
VOLUME 62 2002

The effects of methionine supply upon milk composition and production of dairy cows in mid-lactation

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ABSTRACT

A trial assessing the production response of dairy cows to supplementation with methionine (Met) was conducted indoors over a 21-day period with 26 Friesian cows in mid-lactation and individually fed fresh perennial ryegrass/white clover pasture. Group 1 (n=9) received twice daily oral methionine (15 g/d) that was protected from rumen degradation (RPM; Lactet); Group 2 (n=9) was given a continuous intravenous infusion of free Met (15 g/d; MET-i.v.); and Group 3 (n=8) was given no methionine supplement (Control). Supplementary methionine tended to slightly depress feed intake but milk yield tended to be higher (P=0.08) for the MET-i.v. group compared with the Control and RPM groups (18.5 ± 0.48 vs 17.1 ± 0.45 and 17.2 ± 0.38 kg/d ± SE). Compared with the control group, there was a trend for Met (both MET-i.v. and RPM) to reduce milkfat concentration and to increase the concentration of casein. Neither lactose or whey nitrogen (N) concentrations were affected by the treatments. Methionine supplementation did not affect yields of total protein nor protein components; however, when protein and casein yields were adjusted to equal crude protein intake, Met supplemented cows produced significantly (P=0.05) more total protein and casein per unit of N ingested. These results suggest that post-ruminal Met supplementation improved the efficiency with which dietary protein was converted to casein in the pasture-fed dairy cow. More research is required to investigate which AA, individually or in combination, are likely to limit milk protein synthesis in the mammary gland of the pasture-fed dairy cow.

Keywords: methionine; lactating cow; milk protein; casein.

INTRODUCTION

More than 50% of the protein ingested by the pasture-fed dairy cow can be lost from the rumen as ammonia (Beever *et al.*, 1986). This may limit the amount of protein passing to the small intestine (Black, 1990), and may restrict the availability of amino acids (AA) for milk protein synthesis in the mammary gland. It has been postulated that the identification and post-ruminal supply of the most limiting AA would be an effective way to improve milk protein quality and yield (Erfle & Fisher, 1977).

Several studies have been conducted to determine the effects of supplementation with methionine (Met) upon milk protein yield and composition, to establish if Met is a major limiting AA in lactating dairy cows. The responses obtained have been variable. In some studies an increase in milk protein percentage and protein yield has been observed (Fisher, 1972; Koch *et al.*, 1996; Robert *et al.*, 1996), whilst in others there has been no response in milk protein yield (Papas *et al.*, 1984; Overton *et al.*, 1996). However, most of the data have been obtained with forage:concentrate diets, using different types of forages and different ratios of forage to concentrate, making it difficult to draw firm conclusions. The lack of experimental data on AA utilisation by the dairy cow fed only fresh forage is noticeable. Additionally, there are relatively few reports on the effect of AA supplementation on the proportions of the individual proteins making up the protein fraction of milk.

The objective of this study was to determine whether Met supplementation affected the concentration and yield of individual milk proteins in dairy cows fed sole diets of fresh ryegrass/white clover pasture.

MATERIAL AND METHODS

Animals and diets

Twenty six multiparous dairy cows in mid lactation were maintained and fed indoors at two sites, 15 at the Massey University Dairy Cattle Research Unit (DCRU) and 11 at AgResearch Grasslands (AgR) for a 21-day experimental period. Fresh perennial ryegrass/white clover pasture was cut twice daily and offered to the cows in individual feed bins at 0900 and 1500 h, with approximately 18 kg of dry matter (DM) being offered per cow per day. Water was available *ad libitum*.

The average liveweight of the cows was 482 (SD 31.8) and 471 (SD 57.4) kg at DCRU and AgR, respectively, with the average days in milk (DIM) being 75 (SD 17.0) and 82 (SD 12.6). One jugular catheter was fitted to each animal on day 1 (AgR) and day 2 (DCRU) of the experiment. Daily exercise was allowed for two 2-hour periods (a.m. and p.m.) each day throughout the experiment.

Treatments

Measurements of feed intake and milk yield were made at the end of the first 7 days of the experiment to be used

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as covariates. The treatments were then commenced on day 8 of the experiment and were applied during 14 days.

Three experimental groups were formed with cows of similar liveweight and DIM. Treatments were (1) continuous intra-jugular infusion of 15 g/d of L-methionine dissolved in sterile saline (0.9% w/v NaCl) at a rate of 22.5 ml/h (MET-i.v.); (2) oral administration of 15 g/d of ruminally-protected DL-methionine in tallow divided in two doses (RPM; Lactet, Nippon Soda Co., Ltd., Japan) and (3) Control. Sterile saline was infused into the jugular vein of RPM and control cows at the same rate as the MET-i.v. group. All liquids were infused using a multipurpose peristaltic pump (Desaga PLG, Germany). Methionine-free capsules containing the equivalent amount of tallow in the ruminally-protected product were given to MET-i.v. and Control animals at the same time as RPM cows were dosed.

