Effects of Hydrolyzed Spray Dried Red Blood Cells in Milk Replacer on Calf Intake, Body Weight Gain, and Efficiency

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ABSTRACT

An alternative protein ingredient based on spray-dried, hydrolyzed red blood cells was evaluated in calf milk replacers. Two experiments were conduced to determine the value of the ingredient on intake, growth, and feed efficiency in dairy calves. In experiment 1, Holstein bull calves (n = 120) were fed calf milk replacer containing 0, 11, 22, or 43% of crude protein as spray dried hydrolyzed red blood cells. Calves were fed 454 g/d of experimental milk replacer reconstituted to 12% dry matter plus a conventional calf starter for 28 d. Body weight gain, intake of milk replacer and calf starter, feed efficiency, fecal scores, and days scouring were unaffected by source of protein. In experiment 2, Holstein calves (n = 69) at the University of Minnesota, Crookston and Waseca were fed milk replacer containing 0, 22, or 43% of crude protein as spray dried hydrolyzed red blood cells. Calves were fed 454 g/d of experimental milk replacer reconstituted to 12% dry matter plus a conventional calf starter containing 0 or 25% alfalfa meal for 35 d. No calves died during the study. Body weight gain, feed efficiency, intake of calf starter and milk replacer, fecal scores, and days scouring were unaffected by increasing hydrolyzed red blood cells in milk replacer. Similar performance of all calves indicated that spray dried hydrolyzed red blood cells can replace up to 43% of crude protein from whey protein concentrate without detrimental effects on animal performance.

(**Key words:** calves, milk replacer, alternative protein)

Abbreviation key: CMR = calf milk replacer, **CON** = CMR containing no SDHRBC, **CS** = calf starter, **HIGH** = CMR containing SDHRBC at 43% of CP, **LOW** = CMR containing SDHRBC at 11% of CP, **MED** = CMR containing SDHRBC at 22% of CP, **SDHRBC** = spray-dried hydrolyzed red blood cells, **SDRBC** = spray-dried red blood cells.

INTRODUCTION

Commercial calf milk replacer (**CMR**) formulations have traditionally contained ingredients based on milk, including whey, dried skim milk, and lactose. These formulations are widely used by dairy producers (14) as a means of marketing additional saleable milk. Protein ingredients contribute significantly to the overall cost of CMR; therefore, alternatives to whey and casein proteins may reduce cost of CMR and improve profitability of the heifer rearing enterprise. Furthermore, availability of edible-grade milk proteins may decline in the future as use of these proteins in human foods increases. Alternative protein ingredients, including soy (15, 17, 28), wheat protein (3, 27), fish protein (7, 8, 13), and potato protein (3), have been evaluated and incorporated into some CMR.

More recently, proteins from spray-dried animal plasma (19, 23) and spray-dried red blood cells (**SDRBC**; 1, 32) have been incorporated into CMR formulations to replace 25 and up to 55% of the protein from whey protein, respectively. Blood fractions are widely used in food applications for humans and animals (5, 6, 18, 22). Spray-dried red blood cells are a coproduct of plasma production (22) and are usually less expensive than skim milk or whey protein concentrate per unit of protein. The SDRBC are high in protein (>90%) and have a favorable AA profile, with the exception of Met and Ile (30). Inclusion of

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SDRBC in CMR resulted in rates of BW gain and intake equivalent to CMR containing milk protein, but at lower (1) or similar cost (32). Conversely, Scott et al. (26) reported reduced performance in calves fed 50% of CP as SDRBC. Milk replacers containing SDRBC are a characteristic brown that some dairy producers find unacceptable. Further, the presence of significant Fe in SDRBC contributes to a change in normal fecal color and consistency when calves are fed CMR containing SDRBC. Hydrolysis of SDRBC to reduce Fe may reduce the objectionable characteristics of SDRBC and produce a protein more widely acceptable to dairy producers.

The objectives of this study were to determine the preweaning intake, growth, and health of calves fed CMR containing up to 43% of CP as SDHRBC.

