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Effect of treated alfalfa silage with lactobacillus plant arum and sugar beet pulp molasses on performance of Holstein dairy cows

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ABSTRACT

The purpose of this study was to evaluate the effect of adding Lactobacillus Plantarm and sugar beet pulp molasses on production and blood parameters of dairy cows. Treatments were: 1- alfalfa silage without additives (control), 2- alfalfasilage with beet pulp molasses, 3- alfalfa silage with Lactobacillus Plantarm, 4- alfalfa silage with beet pulp molasses and Lactobacillus Plantarm. Eight cows in third lactation with 25 ± 1.2 kg milk per day were used in this study. The experimental cows were individually inhabited and had free access to fresh water and salt blocks. Data were statistically analyzed in a changeover design with four treatments and four periods of 21 days. The results showed that the use of Lactobacillus Plantarm with and without sugar beet pulp don't have significant effect on dry matter intake. Milk production Showed significant difference between treatments and fourth treatment containing Lactobacillus Plantarm and sugar beet pulp had the highest milk yield and second treatment without Lactobacillus Plantarm had lowest milk yield ($P<0.05$). In the milk composition and production, just milk fat production was increased in the fourth treatment than second treatment ($P<0.05$), other compounds in milk were similar among treatments. Four percent Fat-corrected milk yield also showed significant differences between treatments, and fourth had highest and first and second treatment had the lowest ($P<0.05$). Glucose, Triglycerides, cholesterol and nitrogen showed no significant difference between treatments in blood samples.

KEYWORDS: Alfalfa Silage, Lactobacillus Plantarm, Sugar beet pulp, Dairy cow

INTRODOCTION

Alfalfa (Medicago, sativa L.) is ranked among hard-to-ensile plants due to its high content of crude protein and low content of water-soluble carbohydrates (WSC), which are responsible for increased buffer capacity. Diets with alfalfa silage contain high levels of NPN and other sources of RDP (McDonald et al., 1991). When such diets are fed, ruminal carbohydrate and protein fermentation may be out of synchrony that is, under these conditions, increasing the rate of carbohydrate fermentation could increase microbial protein synthesis and therefore improve the supply of MP to dairy cows (Broderick et al., 2002). The molasses is commonly used to provide readily available energy for lactic acid fermentation. Previous studies showed positive effects of added dry or liquid molasses on FCM, milk fat concentration (Broderick and Radloff, 2004).

Lactic acid bacterial (**LAB**) inoculants are used as additives in ensiling to enhance the preservation efficiency of silage and to improve animal performance. (Weinberg and Muck, 1996). They are used because of their efficient utilization of the water-soluble carbohydrates of the crop, intensive production of lactic acid, and rapid reduction of pH (Weinberg and Muck., 1996). Some LAB inoculants have been reported to improve animal performance by increasing milk yield and feed efficiency (Kung and Muck, 1997; Kung et al., 2003). Kung and Muck (1997) also reported that silage inoculation improved DM intake in 28%, weight gain in 53%, and milk yield in 47% of the studies where these indices of animal performance were measured. Across only the studies where inoculation increased milk yield, the inoculated silages increased milk production 1.36 kg/cow/day compared to untreated silages. They speculated that this could be due to increased propionate concentrations in the rumen, higher digestibility and/or inhibition of growth of undesirable microorganisms (Contreras-Govea et al., 2011).

The objective of this study was to determine the effects of bacterial inoculant and sugar beet pulp molasses as silage additives on production and blood parameters of dairy cows.

