Methionine Deficiency in Early-Weaned Dairy Calves Fed Pelleted Rations Based on Corn and Alfalfa or Corn and Soybean Proteins[1,2]

P. B. DONAHUE, C. G. SCHWAB, J. D. QUIGLEY, III, and W. E. HYLTON
Department of Animal and Nutritional Sciences
New Hampshire Agricultural Experiment Station
University of New Hampshire
Durham 03824

RITZMAN LABORATORY
UNIVERSITY OF NEW HAMPSHIRE
DURHAM, NEW HAMPSHIRE 03824

ABSTRACT

Holstein bull calves weaned at 4 wk of age were assigned to one of three replicated 4 × 4 Latin squares at 5 to 6 wk. Trials were abomasal infusion of 0, .30, .60, and either 90 (Trials 2 and 3) or 1.20 g (Trial 1) L-methionine/kg pelleted starter ration consumed. In Trials 1 and 2, ration ingredients of the ration contributed the following percentages of total crude protein: corn meal 39, alfalfa meal 48, and soybean meal 12; dry matter of rations contained 13.6 and 13.9% crude protein and .26 and .32% sulfur. Infusion of .60 g methionine in Trial 1 tended to depress excretion of nitrogen in urine and increase nitrogen retention. In Trial 2, urine nitrogen excretion and retained nitrogen responded in a quadratic fashion; infusion of .30 and .60, and either .90 (Trials 2 and 3) or 1.20 g (Trial 1) L-methionine/kg pelleted starter ration consumed. In Trials 1 and 2, ingredients of the ration contributed the following percentages of total crude protein: corn meal 39, alfalfa meal 48, and soybean meal 12; dry matter of rations contained 13.6 and 13.9% crude protein and .26 and .32% sulfur. Infusion of .60 g methionine in cretion 9%. Relationship between plasma methionine and infused methionine was linear for all trials. Methionine is the first limiting amino acid for early-weaned calves fed complete pelleted rations based on corn and either alfalfa or soybean proteins.

INTRODUCTION

Published data are limited as to the optimum pattern of absorbable amino acids for growing cattle. However, methionine or lysine generally appear first limiting when rations containing conventional ingredients are fed to growing cattle (2, 3, 6, 7, 8, 13, 14, 15, 22). Moreover, ingredient composition of cereal-based rations appears to determine which of the two amino acids is first limiting. Lysine was first limiting when rations contained no legume protein (2, 6, 7, 13, 14), whereas methionine was first limiting when rations contained 4% or more soybean meal (8, 15).

Abomasal infusion of incremental methionine (0, .30, .60, and 1.20 g L-methionine/kg ration) indicated methionine was first limiting in early-weaned dairy calves fed a ration based on by-product feeds and containing 13.8% crude protein (22). Excretion of urine nitrogen and retention of nitrogen responded in a quadratic fashion to incremental methionine. Greatest responses were with .60 g L-methionine/kg ration; urine nitrogen was depressed 11% and nitrogen retention increased 18% over the negative control infusate.

The purpose of this study was to investigate further the dose-response relationship of incremental methionine infused into the abomasum of early-weaned calves fed complete pelleted starter rations based on corn and either alfalfa or soybean meal proteins. Rations contained 53% corn meal and 35% alfalfa meal (Trials 1 and 2) or 48% corn meal and 13% soybean meal (Trial 3). Excretion of urine nitrogen and nitrogen balance were primary criteria for assessing response to methionine infusions.

MATERIALS AND METHODS

Experimental Design

Twenty-four Holstein bull calves were assigned to three 4 × 4 Latin squares with two

Received May 29, 1984.

[1]Scientific Contribution Number 1290 from the New Hampshire Agricultural Experiment Station.

[2]Supported in part by a grant from Agway Inc., Syracuse, NY.

TABLE 1. Ingredient composition of complete pelleted starter rations.

Ingredient	Trials 1 and 2	Trial 3
	(%)	
Corn meal	53.25	48.85
Alfalfa meal	35.10	5.00
Soybean meal	3.00	12.90
Molasses	3.00	7.00
Oat hulls	2.50	22.45
Dicalcium phosphate	1.50	1.15
Sodium bicarbonate	1.00	1.00
Limestone	...	1.00
Salt	.50	.50
Vitamin A, D, and E premix[1]	.10	.10
Trace mineral mix[2]	.05	.05

[1] Provide 8,800 IU of vitamins A and D and 8.8 IU vitamin E/kg.

