



Effects of Oral Administration of Diffructose Anhydride III on Selected Health and Blood Parameters of Group-housed Japanese Black Calves during the Prewearing Period

Daisaku Matsumoto^{1,2}, Mitsuhiro Takagi^{1,*}, Hiroshi Hasunuma², Yasuo Fushimi², Masayuki Ohtani³,
Tadashi Sato³, Koji Okamoto⁴, Francis Shahada⁵, Tetsuya Tanaka⁶ and Eisaburo Deguchi¹

¹Laboratory of Farm Animal Production Medicine, Kagoshima University Kagoshima 890-0064, Japan

ABSTRACT : Two field studies were conducted to determine the efficacy of diffructose anhydride III (DFA III) as a supplement in colostrum replacer (CR) for improving the general health status (judged on the basis of incidence of enteritis, bronchitis, and pneumonia) of group-housed suckling Japanese Black calves. In a preliminary study, CR supplemented with DFA III (6 g) was orally administered within 24 h of calving to eight individually reared calves fed colostrum (DFA III group) (Exp. 1). Subsequently, CR supplemented with DFA III (6 g) was orally administered twice within 2 and 12 h of calving to four calves (DFA III group) that were not fed colostrum (Exp. 2). In both experiments, the health status of the calves was assessed during the preweaning period. In Exp. 2, hematological and blood-chemistry parameters were analyzed 24 h after the second administration of CR and at 1 wk and 1 month after calving. The results were compared between the DFA III and control groups (without DFA III supplementation, Exp. 1: n = 10, Exp. 2: n = 4). In Exp. 1, the number of calves requiring medications for the treatment of enteritis, bronchitis, and pneumonia during the preweaning period was significantly ($p < 0.05$) lower in the DFA III group than in the control group. In Exp. 2, supplementation of DFA III did not influence the gain in body weight of calves during the pre-weaning period. Calves in the DFA III group tended to require medications for a shorter duration than those in the control group (DFA III: 10.3 d/calf, control: 21.3 d/calf; $p = 0.07$). Significant differences ($p < 0.05$) in the level of mean corpuscular hemoglobin, total protein, total cholesterol, and immunoglobulin (Ig)G were observed between the DFA III and control groups. These differences probably reflect the health and nutritional status of the calves. Additionally, the serum iron and lactoferrin concentrations at 24 h and 1 wk after calving, respectively, differed significantly between the 2 groups. These concentrations might reflect the incidence of infections after calving. The present study revealed that the administration of DFA III as a CR supplement may prevent diseases in group-housed calves during the pre-weaning period. (**Key Words :** Calf, DFA III, Prebiotics, Group Housing, Lactoferrin)

INTRODUCTION

Recently, group housing of preweaned calves has been attracting increasing interest mainly because it can improve

calf welfare and labor efficiency. Group housing is generally practiced for large dynamic groups, often comprising calves of widely varying ages; new young calves are continually added to these groups (Rasmussen et al., 2006). Although varying results have been reported, it is generally accepted that acute and chronic respiratory diseases and diarrhea occur more frequently in group-housed veal calves than in individually reared calves (Maatje et al., 1993). This finding might be attributed to the stress experienced by the calves due to the group dynamics and the difficulty in maintaining hygiene in the group-housing system (Maatje et al., 1993). It is generally acknowledged that in humans, the number of protective lactobacilli tends to decrease during stressful events, whereas the number of coliform bacteria tends to increase (Fuller, 1989). Additionally, a previous report indicated that,

* Corresponding Author: Mitsuhiro Takagi. Tel: +81-99-285-8672, Fax: +81-99-285-8672, E-mail: mtakagi@agri.kagoshima-u.ac.jp

² Shepherd Central Livestock Clinic, Kagoshima 899-1611, Japan.

³ Research Center, Nippon Beet Sugar Manufacturing Co. Ltd., Obihiro 080-0831, Japan.

⁴ Soo Veterinary Clinical Center, Soo Agriculture Mutual Aid Association, Kagoshima 899-8212, Japan.

⁵ Laboratory of Veterinary Public Health, Kagoshima University Kagoshima 890-0064, Japan.

⁶ Laboratory of Emerging Infectious Diseases, Kagoshima University Kagoshima 890-0064, Japan.

Received March 28, 2009; Accepted July 2, 2009

in the case of calves, the protective potential of the microbial gut flora decreases during dietary stress (Cray et al., 1998). In some countries, to prevent the proliferation of opportunistic pathogenic flora in the gut, group-housed calves are routinely treated with prophylactic antibiotics (Timmerman et al., 2005). However, the use of antibiotics as a prophylactic measure to combat infections in calves is generally discouraged in order to avoid drug resistance and ensure food safety (Refsdal, 2000; Wenk, 2000; Jouany and Morgavi, 2007). In Japan, in particular, the addition of antibiotics to milk replacers for prophylactic purpose is strictly prohibited by law.

