A Comparison of Milk Protein Sources in Diets of Calves up to Eight Weeks of Age[1]

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ABSTRACT

Four dietary ratios of dried skim milk and whey protein concentrate as the primary protein sources in milk replacers were evaluated using digestibility, growth performance, health characteristics, and blood measurements of calves that were 12 to 36 h of age to 8 wk of age. Sixteen Holstein bull calves, housed individually within an environmentally controlled facility, were randomly allotted to diets. Protein sources in the milk replacer were expressed as ratios (percentages) of dried skim milk to whey protein concentrate in diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100. There were no significant differences in the mean number of days that calves scoured. Apparent biological value and N retention differed among diets over all weeks. Apparent digestibilities and biological values (percentages) over all weeks were diet 1, 82.50 and 67.71; diet 2, 82.85 and 70.15; diet 3, 83.82 and 67.46; and diet 4, 84.03 and 72.25, respectively. Diets varying in the ratio of dried skim milk to whey protein concentrates had no effect on health, growth, or apparent digestibilities in Holstein calves up to 8 wk of age.
(**Key words**: calf, milk replacers, dried skim milk, whey protein concentrate)

Abbreviation key: **ADG** = average daily gain, **DSM** = dried skim milk, **FE** = feed efficiency, **WPC** = whey protein concentrate.

INTRODUCTION

The utilization of whey proteins is of great interest because of their relatively low cost and abundant production in the cheese industry (4). Whey protein concentrate (**WPC**) has essentially the same proximate analysis as dried skim milk (**DSM**), and whey proteins form the major protein source in milk replacers containing only milk protein (12). Because the rumen, reticulum, and omasum are inactive initially, the calf functions similarly to a monogastric animal for approximately 2 to 3 wk (29). During the first 3 or 4 wk of age, the enzymatic system of the calf is still developing, and the calf cannot digest starch, sucrose, or maltose (16). Also, the calf has difficulty digesting carbohydrates other than lactose and glucose (10, 13, 14, 19, 27). Within 10 min of feeding, a clot is formed in the abomasum as a result of the rennin, pepsin, and hydrochloric acid that act upon the casein protein in digesta. Rennin binds with casein protein, and the curd is slowly digested and emptied from the abomasum into the small intestine for up to approximately 24 h. The clot contracts, and the whey proteins and lactose are released and pass quickly through the abomasum (24). When whey (a nonclotting protein fraction) is present within the milk replacer, no clot is formed within the abomasum (27).

Heinrichs et al. (12) have determined that 66.4% of dairy farmers use milk replacers, and surveys indicate that most farms wean calves at 8 wk of age. Essentially all special-fed veal (usually Holstein bull calves that are raised in individual stalls for approximately 18 wk) are reared entirely on milk replacers. Over the past few years, consumers have demanded nonfat dried milk products and casein derived from skim milk. Several factors, such as the need for countries to reserve milk for human consumption (28) and shortages of skim milk (26), contribute to the need for adequate alternative protein sources in milk replacers. Whey is being utilized for purposes other than dietary products in animal feed. Using whey in drinks for protein fortification is promising (20). Growth, development, and digestive tolerance were improved in premature human infants who were fed formulas with ratios of whey to skim milk of 60:40 rather than 20:80. Studies with foals determined that diets that were predominantly composed of whey caused significant increases in mean BW over time (3). Because of increased human consumption of skim milk products, a need exists for nutritionally complete and economically feasible milk replacers to satisfy the various calf industries. Recently, DSM cost

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approximately four times more than WPC [$5.40 vs. $1.30/kg, respectively; (6)], which significantly affected the cost of the primary protein source in milk replacer diets. The objective of this experiment was to determine the effects of four dietary ratios of DSM and WPC as the primary protein sources in milk replacers. For calves fed such diets, digestibility, growth performance, health characteristics, and blood parameters were measured from 12 to 36 h of age to 8 wk of age.

