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Effect of Rumex Sc on Ruminal Fermentation, Blood Metabolites and Performance of Lactating Dairy Cow

Research Article

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ABSTRACT

This study investigated the effect of Rumex Sc (commercial product which includes of *Saccharomyces cervisiae*, saponin and essential oils) on rumen fermentation, blood glucose, urea, milk yield and milk composition. Animals were offered a basal diet containing alfalfa hay (15.5%), corn silage (24%), beet pulp (7%) and concentrate (53.5%). Additionally, Rumex Sc was included in the experimental diet at a rate of 5 g/day/cow. Sampling of milk, ruminal liquid and blood was conducted for determination of milk composition, fermentation parameters and blood metabolites. Milk yield was significantly increased for the experimental group when compared to the control group ($P<0.05$), but milk composition was not affected by Rumex Sc. The number of protozoa, ammonia nitrogen concentration and pH in the rumen were decreased in the experimental group ($P<0.05$). Concentrations of volatile fatty acids in the rumen were affected to some extent by inclusion of Rumex Sc in the diet. Molar proportion of acetate was decreased and propionate was increased with a corresponding decrease in acetate: propionate ratio. In this study, blood glucose was significantly increased and urea decreased with the addition of Rumex Sc ($P<0.05$). It was concluded that using Rumex Sc can improve the milk yield performance of dairy cows, however further studies are needed.

KEY WORDS dairy cow, milk yield performance, Rumex Sc, ruminal fermentation.

INTRODUCTION

Introduction

Rumex Sc is a commercial product which has three constituent ingredient including *Saccharomyces cervisiae* (a growth promoter for ruminant; Klita *et al*. 1996), saponin (a glycoside compound composed of a steroid (Killeen *et al*. 1998) or triterpenoid (Goetsch and Owens, 1982) nucleus with one or more carbohydrate branches) and essential oils as volatile components responsible for the characteristic aroma of spices. *Saccharomyces cervisiae* has been shown to favorably alter the ruminal environment and pH stability, increase feed intake and increase milk production of dairy cows (Corona *et al*. 1999). Some studies have reported that this yeast prevents the rigorous decrease of ruminal pH in dairy cows (Erasmus *et al*. 1992). Improvements in dry matter intake, milk yield, and milk components have been reported (Piva *et al*. 1993; Corona *et al*. 1999), when cows were fed *Saccharomyces cervisiae*.

Saponin is an active substances found on the surface of wild plants which can increase animal performance by the elimination of the protozoa population. In a study, multiparous cows showed a positive response to saponin in terms of milk yield and composition (Corona *et al*. 1999). Essential oils are the volatile components for the characteristics aroma of species. Essential oils appear to be selective in their antibacterial action, with the spectrum of antibacterial activity varying with components tested (Janssen *et al*.

1986). However Benchaar *et al.* (2007) did not find any effect on the yield and composition of milk (with the exception of an increase in lactose concentration) by feeding essential oils. Thus, it is possible that the concomitant feeding of three substances described above would have favorable effects on ruminal fermentation and protozoa count of lactating dairy cows. Therefore the aim of this study was to investigate the effects of Rumex Sc on the milk yield performance, ruminal fermentation and blood metabolites in lactating dairy cow.

MATERIALS AND METHODS

Animals and diets

Twenty-four multiparous Holstein dairy cows averaging 90 ± 30 days in milk, weighting 600 ± 80 kg were allocated into two groups and used in a changeover design with two 42-day periods. They had free access to water during the experiment. Cows were fed as voluntary total mixed ration (TMR) with or whithout Rumex Sc (0 versus 5 g/day/cow) as has been illustrated in Table 1.

Rumex Sc consisted of a mixture of three component, including *saccharomyces cervisiae*, saponin and essential oil. The adaptation period to the experimental treatments was 10 days, which occurred prior to the commencement of the study. Cows were nourished in accordance with the guidelines of the NRC (2001) in Table 1.

Performance records

Feed consumption was recorded daily by weighting feeds received and refused by cows. Cows were weighed at the beginning and at the end of each experimental period. Cows were milked thrice daily at 0600, 1300 and 2000 h, and milk yield was recorded at each milking. Milk sampling was taken as weekly composites of evening and morning times for analysis of milk composition.

The milk samples were taken from each cow at each milking, pooled on a yield basis, and stored at 4 ˚C with a preservative (dichromate potassium) until analyze d for milk composition.