Recently, growing interest has been shown towards the health-promoting benefits of prebiotics such as mannan oligosaccharide (Heinrichs et al., 2003; Franklin et al., 2005), fructooligosaccharides (Donovan et al., 2002), and lactulose (Fleige et al., 2007). It has been reported that prebiotics positively influence the bacterial flora of the gastrointestinal tract, thereby reducing the incidence of diseases in animals (Fleige et al., 2008). Diffructose anhydride III (DFA III) is a naturally occurring nondigestible disaccharide that is present in commercial roasted chicory and is manufactured from inulin by microbial fermentation (Yokota et al., 1991; Tamura et al., 2004). DFA III promotes calcium absorption in rats (Mineo et al., 2002; Shiga et al., 2003), humans (Shigematsu et al., 2004; Tomita et al., 2007), and cattle (Sato et al., 2007). Furthermore, Minamida et al. (2005; 2006) reported that the oral administration of DFA III in laboratory animals may help maintain a healthy balance of intestinal microbiota, and they suggested that DFA III is a novel candidate prebiotic.

Japanese Black cattle are the most popular breed of beef cattle in Japan. However, compared to other breeds, these cattle are immunologically weak (Inokuma et al., 1995; Ohtsuka et al., 2002) and are thus more prone to diseases during the early postnatal period. The high risk of infections in Japanese Black calves may be ascribed to reduced lymphocyte proliferation (Inokuma et al., 1995; Ohtsuka et al., 2002). On the basis of the findings regarding the possible efficacy of DFA III as a prebiotic (Minamida et al., 2005; 2006), we speculated that the etiotropic effects of DFA III could be applied to group-housed calves that are fed milk replacers and that DFA III administration would help maintain good health in the calves and hence reduce the use of antibiotics.

Therefore, in this preliminary study, we aimed to investigate under commercial farm conditions, the effects of the oral administration of DFA III as a supplement in colostrum replacer (CR) to calves aged ≤ 24 h. We evaluated the health status (judged on the basis of the frequency of medical treatments for infections), gain in body weight, and hematological and blood-chemistry parameters in calves

treated with DFA III and control calves in the preweaning period.

MATERIALS AND METHODS

Two field experiments were conducted to evaluate the effects of DFA III on the health status of calves during the preweaning period. In Exp. 1, also referred to as the preliminary observational field trial, Japanese Black calves from a private farm in Miyazaki Prefecture, Japan, were used. On the basis of the results of Exp. 1, we conducted Exp. 2 in another private farm in Kagoshima Prefecture, Japan, wherein additional assessments, such as blood examination, and a detailed evaluation of the health status were performed.

Experiment 1

We conducted this experiment to evaluate the effects of DFA III on the health status of calves that were reared individually in wooden stalls until weaning. The calves were fed either fresh colostrum from their dams or previously collected frozen-thawed colostrum twice within 2 h and 12 h after birth. Subsequently, the calves were randomly assigned to the DFA III ($n = 8$; all males) and control ($n = 10$; six males and four females) groups. Between 4 and 11 h postpartum, the calves in the former group were administered a CR (containing immunoglobulin (Ig) ≥ 56 g; Calf Support Dash; Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan) supplemented with 6 g DFA III (Nippon Beet Sugar Manufacturing Co. Ltd., Obihiro, Japan). This dose of DFA III is the recommended dose for the prevention of hypocalcemia in dairy cows (Sato et al., 2007). Calves in the control group were fed CR not containing DFA III. The calves were separated from their dams within 3 d postpartum, shifted to individual elevated wood stalls fitted with heat lamps, and fed a milk replacer through a nipple until they were weaned (at approximately 3 months). At the beginning of the experiment, the volume of the milk replacer provided was 4 L/d; it was gradually increased to a maximum of 6 L/d until 1 wk before weaning and then decreased to 3 L/d until weaning, regardless of the body weight and sex of the calves. The intakes of CR and calf starter (total digestible nutrients $>75\%$, crude protein $>25\%$; Calf Manna, Kyodo Shiryō Co. Ltd., Japan) were monitored daily. All calves were injected with vitamin A (VA) solution (50,000 IU; Kawasaki-Mitaka K.K., Tokyo, Japan) and vitamin E (VE) solution (100 IU; Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan) when they were transferred to the individual stalls. Fresh water and the calf starter supplemented with minerals and vitamins were provided *ad libitum*. Thus, calves from both groups were considered to have been exposed to similar levels of stress. The health status was examined in all calves, and blood

samples for the measurement of serum IgG levels were collected before the calves were transferred to the individual stalls.

The general health status, including appetite and fecal consistency, was monitored daily during the entire experimental period by an experienced farm staff. Additionally, a veterinarian routinely visited the herd at least twice a week. Enteritis, bronchitis, and pneumonia were diagnosed on the basis of previously reported clinical criteria such as diarrhea (gruel-like or watery feces), fever (rectal temperature $>39.5^{\circ}\text{C}$), and signs of respiratory disease (severely increased respiratory sounds accompanied by fever and coughing or a grayish to yellowish nasal discharge) (Svensson and Liberg, 2006). Treatment consisting of mainly systematic antibiotic therapy accompanied by supportive therapy was administered according to the clinical diagnosis. Treatment data were recorded for each calf.

