EFFECT OF FEEDING DIRECT-FED MICROBIALS PLUS EXOGENOUS FEED ENZYMES ON MILK YIELD AND MILK COMPOSITION OF HOLSTEIN FRIESIAN COWS

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Abstract. The study was carried out to evaluate the effect of feeding direct-fed microbials (DFM) plus exogenous feed enzymes on milk yield and milk composition of primiparous and multiparous Holstein Friesian cows. Nineteen lactating cows were subjected to two dietary treatments (control and DFM plus enzyme groups) during 305 days. Evening (PM) and total daily milk productions of the cows were 12.7% and 11.5% higher than these of animals in the control group. Morning (AM), PM and total daily milk yields were significantly ($P<0.01$) affected by parity. Multiparous cows produced 16.8%, 15.4% and 16.1% higher AM, PM and total daily milk than primiparous cows. Total daily milk productions expressed as 4% FCM and SCM yields were also significantly affected by diet ($P<0.05$) and parity ($P<0.01$). Although Holstein cows fed diet supplemented with DFM plus enzymes resulted in 163.9 kg and 2.7 kg greater 305-days milk and milk fat productions respectively than cows in control group, the difference was not significant. Feeding of diet with DFM plus enzymes did not have significant influence on the percentages of the milk fat, protein, lactose, ash, dry matter, solids-non-fat as well as density and pH of the milk. Body condition score was significantly affected by parity ($P<0.01$) but not by feeding of DFM plus enzyme supplement. DFM plus enzymes had no apparent effect on count of fecal *Escherichia coli* flora.

Keywords: Holstein Friesian, Direct-fed microbials, feed enzymes, milk yield, milk composition.

Introduction. Modern livestock production requires the use of safe and effective feed additives as rumen manipulators to enhance animal productivity. Nowadays, the use of many growth stimulants including hormones and antibiotics in cattle production has been forbidden by law in many countries due to potential risks such as the spread of antibiotic resistance genes (Hong et al. 2005) or the contamination of milk or meat with antibiotic residues (Aydin et al. 2009). One of the potential alternatives for the banned growth promoters are direct-fed microbials (DFM) also known as probiotics and exogenous feed enzymes. Microorganisms that are used in DFM for ruminants may be classified as lactic acid producing bacteria, lactic acid utilizing bacteria, or other microorganisms including species of *Lactobacillus, Streptococcus, Enterococcus, Bifidobacterium, Propionibacterium* and *Bacillus,* strains of *Prevotella bryantii* and *Megasphaera elsdenii* and yeast products containing *Aspergillus* and *Saccharomyces* (Seo et al. 2010). Although contradictory reports (Soder and Holden, 1999; Boga and Gorgulu, 2007; Weiss et al. 2008) about the effects of DFM, dosages, feeding times and frequencies, strains of DFM, and effects on milk yield of cows are available, there is a growing body of evidence that feeding diets supplemented with DFM can increase milk production of the dairy cattle (Schingoethe et al. 2004; Stein et al. 2006; Lehloenya et al. 2008; Srtenovic et al. 2008, Dutta and Kundu, 2008, Qiao et al. 2009, Boyd et al. 2011).

Effects of feeding DFM and exogenous feed enzymes or both on the milk yield and milk composition of dairy cows in transition periods (from 3 weeks prior to calving to 3 weeks after calving), early or mid-stage of the lactation already have been already reported in literature, however, there is lack of information about the influence of DFM in combination with feed enzymes on the 305-days milk yield and milk composition. Therefore, the study was undertaken to evaluate the effect of feeding DFM plus enzymes on lactation milk yield and milk components of primiparous and multiparous Holstein Friesian cows reared in high altitude and cold climatic conditions of Eastern Turkey.

Material and methods. In this research, 10 primiparous and 9 multiparous (in second and third parity) Holstein Friesian cows from cattle herd of Research Farm of College of Agriculture at Ataturk University, Erzurum, Turkey were used. The research farm is located in longitude 41º 14’ 23.43 E, latitude 39 º 54’ 58.08 N and at an altitude of 1823 m above sea level. The cows were randomly assigned to control or DFM plus enzymes groups based on previous year’s 305-days lactation milk yields for multiparous and lactation averages of their dams for primiparous cows.

