Optimal Concentrations of Lysine, Methionine, and Threonine in Milk Replacers for Calves Less than Five Weeks of Age

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ABSTRACT

The AA requirements of herd-replacement calves less than 5 wk old and fed milk replacers are not clearly defined and have been estimated in a limited number of studies using milk-fed calves ranging from 5 to 20 wk of age. The objective of these 4 studies was to investigate the effect of supplementing milk replacers containing 24 to 28% crude protein (CP; from milk sources) and 17% fat with Lys, Met, and Thr to estimate the optimum requirements for calves less than 5 wk of age. Holstein bull calves (initially 3 and 4 d old, 43 ± 1 kg of body weight, BW) were fed an 18% CP (as-fed) starter ad libitum and weaned at 31 to 32 d of age (28-d studies). Calves were housed in an unheated, curtain-sided nursery. In study 1, 6 milk replacer treatments were fed based on the combination of 3 CP concentrations (24, 26, and 28% CP) each with or without added Lys and Met. In studies 2 and 3, 26% CP and 2.34% Lys milk replacer treatments were fed to test the concentration of Met (0.64, 0.68, and 0.72% Met in study 2 and 0.64, 0.72, and 0.80% Met in study 3). In study 4, 26% CP, 2.34% Lys, and 0.72% Met milk replacer treatments were fed to test the concentration of Thr (1.06, 1.43, and 1.80%). There was a 17% improvement in average daily gain (ADG) in study 1 from adding Lys and Met that was maximized with 2.34% Lys. The ADG response to added Met in studies 2 (linear) and 3 (quadratic) were 13 and 7%, respectively, with a plateau at 0.72% Met. There was no ADG or efficiency response to added Thr in study 4. Formulating 17% fat, whey-based milk replacers fed at 0.68 kg/d to 26% CP, 2.34% Lys, and 0.72% Met appeared optimum based on responses of body weight gain, feed efficiency, and serum concentrations of urea nitrogen, while feeding calves more CP and essential AA did not improved ADG and efficiency. Requirements for calves less than 5 wk old, averaging 48 kg of BW, consuming 204 g of CP/d, and gaining

0.46 kg of BW/d, appeared to be met with 17 g of Lys, 0.31 Met:Lys ratio, 0.54 Met+Cys:Lys ratio, and a Thr:Lys ratio less than 0.60.

Key words: calf, amino acid, milk replacer

INTRODUCTION

The most recent summaries of the AA requirements of calves are Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997), all in calves fed milk and no starter. The estimates of Williams and Hewitt (1979) were in 6- to 14-wk-old calves growing approximately 0.25 kg/d. The estimates of van Weerden and Huisman (1985) were in 5- to 7-wk-old calves growing approximately 0.9 kg/d. The estimates of Toullec (1989) and Gerrits et al. (1997) were in 2- to 5-mo-old calves growing approximately 1 kg/d. Diaz et al. (2001) stimulated the notion of feeding herd-replacement dairy calves more than the 450 to 500 g of milk replacer (MR) powder or approximately 4 L of milk that is conventional in the US system. They fed calves a 30% milk CP, 20% fat MR diet at varying rates up to an maximum consumption of 2.5 kg of dry milk powder daily with no starter feed or weaning of the calf and observed increased daily gains and feed efficiencies with increased intake of powder. Blome et al. (2003) and Bartlett et al. (2006) have reported increased ADG from feeding increasing amounts of CP and increasing ratios of CP to energy in calves fed only milk. More recently, Hill et al. (2006a,b, 2007c) have shown that in calves fed starter, feeding a 26% CP, 17% fat MR at 0.68 kg/d supported more ADG than a 20% CP, 20% fat MR fed at 0.45 kg/d. When the 26% CP, 17% fat MR was fed at rates over 1 kg/d, ADG from 3 d of age through 2 wk postweaning was not increased compared with feeding 0.68 kg of the MR because of reductions in starter intake (Hill et al., 2007c). Feeding a 20% CP MR at rates over 0.45 kg/d did not improve ADG in the same way as a higher CP MR did because CP appeared to be limiting compared with energy (Hill et al., 2006a).

In 20% CP MR fed at 0.45 kg/d, supplementing Lys and Met improved ADG in all whey CP formulas (Hill

Received August 13, 2007.

Accepted February 26, 2008.

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et al., 2007b). Similarly, supplementing Lys, Met, Thr, and Ile has increased ADG in calves fed MR containing milk and soy proteins (Jenkins and Emmons, 1983; Kanjanapruthipong, 1998). Estimates of AA requirements of calves less than 2 mo of age are not available in peer-reviewed form. Additionally, the dairy NRC (2001) does not consider individual AA for calves. Thus, the objective of this series of 4 studies was to estimate the optimal concentrations of Lys, Met, and Thr in MR for calves less than 5 wk of age. Our hypothesis was that Lys, Met, and Thr would be limiting ADG.