[2] Contains a guaranteed minimum of .7% Fe, .5% Cu, .08% Co, .4% Mn, 15% Zn, .3% I, and .04% Se.

replications per treatment. Each period was a minimum of 9 days with at least a 4-day adjustment to treatment followed by a 5-day excreta collection.

Animal Management and Rations

Calves were housed in a unheated barn with tie stalls bedded with sawdust until placed on experiment. Calves were fed 1.8 to 2.3 kg whole or waste milk twice daily from open pails until 3 wk of age. Milk was reduced gradually from 3 wk until weaning at 4 wk of age. A complete pelleted ration[3] was offered for ad libitum intake from approximately 1 wk of age. Abomasal cannulae were placed surgically in 10 to 12 calves for each of the three trials at 5 to 26 days of age according to the procedure of Schwab et al. (22).

When all cannulated calves within a group were weaned (28 to 37 days of age), 10 calves were placed in a temperature-controlled (24 ± 4°C) room. Calves were allowed 7 to 10 days to adjust to digestion crates and experimental rations. Following adaptation, eight calves consuming near equal quantities of feed were selected for study, and abomasal infusions were initiated. Average ages of calves at initiation of infusion were 42 ± 5, 37 ± 3, and 43 ± 2 days, for Trials 1, 2, and 3. Calves managed under the conditions of this study possess functional rumens by 1 to 2 wk after weaning (20).

Infusate solutions were prepared by L-methionine[4] dissolved in tap water (approximately 1800 ml/calf) every 3 days with concentrations adjusted for feed intake and differences in infusion rates. Concentrations of L-methionine were 0, .30, .60, and 1.20 g/kg ration in Trial 1 and 0, .30, .60, and .90 g/kg ration in Trials 2 and 3. Abomasal infusions were continuous for each 9-day period. Solutions were infused via a multichannel cassette pump[5] from graduated cylinders maintained under refrigeration (2°C). Infusion rates were monitored daily.

The ingredient composition of rations is in Table 1. Rations were prepared as complete pelleted feeds with a pellet diameter of 4.4 mm. Rations fed in Trials 1 and 2 were largely corn and alfalfa; corn meal contributed 39%, alfalfa meal 48%, and soybean meal 12% of the total nitrogen. The ration in Trial 3 was formulated to be similar in nutrient content to that fed in Trials 1 and 2 but to contain a maximal amount of soybean meal. In this ration, soybean meal contributed 52%, corn meal 34%, and alfalfa meal 7% of the total nitrogen. Both rations were formulated to contain 13.5% crude protein, 14.0% crude fiber, .8% calcium, and .6% phosphorus (dry matter).

[3] TCR I, Agway Inc., Syracuse, NY.

[4] Nutritional Biochemicals Co., Cleveland, OH.

[5] Monostat brand, Lux Scientific Instrument Corp., New York, NY.

Daily feed allotted to each calf was restricted to that of the animal consuming the least amount to ensure similar intakes of nitrogen across treatments. Feed offered was increased periodically prior to initiation of a treatment and, occasionally, once during a treatment period to allow for increased consumption associated with advancing age; appropriate adjustments of concentration of L-methionine in infusate solutions were at these times. Rations were offered in equal quantities at 0400, 1000, 1600, and 2200 h. Feed refusals, when present, were weighed prior to the 1000-h feeding, and an identical quantity of fresh feed was reoffered at that time.

Measurement, Sample Collection, and Analysis

Feed samples were taken daily during the collection periods, composited by period, and analyzed in triplicate for dry matter (DM, vacuum oven, 60°C). The remaining composite was ground through a 1-mm stainless steel screen and analyzed in triplicate for DM and nitrogen[6] and in duplicate for crude fiber and ether extract (1), acid-detergent fiber (10), and combustible energy with a Parr bomb calorimeter. A composite sample for each trial was analyzed for amino acids by ion exchange chromatography,[7] minerals by atomic absorption spectroscopy,[8] and sulfur turbidmetrically[9] (1). Oxidation of proteins in feed with performic acid (16) prior to hydrolysis permitted complete recovery of cysteine and cystine as cysteic acid; the procedure has been described (22). To analyze for the other amino acids and permit complete recovery of tryptophan, feed samples (13 to 18 mg) were hydrolyzed in 1 ml 3 N mercaptoethanesulfonic acid for 24 h at 110°C (18). Following hydrolysis, samples were centrifuged, the supernatant was adjusted to pH 2.2 with 240 μl 50% NaOH, and 50 μl of sample and 450 μl of sample dilution buffer applied to the column.

