Effect of somatotropin administration and duodenal infusion of methionine and lysine on lactational performance and nutrient flow to the small intestine

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Lack of sufficient methionine and lysine delivered post-ruminally may limit milk production response to bovine somatotropin (bST). To test this hypothesis, four Holstein cows fitted with rumen and duodenal cannulas were used in a 4 × 4 Latin square design with 14 d periods. Treatments were: (1) control, (2) continuous duodenal infusion of 8 g methionine and 24 g lysine/d, (3) injection of 25 mg bST/d and (4) infusion of methionine and lysine plus injection of bST. Infusion of amino acids led to trends for small increases in milk (3%), fat (5·5%), and protein (3·7%) yield. Larger and significant increases (8·7, 14 and 6·9% for milk, fat and protein yield respectively) were achieved with bST administration which also increased milk fat content. Plasma levels of urea-N and essential amino acids were reduced with bST. Duodenal nutrient flow was generally unaffected by treatment. The production response to bST was not enhanced in cows producing an average of 34 kg milk when provided additional methionine and lysine post-ruminally in this short-term study.

Methionine: Lysine: Somatotropin: Nutrient flow

The effect of bovine somatotropin (bST) on stimulating consistent increases in milk production is well documented and has been reviewed by others (Bauman & McCutcheon, 1986; Peel & Bauman 1987). The enhanced milk production occurs within a few days of the onset of bST administration, but there is a lag of several weeks before feed intake increases (Bauman *et al.* 1985) and, in short-term studies, no change in intake has been observed for cows treated with bST (Peel & Bauman, 1987). This rapid increase in milk yield in relation to a delayed response in intake suggests that the nutrient density of the diet may need to be increased. The increased energy secretion in milk when cows are treated with bST occurs at the expense of tissue energy retention (Tyrrell *et al.* 1988). Although labile protein reserves may be substantial (Botts *et al.* 1979), they can be rapidly depleted in early lactation and, thus, the sufficiency of amino acids from body stores may be inadequate to meet the demands of higher milk production when cows are treated with bST (Peel *et al.* 1981). When cows were in negative N balance, they tended to become more negative when treated with bST and the increase in yield of milk protein was less than the increase in milk yield (Tyrrell *et al.* 1988). Cows fed on protein-deficient diets (110 g/kg dry matter (DM)) responded with a smaller increase in milk production when treated with bST than did bST-treated cows fed on protein-adequate diets (160 g/kg DM). Treatment with bST had no

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effect on N retention for either dietary treatment (De Boer & Kennelly, 1989*a*). These findings suggest that diets marginal or deficient in protein or amino acids may limit the milk yield or milk-protein yield response to bST.

Few other studies have examined the effect of manipulating dietary protein for bST-treated cows. Milk yield response was enhanced 1·6–1·8 kg/d by increasing the undegradable protein (UP) from 330 to 400 g/kg total protein (McGuffy *et al.* 1990); however, increasing UP from 360 to 400 g/kg total protein as well as increasing the dietary fat level did not increase milk yield of bST-treated cows (Lormore *et al.* 1990). Winsryg *et al.* (1991) observed no increase in milk yield when the dietary UP was increased for cows treated with bST; however, milk protein and casein contents were elevated for cows fed on the high-UP diet. Supplementation of an 180 g crude protein (CP; N × 6·25)/kg diet with abomasal infusion of casein and glucose did not increase milk yield beyond that achieved by bST alone (Peel *et al.* 1982).

Recent interest has focused on the quantity of amino acids flowing to the small intestine (Schwab, 1989). Lysine and methionine are the two essential amino acids (EAA) that are most probably limiting for high milk production (Schwab, 1989) and may be more limiting for cows treated with bST.

The objectives of the present experiment were to determine whether the short-term milk production response to bST could be enhanced by providing supplemental methionine and lysine post-ruminally and to measure duodenal nutrient flow and plasma amino acid and urea-N levels of cows treated with bST.