The cows in control (n=4 primiparous, n=5 multiparous cows) and DFM plus enzymes (dietary treatment) (n=5 primiparous, n=5 multiparous cows) groups were fed an identical diet comprising of concentrate, corn silage and dry pasture hay. Chemical compositions of the feeds are presented in Table 1. Diets of Holstein Friesian cows in the treatment group were also supplemented daily with 10 g/d/head of DFM (minimum $2x10^{11}$ cfu/kg of *Lactobacillus* sp., minimum

1.8x10^9 cfu/kg of *Saccharomyces cerevisiae*) and exogenous feed enzymes (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). Lactating cows' diet per animal consisted of 5 kg of concentrates, 10 kg of corn silage and *ad libitum* dry hay. All cows also had free access to water. The concentrate feed was offered lactating cows twice a day during morning (AM) and evening (PM) milking in the milking parlour.

Table 1. **Composition and nutritional value of feeds used in this study**

Nutrients, %	Concentrate	Corn Silage	Dry Hay
Dry Matter	88.0	21.8	87.9
Crude Protein	16.0	8.2	7.1
Ether Extract	3.5	2.8	1.0
Crude Cellulose	14.0	21.1	28.4
Crude Ash	9.0	6.3	8.4

Lactating cows were milked twice daily at 6:00 a.m., and 17:00 p.m. Daily (sum of AM and PM) milk yields determined in test-days were recorded and used to calculate 305-days milk yield. Total daily milk production was expressed by three ways, such as (1) uncorrected milk, (2) fat corrected milk (4% FCM), (3) solids corrected milk (SCM). 4% FCM was calculated by using the formula (NRC, 2001);

0.4 x milk, kg/day + 15 x fat, kg/day.

Solids corrected milk (SCM) was derived by using formula of Tyrrell and Reid (1965) as follows;

SCM (kg) = 12.3 (total fat, kg) + 6.56 [kg, solids-non-fat (SNF)]-0.0752 (total kg, uncorrected milk).

Milk samples for chemical analysis were collected from AM milking at monthly intervals in the test-day. Percentages of the milk fat, protein, lactose, ash, dry matter, SNF as well as density and pH of the milk were determined by using a milk analyzer (Lactoscan MMC, Boeckel Co, Hamburg, Germany).

Body condition scores (BCS) of all cows were individually determined by using a 5-item scoring system developed by Edmonson et al. (1989). In this system, the scoring interval was 0.25 and scores varied among 1 (emaciated), 2 (thin), 3 (moderate), 4 (fat), 5 (obese). All cows were scored at monthly intervals through the lactation period.

Most probable number method for determination of fecal *Escherichia coli* counts from fecal samples collected from cows once a week during last 3 weeks of the trial was used. *Escherichia coli* counts were determined according a method reported by Harrigan (1998).

The data were statistically analyzed by the least squares techniques in SPSS statistics software program (SPSS, 2004). A general linear model including fixed effects of diet and parity and their interaction with each other was used in the preliminary statistical analysis. Since the interaction was not statistically significant, it was excluded from the final statistical model. Therefore, the following mathematical model was designed to determine effects of diet and parity on the 4% FCM, SCM, AM, PM and daily total milk productions, 305-days milk and milk fat yields, BCS and *Escherichia coli* counts as well as chemical composition of the milk such as percentages of milk fat, protein, ash, dry matter, non-fat dry matter, lactose, density and pH;

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

Where: Y_{ijk} = Dependent variable, μ = Overall mean, a_i = Diets (I:1, 2; 1:Control, 2:Diet supplemented with DFM plus feed enzymes), b_j = Parity (j = 1, 2; 1 = Primiparous cows, 2 = Multiparous cows), e_{ijk} = Residual.

Table 2. **Least square means and standard error for morning** (AM)**, evening** (PM) **and total daily milk yields as well as fat corrected milk** (4% FCM) **and solids corrected milk** (SCM)

	Daily milk yield, kg				
	AM	PM	Total	4% FCM	SCM
	$\bar{X} \pm S_{\bar{x}}$				
Overall mean	6.65±0.19	5.94±0.17	12.60±0.34	11.87±0.30	11.60±0.27
Diets	NS	*	*	*	**
Control	6.33±0.28	5.59±0.26	11.91±0.51	11.28±0.45	10.95±0.41
DFM plus enzyme	6.98±0.24	6.30±0.23	13.28±0.45	12.47±0.40	12.25±0.36
Parity	**	**	**	**	**
Primiparous	6.14±0.27	5.52±0.25	11.66±0.50	11.15±0.44	10.88±0.40
Multiparous	7.17±0.25	6.37±0.23	13.54±0.46	12.60±0.40	12.33±0.36

