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Research Article

Productive Performance of Lactating Frisian Cows Fed Sugar Beet Leaves Silage Treated with Lactic Acid Bacteria

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Abstract

Objective: This study was carried out to investigate inoculating sugar beet leaves silage with lactic acid bacteria to reduce negative effect of its content of oxalates. **Materials and Methods:** Fifteen lactating Frisian cows weighed 500 ± 30 kg were randomly assigned into three groups (5 cows) using complete random design. The experimental diets were as follow: T1 group was fed 50% (Concentrate Feed Mixture, CFM); 30% corn silage and 20% rice straw, T2 group was fed 50% (CFM); 30% untreated sugar beet leaves silage and 20% rice straw and T3 group was fed 50% (CFM); 30% treated sugar beet leaves silage and 20% rice straw. Dry matter intake and milk yield were recorded and the samples of blood plasma, rumen liquor, milk and feed were analyzed and the data were statistically analyzed according to a completely randomized design using GLM procedure of SAS program. **Results:** The results showed that treated silage with lactic acid bacteria was higher in calcium, lactic acid and acetic acid contents but lower in values of pH, oxalate, cellulose, hemicellulose and butyric acid compared with untreated silage. Roughage DMI decreased with cows fed T2 compared with other groups. Also, nutrients digestibility and nutritive value were decreased in T2 compared with the T1 and T2. However, ruminal TVFA's, acetate acid, CH₄ production and blood glucose decreased significantly ($p < 0.05$) in T2 compared with other groups. T2 recorded the lowest values ($p < 0.05$) for milk yield and energy corrected milk compared with other groups, milk compositions of cows were affected with treatments. **Conclusion:** It is concluded that ensiling sugar beet leaves treated with lactic acid bacteria reduce oxalate contents in sugar beet leaves silage and enhance performance and productivity of lactating Frisian cows.

Key words: Lactating Frisian cows, lactic acid bacteria, oxalate, sugar beet leaves, silage, digestibility, rumen fermentation, blood metabolites

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxalates compounds are widely distributed in many plants throughout the world¹, involving a large number of species, such as *Beta vulgaris*², *Moringa oleifera*³, *Triticum* species⁴, *Acacia* species⁵ and *Pennisetum purpureum*⁶. Sugar beet leaves can be used for ruminants feed such as goats, sheep and cattle as it is or after making silage⁷. Using sugar beet leaves silage for animals feed may contribute in solving some problems regarding resources shortage of animal feeding, especially in summer season and reduction significantly ($p < 0.05$) of feed cost in animal feeding⁸⁻¹⁰.

Oxalate plant contents are the most common causes of decreasing dry matter intake, nutrients digestibility, nutritive values and adverse effects on production performance and health of farm animals¹¹⁻¹⁴. Also, oxalates compounds have antimicrobial effects by acting against bacteria, fungi and protozoa, which have potential negative consequences on rumen microbial fermentation and ruminal function¹⁵. Diets containing oxalate have been shown to cause calcium deficiency absorption in cattle, which consequently reduce milk production and growth^{16,17}.

Oxalate cause rumen and gastrointestinal tract irritation, blockage and damage of the kidney renal tubules by calcium oxalate crystals, which lead to urinary calculi and hypocalcemia. Furthermore, oxalates cause muscular weakness and paralysis¹⁸⁻²¹. Oxalates cause negatively effect on liver functions (ALT and AST activities)^{22,23}.

A number of studies have been investigated the utilization of some biotechnological treatments to decrease negative effects of oxalate. These include lactic acid bacteria, such as *Lactobacillus*, *Enterococcus*, *Eubacterium* and *Bifidobacterium* among others²⁴⁻²⁶. Also, *Enterococcus faecalis* species used oxalate as a carbon source in nutrient poor media²⁷. The aim of this study was to investigate the use of lactic acid bacteria on reduce negative effect of oxalates of diets containing sugar beet leaves and its effect on feed intake, nutrients digestibilities, blood plasma metabolites and milk yield and composition of lactating Frisian cows.

