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# Effect of Feeding Direct-fed Microbial as an Alternative to Antibiotics for the Prophylaxis of Calf Diarrhea in Holstein Calves*

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**ABSTRACT :** The objective of this study was to determine the effect of feeding direct-fed microbials (DFM) on the growth performance and prophylaxis of calf diarrhea during the pre-weaning period as an alternative to antibiotics. A multi-species DFM was formulated including three lactic acid bacteria (*Lactobacillus salivarius* Ls29, *Pediococcus acidilactia* Pa175, and *L. plantarum* Lp177), three *Bacillus* strains (*B. subtilis* T4, *B. polymyxa* T1 and SM2), one yeast, *Saccharomyces boulardii*, and a nonpathogenic *E. coli* Nissle 1917. Lactic acid bacteria and *Bacillus* strains were selected based on the antibacterial activity against various animal pathogens, especially pathogenic *E. coli* using agar diffusion methods *in vitro*. Test and control groups were fed milk replacer and calf starter supplemented with DFM ($10^9$ cfu each of eight species/d/head, n = 29) or with antibiotics (0.1% neomycin sulfate in milk replacer and Colistin 0.08% and Oxyneo 110/110 0.1% in calf starter, n = 15), respectively. Overall fecal score and the incidence rate of diarrhea were reduced in the DFM group compared to the antibiotics one. About 40% of calves in antibiotic group suffered from diarrhea while in DFM group only 14% showed diarrhea. There was no difference in the average daily gain and feed efficiency of two groups. The hematological levels of calves were all within the normal range with no significant difference. In conclusion, the feeding of multi-species DFM during the pre-weaning period could reduce calf diarrhea and there was no difference in the growth performance between the groups, thus showing the potential as an alternative to antibiotics. (**Key Words :** Holstein Neonatal Calves, Calf Diarrhea, Antibiotic, Multi-species Direct-fed Microbial)

## INTRODUCTION

Mortality of young calves has been major problems in dairy farms, and the most common disease among the calves is a diarrhea. During the pre-weaning period, young calves are susceptible to many infectious pathogens causing the primary damage to the intestine. The use of antibiotics benefits the young calves in many ways such as decreased incidence of diarrhea, lower calf mortality, and decreased protein requirements (Morrill et al., 1977; Morrill et al., 1995). Particularly, neomycin which acts mainly in gastrointestinal tract when administered orally (Aschbacher and Feil, 1994), is one of the most generally used antibiotics in milk replacers or calf starters to treat diarrhea in young calves (Zwald et al., 2004). So far, only young calves fed milk replacer and calf starter still receive antibiotics on continual basis.

The overuse of antibiotics in animal husbandry may affect the antibiotic resistance of potential human pathogens (Fey et al., 2000) by exerting the selective pressures which render antibiotics ineffective in controlling bacterial diseases (Amabile-Cuevas et al., 1995). In Korea, most commercial dairy cow farm supplements contain a combination of broad-spectrum antibiotics, such as neomycin, amoxicillin and colistin sulfate or oxytetracycline in milk replacer to prevent calf diarrhea. Due to rising public concern regarding the overuse of antibiotics in animal production, interest in the effects of

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direct-fed microbials (DFM) on animal health and performance has increased. The benefits of DFM feeding was based on the potential for a positive intestinal effect, including the establishment of a desirable gastrointestinal microflora and the prevention of the pathogenic bacterial colonization. The U.S. Food and Drug Administration has required feed producers to utilize the term "direct-fed microbial' (DFM) instead of probiotic (Miles and Bootwalla, 1991) and has confirmed the definition to "a source of live, naturally occurring microorganisms" (Yoon and Stern, 1995). For ruminants, microbial cultures have been used to replace or reduce the use of antibiotics in newborn calves, to the increased milk production in dairy cow, and to improve growth performance, feed efficiency and daily gain in cattle (Krehbiel et al., 2003).

In general, the effect of bacterial DFM (mainly *Lactobacillus* species) fed to calves has been to establish and sustain "normal" intestinal microflora rather than as a growth performance enhancer. Abu-Tarboush et al. (1996) reported that calves fed *L. acidophilus* 27SC had a significantly lower scour index compare with calves fed the control diet. These advantages of DFMs were hypothesized to result from the improvement of intestinal microbial flora in neonatal Holstein calves. Non-pathogenic *E. coli* Nissle 1917 also showed a clear beneficial effect on the prophylaxis and treatment of neonatal calf diarrhea (von Buenau et al., 2005).