* – P<0.05; ** – P<0.01; NS – Non-significant

Results and discussion. PM and total daily milk yields of Holstein Friesian cows in DFM plus enzymes group were significantly (P<0.05) greater than these of animals in control group (Table 2). The PM and total daily milk productions of the cows fed diet containing DFM plus enzymes were 12.7% and 11.5% higher than these of animals in the control group. Likewise, Nocek et al. (2003), Vahora et al. (2006), Ramsing et al. (2009), Phondba et al. (2009) and Heidari Khormizi et al. (2010) reported that feeding DFM and feed enzymes or both increased significantly daily milk yield at rates by 10.4%, 4.3%, 10.0%, 5.1% and 6.6% respectively. On the other hand, results of the several studies (Soder and Holden, 1999; Rihma et al. 2007; Oetzel et al. 2007) demonstrated that supplemental DFM and/or exogenous enzymes had beneficial effect on milk yield in lactating dairy cows, but they were not statistically significant. Beneficial effects of the DFM containing *Lactobacillus* sp. and yeast (*Saccharomyces cerevisiae*) in the current study could be due to lowering pH in small intestines to level that inhibit the growth of pathogenic microbes (Denev et al. 2007), due to production and/or stimulation of enzymes, increase of ruminal digestibility and increase of ruminal end products such as volatile fatty acids (VFA), rumen microbial protein (Seo et al. 2010). Positive effects of the feed enzyme supplements and DFM on the milk production could also be attributed to the improved total tract fiber digestion primarily by increasing fiber digestion in rumen and increased total VFA content as well as individual fatty acids content in total VFA (Das and Singh, 2011; Lopuszanska-Rusek and Bilik, 2011).

Parity also had significant (P<0.01) effects on the AM, PM and total daily milk yields. Multiparous cows produced 16.8%, 15.4% and 16.1% higher AM, PM and total daily milk than primiparous cows (Table 2). Similarly, Lehloenya et al. (2008) reported that daily milk yield of multiparous cows fed yeast and propionibacteria was 43.3% (30 kg/d vs. 44.0 kg/d) greater than that of primiparous cows in control group. Stein et al. (2006) also observed that milk yield increased when multiparous cows were fed DFM. Although interaction between diet and parity was not significant in the present study, maximum amount of AM, PM and total daily milk yields were obtained from multiparous cows fed diet supplemented with DFM plus enzymes (Fig. 1).

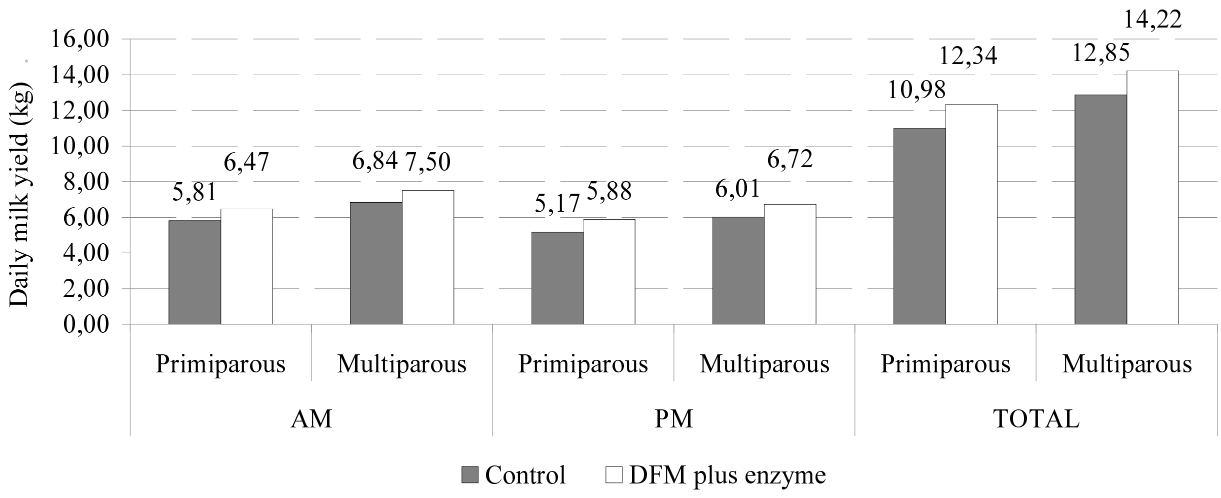

Fig. 1. **Morning** (AM)**, evening** (PM) **and total Daily milk yield of primiparous and multiparous cows in DFM plus enzymes or control groups**

Table 3. **Least square means and standard error for 305-days milk and milk fat yields,** kg

