Feeding an amino acid–formulated milk replacer for Holstein calves during 2 time periods

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ABSTRACT

The milk-fed calf has a requirement for essential amino acids (EAA) instead of crude protein (CP). However, most milk replacers (MR) are still formulated to a CP concentration, and although limited amounts of Lys or Met may be added, these MR are not formulated solely on an AA basis. Previous work has demonstrated that feeding a modified MR balanced for specific EAA concentrations of a 24% CP MR, but reducing CP to 22%, resulted in improved growth performance in calves compared with a 24% CP MR. The 56-d objective was to determine if the hypothesis that an EAA MR formulated to the specific EAA concentrations of a 24% CP MR, but reducing CP to 22 (22AA) compared with a standard 22% CP MR would result in similar or enhanced growth performance, while reducing feed costs. Two 56-d studies using forty 3- to 5-d-old Holstein bull calves per study received in 1 lot starting on May 8, 2018 (late-spring cooler) and June 22, 2018 (summer with heat stress) were blocked by body weight (BW) and randomly assigned to 1 of 2 MR treatments. Milk replacer treatments consisted of a standard 22:20 (CP: fat; 22CP) and a 22:20 MR with greater EAA concentrations of a 24% CP MR (22AA). All MR, containing decoquinat and diflubenzuron, were fed at 0.57 kg/d per calf split into 2 feedings at 0630 h and 1800 h for 0 to 14 d via bucket, increased to 0.85 kg/d through 35 d split in 2 feedings, and fed once per day at 0.41 kg/d in the morning with weaning after 42 d. Calves were housed in straw-bedded hutches with ad libitum access to water and 25% CP pelleted calf starter (CS). All data were analyzed as a randomized complete block design with block within study considered random and week as a repeated measurement. Initial BW was similar across all treatments (39.9 ± 2.32 kg). Average

daily temperature (i.e., heat stress intensity) tended to be greater for calves in study 2 compared with study 1 (20.5 and 22.9°C for study 1 and 2, respectively). The interaction of study and MR effects were nonsignificant. Calf BW, BW gain, average daily gain, CS intake, total dry matter intake (MR plus CS), feed conversions, and EAA intake (MR plus CS) were similar for calves fed both MR. Study 2 (summer heat stress) resulted in more scours incidences compared with study 1, and 22AA MR formulation tended to increase scours possibly due to synthetic AA addition or greater carbohydrate (lactose) inclusion when AA replaced protein sources. Feed costs as MR and total feed costs were lower for calves fed 22AA MR compared with calves fed 22CP MR. The increased Leu, Lys, Met, Thr, and Val concentrations in the 22AA MR compared with the 22CP MR resulted in similar calf growth performance, but the feed cost was lower for calves fed the 22AA MR, which achieved similar growth performance at a lesser cost under current study conditions, but not enhanced as hypothesized.

Key words: amino acids, calf, growth, heat stress, milk replacer

INTRODUCTION

Our previous work (Bai et al., 2020) demonstrated that reducing CP concentration in milk replacer (MR) by using synthetic AA to maintain AA intake equivalent to a higher CP MR improved growth performance and lowered feed cost. The calf requires EAA instead of CP for growth, even though MR are still formulated on a CP basis. The dairy NRC (2001) calf model predicts protein-limited and energy-limited growth performance, but the model does not predict EAA requirements. The use of synthetic EAA provides the opportunity to further define the EAA requirements for optimal growth performance at a potentially lower cost than CP.

Many studies show that calves will consume large amounts of milk or MR when offered (Jasper and Weary, 2002; Vieira et al., 2008; Borderas et al., 2009).

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However, weaning and postweaning ADG slump has plagued the success of some high MR feeding rate (**FR**) programs when calf starter (**CS**) was fed (Bar-Peled et al., 1997; Jasper and Weary, 2002; Hill et al., 2006). This resulted in a series of studies (Jaeger et al., 2020a,b,c) developing a modified accelerated MR program, which increased growth performance compared with calves fed other CP concentrations. Those studies optimized the CP:energy (fat) ratio, but EAA concentrations were not considered. Increased calf growth performance and no CS consumption slump was demonstrated by Bai et al. (2020) when feeding a 22% CP MR balanced to specific EAA concentrations of a 24% CP MR compared with a standard 24% CP formulated MR. Therefore, the development of a modified accelerated MR based on specific EAA formulation should minimize feed costs with the added benefit of supplying more EAA for lean tissue and frame growth during a standard 6-wk weaning program. Our hypothesis was that a 22 EAA MR formulated to the EAA concentration of a 24% CP MR would enhance growth performance at a reduced feed cost compared with a standard 22% CP formulated MR. The study objectives were to determine the growth performance of calves fed commercially available standard 22% CP MR compared with calves fed a 22% CP MR formulated to the EAA concentrations of a 24% CP MR.

MATERIALS AND METHODS

The following 2 experiments reported were conducted using the same facilities, experimental design, procedures, and sample collection as previously published in Bai et al. (2020). The experimental materials and methods are briefly reported below.

Calf Management and Feeding

The 2 experiments were conducted at Casper's Calf Ranch (Freeport, IL). The first experiment started on May 8, 2018 (study 1) and the second experiment started on June 22, 2018 (study 2). The calves were managed and fed according to the guidelines published in the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010), as well as the Chinese Standards for the Use and Care of Research Animals (He et al., 2016).

Both experiments started with forty 3- to 5-d-old Holstein bull calves sourced by a commercial calf buyer (Knueppel Livestock, Shawno, WI) and delivered either on Tuesday (study 1) or Thursday night (study 2). Calves were purchased through several livestock auction barns and represented commingled calves sourced from numerous dairy farms. Calves were assumed to

have been fed colostrum at the dairy farms, but no blood samples were collected for measurement of total serum protein due to the concern of transportation stress and dehydration biasing the results (Guzelbektes et al., 2007). Calves were vaccinated upon arrival with Inforce 3 (Zoetis Inc. Florham Park, NJ) and placed in a chopped wheat straw-bedded calf hutch (measuring 220 × 122 × 38 cm; Calf-Tel Deluxe II, Hampel, Germantown, WI) that was placed on a grass pasture in an open, naturally well-ventilated area. Hutches were spaced 0.6 m apart in rows of 10 hutches. Each hutch had a 183 cm × 122 cm × 107 cm wire panel attached to the front with 2 bucket holders (0.65 m high) for 8-L plastic buckets. One bucket contained ad libitum fresh water and the other bucket contained a pelleted CS. The water bucket was emptied at each feeding and filled with the appropriate experimental MR at the designated FR. Upon completion of milk consumption, the bucket was rinsed and filled with fresh water. Calves were fed the control 22CP MR the next morning after arrival during the night. After the morning feeding, calves were weighed and their frame measurements collected. Calves were then blocked by BW and randomly assigned to 1 of 2 MR treatments. Treatments were: (1) a standard formulation of 22% CP MR (**22CP**) that is commercially available and (2) an AA-formulated MR with the EAA concentrations of a 24% CP MR, but the CP reduced to 22% (**22AA**). All MR contained 20% fat, decoquinate (Deccox, Zoetis Inc., Parsippany, NJ) added at the rate of 41.7 mg/kg (as-is basis) for coccidiosis control, and 16 mg/kg diflubenzuron (as fed MR basis; ClariFly, Central Life Sciences, Schaumburg, IL) for fly control. The ingredient composition of the MR is given in Table 1.

