Responses to protected amino acids or protected protein in dairy cows grazing ryegrass pastures in early or late lactation

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ABSTRACT

Two-hundred-and-four Friesian dairy cows (4-9 years old) were allocated to 1 of 9 groups at calving (n=22-23) in August-September 1995. All cows grazed ryegrass pastures at a stocking rate of 3.1 cows/ha and received some maize silage and barley for the first weeks after calving. In addition, they received one of three experimental supplements in early lactation (weeks 2-11) or mid lactation (weeks 12-22) or late lactation (weeks 23-33). Experimental supplements were 1 kg/d barley only (BAR), 1 kg/d barley with 17 g/d protected methionine/lysine (BML) or 1 kg/d protected protein meal (PPM). Individual pasture intake was estimated twice (5 days) during each lactation stage with sub-groups of cows (n=8) using the alkane technique. Preliminary results for cows in the early and late lactation groups are presented. In cows supplemented in early lactation, supplement intake was lower than anticipated (BAR, BML and PPM: 0.73, 0.67 and 0.41 kg/d respectively). Mean pasture intake (17.4 kg dry matter [DM]/d), milk solid production (2.07 kg/d) or changes in live weight (+23 kg) and body condition (+0.02) did not differ between groups. Gross milk composition and milk protein composition were also unaffected. In cows supplemented during late lactation, supplement intake was 0.83, 0.84 and 0.68 kg /d for groups BAR, BML and PPM respectively. Mean pasture intake (14.3 kg DM/d), milk solid production (1.49 kg/d) and change in live weight (+11 kg) during the period of supplementation did not differ between groups. Although further work with higher levels of supplementation with protein or amino acids is required, these results suggest that neither MP supply nor methionine/lysine supply were limiting milk production in dairy cows grazing ryegrass-based pastures during early or late lactation.

Keywords: dairy cows; pasture; amino acids; metabolisable protein; milk production.

INTRODUCTION

Milk production by individual dairy cows is limited primarily by intake potential and particularly metabolisable energy (ME) intake. There is good international agreement on ME requirements of cows for various levels of production (see de Boer and Bickel, 1988). However, this is not the case for the requirements for metabolisable protein (MP), and predicted MP requirements are significantly lower according to the Agricultural and Food Research Council (AFRC, 1993) than the National Research Council (1989). Similarly, experimental evidence on the extent to which MP supply limits milk production in pasture-fed dairy cows is contradictory. Some studies in New Zealand suggest that MP supply is adequate in grazing dairy cows, as their milk production did not respond to supplementation with undegraded dietary protein (UDP; Brookes, 1984; Penno and Carruthers, 1995). However, the studies by Rogers *et al.* (1980) and Minson (1981) found that increasing the supply of UDP did enhance milk yield, suggesting that MP supply from ryegrass pastures was insufficient to maximise milk production.

The studies referred to above have mostly been conducted with grazing dairy cows in the early lactation period, as this is generally the period of greatest demand for MP. Although MP demand may be reduced in mid or late lactation, the change in feed composition as the lactation season progresses can be expected to result in concurrent reduction in MP supply. There is almost no information on the effects of increasing MP supply, by supplementation with UDP, in grazing dairy cows in mid or late lactation, on milk production and milk quality. Wang *et al.* (1996) reported that the action of condensed tannins in *Lotus corniculatus* significantly increased the efficiency of milk production and increased the secretion rates of milk protein and lactose by some 13% in ewes with twin lambs in mid and late lactation but not in early lactation. These effects were probably due to an increase in absorption of essential amino acids from the small intestine.

Amino acid balance in MP is another factor which may limit milk production. Even when cows absorb an excess of MP, they may still be supplied with an inadequate balance of limiting amino acids, which in turn may constrain milk production. Rogers *et al.* (1979) suggested that in lactating cows given formaldehyde-treated casein (*i.e.* high in UDP) methionine could be a limiting amino acid. Methionine and lysine have been identified as primary limiting amino acids when microbial protein was infused as the only protein source in intragastrically-fed growing sheep (Storm and Orskov, 1984). More recent experimental evidence available from overseas suggests that methionine and lysine are the two amino acids most likely to limit milk production (Rulquin and Verite, 1993). Use of methionine and lysine protected from rumen degradation has resulted in small increases in milk protein production in some European and American studies (*eg*. Robinson *et al.*,

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1995). However, the effects of increasing the supply of these two amino acids on milk production in pasture-fed dairy cows have not been assessed. Effects on milk protein composition (casein, whey etc) and on general performance of grazing dairy cows are also unknown.

