
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

Effect of duodenal infusion of protein or amino acids on nitrogen retention of lambs consuming fresh herbage

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ABSTRACT

Lambs in pens were infused duodenally with increasing amounts of casein or a mixture of four essential amino acids (EAA) to examine effects upon N retention. Eight Dorset Down x Coopworth lambs (39±1.1 kg) each fitted with rumen and duodenal cannulae were housed indoors and offered freshly cut ryegrass/white clover pasture at a rate of 470g fresh herbage/kg$W^{0.75}$(MW)/d. The metabolizable energy intake from herbage was approximately 870 kJ/kg(MW)/d.

Experimental treatments were duodenal infusions of water (control), 3, 6, 9 and 12g casein/kgMW/d and three levels of a mixture of AA (methionine, lysine, histidine and arginine) in amounts equivalent to the supply of these AA in 3, 6 and 9g casein. Treatments were allocated in an 8 x 8 latin square design.

Duodenal non-ammonia N (NAN) flow indicated that 33% of forage protein N intake was lost across the rumen, the mean flow being 1.46gNAN/kgMW/d. Infusions of casein resulted in significant linear increases ($P<0.01$) in N balance up to 6g casein/kgMW/d, the casein N supply at this level being equal to the N lost across the rumen from the basal forage. The increase in N balance with infusion of 6g casein was equivalent to an additional 96% of the N balance observed with the control treatment. No further significant change in N balance was observed with casein infusions greater than 6g/kgMW/d. A small non-significant response to infusions of 4 AA was observed indicating that these 4 essential AA were not solely responsible for the response. These results indicate that liveweight gain responses can be expected if the losses of protein that occur in the rumen of lambs grazing temperate pastures can be reduced.

Keywords Duodenum; protein; amino acids; retention; lambs; fresh herbage.

INTRODUCTION

Animals consuming high quality fresh pasture have been shown to lose up to 40% of ingested nitrogen (N) across the rumen (Ulyatt, *et al.*, 1975; Cruickshank, *et al.*, 1985; Ulyatt, *et al.*, 1988), with duodenal N flow being only 60% of the N eaten. The resulting effect on liveweight gain (LWG) is not known.

Increases in LWG or N retention have been observed in response to increasing post-ruminal protein or amino acid (AA) supply of animals consuming fresh pasture (Barry, 1981; Poppi, *et al.*, 1988). This suggests that ruminants offered fresh high quality herbage may be absorbing insufficient protein or that there are specific limiting AA. Storm and Ørskov (1984) established that methionine (Meth), lysine (Lys), histidine (Hist) and arginine (Arg) were the first four EAA limiting body growth in lambs, where rumen microbial protein was the sole source of nitrogen.

This experiment was undertaken to determine the responses in N balance to duodenal supplements of either casein or mixtures of synthetic AA in lambs offered a basal diet of fresh ryegrass/white clover pasture.

MATERIALS AND METHODS

Animals

Four entire and four cryptorchid Dorset Down x Coopworth lambs of average liveweight 39 (± 1.1) kg were housed indoors in individual metabolism crates. All lambs had previously been surgically prepared with rumen and T-piece duodenal cannulae.

Feeding

Animals were offered freshly cut ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) (60:30) pasture at a rate of 470g fresh pasture/kgMW/d in four feeds between 10 a.m. and 10 p.m. The energy input from herbage was approximately 870 kJ/kg$W^{0.75}$/d. The lambs were weighed every ten days and individual amounts of herbage offered were adjusted for changes in liveweight. The feed offered and feed refusals were weighed and sampled daily throughout the trial. Pooled samples were analyses for dry matter (DM), organic matter (OM), nitrogen (N) and neutral detergent fibre (NDF) content.

Digestibility and digesta flow trial

A seven day digestibility trial was performed at the start of the experiment in early February, 1989, using the eight lambs mentioned above. Total faecal collections were weighed daily, bulked over the seven days for each lamb, sampled and analysed together with herbage samples for DM, OM, N and NDF content.

Immediately following the digestibility trial six lambs were used to estimate the net transfer of ingested N through the rumen. Two markers, Chromium ethylene diamine tetra- acetic acid (Cr-EDTA) and Ytterbium acetate (Yb), were continuously infused into the rumen for a total of six days and duodenal digesta and faecal samples collected every 4 hours on the last two days, as described by Cruickshank (1986). Samples were analysed for DM, OM, ammonia (NH_3N) and N content. Non-ammonia N flow to the duodenum was estimated by the double marker technique of Faichney (1975).