MATERIALS AND METHODS

Animal management and experimental design

Four 2nd lactation Holstein cows, 61 (SD 11) d in milk at the onset of the trial, were used. Cows were fitted with rumen and duodenal cannulas. The latter was a 'Y' plastisol cannula with a 25 mm inside diameter and an 135° internal flange angle and was placed anterior to the bile ampulla. Cows were housed in individual tie stalls and milked twice daily at 06.00 and 17.00 hours.

The experimental design was a 4 × 4 Latin square. Treatments were (on a daily basis): (1) control (C): 4 l tap water infused into the duodenum and an intramuscular injection of 5 ml buffered saline (9 g sodium chloride/l); (2) amino acid infusion (AA): 8 g DL-methionine and 24 g L-lysine hydrochloride (US Biochemical Corp., Cleveland, OH) in 4 l tap water infused into the duodenum; (3) bST injection (bST): intramuscular injection of 25 mg bST (Lilly Research Laboratories, Greenfield, IN) in 5 ml buffered saline; (4) combination (AA + bST): infusion of amino acids plus injection of bST.

The amino acid solution or water alone was infused continuously via a peristaltic pump (model no. 7520-25, Cole Parmer Inc., Chicago, IL) fitted with four pump heads. Bottles (5 l) of amino acid solution were prepared daily with the pump set to deliver 4 l/d. Bottles were changed at approximately 24 h intervals and the amount remaining was measured to monitor the amount infused. The bST was prepared daily by dissolving 25 mg active material into 5 ml of a buffered saline solution and gently agitating at 39° for 15 min before injection.

Experimental periods were 14 d. Cows were fed twice daily at 06.00 and 16.30 hours. The diet consisted of (g/kg DM basis): maize silage 49, chopped grass hay 7, concentrate 44 (Table 1). Feeds were mixed at each feeding and the amount offered was intended to achieve a 10% refusal. Feed intake was recorded during the last 4 d of each period and daily samples of maize silage, grain mix and refusals were obtained and composited at the end of each period.

Table 1. *Ingredients and chemical composition of diet (g/kg DM)*

Ingredients	
Maize silage	494·0
Grass hay	70·0
Shelled maize	214·0
Soya-bean meal	144·0
Maize-gluten meal	18·0
Calcium salts of long-chain fatty acids	18·0
Molasses	15·0
Dicalcium phosphate	5·5
Sodium bicarbonate	5·0
Salt	4·0
Dynamate*	3·5
Calcium carbonate	3·1
Trace mineral and vitamin premix†	2·5
Urea	2·1
Magnesium oxide	1·3
Chemical composition	
Crude protein (nitrogen × 6·25)	168
Degradable protein‡	111
Undegradable protein‡	57
ADF	161
NDF	302
Ca	7
P	5
Mg	3
K	10

DM, dry matter; ADF, acid-detergent fibre; NDF, neutral-detergent fibre.
* Contained (g/kg): Mg 110, S 220, K 180.
† Contained (mg/kg): Ca 59·6 g, Cu 1250, Fe 6249, Mn 3124, Se 93, Zn 12500, vitamin A 315000 Iu, vitamin D 158000 Iu, vitamin E 1400 Iu.
‡ Estimated from National Research Council (1985).

Data collection and sample preparation

Milk production was recorded during the last 7 d and a sample from each morning and afternoon milking was analysed for fat and protein using a Foss 605B Milko-Scan (Foss Electric, Hillerod, Denmark) at the Pennsylvania DHIA Milk Testing Laboratory. A second sample from each milking was composited at the end of each period in proportion to volume, and was frozen (−20°) for subsequent analysis of casein and fatty acid composition.

Duodenal digesta was collected every 3 h during the last 3 d of each period and the sampling time was advanced 1 h daily so that each hour in a 24 h period was represented. The pH was measured immediately by glass electrode and 200 ml were added to a common container for each cow and frozen (−20°).

Rumen samples from the dorsal, ventral and anterior regions for bacterial isolation (Varga *et al.* 1988) were collected at 0, 4, and 10 h after morning feeding on each of the last 3 d of the period. Bacterial isolates were lyophilized before analysis for N and purines. The N:purine ratio was used to calculate the flow of bacterial N to the small intestine (Zinn & Owens, 1986).