The MR feeding time was fixed at 0630 and 1800 h and the feeding sequence was kept consistent with the 22CP fed first, shuttle rinsed, followed by the 22AA. Each MR was accurately weighed (model ACE110, Smart Weigh Inc., Hurricane, WV) and mixed with the appropriate amount of hot water (46°C) using an Urban Milk Shuttle (Urban GmbH and Co., Hamburg, Germany). The shuttle and its computerized mixing, heating, and delivery systems were calibrated to ensure an accurate delivery of a homogeneously mixed MR fed at a temperature of 37.5°C or greater at the correct volume. Each experimental MR was fed at the rate of 0.57 kg/calf daily for the first 14 d, then increased to 0.85 kg/calf daily divided into 2 equal feedings (i.e., morning and evening). For 5 d during wk 5 (d 30–35), Amprolium (Merial, LLC., Duluth, GA) was added at the rate of 10 mg/kg of BW to the experimental MR for the coccidiosis control. Starting on d 36 through d 42, the MR FR was decreased to 0.425 kg/calf fed once per day at the morning feeding to facilitate weaning.

Table 1. Ingredient composition of milk replacers (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP¹

Ingredient	MR ²	
	22CP	22AA
Whey (whey protein concentrate)	58.1	54.5
Fat base	38.8	40.2
Dicalcium phosphate	0.47	0.78
L-Lysine HCl	—	1.07
Decoquate 0.5%	1.00	1.00
Vitamin premix	0.91	0.91
DL-Methionine	0.16	0.45
Organic trace mineral premix ³	0.38	0.38
Bioplus 2B ⁴	0.05	0.05
Clarify 8% ⁵	0.02	0.02
Flavor ⁶	0.06	0.06
Threonine	—	0.29
Stay Strong (oregano) ⁷	0.10	0.10
L-Valine	—	0.11
Vitamin E	0.09	0.09
L-Leucine	—	0.09

¹Tag guarantee of 22% CP, 20% fat, not less than 55,000 IU of vitamin A, 16,500 IU of vitamin D, and 220 IU of vitamin E per kilogram on an as-fed basis. Manufactured by Milk Specialties Global, Eden Prairie, MN.

²Reported as percent of mix.

³Milk Specialties Global.

⁴Chr. Hansen, Milwaukee, WI.

⁵Diflubezuron, Central Life Sciences, Schaumburg, IL.

⁶Milk Specialties Global.

⁷Ralco Nutrition Inc., Marshall, MN.

All experimental MR were fed at 15% solids, and if any calf did not consume its milk, the refused volume was recorded. Starting on d 1, a 2.4-mm mini-pelleted 22% CP CS (as-is basis; Table 2) and water were offered ad libitum throughout the study. All experimental MR were manufactured and supplied by Milk Specialties (Eden Prairie, MN) and CS was manufactured and supplied by Furst-McNess (Freeport, IL). All MR and CS were manufactured in sufficient quantities at 1 time for each experiment to complete the study using the same lots of ingredients (Tables 1 and 2). At the completion of the 56-d experimental period, calves were then dehorned via hot iron, castrated using a scalpel to remove the scrotum bottom, had their testes physically removed by a licensed veterinarian (Bardon Scharping, Lena Veterinary Clinic, Lena, IL), and then sold to a privately owned commercial feed lot.

Weather Data

Weather data were downloaded from a local personal weather station site for Freeport, IL located at 42.301° N, 89.665° W at an elevation of 256.9 m, approximately 5 km from the research location. The temperature-humidity index (**THI**) was calculated according to the

equation of Vitali, et al. (2009) based on the weather station data for daily minimum, average, and maximum THI values. A THI value of <72 was considered no heat stress, 72 to 79 was considered mild, 80 to 89 was considered moderate, and >90 was considered severe heat stress. Although these ranges are based on heat stress in lactating dairy cows, these ranges should be applicable to calves based on limited literature data (Roland et al., 2016).

Feed Intake and Analysis

Starting on d 1, the amounts of CS offered and refusal weights were recorded daily using a digital scale (model ACE110, Smart Weigh Inc., Hurricane, WV). For days that feed was wet (i.e., rain), that day's data was eliminated and the remaining days during that week were compiled into weekly means of CS intake. Samples of each experimental MR and CS were collected weekly and stored frozen at −20°C until composited at the end of the study. Samples collected during wk 1, 2, and 3 or wk 4, 5, and 6 were composited into 2 samples for each experimental MR, and CS samples collected during wk 1, 2, 3, and 4 or wk 5, 6, 7, and 8 were composited into

Table 2. Ingredient composition of pelleted calf starter (CS)¹

Ingredient	CS (%)
Wheat middlings	35.0
Soybean meal, 48	25.5
Corn, fine ground	22.5
Molasses mixer	5.50
Distiller's grains with solubles ²	5.00
Calcium carbonate	2.25
Corn starch	1.25
Salt	1.10
Sodium bentonite	0.50
Soy oil	0.43
Bovine B premix ³	0.25
Vitamin A premix ⁴	0.18
Vitamin E premix ⁵	0.16
Dairy trace mineral premix ⁶	0.13
Vitamin D premix ⁷	0.07
Clarify 0.67% larvicide (diflubenzuron)	0.05
Cherry flavor	0.05
Selenium 0.06%	0.02
Rumensin 198.4 g/kg (monensin)	0.02

¹Manufactured by McNess, Freeport, IL. Reported as percent of mix.

²Fermented corn byproduct.

³Bovine B contains biotin 10.5 mg/kg, choline 167.6 g/kg, folic acid 50.3 mg/kg, niacin 94 g/kg, pantothenic acid 10.5 g/kg, pyridoxine 4.4 mg/kg, riboflavin 4.4 g/kg, thiamine 1.1 g/kg, and vitamin B₁₂ 19.8 mg/kg.