An experiment was conducted to assess the effects of supplementation of grazing dairy cows with protected methionine/lysine or with protected protein meal (high in UDP) on feed intake, milk production and composition, changes in live weight and body condition at three stages of lactation.

MATERIALS AND METHODS

Animals and feeds

Two-hundred-and-four dairy cows were selected, on the basis of age, calving date and health history, from a herd of 380 cows at the Lincoln University dairy farm, Canterbury, New Zealand. At calving (4 August to 18 September 1995), selected cows (4-9 years) were allocated randomly to one of 9 groups with 22 or 23 cows per group. Cows grazed ryegrass-based pastures on a rotational basis and as a separate herd, at a stocking rate of 3.1 cows per ha. In addition to pasture, the basal diet also included supplements of barley meal, maize silage and/or grass silage at various times during the lactation (see Figure 1).

Cows were given one of three experimental concentrates, either in early, mid or late lactation (weeks 2-11, 12-22 or 23-33 after calving respectively; see Table 1). The supplements given were ground barley with 6% molasses (BAR; 1 kg/d) or the same barley with the addition of protected methionine/lysine (BML; 1 kg/d with 17 g of SmartAmine; Rhone-Poulenc, France; providing 6.4 g additional methionine and 4.0 g additional lysine per cow per day) or a protected protein meal (PPM; 1 kg/d; Amino2000, Provimi BV, the Netherlands; based on fish meal and soyabean meal). Supplements were given to individual cows during the afternoon milking and refusals were recorded. For cows given BML, the amino acids were added to and thoroughly mixed with the barley at the time of feeding.

FIGURE 1: Amount of supplements offered to all experimental cows in addition to pasture to make up the basal diet. Barley (△), maize silage (▼) and grass silage (□).

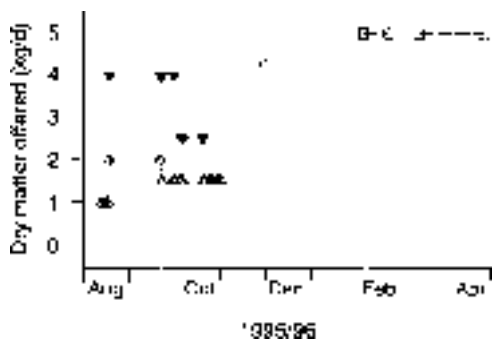

TABLE 1: Treatment groups and amounts of experimental supplements offered to dairy cows grazing ryegrass pastures at different stages of lactation.

Group[1]	Lactation stage weeks	Number of cows	Barley[2] kg/d	Amino[3] Acid g/d	Protein[4] Meal kg/d
BAR-E	2-11	23	1	0	0
BML-E	2-11	23	1	17	0
PPM-E	2-11	23	0	0	1
BAR-M	12-22	23	1	0	0
BML-M	12-22	23	1	17	0
PPM-M	12-22	22	0	0	1
BAR-L	23-33	22	1	0	0
BML-L	23-33	23	1	17	0
PPM-L	23-33	22	0	0	1

[1] BAR, PPM and BML refer to barley, protected protein meal and barley with methionine/lysine respectively.
E, M and L refer to early, mid and late lactation.

[2] Barley contained 94% barley meal and 6% molasses.

[3] Estimated to provide 6.4 g methionine and 4.0 g lysine per day.

[4] Protected protein meal based on fish meal and soyabean meal.

Experimental procedures

Changes in live weight (LW) were assessed in individual cows every three weeks from one week after calving to late April 1996. Changes in body condition score (range 1-10) were monitored from calving to January 1996, once every three weeks.

