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The Effect of Ruminal Fluid Preparations on the Growth and Health of Newborn, Milk-Fed Dairy Calves

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ABSTRACT

The objective of this study was to determine the effect of oral doses of ruminal fluid (RF) on the growth and health of newborn, milk-fed heifer dairy calves (0 to 6 wk of age). Calves given 8 ml of RF each day until weaning gained more weight and had fewer scours than controls that did not receive RF. Because RF that was exposed to oxygen or was autoclaved also gave a response, it is unlikely that the preparations were acting as a probiotic. When the RF was centrifuged to separate the cells (RFC) from the fluid (RFS), both fractions had similar activity, and this result indicated that the response was not nutritional; that is, 1) RFC supplied a small amount of protein (approximately 8 mg/d), but RFS had much less protein, and 2) RFS had volatile fatty acid, but RFC had little if any volatile fatty acid. However, both RFS and RFC had bacterial polysaccharide, and bacterial polysaccharide has strong antigenic properties. In the first three studies, treated calves were given RF preparations each day until weaning (6 wk), but a subsequent experiment indicated that calves given autoclaved RF for only 5 d (d 1 to 5) also had greater body weight gains during the first 2 wk of life and fewer scours than untreated controls. Given that the dosage of RF was small and the material could be autoclaved to prevent disease transmission, RF supplementation could be a practical tool for improving calf health.
(**Key words:** ruminal fluid, calf, growth, health)

Abbreviation key: ARF = autoclaved ruminal fluid, **BPS** = bacterial polysaccharide, **FRF** = fresh ruminal fluid, **PF-RFS** = polysaccharide-free supernatant, **RF** = ruminal fluid, **RFC** = ruminal fluid cell pellet, **RFS** = ruminal fluid supernatant.

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INTRODUCTION

Newborn, milk-fed calves on dairy farms are often severely affected by diarrhea commonly called "scours" (Davis and Drackley, 1998). Dairymen have implemented the following strategies to decrease scours: 1) improvements in sanitation, 2) the use of individual hutches to decrease pathogen transmission, 3) the use of oral antibiotics to combat bacterial infections, and 4) the use of fortified colostrum supplements that may enhance passive immune defenses (Otterby and Linn, 1981). However, recent surveys indicate that calf mortalities still range from 8 to 11% (National Animal Health Monitoring System, 1993, 1996).

Early work indicated ruminal inoculation could have a positive impact on calves (Pounden and Hibbs, 1949a,b). However, the mechanism of action was not defined, and the supporting observations were not quantitative. In some cases, calves with recurrent diarrhea appeared to improve, but this effect was confounded by other dietary differences. Because calves given the cud from mature cows had a denser ruminal flora than uninoculated controls, it appeared that the ruminal contents were simply stimulating normal rumen development.

Ruminal bacteria have a thick coating of bacterial polysaccharide (**BPS**; Costerton et al., 1974), but the impact of this material on the ruminant immune system has largely been ignored. Work with other animals indicates that BPS is not only a trigger for antibody production, but in addition BPS may 1) act as an adjuvant to enhance the potency of other antigens, 2) induce macrophages to release cytokines that affect the differentiation of mammalian cells, and 3) circumvent the normal cascade of immunostimulation to cause an anergy commonly called oral tolerance (Roitt et al., 1998; Tizard, 1996).

The following experiments sought to reexamine the effect of ruminal fluid (**RF**) on the growth and health of newborn, milk-fed dairy calves. RF was fractionated by centrifugation and autoclaved so the mechanism of action could be more precisely defined.

MATERIALS AND METHODS

Calves. A series of four experiments were conducted over a period of 4 yr (1998 to 2001) to examine the effects of RF extracts on calf health and growth. Calves and pregnant cows were housed at the Cornell University Research Center in Dryden, NY. Pregnant, nonlactating, Holstein dairy cattle were fed a TMR (3.8 kg of corn silage, 1.7 kg of haylage, 2 kg of grass hay, 1.2 kg of high-moisture shelled corn, 0.2 kg of whole cottonseed, and 1.1 kg of soybean meal per day) to meet recommendations of the NRC (1989). Several hours before parturition, the pregnant cows were placed in a clean pen that was bedded with sawdust.