MATERIALS AND METHODS

Sixteen Holstein bull calves (two replicates), ranging in age from 12 to 36 h, were obtained from dairy farms in central Pennsylvania. The experimental diets had the following ratios (percentages) of protein sources (DSM to WPC): diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100. Mean initial BW of calves fed diets 1 through 4 were 47.3, 47.3, 48.5, and 45.8 ± 3.2 kg ($P > 0.05$), respectively. All calves were housed in an environmentally regulated room for the duration of the trial. Room temperature was thermostatically maintained between 19.5 and 21°C, and the relative humidity ranged from 65 to 75%. Air movement was controlled and regulated via forced air. Artificial lighting was used from 0630 to 1930 h daily. Initial calf BW were taken 12 h after arrival. Calves were weighed at 0800 h on the first and last day of each collection period (period of at least 5 consecutive d of collection during wk 2, 4, 6, and 8); average daily gain (**ADG**) was calculated for each collection period and over the entire feeding period. Upon arrival, calves were fed 1.9 L of Calf Resorb™ (SmithKline Beecham Animal Health, West Chester, PA) and were placed in group pens. Twelve hours after arrival, all calves were fed 1.9 L of a medicated milk replacer (Purina High Energy Nurse Gro #100™; Purina Mills, Inc., St. Louis, MO) containing 50 mg of oxytetracycline and 105 mg of neomycin base per 3.8 L of reconstituted milk replacer. The calves were weighed, tethered in stalls, and randomly allotted to treatment based on hour of birth and BW. Calves were offered the designated milk replacer diet at 10% of initial BW for the first 2 wk and at 12% of initial BW from wk 3 through 8 of the trial. The ingredients and composition of the four diets are given in Table 1. Milk replacers were analyzed for protein using the

TABLE 1. Ingredient and chemical composition of diet.[1]

	Diet			
Composition	1	2	3	4
Ingredient[2]				
Whey	20.54	19.35	17.81	16.63
DSM	47.69	32.16	16.18	0
WPC	0	16.98	34.35	51.80
7:60 Dry fat blend[3]	19.87	19.10	18.77	18.26
Starter premix[4]	1.13	1.13	1.13	1.13
Neo-terra premix[5]	4.50	4.50	4.50	4.50
Calcium chloride	0.26	0.26	0.26	0
Calcium carbonate	0	0.07	0.09	0.31
Dicalcium phosphate	0	0.31	0.68	1.08
Lysine	0	0.09	0.18	0.27
Ferrous sulfate	0.05	0.05	0.05	0.05
Liquid fat[6]	5.96	6.00	6.00	5.97
Chemical analysis				
CP,[7] %	20.60	21.10	21.10	20.70
Gross energy,[8] cal/g	4745	4726	4719	4761

[1]Ratios (percentages) of dried skim milk (DSM) to whey protein concentrate (WPC): diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100.

[2]As analyzed by Grober Veal Feeds, Inc. (Lehigh Valley, PA).

[3]Provided 7% milk proteins and 60% fat mixture.

[4]Provided all vitamin and trace mineral requirements for day-old calves and supplemental lysine and methionine as per NRC (23) requirements.

[5]Provided 600 g of neomycin base and 300 g of terramycin/tonne of feed.

[6]Contained lard, coconut oil, tallow, lecithin, and synthetic emulsifier (all edible grades).

[7]Determined by the Kjeldahl method (1).

[8]Determined using a bomb calorimeter (Parr Instruments Co., Moline, IL).

Kjeldahl N method (1), and gross energy was analyzed with a bomb calorimeter (Parr Instruments Co., Moline, IL).

All calves were fed warm (38.8 to 40.0°C) milk replacer twice daily at 0700 and 1800 h. Warm water or calf electrolyte solution (0.95 L) was offered from 1100 until 1500 h on the day of arrival and when scouring was prevalent (as diagnosed by a university veterinarian) to prevent dehydration. Amounts of water or electrolyte offered and refused were recorded at each feeding. Calves received 4 ml of Culbac Fast Start™ (*Lactobacillus acidophilus*; TransAgra International, Storm Lake, IA) and 2 ml of GOMAX™ (iron supplement; Farnam Co., Inc., Omaha, NE) each time milk replacer was fed. Feed efficiency (**FE**) was calculated for each collection period and for the entire feeding period.

