Effects of difructose anhydride III on serum immunoglobulin G concentration and health status of newborn Holstein calves during the preweaning period

A. Htun,* T. Sato,† N. Fukuma,† and M. Hanada†

*United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, 020-8550, Japan
†Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan

ABSTRACT

This experiment was performed to investigate the effects of increases in passively acquired immunoglobulin G (IgG) by difructose anhydride (DFA) III supplementation on subsequent serum IgG concentration and health status in calves during the preweaning period. Thirty newborn female Holstein calves were paired by birth order, and 2 calves in each pair were fed 2 L of the same batch of colostrum within 2 h and at 10 h after birth, and followed by 2 L of the same batch of pooled colostrum at 20 h after birth. One calf from each pair was assigned to the control (n = 15) or treatment (n = 15) group. All calves in the treatment group received 18 g of DFA III at each feeding from birth to 7 d of age, whereas calves in the control group did not receive DFA III. Blood samples were collected before feeding at 0, 10, 20, and 36 h, and 4 and 7 d of age, and sampling was repeated at 7-d intervals thereafter until 49 d of age for serum IgG analysis. Calves were monitored daily for diarrhea and respiratory diseases. Serum IgG concentrations peaked at 36 h of age in both groups. Apparent efficiency of IgG absorption and peak serum IgG concentration were higher in the treatment group than in the control group. Using multiple regression analysis, we showed that peak serum IgG concentration in the newborn calves was positively correlated with colostral IgG concentration and DFA III supplementation. Moreover, peak serum IgG concentration (36 h of age) positively influenced subsequent serum IgG concentration until 35 d of age for all calves in both groups. The treatment group had higher serum IgG concentration from 20 h to 21 d of age than the control group. However, we detected no differences between the groups in number of calves with diarrhea or respiratory disease.

Key words: calf, difructose anhydride III, immunoglobulin G, infectious disease

INTRODUCTION

Diarrhea and respiratory disease are the most significant causes of calfhood morbidity and mortality (Johnson et al., 2011; Uetake, 2013). These health problems are associated with failure of passive immunity transfer via colostrum feeding (Paré et al., 1993; Wittum and Perino, 1995; Furman-Fratczak et al., 2011). Thus, provision of adequate serum IgG through consumption of colostrum is well accepted and established as improving the health status of newborn calves (Weaver et al., 2000; Godden, 2008). Absorption of ingested IgG through the intestine of newborn calves is possible for only a short period after birth. Because of the maturation process of the neonatal intestinal epithelium, absorption of colostral IgG is the highest within 2 h after birth, gradually declines with age, and completely ceases during the first 24 to 36 h after birth in newborn calves (Weaver et al., 2000; Furman-Fratczak et al., 2011). Peak serum IgG concentrations are achieved at 36 to 48 h of age (Weaver et al., 2000; Furman-Fratczak et al., 2011) and gradually decline thereafter (Weaver et al., 2000; Chase et al., 2008; Nonnecke et al., 2012). Although the calf’s own immune system becomes active at approximately 1 wk of age (Devery et al., 1979; Hassig et al., 2007; Chase et al., 2008), endogenous immune function does not reach a protective level until approximately 7 wk of age (Hassig et al., 2007; Chase et al., 2008; Nonnecke et al., 2012). Thus, after intestinal absorption of colostral IgG ceases, serum IgG concentration declines until endogenous IgG production begins in the calf (Hassig et al., 2007; Chase et al., 2008; Nonnecke et al., 2012). The most common diseases (diarrhea and respiratory disease) occur before establishment of complete protection by endogenous IgG in calves (Furman-Fratczak et al., 2011; Johnson et al., 2011). The occurrence and incidence of these diseases during the preweaning period were negatively associated with passive immune transfer via colostrum in calves (Paré et al., 1993; Wittum and Perino, 1995; Furman-Fratczak et al., 2011). Therefore, it is important for the improvement of health status to achieve as high a serum IgG concentration as possible in the newborn calves, especially before the adequate...
development of its own immune system, by enhancing passive immunity.

