Effect of difructose anhydride III supplementation on passive immunoglobulin G transfer and serum immunoglobulin G concentration in newborn Holstein calves fed pooled colostrum

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ABSTRACT

Difructose anhydride (DFA) III acts on intestinal epithelial tight junctions and promotes calcium absorption through the paracellular pathway in dairy cows. This trial was done to investigate the effects of DFA III supplementation on passive IgG transfer and serum IgG concentration in calves. Twenty-four newborn Holstein male calves were grouped as 12 pairs, with each pair receiving a separate batch of pooled colostrum (PC) from the first 2 milkings. One calf from each pair was allocated to the treatment group ($n = 12$) and received 18 g of DFA III in each feeding; the other calf was assigned to the control group ($n = 12$) and did not receive DFA III. All calves were fed 2 L of PC per feeding at 1, 10, and 24 h after birth. Prefeeding blood samples taken at 0 h, 10 h, 24 h, 36 h, 4 d, and 7 d were analyzed for serum IgG concentration. Apparent efficiency of IgG absorption in the treatment group was higher compared to that in the control group. Serum IgG concentration of calves in the treatment group was higher for all sampling time points except for 0 h of age. Mean serum IgG concentrations in the treatment and control groups at 36 h of age were 22.3 and 17.7 g/L, respectively. Serum IgG concentration at 36 h of age was positively related with the IgG concentration in the PC in both groups, and it was higher in the treatment group than in the control group at the same IgG concentration in the PC. These results indicate that absorption of ingested IgG by endocytosis as well as a nonselected concentration gradient process of the paracellular pathway of the intestinal epithelium could be enhanced by DFA III supplementation. Supplementation of DFA III in PC containing various IgG concentrations could improve IgG absorption as well as calf serum IgG concentration.

Key words: calf, difructose anhydride III, immunoglobulin G, pooled colostrum

INTRODUCTION

Newborn calves are in an agammaglobulinemic condition at birth because of the spatial characteristics of the bovine placenta. Ingestion and absorption of colostrum IgG are therefore crucial to ensure passive immunity in newborn calves (Weaver et al., 2000). Serum IgG concentration in calves is important and is associated with preweaning mortality and morbidity (National Animal Health Monitoring System, 1996) as well as with long-term productive performance (Wittum and Perino, 1995). Serum IgG concentration of calves should be ≥ 10 g/L at 24 h of age, and a serum IgG concentration < 10 g/L signals a failure of passive transfer (National Animal Health Monitoring System, 1996; Weaver et al., 2000).

Improvement of passive IgG transfer has been attempted by adding different substances including selenium or sodium bicarbonate to colostrum feeding of newborn calves (Kamada et al., 2007; Morrill et al., 2010). In a recent study, oligosaccharide was investigated as a possible way to enhance IgG absorption from colostrum in young animals. Lazarevic et al. (2010) reported that supplementing colostrum fed to Holstein calves and piglets with mannan-oligosaccharide, a gut-active carbohydrate, resulted in an increased serum IgG concentration. They also proposed that gut-active carbohydrates play a role in communication between the gut and immune system of the animals.

Difructose anhydride (DFA) III is a nondigestible plant oligosaccharide that escapes digestion by rumen microbes (Teramura et al., 2015a) and is not easily digested by intestinal flora (Saito and Tomita, 2000). Moreover, DFA III has beneficial effects on nutrient absorption through the paracellular pathway by improving the opening of intestinal epithelial tight junctions (Mineo et al., 2002). Increased mineral absorption has been observed with DFA III supplementation in rats