Urine was collected in polyethylene pails containing 25 ml of 4 N H_2SO_4 and measured volumetrically daily. Aliquots (10%) were composited and stored at 2°C until analyzed for nitrogen[6] and combustible energy (22). Feces from each calf were collected, composited, and frozen (−16°C) daily until analyzed for DM (vacuum oven, 60°C), nitrogen,[6] and combustible energy (22).

Two samples of jugular blood were taken at 0, 2, and 4 h postprandial from each calf on the last day of each collection period. One sample was obtained in a 10-ml evacuated tube containing no additive. The blood was allowed to coagulate at room temperature and then centrifuged (3,500 × *g*, 30 min). Serum was composited for the three sampling times and analyzed for total protein, albumin, urea N, and glucose by a Technicon Sequential Multiple Analyzer.[10] The other blood sample from each calf was taken in a 10-ml evacuated tube[11] containing 15 mg ethylenediaminetetraacetate (EDTA; K_3) and .02 mg potassium sorbate and placed immediately in an ice bath. Within 20 min, samples were centrifuged at 3,500 × *g* for 15 min. Four milliliters of plasma from each sample was deproteinized with 1 ml of 15% sulfosalicylic acid and centrifuged at 3,500 × *g* for 30 min. The deproteinized plasma was composited across time for each calf within period and pH adjusted to 2.2 by 1.5 N LiOH (trace EDTA). Samples were frozen (−20°C) and composited for each pair of calves prior to analysis for free amino acids by ion exchange chromatography.[7]

Statistical Analysis

Data were analyzed as Latin squares. Analysis of variance was by least squares for unbalanced data with animals, periods, and treatments as main effects. Treatment and period effects were subdivided into linear, quadratic, and cubic orthogonal contrasts (23). Means in tables are least squares means. Feed composition was not analyzed statistically.

[6] Apparatus: Model BD-20 block digester and Autoanalyzer II, Sample III, Technicon Industrial Systems, Tarrytown, NY.

[7] Beckman 118CL automated amino acid analyzer, Beckman Instruments, Palo Alto, CA.

[8] Analysis conducted by Forage Testing Laboratory, New York Dairy Herd Improvement Cooperative, Inc., Ithaca, NY.

[9] Analysis by A&L Agricultural Laboratories, Inc., Memphis, TN.

[10] Analysis by New England Clinical Laboratories, Inc., Tilton, NH.

[11] Vacutainer tubes, Becton-Dickinson, Rutherford, NJ.

RESULTS AND DISCUSSION

Chemical composition and apparent digestibility of starter rations are in Table 2. Contents of crude protein in ration DM for Trials 1, 2, and 3 were 13.9, 13.6, and 14.8%. Although content of amino acids was similar among rations, the higher lysine and lower methionine contents in soybean protein relative to cereal proteins is reflected in the ration fed in Trial 3. Percentages of ether extract, crude fiber, acid detergent fiber, and acid detergent fiber nitrogen were lower for the ration fed in Trial 3. With the exception of sodium in Trial 3, content of minerals exceeded that recommended (17) for young calves. The excessive amount of magnesium in the ration fed in Trial 3 probably was due to addition of dolomitic limestone.

TABLE 2. Chemical composition[1] and apparent digestibility of complete pelleted rations.