Difructose anhydride (DFA) III is a plant-derived, indigestible oligosaccharide that can promote calcium absorption through paracellular pathways in the intestine of rats (Mineo et al., 2004) and cattle (Teramura et al., 2015). We previously reported that supplementation of DFA III in colostrum fed to newborn calves improved absorption of colostral IgG and increased serum IgG concentrations (Sato et al., 2012; Htun et al., 2016). It has been suggested that the increase of peak serum IgG concentration after colostrum feeding could be due to improved intestinal paracellular pathway via loosening the tight junctions between intestinal epithelial cells by DFA III (Mineo et al., 2004) for greater IgG absorption in newborn calves (Sato et al., 2012; Htun et al., 2016), because DFA III has been shown to improve paracellular absorption in bovine intestine (Teramura et al., 2015). If DFA III enhances absorption of colostral IgG through a paracellular pathway, peak serum IgG might increase with DFA III supplementation, regardless of IgG concentration in the colos- trum. Moreover, DFA III might also increase subsequent serum IgG concentrations after the peak and contribute to a reduced incidence of diarrhea and respiratory diseases in calves during the preweaning period. However, these expected benefits of DFA III have not yet been investi- gated. Therefore, the aim of this study was to assess whether DFA III improves peak serum IgG concentration, regardless of IgG concentration in colostrum, and to determine the effect of DFA III-associated increases in passively acquired IgG on subsequent serum IgG concentrations and selected health measures (diarrhea and respiratory disease) in calves during the preweaning period.

MATERIALS AND METHODS

Protocols for the experimental procedures and animal care in the present study were approved (no. 27-95) by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine (Obihiro, Hokkaido, Japan). This study was carried out between December 11, 2014, and January 2, 2016, at the Field Science Center of Obihiro University of Agriculture and Veterinary Medicine.

Calf Enrollment

Dams were moved into individual maternity pens on their expected due date or on the day they showed signs of impending parturition. All newborn female calves were separated from their dams approximately 30 min after birth to prevent suckling. Calves were cleaned and dried with a towel, and their navels were sprayed with 7% iodine. Each calf was weighed and put into an individual calf hutch with straw bedding for 7 d. After 7 d, calves were moved to an existing group pen until weaning.

Thirty newborn female Holstein calves were paired (15 pairs) by birth order, and each pair was fed a separate batch of colostrum for the first 2 feedings and pooled colostrum for the third feeding. One calf from each pair was assigned to the control group and the other to the treatment group. All calves in the treatment group were supplemented with 18 g of DFA III (Nippon Beet Sugar Manufacturing Co. Ltd., Obihiro, Japan) at each feeding for 7 d after birth. Calves in the control group were not supplemented with DFA III.

Feed and Feeding Practice

Colostrum was harvested from the first milking of postpartum cows. When sufficient colostrum was harvested from a cow for 1 batch (≥8 L), it was divided into four 2-L aliquots in aluminum zip-lock bags and stored at −20°C until used for the first 2 feedings to a pair of calves. When the amount of harvested colos- trum was less than 8 L, it was stored in a refrigerator (4°C) until the second milking (postpartum) to prepare pooled colostrum for the third feeding to the calves. The pooled colostrum was divided into two 2-L aliquots in aluminum zip-lock bags and stored at −20°C until used for the third feeding to a pair of calves.

Before feeding, frozen colostrum or pooled colostrum was thawed in warm water at 40 to 45°C and poured into a nipple bottle. Calves in each pair were fed 2 L of the same batch of colostrum within 2 h and at 10 h after birth, and followed by 2 L of the same batch of pooled colostrum at 20 h after birth. From 36 h to 4 d of age, all calves were fed 2 L of fresh milk at 0800 and 1630 h. From 5 to 7 d of age, all calves were fed 2 L of milk replacer (150 g/L of Calftop EX, National Federation of Dairy Cooperative Associations, Tokyo, Japan; TDN >103%, CP >28%, crude fat >15%) at 0800 and 1630 h. All calves were fed by nipple bottle from birth to 7 d of age, and 18 g of DFA III was added to each batch of colostrum and milk replacer fed to the calves in the treatment group for 7 d after birth. From birth to 7 d of age, calves received no starter or hay, but water was freely available.

