

Serum IgG and total protein concentrations in dairy calves fed two colostrum replacement products

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Objective—To evaluate effects of 2 commercially available colostrum replacement products on serum IgG and total protein concentrations in dairy calves.

Design—Prospective clinical trial.

Animals—84 Holstein bull calves from a single dairy.

Procedures—Calves were randomly assigned to be given 4 quarts of colostrum (group 1; n = 21), 2 packages of a colostrum replacement product (product A; group 2; 21), 1 package of a different colostrum replacement product (product B; group 3; 21), or 2 packages of product B (group 4; 21). Treatments were given within 3 hours after birth, and blood samples were collected 24 hours later and submitted for determination of serum total protein and IgG concentrations.

Results—Group 1 calves had significantly higher serum total protein and IgG concentrations than did calves in the other 3 groups. However, the percentage of calves with adequate passive transfer (ie, serum IgG concentration > 1,000 mg/dL) was not significantly different among groups 1 (90%), 3 (81%), and 4 (95%). In contrast, only 10% of calves in group 2 had adequate passive transfer. It was predicted that calves fed product B that had serum total protein concentrations > 5.2 g/dL would have serum IgG concentrations > 1,000 mg/dL at least 90% of the time.

Conclusions and Clinical Relevance—Results indicated that product B could be considered as an alternative to colostrum in dairy calves, but product A failed to routinely provide adequate serum IgG concentrations when fed according to label directions. (*J Am Vet Med Assoc* 2006;229:1282–1285)

Proper colostrum management is well recognized as a vital step in preventing disease in neonatal calves, but failure of passive transfer of immunity continues to be a common problem in the US dairy industry.^{1,2} Transfer of passive immunity is generally considered to be adequate if serum IgG concentration is > 1,000 mg/dL in calves that have been fed colostrum.³

Several steps are critical to ensuring adequate transfer of passive immunity in dairy calves. Of these, the most important is ensuring administration of a sufficient quantity of good-quality colostrum during the first few hours of life. The current recommendation is

that 4 L of colostrum with an IgG concentration > 50 g/L and bacterial count < 100,000 colony-forming units/mL be fed within the first 6 to 8 hours of life.³ In instances when only poor-quality or contaminated colostrum is available, dairy managers often turn to colostrum replacements or supplements to avoid failure of passive transfer of immunity in their calves. Colostrum replacements may also be used as a part of biosecurity programs to prevent transmission of disease-causing organisms such as *Salmonella* spp, *Mycobacterium avium* subsp *paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus.

Despite the widespread use of colostrum replacement products in dairy calves, there is little information available on the efficacy of these products. While some serum-based colostrum replacers have been demonstrated to be effective,^{4–6} no milk- or colostrum-based replacer products have been shown to routinely result in serum IgG concentrations \geq 1,000 mg/dL in dairy calves.^{7–11} However, new products have recently become available with higher concentrations of IgG that could possibly be used as alternatives to colostrum. The purpose of the study reported here was to evaluate the effects of 2 commercially available colostrum-based replacement products on serum IgG and total protein concentrations in dairy calves.

Materials and Methods

Study protocol—The study protocol was approved by the North Carolina State University institutional committee on the care and use of laboratory animals. Eighty-four Holstein bull calves obtained from a 1,400-cow dairy in western North Carolina were used in the study. For all calves, parturition had been observed, and calves had been immediately separated from the dams after parturition and were not allowed to nurse.

Calves were randomly allocated to 1 of 4 groups with 21 calves/group. Calves in group 1 were given 4 quarts of colostrum within 3 hours after birth with an esophageal feeder. The dam of each calf in this group was milked out completely immediately after parturition, and each calf was given first-milking colostrum obtained only from its dam. Samples of the colostrum fed to each calf were analyzed by means of radial immunodiffusion to determine IgG concentration.

Calves in group 2 were given 2 packages of a commercially available colostrum replacement product (product A^a) within 3 hours after birth. Product A contained 27 g of IgG/L as fed; the 2 packages of product A fed to each calf contained a total of 100 g of IgG.

Calves in group 3 were given a single package of a second commercially available colostrum replacement product (product B^b) within 3 hours after birth. Product B contained 71.4 g of IgG/L as fed, and the single package fed to each calf contained a total of 100 g of IgG.

Calves in group 4 were given 2 packages of product B within 3 hours after birth. Each calf received a total of 200 g of IgG.

