

## Effect of doses of direct-fed microbials plus exogenous fibrolytic enzymes supplementation on growth, feed efficiency ratio and fecal consistency index of brown swiss and holstein Friesian Calves

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### ABSTRACT

The objectives of this study were to compare the effect of different doses of direct-fed microbials (DFM) plus exogenous fibrolytic enzymes (EFE) additives on the growth performance, feed efficiency ratio and fecal consistency index of Brown Swiss and Holstein Friesian calves. Twenty six calves were assigned to three groups (control, 10 g and 20 g per head/day of DFM plus EFE) according to breeds. Calves in 10 g per head/day of DFM plus EFE group in pre- and post-weaning periods had 20.0 % and 6.3 % higher total weight gains than calves in the control group respectively. However, the effects of breeds and dosages of the supplement on the weights, weight gains and feed efficiency ratios were not significant. Average fecal consistency score of the calves fed a diet supplemented with 10 g head/day of DFM plus EFE had the lowest score ( $P < 0.01$ ) (i.e., less scouring) compared to other treatment groups in pre-weaning period as well as between birth and 6 months of age. The study revealed that the feeding of DFM plus EFE to dairy calves until 6 months of age did not result in statistically significant improvement on the growth traits and feed efficiency ratio. On the other hand, the dose of 10 g head/day of the DFM plus EFE additives might be beneficial for reducing incidence of diarrhea in dairy calves.

**Key words:** Calves, Diarrhea, Direct fed microbials, Fibrolytic enzymes, Weight gains

### INTRODUCTION

In many countries, use of hormones and antimicrobial growth promoters for livestock production due to increasing public concern has recently met with considerable resistance. As a consequence of that fact, most of the traditional growth-promoting substances were forbidden by laws in these countries. Therefore, the use of direct-fed microbials (DFM) and other nontraditional feed additives has risen in response to request for using more “natural” growth stimulants.

According to Nocek and Kautz (2006), the inclusion of a DFM in dairy cow diets has become a generally accepted practice. Numerous studies have shown that cows fed direct-fed microbials have altered intestinal bacterial populations, improved resistance to disease, and enhanced health and performance. However, research on the effects of direct-fed microbials on young calves is much less clear (Quigley, 2011). Many studies (Timmerman *et al.* 2005; Adams *et al.* 2008

and Seo *et al.* 2010) found out that DFM could regulate diarrhea incidence as well as improve weight gain and feed efficiency. However, others (Goncalves *et al.* 2000; Quintero-Gonzalez *et al.* 2003; Frizzo *et al.* 2008) did not reported significant effects of DFM on the growth performance of dairy calves.

Optimum doses of fibrolytic enzymes for large ruminants have not been well established but interestingly, several publications carried out on adult animals have reported that high levels of enzymes resulted in lower milk yields than moderate levels of enzyme treatment (Lewis *et al.* 1999; Kung *et al.* 2000). Over treatment of feeds with enzymes may result in blocking sites that may otherwise be available for microbial enzymatic digestion or may prevent attachment by rumen microbes. More research that will be conducted on the dairy calves is needed in this area.

Distinct effects of feeding DFM or EFE on the growth performance of calves were already reported,

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however, there is still lack of information about influence of feeding various doses of DFM in combination with EFE on the growth characteristics and fecal consistency index of Brown Swiss and Holstein Friesian calves. Therefore, the present study was undertaken to compare the effects of 3 doses of DFM plus EFE on the daily gain, feed efficiency ratio, gains in body measurements and fecal consistency index indicating incidence of diarrhea of dairy calves.

