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Effect of Probiotic and Milk Feeding Frequency on Performance of Dairy Holstein Calves*

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Abstract: Localization of normal microflora in the GIT of ruminants is an important factor, which enhances evolution of their digestive system. Probiotics are one of these options, which contribute in this manner. The present study evaluated single or twice daily milk feeding with and without probiotic on daily calves' performance. Forty Holstein calves weighing 43±5 kg were used in a completely randomized design test to study the effects of probiotic on food intake, blood characteristics and coliform bacteria population of feces. Treatments were as follows: T1) control with feeding four liters of milk twice daily, T2) probiotic with feeding four liters of milk twice daily, T3) control with feeding four liters of milk once daily, T4) probiotic with feeding four liters of milk once daily. From first day to end of the experiment (49 days) T2 and T4 received one-gram probiotic in their milk daily. Water and calf starter were offered free choice. Calves were weighed weekly. Intakes of starter were recorded daily and jugular blood sample were taken every 10 days for recording albumin (A), IgA, IgG and A/G ratio. Feces samples were taken every two weeks. Despite findings in earlier reports the results of this experiment did not confirm the positive effect of probiotic on calf performance. Feed intake mean for calves in treatment 2, was 370.8 g and for treatment 1 was 351.8 g. Body weight gain for group with probiotic (T2, T4) and control (T1, T3) was 441.5 and 422.8 g day^{-1}, respectively. Feed efficiency for probiotic treatments and control groups was 1.3 and 1.4 g g^{-1}, respectively. There was no significant difference ($p>0.05$) between probiotic and non-probiotic groups for any of the performance parameters recorded. Feed intake, body weight gain and Feed efficiency for calves feeding milk once daily and twice daily was 372.4 and 348.8 g, 381.6 and 480.9 g day^{-1}, 1 and 1.4 g g^{-1}, respectively. Feed intake was not significantly different ($p>0.05$) between groups with feeding milk once or twice daily, but body weight gain and feed efficiency were significantly ($p<0.05$) different between the treatments. In conclusion, probiotic used in this study did not affect the performance of dairy calves, but feeding milk once or twice daily influenced their performance.

Key words: Calf, probiotic, milk feeding frequency, performance, blood proteins

Introduction

Due to the current systems of animal rearing, including confinement housing, early weaning and the movement of animal from range of feedlot systems, the normal microflora population of the gut may become out of balance. The aim of feeding probiotics is to correct the deficiencies in the gut microflora and restore the protective effect (Sissons, 1989; Fuller, 1990). Direct- fed microbial, referred

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to as probiotics, are live, naturally occurring bacterial supplements. Many commercially available bacterial DFM for cattle contain lactate- producing organisms from the *lactobacillus* genus (Kung, 1999). Lactic acid bacteria might benefit the host in situations, such as to prevent enteric infections or to act as immunomodulatory agents in other processes (Perdigon *et al*., 1995). The increased BW gain, milk production and total tract digestibility of feedstuffs are some of beneficial effects of probiotics. However, animal responses to probiotics have been inconsistent (Caton *et al*., 1993; Denigan *et al*., 1992; Varel and Kreikemeier, 1994; Williams *et al*., 1986).

Feeding calves viable cultures of species of Lactobacillus and Streptococcus has been reported to decrease the incidence of diarrhea (Abu-Tarboush *et al*., 1996; Agarwal *et al*., 2002). But performance results for neonatal calves consuming bacterial DFM have been variable. Morrill *et al*. (1977), Ellinger *et al*. (1978), Abu- Tarbush *et al*. (1996) Ruppert *et al*. (1997) and Cruywagen *et al*. (1996) reported no improvement in daily gain as a result of feeding lactobacilli. In contrast, Alves *et al*. (2000) reported improved rates of gain when probiotic was added in to milk. Feed efficiency is generally not altered by feeding DFM to young calves (Jenny *et al*., 1991; Abu-Tarbush *et al*., 1996).

Rumen development, the prime objective of any calf rearing enterprise, is to quickly and effectively convert a calf from a high lost, high risk, labour intensive milk diet to an animal that can efficiently convert solid feed in to live weight gain (Schills, 2001). Previous research has shown that feeding milk replacer once daily reduces labour without affecting health, weight gain, or starter consumption by calves (Galton and Braker, 1976). To obtain early rumen development, the first step is to encourage the calf to eat dry matter as soon as possible. It must be stressed however, that once a day milk feeding is perhaps the greatest assets available to assist early rumen development (Schils, 2001). Hopkins (1997) reported that rates of body weight gain were excellent when calves were fed 3.8L of whole milk once daily to weaning at 28 days.

The objective of this study was to evaluate the effect of a probiotic and milk feeding frequency on performance of dairy Holstein calves and also to study the effect of probiotic (Bioplus 2B) on prevention of stress on calves fed milk once a day.