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Colostrum replacement products were fed to calves in groups 2, 3, and 4 by means of an esophageal feeder. Each product was mixed individually for each calf according to label directions. According to the manufacturers, both products A and B had been produced from the first-day colostrum of dairy cows. In general, colostrum had been frozen immediately after collection, and samples had been tested for IgG concentration by means of radial immunodiffusion. Samples of colostrum with adequate IgG concentration were pooled and heat treated, and the colostrum was then spray dried to produce a powder. The powder was subsequently tested by the manufacturer for total IgG content and total bacterial number. Standard bacteriologic techniques were used by the manufacturer to ensure that the product was free from *Salmonella* spp, coliform bacteria, and *Mycobacteria paratuberculosis*.

Measurement of serum IgG and total protein concentrations—A blood sample was collected from the jugular vein of each calf 24 hours after colostrum or colostrum replacement administration. Samples were collected into plain glass tubes and allowed to clot. Serum was removed and stored at -4°C until analyzed.

Serum samples were submitted to an independent laboratory^c for measurement of serum IgG and total protein concentrations; laboratory personnel were blinded to treatment group of the samples. Serum total protein concentration of the samples was determined with a digital temperature-compensating refractometer.^d Before testing of each sample, the refractometer prism was cleaned, and the refractometer was calibrated with distilled water.

Serum IgG concentration of the samples was determined by means of radial immunodiffusion; a bovine IgG sample obtained from the USDA Center for Veterinary Biologics was used as the reference sample. The IgG concentration of the reference sample had been measured by means of a turbidometric assay¹² and reportedly was 3,204 mg/dL. The reference sample was diluted 1:3 with phosphate-buffered saline (0.9% NaCl) solution prior to use in the immunodiffusion assay. Because the radial immunodiffusion assay incorporated an antiserum reactive to heavy and light chains, both IgG₁ and IgG₂ were detected. The assay has been validated for use in calves.¹³

Data analysis—Data are given as mean \pm SD. One-way ANOVA followed by unpaired *t* tests were used to compare serum IgG and total protein concentrations among groups. Values of $P < 0.05$ were considered significant. Data that were not normally distributed were log transformed before ANOVA was performed.

The Fisher exact test was used to compare proportions of calves in groups 2, 3, and 4 with serum IgG concentration $\geq 1,000$ mg/dL with proportion of calves in group 1. For each group, linear regression was used to determine whether there was a linear association between serum IgG concentration and serum total protein concentration. If a significant association was found, 90% prediction intervals were calculated for various total protein concentrations, and the total protein concentration for which the lower limit of the prediction interval for serum IgG concentration was 1,000 mg/dL was determined.

Results

Mean \pm SD IgG concentration of colostrum fed to calves in group 1 was 118 ± 44 g/L. Mean serum IgG and total protein concentrations in calves from group 1 were significantly higher than concentrations for calves in the other 3 groups (Table 1). The distribution of serum IgG concentrations for calves in group 2 was skewed; however, analysis of log-transformed values did not alter the results of statistical analyses. Mean serum IgG and total protein concentrations in calves

Table 1—Mean \pm SD serum IgG and total protein concentrations for calves ($n = 21/\text{group}$) fed colostrum or 1 of 2 colostrum replacement products and proportions of calves with adequate passive immunity.

Group	Serum IgG (mg/dL)	Serum total protein (g/dL)	No. (%) with adequate passive immunity
1 (colostrum)	$2,720 \pm 1,020$	6.2 ± 0.7	19 (90)
2 (product A)	$700 \pm 220^*$	$4.5 \pm 0.5^*$	2 (10)*
3 (product B, 1 package)	$1,160 \pm 290^*$	$5.0 \pm 0.7^*$	17 (81)
4 (product B, 2 packages)	$1,690 \pm 620^*$	$5.6 \pm 0.5^*$	20 (95)

*Value was significantly ($P < 0.05$) different from value for group 1. Adequate passive immunity was defined as serum IgG concentration $\geq 1,000$ mg/dL.