Measurement	Trial 1	Trial 2	Trial 3
Crude protein, %[2]	13.9	13.6	14.8
Ether extract, %[2]	3.7	3.7	2.4
Crude fiber, %[2]	10.4	12.6	8.8
Acid detergent fiber, %[3]	14.5	16.8	12.3
Acid detergent fiber N, % of total N[3]	7.5	7.2	1.4
Amino acids, %[3]			
Arginine	.60	.58	.81
Histidine	.25	.25	.28
Isoleucine	.38	.38	.40
Leucine	1.05	1.04	1.05
Lysine	.48	.46	.61
Methionine	.22	.22	.20
Cystine plus cysteine[4]	.22	.21	.21
Phenylalanine	.54	.54	.61
Threonine	.51	.49	.51
Tryptophan	.28	.23	.21
Valine	.48	.51	.51
Total essential amino acids	4.79	4.70	5.19
Total nonessential amino acids	6.29	6.15	6.95
Total amino acids	11.08	10.85	12.14
Minerals[3]			
Calcium, %	.79	1.04	1.09
Phosphorus, %	.64	.71	.65
Magnesium, %	.18	.19	.74
Potassium, %	1.10	1.27	.66
Sodium, %	.40	.47	.14
Sulfur, %	.32	.26	.34
Iron, ppm	287	330	345
Zinc, ppm	92	99	104
Copper, ppm	31	11	16
Manganese, ppm	55	53	70
Apparent digestibility, %[5]			
Dry matter	71.1	69.4	67.0
Gross energy	69.2	67.0	65.4
Nitrogen	59.2	57.9	70.4

[1] Percent of dry matter. Dry matter content (%) of rations for the three trials was: 92.0, 92.7, 91.0.

[2] Mean of four 5-day composites taken during excreta collection periods.

[3] Composite for the trial.

[4] Measured as cysteic acid (16).

[5] Average of 29 (Trial 1), 25 (Trial), or 30 (Trial 3) digestibility measurements.

Abomasal infusion of methionine had no effect on digestibility of dry matter, gross energy, and nitrogen. The higher digestibility of nitrogen in Trial 3 likely was the result of both a higher content of ration CP and less nitrogen associated with ration fiber.

Trial 1

The effect of infusing 0, .30, .60, and 1.20 g L-methionine/kg ration on nitrogen status of calves fed the ration based on corn and alfalfa meal was investigated in this trial. Three of the potential 32 observations were deleted from statistical analysis because of excessive feed refusals (>10% of that offered) during any 9-day period. In general, the appetite of calves fed this ration containing 35% alfalfa meal (noted also in Trial 2) tended to be depressed as compared to (22). It is possible that saponins (triterpenoid compounds) in alfalfa meal were responsible for reduced feed intake. Rations containing more than 10% alfalfa meal or purified diets containing saponins reduced feed intake in rats (4), chicks (19), pigs (4, 9, 12), and rabbits (4). Although saponin-utilizing bacteria have been isolated from mature ruminants (5, 11), rumen function may not be sufficient in the 6- to 11-wk-old calf to degrade saponins.

The amount of nitrogen retained and excreted in feces and urine did not differ ($P>.05$) for methionine infused (Table 3). The percentage of dietary nitrogen excreted in urine or retained and the percentage of apparently absorbed nitrogen retained also were similar for all treatments. However, infusion of .60 g L-methionine/kg ration tended to lower excretion of nitrogen in urine and increase retained nitrogen. Excretion of nitrogen in urine decreased 14% and retained nitrogen increased 13% as compared to the negative control infusate. These apparent changes of urine and retained nitrogen are similar to those observed by Schwab et al. (22) in calves fed a ration based on cereal by-products and infused abomasally with the same percent of methionine.

Concentrations of metabolites in serum and plasma are in Table 4. Total protein, albumin, urea nitrogen, and glucose in serum were not affected by infusion of incremental methionine. Concentration of total essential amino acids (EAA) in plasma responded in a cubic fashion ($P<.005$) whereas histidine in plasma (as a % of total EAA) responded in both a linear ($P<.05$) and quadratic ($P<.05$) fashion with incremental methionine. There are no apparent biological explanations for these responses. Concentration of total nonessential amino acids (NEAA) in plasma was depressed in a linear fashion ($P<.05$). Infusing incremental methionine into the abomasum in (22) and in Trials 2 and 3 of this study did not affect plasma concentrations of total EAA or total NEAA. The linear decrease

TABLE 3. Utilization of nitrogen by postweaned calves fed a ration based on corn and alfalfa protein and infused abomasally with incremental methionine, Trial 1.