MATERIALS AND METHODS

Holstein bull calves from a single dairy farm were received midday at 2 to 3 d of age after a 3-h transit. Their first MR was fed at the p.m. feeding. The day after arrival at approximately noon, the calves were weighed, blood sampled intravenously for serum protein via a refractometer, and assigned to treatment (d 0, initial BW). Calf management, health protocols, and measurements of BW, starter intake, fecal scoring, body condition scoring, and serum analysis for albumin, alkaline phosphatase, amylase, creatinine, glucose, total protein, and urea-N was conducted as described in Hill et al. (2007b). In study 3, blood was sampled on d 12, 19, and 23 of the study 90 min after the morning MR feeding. In study 4, blood was sampled on d 9, 14, and 23 of the study 90 min after the morning MR feeding. Calves were housed a curtain-sided, naturally ventilated nursery with no added heat in 1.2×2.4 m pens bedded with straw. Calves had access to clean, fresh water at all times. All animals were cared for by acceptable practices as described in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

In each study, single-source Holstein bull calves initially (d 0) 3 to 4 d old and 43 ± 1 kg of BW were weaned at 28 d on-study. A 17% fat MR was fed at 0.681 kg of powder per day to all calves because this rate has been shown to improve ADG over a conventional 20% CP, 20% fat MR while not depressing starter intake or post-weaning ADG (Hill et al., 2006b). The MR were manufactured (Akey, Lewisburg, OH) by altering the concentrations of whey, whey protein concentrate (78% CP as-fed basis), L-Lys, and DL-Met in studies 1, 2, and 3. In study 4, the MR were manufactured by altering the concentrations of whey, skimmed milk (34% CP as-fed basis), L-Lys, DL-Met, and L-Thr. The MR were fed at 0.681 kg of powder per day from d 1 to 28 and divided into 2 equal meals. On d 26 to 28, calves were only fed 0.340 kg of MR powder in the morning to facilitate weaning. The reconstituted MR were 148 g of powder diluted into a final volume of 1 L with warm water. All

calves consumed the amount of MR offered. Measurements were made through d 56, 28 d after weaning. Early weaning at 28 d was used to maximize the contribution of nutrients from MR compared with later weaning when starter intake would have furnished a greater proportion of nutrient intake. An 18% CP starter (as-fed; Hill et al., 2007a) was fed beginning on d 1. The starter contained 37% rolled corn, 35% protein pellet (71% soybean meal, 15% premix of vitamins, minerals, decoquinate, 12% wheat middlings, 2% alfalfa meal), 25% whole oats, and 3% molasses. The starter averaged 88.1% DM, 18.0% CP, 3.7% fat, 0.77% Ca, 0.55% P, 0.92% Lys, 0.29% Met, 0.31% Cys, 0.68% Thr, 1.17% Arg, 3.18% Glu, 0.47% His, 0.71% Iso, 1.44% Leu, 0.84% Phe, 0.23% Trp, 1.65% Tyr, and 0.81% Val.

In study 1, 6 MR treatments were fed based on the combination of 3 CP concentrations (24, 26, and 28% CP as-fed basis) each with or without added Lys and Met (Table 1). In a series of studies where, in addition to MR, calves were fed starter and weaned, Hill et al. (2006a,b, 2007c) reported that calves fed 0.68 kg daily of a 26% CP, 17% fat MR consistently optimized ADG compared with greater and lesser concentrations of CP and fat and feeding rates. However, AA concentrations were not evaluated. The concentrations of Lys and Met in the MR with added Lys and Met were chosen to mimic the ratio of Lys to Met from the 20% CP MR with added Lys and Met used in the study of Hill et al. (2007b), who based their concentrations of Lys and Met on the 2:1 ratio of Lys:Met+Cys suggested by Toullec (1989) for veal calves over 2 mo of age. Concentrations of Lys and Met in MR without added crystalline Lys and Met were those in the basal ingredients. The Lys and Met were included in the MR to provide a ratio of CP to Lys of 11.1 and a ratio of Lys to Met of 3.25. Study 1 was conducted from April through August with 3 groups of 48 calves (144 total calves) entering the research nursery in consecutive 5-wk intervals. The average nursery temperature was 18°C and ranged from -2 to 32°C based on hourly measurements.

In study 2, 3 MR treatments were fed that each contained 26% CP and 2.34% Lys because the MR treatment in study 1 that maximized ADG and efficiency had these concentrations. The 3 MR treatments contained graded concentrations of Met (0.64, 0.68, and 0.72% Met; Table 2). The high Met treatment was 0.72% Met because this was the theoretical concentration in the MR from study 1 that maximized ADG (the MR treatment with more Met did not support greater ADG). The intermediate Met concentration was reduced by 0.04% units based on the 26% CP MR with added Met from study 1. The low Met concentration was reduced another 0.04% units to equally space the theoretical Met concentrations in study 2. This study was conducted

Table 1. Assayed nutrient content of milk replacers containing 3 concentrations of CP with or without added Lys and Met (AA) in study 1¹

Nutrient, % as-fed	24% CP		26% CP		28% CP	
	No AA	AA	No AA	AA	No AA	AA
DM	96.1	96.1	96.1	96.1	96.1	96.1
CP	24.3	24.2	26.1	26.1	28.0	28.0
Fat	17.1	17.0	17.2	17.0	17.2	17.3
Ash	6.5	6.5	6.4	6.4	6.4	6.3
Ca	0.80	0.81	0.79	0.80	0.81	0.79
P	0.61	0.61	0.62	0.61	0.62	0.62
Lys	2.06	2.21	2.20	2.39	2.41	2.59
Met	0.51	0.68	0.56	0.75	0.62	0.80
Cys	0.51	0.52	0.55	0.57	0.59	0.61
Thr	1.71	1.70	1.85	1.84	1.99	2.01
Arg	0.60	0.59	0.65	0.64	0.71	0.69
Glu	4.03	4.00	4.38	4.41	4.71	4.69
His	0.45	0.44	0.49	0.50	0.53	0.53
Ile	1.39	1.35	1.51	1.48	1.60	1.59
Leu	2.40	2.42	2.62	2.62	2.78	2.82
Phe	0.78	0.76	0.83	0.83	0.89	0.86
Trp	0.40	0.43	0.44	0.45	0.47	0.49
Tyr	0.64	0.66	0.71	0.72	0.76	0.75
Val	1.37	1.40	1.51	1.53	1.64	1.62
Calculated DE, ² Mcal/kg	4.88	4.88	4.93	4.93	4.97	4.97

¹Milk replacers (MR) with added Lys and Met were formulated to 2.16% Lys and 0.66% Met in the 24% CP MR, 2.34% Lys and 0.72% Met in the 26% CP MR, and 2.52% Lys and 0.78% Met in the 28% CP MR.