Experiment 2

On the basis of the results of Exp. 1, we conducted the second experiment wherein blood parameters were measured to evaluate the effects of DFA III administration in detail. Calves were separated from their dams immediately after parturition without allowing them to feed on colostrum. The calves were then randomly assigned to the DFA III ($n = 4$; three males and one females) and control ($n = 4$; one males and three females) groups. The animals in the former group were fed CR supplemented with 6 g DFA III twice within 2 h and 12 h of calving. Those in the control group were fed CR not supplemented with DFA III. Each calf was moved to an individual elevated wood stall fitted with a heat lamp and kept there for approximately 7 d. Subsequently, the calves were subjected to group housing and fed using an automatic milk-feeding system on the farm. As was the case in Exp. 1, all calves were injected with VA and VE solutions upon introduction into the individual stalls. New calves were continually introduced into the existing group, and the difference in the ages of the newly introduced and the older calves was approximately 2 to 3 wk. As in Exp. 1, the volume of the milk replacer fed at the beginning of the experiment was 4 L/d; it was gradually increased to a maximum of 6 L/d until 1 wk before weaning and then decreased to 1 L/d until weaning, regardless of the body weight and sex of the calves. Fresh water and a calf starter (total digestible nutrients $>74\%$, crude protein $>19\%$; Meiluck, Meiji Co. Ltd., Tokyo, Japan) supplemented with minerals and vitamins were provided *ad libitum*.

The body weights of the experimental calves were measured immediately after calving, 1 wk after calving, and at weaning (approximately 12 wk after calving). Additionally, blood samples from the jugular vein were

collected 24 h after the second CR intake, 1 wk after calving, and 1 month after calving in order to determine the following: complete blood count (CBC; assessed on F-820; Sysmex, Japan) and levels of blood urea nitrogen (BUN) and serum aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), total cholesterol (T-Cho), glucose (Glu), free fatty acid (FFA), iron (measured on a Labospect 7080 autoanalyzer; Hitachi, Japan), IgG and IgM (measured using radial immunodiffusion method; Metabolic-Eco-System Institute, Japan), VA and VE (measured using high-performance liquid chromatography), and lactoferrin (LF; measured using the latex agglutination turbidimetric immune assay method; Cosmo Bio Co. Ltd., Japan). Additionally, we determined the presence and concentration of LF in the CR fed to the calves. The above tests were performed to monitor hepatic and renal functions, nutritional status, inflammation, and immune status of the calves in the two groups. Furthermore, all health assessments and medical treatments for the examined calves were conducted as described in Exp. 1.

The results obtained for each group are expressed as means \pm SD. In Exp. 1, the numbers of calves that did not receive any medical treatment was compared between the 2 groups by using the chi-square test with Yate's correction. Body weight, daily gain in weight, values of the examined blood parameters, and mean duration (in days) of medical treatment were compared between the 2 groups by using the Student *t* test in order to determine the effects of DFA III supplementation on the calves. *p* values less than 0.05 were considered to indicate a statistically significant difference, while *p* values less than 0.1 were considered to indicate a significant tendency.

RESULTS

Experiment 1

Health status and medical treatments : The mean (\pm SEM) duration of medical treatment per calf was 0.8 ± 0.5 d in the DFA III group and 1.4 ± 0.3 d in the control group. The number of calves not requiring medical treatment during the preweaning period was significantly ($p < 0.05$) higher in the DFA III group (75%, 6/8) than in the control group (20%, 2/10). The mean serum IgG concentrations in all the calves in both the DFA III (25.5 ± 11.2 mg/ml; range, from 7.1 to 42.0 mg/ml) and control groups (24.3 ± 15.1 mg/ml; range, from 9.0 to 60.1 mg/ml) exceeded 10 mg/ml, except in the case of 1 calf in each group (DFA III: 7.1 mg/ml; control: 9.0 mg/ml). Moreover, the difference between the serum IgG concentrations in the 2 groups was not significant. This indicated that almost all of the calves in both the groups possessed IgG in amounts sufficient for the prevention of infections.

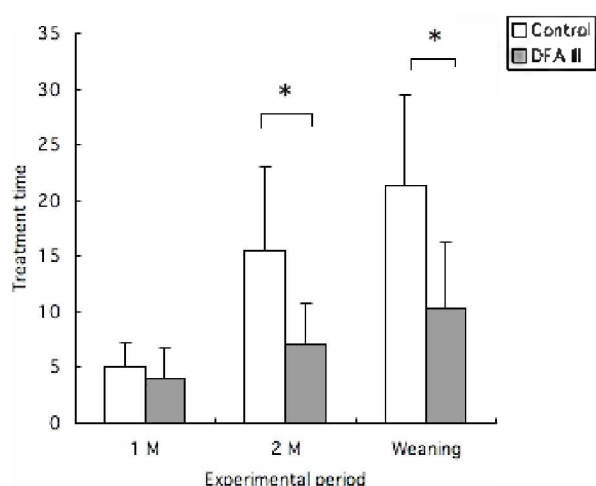


Figure 1. The mean (\pm SD) duration of medical treatment in the calves in the DFA III and control groups at 1 (1 M) and 2 months (2 M) after calving and at weaning. * Significant difference between the DFA III and control groups (2 M; $p = 0.09$, Weaning; $p = 0.07$).

Experiment 2

Effect of DFA III treatment on the growth performance of the calves: Before the start of the experiment, no significant differences in body weight were observed between the DFA III (31.8 ± 5.8 kg) and control (30.8 ± 2.8 kg) groups. In addition, no significant difference was observed in the mean daily gain in body weight between the DFA III (0.5 ± 0.1 kg/d) and the control (0.5 ± 0.1 kg/d) groups during the period from calving to weaning.