Item	Infusate (g L-methionine/kg ration) 0	.30	.60	1.20	SE[1]
n	7	8	6	8	
Dry matter intake, g/day	979	982	981	972	
Nitrogen, g/day					
Intake	22.3	22.4	22.4	22.2	
Infused	0	.03	.05	.11	
Fecal	9.0	8.9	9.3	8.8	.4
Urine	7.0	7.5	6.0	7.0	.5
Retained	6.3	6.0	7.1	6.4	.7
Nitrogen, % of intake					
Urine	31.7	34.3	27.7	32.3	2.4
Retained	27.1	25.6	30.6	26.7	3.1
Nitrogen, % absorbed					
Retained	45.5	41.6	52.6	42.5	4.63

[1] Standard error for n = 8.

TABLE 4. Concentration of selected components in serum, and amino acids in plasma of postweaned calves infused abomasally with incremental methionine, Trial 1.

Metabolites	Infusate (g L-methionine/kg ration) 0	.30	.60	1.20	SE[1]	Orthogonal contrasts[2] L	Q	C
Serum[3]								
Total protein, g/100 ml	6.83	6.76	6.73	6.74	.08	NS	NS	NS
Albumin, g/100 ml	3.32	3.25	3.26	3.19	.04	NS	NS	NS
Urea N, mg/100 ml	6.93	6.25	7.36	6.63	.71	NS	NS	NS
Glucose, mg/100 ml	76.1	74.4	76.7	72.8	1.6	NS	NS	NS
Plasma,[4] µmoles/100 ml								
Total EAA[5]	94.7	81.7	94.1	83.8	2.5	NS	NS	***
Total NEAA[6]	166.4	159.8	161.1	147.7	4.1	*	NS	NS
Plasma, % of total EAA								
Methionine	2.05	2.37	2.74	3.14	.14	***	NS	NS
Arginine	11.44	12.17	11.81	12.05	.46	NS	NS	NS
Histidine	5.43	6.40	6.22	6.28	.15	*	*	NS
Isoleucine	8.97	8.73	8.30	8.71	.17	NS	NS	NS
Leucine	17.29	16.42	16.76	17.03	.49	NS	NS	NS
Lysine	11.08	10.88	10.33	9.23	.50	*	NS	NS
Phenylalanine	7.01	6.90	6.92	6.81	.22	NS	NS	NS
Threonine	9.74	10.19	10.83	10.46	.46	NS	NS	NS
Tryptophan	2.81	2.94	2.73	2.10	.39	NS	NS	NS
Valine	24.19	23.00	23.36	24.22	.48	NS	NS	NS
Plasma, % of total NEAA								
Cystine	1.23	1.27	1.37	1.47	.06	**	NS	NS
Plasma, % of total EAA and NEAA								
Cystathionine	.125	.140	.147	.175	.006	***	NS	NS
Taurine	2.40	2.42	2.92	3.03	.28	NS[7]	NS	NS

[1] Standard error for n = 8 (serum) and n = 4 (plasma).

[2] Linear (L), quadratic (Q), and cubic (C) effects.

[3] Analysis on composite sample from 0, 2, and 4 h postfeeding for each calf.

[4] Analysis on pooled sample from 0, 2, and 4 h postfeeding for each pair of calves.

[5] Essential amino acids include arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

[6] Nonessential amino acids include alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

[7] Nonsignificant ($P>.05$).

* $P<.05$.

** $P<.01$.

*** $P<.005$.

($P<.05$) of plasma concentration of lysine relative to other EAA with increasing methionine infusion may indicate that lysine is limiting following methionine when the described ration is fed. Lysine would be expected to be second limiting in a ration comprised primarily of corn and alfalfa protein for growing cattle (21). Concentrations of methionine relative to total EAA and cystathionine relative to total amino acids in plasma increased ($P<.005$) with increasing amounts of abomasal methionine; concentration of cystine relative to total NEAA also increased ($P<.01$). Elevated cystine and cystathionine in plasma in response to increased methionine availability indicates enhanced catabolism of methionine. Taurine in plasma was not affected significantly by methionine infusions.

Trial 2

This trial replicated Trial 1 except that .90 g L-methionine/kg ration was infused in place of 1.2 g L-methionine. Trial 1 was replicated because of three missing observations and variability in nitrogen balance that we attributed largely to poor feed consumption. Further, there were no indications in either Trial 1 or in (22) that calves would respond positively to 1.20 g of supplemental L-methionine/kg ration via abomasal infusion. Therefore, .90 g L-methionine/kg ration was infused in this trial to determine if this infusate would be excessive.