Feces and urine were collected during wk 2, 4, 6, and 8. During the adjustment phase of the digestibility trial and during the weeks when samples were not collected (wk 1, 3, 5, and 7), calves were housed and tethered (fiber self-adjusting collars) in elevated individual wood stalls. The stalls were 60 cm wide and 191 cm long. Stalls had 61-cm oak dividers from the front of the stalls, and the floors were constructed from vinyl-coated metal. During the collection intervals, calves were tethered and housed in elevated pens (57 cm wide and 147 cm long) made of welded wire that was covered on the inner sides and backs with painted plywood. To collect urine, a funneling device of flexible rubber was placed around the sheath of the penis and secured by two Velcro® (Manchester, NH) straps (5.1 cm wide and 19.4 cm long) over the back of the calf. Feces and urine were collected for five consecutive 24-h periods. Collection began 24 h after calves were placed in the digestion stalls during wk 2, 4, 6, and 8. Urine was collected twice daily at 0800 and 1700 h; 100 ml of 6*N* HCl were added to prevent volatilization of ammonia. The total volume of urine excreted was recorded, and 500-ml samples were frozen for N analysis using the Kjeldahl N method (1). Feces were collected daily at 0800 h from beneath the bottom of the digestion stalls and dried at 55°C for varying times until no moisture remained. Dried samples were weighed, processed in a Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA), and stored for N and DM analyses (1).

Rectal temperatures; examinations of eyes, nose, ears, and navel; persistent cough; and discharge or signs of dehydration were recorded daily. Feces were scored numerically according to a fecal scoring system adapted from Larson et al. (18). Consistency (1 = normal to 5 = constipated), color (1 = white to 7 = black), and fluidity (1 = normal to 4 = watery) were evaluated. When excessive fluidity was noted, calves were treated with 60 cc of bismuth per feeding according to the recommendation of a university veterinarian. All treatments were recorded. In more severe cases, amounts fed were reduced by 50% for one feeding or until diarrhea subsided as per the recommendation of a university veterinarian.

During wk 2, 4, 6, and 8, blood samples were collected via a jugular catheter into three vacutainer vials containing K_3-EDTA, serum separation gel and clot activator, or sodium fluoride (Fisher Scientific Co., Ltd., St. Louis, MO) at 4-h intervals beginning at 2400 h for a 24-h period. Samples were analyzed for hematocrit (22), hemoglobin (Sigma Diagnostics, St. Louis, MO), total serum protein (Atago Clinical Refractometer T2™; Atago Co. Ltd., Tokyo, Japan); triglycerides (Sigma Diagnostics), NEFA (17), blood urea N (Stanbio Laboratory Inc., San Antonio, TX), and glucose (Sigma Diagnostics). Hematocrit, hemoglobin, and total serum protein analyses were completed within 12 h of sample collection. Sodium fluoride tubes were centrifuged at 3300 rpm for 15 min at 4°C, and aliquots were frozen for glucose analysis. Other samples were centrifuged at 2800 rpm for 20 min, and aliquots of serum were frozen for blood urea N, NEFA, and triglyceride an-alyses. All aliquots were stored in sterile glass borosilicate tubes at –20°C until analyzed.

All statistical analyses were conducted using SAS (25). Apparent DM digestibility (as a percentage), apparent biological value (as a percentage) {[grams of N fed – (grams of N in feces and urine)/(grams of N fed – grams of N in feces)] × 100}, and all blood values were analyzed using the general linear models procedure of SAS (25). Apparent DM digestibility, apparent biological values, and all blood values were analyzed on a weekly basis and over all weeks. A chi-square test for significance was used to analyze the fecal score data (25). An adaptation of the Tukey-Krammer procedure for multiple comparisons, assuming the normal approximation of the binomial distribution [$nP > 5$; $n(1 - P) > 5$], was used to determine significant differences in the frequency of symptoms among diets (9). The BW, ADG, and FE data were analyzed using an analysis of covariance procedure; initial BW was a covariate.

RESULTS AND DISCUSSION

Throughout the 8-wk trial, daily fecal scores of all calves were obtained. Of particular importance was the severity of scouring, which was evaluated using the fluidity score. The mean number of days that

TABLE 2. Mean BW change, average daily gain (ADG), feed efficiency (FE), and DMI by diet[1] over 8 wk (n = 4 calves per diet).

Item	Diet 1	2	3	4	SE[2]
BW Change, kg	20.4	18.6	19.3	20.3	2.50
ADG, kg/d	0.39	0.36	0.37	0.39	0.059
FE[3]	0.65	0.58	0.59	0.63	0.069
DMI, g/d	609.8	615	631.7	609.2	0.047

[1]Ratios (percentages) of dried skim milk to whey protein concentrate: diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100.