## MATERIALS AND METHODS

The research was conducted in Research Farm of Agricultural Collage at Ataturk University, Erzurum, Turkey. Sixteen female Brown Swiss and 10 female Holstein Friesian calves were used in this study. The calves were randomly assigned at birth to one of three treatments [control (n=11), 10 g (n=7) and 20 g (n=8) per head/day of DFM plus EFE groups] according to breeds. The dairy calves suckled their dams in order to receive colostrum for 3 days. Then, they were housed in a calf barn that had individual pens furnished by feeders and plastic water buckets. They were weaned at 45 days of age. At the weaning time, the calves were able to consume about 750 g per head/day of the starter. The amount of milk given each calves was kept constant at 8 % of their birth weight through the milk feeding period as suggested by Yanar *et al.* (1994). DFM plus EFE supplement in dried form was purchased from a feed company and used in this research. Young animals in the all treatment groups were offered same basal diet consisting of calf starter and dried hay after first week of calves' ages. Ten or 20 g per head/day of DFM (included minimum  $2 \times 10^{11}$  cfu/kg of *Lactobacillus sp.*, minimum  $1.8 \times 10^9$  cfu/kg of *Saccharomyces cerevisiae*) and EFE ( included 28 000 unit/g of protease, 52 000 unit/g amylase, 14 000 unit/g of cellulase, 1000 unit/g of pectinase, 2000 unit/g of lipase) combination were offered to calves in second and third treatment groups. The DFM plus EFE supplement was given to calves after blending with whole milk during the milk feeding period. After weaning, 10 or 20 g per head/day of DFM plus EFE were offered to the calves by adding into the 250 g per head/day of starters every morning. Then, the rest of the starter was served to the calves, after the starter supplemented with DFM and enzymes was completely consumed by the animals. Two kinds of calf starters were used in this study. Chemical compositions of the starter-I and starter-II as well as dry hay were presented in Table 1. While starter-I was fed by the calves until 4 months of age, starter-II was offered to the young animals between 4 and 6 months of ages. Amount of starter-I and starter-II was limited to 2 kg per head/day. Dry hay was given as *ad libitum* during 6 months. Amount of feed (whole milk, dry hay and calf starters) consumed daily by each calf was also determined throughout the trial.

Body weights and body measurements were determined and recorded at birth, weaning, 4 and 6 months of ages. Body measurements were 1) heart girth, smallest circumference obtained from just behind forelegs with the cow standing square on her legs; 2) height at withers, distance from ground to the highest point of withers; 3) chest depth, vertical distance from the back to the floor of the chest at the shallowest part of the chest; 4) body length, horizontal distance from the front point of shoulder to the end of the pin bones; 5) Cannon bone girth, circumference of the metatarsal bones.

The fecal fluidity score developed by Larson *et al.* (1977) according to physical appearance of feces was used in the present study, in which score 1: feces is firm but not hard (normal); score 2: feces does not hold form, piles but spreads slightly (i.e., soft serve ice milk), score 3: feces spreads readily to about 6 mm depth (i.e., pancake batter), score 4: liquid consistency, splatters (i.e., orange juice).

The data were analyzed statistically by using a 3x2 completely randomized factorial experimental design. Since interactions between breed and doses of DFM plus EFE were not significant in the preliminary statistical analysis, they were excluded from the statistical model. Birth weight was also included in the mathematical model as covariate for analysis of different weights, weight gains, feed efficiency ratio and gains in body measurements. The fecal fluidity scores was also subjected to analysis of variance, since they were found out that they had normal statistical distribution. The ANOVA and Duncan's multiple comparison test were carried out by using SPSS (2004) statistic package program.

## RESULTS AND DISCUSSION

Holstein Friesian calves had 11.7 % (3.81 kg) heavier ( $P < 0.05$ ) birth weight compared to Brown Swiss calves (Fig. 1). However, the weight differences between breeds at weaning, 4 and 6 months of ages were not statistically significant. The result is in accordance with the findings of Ertugrul *et al.* (2000) and Guler *et al.* (2003). Average birth weights of the calves in the different DFM plus EFE treatment groups were not statistically significant, since the calves used in the current study were randomly assigned to the treatment groups. Additionally, weights obtained at

TABLE 1: Chemical compositions of diets used in this study.

| Nutrients (%)           | Starter-I | Starter-II | Dry Hay |
|-------------------------|-----------|------------|---------|
| Dry matter              | 89.6      | 90.5       | 93.9    |
| Crude protein           | 21.0      | 20.0       | 12.0    |
| Crude ash               | 5.1       | 6.0        | 8.5     |
| Ether extract           | 4.9       | 5.4        | 3.5     |
| Acid detergent fiber    | 35.0      | 36.0       | 38.0    |
| Neutral detergent fiber | 40.0      | 42.0       | 48.0    |