After 7 d of age, the experimental calves were moved to a group pen (5 × 7 m) bedded with sawdust, and were kept there with other calves until weaning. The average number of calves in the pen was 9.2 ± 0.4. The milk replacer during the group-housing period was the same as that fed to calves from 5 to 7 d after birth, and the amount was adjusted according to
manufacturer’s recommendations. At the beginning of the group-housing period, all calves received 4.5 L/d of milk replacer, regardless of BW, which was gradually increased to 6 L/d until the BW of the calf reached approximately 77 kg, and then gradually decreased to 1 L/d until weaning, using an automated milk-feeding system (Vario Smart, Förster-Technik GmbH Co. Ltd., Engen, Germany). Calves were gradually weaned at or after 49 d of age, when the BW of calf reached approximately 90 kg. A commercial pelleted calf starter (Calf Manna, Feed One Co. Ltd., Yokohama, Japan; TDN >75%, CP >25%, crude fat >2%) and grass hay were provided ad libitum during the group-housing period, and fresh water was freely available.

Data Collection, Sampling, and Analysis

Calves were weighed at birth and 49 d of age, and were monitored daily for diarrhea and respiratory diseases under veterinary supervision during the entire experimental period. Fecal consistency was classified as described by Quigley et al. (2002), using a scale of 1 = normal fecal consistency, 2 = slightly liquid consistency, 3 = moderately liquid consistency, and 4 = primarily liquid consistency. When a calf showed a fecal score >2 for more than 2 consecutive days, it was diagnosed with diarrhea. Respiratory disease was diagnosed in individual calves with coughing or sneezing symptoms for more than 2 consecutive days, with increased respiratory sounds on lung auscultation (Svensson and Liberg, 2006).

Prefeeding blood samples were collected from individual calves via the jugular vein using blood collection tubes (Venoject II, Terumo Corp., Tokyo, Japan) for serum IgG analysis at 0, 10, 20, and 36 h, and at 4 and 7 d of age. Thereafter, sampling was repeated at 7-d intervals until 49 d of age. Serum was harvested via centrifugation (Hitachi Centrifuge 05P-21, Hitachi Koki Co. Ltd., Tokyo, Japan) at 1,500 × g for 15 min and stored at −30°C until IgG analysis. Subsamples (30 mL) of colostrum and pooled colostrum were taken at the time of colostrum harvesting and pooled colostrum preparation and stored at −30°C until IgG analysis. The concentration of IgG in serum, colostrum, and pooled colostrum samples was determined by single radial immunodiffusion (Institute for Metabolic Ecosystem, Miyagi, Japan).

Apparent efficiency of absorption (AEA) of IgG at 36 h of age was calculated for individual calves based on measured serum IgG concentration, estimated serum volume (8.6% of birth weight), and IgG intake in the first 3 feedings (Quigley et al., 1998) as follows: AEA (%) = [(serum IgG, g/L × serum volume, L)/ingested IgG, g] × 100.

Data Analysis

The results obtained from the individual groups were presented as means (± standard errors). Distributions of the variables were examined with the Shapiro-Wilk test. Mean differences between the groups in dam parity, BW at birth and 49 d of age, age at first feeding, serum IgG concentration, and AEA of IgG were analyzed by paired t-test, because calves in each pair received the same amount of the same batch of colostrum and pooled colostrum. To investigate the factors affecting serum IgG concentration and AEA at 36 h of age, stepwise multi-regression analysis was performed using 6 independent variables (dam parity, birth BW, age at the first feeding, IgG concentration of colostrum and of pooled colostrum, and DFA III supplementation). Correlation coefficients between serum IgG concentration at 36 h of age and subsequent serum IgG concentrations were calculated by Pearson’s procedures. A logistic regression was performed to predict the effect of serum IgG concentration at 36 h of age on the subsequent lowest serum IgG concentration in calves. The numbers of calves with diarrhea and respiratory disease between the groups were compared using Chi-squared tests. Survival analysis was used to compare the difference in age at onset of diarrhea and respiratory disease between the groups. The difference between age at onset of diarrhea (all calves with diarrhea in both groups) and that of respiratory disease (all calves with respiratory disease in both groups) was compared by survival analysis. Power analysis was generated to determine the power value in detection of the differences in the number of calves with diarrhea or respiratory disease and age at onset of diarrhea or respiratory disease between the groups. All data were analyzed using JMP 9 (SAS Institute Inc., Cary, NC). Significance was considered at P < 0.05 unless otherwise noted.