Cows were milked twice daily. Individual milk production and composition were determined from the start of the nutritional treatments for each cow till late April 1996. This was done on a weekly basis while cows were being supplemented and fortnightly after that (afternoon and next morning milking). In addition, all 204 cows were milk sampled on four occasions (afternoon and next morning milking) in early October and late November 1995 and early February and early April 1996. Sampling of milk was by Tru-Test milk meters (Allflex, New Zealand) with a sample size of 5.6 g of milk per litre produced. Milk was preserved (bronopol blue) pending analysis. Separate milk samples were collected without preservative once every three weeks during the supplementation period. These were skimmed by centrifugation, bulked for 6-8 cows in each group and stored at -20 °C pending analysis for the proportions of casein, whey and non-protein-nitrogen (NPN) in milk crude protein (CP).

Individual pasture intake by eight randomly chosen cows in each group was estimated, using the alkane marker technique (Dove and Mayes, 1991). Intake measurements were made mid-way through and towards the end of each supplementation period. In each measurement period, cows were dosed orally immediately after each milking, with a gelatin capsule containing 300 mg of dotriacontane and 300 mg of hexatriacontane for 12 days. Faecal samples were collected after each milking, during days 8 to 12 of the measurement period, and stored at -20 °C pending analysis. Samples of pasture on offer were collected daily from all paddocks grazed during days 7 to 11. Pasture

(approximately 10 g) was cut at ground level with blade shears at 40 to 50 randomly chosen locations in each paddock, and stored at -20 °C pending analysis.

Changes in nutrient composition of all feeds were monitored monthly throughout the experiment. Protein degradability of fresh ryegrass, BAR and PPM was assessed using the dacron bag technique (after Orskov *et al.*, 1980). Fresh ryegrass was collected by hand plucking in late November 1995. Samples of the chopped fresh ryegrass (1 mm chop length; 10 g per bag), BAR or PPM (5 g per bag) were incubated in triplicate for 0, 4, 8, 16, 24, 48 and 72 h in the rumens of two rumen-cannulated beef heifers (350 kg), which were grazing ryegrass-based pastures.

Analytical, mathematical and statistical methods

Individual milk samples were analysed for protein, fat and somatic cell count at the National Milk Testing Laboratory at Hamilton, using an infra-red Foss Scan 4000 (Foss, Denmark). Pooled milk samples were analysed for casein, whey and NPN content as described by New Zealand Dairy Industry (1993).

Organic matter (OM) digestibility and ME content of feeds was estimated according to the in vitro procedure of Jones and Hayward (1975).

Freeze dried faecal samples (1 g; pooled faeces for each cow within a measurement period) and pasture samples (2 g) were analysed for alkanes using the method described by Mayes *et al.* (1986) with some modifications (H. Dove, personal communication).

Feed intake of individual cows was calculated by relating the concentrations of dotriacontane and tritriacontane in herbage and faeces to the daily dose rate of dotriacontane (see Dove and Mayes, 1991). Effective degradability of ryegrass CP was calculated according to McDonald (1981), assuming a rumen outflow rate which is derived from the feeding level L, expressed as a multiple of estimated maintenance requirements (AFRC, 1993).

Treatment effects were analysed within each lactation stage by one-way analysis of variance or covariance. The effects on milk production of supplementation per se (irrespective of supplement type) were assessed for each of four milk sampling periods (early October, late November, early February and early April 1996) by comparing milk production of cows supplemented at the time with that of cows not supplemented at that time.

RESULTS

Preliminary results are reported only, emphasising the main results for the early and late lactation groups.

Feed composition and intake

The ME and CP content of BAR and PPM were estimated to be 13.7 and 13.8 MJ ME/kg DM and 125 and 530 g CP/kg DM respectively. The effective rumen degradability of CP in BAR and PPM at a rumen outflow rate (r) of 0.08/hour was estimated to be 0.84 and 0.52 kg/kg CP respectively. Changes in OM, ME and CP content of pasture on offer through the season are given in Table 2. The effective rumen degradability of CP in ryegrass (at r=0.08/hour) was estimated to be 0.55 kg/kg CP. Maize silage offered in spring and grass silage offered in autumn contained 8.7 MJ ME and 88 g CP and 9.4 MJ ME and 144 g CP per kg DM respectively.

Intake of the experimental supplements was incomplete, in particular for PPM in the early and final stages of the experiment (Table 3). Also the BAR and BML supplements were not eaten completely and the actual intake of methionine and lysine with the BML supplement averaged 5.3 and 3.3 g/d. Table 3 also shows estimates of the DM intake of pasture and supplements for the 8 cows in the groups dosed with alkanes at weeks 11 or 29 of lactation (early and late lactation groups respectively). Intake of DM from pasture and supplements did not differ between groups ($P<0.05$).