Soon after parturition (less than 15 min), the cow was milked and 4 kg of colostrum was given to the newborn calf via a stomach tube. At approximately 12 h after birth, the calf was given another 2 kg of colostrum orally. Calves were randomly allotted in a block design to treatment groups as they were born. Because the studies were relatively short term (approximately 10 wk) and control and treated groups were allotted at the time, season, and time, effects were minimized. Calves were housed outside in individual hutches (approximately 1.7×1.2 m, Cal-Tel Delux; Hampel Corp., Germantown, WI). The hutches were located on clean, coarse gravel that was changed each year to prevent the buildup of disease-causing microorganisms. Calves were given clean bedding (straw) each day, and manure was removed three times per week. Calves were raised under husbandry conditions that are typical of the Cornell Research Center subject to the recommendations of the Cornell Center for Research Animal Resources (IACUC no. 91-32-00).

Calves were fed either milk or nonmedicated milk replacer, and the amounts varied with the experiment. RF preparations and dosage time also varied with the experiments. Calves consumed all of the milk or milk replacer that was provided. All calves were provided with water and a commercial grain mix (Agway Calf Prestarter; Agway, Inc., Syracuse, NY; 22% CP, 81% TDN, 2.0 Mcal/l kg ME, 1.3 Mcal/l kg NE_g) for ad libitum intake starting at 3 d of age. Supplemental hay was not provided.

BW gain. BW gains were assessed according to the guidelines of Larson et al. (1977). Approximately 4 h after birth, the calves were placed in a cart that restrained movement, the cart was placed on a platform scale, and initial BW was the difference between the weight of the cart and the total. Calves that had an initial BW greater than 53 kg or less than 34 kg were not included in the experiments. Thereafter, the calves were weighed in similar fashion approximately 6 h after the morning feeding. The calves were restrained, and the weight measurements were relatively constant during the procedure (<0.25 kg variation).

Scours. The calves were inspected five times per day by the dairy farm workers (approximately five different individuals), and the appearance of feces on the hair surrounding the rectum and in the bedding was scored. In most cases, the calves defecated immediately after they were aroused. In other cases, fecal matter that had not yet dried or crusted over was used as an index of recent defecation. Stools were scored from 1 (normal) to 4 (runny or watery texture and either a white or gray color) according to Larson et al. (1977), and code 4 stools were defined as scours. If calves with scours appeared to be dehydrated (e.g., sunken eyes, sluggish body movements, and loss of skin elasticity), they were supplemented with an aqueous mixture of electrolytes [83% corn sugar, 4.3% sodium chloride, 4.8% potassium chloride, 4.9% sodium bicarbonate, 4% potassium phosphate (wt/vol)].

Ruminal fluid. Fresh ruminal fluid (**FRF**) for experiment 1 was removed from the rumen of a fistulated lactating dairy cow that was housed at the Cornell University Research Center. This fistulated cow was fed the TMR (see above) ad libitum. The fluid was withdrawn from the rumen with a suction device via a pipe that had holes. The holes (6 mm diameter) in the pipe filtered the FRF so that it would not contain large feed particles. In experiments 2, 3 and 4, FRF was taken from a nonlactating fistulated dairy cow that was located on the Cornell campus near our laboratory. This latter fistulated cow was fed timothy hay ad libitum. The FRF for experiments 2, 3, and 4 was brought to the laboratory and placed in a 39°C water bath. After gas production from the fermentation had buoyed feed particles to the top of the flask and protozoa had sedimented to the bottom, FRF containing mixed ruminal bacteria was withdrawn from the center of the flask. The FRF was centrifuged ($10{,}000 \times g$, 30 min, 5°C). The supernatant (**RFS**) was removed, and the cell pellet (**RFC**) was resuspended in an equal volume of 0.9% NaCl. The **ARF** was FRF that had been autoclaved (4 L, 121°C, 40 min). The RFC, RFS, and ARF were dispensed into plastic vials (8 ml) and frozen (–15°C) until use. Bacterial protein in RFC and RFS was analyzed by the method of Bradford (1976) after the cells had been heated to 100°C in 0.2 N NaOH for 15 min (bovine serum albumin as a standard). The RNA and DNA were measured by using an orcinol-$FeCl_3$ reaction (Schneider, 1957) and ribose was used as the standard.