RESULTS

Dam parity ranged from 1 to 8, birth BW ranged from 33.4 to 53.2 kg, and BW at 49 d of age ranged from 71.8 to 92.0 kg, but mean values did not differ between groups (P > 0.05; Table 1). Concentration of IgG in colostrum ranged from 30.6 to 67.4 g/L and concentration of IgG in pooled colostrum ranged from 29.6 to 56.9 g/L, but the mean values for the IgG concentration in colostrum, IgG concentration in pooled colostrum, and total IgG intake from the first 3 feedings did not differ between groups (P > 0.05; Table 1). Change in serum IgG concentration with age is presented in Figure 1. Serum IgG concentration at 0 h (before the first colostrum feeding) for all calves was 0.3 ± 0.2 g/L. After colostrum feeding, the serum IgG concentration
in both groups increased sharply and peaked at 36 h of age. The serum IgG concentrations at 36 h were 20.6 ± 1.2 and 23.7 ± 1.9 g/L in the control and treatment groups, respectively. When a multiple regression analysis was performed to evaluate the effect of DFA III on serum IgG concentration at 36 h of age, colostral IgG concentration and group were accepted as independent variables, and a significant multiple regression equation was obtained (R² = 0.58, P < 0.001; Table 2). The standardized partial regression coefficient values of the colostral IgG concentration and group were 0.734 and 0.228, respectively, which indicate that colostral IgG concentration and DFA III supplement positively affected serum IgG concentration at 36 h of age.

The higher serum IgG concentrations in the treatment group were observed from 20 h to 21 d of age (P < 0.05; Figure 1). After 36 h, serum IgG concentrations gradually decreased to their lowest concentrations at 42 and 35 d of age in the control and treatment groups, respectively (Figure 1). The lowest serum IgG concentrations were 11.0 ± 0.7 and 12.0 ± 0.8 g/L (P > 0.05) in the control and treatment groups, respectively, but serum IgG concentration at 36 h of age (peak concentration) positively affected subsequent serum IgG concentrations until 35 d of age (P < 0.05). After reaching their lowest level, serum IgG concentrations in both groups tended to increase. The mean AEA values at 36 h of age were 27.5 ± 1.2 and 30.7 ± 1.8% for the control and treatment groups, respectively, and the difference between the groups was significant (P < 0.01).

We observed no calf mortality in either group in the present study. The number of calves with diarrhea or respiratory disease did not differ between groups (Table 3), and average age at onset of diarrhea or respiratory disease did not differ between groups (Table 3). Average age at onset of diarrhea (24.8 ± 2.3 d of age for all calves with diarrhea in both groups) was earlier (P = 0.026) than average age at onset of respiratory disease (40.7 ± 1.1 d of age for all calves with respiratory disease in both groups).

### DISCUSSION

**Effect of DFA III on IgG Absorption and Serum IgG Concentration**

Serum IgG concentration developed in a similar pattern in both groups: it sharply increased and peaked

![Figure 1](image.png)

**Figure 1.** Mean (±SE) serum IgG concentrations in calves. Control (n = 15) calves did not receive difructose anhydride (DFA) III; treatment (n = 15) calves received 18 g of DFA III at each feeding for 7 d after birth. Significant differences between the 2 groups for mean values at each time point are indicated by *P < 0.05, **P < 0.01.