	305-Days Milk Yield	305-Days Milk Fat Yield
	$\overline{X} \pm S_{\bar{x}}$	
Overall mean	4155.32±159.41	150.13±6.32
Diets	NS	NS
Control	4073.39±226.58	148.77±8.98
DFM plus enzyme	4237.26±224.30	151.50±8.89
Parity	NS	NS
Primiparous	3836.94±237.10	139.94±9.40
Multiparous	4473.71±214.36	160.32±8.50

NS – Non-significant

Total daily milk productions expressed as 4% FCM and SCM yields were also influenced by diet (P<0.05) and parity (P<0.01) (Table 2), and averaged 11.87 kg/d and 11.60 kg/d across all treatments. Daily 4% FCM and SCM productions of the cows fed diet with DFM plus enzymes supplementation were higher 10.5%, 11.9% than these of the cows in control group. The results are in agreement with findings of Sretenovic et al. (2008) who reported that *Saccharomyces cerevisiae* in combination with probiotic bacteria and enzymes including hemicellulase, protease, cellulose, alpha amylase, β1,3–β1,6-D-Glucan significantly (P<0.01) improved amount of 4% FCM (8.7%). Ramsing et al. (2009) also noted that feeding supplemental DFM cultures at two different levels tended to increase 3.5% FCM by approximately 7.7–14.3% compared with yields from cows that were not supplemented.

Multiparous cows exhibited 13.0% and 13.3% greater 4% FCM and SCM productions above these of primiparous animals (Table 2). Similarly, Lehloenya et al. (2008) reported that daily 4% FCM and SCM yields of multiparous cows in DFM group were 23.1% and 28.6% greater than primiparous cows fed diet containing DFM.

Ramsing et al. (2009) also demonstrated that primiparous Holstein cows had lower (P<0.01) milk yield (28 kg/d) compared with multiparous cows (38 kg/d), and they responded similarly when supplemented with DFM. In the current study, the highest 4% FCM (13.19 kg) and SCM (12.98 kg) yields were obtained from multiparous Holstein Friesian cows who received DFM plus enzymes supplement compared to other treatment groups (Fig. 2).

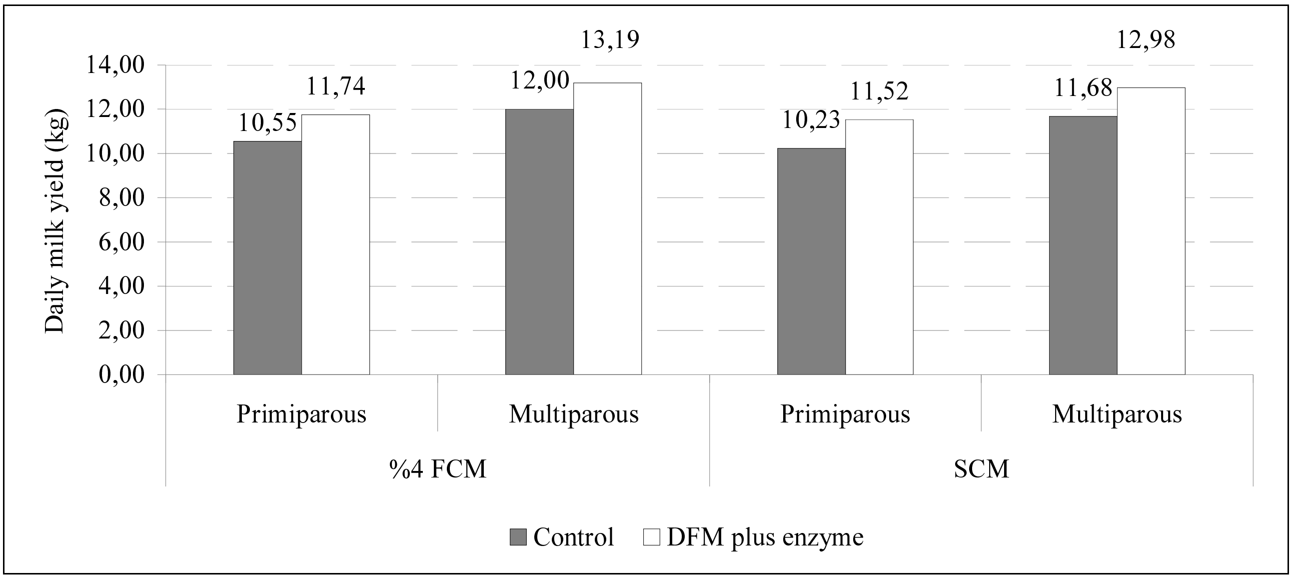

Fig. 2. **FCM% 4 and SCM of primiparous and multiparous cows in DFM plus enzymes or control groups**