Measurements

Feed offered was recorded twice daily (a.m. and p.m. feedings) at 0900 and 1500 h. Any feed refused, either as floor or bin refusals, was recorded and sampled once a day to calculate the average feed intake. Feed was sampled and analysed twice daily for dry matter (DM); the amount of fresh forage offered was calculated using the DM value from the previous day. Pooled samples were prepared for each week, freeze-dried and stored until analysed for crude protein (CP), metabolisable energy (ME) and methionine.

Cows were milked twice daily at 0700 and 1500 h and milk yields recorded daily. Milk samples were taken once weekly on days 7, 14, and 21 at both sites. Morning and afternoon samples were collected, pooled according to yield and subsampled for protein, fat, lactose, total nitrogen, non-casein nitrogen and non-protein nitrogen (NPN) analyses.

Blood samples were taken from the tail vein or artery on days 8, 15, and 21 at both locations following the morning milking. Samples were collected using evacuated tubes containing K-EDTA (Vacutainer, Becton Dickinson) and chilled. Blood was haemolysed with milli-Q water and deproteinised by ultrafiltration (Centrisart, MW cut-off 10,000; Sartorius AG, Germany). Supernatant was collected and stored at -85°C until analysed for free methionine.

Laboratory methods

Feed composition of the composited weekly samples was analysed by near-infrared reflectance spectroscopy (NIRSystems) for CP and ME; methionine in feed was determined using a Waters ion-exchange HPLC system after performic acid oxidation and HCl hydrolysis.

Milk protein, fat and lactose concentrations were analysed using an infrared milk analyser (Milk-O-Scan, Model 104 A/B; Foss Electric, Denmark). Casein, whey protein and NPN where determined as described by Hill (1993). Methionine concentration in blood was determined using a Waters Pico-Tag column method and a Shimadzu LC-10/A HPLC system.

Statistical methods

Data were analysed using the GLM procedure of SAS (SAS Institute, 1985). Measurements obtained in week 3 were analysed as the dependent variable, with the value for the same variable in week 1 used as a covariate. All the data for milk yields and intakes were reduced to weekly averages before the analysis. Intake data were adjusted using liveweight at the beginning of the experiment as a covariate.

Four animals showed signs of clinical mastitis during the third week of the experiment and all the data collected from these animals were not used in the analyses.

RESULTS

Methionine supplementation tended to reduce dry matter intake (DMI) in both RPM and MET-i.v. groups (Table 1). This caused a significantly lower metabolisable energy intake (P<0.05) for those animals in the MET-i.v. group compared with the control group. The Met supple-

TABLE 1: Effect of methionine supplementation on liveweight (LW), LW change, feed intakes and blood methionine concentration.

	Control	Lactet	Methionine infusion	Pooled SEM	Probability
No. cows	7	9	6		
Initial LW (kg)	477	485	472	18.5	P=0.87
LW change (g/d)	173	130	250	165.3	P=0.88
Intakes					
Dry matter (kg/d)	16.7	16.2	15.8	0.23	P=0.09
Crude protein (kg/d)	1.81	1.74	1.72	0.039	P=0.27
Metabolisable energy (MJ/d)	183a	175ab	172b	2.9	P=0.05
Methionine (g/d)[1]	31.2	31.3	30.4	0.65	P=0.63
Methionine (g/d) [2]	31.1	46.3	45.4		
Blood methionine (μmol/l)	24.5a	36.5b§	42.2b§	3.32	P<0.01

Different letters in the same row indicate a significant difference between means *t*-test (P<0.05).

§ *t*-test (P<0.01).

[1] Methionine intake from pasture only.

[2] Total methionine supply.

mentation (15 g/d) represented approximately an extra 50% over the basal Met intake in the forage.

Methionine supplementation did not affect liveweight gain during the experimental period, but tended to increase milk yield (P=0.08; Table 2). There was a trend for methionine supplementation to reduce the concentration of milkfat (Table 2) and to slightly increase the concentration of casein, but there was no effect on the concentration of lactose or whey fractions.

The protein and casein yields (kg/d) were 3% (RPM) and 8% (MET-i.v.) higher for the Met supplemented groups compared to the controls, but the differences were not significant (P>0.05). However, when the yields of total protein, casein, whey and NPN were adjusted to equal CP or ME intake, methionine supplementation significantly (P<0.05) increased the yield of total protein and particularly casein. This resulted in an apparently higher efficiency of utilisation of dietary N (expressed as milk N/N intake x 100) in the methionine supplemented groups (29.7 and 30.0 vs 26.0% for the Control group). Methionine supplementation did not affect the yields of milkfat or lactose, either with or without adjustment to equal CP or ME intake.