TABLE 3: Intake of experimental supplements and effects on pasture intake in dairy cows grazing ryegrass-based pastures in early and late lactation.

Group[1]	Mean supplement intake kg/d[2]	Pasture DM intake kg/d[3]	Supplement DM intake kg/d[3]	Total DM intake kg/d[3]
BAR-E	0.73	17.6	0.68	18.3
BML-E	0.67	17.2	0.71	17.9
PPM-E	0.41	17.5	0.33	17.8
SEM[4]	0.09	1.06	0.11	-
BAR-L	0.83	14.4	0.88	15.3
BML-L	0.84	14.2	0.90	15.1
PPM-L	0.68	14.3	0.89	15.2
SEM	0.05	0.62	0.01	-

[1] BAR, PPM and BML refer to barley, protected protein meal and barley with methionine/lysine respectively.
E and L refer to early and late lactation.

[2] Mean supplement intake for all cows over their respective supplement periods.

[3] For cows dosed with alkanes only (n=8 per group). Feed intake around weeks 11 or 29 of lactation for early and late lactation cows respectively.

[4] Standard error of the mean.

TABLE 2: Temporal changes in chemical composition of ryegrass on offer to dairy cows supplemented with BAR, BML or PPM[1] at different stages of lactation

Measurement	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
OM (g/kg DM)	915	892	830	888	900	895	-	885	885
CP (g/kg DM)	274	273	238	211	160	171	-	183	208
ME (MJ/kg DM)	12.2	11.7	11.0	10.8	9.9	10.2	-	10.1	10.8

[1] BAR, BML and PPM refer to barley, barley with methionine/lysine and protected protein meal respectively.

Milk production and composition

Mean production of milk, milk fat, milk protein and mean somatic cell count of milk are given in Table 4. There were no effects of supplementation on milk production or milk composition in early lactation. Milk protein of early lactation cows contained a mean of 82% casein, 14% whey and 4% NPN, which did not differ between groups (P>0.05). In mid lactation cows, supplementation with BML reduced milk output (l/d; P<0.05) and increased the concentration of milk solids (protein plus fat in kg/d; P<0.05; details not shown), compared to cows given BAR. However, there were no significant differences between groups in production of milk solids and milk protein (P>0.05). Supplementation had no effect on milk production in late lactation cows (P>0.05).

TABLE 4: Effect of supplementation with BAR, BML or PPM on milk production of dairy cows grazing ryegrass-based pastures at different stages of lactation.

Group[1]	Milk volume l/d	Milk protein kg/d	Milk fat kg/d	Milk protein+fat kg/d	Somatic cell count 10^3/ml
BAR-E	26.4	0.92	1.13	2.05	279
BML-E	27.0	0.97	1.14	2.10	364
PPM-E	25.6	0.91	1.14	2.05	103
SEM[2]	0.88	0.03	0.03	0.05	82
BAR-M	21.2	0.76	0.99	1.75	302
BML-M	19.3	0.73	0.94	1.67	390
PPM-M	21.8	0.79	0.98	1.76	202
SEM	0.63	0.02	0.02	0.04	126
BAR-L	17.1	0.62	0.82	1.45	261
BML-L	17.7	0.66	0.86	1.51	218
PPM-L	17.7	0.65	0.85	1.50	227
SEM	0.54	0.02	0.02	0.03	78

[1] BAR, PPM and BML refer to barley, protected protein meal and barley with methionine/lysine respectively.
E, M and L refer to early, mid and late lactation.

[2] Standard error of the mean.

Changes in live weight and body condition

Mean LW of the nine groups of cows at 1 week after calving ranged from 452 to 496 kg, with cows in the PPM-E group being lightest (P<0.05). After correcting for differences in initial LW, there were no differences between early lactation groups in LW change over weeks 1 to 5 after calving (mean -3 kg; P>0.05) or over the next 6 weeks (mean + 26 kg; P>0.05). There were no effects of supplementation in late lactation on LW gain from weeks 23 to 33 of lactation (mean +11 kg; P>0.05).

There were no effects of supplementation in early lactation on change in body condition from weeks 1-5 after calving (mean -0.3 score; P>0.05), nor on change in condition score from weeks 6-12 of lactation (mean +0.3 score; P>0.05).

