Short communication: Effect of difructose anhydride III on serum immunoglobulin G concentration in newborn calves

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ABSTRACT

Difructose anhydride (DFA) III is an indigestible disaccharide that promotes paracellular absorption of calcium, magnesium, and other minerals in the intestine by acting on epithelial tight junctions. This study aimed to elucidate the effect of DFA III on serum IgG concentration. One hundred and twenty Holstein and Holstein/Japanese Black crossbred calves were randomly divided into 4 groups of 30 to receive untreated colostrum (DFA0) or colostrum containing 3, 6, or 18 g of DFA III (DFA3, DFA6, or DFA18, respectively). At 24 h after birth, both serum IgG (ranging from 16.4 to 21.2 mg/mL) and apparent efficiency of absorption (26.0 to 37.2%) showed increases with the amount of DFA III intake. By multiple regression analysis, the standardized partial regression coefficient for DFA III was 0.25, the second highest following that for the colostrum IgG concentration (0.80), indicating a positive effect of DFA III on serum IgG. A positive linear regression was found between colostrum IgG and serum IgG concentrations at 24 h of age. These results indicate that IgG absorption occurred as a nonsaturable process, which might be characteristic of gradient-dependent paracellular transport. Thus, it was concluded that DFA III improves not only minerals but IgG absorption in calves.

Key words: difructose anhydride (DFA) III, immunoglobulin G, colostrum, calf

Short Communication

In newborn animals, ingested immunoglobulin is taken up by intestinal epithelial cells before entering the body system (Staley and Bush, 1985) either via specific receptor-mediated endocytosis or nonspecific endocytosis (Pácha, 2000), depending on the species. Jakoi et al. (1985) reported the isolation and purifi-

cation of an immunoglobulin-binding protein from membranes of neonate rat enterocytes, suggesting that immunoglobulin uptake is a selective process in this species. He et al. (2008) presented neonatal Fc receptor (FcRn)-mediated antibody transport across epithelial cells in rats, revealed by electron tomography. In calves, on the other hand, the apparent lack of cell surface receptors as well as the nonselective and nonspecific nature of protein absorption in the gut has suggested nonselectivity in immunoglobulin uptake as well (Staley and Bush, 1985), although endocytic uptake of immunoglobulin has not been directly demonstrated. More recently, Castro-Alonso et al. (2008) suggested that IgG absorption is mediated by apoptotic enterocytes.

Difructose anhydride (DFA) III is a disaccharide consisting of 2 fructose residues joined by 1,2' and 2,3' linkages and is synthesized enzymatically from inulin (Kikuchi et al., 2009). This new material has been industrially manufactured since 2004. It has half the sweetness of sucrose and physiological characteristics such as low cariogenicity and indigestibility (Kikuchi et al., 2004). In humans, it has been reported that DFA III is rarely digested by the intestinal flora (Saito and Tomita, 2000) and facilitates the paracellular transport of minerals, especially calcium, in the gut by acting on epithelial tight junctions (Mineo et al., 2001). Its benefits for hypocalcemia in dairy cows have also been reported (Sato et al., 2007). Furthermore, Matsumoto et al. (2009) and Takagi et al. (2011) reported that DFA III could be used as a prebiotic for cattle.

Kamada et al. (2007) reported that IgG absorption by newborn calves was enhanced by adding selenium to colostrum, although this phenomenon could not be explained by any identified function of selenium. In the present study, the effect of DFA III on IgG absorption was investigated in newborn calves.

All procedures were done in accordance with Japanese Standards Relating to the Care and Management of Experimental Animals. Difructose anhydride III (with 97% purity; Nippon Beet Sugar Manufacturing Co. Ltd., Tokyo, Japan) was used. Colostrum and milk replacers (Calf top ET; Zenrakuren, Tokyo, Japan)

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were supplemented with 0, 3, 6, or 18 g of DFA III (DFA0, DFA3, DFA6, or DFA18, respectively) and fed for 7 d. One hundred and twenty spontaneous-suckling newborn calves (87 Holsteins and 33 Holstein/Japanese Black crossbreds) were randomly assigned to 1 of the treatments. Calves were separated from their dams immediately after birth before the first suckling occurred, weighed, and housed individually in a pen.

Colostrum was obtained from dams (3.5 L each) within 2 h postparturition. Calves were immediately fed 1.5 L of the colostrum. The remaining 2 L of the colostrum was kept refrigerated, warmed up, and fed at 10 h after birth. Calves were fed 2 L of milk for the third time after blood sampling at 24 h after birth. Thereafter, calves were fed 2 L of milk (for the first 3 d after birth) or milk replacer twice daily at 0630 and 1600 h. Water, calf starter (Nippon Beet Sugar Manufacturing Co. Ltd.) and timothy hay were also supplied, but intakes of starter and hay were negligible during the 7-d study period. Blood samples were obtained at 0 and 24 h after birth and at 0600 h before feeding on d 2, 3, 5, and 7.

