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Amino acid flow at the abomasum in twin-suckling ewes at pasture and the effect of a fishmeal supplement

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ABSTRACT

Fourteen twin-suckling ewes with rumen and abomasal cannulae grazed a ryegrass/clover sward (C; n = 7) or the same sward but with a 500 g/h/d protein supplement (S; n = 7). The trial was designed as a cross-over with two 14 day adaptation periods followed by two five day sampling periods (Days 43-48 and 63-68 after lambing). All ewes were treated with anthelmintic 14 days after lambing. Flow of amino acids (AA) at the abomasum and dry matter (DM) intake were measured during both periods using intra-ruminally infused markers. Diurnal variation in AA flow peaked between 12:00 and 15:00 h and was greatest in the supplemented ewes. Flows of AA were increased by supplementation; Essential AA, +16 %; 128 to 148 g/d, Sulphur-AA, +21.2 %; 3.3 to 4.0 g/d, Branched-chain AA, +15.9%, 47.8 to 55.4 g/d. There was evidence for a lower faecal egg count in supplemented animals, being 670 vs 46 epg, respectively, in C and S groups, (P = 0.08) 21 days after anthelmintic treatment. This work provides information on the extent of enhancement of the supply of amino acids associated with ability to limit the peri-parturient increase in faecal egg count and provides a basis for the development of supplements.

Keywords: amino acids; pasture; parasitized ewes; immunity; bacterial nitrogen.

INTRODUCTION

The peri-parturient breakdown of immunity to nematode parasites plays an important role in the epidemiology of infections in sheep (Houdijk *et al.*, 2005). The work of Donaldson *et al.* (2001) suggested, from indoor studies, that its prevention can be achieved when sheep are offered 130% of current estimates of metabolisable protein (MP) requirement for production (Agriculture and Food Research Council, 1993) and the work of Houdijk *et al.* (2000) suggests that the immune response is more sensitive to MP supply than milk production.

Supplementation studies on typical New Zealand pastures (A.R. Sykes, Unpublished data) suggest that immunity can be maintained at an estimated MP supply of >300g/d. Ultimately a single limiting amino acid (AA) supply or mixture of particular amino acids required by the immune response are likely to be responsible. Certain amino acids such as glutamine (Hoskin *et al.*, 2002), and cysteine (Liu & Karlsson, 2004) have been suggested as critical. However, there are few data on amino acid supply in ewes grazing typical New Zealand pastures during early lactation. This study was designed to provide these data and the changes induced by a protein supplementation strategy which has previously been demonstrated to be effective in reducing the peri-parturient relaxation of immunity.

MATERIALS AND METHODS

Animals, treatments and supplementation

Two weeks prior to lambing, 14 twin-bearing Coopworth ewes weighing 65.1 ± 1.50 kg and fitted

with rumen and abomasal cannulae were randomly allocated to a cross-over treatment design incorporating grazing ryegrass/clover alone (C; n = 7) or ryegrass/clover with 500g/d of a protein supplement (S; n = 7). Supplementation was increased stepwise after lambing until ewes consumed 500 g/d on a group basis. At the same time the ewes were trained to a daily routine of movement to individual pens and the manipulation of equipment for infusion of digesta markers and digesta collection. From day +35, relative to lambing, protein supplementation was provided on an individual basis in the individual pens and on day +38 relative to lambing, digesta marker infusion commenced. On day +43 a five day period of digesta sampling began. Treatment groups were then reversed and a second adaptation period to feeding commenced and lasted for 14 days followed by a five day sampling period.

A protein supplement, consisting of 32% Broll, 24% Barley, 20% Soya, 20% Fishmeal and 4% Molasses (Table 1), was offered daily at 09:00 h. The formulation and rate of supplementation were based on the predictions of Donaldson *et al.* (2001) for MP supply consistent with restoration of immune function during the peri-parturient period. The treatments were anticipated to provide 1.0 x metabolisable energy (ME) requirement in both groups, with 1.0 and 1.3 x MP in C and S ewes, respectively, based on Agriculture and Food Research Council (1993) recommendations for twin suckling ewes. Animals grazed potentially nematode contaminated pastures. In order to minimise possible differences in larval intake as a result of

TABLE 1: Chemical composition of the protein supplement.

Component	Composition
Dry matter	86.1%
Organic matter	91.6%
Crude protein	33.8%
Crude fat	4.2%
Acid detergent fibre	7.1%
Neutral detergent fibre	29.5%
Digestible organic matter	88.9%
Metabolisable energy	135 MJ/kg dry matter

supplementation they were grazed in separate areas behind electric fences to force them to graze to the same sward height. All ewes were drenched with levamisole (7.5 mL/kg LW; Novartis, New Zealand Ltd), on day +14 after lambing.