Health status and medical treatments: We noted the frequency of medical treatments in both groups at 1 and 2 months after calving and at weaning (Figure 1). This frequency did not significantly differ between the 2 groups at 1 month after birth, but significant differences were noted at 2 months ($p = 0.09$) and at weaning ($p = 0.07$). In the DFA III group, 33%, 43%, and 42% of diseases were attributed to respiratory infections at 1 month, 2 months, and weaning, respectively, while the remaining diseases at these time points were attributed to digestive disorders. In the control group, respiratory diseases accounted for 59%, 67%, and 76% of the diseases at 1 month, 2 months, and weaning, respectively, and digestive disorders accounted for the rest at the above time points.

We also determined the total duration of medical treatment and the duration of medical treatment per calf (Table 1). The total duration of medical treatment before weaning was 41 of 347 d and 85 of 379 d in the DFA III and control groups, respectively. This duration tended to be significantly lower in the former group than in the latter group ($p = 0.09$). In addition, the duration of medical treatment per calf in the DFA III group (10.3 d) tended to be lower than that in the control group (21.3 d) ($p = 0.07$).

Blood analysis

The results of hematological and serum biochemical analyses are shown in Figure 2. No significant differences were observed between the DFA III and control groups with regard to the red and white blood cells counts, hemoglobin (Hb) level, and hematocrit values. With regard to the total protein (TP) concentration, no significant differences were observed between the groups at 24 h after birth, but significant differences were noted (DFA III: 6.2 g/dl, control: 5.6 g/dl; $p < 0.05$). Further, in both groups, the TP levels at 1 month after calving were similar to the levels observed at 24 h after birth. Additionally, significant differences in the mean corpuscular hemoglobin (MCH) levels were observed between the groups at 24 h (DFA III: 14.6 pg, control: 13.8 pg) and 1 wk (DFA III: 14.7 pg, control: 13.6 pg) after calving.

The results of serum biochemical analyses are as follows (Figure 3). No significant differences were observed between the DFA III and control groups with regard to the levels of AST, GGT, BUN, T-Cho, Glu, FFA, HP, VA, VE, and IgM. The TP (>4.9 g/dl) and GGT (>300 U/L) concentrations at 24 h after the second CR feeding indicated that all the calves from both groups had consumed sufficient amounts of CR. The iron, LF, and IgG concentrations at 24 h, 1 wk, and 1 month after calving respectively in the DFA III group (20.3 mg/dl, 620 ng/ml, and 8.1 mg/ml respectively) significantly differed from the corresponding concentrations in the control groups (33.5 mg/dl, 945 ng/ml, and 5.1 mg/ml respectively).

DISCUSSION

Although the functional mechanism of prebiotics has not been fully determined, multiple mechanisms of action have been postulated, particularly, enhancement of the

Table 1. Mean (\pm SD) number of days of medical treatment and frequency (%) of diseases in calves in the DFA III and control groups before weaning time

	DFA III (n = 4)	Control (n = 4)
Total duration of the experiment	347	379
Duration of medical treatment (%)	11.8 ^a (41 d)	22.4 ^a (85 d)
Mean number of days of medical treatment per calf	10.3 \pm 5.9 ^b	21.3 \pm 8.1 ^b

^a $p = 0.09$, ^b $p = 0.07$.

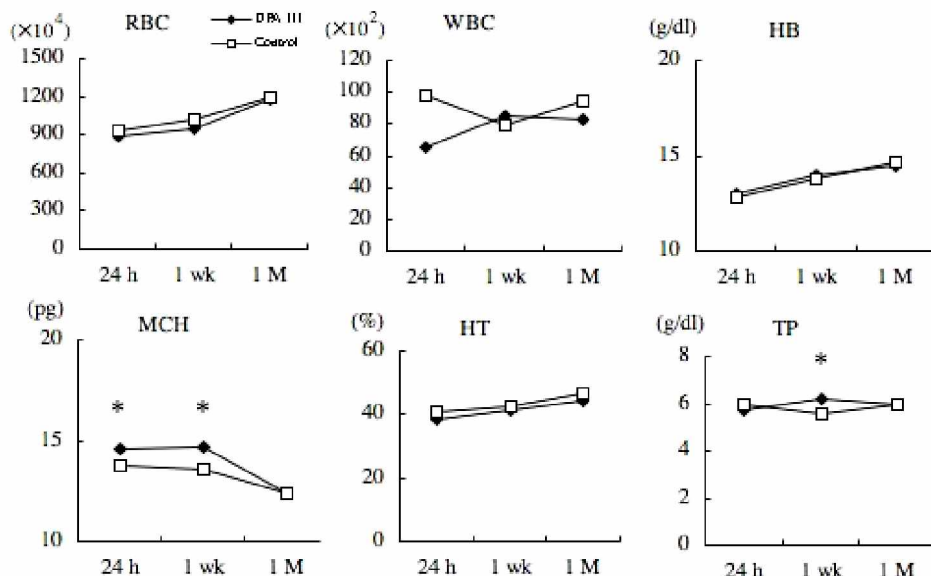


Figure 2. Results of hematological and serum biochemical analyses. Blood samples were obtained 24 h after the second feeding of colostrum replacer and at 1 week (1 wk) and 1 month (1 M) after calving. The red blood cell (RBC) and white blood cell (WBC) counts, levels of hemoglobin (Hb) and total protein (TP), and the mean corpuscular hemoglobin (MCH) and hematocrit (HT) values were measured. * Significant difference between the DFA III and control groups ($p < 0.05$).