The objective of this study was to test the effects of a multi-species DFM supplements on the growth performance and the prophylaxis of calf diarrhea in Holstein neonatal calves compared with conventional antibiotic supplement.

## MATERIALS AND METHODS

### Selection of direct-fed microbials

Three lactic acid bacterial strains were selected based on the growth inhibition of various bacterial pathogens from the lactic acid bacterial collections isolated from pig feces using selective culture procedure (Yun et al., 2009).

An agar diffusion test was used for screening of antibacterial lactic acid bacteria and *Bacillus* (Nowroozi et al., 2004). Briefly, the agar plate was spread with $10^6$ cfu of indicator strains and either 30 μl of heated supernatant of lactobacilli or filtered supernatant of *Bacilli* was applied. Plates were incubated at 37°C and observed for inhibition zones. Indicator strains included *E. coli K88*, *E. coli O157*, *Salmonella galinarum*, *S. enteritidis*, *S. typhimurium*, *S. cholerasuis* and *Enterococcus faecalis* (Table 1). Accordingly, a multi-species DFM of eight strains was formulated to contain *Lactobacillus salivarius* Ls29, *Pediococcus acidilactia* Pa175, *L. plantarum* Lp177, *B. polymyxa* T1, *B. polymyxa* SM2, *B. subtilis* T4, *Saccharomyces boulardii* Sb796, and non-pathogenic *E. coli* nissle1917.

**Table 2.** Ingredient composition of experimental milk replacers

| Ingredient (%) | Milk replacers[1] | |
|---|---|---|
| | Control (Antibiotics) | Test (DFM) |
| Whey protein concentrate | 46.25 | 46.35 |
| Isolated soy protein | 12 | 12 |
| MPF 21/22[2] | 40 | 40 |
| Limestone | 0.4 | 0.4 |
| Dry fat, 7/60[3] | 1.25 | 1.25 |
| Neomycin sulfate | 0.1 | ND[4] |

[1] MR = 400 and 524 g/d of milk replacer was fed on 0 to 10 d and 11 to 30 d, respectively.
[2)]MPF 21/22: skim milk 50%, whey 30%, and oil 20%.
[3] Dry fat, 7/60: whey 60%, oil 40%.
[4] ND = Not determined.

### Animals and experimental procedure

All animal-based procedures were in accordance with the "Guidelines for the Care and Use of Experimental Animals of Seoul National University", which were formulated from the "Declaration of Helsinki and Guiding Principle in the Care and Use of Animals".

Holstein male calves about 4 to 5 d of age were purchased from several local farms. Calves were randomly assigned to groups upon arrival in individual plastic calf condo cages (1.5×2.5 m). For 1 week adjustment period, electrolyte therapy (Eltradd, 3 g/L in drinking water; Byer Animal Health Co., Suwon, South Korea) was performed when calves had fecal scour. Therefore, the experiment was started at approximately 12 d of calf age (experiment 0 d).

**Table 1.** Antimicrobial activity[1] of supernatant of test DFMs on the various pathogenic bacteria

| Item | E.88[3] | E.O157 | S.g | S.en | S.t | S.cho | E.f | L.m | S.a | S.suis |
|---|---|---|---|---|---|---|---|---|---|---|
| L.s29[2] | +++ | +++ | - | +++ | +++ | - | - | + | +++ | - |
| P.a175 | ++ | +++ | - | +++ | ++ | - | - | - | ++ | - |
| L.p177 | - | +++ | - | - | ++ | - | - | + | + | - |
| T1 | + | - | ++ | - | + | + | - | - | + | - |
| SM2 | ++ | + | - | - | - | + | - | +++ | - | - |

[1] Interpretation of zone diameter of inhibition (+++: 6 mm, ++: 4 mm, +: 2-3 mm).
[2] Test strains: L.s: *Lactobaciilus salivarius*, L.p: *Lactobaciilus plantarum*, P.a: *Pediococcus acidilactia*, T1: *Bacillus polymyxa*, SM2: *Bacillus polymyxa*.
[3] Indicator strains: E.88: *E. coli K88*, E.O157: *E. coli O157*, S.g: *Salmonella galinarum*, S.en: *Salmonella enteritidis*, S.t: *Salmonella typhimurium*, S.cho: *Salmonella cholerasuis*, E.f: *Enterococcus faecalis*, L.m: *Listeria monocytogenes*, S.a: *Staphylococcus aureus*, S.suis: *Streptococcus suis*.