DISCUSSION

Although Met did not affect milk production or composition directly, an important finding in this study was the effect of Met supplementation on increasing the yield of casein (approx. 10% over the Control) at constant crude protein intake, demonstrating that supply of this AA was restricting the conversion of pasture protein to casein under the conditions of this experiment. In this study, the efficiency of dietary N utilisation was increased approximately 4 percentage units in the Met-supplemented cows. This result is similar to those reported by Robinson *et al.* (1995) with ruminally protected methionine and lysine.

The trend observed for Met to decrease the DMI was likely the effect of a higher efficiency of utilisation of nutrients, as reflected by the higher milk protein yields per unit of CP intake. Similar results were reported by Barry (1981), who found that abomasal infusion of casein plus Met to growing lambs reduced DM intake but increased body and wool protein deposition. Furthermore, Robinson *et al.*, (1995) suggested that ruminally-protected Met may improve the gross efficiency of dietary N utilisation in dairy cows fed low-protein diets, such as those offered in this trial (approx. 11% CP).

The basal levels of Met in blood are similar to those reported elsewhere (Papas *et al.*, 1984; Illg *et al.*, 1987; Rogers *et al.*, 1987; Donkin *et al.*, 1989). Blood or plasma Met concentration have been used previously to assess the effectiveness of ruminally-protected preparations in delivering Met to the systemic circulation (Papas *et al.*, 1984; Polan *et al.*, 1991). In this study, the treatments increased the basal levels of blood methionine by 50% (RPM) and

TABLE 2: Effect of methionine supplementation on milk production and composition.

	Control	Lactet	Methionine infusion	Pooled SEM	Probability
No. cows	7	9	6		
Milk yield (kg/d)	17.1	17.2	18.5	0.44	P=0.08
Milk composition (%)					
Fat	4.18	3.92	3.91	0.101	P=0.14
Lactose	4.85	4.87	4.89	0.031	P=0.72
Total protein	3.02	3.07	3.00	0.042	P=0.41
Casein	2.26	2.40	2.30	0.055	P=0.19
Whey protein	0.48	0.51	0.47	0.023	P=0.36
Non-protein N	0.019	0.020	0.018	0.0012	P=0.61
Yields of components (kg/d)					
Fat	0.716	0.667	0.702	0.0236	P=0.30
Lactose	0.833	0.835	0.903	0.0238	P=0.12
Total protein	0.511	0.528	0.551	0.0141	P=0.22
Casein	0.389	0.411	0.419	0.0140	P=0.34
Whey protein	0.083	0.087	0.084	0.0045	P=0.82
Non-protein N	0.0035	0.0034	0.0033	0.00026	P=0.92
Yield of N fractions (kg/d) after adjustment to equal crude protein intake					
Total protein	0.503a	0.533ab	0.551b	0.0118	P=0.05
Casein	0.377a	0.418b	0.422b	0.0111	P=0.03
Whey protein	0.083	0.088	0.084	0.0047	P=0.82
Non-protein N	0.0033	0.0035	0.0034	0.00020	P=0.98

Different letters in the same row indicate a significant difference between means *t*-test (P<0.05).

72% (MET-i.v.). The magnitude of the increase in blood Met concentrations in the RPM group is comparable to values reported for sources of ruminally-protected Met (Papas *et al.,* 1984; Rogers *et al.,* 1987; Donkin *et al.,* 1989) demonstrating the effectiveness of Lactet for post-ruminal delivery of Met.

In contrast with several studies (Yang *et al.,* 1986; Casper & Schingoethe, 1988; Schingoethe *et al.,* 1988; Pisulewski *et al.,* 1996; Robert *et al.,* 1996) in which milk protein and/or casein concentration were significantly increased by Met supplementation, in this experiment the responses in those variables were not significant (P>0.05).

Although not significant, the increases in protein yield (3 and 8% for the RPM and MET-i.v. groups, respectively) are comparable with the 5.2% (P<0.05; Schwab *et al.,*1976) and 7.5% (P=0.07; Donkin *et al.,* 1989) reported previously. However, the whey protein and NPN yields were not increased in proportion, indicating a selective effect upon casein. The sole increase in casein (22 g/d for RPM and 30 g/d for MET-i.v.) is comparable with the total increase in milk protein (17 g/d for RPM and 40 g/d for Met-i.v.). Similar effects were observed by Metcalf *et al.* (1996) following the infusion of a mixture of AA to dairy cows fed barley:grass silage and Donkin *et al.*, (1989) after administering ruminally-protected Met to cows fed a corn-based diet.

The results from the present study indicated that methionine increased the efficiency of utilisation of dietary protein in the pasture-fed dairy cow during mid-lactation. However, the mechanisms eliciting this response are still unknown. Therefore, more research is required on the metabolism of AA in the grazing dairy cow in order to assess whether significant and consistent responses in milk protein to AA supplementation can be obtained.

ACKNOWLEDGMENTS

The authors thank the staff at the Nutrition Laboratory, Department of Animal Science and the Animal Research Unit, Massey University; the Analytical Laboratory, AgResearch Grasslands and the Food Science Section, New Zealand Dairy Research Institute for their contribution during the experimental and analytical phases of this work.

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