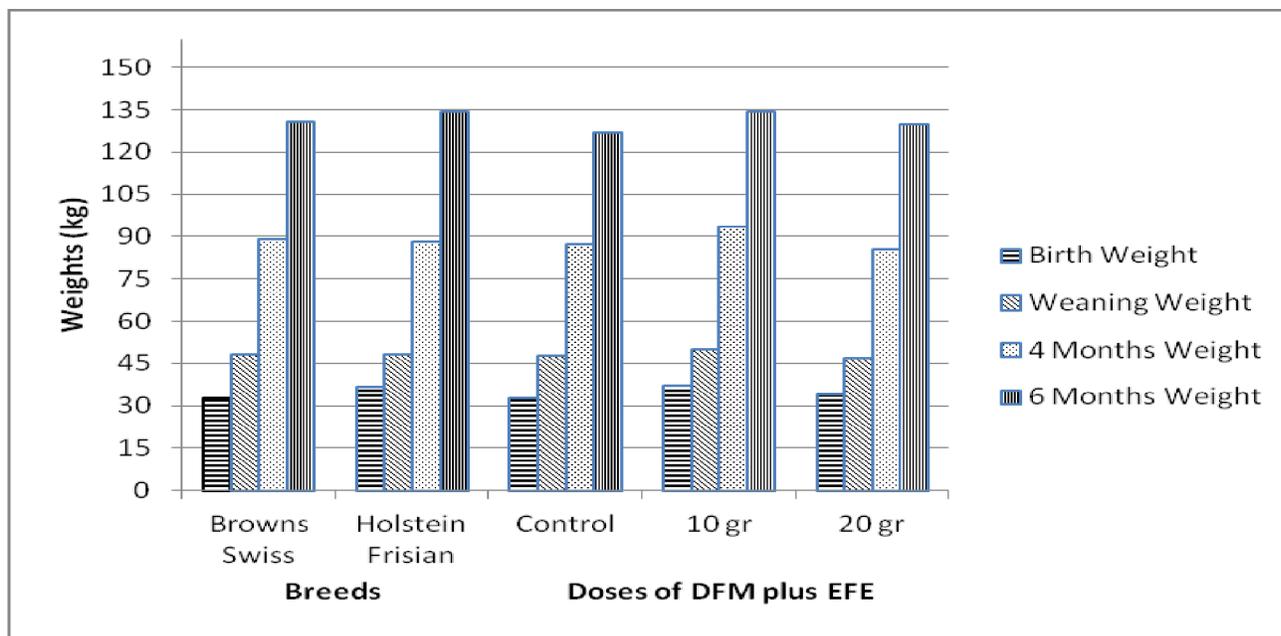


FIG. 1: Live weights obtained at different ages of the dairy calves.

various stages of growth of the calves were not significantly affected by the different doses of DFM plus EFE and breeds. Similarly, Aydin *et al.* (2008) also reported that there were insignificant differences in the body weights of calves in DFM and EFE or control groups at various stages of growth. On the other hand, 4 and 6 month weights of calves in 10 g per head/day of DFM plus EFE group were numerically 6.9 % and 5.7 % greater than these of animals in the control group. Least square means with standard error for total weight gains at pre-weaning and post-weaning periods as well as between birth and 6 months of age are tabulated in Table 2. Even though, in the pre-weaning and post-weaning periods, the calves in 10 g per head/day of DFM plus EFE group had

20.0 % and 6.3 % higher total weight gains than calves in the control group, the effects of breeds and dosages of the supplement on these parameters were not significant. The effect of bacterial DFM on the weight gains of calves has been reported by many researchers with negative or positive results. While Goncalves *et al.* (2000), Kamra *et al.* (2002), Gorgulu *et al.* (2003), Quintero-Gonzalez *et al.* (2003), Aydin *et al.* (2008) and Kim *et al.* (2011) reported no significant improvement in the weight gains of calves fed DFM, Adams *et al.* (2008), Jatkauskas and Vrotniakiene (2010), Frizzo *et al.* (2011), Dimova *et al.* (2013) also stated that weight gains of the calves fed the DFM-supplemented diet was significantly greater than these of the calves consumed the DFM-free diet

TABLE 2: Least square means and standard error for weight gains (kg) in different periods of the growth of dairy calves.