Sampling of ruminal liquor for fermentation profile

Rumen samples were removed on two consecutive days during the final week of each period using a gullet tube (RS-18 Iv, Tomy, Tokyo, Japan).

The pH was measured immediately by a digital pH meter. Rumen liquor was filtered through a four layers burlap fabric and centrifuged at 6000 rpm for 20 min. After centrifuging, 3 mL per cow of ruminal liquor was filtered into a test tube, 3 mL formalin was added for protozoa count and the samples were refrigerated at (4 ˚C). In order to determine ammonia nitrogen, 2.4 ml of ruminal liquor was poured into a tube test, 0.6 mL 5% sulfuric acid was added and the samples were stored in a freezer at -20 ˚C. To determine VFA contents 2.4 mL of ruminal liquor was poured into a tube test and then 0.6 mL meta phosphoric acid was added into respective tubes. The samples were stored at -20 ˚C until time be send to laboratory.

Measurement of VFA, ammonia nitrogen and Protozoa count

Measurement of VFAs was carrying out by using of gas chromatography test (Animal nutrition laboratory, University of Tehran, Karaj, Iran).

Measurement of ammonia nitrogen was conducted by phenol-hypochlorite method (Ceriotti, 1974). In order to estimate protozoa count of rumen fluid, the direct count method under light microscope was used.

Sampling and measurement of blood metabolites

Blood sampling was conducted at the final week of each period.

Table 1 The Ingredients and nutrients composition of the control and experimental diets

Diet composition	% DM	**Diet chemical composition**	Rate
Corn silage	24	DM (%)	59.6
Beet pulp	7	TDN (%)	70.25
Alfalfa	15.5	EE (g/kg)	21.25
Concentrate	53.5	CP (g/kg)	165.25
Concentrate components		RUP (g/kg)	49.65
Cottonseed meal	3	RDP (g/kg)	115.35
Wheat bran	22	NDF (g/kg)	280.5
Barley	10	ADF (g/kg)	213.5
Corn grain	25	NFC (g/kg)	327.5
Soybean meal	15	Ca (%)	0.68
Canola meal	22	P (%)	0.38
Sodium bicarbonate	0.8	K (%)	0.9
Calcium carbonate	0.6	NE_L (Mcal/kg)	1.61
Vitamin-mineral premix	1.3	Cation-Anion balance (meq/100g)	17.4
Salt	0.3		

DM: dry matter; TDN: total digestible nutrition; EE: ether extra; CP: crude protein; RUP: rumen-undegradable protein; RDP: rumen-degradable protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: nonfiber carbohydrates and NE_L: net energy for lactation.

This was conducted by gap tubing of the post-tail vein area. Determination of blood glucose and urea was conducted by spectrophotometry method.

Chemical a nalyses

The dry matter (DM) content of the feeds and orts were determined by drying at 110 ˚C for 24 h and diets were adjusted for weekly changes in the DM content of feeds (AOAC, 1990).

The measurement of feed nitrogen was conducted by Kjeldahl analysis (AOAC, 1990). Crude protein was determined as nitrogen × 6.25. The fat content of the diet was determined using a Soxtec system HT6 apparatus according to AOAC (1990). The concentration of NDF in TMR diet was determined as described by Van Soest *et al.* (1991) without the use of sodium sulfite and with the inclusion of heat-stable α-amylase. The ADF content in TMR diet was determined according to AOAC (1990).

Statistical a nalysis

In this experiment, the statistical analysis of data was conducted by a mixed model procedure of SAS 9.1 program according to the following model:

$$Y_{ijkl} = \mu + P_i + S_j + T_k + SUB_l (S_j) + \varepsilon_{ijkl}$$

Where:
P_i: i^{th} fixed effects of period.
S_j: j^{th} square.
T_k: k^{th} treatment.
SUB_l (S_j): l^{th} random effect of cow within j^{th} square.
ε_{ijkl}: was pooled experimental error.

The means were compared by the Duncan test.

RESULTS AND DISCUSSION

Performance

There were no differences between groups in dry matter intake (Figure 1). In other studies there are inconsistent results by using of yeast in the diets of lactating dairy cow. The different response of feed intake to yeast depend on the content of fermentable carbohydrates and the nature of the diet used (Haddad and Goussous, 2005) as well as yeast type, age of test animals and nutrition method (Grieve, 1979). There were no significant effect on feed intake and body weight changes by saponin (Wilson *et al.* 1998) and essential oil (Benchaar *et al.* 2007).