Calves were fed 3 L/d (containing 400 g of dried diet) of milk replacer formulated to contain 21.81% crude protein and 9.76% fat (Table 3) for the first 10 days and 4 L/d (524 g dried diet) of milk replacer during the 11 to 30 d. The milk replacer was mixed in hot water to disperse the fat component. Cool water was then added to bring temperature to approximately 38°C. Calves were fed twice daily at 8 AM and 5 PM using a plastic bucket. A bucket stand was attached at the front side of individual pens at 50 cm above the floor. At each feeding, a bucket containing milk replacer was fitted into the stand and removed after feeding. Calf starter was offered *ad libitum*, and feed intake was measured as air-dry matter daily. Nutrient composition of milk replacer and calf starter is listed in Table 3. Water was provided free choice and changed twice daily. No hay was fed. Calf condo cages were cleaned throughout the study.

**Experimental design**

The experiment (average temperature: 13°C, relative humidity: 67%) was conducted under field conditions. Animals were fed milk replacer and calf starter supplemented either with DFM (test group; $10^9$ cfu each of eight species/d/head, n = 29) or with antibiotics (control group; 0.1% neomycin sulfate in milk replacer and Colistin 0.08% and Oxyneo 110/110 0.1% in calf starter, n = 15), respectively. DFM includes a combination of three lactic acid bacteria (*L. salivarius* Ls29, *Pediococcus acidilactia* Pa175, and *L. plantarum* Lp177) and three *Bacillus* (*B. subtilis* T4, *B. polymyxa* T1 and SM2), *E. coli* Nissle1917, *Saccharomyces boulardii* in amount of $10^9$ cfu each of eight species/d/head. DFMs were mixed into milk replacer immediately before each feeding.

**Blood collection and analysis**

Blood samples were collected at 3 h after morning feeding (8 AM) on 0, 10, 20 and 30 d of experiment. Blood was sampled by puncture of the jugular vein using evacuated tubes (Vacutainer Systems; Preanalytical Solutions, USA) containing either no anticoagulant for serum separation or K2 EDTA for blood collection. Blood tubes were placed on ice immediately after collection and the collected blood was centrifuged at 3,000 rpm for 15 min at 4°C and plasma was stored at -74°C until it was assayed. Total serum IgG or IgA in samples were determined by ELISA (Bethyl laboratory, Inc, USA) using Nunc immuno plate (MaxiSorb, Nunc Roskilde, Denmark) following the manufacturer's instructions. The values were read at 450 nm using an ELISA reader (GRL 1000, General labs diagnostics, USA). Blood containing K2 EDTA was collected for measurement of red blood cells (RBC), white blood cells (WBC), platelets, hematocrit and hemoglobin by automated hematology analyzer (Sysmex, XE-2100D, Japan).

**Table 3.** Chemical composition of milk replacer and calf starter fed to Holstein calves

| Composition (%) | Chemical composition | |
|---|---|---|
| | Milk replacer | Calf starter |
| Dry matter | 94.45 | 87.72 |
| Crude protein | 21.81 | 16.52 |
| Ether extract | 9.76 | 3.24 |
| Crude fiber | 1.09 | 11.11 |
| Ash | 7.47 | 5.72 |
| Ca | 0.69 | 0.71 |
| P | 0.65 | 0.51 |
| ME(k cal/kg)[1] | 3,800 | ND |
| TDN[2] | ND[3] | 72.15 |

[1] ME = Metabolic energy, calculated from NRC (2001).
[2] TDN = Total digestible nutrients; calculated using the equation of NRC (2001).
[3] ND = Not determined.

**Fecal scoring**

Fecal scoring of fecal fluidity, consistency, odor, and days scoured was conducted daily in the morning (8 AM). Fecal scores based on a four-point scale were recorded using the procedure of Larson et al. (1977). Scoring was as follows: for fecal fluidity, 1 = normal, 2 = soft, 3 = runny, or 4 = watery. A scour day was recorded if fecal fluidity = 3 or 4. The incidence of scours is defined as the number of scouring days during the trial with fecal score >2. A fecal score of 2 was considered to be normal in this experiment.

**Statistical analysis**

Body weight, average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (FE) and diarrhea data were analyzed using the GLM (General Linear Model) procedure of SAS (Statistical Analysis System, V9.1, USA, 2002), and treatment means were compared using the LSD multiple range test. For time and treatment differences, blood serum immunoglobulin and hematological blood concentrations were evaluated using the RANDOM and REPEATED methods of the MIXED procedure (SAS Inst. Inc.). Treatment and time were used as fixed effects and the individual calves were used as random effects. For analyses of differences in time pattern between groups, the interaction (treatment×time) was included in the model.