Bacterial polysaccharide. The FRF that had been centrifuged to remove feed particles ($100 \times g$, 5 min, 5°C) was assayed by the phenol sulfuric acid method

[0.3 ml of phenol, 2.1 ml of 70% (vol/vol) sulfuric acid, 100°C, 10 min, 485 nm; Ashwell, 1966] to estimate total FRF-BPS. The FRF was then centrifuged at higher speed to remove the bacteria (10,000 × *g*, 15 min, 5°C), and the resulting RFS was again assayed by the phenol sulfuric acid method to estimate RFS-BPS. The RFS was then treated with 1% (wt/vol) cetyltrimethyl ammonium bromide to precipitate BPS (Ausubel et al., 1997), and the polysaccharide-free supernatant (**PF-RFS**) was also assayed by the phenol sulfuric acid method to estimate nonspecific RF interference. Preliminary results indicated that cetyltrimethyl ammonium bromide (200 μl of a 1% solution) did not interfere with the phenol sulfuric acid assay. True RFS-BPS was defined as RFS-BPS minus PF-RFS. True RFC-BPS was defined as (FRF-BPS minus PF-RFS) minus true RFS-BPS. Glucose was used as a standard.

Experiment 1. Twenty-four heifer calves were allotted to control (no addition) or the FRF treatment (12 calves per group). Treated calves received 8 ml of FRF per day in the colostrum or morning milk feeding for 6 wk. Both groups of calves were fed equal amounts of whole milk two times per day (approximately 12-h interval between feedings, 4.5 kg/d). The calves were weaned at 6 wk of age. Calves were weighed at birth and at 6 wk of age.

Experiment 2. Thirty-six heifer calves were allotted to control (no addition), RFS, or FRC treatments (12 calves per treatment). Treated calves received 8 ml of RFS or RFC per day in the colostrum or morning feeding for 42 d. All three groups of calves were fed equal amounts of a commercially produced milk replacer (Excelerate, 30% protein, 20% fat; Milk Specialties Company, Dundee, IL) three times per day (approximately 8-h interval between feedings, 7.5 kg/d). The calves were weaned at 6 wk of age. Calves were weighed at birth and at 2, 4, and 6 wk of age.

Experiment 3. A total of 24 heifer calves were allotted to control (no addition) or the ARF treatment (12 calves per treatment). Treated calves received 8 ml of ARF per day in the colostrum or morning milk feeding for 42 d. Both groups of calves were fed equal amounts of whole milk (6 kg/d, two feedings per day). The calves were weaned at 6 wk of age. Calves were weighed at birth and at 2, 4, and 6 wk of age.

Experiment 4. A total of 24 heifer calves were allotted to control (no addition) or the ARF treatment (12 calves per treatment). Treated calves received 8 ml of ARF per day in the colostrum or morning milk feeding for the first 5 d of life. Both groups of calves were fed equal amounts of milk replacer (7.5 kg/d, three feedings per day). The calves were weaned at 6 wk of age. Calves were weighed at birth and at 2, 4, and 6 wk of age.