²Digestible energy; calculated using NRC (2001) equations.

September through November with 48 total calves. The average nursery temperature was 13°C and ranged from -5 to 32°C based on hourly measurements.

Because no plateau in ADG was observed in study 2, study 3 was designed using a greater maximum concen-

tration of Met (0.64, 0.72, and 0.80% Met; Table 3), to further evaluate Met concentration in the MR. The 3 MR treatments fed each contained 26% CP and 2.34% Lys. This study was conducted September through November with 48 total calves. The average nursery tem-

Table 2. Assayed nutrient concentration of milk replacers¹ containing 3 concentrations of Met in study 2

Nutrient, % as-fed	Formulated Met concentration, %		
	0.64	0.68	0.72
DM	96.4	96.4	96.3
CP	25.9	25.8	25.9
Fat	17.7	17.9	18.0
Ash	6.3	6.4	6.4
Ca	0.81	0.79	0.80
P	0.63	0.61	0.62
Lys	2.35	2.36	2.37
Met	0.66	0.69	0.74
Cys	0.54	0.55	0.54
Thr	1.85	1.83	1.84
Arg	0.65	0.64	0.67
Glu	4.38	4.31	4.43
His	0.49	0.50	0.50
Ile	1.51	1.49	1.50
Leu	2.62	2.65	2.66
Phe	0.83	0.81	0.84
Trp	0.44	0.43	0.44
Tyr	0.70	0.74	0.72
Val	1.48	1.48	1.50

¹Each was 4.93 Mcal of digestible energy/kg (as-fed) calculated using NRC (2001) equations.

Table 3. Assayed nutrient concentration of milk replacers¹ containing 3 concentrations of Met in study 3

Nutrient, % as-fed	Formulated Met concentration, %		
	0.64	0.72	0.80
DM	95.9	95.9	95.9
CP	26.2	26.0	26.3
Fat	17.1	17.2	17.1
Ash	6.3	6.4	6.2
Ca	0.78	0.79	0.79
P	0.60	0.62	0.61
Lys	2.30	2.28	2.28
Met	0.63	0.71	0.80
Cys	0.56	0.56	0.55
Thr	1.84	1.83	1.85
Arg	0.69	0.70	0.67
Glu	4.44	4.49	4.35
His	0.48	0.49	0.48
Ile	1.48	1.51	1.47
Leu	2.65	2.68	2.70
Phe	0.80	0.79	0.83
Trp	0.43	0.43	0.43
Tyr	0.73	0.71	0.70
Val	1.50	1.51	1.53

¹Each was 4.93 Mcal of digestible energy/kg (as-fed) calculated using NRC (2001) equations.

Table 4. Assayed nutrient concentration of milk replacers¹ containing 3 concentrations of Thr in study 4

Nutrient, % as-fed	Formulated Thr concentration, %		
	1.06	1.43	1.80
DM	96.8	97.0	96.7
CP	26.5	26.5	26.6
Fat	17.7	17.6	17.7
Ash	6.6	6.7	6.5
Ca	0.82	0.81	0.80
P	0.59	0.62	0.62
Lys	2.32	2.29	2.31
Met	0.73	0.71	0.73
Cys	0.26	0.28	0.28
Thr	1.06	1.48	1.82
Arg	0.88	0.90	0.87
Glu	5.17	5.02	0.51
His	0.66	0.69	0.68
Ile	1.41	1.40	1.38
Leu	2.53	2.41	2.44
Phe	1.20	1.18	1.22
Trp	0.39	0.40	0.41
Tyr	1.17	1.21	1.15
Val	1.70	1.79	1.73

¹Each was 4.93 Mcal of digestible energy/kg (as-fed) calculated using NRC (2001) equations.

perature was 12°C and ranged from -4 to 32°C based on hourly measurements.

In study 4, 3 MR treatments were fed that each contained 26% CP, 2.34% Lys, and 0.72% Met (Table 4). These concentrations were based on the concentration used in studies 1, 2, and 3 that achieved the greatest ADG. The 3 MR treatments evaluated graded concentrations of Thr (1.06, 1.43, and 1.80% Thr). Whey protein from whey and whey protein concentrate was used as the base source of milk protein in studies 1, 2, and 3, resulting in approximately 1.8% Thr and a Lys:Thr ratio of 0.77. This exceeds the ratio of Lys to Thr calculated from Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997) of 0.60 or less. Whey protein is high in Thr relative to skim milk powder. Thus, study 4 was designed using skim milk powder instead of whey protein concentrate to lower the basal Thr in an attempt to better estimate a Thr requirement. Calves in study 4 were all fed the 1.06% Thr MR for the first 9 d, then from d 10 to 28 fed their randomly assigned MR treatment. This initial 9-d period was used to establish a baseline measurement from which to calculate any potential change in selected serum metabolites. This study was conducted November through January with 48 total calves. The average nursery temperature was -6°C and ranged from -8 to 13°C based on hourly measurements.