## RESULTS

**Antimicrobial activities of DFM (direct-fed microbial)**

Lactic acid bacterial strains and *Bacillus* strains were screened for antimicrobial activity by agar diffusion methods against a group of selected bacterial pathogens. Three lactic acid bacteria (*L. salivarius* Ls29, *P. acidilactia* Pa175, *L. plantarum* Lp177) and two *Bacilli* (*B. polymyxa*

**Table 4.** Adjusted means for BW gain and feed efficiency of calves[1]

| Variable[2] | Experiment group | | SE | p-value |
|---|---|---|---|---|
| | Control (Antibiotics) | Test (DFM) | | |
| Calves (no.) | 15 | 29 | | |
| Initial BW (kg) | 44.1 | 43.1 | 0.52 | 0.89 |
| Final BW (kg) | 55.0 | 53.9 | 0.73 | 0.88 |
| ADG (kg) | 0.36 | 0.36 | 0.01 | 0.72 |
| ADFI (kg) | 0.84 | 0.86 | 0.01 | 0.36 |
| FE | 0.424 | 0.415 | 0.01 | 0.90 |

[1] Control and test group was fed milk replacer supplemented with either 0.1% neomycin sulfate or DFM, respectively.
[2] ADG = Average daily gain; FE = Feed efficiency, expressed as kilogram of gain/kilogram of feed.

T1 and SM2) were selected as DFM based on the broad spectrum of antibacterial activity against test pathogens, especially on *E. coli* (Table 1). Accordingly, these DFM strains were used to produce the test DFM. Final DFM contained three other components, *B subtilis* T4, *S. boulardii* and non-pathogenic *E. coli* Nissle 1917.

**Effects of DFM on animal performance**

Mean of average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) in calves of test or control group during the pre-weaning period are given in Table 4. Growth performance parameters ADG (0.36 for both group), ADFI (0.84 vs. 0.86) and FE (0.424 vs. 0.415) was similar between calves fed control (supplemented with antibiotics) or test diet (supplemented with DFM), respectively.

**Effect of DFM on diarrhea frequency**

Mean of diarrhea score by calves fed control or test diet during the pre-weaning period is given in Figure 1. Calf diarrhea was assessed and documented via the fecal score during the 30 d of pre-weaning period. Diarrhea occurred in six calves out of fifteen (40%) in the control group and in 4 calves out of 29 calves (14%) in the test DFM group (data not shown). Diarrhea occurred in calves fed control diet during the entire experiment period, but in calves being fed the DFM diet no sign of diarrhea was found after week 2. Overall fecal score was 0.93 in control and 0.31 in test group (Figure 1).

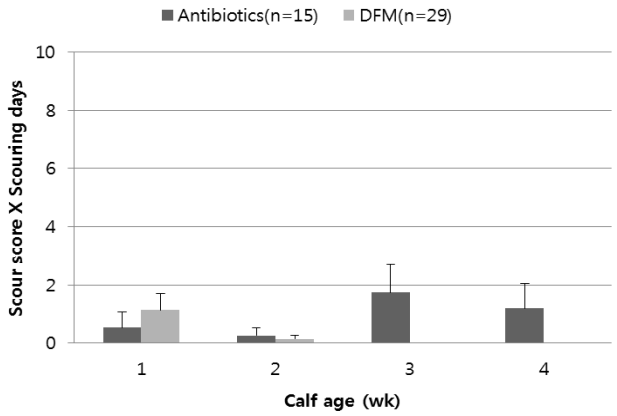


**Figure 1.** Mean (±SE) weekly fecal scores of Holstein calves. Holstein calves of control and test group were fed milk replacer supplemented with either Antibiotics (0.1% neomycin sulfate, n = 15) or DFM (n = 29), respectively. Difference between treatments was not significant (p>0.05).

**Blood biochemical profile**

Blood was collected on 0, 10, 20 and 30 d during the pre-weaning period and immunoglobulin (IgG and IgA) and hematology profile was analyzed (Table 5). Total serum IgG and IgA decreased up to 20 d and thereafter increased in the calves of both control and test group.

Blood hematological profile including RBC, WBC, hematocrit, hemoglobin and platelet were analyzed and the values were all in normal physiological range in animal trial during the 30 d period.