Protein/AA infusion trial

An 8 x 8 latin square design was used. The eight experimental treatments were duodenal infusions of water (Control), four levels of casein infusion (3, 6, 9 and 12g casein/kgMW/d) and three levels of AA infusion. The maximum level of casein infusion was calculated to allow maximum potential N retention, as suggested by Cropper (1987). The AA infusions included Meth, Lys, Hist and Arg and were present in the same proportions (17:44:17:22) as found in casein.

A 10% lactic casein stock solution was prepared (MacLeod, *et al.*, 1982) and individual daily allowances diluted with water to a volume of 2 kg. AAs were weighed individually and added to 2 kg of water on a daily basis. The daily infusate of 2 kg was continuously infused into the duodenum over a 23 hour period. All infusates were stored at +7°C until required.

Treatment periods lasted for 5 days and followed consecutively and quantitative collections of urine and faeces were made over the last 3 days of each treatment period. The length of treatment and collection periods was chosen as a result of previous evidence which showed that production responses to abomasal infusions of casein were very rapid and were evident within the first 24hr of the new treatment being imposed (Ørskov, *et al.*, 1977, Whitelaw, *et al.*, 1986, Fraser, 1988). Sub-samples from these collections were bulked and the resulting samples were analysed for N content. Faecal samples were also analyses for DM content. N balance was calculated by difference from intake of N from herbage and casein or AA and excretions of N in urine and faeces. The data were analysed by analysis of variance.

RESULTS

TABLE 1 Mean values for nitrogen (N) intakes and N balance (mgN/kgMW/d) for lambs consuming fresh ryegrass/white clover pasture and receiving duodenal infusions of water, casein or amino acids.

Treatment	N Input (mgN/kgMW/d)				N Balance (mgN/kgMW/d)
	Forage	Casein	AA	Total	
Water	2311	-	-	2311	431^{a}
3g cas./kgMW/d	2285	395	-	2680	636^{ab}
6g cas./kgMW/d	2270	793	-	3064	845^{bc}
9g cas./kgMW/d	2330	1191	-	3520	902^{c}
12g cas./kgMW/d	2314	1565	-	3880	930^{c}
4AA as in 3g cas.	2267	-	104	2371	546^{a}
4AA as in 6g cas.	2261	-	207	2468	586^{a}
4AA as in 9g cas.	2309	-	309	2617	617^{a}
SED					86

Different alphabetical notations represent significant differences ($P<0.05$).

Herbage characteristics

The herbage fed throughout the experiment was of high N content, 31.0 (±0.96) g N/kg/DM, and high DM, OM, N and NDF digestibilities; 0.77 (± 0.02), 0.77 (± 0.07), 0.75 (± 0.03) and 0.78 (± 0.02) respectively.

The mean values of nitrogen intake (NI) from forage and non-ammonia nitrogen (NAN) (N - NH_3N) flow at the duodenum (g/kgMW/d) were 2.18 (± 0.03) and 1.46 (± 0.05) respectively. Therefore, the mean transfer of forage nitrogen to NAN at the duodenum was 0.67 (± 0.02).

Nitrogen balance

The N balance data are presented in Table 1.

Infusion of casein resulted in significant increases in N balance up to 6g casein/kgMW/d, after which no further significant increases were observed. Increases in N balance were apparent with AA infusion but these were not significant.

Efficiency of utilization of amino acid nitrogen

The linear relationship, y = bx + c, was established for the regression of nitrogen balance (gN/kgMW/d) on the supply of absorbed AAN (gAAN/kgMW/d) from the small intestine, with the slope 'b' being the coefficient of efficiency of utilization of amino acid nitrogen (EOU_{AAN}). The supply of absorbed AAN (Abs. AAN) was calculated as follows:

Abs. AAN = NAN (basal diets) X AAN/NAN (0.8) X T.D. (0.85)
+ Casein N X AAN/Cas.N(0.89) X T.D. (0.95)

TD = True digestibility.

Relationships were established for the regression of N balance on the supply of absorbed AAN, firstly for the control, 3 and 6g casein infusion (treatments A, B and C), secondly for 6, 9 and 12g casein infusion (treatments C, D and E) and thirdly the control and the three levels of AA infusion. The regression equations were as follows:

(1) y = 0.68 (± 0.17) x -0.29 (r=0.65);

(2) y = O.29 (SE 0.16) x +0.29 (r=0.36);

(3) y = 1.07 (SE 0.04) x -0.73 (r=0.61).

Therefore, the EOU_{AAN} when the supply of casein was less than 6 g casein/kgMW/d was 0.68 while increasing the supply of N above this level resulted in a drop in efficiency to 0.29. Although AA infusion did not produce a significant response in N balance, it resulted in increasing EOU_{AAN} to 1.07.