### Table 1. Means (±SE) of dam parity, BW at birth and 49 d, IgG concentration of colostrum, and IgG intake of calves

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam parity</td>
<td>3.5 ± 0.6</td>
<td>2.7 ± 0.5</td>
<td>0.278</td>
</tr>
<tr>
<td>BW at birth, kg</td>
<td>43.9 ± 1.2</td>
<td>43.1 ± 1.2</td>
<td>0.578</td>
</tr>
<tr>
<td>BW at 49 d, kg</td>
<td>79.6 ± 1.3</td>
<td>78.8 ± 1.3</td>
<td>0.609</td>
</tr>
<tr>
<td>Colostrum IgG, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First feeding</td>
<td>50.6 ± 3.1</td>
<td>NA^2</td>
<td></td>
</tr>
<tr>
<td>Second feeding</td>
<td>50.6 ± 3.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Third feeding</td>
<td>42.1 ± 3.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Total IgG intake from first 3 feedings, g</td>
<td>284.2 ± 15.2</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

^1Total number of calves, n = 30. Control (n = 15) calves did not receive difructose anhydride (DFA) III; treatment (n = 15) calves received 18 g of DFA III at each feeding for 7 d after birth.

^2Not applicable.

### Table 2. Results of multiple regression analysis^1 for serum IgG concentration at 36 h of age (R² = 0.58, P < 0.001)

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>β</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum IgG, g/L</td>
<td>0.714</td>
<td>0.734</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group^2</td>
<td>0.261</td>
<td>0.228</td>
<td>0.046</td>
</tr>
</tbody>
</table>

^1r = single correlation between dependent variable and each explanatory variable; β = standardized partial regression coefficient of multiple regression analysis; P-value is for partial regression coefficient of multiple regression analysis.

^2As a dummy variable, values 0 (control group) and 1 (treatment group) were assigned.
at 36 h of age, as reported in previous studies (Weaver et al., 2000; Furman-Fratczak et al., 2011). The peak serum IgG concentration was higher in calves fed colostrum with DFA III than in calves fed colostrum without DFA III, consistent with our previous studies (Sato et al., 2012; Htun et al., 2016). In addition to the peak serum IgG concentration, AEA was higher in the treatment group than in the control group. However, we did not obtain a significant multiple regression equation when stepwise regression analysis was performed to investigate the factors affecting AEA at 36 h of age. Similarly, Halleran et al. (2017) reported that the common range of AEA (20 to 40%) in calves was not affected by colostral IgG concentration or total colostral IgG intake, and suggested that IgG absorption did not appear to be a saturable process in newborn calves. A positive relationship between colostral IgG concentration and serum IgG concentration at 36 h of age (Table 2) supports concentration-gradient absorption for colostral IgG in the intestine of newborn calves. A concentration-gradient, nonsaturable process is characteristic of a paracellular absorption pathway (Bronner, 1998), and bovine intestinal paracellular absorption pathways could be improved by DFA III (Teramura et al., 2015). Therefore, the calves receiving DFA III might absorb more IgG through the intestinal paracellular pathway. These results suggest that DFA III enhances colostral IgG absorption and increases peak serum IgG concentration, regardless of IgG concentration in the colostrum.

Transition Point of Immune System

After its peak, serum IgG concentration gradually decreased and reached the lowest level at 42 and 35 d of age in the control and treatment groups, respectively. After absorption of colostral IgG ceases in the intestine, serum IgG declines until the production of endogenous immunity exceeds passive immunity (Hassig et al., 2007; Chase et al., 2008; Nonnecke et al., 2012). Hassig et al. (2007) investigated the changes of endogenous, maternal, and total serum IgG concentration during the first 182 d of age in Simmental, Swiss Braunvieh, and Holstein Friesian calves using labeled IgG, and demonstrated that endogenous IgG did not exceed passively acquired IgG until approximately 35 d of age. In the present study, the serum IgG concentration at 36 h of age positively affected the subsequent serum IgG concentration until 35 d of age. This dynamic of serum IgG in the present study suggests that there is a transition point in the development of the immune system from passive to active immunity in calves at approximately 35 to 42 d of age, as reported in previous studies (Hassig et al., 2007; Chase et al., 2008).