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(Mineo et al., 2004), humans (Tomita et al., 2007), and cattle (Teramura et al., 2015a,b). In our previous study (Sato et al., 2012), we demonstrated that the addition of DFA III to maternal colostrum improved the efficiency of IgG absorption in 4 experimental groups of Holstein and Holstein/Japanese Black crossbred calves. However, the colostrum fed to calves was from the first milking of individual cows with various IgG masses, and the calves were not the same breed across the groups. Previous studies have reported that ingested IgG mass and breed of calf may affect serum IgG concentration (Hopkins and Quigley, 1997; Jones et al., 2004). Therefore, comparison within the same breed fed the same amount of colostrum containing the same IgG concentration is required to exclude the influence of colostrum quality and breed type and to clarify the effect of DFA III on passive IgG transfer and serum IgG concentration. Good-quality colostrum with IgG >50 g/L cannot be produced by some cows (Weaver et al., 2000; Gulliksen et al., 2008), which may result in colostrum shortage for newborn calves. Feeding of pooled colostrum (PC) made by combining colostrum from the first 2 milkings is one way to resolve a colostrum shortage. However, feeding of PC to newborn calves may result in the failure of passive transfer (Jones et al., 2004). Supplementation with DFA III might improve passive transfer of IgG in newborn calves fed PC. The purpose of this study was to confirm the effects of DFA III on passive IgG transfer and serum IgG concentration in newborn Holstein calves fed PC.

MATERIALS AND METHODS

This study was performed at the Field Science Center of Obihiro University of Agriculture and Veterinary Medicine, Obihiro (Hokkaido, Japan). Animal care, procedures, and experimental protocols were approved (No. 26–7) by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine.

Calf Enrollment

Twenty-four newborn male Holstein calves were divided into 12 pairs alternately by birth order. One calf from each pair was allocated to the treatment group ($n = 12$) and received 18 g of DFA III (Nippon Beet Sugar Manufacturing Co. Ltd., Obihiro, Japan) with each feeding from birth to 7 d of age; the other calf was assigned to the control group ($n = 12$) and did not receive DFA III. The experimental period lasted from birth to 7 d of age.

Dams were housed in an individual maternity pen on their expected due date or on the day they showed signs of parturition. All newborn calves were born under supervision. Newborn calves were left with their dams for 30 min for cleaning and stimulation, but they were removed from the maternity pen before suckling could occur. Calves were cleaned and dried with a towel if they were not dried well by their dams, and their navels were sprayed with 7% iodine solution. Each calf was then weighed and put in an individual calf pen with straw bedding.

Feed and Feeding Practice

Donor cows were milked twice daily by bucket milker at the next standard milking time (0730 and 1600 h) following calving. Milking was done at 4.0 ± 1.1 and 16.0 ± 1.1 h postpartum for the first and second milkings, respectively, and 3.1 ± 0.3 and 3.5 ± 0.2 L of colostrum was obtained from the first and second milkings, respectively, of 8 primiparous and 16 multiparous (1 to 6 parity) cows. Colostrum with a specific gravity >1.045 (Lactodensimeter; Sansyo Co. Ltd., Tokyo, Japan) from the first 2 milkings of donor cows, regardless of parity, was collected and frozen at -20°C until a sufficient amount was obtained for each pair of calves. When sufficient colostrum was obtained, it was thawed in warm water at 40 to 45°C and mixed well in a bucket to prepare PC. Then, PC was placed in six 2-L aluminum zip-lock bags that were identified by batch and stored at -20°C until feeding.

When a calf was born, frozen PC was thawed in warm water at 40 to 45°C and poured into a nipple bottle to feed it. Just before each feeding, 18 g of DFA III was added to the nipple bottle for calves in the treatment group. Two liters of PC from the same batch and with the same IgG concentration was fed to both calves in each pair at 1, 10, and 24 h postpartum. From 36 h to 7 d after birth, all calves were fed 2 L of fresh milk by nipple bottle twice daily at 0800 and 1630 h.

Sampling and IgG Analysis

The body weight of newborn calves was measured about 30 min after birth. Prefeeding blood samples were collected from individual calves via the jugular vein using 9-mL evacuated tubes (Venoject II; Terumo Corporation, Tokyo, Japan) at 0 (before the first PC feeding), 10, 24, and 36 h postpartum and then at 0800 h on days 4 and 7 of age. Serum was separated from the blood via centrifugation (Hitachi Centrifuge 05P-21; Hitachi Koki Co. Ltd., Tokyo, Japan) at $1,300 \times g$ for 15 min and then stored at -30°C until IgG

Table 1. Means ($\pm$ SE) of dam parity, gestation period, birth weight, age at feeding, pooled colostrum volume, IgG concentration of pooled colostrum, and IgG intake of calves fed pooled colostrum¹