Holstein cows received DFM plus enzymes supplement exhibited 163.9 kg and 2.7 kg greater 305-days milk and milk fat productions respectively than cows in control group, but the difference was not statistically significant (Table 3). Similarly, Soder and Holden (1999) suggested that feeding yeast in combination with feed enzymes to dairy cows did not result in significant effect on the total milk yield during first 13 weeks of the lactation. Contrary to these findings, Bruno et al. (2009) reported that supplementation DFM containing *Saccharomyces cerevisiae* yeast culture is beneficial (P<0.05) in improving lactation milk yield during the experiment (120 d).

Percentages of the milk fat, protein, lactose, ash, dry matter, SNF as well as density and pH of the milk were not significantly affected by feeding of diet containing DFM plus enzymes (Table 5). Similar results were also observed by Abo El-Nor and Kholif (1998), Raeth-Knight et al. (2007), Dutta and Kundu (2008) and Moellem et al. (2009). Lack of milk composition responses could be attributed to scarce stimulation effects of DFM and enzymes. Milk composition was also not influenced significantly by the parity of the cows. However, Lehloenya et al. (2008) reported that percentage of milk fat, protein, solids-non-fat contents were significantly affected by the parity.

Table 4. **Least square means and standard error for milk composition**

	Milk fat, %	Solids-non fat, %	Dry matter, %	Density, %	Protein, %	Lactose, %	Ash, %	pH
	$\overline{X} \pm S_{\bar{x}}$							
Overall mean	3.76±0.08	8.53±0.05	12.30±0.10	27.81±0.11	3.01±0.01	4.27±0.02	0.75±0.00	6.45±0.03
Diets	NS	NS	NS	NS	NS	NS	NS	NS
Control	3.84±0.12	8.44±0.08	12.27±0.15	27.68±0.17	3.00±0.02	4.25±0.02	0.75±0.01	6.46±0.04
DFM plus enzyme	3.69±0.11	8.63±0.07	12.32±0.13	27.94±0.15	3.02±0.01	4.29±0.02	0.75±0.01	6.43±0.04
Parity	NS	NS	NS	NS	NS	NS	NS	NS
Primiparous	3.89±0.12	8.53±0.08	12.43±0.15	27.80±0.16	3.01±0.02	4.27±0.02	0.75±0.01	6.43±0.04
Multiparous	3.63±0.11	8.53±0.07	12.17±0.14	27.82±0.15	3.00±0.01	4.26±0.02	0.75±0.01	6.46±0.04

NS – Non-significant

Body condition score was significantly altered by parity (P<0.01) but not by supplementation of DFM plus enzyme (Table 5). The result is in agreement with findings of Soder and Holden (1999) and Lehloenya et al. (2008), Brunono et al. (2009). However, Feeding DFM plus exogenous feed enzymes had no apparent effect on numbers of fecal *Escherichia coli* (Table 4). Likewise, Schwab et al. (1979) and Jenny et al. (1991) reported that fecal *Escherichia coli* flora of calves fed a *Lactobacillus bulgaricus* fermentation product or *Bacillus subtilis* concentrate was not significantly influenced. On the other hand, Ellinger et al. (1980) and Denev et al. (2007) stated that content of fecal *Escherichia coli* of calves, goat and sheep received *Lactobacillus acidophilus* and live yeast products significantly decreased.

Table 5. **Least square means and standard error for body condition score** (BCS) **and count of *Escherichia coli***

	BCS	*Escherichia coli* (log_{10} of count/g)
	$\overline{X} \pm S_{\bar{x}}$	
Overall mean	2.56±0.03	5.87±0.17
Diets	NS	NS
Control	2.58±0.04	5.97±0.25
DFM plus enzyme	2.54±0.04	5.76±0.25
Parity	**	NS
Primiparous	2.45±0.04	5.79±0.25
Multiparous	2.68±0.04	5.94±0.25

** – P<0.01; NS – Non-significant

Conclusion

In conclusion, it could be suggested that feeding a diet supplemented with DFM plus feed enzymes to Holstein Friesian cows significantly increased daily milk yield without causing any adverse effect on the milk composition and BCS. The improving effects of the DFM plus enzymes on the daily and 305-day milk production were higher for multiparous cows compared with primiparous Holstein Friesian cows.

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