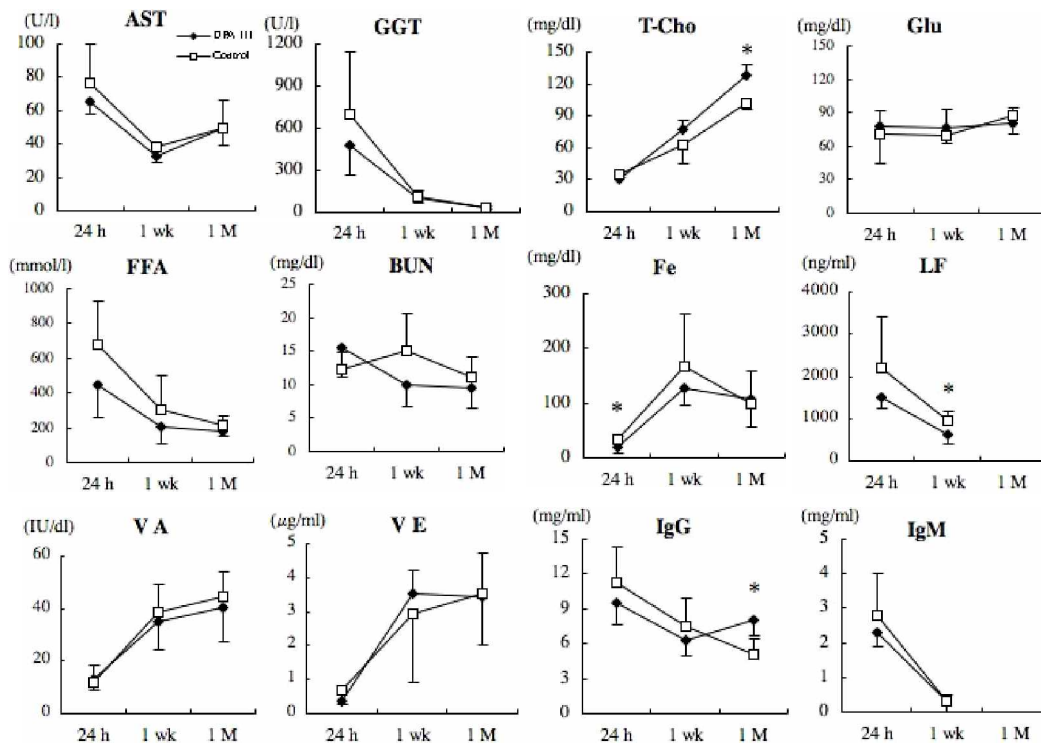


Figure 3. Results of serum biochemical analyses 24 h after the second feeding of colostrum replacer and 1 week (1 wk) and 1 month (1 M) after calving. Hepatic function was assessed by determining the levels of aspartate aminotransferase (AST) and γ -glutamyltransferase (GGT). Renal function was assessed by determining the blood urea nitrogen (BUN) level. Nutritional condition was evaluated by determining the levels of total cholesterol (T-Cho), glucose (Glu) and free fatty acid (FFA). Vitamin intake was examined by determining the levels of vitamin A (VA) and vitamin E (VE). The immune status was assessed by determining the levels of immunoglobulin (Ig)G and IgM. Additionally, the serum iron (Fe) and lactoferrin (LF) concentrations were determined. * Significant difference between the DFA III and control groups ($p < 0.05$).

growth of probiotics in the intestine (Macfarlane et al., 2006). The beneficial effects of prebiotics include prevention of the growth of pathogenic bacteria, production of antimicrobial agents, enhancement of the mucosal barrier function, and alteration of immunoregulation (Novak and Katz, 2006). Several effects of oral administration of DFA III as a supplement have been reported in humans and rats, such as increased intestinal calcium absorption (Mineo et al., 2002; Mitamura et al., 2002; Shiga et al., 2003; Shigematsu et al., 2004) and improved balance of intestinal microbiota resulting in healthier conditions (Minamida et al., 2005; 2006). Recently, Sato et al. (2007) examined the effects of oral DFA III on prepartum dairy cows and found that DFA III prevented decrease in blood calcium concentrations at calving. Hence, they suggested that DFA III supplementation may effectively prevent hypocalcemia in dairy cows. In the present study, we found that oral DFA III within 24 h after calving reduced the total duration of medical treatment in calves; this finding suggests that DFA III may have etiologic effects on the health status of calves in the preweaning period. Hence, it is expected that DFA III functions as a prebiotic in calves- a finding previously suggested in rats (Minamida et al., 2005). The potential role of feed additives such as prebiotics in improving the health and performance of calves is of increasing interest because of the public concern about the use of antimicrobials in cattle production (Timmerman et al., 2005). Therefore, although a significant effect of DFA III on growth performance (daily weight gain in live calves) was not observed in the present study, we recommend that DFA III be fed to calves as a supplemental feed additive colostrum and/or CR within 24 h of calving.