Item	Control	Treatment	P-value
Dam parity	2.8 $\pm$ 0.6	2.9 $\pm$ 0.6	0.914
Gestation period, d	281.8 $\pm$ 1.5	284.0 $\pm$ 1.1	0.243
Birth weight, kg	48.5 $\pm$ 1.9	47.2 $\pm$ 1.7	0.611
Age at pooled colostrum feeding, h			
First feeding	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1	0.907
Second feeding	10.2 $\pm$ 0.1	10.0 $\pm$ 0.0	0.152
Third feeding	24.2 $\pm$ 0.1	24.0 $\pm$ 0.0	0.165
Pooled colostrum volume, L/feeding	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0	1.000
IgG concentration of pooled colostrum, g/L	42.9 $\pm$ 2.9	42.9 $\pm$ 2.9	1.000
IgG intake per feeding, g	85.9 $\pm$ 5.8	85.9 $\pm$ 5.8	1.000
Total from first 3 feedings, g	257.6 $\pm$ 17.4	257.6 $\pm$ 17.4	1.000

¹Total number of calves, n = 24. Control = calves fed pooled colostrum without difructose anhydride (DFA) III; treatment = calves fed pooled colostrum with 18 g of DFA III.

analysis. Subsamples (20 mL) from each batch of PC were taken at the time of PC preparation and stored at -30°C until IgG analysis. The concentration of IgG in both serum and PC samples was determined using a single radial immunodiffusion (Institute for Metabolic Ecosystem, Miyagi, Japan). The assay was previously validated for bovine colostrum and serum IgG analysis (Sato et al., 2012; Osaka et al., 2014).

Calculation of Apparent Efficiency of Absorption

Apparent efficacy of absorption (AEA) of IgG at 10, 24, and 36 h of age was calculated for individual calves using the method of Quigley et al. (1998), based on measured serum IgG, estimated serum volume, and ingested IgG mass at the relevant ages. Serum volume was estimated to be 8.6% of the birth weight of the calf (Quigley et al., 1998).

Statistical Analysis

Results are presented as mean ($\pm$ SE) for all calves. The concentration of IgG in PC was the same for each pair, but differed among pairs; therefore, mean differences between the groups were analyzed using a paired *t*-test. To investigate the effect of IgG concentration in PC on serum IgG concentration in all calves at 36 h of age, Pearson correlations were performed on the 36-h data. If a positive relationship existed between IgG concentrations in PC and serum, single linear regression was performed for each group, and the similarity of the regression lines for both groups was assessed by analysis of covariance (ANCOVA). Differences in AEA among the ages within each group were analyzed by one-way ANOVA using age of calf (i.e., 10, 24, and 36 h) as an explanatory variable. All data analysis was performed using SPSS for Windows Version 16.0 (SPSS

Inc., Chicago, IL). Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS

Dam parity ranged from 1 to 6 and gestation period from 275 to 290 d, but the mean values did not differ between the treatment and control groups (Table 1). Birth weight among all calves ranged from 37.0 to 61.4 kg, but the mean weight did not differ between the groups (Table 1). Age at PC feeding among all calves ranged from 0.8 to 1.5 h for the first feeding, 10.0 to 11.0 h for the second, and 23.9 to 25.1 h for the third, but the mean values for the age at each PC feeding did not differ between the groups (Table 1). Ingested PC volume per feeding was the same for all calves. Some variations occurred in the IgG concentration of PC, which ranged from 29.1 to 61.2 g/L among the 12 batches. Intake of IgG across all pairs ranged from 58.1 to 122.4 g/feeding, and total intake of IgG from the first 3 feedings ranged from 174.4 to 367.2 g, but the mean intake of IgG per feeding and total intake of IgG from the first 3 feedings did not differ between the groups (Table 1).