Materials and Methods

This study was done in Isfahan Milk and Meat Company at summer 2004. Forty Holstein calves (Mean BW 43±5) in groups balanced by sex and weight were randomly assigned at 3 days of age to one of four treatments: 1) 4 L of whole milk fed twice a day without probiotic, 2) 4 L of whole milk fed twice a day with probiotic, 3) 4 L of whole milk fed once a day without probiotic and, 4) 4 L of whole milk fed once a day with probiotic. Throughout the 7 week trial, calves were placed in individual calf hutches with *ad libitum* access to water and starter ration (Table 1). The probiotic was a commercial preparation containing *Bacillus subitillis* (CH 201) and *Bacillus lishniformis* (CH 200) (hr. Hansen, Milwaukee, WI). Treatments 2 and 4 were offered 1 g probiotic once a day providing 3.2×10^9 cfu day^{-1} head^{-1}.

Starter intakes were recorded daily and body weight was measured weekly. Five calves per treatment were blood sampled from the jugular vein in a 10 days interval. Samples were centrifuged (3000 rpm, 20 min) and plasma was frozen at -18 until analyzed for albumin and Immunoglubolins. Fecal samples of 5 calves in each treatment were collected prior to the experiment as well as weeks 2, 4 and 6 for determination of total coliform bacteria. For determination of bacterial count, 10 g of feces

Table 1: Ingredient composition of the starter ration

Item	DM%
Barley	29.3
Corn	20.0
Cottonseed meal	5.0
Soybean meal	27.0
Wheat bran	12.0
Vitamin and mineral Supplement	2.0
Limestone	2.0
Dicalcium phosphate	2.0
Salt	0.7

were diluted in 90 mL of sterile normal saline. Serial dilutions of the original extract were made to achieve a final volume of 0.1 mL plate in McConkey agar (Antec- Iran) and then incubated. After the time and conditions of incubation were optimized coliforms then were counted according to predefined standard procedures.

The study was performed in a completely randomized design and data were analyzed using the proc. GLM of SAS (1999). Treatment effects were declared significant at $p<0.05$ and trend were discussed when $p<0.10$. Means were compared using Duncan's where F test for the treatment effect was significant.

Results and Discussion

Feed intake during the trial was higher ($p<0.10$) for calves Mean Feed intake during the trail was higher ($p<0.10$) for calves receiving milk twice daily plus probiotic and those receiving milk once daily without probiotic compared to treatment 1 and 4 (Table 2). Probiotic did not influence feed intake in this experiment ($p> 0.10$) (Table 3). This result is in contrast with those reported by Higginbotham and Bath (1993) and Ruppert *et al.* (1994).

According to the Ruppert *et al.* (1994), when the diet was supplemented with a probiotic and when calves kept under stressful conditions, feed intake of calves (2 to 28 days) was higher than that of calves in the negative control group. Jenny *et al.* (1991) also observed no difference in dry matter intake of solid feed when *B. Subitilis* was fed to calves.

Average daily starter intake was not affected ($p>0.10$) by milk feeding frequency (Table 4). Average Daily Gain (ADG) was significantly different ($p<0.05$) for calves fed milk twice daily plus probiotic than calves fed milk once daily plus probiotic. Windschitl *et al.* (1991) reported that ADG was no statistically different between the control and probiotic treatments and it 1.94 Ibs/day for both of them.

Average daily gain was unaffected by probiotic (Table 3). Daily gain did not differ among calves receiving lactic acid- producing bacteria vs. no DFM (Krehbiel *et al.*, 2001). Previous reports (Cerna *et al.*, 1991; Higginbothem *et al.*, 1997; Panda *et al.*, 1995.) have noted improvement in gain when Lactobacillus products were added to milk or milk replacer fed to dairy calves, whereas other researchers (Cruywagen *et al.*, 1996; Windschitl *et al.*, 1991) have found no improvement in growth. Abe *et al.* (1995) reported that BW gain of calves in the group fed probiotic (*B. pseudolongum* and *L.acidophilus*) was greater than that of the control group. *Bifido bacterium pseudolongum* has been isolated from many kinds of animals, including calves, piglets, chickens, dogs and others, suggesting that this bacterial species has a wide host specificity and might be commercially useful as a probiotic

Table 2: Effect of probiotic and milk feeding frequency on performance and coliform numbers in the feces of calves

Item	Control fed milk twice daily	Probiotic fed milk twice daily	Control fed milk once daily	Probiotic fed milk once daily	SE[a]
DMI (g day^{-1})	298.7[e]	412.3[d]	415.9[d]	298.2[e]	48.6
ADG (g day^{-1})	438b[bc]	522.8[b]	407.1[bc]	356.1[c]	41.7
FE (g g^{-1})	1.5[d]	1.3[d]	0.98[e]	1.2[d]	0.44
BW	58	60	57	58	1.1
Feces coliforms($\times10^5$)	0.35	2.1	1.3	0.99	0.95[a]

Standard error, [b,c] Within a row, means without a common superscript letter differ (p<0.05), [d,e] Within a row, means without a common superscript letter differ (p<0.10)

Table 3: Effect of probiotic on performance and coliform numbers in the feces of calves