Metabolic evaluation revealed significant differences in some blood parameters between the calves receiving DFA III supplements and those not receiving these supplements. These differences might reflect the health status of the calves during the weaning period. The MCH was significantly higher in DFA III group than in the control group. This suggests that DFA III increases Hb production and maintains the MCH within the normal range. Further, supplemental DFA III may accelerate the shift of iron into the Hb in the blood more efficiently in the early stages after calving than in the late stages. Further, the serum iron concentration at 24 h after calving was significantly lower in the DFA III group than in the control group. Iron deficiency is usually primary and most likely to occur in newborn animals because the milk of the dam, which is the exclusive source of iron for these animals, is poor in iron (Radostits, 2000b). In the present study, the calves in both the DFA III and control groups were fed the same type and volume of CR at the same time points after calving. Additionally, it has been reported that plasma iron is independent of the parity or sex of the calf (Kume and

Tanabe, 1996). Although the normal range of serum iron concentrations in neonatal Japanese Black neonatal calves has not yet been clinically determined, we speculate that the difference in the serum iron concentration between the DFA III and control groups could not be attributed to the composition of the CR. It has been suggested that in dairy cattle, the plasma iron concentration decreases during acute-phase immunological responses in diseases such as mastitis. This is because of the increased secretion of binding proteins such as LF in milk; these proteins decrease the amount of available iron and thus reduce the availability of divalent iron, which is required for bacterial growth (Andrieu, 2008). Therefore, the differences in the iron concentrations observed in this study may reflect differences in infection status between the groups within 48 h after parturition. Interestingly, at 1 wk after calving, significant differences were observed in the TP and LF concentrations between the groups. LF is an 80-kDa member of the transferrin family of iron-binding glycoproteins. Studies on LF in humans, experimental animals, and cattle have reported the accumulation of LF, a functional glycoprotein (Yamauchi et al., 1998; Talukder et al., 2003; Legrand et al., 2004; Teraguchi et al., 2004). Talukder et al. (2003) reported that the concentration of plasma LF in neonatal calves is 238.0 ng/ml before and 1,696 ng/ml at 12 h after feeding on their dams' colostrums. Thus, the plasma LF concentration steeply increases after the intake of colostrum. The serum LF concentration at 24 h after the second CR feeding in the present study was consistent with the concentration mentioned in the abovementioned study. This finding suggests that DFA III does not disturb intestinal LF absorption in calves. Some previous studies have also suggested that LF may be a marker of early-stage inflammation due to infectious diseases (Bennet and Kokocinski, 1978; Lash et al., 1983; Maaks et al., 1989). Since the CR fed to the calves contained the same amount of LF, it was suggested that the abundant LF in the colostrum served to mediate bacteriostatic effect and the regulation of iron absorption in newborn calves (Kume and Tanabe, 1996). Therefore, the differences in LF concentrations between the groups may reflect the differences in infection status between the groups. It is well known that calves are immunologically competent at birth, but endogenous antibody production does not usually reach protective levels until 1 month after calving. Additionally, in the case of calves, the level of passive IgG declines slowly and reaches the lowest value at 60 d after calving (Radostits et al., 2000a). In the present study, the IgG concentration at 1 month after calving in the DFA III group was significantly higher than that in the control group. This difference clearly indicates the superior immunological status of the calves in the DFA III group at 1 month after

calving, and this finding is consistent with the difference in duration of medical treatment between the groups at 2 months after calving and at weaning.

At birth, calves do not have Igs in their blood streams and rely on the colostrum for immunity; passive immunity is critical to the survival and health of neonatal calves until they undergo weaning (Besser and Gay, 1994; Wittum and Perino, 1995). However, although feeding on colostrum soon after calving offers important nutritional and immunological benefits to calves, it is also the first instance at which calves are exposed to pathogens (Swan et al., 2007). It has been suggested that commercially available CR products may be a viable alternative to maternal colostrum and could serve as a very effective tool to prevent disease transmission via colostrum (Swan et al., 2007). Furthermore, colostrum leukocytes have been shown to migrate to the peripheral blood in neonatal cattle (Reber et al., 2008). Although the relationship between calf bovine leucosis virus (BLV) infection and colostrum ingestion remains unclear (Meas et al., 2002; Nagy et al., 2007), the virus may be transmitted vertically during suckling of colostrum contaminated with infected lymphocytes. A recent study suggested that the risk of treatment, duration of treatment, and risk of mortality during the preweaning period did not differ between calves fed maternal colostrum and those fed CR (Swan et al., 2007). Therefore, CR may be critical for preventing or controlling disease transmission in herds infected with BLV. Our results strongly suggest the efficacy of CR supplementation with DFA III in calf herds in which vertically transmitted infections warrant control/eradication.

In conclusion, our findings suggest that the addition of DFA III as a supplemental prebiotic to CR and administering this supplemented CR to calves immediately after calving prevents disease in group-housed calves. Our results also support the observation that the serum LF concentration soon after calving may be an indicator of inflammation and predict the health status of calves during the preweaning period. An in-depth study of the effects of DFA III administration on the health status of calves is crucial for the use of DFA III as a daily supplement during the entire preweaning period. Further research is required to elucidate the detailed mechanism of action of DFA III as a prebiotic.