|                       | N  | Gains at pre-weaning period | Gains at post-weaning period | Gains between birth and 6 months of age |
|-----------------------|----|-----------------------------|------------------------------|---|
|                       |    | $\bar{X} \pm S_{\bar{x}}$   | $\bar{X} \pm S_{\bar{x}}$    | $\bar{X} \pm S_{\bar{x}}$               |
| Overall Mean          | 26 | 14.47±0.80                  | 81.93±2.21                   | 97.09±2.32                              |
| Breeds                |    | ns                          | ns                           | ns                                      |
| Brown Swiss           | 16 | 14.32±1.01                  | 82.44±2.81                   | 95.96±2.95                              |
| Holstein Friesian     | 10 | 14.62±1.24                  | 81.42±3.65                   | 98.21±3.61                              |
| Doses of DFM plus EFE |    | ns                          | ns                           | ns                                      |
| Control               | 11 | 13.71±1.23                  | 79.05±3.56                   | 92.24±3.59                              |
| 10 g                  | 7  | 16.45±1.48                  | 84.04±4.36                   | 103.02±4.31                             |
| 20 g                  | 8  | 13.25±1.38                  | 82.69±3.70                   | 96.00±4.02                              |

Mean weight gains at pre-weaning and post-weaning periods as well as between birth and 6 months of ages are adjusted for covariate (birth weight). \*\*, P< 0.01, \*, P< 0.05, ns: Nonsignificant

throughout the trials. Thakur *et al.* (2010) compared effect of the two levels of (1.5 and 3.0 g mixture/kg DM of TMR) exogenous fibrolytic enzymes supplementation on the body weight gain, and concluded that supplementation of enzymes at 1.5 g mixture/kg DM caused a higher growth rate than 3.0 g mixture/kg DM in Murrah buffalo calves. Similarly increasing doses of DFM plus EFE in the present study did not result in a linear increase in total weight gain in the various parts of the growth period of calves. The maximum weight gain was obtained from calves consumed diet supplemented with 10 g per head/day of DFM plus EFE instead of animals received 20 g per head/day. Likewise, Orr *et al.* (1988) suggested that feeding a high dose of *L. acidophilus* to feeder calves ( $10^{10}$  cfu per head/day) had no effect on weight gain when compared to feeding a lower dose ( $10^6$  cfu per head/day). Additionally, Malik and Bandla (2010) reported that dose was not important for the probiotics of *L. acidophilus*, whereas, higher dose of  $3 \times 10^9$  cfu/flask was more effective with the strains of *S. cerevisiae* in buffalo calves.

Rate of growth for Brown Swiss and Holstein Friesian calves at pre-weaning and post-weaning periods were comparable. The result is in agreement with the finding of Guler *et al.* (2003). On the other hand, Ugur (2003) reported that daily weight gains between birth and 6 months of ages were higher in favor of Brown Swiss calves, while others noted that Holstein Friesian calves had greater weight gain than Brown Swiss calves (Yanar *et al.* 1994, Ertugrul *et al.* 2000).

Amounts of dry matter intake of feeds including milk, hay and starter I and II per kg weight gain from birth to 6 months of age are presented in Figure 2. Brown Swiss calves had 33.3 % and 19.0 % lower ( $P < 0.01$ ) feed conversion ratio for starters and total feed respectively than Holstein Friesian calves. However, levels of DFM plus EFE supplements did not result in significant influence on the feed efficiency ratio calculated in a period between birth and 6 months of age. Similar findings are also reported by Bakhshi *et al.* (2006), Frizzo *et al.* (2008), Aydin *et al.* (2008) and Kim *et al.* (2011) who did not observed significant influence of the feeding of DFM on the feed efficiency ratio. However, Timmerman *et al.* (2005) and Frizzo *et al.* (2011) suggested that DFM had beneficial effect on the feed efficiency ratio in calves, especially, when the calves were stressed and disease incidence was significant.

Breed had significant ( $P < 0.01$ ) influence on the amounts of starter and total feed consumed per kg weight gain, and Brown Swiss calves consumed 0.86 kg of starter and 0.90 kg of total feed less than Holstein Friesian calves per kg weight gain (Figure 2). The result is in accordance with the finding of Guler *et al.* (2003).