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MATERIALS AND METHODS

Experimental Design

Experiments were conducted in accordance with a research protocol approved by the Gorgan University of Agricultural Sciences and Natural Resources College of Animal Science. Eight multiparous Holstein cows (mean ± SD) of 630 ± 10 Kg of BW, 160 ± 15 DIM and 25 ± 1.2 Kg/d of milk were blocked into 4 groups of 2 by parity and milk yield. Dietary treatments were a 1:control diet without additives, 2:alfalfa silage with 20% sugar beet pulp molasses, 3: alfalfa silage with LP (*Lactobacillus plantarum* MTD/1 inoculant; Ecosyl Products Ltd., Yorkshire, UK), 4: alfalfa silage with sugar beet pulp molasses and LP diets. Cows were housed in tie-stalls with free access to water and salt blocks. Four diets were formulated by nutrient requirements of dairy cows (NRC 2001; Table 1). Diets were offered ad libitum twice daily at 0630 and 1830 h. Experimental periods (n = 4) consisted of 14 d of diet adaptation and 7 d of data collection. Cows were milked 2 times daily at 0600 and 1800 h, and milk yield and feed intake were recorded daily. Milk samples were collected (a.m. and p.m.) on d 21 of each period from all the cows. At collection, all milk samples were preserved with potassium bicarbonate. Individual a.m. and

P.m. milk samples were analyzed for fat, protein, lactose, SNF by infrared analysis (Ag-Source, Verona, WI). Blood was sampled from the jugular vein of each cow at 4 h after feeding on d 21 of each period. Whole blood was immediately used for hematological estimation. Plasma was separated from an others blood samples (heparinized test tube) by centrifugation at 4000 rpm for 20 minutes whereas a part was used for enzyme determination, while the other part was kept frozen at -20°C until other biochemical analysis. The biochemical constituents of plasma consist of glucose, triglycerides, cholesterol and blood urea nitrogen (BUN), were determined calorimetrically using the specific kits.

Table 1. Ingredients and chemical composition of dietary treatments (DM basis)

	Dietary treatment*			
	1	2	3	4
Alfalfa silage	15	15	15	15
Wheat straw	10	5	10	5
Corn silage	21.3	10	21.3	10
Barley grain	15	15	15	15
Corn dry	15	15	15	15
Soybean meal	10	6.3	10	6.3
Cottonseed meal	7	7	7	7
Wheat bran	5	5	5	5
Sugar beet pulp	0	20	0	20
oystershell	0.6	0.6	0.6	0.6
Mineral and vitamin mix	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.3	0.3	0.3	0.3
Chemical composition				
CP	16	15.6	16	15.6
ADF	23.1	22.5	23.1	22.5
NDF	36	35.9	36	35.9
Ca	0.6	0.66	0.6	0.66
P	0.4	0.37	0.4	0.37
NE_LMcal/kg of DM	1.58	1.59	1.58	1.59

***1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.**

The inoculant was applied to third cut alfalfa at 1.0 × 10^5 cfu/g of the crop (in liquid form) at the forage harvester. Alfalfa was wilted in the field to approximately 35% DM. The inoculated treatments were harvested first from alternate windrows chopped to a theoretical chop length of 10 mm and ensiled in an oxygen-limiting tower silos. Later the same day the untreated alfalfa was harvested with the same equipment and ensiled in another oxygen-limiting tower silo. The 2g of each treatments were collected and were immediately frozen at −20°C for later analysis. Samples were dried at 60°C for 48 h in a forced draft oven, ground to pass a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), and analyzed for DM (105°C for 8 h) and ash (512°C for 8 h),(table 2). Concentrations of NDF and ADF were measured using the method of Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Analysis of NDF was done without the inclusion of sodium sulfite and with the use of a heat-stable α-amylase. Nitrogen was determined by rapid combustion using a

Macro elemental N analyzer (Vario MAX CN, model ID 25.00-5003; Elementar, Hanau, Germany), and CP was calculated as N × 6.25. The pH was measured with a glass electrode pH meter (MP230; Mettler Toledo, Greifensee, Switzerland).