The effects of Rumex Sc on milk yield and composition are shown in Table 2. Addition of Rumex Sc to diet affect milk yield, significantly (P<0.05).

The average of milk yield was 31.9 and 33.3 kg/day for control and experimental groups, respectively.

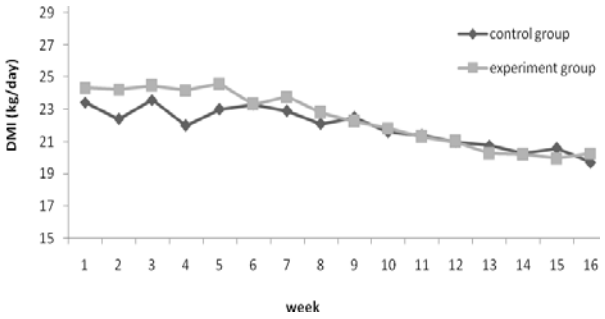

Figure 1 Dry matter intake of Holstein cows fed a control diet or the same diet supplemented with Rumex Sc

Aramble and Kent (1990) reported thayeast stimulates the rumen micro organisms resulting in the improvement of fiber digestion, following the increase of the feed intake and subsequent increase of milk production. Other workers (Dann *et al.* 2000) showed that cows fed yeast reached the production peak sooner than cows fed by control diet (without yeast).

Table 2 Effect of the Rumex Sc on milk yield and composition of dairy cows fed control (C) diet or the same diet supplemented with Rumex Sc

Variable	Diet		SEM	P-value
	Control	Rumex Sc		
Milk kg/d	31.9	33.3	0.55	0.02
Fat corrected milk (3.5 %) kg/d	30.1	31.5	0.61	0.01
Milk fat %	3.51	3.47	0.06	0.17
Milk fat kg/d	1.15	1.12	0.04	0.09
Milk cp %	3.17	3.20	0.02	0.08
Milk cp kg/d	1.01	1.07	0.01	0.11
Solid not fat %	8.88	8.91	0.03	0.39
Total solid %	3.88	3.97	0.01	0.21
Lactose %	4.70	4.69	0.01	0.59
Lactose kg/d	1.50	1.53	0.56	0.38
Body weight changes kg/d	0.38	0.26	0.17	0.54

SEM: standard error of the means.

It is possible that *Saccharomyces cervisiae* via the substitution of beneficial microorganisms in the rumen, increases the supply of microbial protein in the duodenum, decreases the production of methane and ethanol, improves the digestion of nutrient and accordingly increases milk production (Aramble and Kent, 1990).

The concentration of milk fat was not affected by experimental groups. Unlike our results, Besong *et al.* (1996) observed that the addition of yeast to diet caused an increase in milk fat concentration.

These results can be related to difference in diet composition, since the concentration of milk fat positively has a

high correlation with the concentration of NDF. In some studies, increases in the content of milk protein can be due to decreasing ammonia nitrogen, subsequently conducted to the synthesis pathway of true protein (Wang *et al*. 1998). Jouany (1996) reported that saponin and essential oils (Hristov *et al*. 1991) suppress the degradability of diet protein, increase protected protein flow, and thus increase the efficiency of production.

However, concomitant use of these three substances did not result in a significant effect on milk composition (Table 2).

Ruminal measurements

Addition of Rumex Sc (5 g/day/cow) to diet resulted in a decrease (P<0.05) in rumen fluid pH. The measurement of pH for control and experimental groups were 6.43 and 6.35, respectively (Table 3).

Table 3 The effect of dietary treatments on fermentation profile, ammonia nitrogen and protozoa count in the rumen of dairy cows

Variable	Diet		SEM	P- value
	Control	Rumex Sc		
Ruminal pH	6.43	6.35	0.03	0.03
Acetate (mol/100 mol)	69.82	69.01	0.26	0.01
Propionate (mol/100 mol)	8.11	8.68	0.18	0.01
Butyrate (mol/100 mol)	14.6	14.41	0.16	0.28
Valerate (mol/100 mol)	2.08	1.98	0.49	0.16
Iso-valerate (mol/100 mol)	2.48	2.55	0.07	0.35
Iso-butyrate (mol/100 mol)	0.94	1.02	0.05	0.11
Acetate/propionate	8.62	8.12	0.20	0.02
Total VFA (mmol/L)	98.17	95.50	0.29	0.29
Ammonia nitrogen (mmol/L)	5.14	5.02	0.05	0.04
Ruminal protozoa count ($\times 10^5$)	3.88	3.41	0.17	0.01

SEM: standard error of the means.