[2]Standard error within diets.

[3]Kilograms of gain per kilogram of DMI.

calves scoured according to diet was diet 1, 3.5 d; diet 2, 3.5 d; diet 3, 4.0 d; and diet 4, 2.25 d. The standard error within diets was 2.25 d. The number of days that calves scoured per week varied, and all scouring occurred during or before wk 4. The frequency of the number of days that calves scoured was not significant ($P > 0.05$) as determined by a chi-square statistic for differences among diets ($\alpha = 0.05$; $\chi^2 = 2.1438$ with 3 df; $P = 0.5422$). The number of days that calves scoured according to diet was similar to the number of days reported by Donnelly et al. (8). Those researchers also observed no difference in the mean number of days that calves scoured when DSM and diets based on whey were compared. Therefore, diets with high concentrations of DSM or WPC did not affect the amount of scouring for calves from wk 1 through 8 of age.

All calves were weighed once weekly. Final mean BW gains for calves fed diets 1 through 4 were 20.4, 18.6, 19.3, and 20.3 ± 3.22 kg, respectively, over the 8-wk period. Means and standard deviations for BW changes, ADG, FE, and DMI are presented in Table 2. All calves readily consumed the total milk replacer offered during each feeding. There were no significant differences in mean BW change, ADG, and FE among diets when $\alpha > 0.05$. All calves had similar rates of BW gain over time, and all BW gains were in the range of expected values for calves of similar age fed similar diets (11).

A summary of means for blood measurements by diet is presented in Table 3. There were no discernible trends caused by dietary treatment.

Tables 4 and 5 include means and standard errors within diets for apparent digestibilities of CP and apparent biological values (percentages) at wk 2, 4, 6, and 8 and over all weeks. Collection days were omitted from the analyses when urine leaked excessively and when urine and feces were mixed (wk 2, 9 d per diet were omitted; wk 4, 3 d per diet were omitted; wk 6, no days were omitted; and wk 8, 2 d per diet were omitted). The dietary means for apparent digestibility of CP at wk 2 were not different (Table 4). Relatively low digestibilities of CP were expected for calves at this age because most calves scoured during wk 2. These apparent digestibilities were similar to, but slightly lower, than those reported by Brown et al. (2) for calves of the same age. There were no differences in apparent digestibili-

TABLE 3. Mean blood measurements by diet[1] over all weeks (n = 196 observations for each diet).

Blood measurement[2]	Diet 1	2	3	4	SE[3]
Hb, mg/dl	12.5	10.9	11.2	10.7	0.149
Hct, %	39.2	34.1	35.7	34.0	0.450
Total protein, g/dl	4.8	4.7	4.7	4.9	0.029
Triglyceride, mg/dl	10.5	14.0	10.1	13.8	0.756
NEFA, meq/dl	183.9	189.1	185.2	190.6	7.06
BUN, mg/dl	8.0	7.0	7.3	7.7	0.168
Glucose, mg/dl	106.8	112.9	110.4	111.7	2.54

[1]Ratios (percentages) of dried skim milk to whey protein concentrate: diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100.

[2]Hb = Hemoglobin, Hct = hematocrit, and BUN = blood urea N.

[3]Standard error within diets.

TABLE 4. Means and standard errors for apparent digestibility of CP of each diet[1] by week.

	Diet				
Item	1	2	3	4	SE[2]
wk 2					
Apparent digestibility, %	72.50	67.83	61.13	72.49	6.33
Apparent biological value, %	45.49[ab]	58.25[ab]	32.08[b]	69.11[a]	0.10
Observations, no.	11	19	11	17	
wk 4					
Apparent digestibility, %	77.16	83.17	83.35	83.88	2.67
Apparent biological value, %	69.19[b]	73.38[ab]	73.42[ab]	76.51[a]	2.92
Observations, no.	23	17	20	17	
wk 6					
Apparent digestibility, %	89.88	87.38	88.96	90.53	1.33
Apparent biological value, %	71.93[b]	77.51[a]	76.04[ab]	74.95[ab]	0.02
Observations, no.	20	20	21	22	
wk 8					
Apparent digestibility, %	90.00	91.46	89.39	88.35	1.18
Apparent biological value, %	68.30	71.23	72.31	69.70	2.25
Observations, no.	20	20	20	18	

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]Ratios (percentages) of dried skim milk to whey protein concentrate: diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100.