Digesta flow was determined using Yb-marked insoluble DM (Glenn *et al.* 1989). The marker was given intraruminally at 06.00 and 16.30 hours on days 4–14 of each period (240 g insoluble DM/d). Measured Yb concentration was 8·3 mg/g insoluble DM.

Blood was drawn from the tail vein or artery into heparinized tubes 3 h after the morning

feeding on days 13 and 14, centrifuged at 3000 ***g*** for 15 min and the resulting plasma was frozen for urea-N analysis. For amino acid analysis, 2 ml plasma were added to a centrifuge tube containing 0·2 ml sulphosalicylic acid (5 g/l) and centrifuged at 4000 ***g*** for 15 min and 1 ml plasma was withdrawn and frozen.

Analytical procedures

Feed and refusals were prepared for analyses by drying at 55° for 48 h and grinding through a Wiley mill (1 mm screen) and analysed for crude protein (CP) and ash (Association of Official Analytical Chemists 1980), acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) (Robertson & Van Soest, 1981). Mineral analyses of maize silage and grain mix were conducted by Skyview Laboratories Inc., Jennerstown, PA.

Duodenal digesta was thawed at room temperature and a portion was withdrawn while the digesta was being agitated with a mechanical stirrer. This sample was used to determine NH_3-N (Chaney & Marbach, 1962). Digesta was immediately re-frozen and approximately 1 l was lyophilized, ground in a Wiley mill (1 mm screen) and analysed for purines, CP, amino acids and Yb concentration (Hart & Polan, 1984).

For amino acid analysis of duodenal samples, 150 mg were hydrolysed with 6 M-HCl (gassed with N_2 for 15 min) at 110° for 22 h in sealed tubes. Hydrolysates were allowed to cool and passed through a 0·45 μm pore filter (Millipore Corp., Bedford, MA) and filtrates were diluted with sodium citrate buffer (pH 2·2). Analysis was done on a Beckman 119CL amino acid analyzer (Beckman Instrument Inc., San Ramon, CA).

Plasma urea-N was determined using the Technicon AutoAnalyzer (Technicon Corp., Tarrytown, NY). Analysis of plasma for free amino acids was with a Biotronik LC 5000 amino acid analyzer (Biotronik, Strasse, FRG). Plasma growth hormone (GH) analysis was by the method described by Donkin *et al.* (1989), except antisera were provided by R.S. Kensinger (The Pennsylvania State University, University Park, PA).

Casein proteins were separated by polyacrylamide gel electrophoresis (Ng-Kwai-Hang & Kroeker, 1984). Extraction of milk fatty acids was according to the double-extraction technique of Christopherson & Glass (1969), the methyl esters were separated on a gas–liquid chromatograph (Perkin-Elmer 8410; Perkin-Elmer Corp., Norwalk, CT).

Statistical analysis

Data were analysed as a 4×4 Latin square using the ANOVA procedure of SAS Institute Inc. (1985) with cow, period and treatment as the effects in the model. The sum of squares for treatment was partitioned into single df orthogonal contrasts to determine the effect of amino acid infusion, bST injection, and amino acid $\times$ bST interactions.

RESULTS

Feed composition

Ingredient and chemical composition of the diet are listed in Table 1. The diet was formulated to meet National Research Council (1989) requirements for cows milking 40 kg milk. The ADF level was lower than anticipated primarily due to a lower ADF content in the grain and hay mixture than was initially calculated from the ingredients. Estimated degradability of protein (National Research Council, 1985) was 0·66.