Results of the nitrogen balance study are in Table 5. There were seven missing observations in this trial because of poor consumption of feed by two calves and death of one calf by bloat. In contrast to Trial 1, quadratic responses ($P<.05$) in excretion of urine nitrogen and in retained nitrogen occurred with infusion of incremental methionine. The decrease of daily excretion of urine nitrogen and the increase of retained nitrogen were maximized at .30 g L-methionine/kg ration. The abomasal infusion of .30 and .60 g L-methionine/kg ration depressed excretion of nitrogen in urine 12 and 9% as compared to the negative control infusate. These responses are similar to the response with infusion of .60 g L-methionine in Trial 1 and suggest that methionine is the first-limiting amino acid for calves fed the described ration under the imposed experimental conditions. However, improvement of nitrogen utilization with .30 g L-methionine in this trial and lack of such an effect in Trial 1 is unexplained as rations and all other experimental conditions were as similar as possible.

As in Trial 1, total protein, albumin, urea nitrogen, and glucose in blood serum were not affected by methionine treatments (Table 6). Concentrations in plasma of total EAA and total NEAA also did not differ with methionine infusion (Table 6). Plasma methionine increased in linear fashion ($P<.05$) to incremental infusion of methionine. No orthogonal contrasts were significant for other plasma amino acids. However, the abomasal infusion of .30 and .60 g L-methionine/kg ration tended to depress lysine in plasma similar to Trial 1. Cystathionine was not measured in this trial.

TABLE 5. Utilization of nitrogen by postweaned calves fed a ration based on corn and alfalfa protein and infused abomasally with incremental methionine, Trial 2.

Item	Infusate (g L-methionine/kg ration)					Orthogonal contrasts[2]		
	0	.30	.60	.90	SE[1]	L	Q	C
n	7	6	6	6				
DM intake, g/day	922	929	921	924				
Nitrogen, g/day								
Intake	21.1	21.3	21.1	21.2				
Infused	0	.03	.06	.08				
Fecal	8.8	8.5	9.1	9.3	.3	NS[3]	NS	NS
Urine	7.0	6.2	6.4	7.8	.6	NS	*	NS
Retained	5.3	6.6	5.6	4.2	.6	NS	*	NS
Nitrogen, % of intake								
Urine	34.2	29.8	30.9	37.5	2.7	NS	NS	NS
Retained	23.6	30.0	25.5	17.9	3.3	NS	NS	NS
Nitrogen, % of absorbed								
Retained	39.7	49.1	44.2	30.4	5.7	NS	NS	NS

[1] Standard error for n = 7.

[2] Linear (L), quadratic (Q), and cubic (C) contrasts.

[3] Nonsignificant ($P>.05$).

* $P<.05$.

TABLE 8. Concentration of selected components in serum, and amino acids in plasma of postweaned calves infused abomasally with incremental methionine, Trial 3.

Metabolites	Infusate (g L-methionine/kg ration)				
	0	.30	.60	.90	SE[1]
Serum[2]					
Total protein, g/100 ml	6.92	6.87	7.06	7.04	.10
Albumin, g/100 ml	3.38	3.37	3.40	3.45	.03
Urea N, mg/100 ml	12.50	11.43	12.07	11.87	.41
Glucose, mg/100 ml	82.9	80.7	84.4	85.6	2.3
Plasma,[3] μmoles/100 ml					
Total EAA[4]	85.2	86.0	90.5	97.0	6.7
Total NEAA[5]	140.2	149.5	151.8	152.5	3.6
Plasma, % of total EAA					
Methionine[6]	2.78	3.26	3.64	3.71	.22
Arginine	15.29	15.36	15.04	15.55	.52
Histidine	6.47	6.37	6.95	6.07	.70
Isoleucine[6,7]	16.72	12.78	10.21	12.94	1.18
Leucine	14.81	14.01	14.99	14.05	.70
Lysine	10.08	10.53	10.73	10.44	.43
Phenylalanine	5.57	5.87	5.57	5.75	.23
Threonine[8]	8.31	10.09	9.96	10.84	.32
Tryptophan	2.76	2.80	2.45	2.63	.47
Valine	18.49	18.87	20.43	18.49	.80
Plasma, % of total NEAA					
Cystine	2.94	2.70	2.83	3.00	.17
Plasma, % of total EAA and NEAA					
Taurine	2.80	2.96	4.27	4.13	.63

[1] Standard error for n = 8 (serum) and n = 4 (plasma).