Item	Control	Probiotic	SE [a]
DMI (g day^{-1})	351.8	370.8	35
ADG (g day^{-1})	422.8	441.5	30
FE (g g^{-1})	1.4	1.3	0.3
Feces coliforms ($\times10^5$)	0.8	1.6	0.6

[a]Standard error

Table 4: Effect of milk feeding frequency on performance and coliform numbers in the feces of calves

Item	Fed milk once daily	Fed milk twice daily	SE[a]
DMI (g day^{-1})	372.4	348.8	35
ADG (g day^{-1})	381.6[b]	480.9[a]	29
FE (g g^{-1})	1[b]	1.4[a]	0.31
Feces coliforms ($\times10^5$)	1.1	1.2	0.6[a]

Standard error., [b,c] Within a row, means without a common superscript letter differ (p<0.05)

(Mitsuoka and Kaneuchi, 1977; Rasic and Kurmann, 1983). But average daily gain was not different significantly when calves fed replacer containing the *B. subitilis* concentrate (Jenny *et al*., 1991). It has been suggested that at time of stress, such as weaning, the balance of intestinal bacteria may become disturbed and performance reduced (Fuller, 1989; Schwab *et al*., 1980).

Average daily gain was affected (p<0.05)by milk feeding frequency. Calves that fed milk twice daily showed the highest gain during the trial (Table 4). Stanley *et al*. (2002) however, reported that milk replacer feeding frequency did not affect BW.

Researchers in the UK found no effect of feeding frequency (1, 2, 4 or 6 times daily) on growth rates or energy balance when calves were less than 28 days of age (Quigley, 2001). In addition Hopkins (1997) reported that rates of body weight gain were excellent when calves were fed 3.8 L of whole milk once daily to weaning at 28 days.

Feed efficiency was different among treatments and calves fed milk once daily without probiotic have the largest FE (Table 2). Probiotic did not influence (p>0.10) feed efficiency in this study (Table 3). Abe *et al*. (1995) however, reported that feed conversion of the calves fed *bifidobacteria* or *lactobacilli* was superior to that of the control group.

Schwab *et al*. (1980) reported that when calves were fed a Lactobacillus fermentation product there was a trend toward improved feed efficiency. But Jenny *et al*. (1991) observed no differences in Feed efficiency when *B. subitilis* was fed to calves.

Variability among calves in early growth and acceptance of dry feeds make it difficult to show benefit of probiotics when effects are likely only in animals in which the gut microflora is out of balance. Feed efficiency was affected by (p<0.05) milk feeding frequency and it was greater for those fed milk twice daily than for calves fed milk once daily (Table 4).

Table 5: Effect of probiotic and milk feeding frequency on blood parameters

Item	Control fed milk twice daily	Probiotic fed milk twice daily	Control fed milk once daily	Probiotic fed milk once daily	SE [a]
Albumin (%)	51.7^{bcd}	50.3^{bc}	48.2^{ce}	52.2^{b}	1.3
α-globulin	15.2	14.4	15.4	13.8	0.74
β-globulin	17.4	17.7	19.3	17.6	0.84
γ-globulin	15.6	17.5	16.5	16.3	1.3
Albumin/globulin	1.1	1	1.6	1.1	0.25

[a] Standard error., [b,c] Within a row, means without a common superscript letter differ ($p<0.05$), [d,e] Within a row, means without a common superscript letter differ ($p<0.10$)

Treatments had no effect on the occurrence of diarrhea and fecal counts of coliforms (Table 2). Fecal counts of coliform were affected ($p>0.10$) neither by probiotic (Table 3) nor milk feeding frequency (Table 4). Previous researchers (Gilliland *et al*., 1980; Abu *et al*., 1996) have suggested that animals experiencing normal stools are less likely to be shedding coliform in feces. Fecal shedding of coliform has generally not increased when calves were not experiencing diarrheas (Gilliland *et al*., 1980; Abu *et al*., 1996). Schwab *et al*. (1980) observed no apparent effect of *L. bulgaricus* fermentation product on numbers of fecal Latobacilli and coliforms. Abe *et al*. (1995) reported that the addition of bifidobacteria to the diets of calves reduced fecal scours. In this study it seems, the gut microflora was in balance, therefore, not as readily affected by the use of probiotic.

Blood plasma immunoglobulins were not affected by treatments and only in calves fed milk once daily without probiotic. Albumin was lower than other groups ($p<0.05$, $p<0.10$) (Table 5). Albumin was influenced by probiotic treatment in the group which was under one time milk feeding ($p< 0.05$). In addition the group which received milk frequently showed change in plasma albumin level ($p<0.05$). Mcknight *et al*. (1991) and Morril *et al*. (1995) observed no apparent effect of probiotic on blood parameters.

The effect of feeding regimen and stress appeared to complicate the observation of absolute effects of probiotics on calf performance and, according to Fuller (1989), probiotics are only effective when animals are stressed by the presence of a microbial population that depresses growth. Management and feeding condition in our study were good and probiotic might have had different effects under sub optimal conditions.

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