Approximately 110% of the estimated feeds needed for each study were manufactured at one time. Samples were collected from every other bag (22.7 kg) of feed at the time of manufacture. Composites were analyzed

(AOAC, 2000) before the animal phase of the studies for DM (oven method; method 930.15), CP (Kjeldahl method; method 988.05), fat (alkaline treatment with Röse-Gottlieb method; method 932.06), ash (muffle furnace method; method 923.03), Ca, P (NIR method; method 989.03), and AA by HPLC. Amino acids were determined after acid hydrolysis (method 982.30 E[a]; AOAC, 2000). Total sulfur AA were determined after performic acid oxidation and acid hydrolysis (method 982.30 E[b]; AOAC, 2000). Tryptophan content was determined after alkaline hydrolysis (method 982.30 E[c]; AOAC, 2000).

Data from each study were analyzed separately as completely randomized block designs using the GLM procedure of SAS (version 8, SAS Institute Inc., Cary, NC). Single estimates for the growth measures of ADG, average starter intake and feed efficiency, change in BCS and hip width, average fecal score, total days requiring medical treatment, and total days when fecal score exceeded 2 were calculated for the 28-d milk-fed period and 28-d postweaning period of each study and used in the analysis. The statistical model included terms for treatment, calf, and experimental error. Means for treatment's concentration of CP in study 1 and the concentration of AA tested in studies 2, 3, and 4 were separated using linear and quadratic contrast statements. Serum metabolite data from study 3 were analyzed using a mixed model as repeated measures. The model included terms for treatment, day, and the interaction of treatment and day. Calf nested within treatment was included as a random effect that was used to test the main effect of treatment. Day was modeled as a repeated measurement using an autoregressive type 1 covariance structure. Similar procedures were used to analyze serum metabolite data from study 4 with the following exceptions. Because all calves were fed a common diet for the first 9 d of the study, serum metabolite measures from d 9 were included as a covariate when analyzing data from study 4. Data reported are least squares means for the experimental unit (calf) in all studies.

RESULTS

No calves died or were removed from the studies. In each study, there were no differences ($P > 0.05$) in initial BW, serum protein, hip widths, or BCS. Change in hip width and BCS, abnormal fecal score days, medical days, and average fecal score did not differ ($P > 0.05$) among treatments in any study.

Assayed nutrients for the MR fed in study 1 are reported in Table 1. In study 1, intake of CP, apparent digestible protein (ADP), Lys, Met, and Thr increased linearly ($P < 0.05$) with increasing concentration of CP

Table 5. Effect of 3 concentrations of CP with or without added Lys and Met (AA) on ADG, intake, and efficiency of calves for 28 d in study 1

Item	24% CP		26% CP		28% CP		SEM	
	No AA	AA	No AA	AA	No AA	AA	CP	AA
Calves, n	24	24	24	24	24	24	—	—
Initial serum protein, mg/dL	5.0	5.2	5.0	5.3	5.2	5.2	0.2	0.1
Initial weight, kg	42.5	42.4	43.5	42.0	42.5	42.7	0.8	0.7
ADG, ^{1,2,3} kg/d	0.376	0.446	0.415	0.484	0.447	0.468	0.020	0.018
Starter intake, kg/d	0.143	0.127	0.143	0.153	0.152	0.135	0.017	0.016
Milk replacer intake, kg/d	0.645	0.645	0.645	0.645	0.645	0.645	—	—
Feed efficiency ^{1,2,3,4}	0.472	0.580	0.523	0.608	0.556	0.596	0.019	0.016
Digestible energy intake, Mcal/d	3.59	3.54	3.62	3.65	3.68	3.62	0.51	0.48
CP intake, ¹ kg/d	0.182	0.179	0.194	0.196	0.208	0.205	0.022	0.020
ADP ⁵ intake, ¹ g/d	0.165	0.162	0.176	0.177	0.189	0.186	0.020	0.018
Lys intake, ^{1,2} g/d	14.6	15.4	15.5	16.8	16.9	17.9	1.9	1.6
Met intake, ^{1,2} g/d	3.7	4.8	4.1	5.3	4.5	5.6	0.6	0.5
Thr intake, ¹ g/d	12.7	12.5	13.6	13.7	14.6	14.5	1.6	1.4
Energy-allowable ADG, ⁶ kg/d	0.55	0.54	0.56	0.57	0.57	0.57	—	—
Protein-allowable ADG, ⁶ kg/d	0.59	0.58	0.64	0.65	0.70	0.70	—	—

¹Increased linearly with CP ($P < 0.05$).²Differed with AA supplementation ($P < 0.05$).³CP and AA interacted ($P < 0.05$).⁴Gain divided by milk replacer (MR) plus starter intake.⁵Apparent digestible protein intake ($0.93 \times \text{MR CP}$ and $0.75 \times \text{starter CP}$).⁶Predicted ME- and ADP-allowable gain using NRC (2001) submodel.

in the MR (Table 5). Calf ADG and efficiency increased linearly ($P < 0.05$) with increasing concentration of CP in the MR. Intake of Lys, Met, and Thr was greater ($P < 0.05$) in calves fed the MR with added Lys and Met than in calves fed the MR without added Lys and Met. There was an interaction of concentration of CP and

AA ($P < 0.05$) for ADG and efficiency with calves fed the 28% CP MR with added Lys and Met not increasing in ADG and efficiency compared with calves fed the 28% CP MR without added Lys and Met. However, the main effect of added AA was also significant ($P < 0.05$) because calves fed MR at lower CP concentrations with

Table 6. Effect of 3 concentrations of Met on ADG, intake, and efficiency of calves for 28 d in study 2