Milk production, composition and DM intake

Treatment with bST increased ($P = 0{\cdot}01$) milk yield 8·7% (Table 2). Fat-corrected milk (FCM), and yield of fat and protein were also increased with bST (Table 2). Amino acid infusion tended to increase milk yield ($P = 0{\cdot}11$), FCM ($P = 0{\cdot}12$), and the yield of fat

Table 2. *Milk production, composition and dry matter intake by cows given control (C), duodenal infusion of methionine and lysine (AA), injection of bST (bST), or the combination of AA + bST*†

Treatment...	C	AA	bST	AA + bST	SE	Statistical significance (P=) of effect of*: AA	bST
Milk (kg/d)	31·5	32·8	34·6	35·3	0·53	0·11	0·002
4% Fat-corrected milk (kg/d)	30·4	31·5	34·0	35·9	0·84	0·12	0·003
Fat: g/kg	37·9	37·5	38·5	40·5	0·99	NS	0·11
kg/d	1·19	1·23	1·33	1·43	0·04	0·12	0·005
Protein: g/kg	29·3	29·6	28·7	29·1	0·30	NS	NS
kg/d	0·92	0·96	0·99	1·02	0·02	0·15	0·03
Dry matter intake (kg/d)	20·0	19·8	19·5	20·6	0·59	NS	NS

NS, not significant ($P < 0·15$).
* The interaction between AA and bST was not significant ($P > 0·15$).
† For details of treatments and procedures, see pp. 50–52.

Table 3. *Casein content of milk from cows given control (C), duodenal infusion of methionine and lysine (AA), injection of bST (bST), or the combination of AA + bST**†

Treatment...	C	AA	bST	AA + bST	SE
Casein (g/kg total protein)	786	771	776	779	11·6
Casein fractions (g/kg casein)					
α-Casein	558	539	579	563	13·1
β-Casein	336	353	321	327	12·6
K-Casein	100	101	94	101	3·6
Γ2-Casein	6·7	7·3	5·8	9·0	1·4

* Treatment contrasts were not significant ($P > 0·15$).
† For details of treatments and procedures, see pp. 50–52.

($P = 0·15$), and protein ($P = 0·15$). However, fat and protein content were not affected by AA ($P > 0·15$). The AA + bST treatment did not increase milk yield or composition above that of bST alone.

Injection of bST increased the proportion of long-chain fatty acids (28·8 *v.* 22·6; $P = 0·05$, and decreased the proportion of short-chain fatty acids (24·5 *v.* 32·8; $P = 0·04$) in milk. AA had no effect on the fatty acid composition of milk.

Casein relative to total protein or casein fractions (Table 3) were not affected by treatment.

Intake of DM averaged 20·0 kg/d and was similar across all treatments.

Duodenal nutrient flow

Generally, there was no effect of either bST administration or duodenal infusion of amino acids on flow of nutrients to the small intestine (Table 4). Glycine flow (values not shown) was increased with bST (181 *v.* 157 g/d; $P = 0·04$). Glycocholic acid present in bile may make a significant contribution to glycine flow (Wanderley *et al.* 1988) but this would imply increased contamination of samples from biliary secretions in bST-treated cows. However, duodenal pH was 2·88 for bST-treated cows and 2·82 for controls, indicating that samples

Table 4. *Duodenal flows (g/d) for cows given control (C), duodenal infusion of methionine and lysine (AA), injection of bST (bST), or the combination of AA + bST*†

Treatment...	C	AA	bST	AA + bST	SE	Statistical significance ($P=$) of effect of*: AA	bST
Duodenal flow							
Dry matter (kg/d)	12·5	13·6	12·2	12·3	0·74	NS	NS
Nitrogen	464	498	509	503	20·74	NS	NS
Ammonia	16·6	17·9	14·1	16·8	1·26	NS	NS
Non-NH_3-N	447	478	495	487	20·56	NS	NS
Bacterial N	179	188	202	199	9·82	NS	0·15
Amino acids							
Arginine	107	115	119	119	4·88	NS	NS
Histidine	54	58	57	57	2·03	NS	NS
Isoleucine	115	124	125	126	9·84	NS	NS
Leucine	247	267	256	259	9·84	NS	NS
Lysine	163	188	181	196	8·06	0·05	NS
Methionine	44	49	47	50	2·19	0·13	NS
Phenylalanine	120	129	128	131	4·27	NS	NS
Threonine	116	124	126	127	4·67	NS	NS
Valine	130	143	143	144	5·18	NS	NS
Total essential amino acids	1098	1197	1183	1208	43·2	NS	NS