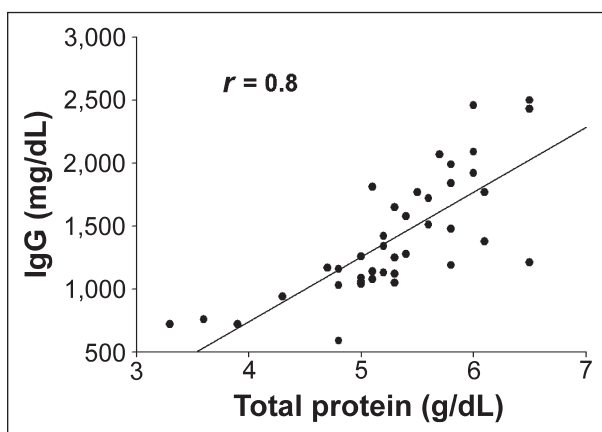


Figure 1—Scatterplot of serum IgG and total protein concentrations in calves fed a colostrum replacement product (groups 3 and 4).

from group 4 were significantly higher than concentrations for calves in groups 2 and 3.

The proportion of group 2 calves with adequate passive immunity (ie, serum IgG concentration $\geq 1,000$ mg/dL) was significantly lower than the proportion of group 1 calves with adequate passive immunity (Table 1). However, the proportions of groups 3 and 4 calves with adequate passive immunity were not significantly different from the proportion of group 1 calves with adequate passive immunity.

There were significant linear associations between serum IgG concentration and serum total protein concentration for calves in groups 1 ($r = 0.9$), 3 ($r = 0.7$), and 4 ($r = 0.9$), but only a weak linear association between concentrations for calves in group 2 ($r = 0.1$). When data for groups 3 and 4 were combined and 90% prediction intervals were calculated for various protein concentrations, it was determined that calves fed product B that had serum total protein concentrations > 5.2 g/dL would have serum IgG concentrations $> 1,000$ mg/dL at least 90% of the time (Figure 1).

Discussion

Results of the present study indicate that product B could be considered as an alternative to colostrum in dairy calves, as the rate of adequate passive immunity for calves fed product B was similar to the rate for calves fed colostrum. However, product A should not be considered adequate for use as a colostrum replace-

ment product because the rate of adequate passive immunity for calves fed product A was significantly lower. For calves fed product B, a serum total protein concentration > 5.2 g/dL could be used to predict which calves had adequate serum IgG concentrations.

In the present study, the proportions of calves with adequate passive immunity (ie, serum IgG concentration $\geq 1,000$ mg/dL) were not significantly different among groups 1 (calves fed colostrum), 3 (calves fed 1 package of product B), and 4 (calves fed 2 packages of product B). Therefore, our findings suggest that feeding calves either 1 or 2 packages of product B will result in adequate serum IgG concentrations.

However, calves in group 4 (calves fed 2 packages of product B) did have higher IgG concentrations, compared with calves in group 3 (calves fed 1 package of product B). This would be expected, as a previous study⁷ has shown that serum IgG concentration is positively correlated with mass of IgG fed in colostrum replacer products, and calves in group 4 in the present study received twice as much IgG, compared with calves in group 3.

In contrast, the proportion of calves with adequate passive immunity was significantly lower for group 2 (calves fed 1 package of product A) than for group 1, indicating that product A is unacceptable as a colostrum replacement product. This is in agreement with results of a previous study⁸ in which 2 packages of a similar product were fed to calves within 2 hours after birth and again 12 hours after birth. Calves in that study had a mean serum IgG concentration < 500 mg/dL at 24 and 48 hours of age. There was a difference between studies, in that the product used in the previous study was reported to contain 31.5 g of IgG in each package, whereas the product used in the present study was reported by the manufacturer to contain at least 50 g of IgG in each package. However, in both studies, calves received ≥ 100 g of IgG within the first 12 hours after birth.

Calves in groups 2 and 3 in the present study each received approximately 100 g of IgG, yet there was a significant difference in proportion of calves with adequate passive immunity between groups. This suggests that simply measuring the mass of IgG provided by a colostrum replacement product is an inadequate measure of its efficacy. Various studies^{6,9,11,14} have analyzed the effects that various factors, including source of IgG, method of IgG fractionation, amount and type of non-IgG protein, and the presence of fat and lactose, have on the efficiency of IgG absorption in calves fed colostrum replacement products and supplements. Results of these studies suggest that serum-derived IgG is more efficiently absorbed than colostrum-derived IgG, depending on the method of IgG separation.⁶ Furthermore, 1 study¹⁰ found that addition of some colostrum supplements reduced the absorption of IgG from natural colostrum. Therefore, each colostrum replacement product and colostrum supplement should be separately tested for efficacy prior to use.