ACKNOWLEDGMENTS

The authors thank Nippon Zenyaku Kogyo Co. Ltd. for donating the colostrum replacers and Mrs. Munehiro Fujisawa, Takeyoshi Kusayanagi, and Keiichi Kambara, Fujitechs Co. Ltd., Japan, for measuring the LF concentrations.

REFERENCES

- Andrieu, S. 2008. Is there a role for organic trace element supplement in transition cow health? *Vet. J.* 176:77-83.
- Bennett, R. M. and T. Kokocinski. 1978. Lactoferrin content of peripheral blood cells. *Br. J. Haematol.* 39:509-521.
- Besser, T. E. and C. C. Gay. 1994. The importance of colostrum to the health of the neonatal calf. *Vet. Clin. North Am. Food Anim. Pract.* 10:107-117.
- Cray, W. C., T. A. Casey, B. T. Bosworth and M. A. Rasmussen. 1998. Effect of dietary stress on fecal shedding of *Escherichia coli* O157: H7 in calves. *Appl. Environ. Microbiol.* 64:1975-1979.
- Donovan, D. C., S. T. Franklin, C. C. L. Chase and A. R. Hippen. 2002. Growth and health of Holstein calves fed milk replacers supplemented with antibiotics or enteroguard. *J. Dairy Sci.* 85:947-950.
- Fleige, S., W. Preißinger, H. H. D. Meyer and M. W. Pfaffl. 2007. Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. *Animal* 1:367-373.
- Fleige, S., W. Preißinger, H. H. D. Meyer and M. W. Pfaffl. 2008. The immuno-modulatory effect of lactulose on *Enterococcus faecium* fed pre-ruminant calves. *J. Anim. Sci.* (In press).
- Franklin, S. T., M. C. Newman, K. E. Newman and K. I. Meek. 2005. Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. *J. Dairy Sci.* 88:766-775.
- Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* 66:365-378.
- Heinrichs, A. J., C. M. Jones and B. S. Heinrichs. 2003. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. *J. Dairy Sci.* 86:4064-4069.
- Inokuma, H., T. Yoshida and T. Onishi. 1995. Development of peripheral blood mononuclear cell response to mitogens in Japanese Black newborn calves. *J. Vet. Med. Sci.* 57:971-972.
- Jouany, J. P. and D. P. Morgavi. 2007. Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. *Animal* 1:1443-1466.
- Kume, S. and S. Tanabe. 1996. Effect of supplemental lactoferrin with ferrous iron on iron status of newborn calves. *J. Dairy Sci.* 79:459-464.
- Lash, J. A., T. D. Coates, J. Lafuze, R. L. Baehner and L. A. Boxer. 1983. Plasma lactoferrin reflects granulocyte activation *in vivo*. *Blood* 61:885-888.
- Legrand, D., E. Ellass, A. Pierce and J. Mazurier. 2004. Lactoferrin and host defense: An overview of its immuno-modulating and anti-inflammatory properties. *Biometals* 17:225-229.
- Maaks, S., H. Z. Yan and W. G. Wood. 1989. Development and evaluation of luminescence based sandwich assay for plasma lactoferrin as a marker for sepsis and bacterial infections in pediatric medicine. *J. Biolumin. Chemilumin.* 3:221-226.
- Maatje, K., J. Verhoeff, W. D. Kremer, A. L. Crujisen and T. S. van den Ingh. 1993. Automated feeding of milk replacer and health control of group-housed veal calves. *Vet. Rec.* 133:266-270.
- Macfarlane, S., G. T. Macfarlane and J. H. Cummings. 2006. Probiotics in the gastrointestinal tract. *Review. Aliment. Pharmacol. Ther.* 24:701-714.
- Meas, S., T. Usui, K. Ohashi, C. Sugimoto and M. Onuma. 2002.