MATERIALS AND METHODS

General

Liquid red blood cells were collected from abattoirs following collection of whole blood and centrifugation to remove plasma. All blood was collected in USDA inspected facilities and was inspected for human consumption. Red blood cells were chilled to 4°C, transported to the laboratory, and hydrolyzed using a proprietary procedure. Resulting hydrolysate (P.E.P.; American Protein Corporation, Ames, IA), was spray dried to produce a tan, soluble, freely flowable powder containing approximately 85% protein (air-dry basis). The spray-dried material contained approximately 9% Lys, 1.6% Met, 11.1% Leu, and 0.2% Ile. In vitro pepsin digestibility (0.002% pepsin) was >98%. The spray-dried hydrolysate was stored at room temperature prior to inclusion in CMR formulations.

Experiment 1

Holstein bull calves (n = 120) were purchased from area dairies or sale barns and shipped to the APC Calf Research Unit in Ames, IA. Calves were approximately 1 to 5 d of age on arrival, although actual date of birth was not determined. Calves were received in two groups on two consecutive days. Samples of jugular blood were collected into evacuated tubes from each calf upon arrival, and a subsample was collected for measurement of hematocrit. Tubes were then centrifuged and serum was separated and frozen (–20°C) until analyzed for IgG by turbidimetric immunoassay (11).

Calves were weighed on arrival, fed 1 dose (454 g) of a colostrum supplement product (Lifeline Calf Nutritional Colostrum Supplement, American Protein Corporation) and then were assigned to receive one experimental CMR with 0 (**CON**), 11 (**LOW**), 22 (**MED**), or 43% (**HIGH**) of CP from SDHRBC. Other ingredients in experimental CMR included whey (47 to 49% on an air-dry basis), 75% whey protein concentrate, dry fat blend (33%), vitamins, minerals, emulsifier, and AA (4 to 5%). All CMR were formulated to contain 20% CP, 20% fat, 0.8% Ca, 0.7% P (air-dry basis), and to meet or exceed NRC requirements (21) for vitamins and minerals. The CMR were supplemented with crystalline Lys, D,L-Met, Ile, Thr, and Trp to meet predicted AA requirements of milk fed calves (30).

Calves were housed in individual fiberglass hutches and fed CMR (227 g) twice daily at approximately 0700 and 1600 h. Experimental CMR was mixed in hot water (approximately 50°C) to disperse fat. Cool water was then added to bring temperature to approximately 39°C and DM to 12%. Reconstituted CMR was fed to calves in individual nipple bottles. The amount of CMR offered and refused was measured at each feeding. Commercial textured calf starter (**CS**; Cargill Herd Builder, Cargill, Inc., Minnetonka, MN) was fed once daily ad libitum, and feed refusals were measured daily. Water was available at all times and no hay was fed. Hutches were bedded with straw throughout the study. Samples of CMR and CS were collected weekly and stored (–20°C) prior to analysis for CP, ether extract and ash (2), minerals (inductively coupled plasma emission spectroscopy), gross energy by bomb calorimetry (Parr Instrument Co., Moline, IL), and AA (HPLC following hydrolysis in 6 N HCl) by a commercial laboratory (Silliker Laboratories of Iowa, Cedar Rapids, IA).

Calves were weighed once weekly. Fecal consistency was subjectively scored once daily using a scale of 1 = normal fecal consistency to 4 = severe scours. When fecal material was unavailable for scoring, calves were assigned a missing value. A scour day was defined when calves had a fecal score >2. Scours were treated with intramuscular injections of antibiotics for 3 d and electrolyte therapy until signs of disease abated.