Table 2. Nutrient composition of alfalfa silage

Component	treatment*			
	1	2	3	4
DM, %	33	35	32.3	34.5
CP, % of DM	16	14.3	15.6	14.5
Ash, % of DM	9.6	9.9	9.3	9.4
NDF, % of DM	43.3	38.9	42.5	37.8
ADF, % of DM	34.2	31.9	35	32.8
pH	4.7	4.56	4.64	4.48

***1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.**

Statistical Analysis

The experiment had a changeover design. All data were analyzed using the MIXED procedures of SAS (SAS Institute, 2002). The experiment were analyzed according to the model:

$$Y_{ijkm} = \mu + A_i + B_j + (AB)_{ij} + P_k + L_m + e_{ijkm}$$

where Y_{ijkm} represents the observation for dependent variables; μ is the overall mean;A_i effect of sugar beet pulp molasses i;B_j effect of *Lactobacillus Plantarm* j;AB_{ij}interaction between sugar beet pulp molasses i and *Lactobacillus Plantarm* j; P_k effect of period; L_m effect of animal random; and e_{ijkm}residual error. The Tukey test was used to identify differences ($P < 0.05$) between means.

RESULTS AND DISCUSSION

Dry matter intake

Dry matter intake of dairy cows was not affected by inoculation (table 3).

Our results are in accordance with other studies, which found no improvement in DM intake of lactating cows (martel et al., 2011; baurhoo et al., 2014). However, some researchers have reported improved DM intake when inoculated grass (Rooke and Kafilzadeh 1994). The increased DM intake of inoculated silages has been attributed to improvement in fermentation parameters and / or enhancement in NDF digestibility (Keady and Steen, 1995).

Table 3. Effect of feeding alfalfa silage on DMI, milk production and composition

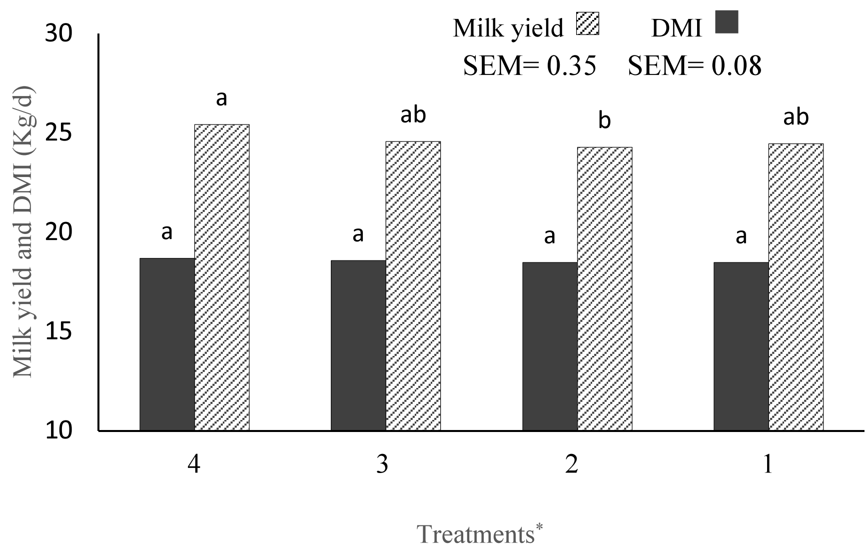
Item	treatment*				SEM	*P*-value
	1	2	3	4		
DMI, kg/d	18.47	18.46	18.56	18.67	0.08	0.15
Milk	24.45[ab]	24.27[b]	24.65[ab]	25.41[a]	0.35	0.04
4% FCM,kg/d	22.13[ab]	21.88[b]	22.51[ab]	23.49[a]	0.45	0.03
Lactose	4.81	4.81	4.80	4.83	0.008	0.15
Protein	3.23	3.22	3.21	3.25	0.04	0.94
Fat, %	3.37	3.34	3.42	3.49	0.07	0.37
Fat, kg/d	0.82[ab]	0.80[b]	0.83[ab]	0.88[a]	0.02	0.05
SNF	8.37	8.35	8.49	8.50	0.14	0.62

***1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.**

Milk yield

Milk production Showed significant difference between treatments and fourth treatment containing *Lactobacillus Plantarm* and sugar beet pulp had the highest milk yield and second treatment without *Lactobacillus Plantarm* had lowest milk yield (P<0.05). Kung et al. (2003) reported positive effect of silage inoculation on milk yield. Improvement in milk yield was for most part attributed to increased metabolisable energy intake, either due to increased dry matter intake (Kung et al., 1993). The effect of molasses on milk yield of dairy cows is inconsistent. Martel et al. (2011) reported that dietary molasses (up to 5% of the diet DM) decreased milk yield. Feeding dietary molasses with alfalfa silage-based diets up to 8% of the diet DM had no effect on milk yields (Broderick and Radloff, 2004).