Statistics. The statistical analyses were performed by SAS (SAS Inst., Cary, NC) (SAS, 1999). In the first analysis, the GLM procedure was used to analyze the weight gain in a completely randomized design with analysis of covariance as described by Kuehl (2000) for each period of growth. Initial BW was used as the covariate. The interaction between treatments and the covariate was used to check the uniformity of the slopes among treatments by using the sequential sum of squares (Littell et al., 1991); the interaction and (or) the covariate were removed from the statistical model if not significant at $P < 0.05$. The partial sum of squares was used in the analysis of covariance to test treatments (Littell et al., 1991). The statistical model is as follows:

$$Y_{ij} = \mu + \tau_i + \beta(X_{ij} - \overline{T}..) + e_{ij}$$

where Y is the BW gain in each period, mμ is the overall BW gain mean, τ_i is the fixed effect of treatments, β_i is the coefficient for the linear regression of Y on X, X is the initial BW, and e_{ij} is the independent, identical, and normally distributed random experimental error.

In the second analysis, the weight gain of each growth period was analyzed as a repeated measure design for all experiments (except experiment 1). The Mauchly sphericity test of the Proc GLM (SAS Inst., Cary, NC) (SAS, 1999) was used to test the variance-covariance matrix and a univariate ANOVA was performed if $P > 0.05$. The treatment comparison was performed by contrast analysis. The split-plot statistical model used (Kuehl, 2000) is as follows:

$$Y_{ijk} = \mu + \alpha_i + d_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where μ is the general mean, α_i is the fixed effect of treatment, d_{ik} is the random experimental error for calves within treatments to test treatment effect, β_j is the effect of time (period of growth), $(\alpha\beta)_{ij}$ is the interaction between treatment and time, and e_{ijk} is the normally distributed random experimental error.

In the third statistical analysis, all experiments were analyzed together, and treatments had two levels, i.e., control and treated, which consisted of calves that received any form of RF. Experiments were considered as blocks. Experiments 2, 3, and 4 were used to investigate the effect of RF on weight gain of each week period. All experiments were used in the overall weight gain (0 to 6 wk). In this overall analysis, milk intake was used as a covariate, and the analysis was similar to that described above. Because a preliminary study indicated an interaction between treatment and

Table 1. The effect of ruminal fluid (RF) preparations on the number of calves with scours and the duration of scours in newborn calves.

	Growth period (wk)			Scour (days)[1]
	0 to 2	2 to 4	4 to 6	
Experiment 1				
Control	10	3	5	2.67[a]
FRF[2] (42 d)	6	1	0	0.83[b]
Experiment 2				
Control	12	5	2	2.75[a]
RFS[2] (42 d)	4	3	0	0.58[b]
RFC[2] (42 d)	5	1	0	0.50[b]
Experiment 3				
Control	4	7	5	1.83[a]
ARF[2] (42 d)	3	1	0	0.33[b]
Experiment 4				
Control	12	3	2	3.67[a]
ARF (5 d)	3	1	0	0.42[b]

[1]Mean values within a column for each experiment with different superscripts differ ($P < 0.05$, Kruskal-Wallis test).

[2]FRF = Fresh ruminal fluid, RFS = ruminal fluid supernatant, RFC = ruminal fluid cell pellet, ARF = autoclaved ruminal fluid.

the covariate (milk intake), a model with unequal slopes was used (Littell et al., 1999). All analyses were performed by Proc MIXED (SAS Inst., Cary, NC), (SAS, 1999) and the statistical model is as follows:

$$Y_{ij} = \alpha_i + \beta_i X_{ij} + b_j + e_{ij}$$

where α_i is the intercept of the ith treatment effect, β_i is the slope of the regression of weight gain on milk intake of the ith treatment, b_j is the random effect of experiments, and e_{ij} is the experimental random error.

The plot of studentized residues against the predicted values from the analysis of covariance was used to identify outliers, and the plot of the studentized residues against treatments was analyzed to test the assumption of identical variance (Kuehl, 2000). The normal distribution was also investigated (not shown).