- Vertical transmission of bovine leukemia virus and bovine immunodeficiency virus in dairy cattle herds. *Vet. Microbiol.* 84:275-282.
- Minamida, K., M. Ohashi, H. Hara, K. Asano and F. Tomita. 2006. Effects of ingestion of difructose anhydride III (DFA III) and the DFA III-assimilating bacterium *Ruminococcus* products on rat intestine. *Biosci. Biotechnol. Biochem.* 70:332-339.
- Minamida, K., K. Shiga, I. N. Sujaya, T. Sone, A. Yokota, H. Hara, K. Asano and F. Tomita. 2005. Effects of difructose anhydride III (DFA III) administration on rat intestinal microbiota. *J. Biosci. Bioeng.* 99:230-236.
- Mineo, H., H. Hara, N. Shigematsu, Y. Okuhara and F. Tomita. 2002. Melibiose, difructose anhydride III and difructose anhydride IV enhance net calcium absorption in rat small and large intestinal epithelium by increasing the passage of tight junctions *in vitro*. *J. Nutr.* 132:3394-3399.
- Mitamura, R., H. Hara, Y. Aoyama and H. Chiji. 2002. Supplemental feeding of difructose anhydride III restores calcium absorption impaired by ovariectomy in rats. *J. Nutr.* 132:3387-3393.
- Nagy, D. W., J. W. Tyler and S. B. Kleiboeker. 2007. Decreased periparturient transmission of bovine leucosis virus in colostrums-fed calves. *J. Vet. Intern. Med.* 21:1104-1107.
- Novak, J. and J. A. Katz. 2006. Probiotics and prebiotics for gastrointestinal infections. *Curr. Infect. Dis. Rep.* 8:103-109.
- Ohtsuka, H., S. Komatsu, S. Kon-nai, S. Fukuda, Y. Kuku, T. Yoshino, M. Koiwa and S. Kawamura. 2002. Comparison of peripheral leukocytes in Japanese Black and Holstein calves. *J. Jpn. Vet. Med. Assoc.* 55:789-795 (in Japanese, with English abstract).
- Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. 2000a. Diseases of the newborn. Neonatal infection. In: *Veterinary Medicine*, 9th ed. W.B. Saunders Company, Pennsylvania. pp. 134-146.
- Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. 2000b. Diseases caused by nutritional deficiencies. Iron deficiency. In: *Veterinary Medicine*, 9th Ed. W.B. Saunders Company, Pennsylvania pp. 1505-1509.
- Rasmussen, L., M. B. Jensen and L. L. Jeppesen. 2006. The effect of age at introduction and number of milk-portions on the behavior of group housed dairy calves fed by a computer controlled milk feeder. *Appl. Anim. Behav. Sci.* 100:153-163.
- Reber, A. J., D. C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K. A. Holbert, L. Marshall and D. J. Hurley. 2008. Transfer of maternal colostrum leukocyte promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. *Vet. Immunol. Immunop.* 123:305-313.
- Refsdal, A. O. 2000. To treat or not treat: A proper use of hormones and antibiotics. *Anim. Reprod. Sci.* 60-61:109-119.
- Sato, T., T. Nakai, H. Sadoya, M. Ohtani, M. Hanada and M. Okamoto. 2007. Effect of difructose anhydride III on hypocalcemia in dairy cows after calving. *Anim. Sci. J.* 78:37-43 (in Japanese, with English abstract).
- Shiga, K., H. Hara, G. Okano, M. Ito, A. Minami and F. Tomita. 2003. Ingestion of difructose anhydride III and voluntary running exercise independently increase femoral and tibial bone mineral density and bone strength with increasing calcium absorption in rats. *J. Nutr.* 123:4207-4211.
- Shigematsu, N., Y. Okuhara, T. Shiomi, F. Tomita and H. Hara. 2004. Effect of difructose anhydride III on calcium absorption in humans. *Biosci. Biotechnol. Biochem.* 68:1011-1016.
- Svensson, C. and P. Liberg. 2006. The effect of group size on health and growth rate of Swedish dairy calves housed in pens with automatic milk-feeders. *Prev. Vet. Med.* 73:43-53.
- Swan, H., S. Godden, R. Bey, S. Wells, J. Fetrow and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrums replacer. *J. Dairy Sci.* 90:3857-3866.
- Talukder, M. J., T. Takeuchi and E. Harada. 2003. Receptor-mediated transport of lactoferrin into the cerebrospinal fluid via plasma in young calves. *J. Vet. Med. Sci.* 65:957-964.
- Tamura, A., T. Shiomi, N. Tamaki, N. Shigematsu, F. Tomita and H. Hara. 2004. Comparative effect of repeated ingestion of difructose anhydride III and palatinose on the induction of gastrointestinal symptom in human. *Biosci. Biotechnol. Biochem.* 68:1882-1887.
- Teraguchi, S., H. Wakabayashi, H. Kuwata, K. Yamauchi and Y. Tamura. 2004. Protection against infections by oral lactoferrin: Evaluation in animal models. *Biometals* 17:231-234.
- Timmerman, H. M., L. Mulder, H. Everts, D. C. van Espen, E. van der Wal, G. Klaassen, S. M. Rouwers, R. Hartemink, F. M. Rombouts and A. C. Beynen. 2005. Health and growth of veal calves fed milk replacers with or without probiotics. *J. Dairy Sci.* 88:2154-2165.
- Tomita, K., T. Shiomi, Y. Okuhara, A. Tamura, N. Shigematsu and H. Hara. 2007. Ingestion of difructose anhydride III enhances absorption and retention of calcium in healthy men. *Biosci. Biotechnol. Biochem.* 71:681-687.
- Wenk, C. 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. *Review. Asian-Aust. J. Anim. Sci.* 13:86-95.
- Wittum, T. E. and L. J. Perino. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. *Am. J. Vet. Res.* 56:1149-1154.
- Yamauchi, K., H. Wakabayashi, S. Hashimoto, S. Teraguchi, H. Hayasawa and M. Tomita. 1998. Effect of orally administered bovine lactoferrin on the immune system of healthy volunteers. *Adv. Exp. Med. Biol.* 443:261-265.
- Yokota, A., S. Hirayama, K. Enomoto, Y. Miura and F. Tomita. 1991. Production of inulin fructotransferase (de-polymerizing) by *Arthrobacter* sp. H65-7 and preparation of DFA III from inulin by the enzyme. *J. Ferment. Bioeng.* 72:258-261.