Similar to results of the present study, it has been reported previously that serum total protein concentrations are lower in calves fed colostrum replacement products than in calves fed colostrum.^{4,9} Because mea-

surement of serum total protein concentration is a common and convenient method of screening calves for adequate passive immunity, it is useful to know how total protein concentration and serum IgG concentration are associated in calves fed colostrum replacement products. Unfortunately, a significant association was not found between total protein concentration and serum IgG concentration in calves fed product A. However, strong associations were found in both groups of calves fed product B, and we calculated that one could be 90% confident that a calf fed product B that had a serum total protein concentration > 5.2 g/dL would have a serum IgG concentration $> 1,000$ mg/dL. Additional research is needed to determine whether this value applies only to product B or can be used for calves fed other colostrum replacement products.

Mean serum IgG and total protein concentrations for group 1 calves in the present study, which were fed colostrum, were significantly higher than mean concentrations for calves in the other 3 groups. This difference was likely attributable to the high IgG concentration of the colostrum that was fed (mean \pm SD, 118 ± 44 g/L). The colostrum IgG concentration in the present study was substantially higher than expected for the breed. A previous study,¹⁵ for instance, reported a mean colostrum IgG concentration of 41.2 g/L for 19 Holstein cows, and a study¹⁶ of 919 Holstein cows examined over a 4-year period reported a mean colostrum IgG concentration of 48.2 g/L, with $> 80\%$ of the cows producing colostrum with an IgG concentration < 65 g/L.

Many factors affect colostrum IgG concentration, one of which is time between calving and colostrum collection. A study¹⁷ of 13 Holstein cows, for instance, found that colostrum IgG concentration was significantly lower when colostrum was collected > 6 hours after calving, compared with 2 hours after calving. In that study, mean IgG concentration of colostrum collected 2 hours after calving was 113 g/L. Two studies^{18,19} of colostrum collected within 3 hours after calving from 55 cows and 101 cows found mean colostrum IgG concentrations of 69 and 79 g/L, respectively, and a study²⁰ of colostrum collected a mean of 3 hours after calving reported a mean colostrum IgG concentration of 89 g/L. In the present study, colostrum was collected immediately after calving, and this early collection time may account for the high colostrum IgG concentration. Parity^{1,17,18,21} and season^{20,22,23} may also have an effect on colostrum IgG concentration and may have played a role in the present study.

Mean serum IgG concentration (2,720 mg/dL) for group 1 calves in the present study was higher than values previously reported for dairy calves that were given 4 L of colostrum. Previous studies,^{10,24,25} for instance, have reported concentrations ranging from 1,200 to 1,900 mg/dL for calves given 4 L of colostrum immediately after birth or 2 L immediately after birth and an additional 2 L 12 hours later. Mean serum IgG concentrations for calves fed product B in the present study (groups 3 and 4) were closer to these previously reported values.

Although it might be assumed that calves with higher serum IgG and total protein concentrations

would be less likely to develop various diseases, previous research has suggested that there is little additional protective effect associated with concentrations substantially higher than some threshold level. In 1 study,²⁶ for instance, mortality risk among calves at a calf-rearing facility did not decrease as serum total protein concentration increased above a concentration of 5.5 g/dL. Similarly, when other measures are used to identify calves with failure of passive transfer, only those calves with failure of passive transfer have an increased risk of death, and the mortality risk is not reduced as serum IgG concentration is increased.^{27–29} Although calf growth and future milk production have been shown to be positively correlated with neonatal serum IgG concentration,^{28,29} the cause of this effect is unknown.

Results of the present study indicate that product A is not an effective colostrum replacement product because few calves had serum IgG concentrations > 1,000 mg/dL after being fed product A. Yet because some IgG was transferred to the calves, product A could possibly be used as a colostrum supplement when colostrum quality is inadequate. However, product B appeared to be an effective colostrum replacement product that could be used during times of low colostrum reserves or excessive bacterial contamination of colostrum or to prevent transmission of disease-causing organisms through colostrum.

- a. Immu-Start 50, Imu-Tek Animal Health Inc, Fort Collins, Colo.
- b. Land O' Lakes colostrum replacement, Land O' Lakes Inc, Saint Paul, Minn.
- c. Prairie Diagnostic Services, Saskatoon, SK, Canada.
- d. Model 300027, SPER Scientific Ltd, Scottsdale, Ariz.

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