NS, not significant ($P > 0·15$).
* The interaction between AA and bST was not significant ($P > 0·15$).
† For details of treatments and procedures, see pp. 50–52.

were not contaminated. There was a tendency for the flow of bacterial N to be increased with bST ($P = 0·15$). Duodenal flow measurements reported in Table 4 are exclusive of AA; however, lysine flow was increased ($P = 0·05$) and there was a tendency for methionine flow to increase ($P = 0·13$) with AA. This was probably due to retention of some of the infusate anterior to the cannula between sampling times.

Plasma profiles

bST increased the level of GH in plasma ($P = 0·001$) and AA increased plasma levels of lysine and methionine ($P < 0·01$; Table 5). Urea-N levels were reduced ($P = 0·06$) in bST-treated cows as were total EAA ($P = 0·06$). The urea cycle intermediates, citrulline, ornithine, and arginine also tended to be lower ($P < 0·15$) for the bST treatments.

Ornithine and arginine, but not citrulline, were increased ($P < 0·07$) with AA. Histidine concentration was reduced in cows injected with bST, but not when bST-treated cows were also infused with amino acids, resulting in a significant interaction effect ($P = 0·02$).

DISCUSSION

The milk yield increase with bST administration was similar to the increase in other short-term studies (Peel & Bauman, 1987). The combination of amino acids plus bST did not increase milk yield or composition above that of bST alone, indicating that the production response to bST was not enhanced by providing additional lysine and methionine post-ruminally. These results are in agreement with those of Peel *et al.* (1982) who reported that

Table 5. *Plasma levels of growth hormone (ng/ml), urea (mg/l) and amino acids (mg/l) for cows given control (C), duodenal infusion of methionine and lysine (AA), injection of bST (bST), or the combination of AA + bST*†

Treatment...	C	AA	bST	AA + bST	SE	Statistical significance (P=) of effect of: AA	bST	AA × bST*
Growth hormone (mg/l)	2·0	1·8	34·9	31·0	5·45	NS	0·001	NS
Urea-nitrogen (mg/l)	158	163	139	145	7·9	NS	0·06	NS
Amino acids (mg/l)								
Arginine	10·1	10·8	6·2	9·1	0·7	0·05	0·01	NS
Histidine	4·1	4·0	2·0	4·5	0·4	0·03	0·11	0·02
Isoleucine	14·3	12·4	9·1	13·2	1·6	NS	NS	0·11
Leucine	19·4	16·4	12·5	17·8	1.8	NS	NS	0·06
Lysine	8·1	13·3	5·1	12·3	1·6	0·01	NS	NS
Methionine	3·6	4·8	2·3	4·7	0·5	0·01	NS	NS
Phenylalanine	4·9	4·6	3·6	4·9	6·0	NS	NS	NS
Threonine	8·3	8·7	6·8	4·8	1·1	NS	0·05	NS
Valine	19·4	16·8	13·0	16·3	1·6	NS	0·07	0·12
Total essential amino acids	92·1	91·8	60·5	87·4	7·8	0·14	0·06	0·13
Urea cycle‡	28·9	30·0	21·9	27·9	2·6	NS	0·13	NS

NS, not significant ($P > 0·15$).
* Interaction between AA and bST.
† For details of treatments and procedures, see pp. 50–52.
‡ Citrulline + ornithine + arginine.

abomasal infusion of casein and glucose stimulated a small non-significant increase in milk and protein yields but, when combined with GH did not increase the response above the level achieved by GH alone. In a study completed while the current trial was in progress, Lynch *et al.* (1991) observed no additional milk production response to post-rumen lysine and methionine supplementation from cows injected with bST.