Blood samples were collected into vacuum tubes via the jugular vein. The serum was separated and stored at -30°C until assayed. Colostrum and serum IgG concentrations were determined by single radial immunodiffusion (The Institute for Metabolic Ecosystem, Miyagi, Japan). The apparent efficiency of absorption (AEA%) of IgG was calculated from the IgG intake for the first 24 h and the estimated blood IgG volume at 24 h, based on measured serum IgG with the blood volume of 8.6% of the birth BW (Quigley et al., 1998).

The experimental data were statistically analyzed using JMP 9 (SAS Institute Inc., Cary, NC). The numbers of calves were analyzed for each breed for genetic significance by chi-squared contingency table analysis. Other parameters were analyzed by one-way ANOVA using DFA III treatment as an explanatory variable. Nine variables (i.e., the amount of DFA III intake, colostrum IgG concentration, BW, the time between the first feeding and the second blood sampling, the time between the second feeding and the second blood sampling, breed, sex, colostrum protein concentration, and specific gravity of colostrum) were analyzed by stepwise regression for their effects on serum IgG concentration at 24 h of age, and multiple regression analysis was performed using 5 of these variables (the amount of DFA III intake, colostrum IgG, BW, the time between the second feeding and the second blood sampling, and sex) as explanatory variables. The standardized partial regression coefficient for each variable was determined to compare the effect of the variables on IgG concentration. The correlation between colostrum IgG and serum IgG concentrations at 24 h was analyzed by simple re-

gression analysis for all calves and for each treatment according to the amount of DFA III. Analysis of covariance was also used to compare the effect of DFA III intake on IgG concentration among the treatments.

In Table 1, the breed, BW, timing of first feeding, and timing of second feeding, which are considered to affect serum IgG absorption, did not differ among the treatments. The IgG concentration in colostrum ranged from 19.7 to 140.4 mg/mL, and the amount of IgG ingested during the first 24 h of life ranged from 69 to 491 g. Mean IgG intake did not differ ($P = 0.79$) among the treatments. The serum IgG level at 24 h of age ranged from 4.4 to 51.7 mg/mL, showing a slight tendency to increase with the amount of DFA III intake ($P = 0.053$). The AEA% increased significantly with the amount of DFAIII intake ($P < 0.001$).

To identify the effect of DFA III on serum IgG concentration at 24 h of age, a multiple regression analysis was performed using the 5 variables, and a significant multiple regression equation was obtained ($r^2 = 0.69$, $P < 0.001$; Table 2). The regression showed the serum IgG concentration at 24 h of age was positively affected by colostrum IgG, the amount of DFAIII intake, and time from second feeding to second blood sampling, and affected by BW negatively. The standardized partial regression coefficient of the equation was highest for colostrum IgG and the standardized partial regression coefficient for the amount of DFAIII intake was 0.25. This result suggests that serum IgG concentration at 24 h of age can be improved by increasing DFAIII intake, although the serum IgG concentration is strongly affected by colostrum IgG.

As shown in Figure 1, the serum IgG concentration at 24 h of age showed a positive linear correlation with the colostrum IgG concentration, when all data were combined across the treatments ($r^2 = 0.54$, $P < 0.001$). Stott and Fellah (1983) have reported a similar result and shown that serum IgG levels were more significantly affected by the colostrum IgG concentration than the amount of colostrum ingested. When relationships between serum IgG and colostrum IgG according to the amount of DFA III given were investigated by ANOVA, the intercept of the regression lines increased significantly according to the increase of DFA III supplement ($r^2 = 0.63$, $P < 0.001$). However, no significant difference was observed in the slope of the regression lines among the treatments.

By studying the kinetics of calcium absorption in the rat, Pansu et al. (1983) successfully demonstrated 2 distinct calcium absorption processes: a saturable process and a nonsaturable process. According to their study, the saturable process is a transcellular pathway that is dependent on calcium-binding protein and can be represented by a Michaelis-Menten function in which

Figure 1. Regression lines of colostrum IgG versus serum IgG at 24 h of age in calves treated with 0 g (DFA0; $R^2 = 0.72$), 3 g (DFA3; $R^2 = 0.70$), 6 g (DFA6; $R^2 = 0.38$), and 18 g (DFA18; $R^2 = 0.68$) of difructose anhydride (DFA) III.

absorption eventually approaches the maximum. On the other hand, the nonsaturable process is predominantly a paracellular, gradient-dependent absorption and can be represented by a regression line. The linear increase of serum IgG with IgG concentration in the colostrum suggests that IgG absorption is based on the nonsaturable process. The evidence that proteins are nonselectively absorbed in newborn calves (Staley and Bush, 1985) also supports the nonsaturable process of IgG absorption. It has been proposed that IgG uptake

occurs via endocytosis by intestinal epithelia (Staley and Bush, 1985). However, the effect of DFA III on endocytosis has not been reported.