Data were analyzed by ANOVA using SAS (25). Data were summarized over the 28-d study and analyzed using a completely random design. Weekly BW, intake, fecal scores, and efficiency data were analyzed using a mixed model ANOVA as a completely random design. Calf within treatment was the random variable and was used as the error term to test treatment effects. Initial BW and serum IgG at 1 d were evaluated as covariables, but neither explained a significant ($P > 0.05$) amount of variation in the model. Therefore, unadjusted least squares means are reported. Single degree of freedom contrasts were used to compare linear, quadratic, or cubic effects of SDHRBC protein. Significance was declared at $P < 0.05$.

Experiment 2

Sixty-nine calves (36 bulls) were used at the Waseca (n = 39) and Crookston (n = 30) Research and Outreach

Centers. Experiments at both sites were approved by the University of Minnesota Institutional Animal Care and Use Committee. Calves at Waseca were housed in individual elevated metal crates in an environmentally controlled room. No bedding was used. Calves at Crookston were housed in individual calf stalls within a controlled environment calf room. Sunflower hulls were used as bedding.

Calves were fed colostrum and transition milk for the first 3 d of life, then were assigned to receive one of three levels of SDHRBC (0 [CON], 22 [MED] or 43% [HIGH] of CP, replacing 75% whey protein concentrate) from d 4 to 38. The CMR were formulated as in experiment 1, except all CMR contained neomycin and oxytetracycline (400 and 200 mg/454 g of CMR, respectively). The procedure for reconstituting CMR was as described for experiment 1. Calves were fed 227 g of CMR powder twice daily in two equal feedings at approximately 0730 and 1600 h from individual metal buckets. In addition, calves were fed CS containing 0 or 25% alfalfa meal (as part of a separate study) ad libitum throughout the study. Starters were balanced across treatments and were offered daily. Starter refusals were measured twice weekly. Water was available at all times and no hay was offered. Fecal consistency was scored as in experiment 1. Samples of CMR and CS were stored (–20°C) prior to analysis for DM, CP, fat, and ash (2) and selected minerals (inductively coupled plasma emission spectroscopy).

Data collection included intake of CMR, CS, fecal scores, morbidity and mortality, as in experiment 1. However, the amount of CS refused was measured twice weekly.

Data were pooled over the 35-d feeding period and analyzed as a randomized complete block experimental design using PROC GLM of SAS. Sex of calf and location (Waseca, Crookston) were included in the model as blocking factors. Initial BW and serum IgG at d 1 were evaluated as covariables, but neither explained a significant amount of variation in the model. Therefore, unadjusted least squares means are reported. Weekly BW were analyzed as in experiment 1. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

General

Method of hydrolysis differed somewhat from the method reported by Duarte et al. (10) and, therefore, chemical composition of resulting material varied somewhat (10). Hydrolysis of red blood cells reduced molecular weight of proteins. Prior to hydrolysis, >98% of protein was >22 kDa. Following hydrolysis, >85% of protein was <2 kDa, indicating extensive reduction in molecular weight of the protein. All CMR produced with SDHRBC were white to off-white. The products were stable with a neutral odor, and empirical observation indicated that solubility of CMR did not vary markedly with inclusion of SDHRBC. There were no differences in acceptance or palatability of CMR containing SDHRBC at any location. Fecal color and consistency of calves fed SDHRBC did not differ from that of calves fed CMR containing no SDHRBC.

Experiment 1

Serum IgG and hematocrit concentrations on arrival averaged 7.5 g/L and 33.8%, respectively. The relatively low serum IgG concentrations indicated inadequate colostrum feeding during the first 24 h of birth. Most calves (69.1%) had serum IgG <10 g/L and 10 calves had no measurable IgG by turbidimetric immunoassay. A lack of passive immunity has been associated with increased morbidity and mortality (9, 31) and impaired efficiency of BW gain during the growing period (29, 31). Calf morbidity and mortality was indicative of the serum IgG concentrations and number of calves with failure of passive transfer. Twelve calves died during the study and were not replaced. Mortality tended ($P < 0.10$) to be higher on LOW and HIGH treatments; however, the cause is unclear. Mean days scouring, fecal score, and number of treatments per calf were unaffected by treatment (Table 1) and were 6.75 d, 2.14, and 3.61 treatments, respectively.