[2] Analysis on composite sample from 0, 2, and 4 h postfeeding for each calf.

[3] Analysis on pooled sample from 0, 2, and 4 h postfeeding for each pair of calves.

[4] Essential amino acids include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalamine, threonine, and valine.

[5] Nonessential amino acids include alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

[6] Linear effect of treatment ($P<.05$).

[7] Quadratic effect of treatment ($P<.05$).

[8] Linear effect of treatment ($P<.005$).

TABLE 9. Effect of advancing age of calves on methionine and lysine in plasma.

Item	Period of trials				SE[1]	Orthogonal contrasts
	1	2	3	4		
	(% of total essential amino acids)					
Trial 1						
Methionine	2.31	2.64	2.54	2.81	.14	NS[2]
Lysine	11.66	10.62	9.56	9.68	.50	Linear, $P<.05$
Trial 2						
Methionine	2.21	2.24	2.14	2.39	.15	NS
Lysine	12.80	10.89	11.67	9.89	.33	Linear, $P<.01$
Trial 3						
Methionine	2.84	3.42	3.56	3.58	.22	NS
Lysine	13.06	10.64	9.35	8.74	.43	Linear, $P<.005$

[1] Standard error for n = 4.

[2] Nonsignificant ($P>.05$).

TABLE 10. Estimation of curde protein (CP) in ration dry matter (DM) that was spared with optimal L-methionine[1] infused into the abomasum of postweaned calves in (22) and the present trials.

Experiment	N ingredients in ration	When no methionine was infused: Intake of DM	Intake of N	Retained N	Retained N	Response in retained N w/optimum L-Met	CP computed to yield same response	CP computed to yield same response	CP in basal ration	CP spared
		(g)	(g)	(g)	(% of intake)	(g)	(g)	(% of ration DM)	(%)	(% units)
(Column)		1	2	3	4	5	6	7	8	9
Ref. (22)	Cereal byproducts	1085[2]	24.0[2]	7.1[2]	29.6[3]	+1.3[2]	27.4[4]	16.4[5]	13.8[6]	2.6[7]
Trial 1	Corn/alfalfa meal	979	22.3	6.3	28.3	+ .8	17.7	16.0	13.9	2.1
Trial 2	Corn/alfalfa meal	922	21.1	5.3	25.1	+1.3	32.4	17.8	13.6	4.2
Trial 3	Corn/soybean meal	1088	25.0	8.1	32.4	+ .7	13.5	15.6	14.8	.8

[1] Quantity of abomasally infused L-methionine yielding maximum responses in retained nitrogen (N) in (22) and Trials 1, 2, and 3 of the present study was .6, .6, .3, and .9 g/kg ration consumed.

[2] Values obtained from Table 7 in (22) and Tables 3, 5, and 7 in present study.

[3] (Column 3/column 2) X 100.

[4] (Column 5/column 4) X 6.25 X 100.

[5] [(Column 2 X 6.25) + column 6] /column 1 X 100.

[6] From Table 2 in (22) and Table 2 in present study.

[7] Column 7 – column 8.

young calves, relative to methionine, must increase with advancing age.

The fundamental reason for supplementing the absorbable amino acid pool with the first-limiting amino acid is to enhance the pattern of amino acids for protein synthesis and thus spare dietary protein. Although the interaction between levels of dietary protein and abomasally infused methionine on growth or feed efficiency was not examined in this study and (22), the amount of dietary crude protein that apparently was spared with the optimum infused methionine ranged from 1 to 4 percentage units (Table 10, column 9). It is uncertain whether these estimates represent maximums for calves under described experimental conditions because it is not known whether content of ration protein was low enough not to exceed their requirement for the second limiting EAA. Although ration methionine was lowest in Trial 3 (Table 2), crude protein (14.8% of DM) probably was sufficiently high to alleviate part of the methionine deficiency. Optimum supplemental methionine supplied abomasally in (22) and in Trials 1 and 2 of our study ranged from .30 to .60 mg/kg ration consumed. These amounts are in 2 to 4 percentage units of crude protein in the rations described and agree with estimates in Table 10 for those trials.