Because a preliminary analysis of the number of days that calves had scours (scour days) was not normally distributed, a nonparametric test using the Proc NPAR1WAY of SAS (SAS Inst., Cary, NC) (SAS, 1999) was selected to compare the distributions of each treatment (Snedecor and Cochran, 1971). Treatment comparisons were done by the Wilcoxon score and Kruskal-Wallis tests (SAS Inst., Cary, NC) (SAS, 1999) without the continuity correction.

RESULTS

Experiment 1. A preliminary experiment was conducted to ascertain the effect of FRF on the BW gain and scours. Control calves that did not receive FRF had an initial BW of 41.7 ± 1.3 kg, and the total weight gain (0 to 6 wk) was 16.5 ± 1.0 kg. Treated calves (n = 12) that were given the same amounts of milk and FRF for 42 d had an initial BW of 43.4 ± 4.9 kg, and the total weight gain (0 to 6 wk) was 24.3 ± 1.1 kg. The interaction between treatment (FRF) and the covariate (initial BW) was not significant ($P > 0.05$), the slope of the observed variable on initial weight (covariate) was similar, outliers were not identified, and treated calves gained more weight than the controls ($P < 0.05$). None of these control calves died, but most of them (11 of 12) eventually scoured, and the average number of days that each calf had scours was 2.67 (Table 1). Most of the FRF-treated calves scoured (8 of 12), but the average number of scour days was threefold less ($P < 0.05$).

Experiment 2.] Because results from experiment 1 indicated that FRF had a positive impact, we fractionated the RF and determined BW at 2-wk intervals. Control calves gained more weight in the second (2 to 4 wk) and third growth periods (4 to 6 wk) than the first period (0 to 2 wk), and the total gain was 23.7 kg (Table 2). All of the control calves (12 of 12) scoured during the first 2-wk period, but scours declined as the calves became older (Table 1). Control calves had 2.75 scour days per calf.

Calves given RFS gained more weight than the untreated controls ($P < 0.05$), and this overall advantage (0 to 6 wk) could be explained by an improvement in the first 2 wk ($P < 0.05$). During the second and third growth periods, RFS-treated calves did not gain more weight than the untreated calves ($P > 0.05$). RFS-treated calves had fewer scours than untreated calves (Table 1, $P < 0.05$).

Table 2. Comparison of initial BW (IBW) and BW gain (BWG) during each period of growth for experiments 2, 3, and 4.

	IBW (kg)	BWG (kg)			BWG contrasts[1]	
	0 wk	0 to 2 wk	2 to 4 wk	4 to 6 wk	L	Q
Experiment 2						
Control (C)	45.1	3.7	10.0	10.0	***	***
RFC[2] (42 d)	42.9	6.7	10.7	9.2	***	***
RFS[2] (42 d)	42.9	7.2	9.8	9.7	***	NS
Contrasts						
IBW C × (RFC + RFS)	NS					
IBW RFC × RFS	NS					
Gain C × (RFC + RFS)		***	NS	NS		
Gain RFC × RFS		NS	NS	NS		
Experiment 3						
Control (C)	43.8	3.3	8.2	10.0	***	NS
ARF[2] (42 d)	43.9	7.2	9.5	9.3	NS	NS
Contrasts						
IBW C × ARF	NS					
Gain C × ARF		***	NS	NS		
Experiment 4						
Control (C)	44.4	2.4	8.9	10.8	***	**
ARF (5 d)	45.3	4.8	9.2	10.8	***	NS
Contrasts						
IBW C × ARF	NS					
Gain C × ARF		**	NS	NS		

[1]Polynomial contrasts: L = linear and Q = quadratic effects.

[2]RFC = Ruminal fluid cell pellet, RFS = ruminal fluid supernatant, ARF = autoclaved ruminal fluid.

**$P < 0.01$.

***$P < 0.001$.

NS = Nonsignificant.

Calves given RFC that had been resuspended in a similar volume of sodium chloride gained more weight in the first growth period (0 to 2 wk, $P < 0.05$), but the overall gain (0 to 6 wk) was not statistically improved relative to the control calves ($P = 0.06$). The RFC-treated calves had fewer scours than untreated calves (Table 1, $P < 0.05$).