## DISCUSSION

In the animal husbandry fields, the use of antibiotics can disturb the microbial balance responsible for animal resistance to disease. Probiotic DFM may be an alternative to reduce the overuse of antibotics and to yield positive effects on animal performance through the antimicrobial activity against pathogenic bacteria and the stimulation of immune system (Fuller, 1981; Salminen et al., 1998). Krehbiel et al. (2001) reported that the direct-fed microbials as probiotics have been used to replace antibiotics in neonatal calves. Probiotic microorganisms used in piglets decreased pathogenic bacteria in the gastrointestinal tract and increased body weight gain (Pollman et al., 1980; Ratcliffe et al., 1986). In this study, we selected three lactic acid bacteria and three *Bacilli* as DFM components based on their strong inhibition of various pathogenic bacteria especially *E. coli*. In a metaclinical review study, Contrable (2004) showed calves with diarrhea often have small intestinal overgrowth with *E. coli* regardless of the cause of

**Table 5.** Effect of serum immunoglobulins and hematological values in Holstein calves fed milk replacer supplemented with either antibiotics[2] (control, n = 15) or DFM (test, n = 29)

| Item[3] | Treatment | Experiment (d)[1] | | | | SE | p-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 | | Treatment (Trt) | Time (T) | Trt×T |
| IgG (mg/ml) | Control | 6.89 | 6.31 | 5.76 | 5.92 | 0.12 | 0.30 | 0.04 | 0.81 |
| | Test | 6.25 | 6.28 | 5.40 | 5.87 | - | - | - | - |
| IgA (μg/ml) | Control | 200.30 | 68.04 | 66.71 | 77.47 | 6.83 | 0.57 | <0.01 | 0.96 |
| | Test | 197.34 | 80.43 | 71.67 | 90.09 | - | - | - | - |
| RBC (M/μl) | Control | 7.55 | 8.10 | 7.75 | 7.96 | 0.09 | 0.21 | 0.36 | 0.94 |
| | Test | 7.49 | 7.77 | 7.39 | 7.74 | - | - | - | - |
| WBC (K/μl) | Control | 9.71 | 7.80 | 8.15 | 8.27 | 0.19 | 0.50 | 0.21 | 0.38 |
| | Test | 8.33 | 8.23 | 7.65 | 8.60 | - | - | - | - |
| Hb (g/dl) | Control | 9.87 | 10.33 | 9.63 | 9.89 | 0.13 | 0.09 | 0.45 | 0.99 |
| | Test | 9.54 | 9.73 | 9.14 | 9.42 | - | - | - | - |
| Hct (%) | Control | 30.96 | 32.06 | 29.73 | 29.75 | 0.38 | 0.07 | 0.24 | 0.93 |
| | Test | 29.79 | 29.93 | 27.98 | 29.03 | - | - | - | - |
| Plt (K/μl) | Control | 449.13 | 356.80 | 376.06 | 352.73 | 8.99 | 0.34 | 0.11 | 0.42 |
| | Test | 415.00 | 405.13 | 395.79 | 390.03 | - | - | - | - |

[1] Experiment was started at approximately 12 d of calf age (experiment 0 d). The calves were fed colostrums for 4 d, approximately, and moved to individual experiment cage. After 1 week adjustment period, experiment was started. The calves were fed 400 g/d (3 L/feeding) and 524 g/d (4 L/feeding) of diet during 0 to 10 d and 11 to 30 d, respectively.
[2] Antibiotics: neomycin sulfate 0.1%.
[3] RBC = Red blood cell, WBC = White blood cell, Hb = Hemoglobin, Hct = Hematocrit, Plt = Platelet.

the diarrhea and 30% of systemically ill calves with diarrhea have predominantly *E. coli* bacteremia. Thus, he concluded that antimicrobial treatment of calves for diarrhea should be focused on *E. coli* both in the small intestine and in blood. The administration of *Bacillus subtilis* spores suppressed *E. coli* O78:K80 infection in poultry (La Ragione et al., 2001) and tended to have a positive feed efficiency during wk 1 to 4 in post-weaning calves (Jenny et al., 1991). A yeast *Saccharomyces boulardii* showed a beneficial effect in antibiotic-associated diarrhea in a human clinical study (Cremonini et al., 2002; Vanderhoof and Young, 2002). The *E. coli* Nissle 1917 strain has been well recognized in human medicine since 1917 (von Buenau et al., 2005). *In vitro* the *E. coli* strain Nissle 1917 was reported as antagonistically active against *Salmonella spp*. and *Shigella spp*. (von Buenau et al., 2005). Nonpathogenic *E. coli* strain 1917 displaces enteropathogenic *E. coli*, *S. typhimurium* and *Candida albicans in vivo* (Lorenz and Schulze, 1996; Kuzela et al., 2001). The administration of *E. coli* Nissle 1917 showed positive effect on the prophylaxis and treatment of calf diarrhea under field conditions (von Buenau et al., 2005). Therefore, a multi species DFM was formulated to include three lactic acid bacteria and three *Bacillus strains* selected in this study, and a yeast *S. boulardii* and a non-pathogenic *E. coli* strains which was tested by other group.