Figure 1. Relationship between milk yield with DMI (kg/d).

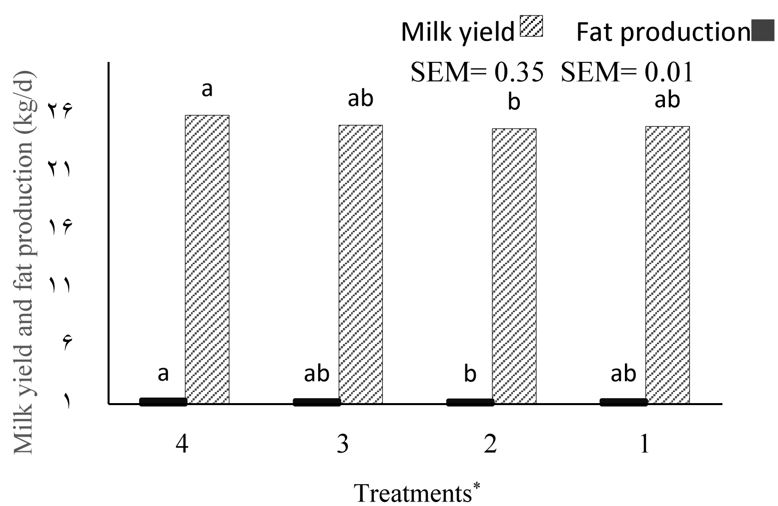


*1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.

Milk composition

Milk components, just milk fat production was increased in the fourth treatment than second treatment ($P<0.05$), suggesting this increased due to increase milk yield, other components in milk was similar for cows fed untreated and inoculated alfalfa silage diets (table 3). Our results agree with other studies that found no effect of inoculation on milk composition (kung et al., 1993). Mohammed et al. (2012) showed milk fat depression was associated with an increase in the relative population size of the bacterium *Megasphaera elsdenii*.

Figure 2. Relationship between milk yield with fat production(kg/d).



*1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.

Blood parametrs

Glucose, triglycerides, cholesterol and blood urea nitrogen showed no significant difference between treatments in blood samples (table 4). Vatandoost et al. (2011) Showed no significant effect of adding *Lactobacillus* on glucose. Dehghan-Banadaky et al.(2012) showed

concentrations of glucose was greater when live yeast was supplemented compared with the control. Increasing fibre digestion in the live yeast group could improve the energy status of cows and cause higher blood glucose concentration via decreasing glucose uptake in adiposity. Hafez et al. (2012) reported silage inoculation dont have effect on cholesterol and blood urea nitrogen.

Table 4. blood metabolites (mg/dl).

Item	treatment*				SEM	*P*-value
	1	2	3	4		
Glucose	50.12	55.25	52.37	53.25	3.61	0.45
triglycerides	13.75	14.37	14	14	1.45	0.97
cholesterol	184.75	200.25	177.25	186.75	9.45	0.31
urea nitrogen	12.87	13.87	13.25	13.37	1.09	0.74

***1: alfalfa silage without additives (control), 2: alfalfasilage with beet pulp molasses, 3: alfalfa silage with *Lactobacillus Plantarm*, 4: alfalfa silage with beet pulp molasses and *Lactobacillus Plantarm*.**

CONCLUSIONS

This study evaluated the effect of alfalfa ensiled with or without the inoculant *L. plantarum* MTD/1 and sugar beet pulp molasses on intakes, production and blood parameters of dairy cows. No effect of the inoculant on DMI, Milk composition and Blood parametrs occurred. Data from this study suggest inoculantion can improve milk yield and milk fat production.

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