Concentrations of CP and fat analyzed in CMR slightly exceeded formulated values (Table 2). The amount of Met in all CMR was slightly lower than formulated, which was due to a slightly smaller than expected amount of Met in the whey protein concentrate. Amounts of Lys, Thr, Ile, and Leu met or exceeded formulated values. Gross energy was 4.9 Mcal/kg of DM and was similar to other reports of gross energy in commercial CMR formulations (16). Recommended digestible energy concentration of commercial CMR is 4.19 Mcal/kg of DM (21); digestibility of 85% of the CMR used in this study would provide recommended amounts of digestible energy.

Initial BW, final BW, and BW gain during the 28-d experimental period were unaffected by treatment (Table 1). Mean initial and final BW were 42.7 (SE = 0.4) and 51.9 (SE = 0.7) kg, respectively. Rates of BW gain of calves during the initial 28 d of life are often limited to <500 g/d by feeding management, pathogen exposure, and environmental factors (29). Consequently, use of antibiotics and other feed additives to reduce the effects of pathogen exposure have been incorporated into CMR formulations (20, 24). Weekly BW gains (Figure 1) were unaffected by treatment and indicated that BW gains during the first wk of the study were minimal. The greatest increases in BW gain generally occurred during the

Table 1. Least squares means of morbidity, mortality, BW, intake, and feed efficiency in calves fed experimental calf milk replacer (CMR), experiment 1.

	Treatments[1]					Contrasts[2]		
Item	CON	Low	Med	High	SE	L	Q	C
No. of calves at start	30	30	30	30	. . .	. . .	. . .	. . .
No. of calves at end	29	25	28	26	. . .	. . .	. . .	. . .
Mortality, %	3.3	16.7	6.7	13.3	5.4	NS	NS	0.10
Hematocrit at arrival, %	33.3	33.3	36.1	32.6	1.3	NS	NS	NS
Serum IgG at arrival, g/L	5.5	9.1	7.9	7.5	0.9	NS	0.02	NS
BW, kg								
0 d	41.8	43.1	43.3	42.7	0.8	NS	NS	NS
28 d	52.3	51.8	51.6	51.8	1.4	NS	NS	NS
BW gain, 0 to 28 d, g	375	313	296	326	40	NS	NS	NS
DM intake, g/d								
Total	818	776	743	777	35	NS	NS	NS
CMR	439	440	441	440	1	NS	NS	NS
Calf starter (CS)	378	336	303	337	35	NS	NS	NS
Feed cost,[3] $/d								
Total	0.77	0.74	0.71	0.69	0.01	0.01	NS	NS
CMR	0.67	0.65	0.63	0.60	0.01	0.01	0.01	NS
CS	0.10	0.09	0.08	0.09	0.01	NS	NS	NS
ADG:DM intake, g/kg	320	309	277	321	55	NS	NS	NS
ADG:Feed cost, g/$[3]	451	412	398	447	52	NS	NS	NS
Treatments, n	3.8	4.0	3.1	3.6	1.6	NS	NS	NS
Days scouring	7.0	7.3	6.6	6.1	0.8	NS	NS	NS
Fecal score[4]	2.14	2.20	2.07	2.11	0.06	NS	NS	NS

[1]CON = CMR containing no spray-dried hydrolyzed red blood cells (SDHRBC); LOW = CMR containing SDHRBC at 11% of CP; MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

[2]Contrasts: L = Linear effect, Q = quadratic effect, C = cubic effect of inclusion of SDHRBC in CMR.

[3]Cost of feed: CON = $1.52/kg; LOW = $1.47/kg; MED = $1.42/kg; HIGH = $1.36/kg; CS = $0.275/kg.

[4]Fecal scores: 1 = Normal fecal consistency; 2 = slightly liquid feces; 3 = moderately liquid feces; 4 = severely liquid feces.

last 2 wk of the study when intake of CS became significant.