DISCUSSION

The high quality of the ryegrass/white clover pasture in this experiment, as indicated by the high N content (31.0g N/kg DM, which converts to 34.9 g N/kg OM) and DM, OM and N digestibilities (0.77, 0.77 and 0.75 respectively), resulted in a 33% loss of ingested N across the rumen. This is in agreement with studies in both cattle and sheep (Beever, *et al.*, 1986; Cruickshank, 1986; Ulyatt, *et al.*, 1988), where it has been indicated that losses of ingested fresh forage N would occur across the rumen when the N content of the forage exceeds 25.5g N/kg OM.

The objective of this experiment was to determine the effect of duodenal casein N infusions on N balance of lambs consuming high quality pasture.

The highly significant (P<0.01) increases in N balance observed with duodenal infusion of casein in this trial indicates that the supply of protein or EAA from this herbage was limiting for growth in lambs. This is supported by the results of Black *et al.* (1979), Barry (1981) and Poppi *et al.* (1988), who observed increases in LWG of lambs in response to increased supply of protein to the duodenum of lambs consuming fresh pasture. Protein may have been used as a precursor for glucose synthesis but Black *et al.* (1979) found little response to glucose infusion, so it is more probable that it is an amino acid response.

The linear response in N balance to increasing casein infusion up to 6g casein indicates that protein supply was limiting. Black and Griffith (1975) stated that, when energy supply was constant and N supply was limiting, N balance was linearly related to the supply of absorbed AAN, but, when N supply was not limiting, no response in N balance was obtained to increasing AAN supply indicating that energy supply was the limiting factor. This would suggest that the lack of response in N balance with infusions greater than 6g casein was due to limiting energy supply. The supply of N from 6g casein was found to be equivalent to 34%

of the N intake from pasture. Therefore, responses in N balance can be expected to increase the absorbed AA supply to the small intestine up to a level equivalent to the protein N lost in passage of digesta across the rumen.

The efficiency of utilization of AAN (EOUAAN) in this trial, 0.68, at low levels of casein infusion is similar to the value of 0.80 established by Storm and Ørskov (1984) for the EOU_{AAN} of rumen microbial protein (RMP). However, this value, 0.68, is in contrast with the low value of 0.51 obtained by Barry (1981), who gave abomasal infusions of casein and methionine to lambs consuming fresh ryegrass/white clover pasture. The latter value, may be a reflection of the use of a single level of casein infusion. If increasing amounts of casein infusion had been used instead of a single level, a response in protein deposition similar to that shown in the present experiment may have been obtained. The data of Storm and Ørskov also support the findings that, at constant energy input, a reduced response in N balance to high levels of N input is due to limiting energy supply.

The EOUAAN obtained with EAA infusion was found to be approximately 1.0. This increase in EOUAAN relative to that of low levels of casein infusion, 0.68, indicates that an AA deficiency had been alleviated to a certain extent but this was not enough to produce significant responses in N balance.

Since protein supply to the lamb in this experiment was found to be limiting for high quality pastures, it is possible that the supply of one or more EAA may have been limiting. Storm and Ørskov, (1984) found that Meth, Lys, Hist and Arg were the first four limiting AA for growth in lambs, where rumen microbial protein was the sole source of N. Other studies (Reis and Schinkel, 1964) have shown that deficiencies in the supply of sulphur AA from forage based diets restricts both wool growth and protein deposition in the wool-free body. The four AA mentioned above were, therefore, infused duodenally in quantities equivalent to those in casein. Although N balance did show a slight increase with AA infusion this was not significance. Theoretically, if these four AA were limiting, N balance would be similar to that of the equivalent casein infusion (balanced for the 4AA; Table 1). This suggests that some other AA(s) were limiting for growth. The response in N balance obtained with the infusion of the highest level of AA infusion was similar to that of 3g casein infusion. This may indicate that the response was due to a deficiency of all AA. However, the increase in N balance to AA infusion was low and non-significant. The difference in response to casein or AA infusion is more likely to be due to limitations in the essential AA supplementation used in this experiment, possibly the ommission of threonine. Nimrick, *et al.* (1970) suggested that the first three limiting AA for growth in lambs fed urea as the sole source of N were Meth, Lys and Threonine (Thr) and Storm and Ørskov, (1984) indicated that Thr was the fifth limiting AA.

CONCLUSIONS

It was concluded that N retention will increase in response to increasing absorbed AA supply from the intestines at levels equal to the protein N lost in passage of digesta across the rumen. AA infusion had a small but non-significant effect on nitrogen balance indicating that other AA(s) may be limiting for growth.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs J. Nahkies, Mr D. Herrick and Mr S. Kirsopp for their help with running the trial, Mr P. Isherwood, Mr G. Elmsley and Mr J. Thackwell for assistance with chemical analysis and all other members of the Animal Science Department who helped with sampling during the trial.

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