In summary, methionine limits utilization of dietary and absorbed nitrogen in early-weaned calves fed complete pelleted starter rations as described in this study and (22), at least until 11 wk of age. Whether or not sufficient dietary protein would be spared under conventional rearing practices for consideration to be given to commercial supplementation of starter rations with ruminally protected methionine remains to be determined.

ACKNOWLEDGMENTS

The authors are grateful to R. P. Blakey, C. K. Bozak, and R. A. Murray for their technical assistance.

REFERENCES

1 Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. Assoc. Offic. Anal. Chem., Washington, DC.

2 Burris, W. R., J. A. Boling, N. W. Bradley, and A. W. Young. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699.

3 Chalupa, W., J. E. Chandler, and R. E. Brown. 1972. Amino acid nutrition of growing cattle. Fed. Proc. 31:681. (Abstr.)

4 Cheeke, P. R., J. H. Kinzell, and M. W. Pedersen. 1977. Influence of saponins on alfalfa utilization by rats, rabbits, and swine. J. Anim. Sci. 46:476.

5 Clark, R.T.J., and C.S.W. Reid. 1974. Foamy bloat of cattle. A review. J. Dairy Sci. 57:753.

6 Devlin, T. J., and W. Woods. 1964. Nitrogen metabolism as influenced by lysine administration in and posterior to the rumen. J. Anim. Sci. 23:872. (Abstr.)

7 Devlin, T. J., and W. Woods. 1965. Nitrogen metabolism as influenced by lysine administration posterior to the rumen. J. Anim. Sci. 24:878. (Abstr.)

8 Fenderson, C. L., and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759.

9 Gard, D. I., D. E. Becker, S. W. Terrill, H. W. Norton, and A. V. Nalbandou. 1955. Sources of unidentified factors for the pig. J. Anim. Sci. 14:532.

10 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, and some procedures). USDA Agric. Handbook 379, US Dep. Agric., Washington, DC.

11 Gutierrez, J., and R. E. Davis. 1962. Isolation of saponin-digesting bacteria from the rumen of bloating cattle on ladino clover pasture. J. Anim. Sci. 21:819.

12 Hanson, L. F., D. E. Becker, S. W. Terrill, A. H. Jensen, and H. W. Norton. 1956. The inhibitory effect of dehydrated alfalfa meal in the diet of swine. J. Anim. Sci. 15:830.

13 Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242.

14 Hill, G. M., N. W. Bradley, and J. A. Boling. 1977. Postruminal infusion of methionine and lysine in growing steers. J. Anim. Sci. 45(Suppl. 1):240. (Abstr.)

15 Komarek, R. J., and R. A. Jandzinski. 1979. Effect of diet upon supplemental postruminal methionine needs of steers. J. Anim. Sci. 45:383. (Abstr.)

16 Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238:235.

17 National Research Council. 1978. Nutrient requirements of domestic animals. III. Nutrient requirements of dairy cattle. 5th ed. Natl. Acad. Sci., Washington, DC.

18 Penke, B., R. Ferenczi, and K. Kovacs. 1974. A new acid hydrolysis method of determining tryptophan in peptides and proteins. Anal. Biochem. 60:45.

19 Peterson, D. W. 1950. Effect of sterols on the growth of chicks fed high alfalfa diets or a diet containing quillaja saponin. J. Nutr. 42:597.

20 Quigley, III, J. D., C. G. Schwab, and W. E. Hylton. 1985. Development of rumen function in calves: Nature of protein reaching the abomasum. J. Dairy Sci. 68:694.

21 Richardson, C. R., and E. E. Hatfield. 1978. The

limiting amino acids in growing cattle. J. Anim. Sci. 46:740.

22 Schwab. C. G., S. J. Muise, W. E. Hylton, and J. J. Moore, III. 1982. Response to abomasal infusion of methionine of weaned dairy calves fed a complete pelleted starter ration based on by-product feeds. J. Dairy Sci. 65:1950.

23 Steel, R.G.D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, NY.