Duarte et al. (10) reported markedly reduced growth of rats fed diets containing a hydrolyzed fraction of processed bovine red blood cells containing reduced amounts of heme. Although CP digestibility of the fraction was 92%, N retention was only 14% of the N retention of control diets containing casein. Reduced growth and N retention was attributed to AA deficiency in the fraction, which contained 0.14 and 0.86% of total protein as Ile and

Table 2. Chemical composition[1] (DM basis) of experimental diets, experiment 1.

	Treatments[2]				
Nutrient	CON	LOW	MED	HIGH	Calf starter
DM, %	97.07	97.15	97.18	97.18	86.48
CP, %	21.22	21.07	21.07	20.92	24.24
Fat, %	22.05	22.22	21.86	21.12	4.95
Ash, %	7.64	6.90	7.41	8.28	4.53
ADF, %	. . .	. . .	. . .	. . .	5.93
Gross E, Mcal/kg	4.928	5.009	4.885	4.945	4.853
Methionine, %	0.3	0.4	0.4	0.5	0.5
Lysine, %	2.0	2.1	2.0	2.0	0.9
Threonine, %	1.3	1.7	1.5	1.4	0.9
Isoleucine, %	1.0	1.1	1.0	0.7	0.9
Leucine, %	1.4	2.0	2.0	2.2	3.4

[1]Data from Silliker Laboratories of Iowa, Cedar Rapids, IA.

[2]CON = Calf milk replacer (CMR) containing no spray-dried hydrolyzed blood cells (SDHRBC); LOW = CMR containing SDHRBC at 11% of CP; MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

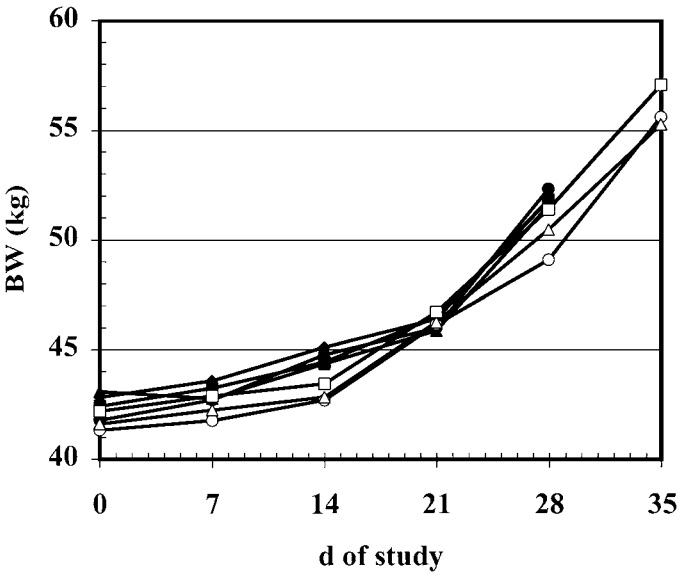

Figure 1. Least squares means of BW of calves fed CON (●), LOW (◆), MED (▲), and HIGH (■), at Iowa and CON (○), MED (△), and HIGH (□) at Minnesota. Standard error of means were 1.1 and 0.8 kg at Iowa and Minnesota, respectively. CON = calf milk replacer (CMR) with no spray-dried hydrolyzed red blood cells (SDHRBC); LOW = CMR containing SDHRBC at 11% of CP; MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

Met, respectively. In the present study, supplemental Ile and Met were included in the diet to meet estimated AA requirements for milk fed calves (30). Amino acid supplementation may have overcome AA deficiencies in the SDHRBC and allow acceptable growth rates.

Intake of DM was unaffected by treatment (Table 1). Mean intake of CMR was 440 g of DM/d (SE = 1). Refusals of CMR were minimal and were limited to calves with serious morbidity. Starter DM intake increased with increasing age (Figure 2) to exceed 1 kg/d by d 28. Calves are prepared for weaning when they consumed 1 to 2% of birth BW as dry feed (12). In the present study, calves reached CS intake of 1% of initial BW (427 g/d) by 17 to 18 d of age for all treatments.