Results of this study and compatible results of other studies showed low protozoa count in cows fed Rumen Sc containing diet. Protozoa are able to stabilize ruminal pH via a reduction in the fermentation rate of highly digestible diets and thus, a decrease in protozoa count result in a decrease of pH (Klita *et al*. 1996).

As shown in Table 3, the concentration of ammonia nitrogen decreased (P<0.05) in the rumen liquid of dairy cows which were fed Rumex Sc (5 g/day/cow). Reduced ammonia nitrogen concentrations in the rumen are typical when protozoa are inhibited (Van Soest, 1991), presumably as a result of depressed bacterial lyses.

Inhibition of bacterial lysis is probably only partially responsible for the decreased ruminal ammonia nitrogen concentrations observed in defaunated animals (Wang *et al*. 1998).

The effect of yeast on ruminal ammonia in this study, likely, resulted from a decrease in bacterial lyses (as a consequence of inhibited protozoa growth). Weidmeier *et al*. (1987) stated that decreases in ammonia nitrogen can be due to an increase in the passage rate of nutrients in the rumen that result in decreases in accessible time for the fermentative microorganisms of substance, and even can be related to a decrease in the degradation of diet protein.

As shown in Table 3 addition of Rumex Sc to the diet caused a decrease in the number of rumen protozoa (of 3.88×10^5 to 3.41×10^5) (P<0.05). Jouany (1996) proposed that the ciliated protozoa of rumen have a significant role in the cycle of microbial nitrogen and also the efficiency of microbial synthesis.

A Significant decrease in the number of the protozoa improves the use of dietary nitrogen and increases the outflow of microbial protein to the intestine.

Enjalbert *et al*. (1999) believed that yeast causes stimulation of consumer bacteria of lactic acid such as *Slenomonas ruminantum* and *Megasfera elsedni* avoiding sudden decreases in acidity thus, providing favorable conditions for the growth and activity of microorganism.

Klita *et al*. (1996) stated that decreases in the number of protozoa are accomplished via decreases in pH by saponins which result in the decrease in the number of protozoa. Killeen *et al*. (1998) remarked that the antibacterial properties of saponin seem to explain the opposition of saponin against positive germ bacteria and the separated properties of cellular membrane.

Our result showed that diets supplemented with Rumex Sc did not have any effect on total yield of volatile fatty acids. Nevertheless this substance (Rumex Sc) resulted in decrease in acetic acid, acetate to propionate ratio and increase in propionic acid (P<0.05) concentration. Regression analysis showed that there are correlation between the dose of yeast and VFA production (Sullivan and Martin, 1999).

Erasmus *et al*. (1992) attributed increases in the amount of the ruminal VFA to increases in microbial activity. The increase in the number of ruminal microorganisms and their activity result in the faster fermentation of feed in the rumen which caused increased VFA production in rumen greater than their absorption by ruminal parapet; however, in this study, we saw the inverse result. Saponin was probably decreased and fiber and starch digestive bacteria increased by lower pH respectively and so the acetate/propionate ratio decreased.

Blood metabolites

The addition of Rumex Sc to the diet resulted in an increase

in blood glucose (P<0.05; Table 4). Rumex Sc had a significant affect on urea concentration, causing a decrease in blood urea.

Table 4 Blood parameters of dairy cows fed control (C) diet or the same diet supplemented with Rumex Sc

Variable	Diet		SEM	P-Value
	Control	Rumex Sc		
Glucose (mg/dL)	57.54	63.42	0.10	0.01
Urea (mg/dL)	49.58	44.92	0.12	0.01

SEM: standard error of the means.

The observed decrease in our study may be due to a decrease in ammonium concentration introduced to microbial protein synthesis pathway.

CONCLUSION

Addition of Rumex Sc to the diet of Holstein dairy cows resulted in an increase in propionic acid, and a decrease in acetic acids as well as the acetic / propionic ratio. In this experiment, ammonia nitrogen, pH and protozoa count were decreased, significantly by inclusion of Rumex Sc. Cows fed Rumex Sc showed an increase in milk yield but milk composition was not affected. These results suggest that the addition of Rumex Sc can improve the milk yield performance of dairy cows, however further studies are needed.

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