Hassig et al. (2007) demonstrated that total serum IgG concentration was derived from both passively acquired and endogenous IgG from 2 to ~170 d of age in calves. Furman-Fratczak et al. (2011) reported that serum IgG concentrations at 30 to 60 h of age were significantly correlated with subsequent serum IgG concentrations at 21 to 28 d of age. The present study demonstrated that serum IgG concentration at 36 h of age was increased by DFA III supplementation and positively correlated with subsequent serum IgG concentration until 35 d of age. These results reinforce the importance of enhancing passive immunity until the transition point, and serum IgG concentration of newborn calves before the transition point could be increased by feeding DFA III with colostrum.
the risk of infectious disease in newborn calves (Chase et al., 2008). The average lowest serum IgG concentrations after the peak were <10 g/L in Holstein calves (Villarroel et al., 2013; Dunn et al., 2017) and 11.8 g/L in Simmental, Swiss Braunvieh, and Holstein Friesian calves (Hassig et al., 2007). These lowest serum IgG concentrations were similar to that of the present study. In our study, the lowest serum IgG concentration positively correlated with 36-h serum IgG concentration, but the relationship was not linear and a significant logistic equation was obtained (Figure 2). The lowest serum IgG concentration increased sharply with increases in serum IgG at 36 h of age when it was <20 g/L, but the lowest serum IgG concentration reached a plateau of 12.0 g/L when serum IgG at 36 h of age was >25 g/L. This equation suggests that serum IgG concentration at the transition point of the immune system can be increased up to 12 g/L by increasing the peak serum IgG level but that it is difficult to increase the lowest serum IgG concentration above 12 g/L by enhancing passive immunity. Development of the endogenous immunity system may be another considerable issue to increase the lowest serum IgG concentration and reduce the risk of incidence of infectious diseases in newborn calves.

**Potential for DFA III to Develop the Intestinal Immune System**

It is well recognized that indigestible oligosaccharides are nutrients that can improve intestinal immunity. Schley and Field (2002) reported that indigestible oligosaccharides have immune-enhancing effects by facilitating the direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the gut-associated lymphoid tissue of the intestine and through production of short-chain fatty acids in the intestine. Suzuki et al. (1998) demonstrated that long-term feeding of DFA III to laboratory animals resulted in increased production of short-chain fatty acids, including lactic acid, in the lower intestine. Minamida et al. (2005) also demonstrated that long-term supplementation of DFA III in rats revealed health-promoting prebiotic properties, such as proliferation of healthier intestinal microbiota and lowering of pH through production of short-chain fatty acids in the lower intestine. Although DFA III had little effect on serum IgG concentration after the transition point of the immune system in the present study, it might be effective in improving intestinal immunity in calves. Most oligosaccharides are degraded in the rumen, but DFA III can escape microbial degradation in the rumen and flow into the intestine (Teramura et al., 2015). Thus, further work is needed on the contribution of DFA III to the development of the intestinal immune system in newborn calves.

**Effect of DFA III on Health Conditions During the Preweaning Period**

The sample size of the present study was too small to provide sufficient power for assessment of health status in calves. Nevertheless, we observed no differences in health status (diarrhea and respiratory disease) in calves between the groups. Previous studies using large sample sizes demonstrated that Holstein (Paré et al., 1993) and Polish Holstein-Friesian (Furman-Fratczak et al., 2011) calves with higher passive serum IgG concentration had lower rate and intensity of diarrhea. Moreover, a previous study using large number of calves reported that passive immune status was important for reducing the risk of morbidity and mortality in calves during the preweaning period (Wittum and Perino, 1995). Calves receiving DFA III in the current study had higher peak serum IgG concentrations, which positively influenced the subsequent serum IgG concentration until 35 d of age. Age at onset of diarrhea was earlier (before 35 d of age) than age at onset of respiratory disease, as reported in previous studies (Svensson et al., 2003; Villarroel et al., 2013). Enhancing passive serum IgG concentration may be effective for reducing infectious diseases that appear before the transition point of the
immature system, because passively acquired IgG affects subsequent serum IgG concentration until the transition point.

CONCLUSIONS

Supplementation with DFA III in newborn calves increased peak serum IgG concentration obtained via colostrum, regardless of IgG concentration in the colostrum, and peak serum IgG positively affected subsequent serum IgG concentration until 35 d of age in calves. However, the effect of DFA III on incidence of diarrhea and respiratory disease in calves was not clear. Therefore, further studies using a larger sample of calves are necessary to confirm the effect of DFA III-associated increases in passively acquired IgG on health status in calves.

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