Daily feed costs from CMR decreased with increasing SDHRBC in the formula (Table 1). This was reflected in similar decreases in overall daily feed costs from $0.77/d for calves fed CON to $0.69/d for calves fed HIGH. Feed costs were estimated with market prices for ingredients and estimated manufacturing cost for SDHRBC. Daily gain per feed cost dollar was not significantly different among treatments due to the variation in BW gain. Feed efficiency was unaffected by treatment but increased with increasing age (data not shown). Mean efficiency was 326 g of BW gain/kg of DM intake (SE = 25 g/kg).

Experiment 2

Compositional analyses for the respective CMR used in experiment 2 (Table 3) were similar to those in experiment 1 except for fat, which was slightly lower. Ash was also slightly higher, due to the inclusion of antibiotics in the CMR. The commercial CS used in experiment 2 were formulated to contain 0 or 25% alfalfa meal providing similar nutrient composition (Table 3). Both CS were medicated with lasalocid at 37.4 mg/kg. The CS used in experiment 2 had similar nutrients to those used in experiment 1, except for higher ADF. The estimated metabolizable energy of CS was 2.64 Mcal/kg. The DM intake of CS by calves in experiment 2 was 15.7% lower than calves in experiment 1. The difference is consistent with expectations for inside versus outside housing in the Midwest winter months (4).

Intake of CS by calves fed 0 or 25% alfalfa meal did not differ (data not shown) which was reflected by similar preweaning BW gain. Both CS were pelleted, which provided a consistent physical form and high quality CS.

Calves at Minnesota were healthy throughout the trial and there was no mortality (Table 4). Concentrations of IgG in serum of calves were higher in Minnesota than in Iowa, although mean serum IgG of calves fed HIGH was <10 g/L. Location effect (Waseca vs. Crookston) was significant for many variables measured. Although management during the study was similar between the locations, normal differences in housing, climate, level of

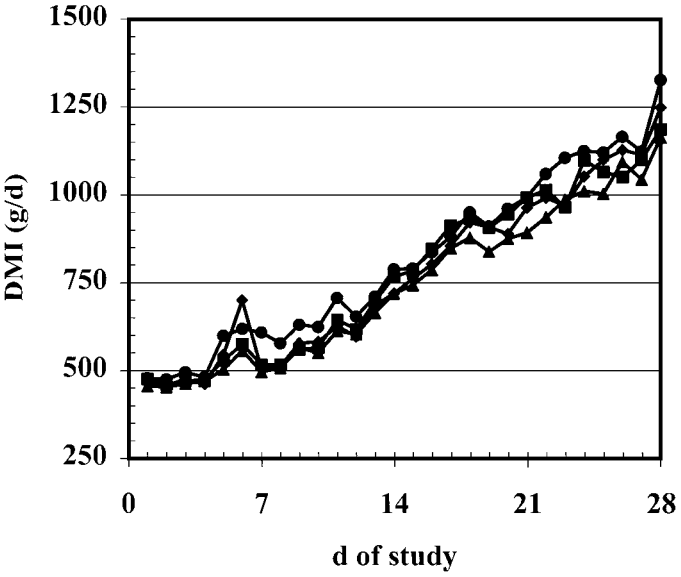

Figure 2. Least squares means of total DMI of calves fed CON (●), LOW (◆), MED (▲), and HIGH (■) at Iowa. Standard error of each weekly mean was 50 g. CON = Calf milk replacer (CMR) with no spray-dried hydrolyzed red blood cells (SDHRBC); LOW = CMR containing SDHRBC at 11% of CP; MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

Table 3. Chemical composition of experimental diets[1], experiment 2.