The effect of bacterial DFM on the performance of calves has been reported by many researchers with positive or negative results. Morrill et al. (1977), Ellinger et al. (1978), and Abu-Tarboush (1996) had negative effects in daily gain response by feeding lactic acid bacteria. Krehbiel et al. (2001) also did not get positive results in daily weight gains in a DFM group compared to the control in a trial involving 466 calves fed $5.0\times10^9$ cfu of lactic acid bacteria (*E. faecium*, *L. acidophilus*, *B. thermophilum*, and *B. longum*).

On the other hand, Bechman et al. (1977) reported 17% improvement of gain when $2.5\times10^{11}$ cfu/d of *L. acidophilus* was added to milk or milk replacer. In a review of the efficacy of bacterial DFM containing various *Lactobacilli* including *L. acidophilus*, *L. plantarum*, *L. casei*, and *S. faecium*, Fox (1988) summarized that DFM supplements had positive effects of a 13.2% increase in ADG, 2.5% increase in feed consumption, and a 6.3% improvement in feed efficiency. Similarly, Gill et al. (1987) reported a 9.3% increase in ADG, 9.5% improvement in feed efficiency, and a 10.9% reduction in morbidity during a 28-d pre-weaning period by feeding a bacterial DFM.

In this study, control or test diet was supplemented with antibiotics or DFM. So the comparison was between antibiotics and DFM supplement affecting on the growth performance and prophylaxis of calf diarrhea. Average daily gain (0.36 for both group) and feed efficiency (0.424 vs. 0.415) was similar between calves fed control and test diet, respectively. Overall diarrhea scour score of test DFM group (0.31) was much lower than the control antibiotic group (0.93). Frequency of diarrhea in DFM group (14%) was much lower than in antibiotic group (40.0%) (Table 4). In this study, we tested the effects of DFM supplements as an alternative to antibiotics. In Korea, milk replacers for the

receiving dairy calves contain one or more antibiotics as a growth enhancer and for prophylactic purpose. Thus it is not relevant to compare with above mentioned animal test results in which control group was fed milk or milk replacers without any antibiotics.

In the neonatal calves, the microbial flora of gastrointestinal tract is sensitive; abrupt changes in diet or the environment can cause alternation in microbial populations in the gastrointestinal tract (Savage, 1977). Tannock (1983) reported that stress often can cause an increased diarrhea in neonatal calves, which is related with declines in the population of *Lactobacillus* in the gut. Viable cultures of *Lactobacillus* and *Streptococcus* in calves have been suggested to decrease the incidence of diarrhea (Bechman et al., 1977; Maeng et al., 1987; Fox, 1988). Calves fed *L. acidophilus* 27SC had a significantly lower diarrhea compared with calves fed the control feed, which confirmed the positive effect of lactobacilli in decreasing the incidence of calf diarrhea but there was no improvement in average daily gain on *Lactobacillus* supplement (Abu-Tarboush et al., 1996).

In this study, overall diarrhea score was higher in antibiotic group compared to DFM group (Figure 1) which indicates that DFM supplement has a clear prophylactic effect better than the antibiotic in Holstein neonatal calves during the pre-weaning period. Accordingly, the customary Korean antibiotics supplemented in milk replacers can be replaced with DFM to improve fecal scour score in calves.

At birth, neonatal calves do not have Immunoglobulins (IgG and IgA) in their blood streams and rely on the maternal colostrum for immunity and thus passive immunity is important to disease control of neonatal calves before weaning (Besser and Gay, 1994; Wittum and Perino, 1995). For a successful passive transfer, the adequate serum IgG concentration of neonatal calves was ≥10.0 mg/ml between 1 and 2 d of age (NAHMS, 1993). It is well known that calves are immunologically competent, but endogenous antibody production does not usually reach protective concentrations until 1 month after birth.

In this study, IgG level reached the lowest point at 20 d (32 d of calf age) and increased at 30 d (42 d of calf age). Serum IgA concentration was maintained from 10 to 30 d (22 to 42 d of calf age) in both groups, in contrary to the sudden drop from 0 to 10 d (12 to 22 d of calf age). These results are in accordance with past research. Heinrichs et al. (2009) reported that the fecal IgA level declined as calf age increased, with the highest level in week 2 ($p<0.01$) for all calves. Intestinal IgA is required to eliminate invading pathogens from the rumen of gastrointestinal tract (Underdown, 1986).