⁴Vitamin A premix contains 11,050,072 IU/kg.

⁵Vitamin E premix contains 44,750 IU/kg.

⁶Trace mineral premix contains cobalt 1,350 mg/kg, copper 23,500 mg/kg, iodine 2,000 mg/kg, manganese 100,000 mg/kg, selenium 510 mg/kg, and zinc 125,000 mg/kg.

⁷Vitamin D premix contains 8,375,055 IU/kg.

2 lots. One water sample was collected from the water supply used for both mixing MR and free choice water offered. Feed and water samples were submitted to Cumberland Valley Analytical Services (Waynesboro, PA) for nutrient analyses. Samples of MR were analyzed using the following AOAC International (2019) methods for DM (930.15), CP (990.03), acid hydrolysis fat (954.02), NDF (2002.04), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), K (985.01), S (923.01), Na (985.01), Cu (985.01), Fe (985.01), Mn (985.01), and Zn (985.01). Calf starter samples were analyzed for DM (930.15), CP (990.03), soluble protein (Krishnamoorthy et al., 1982), NDF (2002.04), ADF (973.18), lignin (973.18), NDF insoluble protein (2002.04 without sulfite and 976.06), ADF insoluble protein (973.18 and 976.06), water soluble sugar (DuBois et al., 1956), starch (Hall, 2009), fat (2003.05), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), K (985.01), S (923.01), Na (985.01), Cu (985.01), Fe (985.01), Mn (985.01), and Zn (985.01). Chloride for both MR and CS samples were extracted with 0.5% nitric acid and analyzed by potentiometric titration with silver nitrate (Metrohm 848 Titrino Plus, Metrohm, Riverview, FL). Nonfiber carbohydrates were calculated using the following equation: $NFC = \{100 - [(NDF - NDF \text{ insoluble protein}) + CP + \text{fat} + \text{ash}]\}$, and ME (Mcal/kg) was calculated for MR and CS using the equations of Quigley (2007) and NRC (2001). Samples of experimental MR and CS were sent to the Agricultural Experiment Station Chemical Laboratories at the University of Missouri–Columbia for AA analysis (AOAC International, 2019; method 982.30E). For all AA except cystine, methionine, and tryptophan, a test portion was hydrolyzed in 6 M HCl at 110°C in an atmosphere free of oxygen for 24 h. The hydrolysate was filtered to remove carbon and brought to a specified volume. An aliquot of the filtrate was evaporated to dryness under vacuum. The AA residue was dissolved in a buffer and injected into an AA analyzer. For cystine and methionine, a test portion was mixed with cold performic acid and allowed to stand overnight between 0 and 5°C to convert methionine to methionine sulfone and cysteine and cystine to cysteic acid. The performic acid mixture was then neutralized with cold hydrobromic acid and evaporated to dryness under vacuum. The residue was then hydrolyzed in acid as described previously. For tryptophan, a test portion was hydrolyzed in 4 M NaOH under vacuum for 22 h at 110°C. The hydrolysate was neutralized with HCl and diluted to a specified volume using a buffer. Aliquots of the prepared hydrolysates were injected onto an AA analyzer (Hitachi Model 8900, Hitachi, Schaumburg, IL) that used the classical ion exchange resolution and ninhydrin postcolumn derivatization method developed by Moore and Stein (1963).

To evaluate MR solubility, we mixed a typical MR feeding of 0.425 kg with 2.85 L of 110°C water, let it sit for 5 to 10 min (approximate consumption time by calf), and then filtered it through 4 layers of cheesecloth. The cheesecloth was dried and weighed before and after filtering the individual MR samples to determine the amount of residue remaining on the cheesecloth. Each experimental composite MR sample was replicated 3 times.

Body and Health Measurements

Body weights were measured weekly using a Wrangler Jr. digital scale (Digi-Star, LLC, Fort Atkinson, WI) placed on a 1.2 m × 2.4 m sheet of 1.9 cm thick sheet of plywood towed by a John Deere 825 Gator (John Deere, Moline, IL). Body weights were taken after the morning feeding starting at approximately 0900 h each week. Hip height (**HH**) and withers height (**WH**) were measured using a Ketchum Teletape with a level mounted on top (Ketchum Manufacturing Inc., Brockville, Canada). Body length (**BL**) and heart girth (**HG**) were measured using a Nasco dairy calf weigh tape (Nasco, Fort Atkinson, WI). Body measurements were taken the same time as BW, but only every 4 wk (i.e., wk 0, 4, and 8).

Calf health, along with fecal, nasal, and ear/eye scores were monitored daily during the experiments. Health scores were visually assessed before the evening feeding according to the University of Wisconsin calf health scoring chart (McGuirk, 2013) based on a scale of 0 to 3. Fecal scores were established as 0 (normal), 1 (semi-formed or pasty), 2 (loose but stays on top of bedding), and 3 (watery or sifts through bedding). Nasal scores were established as 0 (normal serous discharge), 1 (small amount of unilateral cloudy discharge), 2 (bilateral, cloudy, or excessive mucus discharge), and 3 (copious bilateral mucopurulent discharge). Eye/ear scores were established as 0 (normal), 1 (small amount of ocular discharge with ear flick or head shake), 2 (moderate amount of bilateral ocular discharge or slight unilateral droop), and 3 (heavy ocular discharge, head tilt, or bilateral droop). In the case of illness, body temperature was measured using a rectal thermometer (Zoe+Ruth, Portland, OR) and appropriate medical treatments prescribed by a licensed veterinarian (Brandon Scharping, Lena, IL) were administered if needed. All health incidents and treatments were recorded during the study.

Economic Analysis

The time point of March 2019 was selected to calculate the feed costs to raise the calves. Retail prices of

MR and CS were based on Furst-McNess's retail price list to the dairy producer and did not include freight costs. Data on BW gain, MR intake, and CS intake were used with retail product pricing to calculate the feed costs for MR, CS, total DMI, and dollars per kilogram of BW gain. Retail MR prices were \$2.91 and \$2.78/kg, and CS was \$0.43/kg. Per the study's hypothesis, using synthetic AA to meet the AA specifications in the MR formulation while reducing CP concentration caused a reduction in the ingredient cost, resulting in a more economical MR that meets the AA concentrations of a 24% CP MR, (i.e., \$2.91 versus \$2.78 for 22CP and 22AA, respectively).

