36 kg) infused intraruminally with VFA and intravenously with increasing levels of Leu, observed an improvement in N-balance with infusions of up to 4 to 6 g Leu lamb^{-1} d^{-1} and a deterioration in N-balance with above 6 g Leu lamb^{-1} d^{-1} infusion. In our work, the infusion of 2.1 g lamb^{-1} day^{-1} in addition to an estimated 4 g of Leu absorbed from the gastrointestinal tract had no effect on N-balance. Although Leu or Ile infused alone did not affect protein utilization, these amino acids infused conjointly, improved ($P<0.05$) N-balance by 42% in comparison with control and by 31% in comparison with treatment where Leu was infused alone. Infusion of all three BCAA also improved N-retention but not to the extent observed in Leu plus

TABLE 3

Influence of Leu, Ile and Val infused in combination on urinary N excretion and N-retention in growing lambs – Square II

Item	Treatment				SE
	control	Leu	Ile	Val	
N excreted in urine, g day^{-1}	7.06	7.13	6.35	6.45	0.25
% of N intake[1]	45.5	46.0	41.0	41.6	1.64
N retention, g day^{-1}	2.75^{a}	2.97^{a}	3.90^{b}	3.3^{ab}	0.21
N retention: N intake, %	17.7^{a}	19.2^{a}	25.1^{b}	21.3^{ab}	1.37

[1] daily N intake in the diet + N infused (14.7 g + 0.6 g = 15.5 g)
a, b = $P < 0.05$

Ile treatment. These results confirm the observations of Kishi et al. (1980) who noted an improved N-balance when BCAA were infused into rats at a rate estimated to be 40% over requirement.

CONCLUSIONS

Leucine, Ile and Val infused individually with glucose did not significantly alter N-utilization in growing lambs. However, when Leu was infused with Ile, a significant improvement in N-utilization was noted. This may indicate that these two BCAA together are limiting amino acids in growing lambs. The simultaneous infusion of glucose probably minimized the utilization of the amino acids for gluconeogenesis and reduced body protein degradation.

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