Least square means with standard error for gains in body measurements between birth and 6 months of age are depicted in Table 3. Breeds had only significant ( $P < 0.05$ ) influence on the gain in chest depth. The rest of the gains in body measurements were not significantly affected by breeds

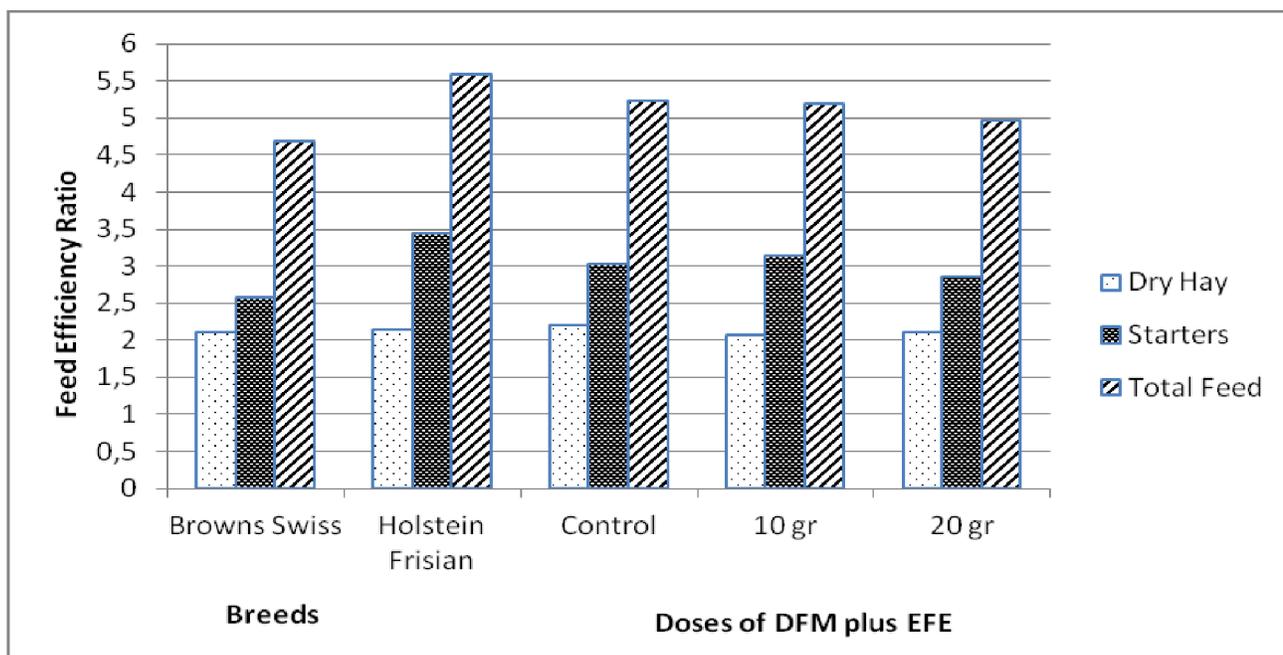


FIG.2: Feed efficiency ratio of the dairy calves between birth and 6 month ages.

TABLE 3: Least square means and standard error for gains in the body measurements (cm) of dairy calves between birth and 6 month of ages.

|                       | N  | Height at Withers         | Body Length               | Chest Depth               | Heart Girth               | Cannon Bone Girth         |
|-----------------------|----|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                       |    | $\bar{X} \pm S_{\bar{x}}$ |
| Overall Mean          | 26 | 25.53±0.75                | 22.57±0.66                | 14.83±0.52                | 41.24±1.20                | 2.66±0.16                 |
| Breeds                |    | ns                        | ns                        | *                         | ns                        | ns                        |
| Brown Swiss           | 16 | 24.83±0.96                | 23.04±0.83                | 13.67±0.67                | 40.25±1.52                | 2.89±0.20                 |
| Holstein Friesian     | 10 | 26.23±1.17                | 22.11±1.02                | 16.00±0.82                | 42.24±1.87                | 2.44±0.25                 |
| Doses of DFM plus EFE |    | ns                        | ns                        | ns                        | ns                        | ns                        |
| Control               | 11 | 24.46±1.17                | 22.43±1.02                | 15.02±0.81                | 38.64±1.85                | 2.49±0.24                 |
| 10 g                  | 7  | 26.96±1.40                | 23.79±1.22                | 15.17±0.98                | 41.79±2.23                | 2.75±0.29                 |
| 20 g                  | 8  | 25.19±1.31                | 21.50±1.14                | 14.31±0.91                | 43.31±2.08                | 2.75±0.27                 |