Measurement	Formulated Met concentrations, %			SEM
	0.64	0.68	0.72	
Calves, n	16	16	16	—
Initial serum protein, ¹ mg/dL	5.1	4.7	5.1	0.2
Initial wt, kg	42.7	41.9	43.5	1.0
ADG, ² kg/d	0.418	0.426	0.473	0.03
Starter intake, ³ kg/d	0.135	0.113	0.194	0.016
Milk replacer intake, kg/d	0.645	0.645	0.645	—
Feed efficiency ⁴	0.534	0.557	0.565	0.036
Digestible energy intake, Mcal/d	3.60	3.53	3.78	0.49
CP intake, kg/d	0.191	0.187	0.202	0.019
ADP intake, ⁵ g/d	0.174	0.170	0.181	0.017
Lys intake, g/d	16.4	16.3	17.1	0.2
Met intake, ⁶ g/d	4.6	4.8	5.4	0.4
Thr intake, g/d	13.5	13.1	14.1	1.5
Energy allowable ADG, ⁷ kg/d	0.49	0.47	0.53	—
Protein allowable ADG, ⁷ kg/d	0.64	0.63	0.66	—

¹Quadratic relationship (trend) to Met ($P < 0.06$).²Linear relationship to Met ($P < 0.02$).³Quadratic relationship to Met ($P < 0.012$).⁴Gain divided by milk replacer (MR) plus starter intake.⁵Apparent digestible protein intake ($0.93 \times \text{MR CP}$ and $0.75 \times \text{starter CP}$).⁶Linear relationship to Met ($P < 0.05$).⁷Predicted ME and ADP allowable gain using NRC (2001) submodel.

Table 7. Effect of 3 concentrations of Met on ADG, intake, and efficiency of calves for 28 d in study 3

Item	Formulated Met concentration, %			SEM
	0.64	0.72	0.80	
Calves, n	14	14	14	—
Initial serum protein, mg/dL	5.0	5.2	5.1	0.18
Initial weight, kg	42.0	42.2	42.4	1.4
ADG, ¹ kg/d	0.423	0.456	0.442	0.009
Starter intake, kg/d	0.158	0.160	0.181	0.025
Milk replacer intake, kg/d	0.645	0.645	0.645	—
Feed efficiency ^{2,3}	0.529	0.569	0.540	0.018
DE intake, Mcal/d	3.67	3.68	3.74	0.54
CP intake, kg/d	0.197	0.197	0.202	0.023
ADP intake, ⁴ g/d	0.180	0.178	0.182	0.021
Lys intake, g/d	16.3	16.2	16.4	0.2
Met intake, ⁵ g/d	4.6	5.1	5.7	0.6
Thr intake, g/d	13.7	13.7	14.1	1.6
Energy-allowable ADG, ⁶ kg/d	0.50	0.50	0.52	—
Protein-allowable ADG, ⁶ kg/d	0.65	0.65	0.66	—

¹Quadratic relationship to Met ($P < 0.01$).

²Gain divided by milk replacer (MR) plus starter intake.

³Quadratic relationship to Met ($P < 0.05$).

⁴Apparent digestible protein intake ($0.93 \times \text{MR CP}$ and $0.75 \times \text{starter CP}$).

⁵Linear relationship to Met ($P < 0.05$).

⁶Predicted ME and ADP allowable gain using NRC (2001) submodel.

added AA had greater ADG and efficiency than calves fed the MR without added Lys and Met. In study 1, the MR with 26% CP and added Lys (2.34%) and Met (0.72%) supported the greatest ADG. The difference in ADG between calves fed the 26% CP MR with and without Lys and Met was 16%. The calves fed the MR with 26% CP and added Lys and Met had 8% greater ADG than the calves fed the 28% CP MR without added no Lys and Met. The 28-d postweaning ADG (0.87 kg/d), starter intake (1.90 kg/d), and other performance measures of the calves did not differ ($P > 0.05$) among treatments.

Assayed nutrients for the MR fed in study 2 are reported in Table 2. In study 2, Met intake and ADG increased linearly ($P < 0.05$) and starter intake responded quadratically ($P < 0.05$) to concentration of Met in the MR (Table 6). Starter intake decreased at the first addition of Met (0.68%) but was greatest for calves fed the high (0.72%) concentration of Met. The 28-d postweaning ADG (0.73 kg/d), starter intake (1.83 kg/d), and other performance measures of the calves did not differ ($P > 0.05$) among treatments.

Assayed nutrients for the MR fed in study 3 are reported in Table 3. In study 3, ADG and feed efficiency responded quadratically ($P < 0.05$) with increasing concentrations of Met in the MR (Table 7). Maximum response was achieved with the intermediate concentration of 0.72% Met and there was no increase in ADG or efficiency with the high concentration of 0.80% Met. Serum concentrations of urea nitrogen and creatinine responded in a quadratic manner ($P < 0.05$) to Met

concentration in the MR (Table 8). Calves fed the low concentration of 0.64% Met had the greatest serum concentration of urea nitrogen, indicating inefficient use of dietary N, and lowest concentration of creatinine, indicating less muscle mass, relative to the greater concentrations of Met. Other serum metabolites measured did not differ ($P > 0.05$). The 28-d postweaning ADG (0.76 kg/d), starter intake (1.88 kg/d), and other performance measures of the calves did not differ ($P > 0.05$) among treatments.