Experiment 3. Because results from experiment 2 indicated even RFS could have a positive impact, we then examined the effect of ARF. Control calves had a total gain of 21.5 kg (Table 2), and the average number of scour days was 1.83 (Table 1). Calves given ARF gained more weight than untreated controls (0 to 6 wk, $P < 0.05$), and this advantage could be explained by an improvement in the first time period ($P < 0.05$, Table 2). Calves that received ARF had fewer scours than untreated controls (Table 1, $P < 0.05$).

Experiment 4. Because results from experiment 3 indicated even ARF could have a positive impact, we then decided to decrease the treatment period from 42 to 5 d. Control calves had a total gain of 22.0 kg (Table 2), and the average number of scour days was 3.67 (Table 1). Calves given ARF for only 5 d gained more weight ($P < 0.05$) in the first growth period (0 to 2 wk), but the overall gain (0 to 6 wk) was not statistically improved ($P = 0.14$). Calves that received ARF for 5 d had fewer scours than untreated controls ($P < 0.05$).

Pooled experiments. The analysis of pooled BW gains from experiments 2, 3, and 4 are shown in Table 3. Similar to the individual analysis of each experiment, the analysis of pooled experiments indicated the administration of RF affected the first 2 wk of growth ($P < 0.05$), but RF had no effect on gain in subsequent time periods ($P > 0.05$). Pooled results indicated that 94% of the control calves had scours (average scour days were 2.8), whereas only 55% the treated calves had scours (average scour days were 0.5).

DISCUSSION

Our experiments were conducted over a 4-yr period, and during this time the standard management procedures at the Cornell Research Center changed as follows: 1) calves in the first and third studies were given whole milk, but experiments 2 and 4 were conducted with commercial milk replacers, 2) the intake of milk or milk replacer was varied from 4.5 to 7.5 kg/d, and 3) calves in experiments 1 and 3 were fed twice daily, but calves in experiment 2 and 4 were fed three times per day. However, within each experiment, control and

Table 3. Analysis of BW gain (BWG) for experiments 2, 3, and 4 together.

	Weight gain (kg), wk			Contrasts[1]	
	0 to 2	2 to 4	4 to 6	L	Q
Control (C)	3.6	9.5	10.7	***	***
RF-Treated	6.9	10.3	10.2	***	***
Contrasts[1]					
C × Treated	***	NS	NS		

[1]Polynomial contrasts: L = linear and Q = quadratic effects.

[2]RF = Ruminal fluid.

**$P < 0.01$.

***$P < 0.001$.

NS = Nonsignificant.

RF-treated calves always were given the same amount and type of milk.

It has long been recognized that calf growth experiments often have an inherently high degree of variation (Kertz et al., 1984); therefore, we used relatively large numbers of calves (n = 12 per treatment). However, RF-dependent improvements in BW gain were much greater for experiment 1 than experiments 2 through 4 (Table 1). The RF-dependent improvements in BW gain for experiments 2 through 4 were 13, 20, and 12%, respectively (Table 1), but experiment 1 seemed to have a 49% increase in growth.

To examine if the control group of experiment 1 was abnormal, we compared it to another independent group of 12 calves. These other calves were fed the same amount of milk (4.5 g/d), were raised during the same period, had identical management and did not receive RF; however, they gained more weight than the original controls (19.7 ± 1.5 vs. 16.5 ± 1.0 kg, respectively; $P < 0.05$), although the number of scour days was similar (2.51 vs. 2.67, respectively; $P > 0.05$). When this independent control was compared to the RF-treatment group, the improvement was only 23%, a value that more closely resembled the effects seen in the other experiments.