Measurement of intake and amino acid flows at the abomasum

The double marker method of Faichney (1980) was used. For five days before and during the sampling periods ewes received a continuous intraruminal infusion of chromium-ethylenediaminetetra acetic acid (EDTA) at the rate of 2.77 g/d in one litre of solution and ytterbium acetate at the rate of 150 mg/d in one litre of solution pumped by peristaltic pumps from reservoirs held on the sheep's backs. Digesta sampling provided samples at three hourly intervals during the 24 hour day but was done at intervals of 12 and 15 hours over the five days to reduce disturbing the pattern of grazing. Abomasal samples were collected into 500 mL plastic containers. Samples were immediately placed on ice before transfer and storage at -20 °C until analysis. The samples were freeze-dried and ground to pass through a 0.5 mm sieve (Tecator 1094 homogenizer, France) prior to analysis for amino acids.

To estimate nutrient intake, an alkane pellet (C32; 150 mg) was placed onto the rumen mat via the rumen cannula at 09:00 h daily for three days prior to, and during, sampling.

Faecal and pasture sampling

On day -17, relative to lambing and weekly thereafter a faecal sample was taken to determine the concentration of gastrointestinal parasite eggs (FEC) being shed in the faeces. During the five day digesta sampling period, faecal samples were taken daily and bulked for each animal, frozen and later analysed for alkane concentration. Pasture samples were obtained twice weekly during digesta sampling and stored at -20°C pending analysis. Pasture sampling for alkane analysis was also done two days prior to digesta sampling and two days before the end of sampling, during both periods. Supplement was sampled daily during feeding, bulked and stored until analysed for chemical composition.

Analyses

FECs were measured according to the method of the Ministry of Agriculture, Fisheries and Food (1979). Pasture and supplement samples were analysed for CP and ME by near infrared spectroscopy (NIR Systems Model 5000, Foss, Hillerød, Denmark). Pasture and faecal samples were analysed for alkanes according to the method of Dove *et al.* (1996) and estimates of DM intake made according to the formula of Dove and Mayes (1991). Metabolisable protein (MP) supply was estimated according to Agriculture and Food Research Council (1993) recommendations. Chromium and ytterbium were determined in the abomasal digesta by an atomic absorption spectrophotometer (Perkin-Elmer, 5100 PC, Norwalk, Connecticut, USA), the former according to Murthy *et al.* (1971) and the latter according to the method of Siddons *et al.* (1985). Freeze-dried abomasal digesta samples were analysed, after hydrolysis, for 10 amino acids by high performance liquid chromatography according to the methods of Heems *et al.* (1998) and Carducci *et al.* (1996). Cysteine and methionine were analysed according to modified methods of McNabb *et al.* (1993), hydrolysis being undertaken after the amino acids were oxidised with performic acid.

TABLE 2: Nutrient intakes by twin rearing ewes grazing spring pasture alone (C) or pasture and a protein supplement (S) during Periods 1 and 2 (Days 43-48 and 63-68) after lambing.

Parameter	Treatment		Period		Significance of main effects	
	C	S	1	2	Treatment	Period
Dry matter intake (g/d)	2,629 ± 140	2,676 ± 135	2,874 ± 135	2,431 ± 140	NS	*
Metabolisable energy intake (MJ ME/d)	29.6 ± 1.5	30.9 ± 1.5	34.3 ± 1.4	26.2 ± 1.5	NS	***
Metabolisable protein supply (g/d)	309 ± 13	434 ± 13	400 ± 13	344 ± 13	***	**
Metabolisable protein relative to requirements	1.0	1.4	1.3	1.1		

Statistical analysis

Time series data was analysed as repeated measures using Proc Mixed procedure (SAS, 2002-2003) with ewe x treatment x period as a random effect. Results are reported as least squares means. For FEC, statistical inference was based on log transformed ($\log_{10}(\text{FEC} + 1)$) data.

RESULTS

Pasture, nutrient and amino acid intake

One C group ewe was removed from the trial due to failure of the abomasal cannula. Dry matter and nutrient intakes are shown in Table 2. Dry matter and ME intakes were similar ($P > 0.05$) between C and S animals. Estimated MP supply was significantly ($P < 0.001$) lower for C than S ewes. Intakes of DM ($P < 0.05$), ME ($P < 0.001$) and MP intake ($P < 0.01$), were higher during sampling Period 1 than sampling Period 2.

Abomasal dry matter and amino acid flows

Flows of abomasal AA are shown in Figures 1 and 2, and Table 3. Supplementation significantly ($P < 0.001$) increased flows of the measured AA. This increase occurred immediately after offering the supplement at 09:00 h and lasted up to 21:00 h (Figures 1 and 2).