As presented in Table 2, serum IgG concentration measured at 0 h (before the first PC feeding) was 0 g/L and was uniform for all calves. In both groups, the mean serum IgG concentration increased from 0 h of age and reached the highest concentration at 36 h of age. After 36 h of age, serum IgG concentrations started to decline in a similar manner in both groups. Serum IgG concentration in the treatment group was higher ($P < 0.01$) than in the control group for all sampling time points except for 0 h of age (Table 2). At 10 h of age, average serum IgG concentrations in both groups exceeded 10 g/L, with the exception of one calf in the control group. Mean AEA in the treatment

group was higher ($P < 0.05$) than in the control group (Table 2).

DISCUSSION

We observed higher serum IgG concentrations in the treatment group than in the control group, except at 0 h of age (Table 2). These results are consistent with our previous study (Sato et al., 2012) in which supplementation of maternal colostrum with DFA III resulted in increased serum IgG concentration in Holstein and Holstein/Japanese Black crossbred calves. The serum IgG concentration in newborn calves may be affected by age at colostrum feeding (Osaka et al., 2014), ingested colostrum volume (Conneely et al., 2014), ingested IgG mass (Hopkins and Quigley, 1997), colostrum IgG concentration (Sato et al., 2012), and breed and sex of calves (Jones et al., 2004). To exclude these factors, this experiment was carried out using Holstein male calves fed the same amount of PC containing the same IgG concentration at the same age. Moreover, birth weight of the calves, dam parity, and gestation period did not differ between the groups (Table 1). In summary, it appears that the higher serum IgG concentrations in the treatment group compared with the control group were due to the DFA III supplementation.

In this study, we observed a positive linear regression between IgG concentration in PC and serum IgG concentration at 36 h in both groups ($r = 0.64$, $P = 0.026$ and $r = 0.50$, $P = 0.098$ for the treatment and control groups, respectively, Figure 1). Similar results were observed in our previous study (Sato et al., 2012). Although the slope of the regression lines did not differ between the groups, the intercept value of the regres-

Table 2. Means ($\pm$ SE) of serum IgG concentration and apparent efficiency of IgG absorption of calves fed pooled colostrum¹

Item	Control	Treatment	P-value
Serum IgG, g/L			
0 h of age	0.0	0.0	
10 h of age	11.5 $\pm$ 0.9	13.8 $\pm$ 0.8	<0.001
24 h of age	17.5 $\pm$ 1.5	21.3 $\pm$ 1.5	<0.001
36 h of age	17.7 $\pm$ 1.4	22.3 $\pm$ 1.4	<0.001
4 d of age	15.7 $\pm$ 1.4	19.9 $\pm$ 1.2	<0.001
7 d of age	14.5 $\pm$ 1.3	18.6 $\pm$ 1.1	0.001
AEA, ² %	29.5 $\pm$ 2.7	36.1 $\pm$ 2.7	0.026

¹Total number of calves, n = 24. Control = calves fed pooled colostrum without difructose anhydride (DFA) III; treatment = calves fed pooled colostrum with 18 g of DFA III.

²Apparent efficiency of absorption (AEA) of IgG at 36 h of age, calculated as serum IgG at 36 h (g/L) $\times$ serum volume (8.6% of birth weight, kg) (L) $\div$ ingested IgG (g).

sion line was higher ($P = 0.012$) for the treatment group than for the control group. This finding suggests that serum IgG concentration of newborn calves could be increased by DFA III supplementation regardless of the IgG concentration in colostrum, and IgG absorption across the intestinal epithelium in newborn calves could be increased by enhanced IgG flow through the small intestine.

Pansu et al. (1983) reported that calcium absorption in rat intestine follows 2 different pathways: a paracellular pathway, mediated by a nonsaturable process, and a transcellular pathway, mediated by a saturable process. The transcellular pathway down-regulates absorption when calcium intake is increased, whereas the paracellular pathway is positive for absorption when calcium intake is increased (Bronner, 1998). Although Heinrichs and Elizondo-Salazar (2009) proposed that nonselective endocytosis is a transport mechanism for IgG absorption across the intestinal epithelium, the effect of DFA III on endocytosis has not yet been proven. It is difficult to explain the increase in serum IgG concentration in the treatment group solely by endocytotic transport of IgG across the intestinal epithelium because serum IgG increased with IgG concentration in PC in the present study (Figure 1) and in our previous study (Sato et al., 2012). Because the concentration gradient absorption is a characteristic of a paracellular pathway, some IgG could have been absorbed through the paracellular pathway. It has been reported that DFA III enhances the opening of intestinal epithelial tight junctions (Mineo et al., 2002) and thereby increases mineral absorption in cattle (Teramura et al., 2015b). Teramura et al. (2015a) reported that DFA III enhances calcium absorption through the paracellular pathway of everted bovine intestinal sacs. Thus, more IgG might have been absorbed by calves fed DFA III, resulting in higher serum IgG concentration in calves