DISCUSSION

Cows supplemented with PPM or BML did not produce more milk solids or milk protein than cows supplemented with BAR at any stage of their lactation. Similarly, there were no indications that milk protein quality (as assessed by the proportions of casein, whey and NPN) was altered by PPM or BML. Unfortunately, the amount of experimental supplement consumed was lower than the planned 1 kg/cow/d, particularly for the PPM cows in early lactation and to a lesser extent in late lactation. This may be due to a poor palatability of the fish meal component of PPM. However, also the BAR and BML supplements were not eaten entirely.

Given the low level of supplement intake (PPM: 0.43-0.68 kg/d; BML: 5.3 and 3.3 g/d of methionine and lysine respectively), the possibility cannot be excluded that differences in MP and/or methionine/lysine supply between groups were too small to be effective. On the other hand, mid-lactation cows given BML produced 9% less milk (l/d) than cows given BAR, albeit with a 4% higher concentration of milk solids. Therefore, the possibility that the low level of supplementation with BML adversely affected amino acid balance cannot be excluded also. This and the extent to which essential amino acids other than methionine/lysine limit milk production and composition or other aspects of animal performance remains to be investigated.

Although the level of supplement intake was lower than planned, supplementation per se (irrespective of supplement type) was still found to significantly increase production of milk solids around weeks 13 and 23 of lactation (late November and early February) from 1.90 to 2.03 kg/d (P<0.001) and from 1.41 to 1.49 kg/d (P<0.01) respectively. Supplementation had no significant effect on production of milk solids around week 7 of lactation (early October; unsupplemented vs supplemented means: 2.05 vs 2.02 kg/d; P>0.05), nor around week 32 of lactation (early April; 1.48 vs 1.56 kg/d; P>0.05). Around week 32, milk protein output was increased by supplementation, however (from 0.64 to 0.69 kg/d; P<0.05).

The observed responses for cows in mid to late lactation, irrespective of supplement type, can be taken to suggest that total nutrient intake was the primary factor limiting milk production. Intake of ME by early and late lactation cows was calculated from estimates of DM intake and ME content of the pasture on offer. The estimates for feed DM intake are considerably higher than predicted by overseas feeding standards (e.g. AFRC, 1993), although it was similar to predictions for New Zealand dairy cows published by Holmes and Wilson (1987). Hexatriacontane was used as a faecal marker in this study to enable estimation of OM digestibility of the diet in individual cows. However, this has not been accomplished, apparently owing to difficulties with faecal recovery of hexatriacontane. Based on DM intake and the ME content of pasture on offer, mean ME intake from the total diet was estimated to be 195 MJ/d for early lactation cows and 156 MJ/d for late lactation cows. This is 15-20 MJ/d less than the predicted ME requirements to sustain the

observed level of production (milk and LW change; AFRC, 1993). As the ME content of pasture on offer was measured in herbage cut at soil level, it is likely that cows were able to select herbage with a higher ME content than that shown in Table 2. Given the level of feed DM intake and the discrepancy between estimates for ME intake and ME requirements, it appears that the ME content of the pasture consumed must have been at least 1 MJ/kg DM higher than that of the pasture on offer.

It has been suggested that diets providing protein in excess of requirements may reduce dairy cow performance and may impact negatively on animal health (e.g. Hibbitt, 1984; Wilson *et al.*, 1995). The N in excess absorbed CP (in the form of ammonia from rumen degradable protein [RDP] and in the form of amino acids from MP) is detoxified in the liver to urea. Hepatic ureagenesis from ammonia requires energy (see Martin and Blaxter, 1965) and may have implications for amino acid partitioning also (Reynolds, 1992; Lobley *et al.*, 1995). Calculations on the extent to which protein (in the form of RDP and MP) was provided in excess of requirements were made for BAR cows in the early lactation group (Table 5). Such calculations depend on the accurate estimation of rumen degradability of feed proteins, which was estimated here using the dacron bag method (McDonald, 1981; AFRC, 1993).

TABLE 5: Estimation of the amount and nature of excess protein supplied to dairy cows[1] grazing ryegrass-based pastures and supplemented with BAR in early lactation, as affected by changes in effective rumen degradability of ryegrass CP.