Faecal egg count

The trends in FEC are shown in Figure 3. Twenty one days after de-worming, the FEC of C rose rapidly compared to S animals (670 vs 46, respectively; $P = 0.08$).

DISCUSSION

This trial was designed to quantify AA supply at pasture and during protein supplementation expected to result in a reduction in FEC as anticipated from the work of Donaldson *et al.* (2001). MP supply was estimated to be 1.4 times the Agriculture and Food Research Council (1993) recommendations in S ewes while it was 1.0 x MP requirement in C ewes (Table 2).

The diurnal fluctuation in AA flow in C ewes (Figures 1 and 2), which was greatest around midday and late evening, was similar to that observed by Dove *et al.* (1988) in one of the few previous studies with lactating ewes on pasture. There are no data on AA flow rates in parasitized sheep on pasture. When compared with *T. colubriformis*-infected lambs offered fresh forages indoors (Birmingham *et al.*, 2008) AA flows, adjusted for metabolic body weight; $LW^{0.75}$, were greater in the present sheep (5.8 vs 3.8 g/d/kg for essential AA and 10.6 vs 8.3 g/d/kg for total AA). When the flow of amino acid of C ewes was expressed per unit of DM intake flows (91.9g/d/kgDM) were virtually identical to rates

FIGURE 1: Abomasal total amino acid flows in parasitized twin-rearing ewes grazing spring pasture with or without a protein supplement.

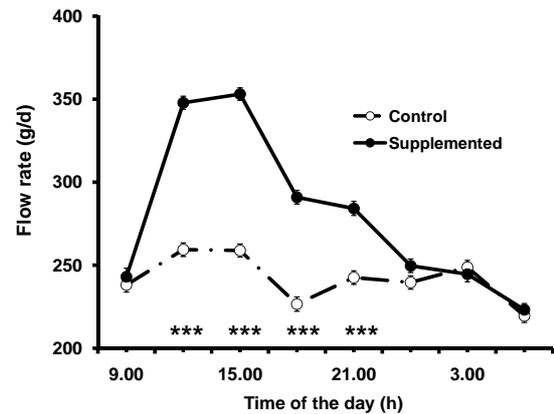


FIGURE 2: Abomasal essential amino acid flows in parasitized twin-rearing ewes grazing spring pasture with or without a protein supplement.

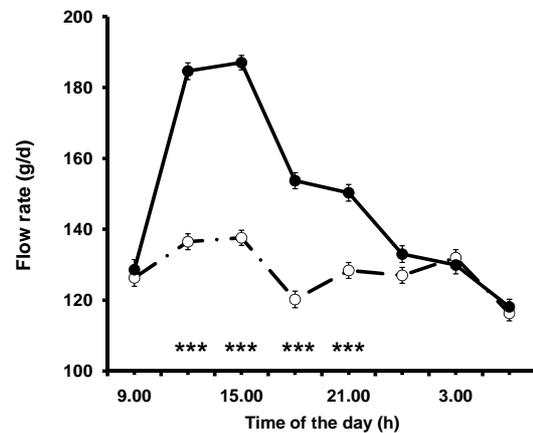
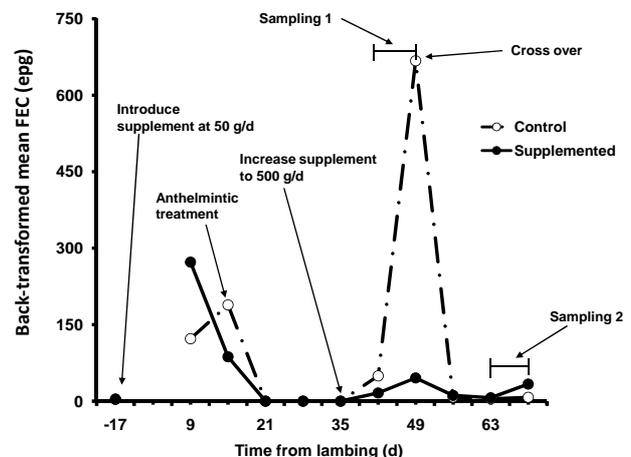


FIGURE 3: Back-transformed faecal egg counts of twin-rearing ewes grazing spring pasture with or without a protein supplement.



observed by Kolver *et al.* (1999) in lactating dairy cows offered an *ad lib* allowance of ryegrass/white clover or only ryegrass (93.0 and 97.6 g/d/kg DMI, respectively). Relative flow rates of individual amino acids also appear typical; especially when

TABLE 3: Flow of amino acids through the abomasum (g/d) in twin rearing ewes grazing spring pasture alone (C) or pasture and a protein supplement (S) during Periods 1 and 2 (Days 43-48 and 63-68) after lambing. B = Branched chain amino acid, E = Essential amino acid, S = Sulphur amino acid. SEM = Standard error of the mean.