Figure 1. Regression lines of serum IgG concentration at 36 h of age on pooled colostrum IgG concentration ($r = 0.67$, $P = 0.002$): — and $\blacklozenge$ = pooled colostrum with 18 g of difructose anhydride (DFA) III ($r = 0.64$, $P = 0.026$); --- and $\square$ = pooled colostrum without DFA III ($r = 0.50$, $P = 0.098$).

receiving DFA III than in control calves in the present study. Therefore, the paracellular pathway through the intestinal epithelium of newborn calves has an important role for IgG absorption, but transmural permeability of intestinal epithelium becomes narrower at a later age (Wisser and Horster, 1978). The AEA in both groups declined with age (Figure 2).

The AEA is useful for a better understanding of the nature of IgG absorption. It is influenced by the age at first colostrum feeding (Weaver et al., 2000; Osaka et al., 2014) and by the volume of the ingested colostrum (Conneely et al., 2014). In this study, average AEA was $43.1 \pm 3.6\%$ and $51.4 \pm 3.3\%$ at 24 h of age and $29.5 \pm 2.7\%$ and $36.1 \pm 2.7\%$ at 36 h of age for the control and treatment groups, respectively. These AEA values were higher than those of Hopkins and Quigley (1997) and Jones et al. (2004) and lower than those of Conneely et al. (2014). Conneely et al. (2014) reported that the average AEA at 48 h of age was 38% when newborn calves were fed 3.2 L of colostrum within 2 h of age. However, the calves in our study received 2 L of PC around 1 h of age and another 2 L at 10 h of age. Hopkins and Quigley (1997) also reported that the average AEA at 24 h of age was 40% when calves were fed 1.9 L of maternal colostrum at 1.3 h of age and a further 1.9 L at 10 to 12 h after the first feeding. Moreover, Jones et al. (2004) reported that the average AEA of calves at 24 h of age was 20% when the calves received 1.4 L of PC within 2 h of age and a further 1.4 L within 14 h after birth. The high AEA in our study compared with that of Hopkins and Quigley (1997) and Jones et al. (2004) was probably due to earlier consumption and larger volumes for the first and second PC feedings. Osaka et al. (2014)

reported that AEA of newborn calves decreased with a delay in the first colostrum feeding, and Conneely et al. (2014) demonstrated that AEA in newborn calves was influenced by colostrum volume.

The serum IgG concentration increase by 36 h of age, but the increase from 24 to 36 h of age was small in both groups in this study. Absorption of ingested IgG in newborn calves may occur until 24 to 36 h of age (Weaver et al., 2000). Also, AEA in both groups decreased with aging (Figure 2). These results suggest that IgG absorption after 24 h of age is limited and DFA III cannot extend the absorption period of IgG in newborn calves, although it can enhance IgG absorption within 24 h after birth.

CONCLUSIONS

Difructose anhydride III supplementation of PC resulted in an increase in AEA and serum IgG concentration in newborn calves fed PC containing various IgG concentrations. The current study suggests that absorption of ingested IgG by endocytosis as well as by the paracellular pathway of the intestinal epithelium could be enhanced by DFA III supplementation. These findings are of practical importance in neonatal calf management for achieving successful passive IgG transfer by DFA III supplementation of PC (combination of colostrum from the first and second milkings). This supplementation could alleviate the need for high-quality colostrum.

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Figure 2. Apparent efficiency of absorption (AEA) of IgG at 10, 24, and 36 h of age in calves fed pooled colostrum without difructose anhydride (DFA) III (control) or pooled colostrum with 18 g of DFA III (treatment). Values within each group followed by different letters (a–c) are significantly different ($P < 0.05$).

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