Measurement	Effective CP degradability in rumen[2]		
(kg/d)	0.55	0.70	0.85
ERDP supply[3]	2.10	2.65	3.19
Max. ERDP use[4]	2.05	2.05	2.05
ERDP excess	0.05	0.60	1.14
MP supply	2.80	2.28	1.80
MP demand[5]	1.68	1.68	1.68
MP excess	1.12	0.60	0.12
Total protein excess[6]	1.17	1.20	1.26

[1] Base on pasture intake of cows dosed with alkanes (n=8 per group) around week 11 of lactation (November 1995).

[2] Effective rumen degradability of ryegrass CP (kg/kg CP) at a rumen outflow rate of 0.08/hour.

[3] Effective RDP supply, calculated as per AFRC (1993).

[4] Based on total estimated fermentable ME intake and the requirements for ERDP/MJ FME at a rumen outflow rate of 0.08/hour (AFRC, 1993).

[5] MP demand was estimated as per AFRC (1993), taking into account LW, LW change, milk yield and composition. Rumen outflow rate was assumed to be 0.08/hour. Efficiency of MP use for maintenance (knm) was assumed to be 0.7.

[6] Sum of ERDP excess and MP excess.

Our estimate of a rumen degradability of ryegrass CP of 0.55 kg/kg CP was considerably lower than published values (e.g. 0.67 kg/kg CP for high-quality ryegrass; AFRC, 1993). Discrepancies in the apparent CP loss from dacron bags incubated in the rumen for 4 and 8 h may have contributed to this low estimate (details not shown). When these values were omitted from the calculations, effective CP degradability was increased to 0.61 kg/kg CP. It is also possible that the water-soluble CP component (0 h incubation; mean 0.35 kg/kg CP, n=7) was underestimated, although our value is in agreement with other published estimates (AFRC, 1993).

Using the low estimate of rumen degradability for ryegrass CP (0.55 kg/kg CP), the supply of effective RDP was calculated to have been close to adequate relative to the estimated supply of fermentable ME (effective RDP and fermentable ME as defined by AFRC, 1993). Due to the large amount of feed protein which would be escaping rumen fermentation at a degradability of 0.55, total MP supply (from microbial protein and UDP) was calculated to have been 2800 g/d, or over 1100 g/d in excess of estimated MP requirements. Similar calculations were made using higher rumen degradabilities of ryegrass CP (0.70 and 0.85 kg/kg CP respectively; Table 5). In all these cases, effective RDP is provided well in excess of the amount which can be used for microbial protein synthesis given the availability of fermentable ME. Even at the highest rumen degradability (0.85), however, calculated MP supply was greater than predicted MP demand (excess 120 g/d). It can be calculated that MP supply would have been adequate for these cows as long as effective rumen degradability of ryegrass CP was no more than 0.88-0.90 kg/kg.

The estimate of effective ryegrass CP degradability clearly also has little effect on the total amount of excess protein which was estimated to have been absorbed by early lactation cows (mean 1.2 kg/d). Estimates of the energy cost for conversion of ammonia-N to urea-N range from 15 to 50 kJ/g N (Martin and Blaxter, 1965; Twigge & van Gils, 1984). Ureagenesis from 1.2 kg excess protein can therefore be expected to require 2.9-9.6 MJ ME, which is equivalent to the ME needed for production of 0.6-1.9 kg milk. Rumen degradability of pasture protein clearly affects the make-up of the excess protein (i.e. RDP vs MP) metabolised by the animal. It is unclear if the form in which the excess protein is presented to the liver (ammonia from RDP vs amino acids from MP) differentially affects animal metabolism and/or animal production. The implications of the source of excessive protein intake for animal metabolism and production require further investigation.

In view of the experimental results and our calculations on MP supply/demand, we conclude that it appears unlikely that milk production was limited by MP supply in our early-lactation cows. A significant excess in the supply of MP would reduce the likelihood of a "single" amino acid deficiency (e.g. methionine/lysine). No production responses to an increased MP supply or methionine/lysine supply were recorded in our late lactation cows. This work suggests that further dose response studies to changes in MP supply and/or methionine/lysine supply are required with grazing dairy cows, in particular in mid to late lactation. The implications of excessive protein intake for animal metabolism and production also require further investigation.

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