Statistical Analysis

All data were checked for normality and outliers using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) before any statistical analyses were conducted. Two calves from study 2 were identified as statistical outliers, and their individual growth data indicated that these 2 calves were stunted for unknown reasons. The box and whisker plots and the Shapiro Wilk test were then used to verify that the remaining data were normally distributed ($P > 0.10$). All data were then subjected to least squares ANOVA for a randomized complete block design (Steele and Torrie, 1980) in a 2×2 factorial arrangement of treatments via the MIXED procedure of SAS with study week as a repeated measure ANOVA. The statistical model used was as follows:

$$Y_{ijk} = \mu + Rep(Study)_i + Study_j + MR_k \\ + (Study_j \times MR_k) + W_l + (MR_k \times W_l) \\ + (Study_j \times W_l) + (Study_j \times MR_k \times W_l) \\ + Cov + e_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, $Rep(Study)_i$ = replication nested within study, $Study_j$ = study, MR_k = MR_k, $Study_j \times MR_k$ = study by MR interaction, W_l = week of study, $Study_j \times W_l$ = study by week interaction, $MR_k \times W_l$ = MR by week interaction, $Study_j \times MR_k \times W_l$ = study by MR by week interaction, Cov = covariate (initial measurement when appropriate), and e_{ijk} is the residual random error. Study, MR, week, and all interactions were considered fixed effects, and replication nested with study was considered a random effect. Study week was considered a repeated measurement in time having an autoregressive covariance structure. Least squares means were separated by PDIF statement when the F-test for MR was significant. All other data were summarized using

the same model described above, but excluded week. Initial BW and frame measurements were tested as a covariate for their respective parameters, but did not improve statistical significance ($P > 0.15$), and therefore excluded from the model. Significance was declared at $P < 0.05$ and trends at $0.05 < P \leq 0.10$. Daily feed intake and orts measurements were compiled as weekly averages and DM and AA intakes were calculated. Each daily fecal, nasal, and eye/ear scores were summarized by tallying (by week) the number of days with a specific score (i.e., number of days with a score of 0) and analyzed as weekly averages. The second method was to total the number of days of a specific score for the entire 6-wk MR phase of the study.

RESULTS AND DISCUSSION

Mixability and Nutrient Composition of Water and Feeds

The remaining residue amount after mixing a typical MR feeding (~0.41 kg) with 110°C water was similar ($P > 0.93$) for calves fed both MR (0.84 and 0.83% for 22CP and 22AA, respectively; SEM = 0.07). Mixability is always a concern with the development of new MR because residues left in the container after feeding are a concern both to the calf (unable to consume all of the allotted nutrients) and the person feeding the calves (who notices the residue remaining). Both MR mixed easily and stayed in suspension to ensure accurate and precise feeding.

The water nutrient composition was typical of water sourced from a northern Illinois well with a pH of 7.08 and the following concentrations: nitrate = 2.42 mg/kg, total dissolved solids = 374 mg/kg, Ca = 76.5 mg/kg, Mg = 39.8 mg/kg, Na = 6.9 mg/kg, Cl = 14 mg/kg, P < 0.10 mg/kg, K < 1 mg/kg, Fe and Mn < 0.05 mg/kg, and Cu and Zn < 0.01 mg/kg. The water was considered hard water (360 mg/kg) due to the high Ca and Mg concentrations, which are related to the total dissolved solids. Total dissolved solids above 960 mg/kg can limit water intake by livestock, but the amount of Ca and Mg in the water would not be likely to affect water intake or growth performance by dairy cattle (Beede, 2012). The trace minerals Fe, Mn, Zn, and Cu are known to limit water intake and livestock performance if concentrations are excessive (Beede, 2012), but in this study all trace minerals were below detection limits of the laboratory's analytical procedures.

The nutrient composition of the 2 MR fed to the calves in this study indicated that the nutrient composition met or slightly exceeded formulated specifications for CP and minerals (Table 3). There was a small trend ($P < 0.10$) for higher DM concentration

of the 22AA MR, probably due to the replacement of whey protein concentrates with synthetic EAA. There were no differences ($P > 0.10$) in CP, SP, NDF, Mn, Zn, and Cu concentrations among the 2 MR. However, concentrations of ME, K, and Na tended to be lower ($P < 0.10$), and ADF, Ca, P, Mg ($P < 0.05$), and ash ($P < 0.01$) were lower for MR 22AA compared with MR 22CP. In contrast, NFC concentrations were higher ($P < 0.05$) for MR 22AA compared with MR 22CP. Substitution of synthetic EAA to meet formulation specifications instead of whey protein concentrate reduced whey protein concentrate inclusion rates, allowing for greater inclusion of carbohydrates (i.e., lactose) because fat concentration is fixed. These nutrient concentrations would meet or exceed the nutrient requirement guidelines for neonatal calves (NRC, 2001). The mini-pelleted CS met or exceeded formulation specifications ($>25.2\%$ CP, DM basis) to provide the nutrients to meet or exceed the NRC (2001) nutrient requirement guidelines for growing Holstein dairy calves (Table 3).

Formulating a MR to Leu, Lys, Met, Thr, Trp, and Val specifications of a 24% CP while reducing CP to 22% resulted in EAA concentration differences (22AA) compared with formulating a standard 22% CP MR (22CP) without AA specifications (Table 4). For example, the concentrations of Arg, His, and Ile were higher

for MR 22CP compared with MR 22AA. Formulating to the specific specifications of Leu, Lys, Met, Thr, Trp, and Val ensured that the animals' requirements for these EAA responsible for growth were closer to being met for optimal growth compared with MR 22 CP. The 22 CP MR may or may not meet the animals' specific EAA requirements due to changes in ingredients, and especially ingredient prices, to meet the formulation specification of a 22% CP MR (22CP), as these can change with reformulation. Bai et al. (2020) reported that formulating MR only to CP specifications can result in variable AA concentrations from batch to batch within each MR due to use of different CP ingredients based on costs, ingredient availability, and nutrient variability. Thus, formulating a MR to specific EAA concentrations (Leu, Lys, Met, Thr, Trp, and Val) ensures that the EAA requirements of the calf can be better managed without over- or under-supplementation of any specific EAA, with the potential of reducing the CP concentration and ultimately reducing ingredient costs. It is interesting to note that formulation of the 22AA MR required the addition of 5 of the 6 synthetic AA offered to the formulation matrix, and the total EAA concentrations were similar at the conclusion ($P > 0.10$). Methionine and Lys are usually considered the first 2 limiting AA (Hill et al., 2007); however, given

Table 3. Nutrient composition of milk replacer formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP and pelleted calf starter¹