Assayed nutrients for the MR fed in study 4 are reported in Table 4. In study 4, Thr intake increased linearly ($P < 0.05$) with Thr concentration of the MR (Table 9). There were no differences ($P > 0.05$) for ADG, starter intake, or efficiency relative to Thr concentration of the MR. Serum metabolites are reported in Table 10. Serum urea nitrogen concentrations responded quadratically ($P < 0.01$) to concentration of Thr in the MR. Calves fed MR with 1.80% Thr had a greater concentration of serum urea nitrogen than calves fed the MR with 1.06 and 1.43% Thr. Serum alkaline phosphatase responded quadratically ($P < 0.05$) to concentration of Thr in the MR with calves fed the MR with 1.43% Thr having the greatest increase. Other serum metabolites measured did not differ ($P > 0.1$). The 28-d postweaning ADG (0.88 kg/d), starter intake (2.28 kg/d), and other performance measures of the calves did not differ ($P > 0.05$) among treatments.

DISCUSSION

In study 1, 2.34% Lys (0.48 g of Lys/Mcal of digestible energy, DE) in the MR maximized ADG and efficiency.

Table 8. Effect of 3 concentrations of Met on selected serum constituents of calves in study 3

Item	Formulated Met concentration, %			SEM
	0.64	0.72	0.80	
Urea nitrogen, ¹ mmol/L	4.2	2.6	2.5	0.14
Creatinine, ² μ mol/L	82.2	93.5	88.0	2.8
Alkaline phosphatase, U/L	223	240	235	20
Albumin, g/L	13.6	13.6	13.8	0.36
Total protein, g/L	51.7	51.7	51.9	0.79
Glucose, mmol/L	5.2	5.3	5.3	0.20

¹Quadratic relationship to Met ($P < 0.01$).²Quadratic relationship to Met ($P < 0.05$).

This calculated to 4.6 g of Lys/Mcal of DE consumed from both the MR and starter. In study 2, a maximum ADG response to Met was not achieved. One anomaly of this study was that starter intake responded quadratically to Met in the MR with the 0.68% Met (intermediate) treatment having a lower intake. No other study had a change in starter intake because of treatment. If starter intake of this intermediate Met treatment in study 2 had been the average of the low and high Met treatments, this added starter intake would have increased ADG to approximately 0.46 kg/d (per equations of NRC, 2001) perhaps resulting in a quadratic relationship of ADG to Met in the MR rather than the linear relationship observed. The ADG response would have then approached a plateau, indicating that 0.68% Met was near the optimum. In study 3, 0.72% Met or a Met to Lys ratio of 0.31 appeared near optimum.

In study 4, ADG, starter intake, and efficiency did not change with Thr concentration, which could indi-

cate that 1.06% Thr or a Thr to Lys ratio of 0.45 is adequate. However, the change in serum alkaline phosphatase might also indicate that the low concentration of 1.06% Thr was deficient, supporting less bone formation, and that the intermediate concentration of 1.43% Thr (0.60 Thr to Lys ratio) was adequate. The 1.43% Thr concentration was selected to be approximately equal to the 0.6 ratio of Thr to Lys from both Williams and Hewitt (1979) and Toullec (1989). The Cys concentration of skim milk is low relative to whey protein concentrate. The MR in study 4 had approximately 0.27% Cys whereas those in studies 1, 2, and 3 had approximately 0.55% Cys. The concentrations of 2.34% Lys, 0.72% Met, and 0.55% Cys yield a Met+Cys to Lys ratio of 0.54, which is greater than estimates from Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997), which ranged from 0.41 to 0.51. However, the Met+Cys to Lys ratio in study 4 was approximately 0.42, similar to the estimate of

Table 9. Effect of 3 concentrations of Thr on ADG, intake, and efficiency of calves for 28 d in study 4

Item	Formulated Thr concentration, %			SEM ¹
	1.06	1.43	1.80	
Calves, n	16	16	16	—
Initial serum protein, mg/dL	5.0	5.0	4.9	0.2
Initial weight, kg	45.1	44.9	45.9	1.5
ADG, kg/d	0.569	0.576	0.563	0.043
Starter intake, kg/d	0.284	0.286	0.272	0.034
Milk replacer intake, kg/d	0.638	0.638	0.638	—
Feed efficiency ²	0.614	0.620	0.616	0.031
Digestible energy intake, Mcal/d	4.03	4.03	3.99	0.54
CP intake, kg/d	0.220	0.221	0.219	0.023
ADP intake, ³ g/d	0.196	0.196	0.194	0.019
Lys intake, g/d	17.4	17.2	17.2	0.2
Met intake, g/d	5.5	5.4	5.5	0.6
Thr intake, ⁴ g/d	10.1	12.8	14.8	1.6
Energy-allowable ADG, ⁵ kg/d	0.29	0.30	0.28	—
Protein-allowable ADG, ⁵ kg/d	0.71	0.71	0.70	—

¹No differences among Thr concentrations ($P > 0.05$).²Gain divided by milk replacer (MR) plus starter intake.³Apparent digestible protein intake ($0.93 \times \text{MR CP}$ and $0.75 \times \text{starter CP}$).⁴Linear relationship to Thr ($P < 0.05$).⁵Predicted ME and ADP allowable gain using NRC (2001) submodel.

Table 10. Effect of 3 concentrations of Thr on selected serum constituents of calves in study 4

Item	Formulated Thr concentration, %			SEM
	1.06	1.43	1.80	
Urea nitrogen, ¹ mmol/L	3.2	3.1	3.8	0.14
Creatinine, μ mol/L	81.2	83.6	79.7	3.2
Alkaline phosphatase, ¹ U/L	116	191	173	7.1
Albumin, g/L	13.8	13.9	13.6	0.23
Total protein, g/L	58.9	58.4	57.3	0.62
Glucose, mmol/L	6.0	5.6	5.7	0.26

¹Quadratic relationship to Thr ($P < 0.01$).