\*\*: $P < 0.01$ , \*: $P < 0.05$ , ns:Nonsignificant

and doses of DFM plus EFE additives. Likewise, Mokhber-Dezfouli (2007) and Frizzo *et al.* (2008) reported that gains in the heart girth and height at withers of the dairy calves in the DFM and control groups were not significantly different each other. Influence of the breeds on the gains of body measurements was significant only for gain in the chest depth, however, the rest of the gains in the body measurements of Brown Swiss and Holstein Friesian calves were comparable. The result is in agreement with the finding of Guler *et al.* (2003).

Results concerning growth rate, feed efficiency ratio and gain in the body measurements that are available from the literature on DFM treatments often appear to be contradictory. This may be due to variations in the source of DFM, concentration and/or type of viable cells in the DFM supplements and their consistency, survivability and

metabolic capacity in the host gut. Influence of feed processing (e.g., steam conditioning, pelleting) on the survivability of the DFM in the final prepared diet, intestinal microbial balance, and differences in the calf rearing systems could also play important roles in the inconsistent responses observed in these studies.

Least square means with standard error for fecal consistency scores of Brown Swiss and Holstein calves are presented in Table 4. The fecal consistency scores of Holstein Friesian calves between birth and 6 months of age was significantly ( $P < 0.01$ ) higher than those of Brown Swiss calves. In addition, different dosages of DFM plus EFE resulted in significant ( $P < 0.01$ ) difference in the fecal consistency scores in the pre-weaning period and between birth and 6 months of age. Average fecal consistency score of the calves fed a diet supplemented with 10 g head/day of

TABLE 4: Least square means and standard error for fecal consistency scores of calves.

|                       | N  | Fecal consistency scores  |                           |                                |
|-----------------------|----|---------------------------|---------------------------|--------------------------------|
|                       |    | At pre-weaning period     | At post-weaning period    | Between birth and 6 months age |
|                       |    | $\bar{X} \pm S_{\bar{x}}$ | $\bar{X} \pm S_{\bar{x}}$ | $\bar{X} \pm S_{\bar{x}}$      |
| Overall Mean          | 26 | 1.93±0.04                 | 1.07±0.02                 | 1.70±0.02                      |
| Breeds                |    | ns                        | **                        | **                             |
| Brown Swiss           | 16 | 1.89±0.05                 | 1.01±0.02                 | 1.65±0.02                      |
| Holstein Friesian     | 10 | 1.97±0.06                 | 1.14±0.03                 | 1.75±0.03                      |
| Doses of DFM plus EFE |    | **                        | ns                        | **                             |
| Control               | 11 | 2.90±0.06 <sup>a</sup>    | 1.10±0.03                 | 2.51±0.02 <sup>a</sup>         |
| 10 g                  | 7  | 1.07±0.08 <sup>b</sup>    | 1.04±0.03                 | 1.00±0.03 <sup>b</sup>         |
| 20 g                  | 8  | 1.83±0.07 <sup>c</sup>    | 1.08±0.03                 | 1.30±0.03 <sup>c</sup>         |

\*\*: $P < 0.01$ , \*: $P < 0.05$ , ns:Nonsignificant

DFM plus EFE had the lowest score (i.e., less scouring) compared to other treatment groups in pre-weaning period as well as between birth and 6 months of age (Table 4). Lower fecal consistency index indicated that diarrhea incidence was significantly reduced compared to control group during the present experiment. Similarly, Agarwal *et al.* (2002), Seo *et al.* (2010) and Kim *et al.* (2011) determined the consistency score of the feces of the calves and reported a significant decrease of incidence and duration of diarrhea in the young animals of DFM fed groups as compared to control group. Additionally, Foster *et al.* (2003), Jatkauskas and Vrotniakienė

(2010) have shown that supplementation of DFM into the diet of the calves resulted in a reduction of the incidence of diarrhea in the dairy calves.

From this study, it could be concluded that although the feeding of DFM plus EFE improved numerically growth performance of the dairy calves, the differences were not statistically significant. However, dose of 10 g head/day of the DFM plus EFE additives could be beneficial for reducing incidence of diarrhea in Brown Swiss and Holstein Friesian female calves.

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