[2]Standard error within diets.

ties of CP at wk 4 and 6. The apparent digestibility of CP of diet 4 (0:100, DSM to WPC) was 90.5%, which corresponded to similar values determined by Toullec et al. (28), who reported a 90.7% digestibility of CP for a WPC diet at wk 5. These mean digestibilities of CP were slightly higher than the 87.5% reported by Dawson et al. (7).

Apparent digestibilities of DM were not different among diets 1 through 4 at wk 8 and averaged 90.00, 91.46, 89.39, and 88.35 ± 1.18%, respectively. The reasons for the slight decrease in apparent digestibility of DM from wk 6 to 8 for diet 4 (0:100, DSM to WPC) are unclear. However, digestibilities of DM were still comparable with the mean digestibility reported (87.0%) for newborn calves by Jarrige (15). The apparent digestibilities of DM for diets 1 through 4 over all weeks were 82.50, 82.85, 83.82, and 84.03 ± 1.77%, respectively ($P > 0.05$). Digestibility values were also calculated after data were omitted for wk 2 because most of the excrement contamination and scouring was noted during this period. The apparent digestibilities of DM for diets 1 through 4 over wk 4, 6, and 8 were 85.54, 87.69, 87.94, and 87.93 ± 0.93%, respectively. Apparent digestibility of DM was not different among diets for all weeks or when data from wk 4, 6, and 8 were combined. No trend was found for apparent biological value or N retention as changes occurred in the ratios of protein sources. Therefore, DSM and WPC are comparable protein sources for milk replacer diets based on apparent digestibilities of DM.

TABLE 5. Means and standard errors for apparent digestibility of CP and biological value of diets[1] for wk 4, 6, and 8.

	Diet				
Item	1	2	3	4	SE[2]
wk 4, 6, and 8					
Apparent digestibility, %	85.54	87.69	87.94	87.93	0.93
Apparent biological value, %	69.11[b]	73.98[a]	73.93[a]	73.71[a]	1.21
Observations, no.	74	76	72	74	

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]Ratios (percentages) of dried skim milk to whey protein concentrate: diet 1, 100:0; diet 2, 67:33; diet 3, 33:67; and diet 4, 0:100.

[2]Standard error within diets.

Mean apparent biological values were affected ($P < 0.05$) by diet at wk 2, 4, and 6 (Table 4), which may be partially attributed to the incomplete urine and feces collection and separation during the collection intervals. Although we attempted to separate feces from urine throughout the experiment, some mixing might have occurred, especially during wk 2 when scouring was most frequent. Biological values increased markedly after wk 2. It is difficult to determine why the biological values at wk 4 and 6 were higher than those at wk 8.

There is little published research that identifies the biological values of DSM and WPC. However, Church and Pond (5) reported a biological value of 69% for the milk casein fed to adult rats (at an unidentified percentage). This biological value is similar to the values calculated at wk 8 in the present study for diets 1 and 2, which contained high portions of DSM. Maynard and Loosli (21) reported a biological value of 85% for milk, which is greater than those in the present study. At wk 8, no significant differences were found among diets in apparent biological value; means for diets 1 through 4 were 68.3, 71.2, 72.3, and 69.7 ± 2.25%, respectively. Because separation of urine and feces was better at wk 8, the apparent biological values for that week should be the most reliable of the study, reflecting that, at 8 wk of age, diets containing DSM or WPC as the major sources of protein have biological values similar to those for the preweaned dairy calf.

CONCLUSIONS

Over the past three decades, technology has led to important changes in the types and qualities of protein sources used in milk replacers for calves. Because of the increased demand for casein, there is a need to determine alternative sources of milk protein and other proteins that are nutritionally complete and financially practical. The milk manufacturing industries provide the by-product protein sources that can be used for rearing young animals. Dried skim milk and WPC are comparable alternative sources of protein in milk replacer diets for calves from 12 to 36 h of age to 8 wk of age. Under the conditions of this study, because of the reduced cost of WPC compared with DSM, WPC is nutritionally acceptable and also a more economically feasible replacement for DSM in dairy calves that are 1 to 8 wk of age.

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