Item	Milk replacer		SEM	<i>P</i> -value ²		
	22CP	22AA		Study	Calf starter	SD
N	4	4	—	—	8	—
DM, %	97.1	97.7†	0.17	0.12	87.2	0.68
CP, %	22.4	22.6	0.18	0.20	25.5	0.48
SP, ³ % of CP	21.6	21.9	0.16	0.37	21.6	2.71
ADF, %	1.22	0.73*	0.13	0.23	8.04	0.29
NDF, %	1.23	1.60	0.29	0.34	21.1	1.24
NFC, %	56.7	63.3*	1.75	0.12	40.5	1.40
Starch, %	—	—	—	—	25.0	1.03
Crude fat, %	—	—	—	—	3.76	0.16
ME, ⁴ Mcal/kg	4.82	4.89†	0.03	0.03	2.75	0.04
Ash, %	9.86	7.49**	0.34	0.74	9.20	0.81
Ca, %	1.16	1.11*	0.01	0.16	1.51	0.18
P, %	0.80	0.72*	0.02	0.74	0.80	0.03
Mg, %	0.15	0.14*	0.003	0.13	0.37	0.02
K, %	2.17	1.68†	0.15	0.17	1.53	0.25
Na, %	0.86	0.60†	0.84	0.18	0.56	0.12
Fe, mg/kg	86.0	103.0†	5.59	0.91	269	31.4
Mn, mg/kg	36.0	33.3	4.07	0.24	190	24.7
Zn, mg/kg	110.8	116.5	5.03	0.01	287	93.8
Cu, mg/kg	13.3	10.8	1.07	0.76	53.4	13.6

¹Analyses conducted by Cumberland Valley Analytical Services (Waynesboro, PA).

²*F*-test for probability that study 1 versus study 2 differ.

³SP = soluble protein.

⁴Metabolizable energy was calculated using equations from Quigley (2007) or NRC (2001).

†, *, **Means differ, $P < 0.10$, $P < 0.05$, $P < 0.01$, respectively.

Table 4. Amino acid composition of milk replacer formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP and pelleted calf starter¹

Item	Milk replacer		SEM	$P <^2$		SD
	22	22AA		Study	Calf starter	
N	4	4	—	—		
DM, %	96.5	97.0**	0.04	0.01	88.1	0.57
AA, % of DM						
Arg	0.61	0.58**	0.004	0.47	1.61	0.09
His	0.46	0.44**	0.003	0.01	0.66	0.04
Ile	1.39	1.31**	0.004	0.01	1.04	0.05
Leu	2.25	2.32**	0.01	0.81	1.93	0.03
Lys	1.99	2.71*	0.16	0.69	1.34	0.07
Met	0.60	0.84*	0.03	0.25	0.35	0.01
Phe	0.78	0.75	0.01	0.58	1.18	0.04
Thr	1.48	1.60†	0.03	0.32	0.88	0.02
Trp	0.40	0.38*	0.004	0.02	0.30	0.02
Val	1.36	1.41†	0.01	0.22	1.22	0.05
Total EAA, % of DM	11.3	12.3	0.18	0.11	10.5	0.37
Ala	1.08	1.02**	0.005	0.06	1.20	0.02
Asp	2.26	2.13**	0.01	0.87	2.33	0.13
Cys	0.49	0.45**	0.004	0.01	0.41	0.02
Gly	0.51	0.47**	0.002	0.01	1.09	0.05
Glu	3.61	3.43**	0.01	0.01	4.33	0.18
Pro	1.30	1.26	0.02	0.03	1.42	0.04
Ser	0.98	0.93*	0.01	0.01	0.97	0.04
Tyr	0.67	0.63*	0.01	0.09	0.82	0.02
Total NEAA, % of DM	11.0	10.5	0.06	0.13	12.8	0.48
Total AA, % of DM	22.4	22.7	0.21	0.87	23.3	0.84
CP, %	22.6	22.8*	0.05	0.01	24.8	0.24

¹Analyses conducted by University of Missouri.² F -test for probability that study 1 versus study 2 differ.†, *, **Means differ, $P < 0.10$, $P < 0.05$, $P < 0.01$, respectively.

further reductions in CP concentrations could result in another EAA becoming limiting (i.e., Leu, Thr, Trp, and Val). If these EAA are critical for calf growth performance, then formulating for these specific EAA will ensure consistent animal growth performance over time, (i.e., manufacture batch to batch). For example, calves fed the 22CP MR would have greater intake of several lesser limiting important EAA than calves fed the 22AA by formulating to a CP specification.

The non-EAA concentrations of Ala, Asp, Cys, Gly, Glu, Ser, and Tyr were lower ($P < 0.05$) for MR 22AA compared with MR 22CP, and concentrations of Pro, total non-EAA, and total AA concentrations were similar ($P > 0.10$). It would be expected that formulating to specific EAA concentrations using synthetic EAA would reduce the non-EAA concentrations by altering the inclusion amounts of whey and milk protein ingredients (Table 1).

Weather Data

Although the timeline for these studies does not precisely match up with a spring versus summer weather comparison, the maximum, average, and minimum dai-

ly temperatures and calculated THI tended ($P < 0.13$) to be higher during study 2 compared with the study 1 time period (Table 5). Thus, calves in study 2 were experiencing greater heat stress than calves in study 1. During study 2, the maximum daily temperatures tended to be higher ($P < 0.13$), but the daily nighttime temperatures also tended to be higher ($P < 0.11$) compared with study 1, which can affect the calf's ability to dissipate heat. Calves in both studies experienced minimum THI values that indicated no heat stress, but calves in study 2 experienced moderate heat stress (THI >80) compared with study 1 calves. Spiers et al. (2001) reported that livestock typically dissipate heat during nighttime, such that nighttime cooling is important for coping with daytime heat stress. Although the maximum daily dew point temperatures were similar ($P > 0.19$) for study 1 compared with study 2, the average and minimum daily dew point temperatures tended ($P < 0.12$) to be higher for calves in study 2 compared with calves in study 1. Maximum, average, and minimum relative humidity and windspeed measurements were similar ($P > 0.15$) for both studies. Maximum, average, and minimum barometric pressure were higher ($P < 0.06$) in study 2 compared with study

Table 5. Weather data when studies 1 and 2 were conducted

Measurement	Study		SEM	<i>P</i> -value ¹
	1	2		<i>F</i> -test
Maximum daily temperature, °C	26.0	28.4	1.04	0.13
Average daily temperature, °C	20.5	22.9	0.97	0.10
Minimum daily temperature, °C	15.3	17.7	0.95	0.11
Maximum dew point, °C	18.1	19.7	0.84	0.20
Average dew point, °C	15.3	17.5	0.92	0.12
Minimum dew point, °C	12.0	15.0	1.08	0.07
Maximum humidity, %	92.5	92.9	1.02	0.77
Average humidity, %	74.6	73.4	1.94	0.67
Minimum humidity, %	55.3	51.3	2.39	0.27
Maximum windspeed, km/h	23.7	22.2	1.10	0.34
Average windspeed, km/h	12.1	10.3	0.88	0.16
Minimum windspeed, km/h	2.51	1.47	0.54	0.20
Maximum barometric pressure, mm	742.4	744.1	0.59	0.06
Average barometric pressure, mm	740.6	742.7	0.72	0.06
Minimum barometric pressure, mm	738.8	741.3	0.61	0.02
Maximum THI ²	78.0	82.1	1.76	0.12
Average THI	67.2	71.0	1.48	0.10
Minimum THI	59.2	62.3	1.30	0.12
Precipitation, cm	0.82	0.32	0.19	0.10

¹*F*-test for probability that study 1 versus study 2 differ.²THI = temperature-humidity index.