Also, the calves begin to develop certain microflora of gastrointestinal tract at wk 2 of age, and thus serum IgA secretion is high at this point and the maternal IgA decreases quickly in blood (Heinrichs et al., 2009). IgA concentration decreased until wk 4 and then remains stable. In humans, Fukushima et al. (1998) reported that when infants were fed probiotics for 20 days the IgA production peaked at d 8 and declined thereafter. In piglets, fecal IgA levels after 14 d of age declined (Scharek et al., 2005). However, both groups showed no statistical difference in their IgA level over time. In this study, the addition of DFM did not affect RBC, WBC, hemoglobin, hematocrit and platelet during the pre-weaning period. The hematological values in both groups were within the normal physiological range as previously described by Knowles et al. (2000) and there were no significant difference among treatments.

Therefore, the present results may indicate that the feeding of DFM to calves may have beneficial potential as an alternative to antibiotics for the prophylaxis of calf diarrhea in Holstein neonatal calves.

In conclusion, a multi species DFM supplemented to milk replacer showed better prophylactic effects on scour reduction compared with an antibiotic supplement group, indicating that DFM could effectively replace antibiotics in milk replacer. A further study is needed to prove the efficacy of using this DFM in commercial farms on a larger scale.

## REFERENCES

Abu-Tarboush, H. M., M. Y. Al-Saiady and A. H. Keir El-Din. 1996. Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young dairy calves. Anim. Feed Sci. Technol. 57:39-49.

Amabile-Cuevas, C., M. Cardenas-Garcia and M. Ludgar. 1995. Antibiotic resistance. Am. Sci. 83:320-329.

Aschbacher, P. W. and V. J. Feil. 1994. Neomycin metabolism in calves. J. Anim. Sci. 72:683-689.

Bechman, T. J., J. V. Chambers and M. D. Cunningham. 1977. Influence of *Lactobacillus acidophilus* on performance of young dairy calves. J. Dairy Sci. 60:74.

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.

Contrable, P. D. 2004. Antimicrobial use in the treatment of calf diarrhea. J. Vet. Intern. Med. 18:8-17.

Cremonini, F., S. Di Caro, L. Santarelli, M. Gabrielli, M. Candelli, E. C. Nista, A. Lupascu, G. Gasbarrini and A. Gasbarrini. 2002. Probiotics in antibiotic-associated diarrhoea. Dig. Liver Dis. 34:78-80.

Ellinger, D. K., L. D. Muller and P. J. Glantz. 1978. Influence of feeding fermented colostrum and *Lactobacillus acidophilus* on fecal flora and selected blood parameters of young dairy calves. J. Dairy Sci. 61:126.

Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo and S. H. Hinrichs. 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N. Engl. J. Med. 342:1242-1249.

Fox, S. M. 1988. Probiotics intestinal inoculants for production animals. Vet. Med. 83:806-830.

Fukushima, Y., Y. Kawata, H. Hara, A. Terada and T. Mitsuoka. 1998. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. Int. J. Food Microbiol. 42:39-44.

Fuller, R. 1981. Probiotics in human medicine. Gut. 32:439-451.

Gill, D. R., R. A. Smith and R. L. Ball. 1987. The effect of probiotic feeding on health and performance of newly-arrived stocker calves. Okla. Agr. Exp. Stn. MP-119, 202-204.

Heinrichs, A. J., C. M. Jones, J. A. Elizondo-Salazar and S. J. Terrill. 2009. Effects of a prebiotic supplement on health of neonatal dairy calves. Livest. Sci. 125:149-154.

Jenny, B. F., H. J. Vandijk and J. A. Collins. 1991. Performance and fecal flora of calves fed a *Bacillus subtilis* concentrate. J. Dairy Sci. 74:1968-1973.

Knowles, T. G., J. E. Edwards, K. J. Bazeley, S. N. Brown, A. Butterworth and P. D. Warriss. 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age. Vet. Rec. 147:593-598.

Krehbiel, C. R., B. A. Berry, J. M. Reeves, D. R. Gill, R. A. Smith, D. L. Step, W. T. Choat and R. L. Ball. 2001. Effects of feed additives fed to sale barn-origin calves during the receiving period: Animal performance, health and medical costs. Okla. Agr. Exp. Stn. Available: http://www.ansi.okstate.edu/research/2001rr/27/27.htm

Krehbiel, C. R., S. R. Rust, G. Zhang and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81:120-132.