The most consistent response to post-rumen supplementation of lysine and methionine has been an increase in content or yield of milk protein (Schwab, 1989). Response to post-rumen supplementation appears highly dependent on dietary protein level relative to requirements and the types of ingredients fed. The greatest responses have occurred in animals fed on low-protein diets in order to assess which amino acids were limiting (Schwab *et al.* 1976). Response to supplemental lysine and methionine has generally been greatest when a high proportion of the dietary protein was supplied by maize products (Schwab *et al.* 1976; Donkin et al. 1989) where lysine appeared to be the first limiting amino acid. In contrast, methionine supplementation of diets containing soya-bean products has improved lactational performance (Schingoethe *et al.* 1988). In the current trial calculated contributions to the total ration protein were: maize products (maize silage, maize grain, and maize gluten meal) 0·47, soybean meal 0·46, hay, molasses, and urea 0·07, and with this distribution either lysine or methionine could have been limiting.

Milk protein or casein composition was not altered. This is in contrast to Donkin *et al.* (1989) who observed an increase in milk protein content, α- and β-casein and a decrease in K-casein when mid-lactation cows were fed on protected methionine and lysine and a maize-based diet. The increase in the proportion of long-chain fatty acids in milk with bST

injection is consistent with findings of Bitman *et al.* (1984), probably indicating that body stores of lipid were being mobilized to support the increase in milk production.

The intake of DM was not affected by either AA or bST. In short-term studies bST has been shown to have little effect on intake (Peel & Bauman, 1987), but intake tends to increase in bST-treated cows after 4–6 weeks (Bauman *et al.* 1985). Positive responses to post-rumen administration of amino acids are partially accounted for by increases in feed intake (Schwab, 1989).

To assess the potential for either methionine or lysine to be limiting, the proportional flow of these two amino acids of the total EAA was calculated. Lysine constituted 0·15 × EAA flow across treatments and methionine was 0·039 × EAA flow. Schwab (1989) reported that lysine was the first limiting amino acid for peak- and early-lactation cows when lysine flow was 0·13 × EAA flow and methionine was second limiting or co-limiting when it constituted 0·039 × EAA flow. In the studies cited (Schwab, 1989), lysine flow was similar (175–185 g/d) but methionine flow was higher (55–58 g/d) than in the present study. Thus, methionine may have been more limiting than lysine and this is consistent with other studies where soya-bean meal supplied a high proportion of the CP (Schingoethe *et al.* 1988).

Plasma urea concentrations were decreased in GH-treated mid-lactation cows but not in cows at peak lactation (McDowell *et al.* 1987). When measures of adipose tissue mobilization have been made simultaneously with some measure of amino acid oxidation in bST-treated animals there is a strong inverse relationship (Eisemann *et al.* 1986; McDowell *et al.* 1987) suggesting that lipid reserves are preferentially being oxidized, thus sparing amino acids for productive functions (Bauman & McCutcheon, 1986). The greater ability of liver from bST-treated cows to convert propionate to glucose (Pocius & Herbein, 1986) and a greater capacity to conserve glucose (De Boer & Kennelly, 1989*b*) are other mechanisms whereby amino acids could be spared in bST-treated animals.

Since the ruminal supply of total EAA to the small intestine was similar across treatments, the decrease in EAA and branched-chain amino acid concentrations in plasma with bST treatment could have been due to their increased uptake by the mammary gland.

Conclusions

Providing 8 g methionine and 24 g lysine post-ruminally led to small increases in milk, fat, and protein yields. Injecting cows with bST stimulated larger increases in milk and component production but the response to bST was not enhanced by amino acids in cows producing an average an 34 kg milk. Duodenal nutrient flow was generally not affected by treatment. Plasma levels of urea-N and EAA and duodenal NH_3-N levels were decreased with bST. A greater proportion of long-chain fatty acids in milk of bST-treated cows was probably indicative of mobilization of lipid reserves. This may have provided oxidative fuel for higher milk production and, thus, spared amino acids for other productive functions. Methionine appeared to be more limiting than lysine in this trial, and the production response to bST may have limited by methionine or other amino acids that became co-limiting. Further investigation is needed to determine if the long-term response to bST could be enhanced with additional amino acids.

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