1. The amount of precipitation tended ($P < 0.10$) to be greater for study 2 compared with study 1, indicating that the 2018 summer was hotter and drier than late-spring 2018.

Growth Performance

Both studies started out with 40 calves (20 per treatment). Study 1 lost 1 calf due to respiratory disease and 1 calf became an outlier and eliminated due to insufficient CS consumption during weaning (causing BW loss). The calf was placed back on MR to gain BW. During study 2, when the summer heat resulted in moderate heat stress, 3 calves were lost with 1 due to respiratory disease, 1 due to dehydration from lack of drinking (cause unknown), and 1 due to scours. Also, 2 calves were deemed as statistical outliers due to very low BW gains. This resulted in 2 calves lost or removed when fed 22CP MR and 5 calves lost when fed the 22AA MR over the course of the 2 studies.

The interaction of study by MR was nonsignificant ($P > 0.12$) for BW measurements and ADG calculations (Table 6). The interaction of study by week was significant ($P < 0.01$), but the interaction of study by MR by week was nonsignificant ($P > 0.20$). Initial weekly, final, and study average BW were similar ($P > 0.24$) for both studies, even though we would expect heat stress to have had more of an effect on BW in study 2 than study 1. The study effect approached ($P < 0.07$) significance due to the heat stress of study 2,

which reduced the ADG of calves during the first 14 d of the study. There was a tendency ($P < 0.07$) for the 0 to 14 d ADG to be affected by heat stress (data not shown), but the slower start did not carry through the rest of the study, and resulted in an overall similar 0 to 56 d ADG ($P > 0.17$) among study. Bateman et al. (2012) reported a decline in ADG as environmental temperature increased (approximately 1% ADG change per 1°C). Thus, heat stress increases the calf's maintenance requirement, which Hill et al. (2012) partially improved by feeding more MR.

The initial, weekly, and final calf BW, BW gain, and ADG were similar ($P > 0.25$) for calves fed both MR (Table 6). Increasing the EAA concentration of the 22AA MR to that of a 24% CP MR, but with a reduced CP concentration to 22% CP, did not improve calf growth performance in this study. Bai et al. (2020) reported feeding an EAA balanced 22% CP MR with the EAA concentrations of a 24% CP improved ADG during the crucial first 2 wk of life compared with a 20, 22, and 24% CP MR. Getting calves started correctly and growing during the first weeks of life by minimizing or eliminating disease and stress challenges is crucial to calf growth performance. Our data here numerically demonstrated an 18% ADG improvement with large variation during the first 14 d, which supports the work of Bai et al. (2020). Average daily gains for the remaining periods and overall study ADG were similar ($P > 0.17$) for calves fed both MR. These data demonstrate that formulating an 22% CP AA MR with the EAA

Table 6. Body weight, BW gains, and ADG for calves fed a milk replacer (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Measurement	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
Number of calves	38	35	—	—	—	—
Death loss, number	2	5	—	—	—	—
BW, kg						
Week 0, initial	39.9	39.9	0.59	0.63	0.92	0.35
Week 1	42.1	42.0	0.59			
Week 2	42.8	43.3	0.59			
Week 3	48.7	48.9	0.59			
Week 4	53.0	53.3	0.59			
Week 5	57.6	57.7	0.59			
Week 6	61.2	60.7	0.59			
Week 7	65.4	64.9	0.59			
Week 8, final	71.2	70.3	0.59			
Study average	53.5	53.5	0.65			
Study BW gain, kg	31.2	30.4	1.13	0.25	0.57	0.20
ADG						
0–14 d, g/d	207.1	245.5	22.8	0.07	0.25	0.83
0–28 d, g/d	468.9	478.0	16.2	0.27	0.69	0.13
0–42 d, g/d	507.3	496.4	19.0	0.39	0.65	0.24
0–56 d, g/d	568.6	552.4	20.6	0.18	0.55	0.22

¹Probability of *F*-test for effects of study, MR, and interaction.

concentration of a 24% CP did not improve calf growth performance compared with calves fed a 22CP formulated MR.

Dry Matter Intake, Feed Efficiency, and AA Intake

Per experimental design, the MR DMI was the same for both MR treatments (Table 7). There was a significant ($P < 0.01$) study by week interaction (0.02, 0.05, 0.09, 0.14, 0.21, 0.61, 1.39, and 1.85 kg/d for study 1 compared with 0.03, 0.05, 0.07, 0.13, 0.21, 0.53, 1.22, and 1.78 kg/d for study 2 for wk 1–8, respectively) for CS DMI, which demonstrated that calves fed CS in study 2 consumed less CS DMI with experimental week compared with calves fed CS in study 1. The reduction in CS DMI would be related to the heat stress experienced, which was visually observed during study 2. Chester-Jones et al. (2008) reported reduced starter intake during May through September compared with other months. McKnight (1978) reported reduced starter intake in summer compared with winter or fall. The study by MR interaction was nonsignificant ($P > 0.10$) for CS DMI and total DMI (MR plus CS). Both the study effect and MR effect were nonsignificant ($P > 0.38$) for CS DMI and total DMI. Heat stress and MR source demonstrated no effect on CS DMI and total DMI.

The study by MR interaction for feed conversion was nonsignificant ($P > 0.10$) across all summarized phases (Table 7). A study trend ($P < 0.09$) effect was detected for the 0 to 14 d and 0 to 42 d MR feeding phase for feed

conversion expressed as kilogram of BW gain per total DMI for calves fed in study 2 compared with calves fed during study 1. Because CS intake was affected by heat stress, study 2 calves demonstrated improved feed conversions compared with study 1, which resulted in a significant ($P < 0.05$) study-by-week interaction for CS DMI. Hill et al. (2012) reported similar feed conversions for calves fed additional MR during summer heat stress. Marai et al. (1995) reported lower feed conversions for calves fed in summer compared with calves fed in the winter. Calculated feed conversions from the data of McKnight (1978) indicated lower feed conversion for summer-fed calves compared with calves fed during the other seasons.