Kuzela, L., M. Kascak and A. Vavrecka. 2001. Induction and maintenance of remission with nonpathogenic *Escherichia coli* in patients with pouchitis. Am. J. Gastroenterol. 96:3218-3219.

La Ragione, R. M., G. Casula, S. M. Cutting and M. J. Woodward. 2001. *Bacillus subtilis* spores competitively exclude *Escherichia coli* O78:K80 in poultry. Vet. Microbiol. 79:133-142.

Larson, L. L., F. G. Owens, J. L. Albright, R. D. Appleman, R. C. Lamb and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60:989-991.

Lorenz, A. and J. Schulze. 1996. Establishment of *E. coli* Nissle 1917 and its interaction with *Candida albicans* in gnotobiotic rats. Mikroo¨kologie und Therapie. 24:45-51.

Maeng, W. J., C. W. Kim and H. T. Shin. 1987. Effect of a lactic acid bacteria concentrate (*Streptococcus faecium* Cernelle 68) on growth rate and scouring prevention in dairy calves. J. Dairy Sci. 9:204-210.

Miles, R. D. and S. M. Bootwalla. 1991. Direct-fed microbials in avian. In Direct-Fed Microbials in Animal Production: A Review of Literature. Nat. Feed Ingred. Assoc., West Des Moines, IA, p. 117.

Morrill, J. L., A. D. Dayton and R. Mickelson. 1977. Cultured milk and antibiotics for young calves. J. Dairy Sci. 60:1105-1109.

Morrill, J. L., J. M. Morrill, A. M. Feyerham and J. F. Laster. 1995. Plasma protein and probiotics as ingredients in milk replacer. J. Dairy Sci. 78:902-907.

NAHMS. 1993. Transfer of maternal immunity to calves. USDA:APHIS:VS, Fort Collins, Co.

Nowroozi, J., M. Mirzaii and M. Norouzi. 2004. Study of *Lactobacillus* as probiotic bacteria. Iranian J. Publ. Health. 33: 1-7.

Pollman, D. S., D. M. Danielson and W. B. Wren. 1980. Influence of *Lactobacillus acidophilus* inoculum on gnotobiotic and conventional pigs. J. Anim. Sci. 51:629-637.

Ratcliffe, B., C. B. Cole, R. Fuller and M. J. Newport. 1986. The effect of yoghurt and milk fermented with a strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. Food Microbiol. 3: 203-211.

Salminen, S., M. A. Deighton, Y. Benno and S. L. Gorbach. 1998. Lactic acid bacteria in health and disease. In: Lactic Acid Bacteria: Microbiology and Functional Aspects (Ed. S. Salminen). Marcel Dekker Pubishers, New York, pp. 211-253.

Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 31:107-133.

Scharek, L., J. Guth, K. Reiter, K. D. Weyrauch, D. Taras, P. Schwerk, M. F. G. Schmidt, L. H. Wieler and K. Tedin. 2005. Influence of a probiotic Enterococcus faecium on development of the immune system of sows and piglets. Vet. Immunol. Immunopathol. 105:151-161.

Tannock, G. W. 1983. Effect of dietary and environmental stress on the gastrointestinal microbiota. Human Intestinal Microflora in Health and Disease (Ed. D. Hentges). Academic Press, New York, pp. 517-539.

Underdown, B. J. 1986. Immunoglobulin A, strategic defense initiative at the mucosal surface. Annu. Rev. Immunol. 4:389-417.

Vanderhoof, J. A. and R. J. Young. 2002. Probiotics in pediatrics. Pediatrics 109:956-958.

von Buenau, R., L. Jaekel, E. Schubotz, S. Schwarz, T. Stroff and M. Krueger. 2005. *Escherichia coli* strain nissle 1917: Significant Reduction of Neonatal Calf Diarrhea. J. Dairy Sci. 88:317-323.

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.

Yoon, I. K. and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. Asian-Aust. J. Anim. Sci. 8:533-555.

Yun, J. H., K. B. Lee, Y. K. Sung, E. B. Kim, H. G. Lee and Y. J. Choi. 2009. Isolation and characterization of potential probiotic lactobacilli from pig feces. J. Basic Microbiol. 49: 220-226.

Zwald, A. G., P. L. Ruegg, J. B. Kaneene, L. D. Warnick, S. J. Wells, C. Fossler and L. W. Halbert. 2004. Management practices and reported antimicrobial usage on conventional and organic dairy farms. J. Dairy Sci. 87:191-201.