Researchers must often balance the advantages of highly controlled experiments with the reality of farm practices. We could have raised our calves in metabolism stalls where environmental temperature, sanitation, and the intake of grain could be rigorously controlled and monitored, but we chose to raise our calves in outdoor hutches that closely mimicked conditions on modern farms. Control and RF-treated calves were always provided with same amount of milk, but water and grain were provided free-choice. Because some of the grain spilled onto the ground, rain water sometimes leaked into the grain bucket, and the grain consumptions were low, it was not possible to determine the grain intake precisely.

The suggestion that RF-treated calves simply consumed more grain than the untreated controls does not diminish the potential benefit of RF as a supplement. In addition, improvements in BW gain and decreases in scours were primarily observed in the first 2 wk when grain consumption was very low and the calves were deriving almost all of their nutrition from milk. Kertz et al. (1984) monitored the grain intake of 335 neonatal calves, and grain intake during the first 2 wk was less than 2 kg for each calf. Because improvements were only observed in the first 2-wk period when BW gains were low, dehydration or gut fill differences caused by scours could have been a factor.

We did not determine the cause of scours in our calves because specific causes of diarrhea in calves are difficult to establish (Davis and Drackley, 1998; Steiner et al., 1997; Waltner-Toews et al., 1987). However, previous workers used fecal appearance as an index of calf scours and health, and we used a similar scoring system (Larson et al., 1977). Kertz et al. (1984) reported that 95% of their neonatal calves had scours, and the average number of scour days per calf for the control group was 4.5. In our studies, 94% of the control calves had scours (average scour days were 2.8) whereas only 55% the treated calves had scours (average scour days were 0.5).

All of our calves were given colostrum immediately after birth via stomach tube, and this practice should have ensured passive immunity (Morin et al., 1997). Some researchers have fortified colostrum by vaccinating cows with viral and bacterial antigens before calving (Acres et al., 1979; Saif et al., 1983; Snodgrass, 1982), but we did not use fortified colostrum in our studies. Fortified colostrum can increase BW gain and decrease diarrhea if calves are given the same pathogens, but in actual field conditions, fortified colostrum is often without effect (Tizard, 1996).

Based on the previous literature (Pounden and Hibbs, 1949a,b), we had originally hypothesized that

Table 4. The composition of ruminal fluid preparations that had been centrifuged to create cellular (RFC) and supernatant (RFS) fractions.

Material	RFC	RFS
VFA		
Acetate (m*M*)	<2	48 ± 2
Propionate (m*M*)	<1	13 ± 2
Butyrate (m*M*)	<0.5	8 ± 1
Cellular components		
Protein (μg/ml)	1490 ± 65	73 ± 25
RNA (μg/ml)	190 ± 27	<10
DNA (μg/ml)	16 ± 5	<10
BPS[1] (μg hexose equiv./ml)	333 ± 10	260 ± 19

[1]Bacterial polysaccharide.

FRF might act as a probiotic, and FRF decreased scours ($P < 0.05$) and increased BW gain ($P < 0.05$). However, subsequent experiments indicated that cells harvested by centrifugation, resuspended in sodium chloride, and frozen aerobically without a cryoprotectant could promote growth ($P < 0.05$) and decrease scours ($P < 0.05$). The idea that FRF was a probiotic was further contradicted by the observation that RFS or even ARF increased BW gain ($P < 0.05$) and decreased scours ($P < 0.05$).

RF has microbial protein, VFA, and vitamins, but it is very unlikely that our response was nutritional (Table 4). The amount of bacterial protein was very small (approximately 8 mg/d), and even RFS that had been centrifuged to remove virtually all of the bacteria had activity (0 to 2 wk, $P < 0.05$). Because RFC also had activity (0 to 2 wk, $P < 0.05$), the benefit could not be explained by VFA from the fluid phase.

When FRF was harvested by centrifugation, there was a distinct layer of polysaccharide (slime) directly above the cell pellet, and RFS was clear. Subsequent work, however, indicated that even RFS had an abundance of BPS that could be precipitated by cetyltrimethyl ammonium bromide (Table 4), an anionic detergent that has been used to precipitate BPS and "clean up" DNA preparations (Ausubel et al., 1997). Because BPS are potent antigens and retain activity after autoclaving (Tizard, 1996), it appeared that BPS was the active ingredient in RF.