The study by MR interaction, study main effect, and MR main effect for EAA intake were similar ($P > 0.35$) for all calves (MR plus CS; Table 8). Even though the EAA concentrations were greater for many of the EAA (Table 4) for calves fed the 22AA MR, the total EAA intakes were similar ($P > 0.10$) due to slight numerical differences in CS DMI and total DMI that overcame the differences in EAA intake during the MR feeding phase. In this study, the 22AA MR EAA formulation did not improve EAA intake.

Frame Measurements

The study by MR interaction was nonsignificant ($P > 0.10$) for all body frame measurements of HH, WH, HG, and BL (Table 9). Initial and final hip heights were similar ($P > 0.11$) for calves fed in both studies,

Table 7. Dry matter intake of milk replacer (MR) and calf starter, and calculated feed conversions for calves fed a milk replacer (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Measurement	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
MR DMI, kg/d						
Weeks 1 and 2	0.55	0.55	—	—	—	—
Weeks 3, 4, and 5	0.82	0.82	—	—	—	—
Week 6	0.41	0.41	—	—	—	—
Calf starter DMI, kg/d						
Week 1	0.03	0.02	0.04	0.39	0.45	0.76
Week 2	0.06	0.04	0.04			
Week 3	0.10	0.06	0.04			
Week 4	0.17	0.10	0.04			
Week 5	0.23	0.19	0.04			
Week 6	0.60	0.54	0.04			
Week 7	1.33	1.29	0.04			
Week 8	1.83	1.80	0.04			
Study average	0.54	0.50	0.04			
Total DMI, kg/d						
Week 1	0.58	0.57	0.04	0.33	0.49	0.70
Week 2	0.60	0.59	0.04			
Week 3	0.92	0.89	0.04			
Week 4	0.99	0.93	0.04			
Week 5	1.05	1.01	0.04			
Week 6	1.01	0.95	0.04			
Week 7	1.33	1.29	0.04			
Week 8	1.83	1.80	0.04			
Study average	1.04	1.00	0.04			
Total DMI						
0–56 d, kg	58.1	56.2	1.70	0.29	0.37	0.63
Gain/DMI, kg/kg						
0–14 d, g/d	0.35	0.42	0.04	0.09	0.20	0.73
0–28 d, g/d	0.61	0.64	0.02	0.26	0.21	0.18
0–42 d, g/d	0.59	0.60	0.01	0.06	0.41	0.12
0–56 d, g/d	0.54	0.54	0.01	0.45	0.83	0.11

¹Probability of *F*-test for effects of study, milk replacer, and interaction.

but the gain (final – initial) was greater ($P < 0.01$) for study 1 compared with study 2 (data not shown). The initial, final, and gains in WH were similar ($P > 0.35$) for calves in both studies. In contrast, differences were observed for study 1 compared with study 2 for

initial and final HG, but the gain was similar ($P > 0.88$) across both studies. The initial, final, and BL gains were greater ($P < 0.01$) for calves in study 1 compared with calves in study 2 (data not shown). Calves sourced from auction barns for study 2 were

Table 8. Amino acid intake (milk replacer plus calf starter) for calves fed a milk replacer (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Item, g/d	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
Arg	11.6	11.0	0.59	0.38	0.47	0.75
His	5.8	5.5	0.27	0.39	0.48	0.73
Ile	12.1	11.8	0.42	0.40	0.50	0.70
Leu	22.0	21.4	0.78	0.40	0.50	0.71
Lys	20.7	20.3	0.54	0.42	0.52	0.67
Met	6.0	6.1	0.14	0.43	0.54	0.65
Phe	10.1	9.7	0.48	0.38	0.48	0.73
Thr	12.7	12.4	0.35	0.41	0.52	0.68
Trp	3.5	3.4	0.12	0.40	0.50	0.70
Val	13.6	12.2	0.49	0.40	0.50	0.70
Total EAA	117.9	114.4	4.24	0.39	0.50	0.70

¹Probability of *F*-test for effects of study, MR, and interaction.

Table 9. Body frame measurements for calves fed a milk replacer (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Measurement, cm	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
Hip height						
Initial	80.9	81.1	0.51	0.25	0.70	0.24
Final	89.9	89.6	0.61	0.11	0.58	0.40
Gain	8.94	8.38	0.37	0.01	0.31	0.72
Withers height						
Initial	76.3	76.7	0.46	0.36	0.33	0.26
Final	84.7	85.0	0.51	0.92	0.64	0.76
Gain	8.38	8.25	0.41	0.36	0.81	0.52
Heart girth						
Initial	78.3	78.2	0.47	0.01	0.75	0.94
Final	94.8	93.8	0.85	0.01	0.22	0.73
Gain	16.5	15.6	0.60	0.89	0.27	0.77
Body length						
Initial	52.5	56.3	0.58	0.01	0.48	0.64
Final	62.8	62.7	0.63	0.01	0.89	0.73
Gain	10.3	9.8	0.66	0.01	0.59	0.59

¹Probability of *F*-test for effects of study, MR, and interaction.

smaller ($P < 0.01$) in both HG and BL, and HH and wither heights were numerically lower, which indicated that calves sourced for study 2 were smaller possibly due to maternal heat stress. Calves experiencing heat stress during study 2 gained less HH and BL. Marai et al. (1995) reported reduced Holstein calf growth rates during the summer compared with winter. Collier et al. (1982) and Wolfenson et al. (1988) reported that calf birth weights were lower when dairy cows experienced heat stress during the dry period. Tao and Dahl (2013) reported that maternal heat stress may have carryover effects on subsequent newborn calf growth.

Calves fed either MR were similar ($P > 0.20$) in initial and final HH, wither height, HG, and BL gains (Table 9). In this study, increasing the AA supply through the MR did not influence body frame measurements. Amino acids are required for frame growth, and this is the most efficient time in the animal's life cycle to grow the skeletal frame (Le Cozler et al., 2008). Bai et al. (2020) reported similar frame growth for neonatal calves fed a 22% CP and 22% CP containing specific EAA concentration of a 24% CP MR. Jaeger et al. (2020c) reported that feeding a modified accelerated MR program resulted in greater hip width growth rates. Thus, it takes both EAA and energy in the proper balance to drive frame growth.