Parameter	Treatment		Period		SEM	Significance of main effects			
	C	S	1	2		Treatment	Time	Period	Treatment x Time
Arginine (E)	15.9	18.5	18.6	15.7	0.1	***	***	***	***
Aspartate	16.6	20.6	16.6	20.6	0.6	***	***	**	***
Cysteine (S)	1.62	1.93	1.90	1.65	0.03	***	***	***	***
Glutamine	16.5	19.3	19.3	16.5	0.2	***	***	***	***
Histidine (E)	15.4	17.6	17.8	15.2	0.2	***	***	***	***
Isoleucine (B,E)	15.8	18.2	18.4	15.5	0.1	***	***	***	***
Leucine (B,E)	16.2	19.0	19.1	16.1	0.1	***	***	***	***
Lysine (E)	16.2	19.0	19.1	16.1	0.1	***	***	***	***
Methionine (E,S)	1.68	2.08	2.01	1.75	0.05	***	***	**	***
Phenylalanine (E)	15.8	18.2	18.5	15.5	0.1	***	***	***	***
Threonine (E)	15.9	18.3	18.6	15.6	0.1	***	***	***	***
Valine (B,E)	15.8	18.1	18.4	15.5	0.1	***	***	***	***
Total essential amino acids	128.0	148.1	149.8	126.4	0.8	***	***	***	***
Total sulphur amino acids	3.3	4.0	3.9	3.4	0.08	***	***	***	***
Total branched-chain amino acids	47.8	55.4	56.0	47.2	0.3	***	***	***	***
Total amino acids	241.7	279.6	283.2	238.1	1.6	***	***	***	***

supplemented ewes are compared with 18 month-old wethers offered *Lotus corniculatus* (Waghorn *et al.*, 1987). Supplementation significantly increased flows of amino acids; the increase for essential AA was 16% though the changes were not consistent amongst all amino acids. Flow rates of leucine, lysine, methionine, cysteine, glutamine and aspartate were increased by about 20%, while those of threonine, valine, isoleucine, phenylalanine, histidine, arginine and taurine increased by only 14% (Table 3).

The complex experimental demands to enable measurement of AA flow are incompatible with the large animal numbers required for parasitology Figure 3 studies. Nevertheless the evidence in for more rapid increase in FEC in unsupplemented sheep following anthelmintic treatment and its reduction when treatments were reversed, is consistent with the previously recorded benefits of high MP supply. Of the S-amino acids which were most enhanced by the supplement, cysteine (Shoveller *et al.*, 2005), and methionine which is important for replenishing the supply of cysteine (Liu *et al.*, 2000), are important in the synthesis of glutathione, a component of anti-oxidant defences (Grimble *et al.*, 1992). Glutamine, which increased function of macrophages in early-weaned mice (Rogerio *et al.*, 2008), is also a precursor for glutathione synthesis. Glutamate has been reported as important in cytokine production and regulation of the immune response (Wu *et al.*, 2004). Both glutamate and glutamine carbon skeletons can be

utilised equally well in pathways of mucosal intermediary metabolism (Reeds & Burrin, 2001). Leucine sequestration across the gastrointestinal tract from both arterial blood and digesta during absorption was increased by 24% in nematode-infected sheep (Yu *et al.*, 2000), indicating that it may have particular importance for mucosal immune function.

If hypothetically, one considers these augmented flow rates of AA to represent the needs of the immune response, one can consider the extent to which pasture intake might need to increase to achieve these without need for supplementation. On this basis it can be calculated that DM intake would have to increase to 3.2 kgDM/d for most AAs, 3.8 kgDM/d for phenylalanine, arginine, histidine and glutamine but to 9.0 kgDM/d for the sulphur AAs. This is well above the theoretical maximum intake of 2.6kgDM/d (4% of bodyweight). A prediction that sulphur AA are therefore the most likely limiting AA is consistent with the prediction of methionine to be limiting for milk production from pasture diets (Kolover *et al.*, 1999; Kolover 2003). The lack of a difference in lamb growth rates (O.R. Madibela, Unpublished data) indicates that milk production was not limited by protein supply. However, given the trend for greater FEC in unsupplemented ewes, immune responses are more sensitive than lactation to MP supply, as previously observed by Houdijk *et al.* (2000).

CONCLUSION

This trial provided information on the possible extent of enhancement of AA supply associated with limitation of the peri-parturient increase in FEC and provides a basis to develop appropriate supplements to enhance a breeding ewe's immune system around lambing.

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