In the 1990s, Nosky and Worthington developed a product based on mycobacterium cell walls under the trade name Immunoboost (Veterphram Research Inc, Athens, GA, and Chino Corona Veterinary Services, Chino, CA). Their work indicated that calves given intravenous, intramuscular, and subcutaneous injections of Immunoboost during the first 24 h of life had fewer scours and higher average daily gain than untreated controls. Oral administration was not tested, but the authors noted that Immunoboost-treated calves required 17% less antibiotic treatment.

Calves can die from microbial infection, but water loss and dehydration are even more apt to cause mortality (Davis and Drackley, 1998; Tizard, 1996). Newborns are very prone to diarrhea, and this condition is triggered by agents that irritate the intestine (Guyton, 1971). Intestinal irritation increases secretion, motility, and stool volume. As the animal becomes older and the intestine is repeatedly exposed to irritants and antigens, the intestinal tissues become desensitized, and the frequency of diarrhea declines (Ernst et al., 1988).

Intestinal desensitization (sometimes called oral tolerance) is a localized phenomenon that is mediated by circulating immunoglobulins and the macrophages (Fahmi and Chaby, 1993, 1994). When macrophages are presented with antigens bound to immunoglobulins, they secrete cytokines that can directly affect mammalian cells (Kaufman et al., 2000). Cytokines appear to accelerate intestinal maturation and desensitization, and this process is dose dependent. Studies with food allergens have shown that low doses invoke limited suppression, but large doses can provoke clonal anergy and immunotolerance (Roitt et al., 1998; Tizard, 1996).

Because RF has a highly diverse population of bacteria and other microorganisms (Krause and Russell, 1996), it would contain dozens, perhaps hundreds, of different BPS molecules. The activity of RF does not seem to be highly diet dependent. The FRF (experiment 1) was obtained from a cow fed a typical dairy cattle ration, but the cow that served as a donor for experiments 2, 3, and 4 was fed only timothy hay. Additional work is needed to see if RF from cattle fed diets containing mostly grain also has activity. None of the calves in our studies died, the scours were relatively mild, and there were no lasting effects on health. Further work is needed to see if RF can also succeed in preventing scours in the face of a more thorough disease challenge.

We originally gave the calves RF preparations each day until weaning (6 wk), but the improvement in BW gain and decrease in scours was greatest during the first 2 wk of life ($P < 0.05$, Table 1). Because the improvement in gain merely carried over into subsequent time periods (Tables 1 and 3), we decided to decrease the dosage time from 42 to 5 d (experiment 4). Calves given ARF for only 5 d also responded, and this result is consistent with the idea that RF is most beneficial to newborn calves that do not have a fully developed immune system.

The use of RF preparations as an oral supplement is an organic method of improving the health of the calf. In nature, the cow frequently licks the muzzle of the calf, and she has RF in her saliva. When calves are removed from the cow immediately after birth, this natural means of antigenic transfer is no longer possible. It has long been recognized that calves removed immediately from the mother and reared in isolation do not develop populations of ruminal protozoa (Hungate, 1966), but the potential impact of RF on the health of the calf has largely been ignored.

The observation that autoclaved RF preparations decrease scours as well as increase BW gain has the following practical relevance: 1) RF preparations could be given orally via the milk, 2) RF contains naturally occurring nonpathogenic bacteria, 3) RF can be autoclaved to eliminate the chance of disease transmission, and 4) the time needed to demonstrate a response was relatively short (as little as 5 d).

CONCLUSIONS

Newborn dairy calves that were given daily doses of RF gained more weight and had fewer scours than untreated controls. Because even autoclaved preparations resulted in a positive response, RF preparations were not acting as a probiotic.

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