0.41 from van Weerden and Huisman (1985). If the Met+Cys to Lys ratio was a limiting nutrient, it may not have allowed for a proper evaluation of Thr concentrations. Another difference in study 4 and the previous studies was the low temperature of -6°C . This low temperature did not result in lower ADG compared with the previous studies. In fact, ADG and starter intake of calves in study 4 appeared greater than in the other studies. Cold is expected to increase intake and to increase maintenance energy requirements and not affect ADP requirements (NRC, 2001). Predicted energy-allowable ADG were approximately 0.30 kg/d for calves in study 4 or approximately half of actual ADG. A micro-environment considerably warmer than -6°C within the straw bedding of the calves might have reduced any cold stress of the calves (Hill et al., 2007d), and temperature should not have affected the results of the Thr treatments.

The optimum concentrations of Lys (2.34%) and Met (0.72%) and the average concentration of AA, their daily intake from MR, and their ratio to Lys from studies 1, 2, and 3 where whey-based MR were fed are shown in Table 11. Similar data from study 4, in which the MR with skim milk were fed using 1.84% Thr, are also shown in Table 11. Also, the calculated AA ratios to Lys from Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997) are listed in Table 11. For studies 1, 2, and 3, the ratios to Lys for His (0.21), Arg (0.28), and Phe+Tyr (0.66) were lower than ratios to Lys calculated from Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997). The ratios to Lys for Val (0.64) and Ile (0.64) are lower than ratios to Lys calculated from Toullec (1989) but greater than values from Williams and Hewitt (1979), van Weerden and Huisman (1985), and Gerrits et al. (1997). For study 4, the ratios to Lys for Arg (0.38), His (0.29), Ile (0.60), Leu (1.05), and Met+Cys (0.42) were lower than some of the ratios calculated from the literature. The AA ratios determined in studies 1, 2, and 3 using whey-based MR and in study 4 using skim-milk-based MR were different from the literature in which

skim-milk-based MR were fed. We are not aware of attempts to measure a response to supplementing His, Arg, Phe, Tyr, Val, Leu, and Ile in whey-based calf MR, and these AA are presently not commercially cost effective as synthetic AA.

Starter nutrients would be subjected to some degradation in the immature rumen and should have a lower postruminal digestibility than milk protein. Yet by design, calves were weaned early to minimize the contribution of starter to nutrient intake during the 28-d preweaning period. Starter intake averaged 0.15 kg daily in studies 1, 2, and 3. Using this intake and the average CP and AA concentrations of the starters, the starters only contributed approximately 13% of CP (27 g) and 8% of Lys (1.4 g), Met (0.4 g), and Thr (0.5 g) consumed by the calves each day. Total daily intakes were approximately 204 g of CP, 17 g of Lys, 5 g of Met, 13 g of Thr, and 9 g of Met+Cys in calves gaining approximately 0.46 kg/d and fed the 26% CP with synthetic Lys and Met. In study 1, calves fed the 28% CP MR with synthetic Lys and Met consumed 9 to 11% more Lys, Met, Cys, and Thr and 4 to 12% more of the other AA than calves fed the 26% CP MR with synthetic Lys and Met. All of the AA were approximately adequate in the 26% CP MR with synthetic Lys and Met, because calves fed the 26 and 28% CP MR with synthetic Lys and Met had similar ADG (0.484 and 0.468 kg/d) and efficiency (0.608 and 0.596).

The daily intakes of 204 g of CP, 17 g of Lys, 5 g of Met, 13 g of Thr, and 9 g of Met+Cys in 48-kg calves gaining 0.46 kg of BW/d in the current trials were greater than estimates of Gerrits et al. (1997) in calves gaining 0.932 kg/d, implying less efficient use of nutrients in the current studies. Gerrits et al. (1997) estimated requirements of 16.3 g of Lys/d, 4.2 g of Met/d, 10.8 g of Thr/d, and 7.6 g of Met+Cys/d in 90-kg veal calves consuming 263 g of CP/d. The estimates of Gerrits et al. (1997) for these AA increased 11% for 90-kg calves consuming 352 g of CP/d and gaining 1.056 kg/d. Williams and Hewitt (1979) estimated that 6- to 14-wk-old, 50- to 58-kg BW calves gaining 0.25 kg/d required 7.8 g of Lys. The housing of calves in the current

Table 11. Amino acid concentrations in milk replacer (MR), intake from MR, and ratio to Lys in the current studies (studies 1, 2, and 3 were whey-based diets; study 4 was a diet based on skim milk protein) and ratio to Lys in selected literature studies¹