Health Performance

There was no study by MR interaction ($P > 0.58$) for fecal scores, whether expressed as total days for the study or number of days during the week for the 6 wk that MR treatments were fed (Table 10). Calves in study 1 demonstrated more ($P < 0.01$) days with fecal

score of 0 and less ($P < 0.01$) days with fecal score 2 than calves fed during study 2, which demonstrated that calves in study 2 experienced more scours due to heat stress (data not shown). Heat stress has been shown to increase the incidence of scours (Roland et al., 2016). Calves with fecal scores of 2 and 3 were considered to have scours, and fecal scores of 0 and 1 were considered normal with no scours.

Calves fed the 22AA MR demonstrated fewer fecal scores of 0 for total days ($P < 0.03$) or number of days during the week ($P < 0.01$) compared with calves fed 22CP MR. Total days of fecal score 2 or 3 and number of days during the week were similar ($P < 0.15$) for calves fed 22AA MR compared with calves fed 22CP. Calves fed 22AA MR tended to have greater ($P < 0.06$) total days of fecal score 3 and number of days during the week ($P < 0.07$). Thus, calves fed the 22AA MR with greater concentrations of synthetic EAA demonstrated more scours than calves fed lower concentrations of synthetic EAA. The use of synthetic EAA reduced inclusion rate of all milk protein ingredients, allowing for increased NFC concentrations for the 22 AA MR. The increased NFC concentration was attributed a greater lactose concentration (Table 3). Davis and Drackley (1998) cited research that high lactose intake can lead to an increase incidence of scours. Whole milk on a DM basis would contain less than 40 to 45% lactose (NRC, 2001; Berends et al., 2020), thus increasing lactose concentrations could influence scour incidences. Berends et al. (2020) reported that exchanging fat for lactose in MR reduced the number of health events.

There were no study by MR interactions ($P > 0.50$), study, or MR effects on nasal and eye/ear scores (data not shown). Overall, calves were healthy; feeding a

Table 10. Fecal scores for calves fed a milk replacer (MR) formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Score	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
Total days of fecal scores ²						
Score = 0	25.0	22.4	0.81	0.01	0.03	0.59
Score = 1	10.3	11.4	0.75	0.11	0.24	0.93
Score = 2	5.2	5.9	0.48	0.01	0.26	0.87
Score = 3	1.0	1.7	0.33	0.78	0.06	0.27
Fecal scores, d/wk						
Score = 0	4.2	3.7	0.13	0.01	0.01	0.57
Score = 1	1.7	1.9	0.12	0.05	0.15	0.94
Score = 2	0.9	1.0	0.08	0.01	0.26	0.86
Score = 3	0.2	0.3	0.06	0.72	0.07	0.24

¹Probability of *F*-test for effects of study, MR, and interaction.²Fecal score = 0–3; 0 = normal, ≥2 = scours.

22CP or 22AA MR resulted in similar ($P > 0.15$) nasal and eye/ear scores (data not shown), but the calves fed 22AA MR demonstrated lower fecal scores.

Economic Analysis

The MR feeding cost (MR DMI × \$/kg) was the same for study 1 and 2 due to the same feeding rates, but feeding the 22AA MR resulted in a \$0.07 per calf per day savings in feed costs compared with the calves fed 22CP (Table 11) for the 42 d MR feeding phase. The total MR cost per calf per the 42 d MR feeding period was greater ($P < 0.01$) for calves fed 22CP than calves fed 22AA MR, which is approximately a 4.7% reduction in MR feed costs. Bai et al. (2020) reported that calves fed a specific EAA-formulated MR of a 24% CP, but reduced CP to 22% (similar to the AA MR treatment in this study) resulted in a lower cost per kilogram of BW gain compared with calves fed a 24% CP MR.

The study by MR interaction, study effect, and MR effect were similar ($P > 0.38$) for calves fed both MR

treatments. The total daily cost of CS (DMI of CS × \$/kg of CS) per calf was similar ($P > 0.10$) for calves fed both treatments totaling, \$14.71 and \$13.71 due to the nonsignificant numerically lower CS intake for calves fed 22AA MR. Total feed costs per day and per study calf (MR + CS) were lower ($P < 0.01$) for calves fed AA MR compared with calves fed 22CP MR. The total feed cost per calf per experiment was \$4.79 for calves fed 22AA MR compared with calves fed 22CP MR, however the numerical differences in BW gains, CS intake, and total feed intake resulted in a similar total feed cost per kilogram of BW gain ($P > 0.44$) for calves fed both treatments. In our study, we found that feeding calves an EAA formulated MR (22AA) compared with a CP formulated MR demonstrated the hypothesis of lowered feed cost per day with similar growth performance, but resulted in similar feed cost per kilogram of BW gain under current study conditions. The differences in feed costs resulted in small numerical differences between treatments in growth performance and the effects of heat stress resulted in lower feed costs on a per day basis, but the total cost

Table 11. Cost (\$) per day for milk replacer (MR), pelleted calf starter (CS), and total cost per day and total cost per kilogram of BW gain for calves fed a MR formulated for 22% CP (22CP) or containing greater AA concentrations (22AA) equal to 24% CP

Item	MR		SEM	Effects and interaction, <i>P</i> -value ¹		
	22CP	22AA		Study	MR	Study × MR
MR, \$/d, 0–42 d	1.92	1.85	<0.01	—	0.01	—
Total MR, \$/calf	83.20	79.44	0.06	—	0.01	—
CS, \$/d, 0–56 d	0.23	0.22	0.02	0.39	0.45	0.76
Total CS, \$/calf	14.71	13.71	0.80	0.26	0.34	0.69
Total feed cost, \$/d,	1.67	1.60	0.02	0.56	0.01	0.46
Total feed cost, \$/calf	97.94	93.15	0.80	0.43	0.01	0.38
Total feed cost, \$/kg of BW gain per calf	3.25	3.16	0.10	0.23	0.45	0.27

¹Probability of *F*-test for treatment.

of gain was similar. Thus, the study hypothesis was partially confirmed in that similar growth performance can be achieved with a lesser feed cost, but the total cost per kilogram of BW gain was similar.

CONCLUSIONS

The study objective was partially achieved by feeding a MR containing greater concentrations of EAA using synthetic EAA, but the same 22% CP (equal to 24% CP EAA MR), while reducing the CP concentration for achieving similar performance at a reduced cost. The use of synthetic EAA to supply key limiting AA can be used at concentrations to meet the EAA requirements for growing neonatal Holstein calves instead of CP, and reducing the cost of more expensive MR ingredients reduces the total feed cost per calf. This study demonstrated similar growth performance, feed intake, feed conversions, and frame growth for both MR. Therefore, similar growth performance can be achieved by feeding an EAA-fortified MR with less CP, which reduced feed costs under current study conditions.

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