	Treatment[2]			Alfalfa in starter	
Nutrient	CON	MED	HIGH	0%	25%
DM, %	98.29	98.30	98.22	91.4	91.2
			(% of DM)		
CP	19.85	19.16	21.11	20.2	21.6
Fat	19.40	19.48	19.32	3.6	4.4
Ash	8.94	9.09	8.97	9.7	10.3
ADF	. . .	. . .	. . .	20.3	19.1
Calcium	1.07	1.08	0.90	1.7	1.7
Phosphorus	0.87	0.88	0.79	0.4	0.5
Potassium	1.56	1.58	1.55	1.5	1.2

[1]Data from University of Minnesota.

[2]CON = Calf milk replacer (CMR) containing no spray-dried hydrolyzed red blood cells (SDHRBC); MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

pathogen exposure, and environmental factors probably contributed to the observed location differences.

Calf BW at 4 and 38 d and BW gain during the study were unaffected by treatment (Table 4). Body weight gains were generally greater in Minnesota than in Iowa (407 vs. 328 g/d), but calves in Minnesota were fed CMR for an additional week. Weekly BW at both locations (Figure 1) indicated similar BW and rates of BW gain during the first 4 wk of each study; increased BW gain occurred from 28 to 35 d of age.

Intake of DM, CMR, and CS, efficiency of BW gain and fecal scores were unaffected by treatment (Table 4). Number of days treated tended to be higher when calves were fed MED. Feed cost per day and BW gain per dollar of feed cost were reduced when calves were fed increasing amounts of SDHRBC (Table 4). Efficiency of BW gain tended ($P < 0.13$) to be improved with increasing SDHRBC.

Animal intake and growth in calves fed SDHRBC were similar to performance of calves fed SDRBC replacing

Table 4. Least squares means of morbidity, mortality, BW, intake, and feed efficiency in calves fed experimental calf milk replacer (CMR), experiment 2.

	Treatments[1]				Contrasts[2]	
Item	CON	MED	HIGH	SE	L	Q
No. of calves at start	23	23	23	. . .	. . .	. . .
No. of calves at end	23	23	23	. . .	. . .	. . .
Serum IgG at 24 h, g/L	11.3	10.0	9.5	1.2	NS	NS
BW, kg						
4 d of age	41.3	41.6	42.2	1.2	NS	NS
38 d of age	55.5	55.3	57.5	1.2	NS	NS
BW gain, 4 to 38 d, g/d	405	392	437	28	NS	NS
DM intake, g/d						
Total	743	710	730	27	NS	NS
CMR	433	435	436	1	NS	NS
Calf starter (CS)	311	275	294	27	NS	NS
Cost per day,[3] $						
Total	0.79	0.73	0.72	0.01	0.01	0.06
CMR	0.69	0.65	0.62	0.01	0.01	0.01
CS	0.10	0.09	0.09	0.01	NS	NS
ADG:DM intake, g/kg	532	550	597	30	0.13	NS
ADG:Cost kg/$[3]	504	530	606	33	0.03	NS
Treatment days	1.4	2.5	1.8	0.4	NS	0.06
Fecal score[4]	1.16	1.23	1.21	0.03	NS	NS

[1]CON = CMR containing no spray-dried hydrolyzed red blood cells (SDHRBC); MED = CMR containing SDHRBC at 22% of CP; HIGH = CMR containing SDHRBC at 43% of CP.

[2]Contrasts: L = linear effect, Q = quadratic effect of SDHRBC in CMR.

[3]Cost of feed: CON = $1.52/kg; MED = $1.42/kg; HIGH = $1.36/kg; CS = $0.275/kg.

[4]Fecal scores: 1 = normal fecal consistency; 2 = slightly liquid feces; 3 = moderately liquid feces; 4 = severely liquid feces.

55% of whey protein over a 35-d preweaning period (32) and equal to, or greater than performance of calves fed animal plasma to replace 25% whey protein (19, 23). Results of these studies indicates that SDHRBC can effectively replace up to 43% of the CP from whey and can support acceptable rates of intake and BW gain. Calf milk replacers containing SDHRBC have characteristics of CMR containing whey proteins but at lower cost.

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