Item	Studies 1, 2, and 3			Study 4			Williams and Hewitt (1979)	van Weerden and Huisman (1985)	Toullec (1989)	Gerrits et al. (1997)
Calf age, wk	0 to 4			0 to 4			6 to 14	5 to 7	8 to 20	8 to 20
Cal ADG, kg	0.45 to 0.47			0.57			0.25	0.9	1.0	1.0
AA	%, as fed ²	Intake, g/d	Ratio to Lys	%, as fed ³	Intake, g/d	Ratio to Lys	Ratio to Lys			
Lys	2.34	15.9	1.00	2.34	15.9	1.00	1.00	1.00	1.00	1.00
Met	0.72	4.9	0.31	0.72	4.9	0.31	0.27	—	—	0.25
Cys	0.55	3.8	0.24	0.27	1.9	0.12	0.20	—	—	0.21
Thr	1.84	12.5	0.79	1.45	9.9	0.62	0.63	0.50	0.60	0.66
Arg	0.66	4.5	0.28	0.88	6.0	0.38	1.09	0.33	0.44	0.40
Glu	4.40	30.0	1.88	3.57	24.3	1.52	—	—	—	—
His	0.49	3.3	0.21	0.68	4.6	0.29	0.38	0.25	0.31	0.39
Ile	1.49	10.2	0.64	1.40	9.5	0.60	0.44	0.62	0.67	0.43
Leu	2.65	18.0	1.13	2.46	16.8	1.05	1.08	0.87	1.02	1.12
Phe	0.82	5.6	0.35	1.20	8.2	0.51	—	—	—	0.59
Trp	0.44	3.0	0.19	0.40	2.7	0.17	0.13	0.11	0.09	0.12
Tyr	0.72	4.9	0.31	1.18	8.0	0.50	—	—	—	0.38
Val	1.51	10.2	0.64	1.74	11.8	0.74	0.62	0.61	0.71	0.63
Met+Cys	1.27	8.7	0.54	0.99	6.7	0.42	0.47	0.41	0.51	0.46
Phe+Tyr	1.54	10.5	0.66	2.37	16.1	1.01	0.95	0.88	0.71	0.97

¹No starter, only MR was given to calves in the studies from the literature.

²Nutrient concentrations in the whey-based MR that maximized ADG.

³Nutrient concentrations in the skim-based MR in study 4 with the intermediate concentration of Thr.

studies in an unheated environment, their young age, the consumption of starter, and their lower intake of MR may explain their less efficient use of AA (NRC, 2001) compared with calves from the previous literature cited.

The NRC (2001) calf submodel was used to predict energy- and ADP-allowable ADG for each study. For these predictions, average BW, MR and starter intake, and average environmental temperature were used as inputs to the model. In each study, energy-allowable ADG was lower than ADP-allowable gain indicating that the model predicted energy to be the growth-limiting nutrient. The NRC model does not account for AA, so the model could not predict the differences in ADG observed from Lys and Met concentrations in the MR. The observed ADG increased 0.07 kg/d between the calves fed the 24 and 28% CP MR with no added Lys and Met in study 1. However, the NRC (2001) calf submodel did not predict a response to increasing CP for these studies because energy was predicted to limit ADG. In studies 1, 2, and 3, predicted ADG was greater than measured ADG. Environmental temperatures were set to 18, 13, and 12°C in studies 1, 2, and 3, respectively. In study 4, measured ADG was greater than predicted ADG. Average temperature in study 4 was –6°C, which increased the maintenance energy requirement by 40% and therefore would lower the energy-allowable gain. The difference in energy-allowable ADG when the temperature changed from 20°C (ther-

monneutral) to –6°C was 0.36 kg/d. Temperatures of 10 and 11°C would have yielded predicted energy-allowable ADG of 0.52 and 0.59 kg/d, respectively, closer to the actual ADG of 0.57 kg/d. For these studies, the NRC (2001) model did not accurately predict ADG and was not responsive to the changes resulting from increasing the CP of the diet. Although the environmental temperature adjuster decreases predictions of ADG from the NRC (2001) calf submodel, other errors in the model structure must occur. The overestimation by NRC (2001) could be explained by either an underestimation of the energy requirement for maintenance or an overestimation of the digestibility of the nutrients for very young calves.

The NRC (2001) uses a fixed and high biological value for the CP of milk protein-based MR (0.80) and a high conversion of CP to ADP (0.93) and states that those values are too high for very young calves. This is confirmed by the results of the current studies and this topic has been discussed by Van Amburgh and Drackley (2005). Assuming that the conversion of CP from whey to ADP is 85% in very young calves (Tanan, 2005), a biological value can be calculated for each treatment so that the requirement of ADP for the observed ADG matches the supply of ADP. The improvement in ADG observed with the supplementation of synthetic AA cannot be predicted by the NRC (2001) system. In study 1, the CP of the MR with 24 and 26% CP without synthetic AA and the 28% CP MR with or without synthetic

AA would present a biological value of 0.66 to 0.68. For the MR with 24 and 26% synthetic AA, the biological value would be approximately 0.75. Those values are similar to the range in biological values (0.67 to 0.72) from Terosky et al. (1997), as referenced in NRC (2001), using both whey and skim milk protein. Those biological values are also similar to estimates of Diaz et al. (2001), Blome et al. (2003), and Bartlett et al. (2006) that ranged from 0.63 to 0.75 using whey proteins.

CONCLUSIONS

The response to added Lys and Met in study 1 was large (approximately 17%). The responses to added Met in studies 2 and 3 were 13 and 7%, respectively; a smaller response than in study 1, although the Met deficiency was smaller. These large responses to added AA show the need to formulate MR for Lys and Met, and not just CP. Feeding calves 0.68 kg of a whey-based MR with synthetic Lys and Met that was 26% CP, 17% fat, 2.34% Lys, 0.72% Met, 1.27% Met+Cys, and 1.8% Thr maximized ADG and efficiency. Feeding calves 4 to 11% more CP and all essential AA did not improve ADG and efficiency. For calves less than 5 wk old, averaging 48 kg of BW, consuming 204 g of CP/d, and gaining 0.46 kg of BW/d, their requirements appeared to be met with 17 g of Lys, 0.31 Met to Lys ratio, 0.54 Met+Cys to Lys ratio, and a Thr to Lys ratio less than 0.60.

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