Tight junctions have been considered to function as a strong barrier against paracellular transport by tightly holding adjacent epithelial cells together. However, it is now known that there are various types of claudins, a major protein family that makes up the tight junctions, and that the barrier function of the tight junctions is determined by the combination and the number of claudins (Furuse et al., 2001; Van Itallie et al., 2001; Amasheh et al., 2002). In addition, one tight junction strand can be connected to or disconnected from other strands at the ends and on the sides to allow slow transport of macromolecules (Sasaki et al., 2003). Pansu et al. (1983) also demonstrated that nonsaturable calcium uptake was highest at birth and decreased over time, and Wisser and Horster (1978) showed that transmural permeability was higher in an immature intestine compared with a mature one. The earlier calves are fed colostrum, the more efficient IgG absorption becomes, and IgG absorption occurs only during the first 24 to 36 h of life.

After 24 h of life, serum IgG concentration tended to be higher with increasing amounts of DFA III intake (Table 1). Serum IgG showed similar kinetics in all treatments, reaching a maximum on the second day and then decreasing after that. Thus, it was thought

Table 1. Means and SE of study parameters in calves fed colostrum with or without difructose anhydride (DFA) III

Parameter	Treatment ¹				SE	P-value
	DFA0	DFA3	DFA6	DFA18		
Number of calves	30	30	30	30		0.48
Holstein	24	21	23	19		
Holstein × Japanese Black	6	9	7	11		
Birth BW, kg	42.3	40.8	42.7	41.3		0.49
Holstein	43.2	41.1	44.2	43.2		0.22
Holstein × Japanese Black	38.7	40.0	37.5	37.9		0.70
Age at feeding, h						
First feeding	1.2	1.1	1.1	1.3	0.1	0.60
Second feeding	10.1	10.4	9.8	10.2	0.2	0.38
Age at blood sampling, h						
First blood sampling	0.8	0.8	0.9	0.9	0.1	0.77
Second blood sampling	24.8	24.8	24.6	24.7	0.2	0.93
Colostrum IgG, mg/mL	65.9	61.1	62.3	58.7	5.0	0.79
IgG intake, g	230.6	214.1	218.2	205.5	17.5	0.79
Serum IgG, mg/mL						
0 h	0.0	0.3	0.0	0.0	0.1	0.18
24 h	16.4	17.1	21.0	21.2	1.6	0.053
2 d	17.2	17.3	21.3	21.4	1.5	0.068
3 d	15.2	16.0	18.8	19.8	1.4	0.060
5 d	14.3	15.1	17.9	19.3	1.4	0.037
7 d	13.2	13.7	16.5	17.6	1.3	0.039
AEA, ² %	26.0	29.7	36.2	37.2	1.6	<0.001

¹DFA0 = 0 g of DFA III; DFA3 = 3 g of DFA III; DFA6 = 6 g of DFA III; DFA18 = 18 g of DFA III.

²Apparent efficiency of IgG absorption, calculated as serum IgG at 24 h × BW at birth (kg × 8.6; serum volume as a percentage of BW) divided by IgG intake.

Table 2. Multiple regression analysis for serum IgG concentration at 24 h of age ($R^2 = 0.69$)

Variable	r	β^1	P-value
DFA III intake, ² g	0.32	0.25	<0.001
Colostrum IgG, mg/mL	0.26	0.80	<0.001
BW, kg	-0.44	-0.27	<0.001
Time from second feeding to second blood sampling, h	0.64	0.11	0.034
Sex ³	-1.11	-0.13	0.017

¹The standardized partial regression coefficient of multiple regression analysis.²DFA = difructose anhydride.³As a dummy variable, values 1 (female) and -1 (male) were assigned.

that intestinal closure for IgG appeared occurred after 24 to 48 h of age, and was not influenced by the amount of DFA III.

Difructose anhydride III supplementation resulted in increased serum IgG levels as well as increased AEA% at 24 h after birth. These results indicate that DFA III alleviates failure of transfer of passive immunity and has some advantages in health and viability of newborn calves.

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