MATERIALS AND METHODS

Bacterial strains: Lactic acid bacteria were used for degrading oxalic acid as carbon source in the growth media. The lactic acid bacteria were obtained from the dairy microbiology laboratory at National Research Centre, Giza, Egypt. Eleven strains of lactic acid bacteria were tested for degrading oxalic

Table 1: Effect of lactic acid bacteria on *in vitro* oxalic acid degradation

Strains	Oxalate degradation (%)
<i>Lactobacillus cremoris</i>	Nil
<i>Lactobacillus casei</i>	Nil
<i>Lactobacillus rhamnosus</i>	Nil
<i>Lactobacillus reuteri</i>	Nil
<i>Lactobacillus bulgaricus</i>	Nil
<i>Lactobacillus acidophilus</i>	Nil
<i>Streptococcus thermophilus</i>	Nil
<i>Bifidobacterium breve</i>	8.3
<i>Lactobacillus gasserii</i>	8.3
<i>Leuconostoc mesenteroides</i>	12.5
<i>Lactobacillus plantarum</i>	12.5

acid into (Table 1). Lactic acid bacteria were cultured anaerobically on MRS broth medium containing 0.05% (w/v) L-cysteine at 37 °C for 24 h. Tested MRS medium was prepared to evaluate the oxalate degrading property which contained 5 mmol L⁻¹ oxalic acid and 0.05% (w/v) L-cysteine 28. Lactic acid bacteria strains (*Lactobacillus cremoris*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Lactobacillus gasserii*, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*) were cultured for 5 days²⁸. Subsequently, the bacterial cultures were processed as described by Federici *et al.*²⁹. The oxalate concentration was measured according to AOAC³⁰.

Silage making: Sugar beet leaves were wilted for 48 h to reduce the moisture content to 65-70% before ensiling, the plant was chopped and molasses was added at a level of 5% of fresh weight basis. In addition, 3% calcium carbonate and 2% lactic acid bacterial solution (*Lactobacillus plantarum*, *Lactobacillus gasserii*, *Leuconostoc mesenteroides* and *Bifidobacterium breve*) were added during ensilage (treated silage), or without lactic acid bacteria (untreated silage). The material was ensiled in plastic bags for two months before feeding to the cows. After ensilage period, samples were collected for chemical analysis and oxalic acid was determination.

Harvesting corn when the entire plant is between 65 and 70% moisture and kernel milk stage is 80% will provide the best combination of dry matter yield and digestibility. It is also the moisture level where the best silage fermentation occurs, the plant was chopped; molasses and urea were added for corn at 5 and 3% of fresh weight basis, respectively. The material was ensiled in plastic bags for two months before feeding. After ensilage period, samples were analyzed as described earlier.

Animals and diets: Fifteen lactating Frisian cows at second to fifth season of lactation and weighing; 500±30 kg were divided randomly into three groups (5 animals) using complete random design, the experimental group were homogenized according to previous lactation season productivity. The experimental periods started 2 week after parturition and continued until 90 days of lactation. Cows were individually fed as per recommendations of NRC³¹. The experimental diets for T1 group was 50% (CFM); 30% corn silage and 20% rice straw, for T2 group was 50% (CFM); 30% untreated sugar beet leaves silage and 20% rice straw, for T3 group was 50% (CFM); 30% treated sugar beet leaves silage and 20% rice straw. The CFM consisted of 39% yellow corn, 25% wheat bran, 6% sugar beet pulp, 16% soybean meal, 8% cottonseed meal, 3.2% molasses, 1.5% limestone, 0.8% salt, 0.5% Premix1 and was formulated to provide (per kilogram of DM) 400,000 IU of vitamin A, 80,000 IU vitamin D, 2,000 IU of vitamin E, 5,000 mg Zn, 40 mg Se, 2,000 mg Mn, 2,000 mg Fe, 20 mg Co, 1,200 mg Cu. The chemical composition of feedstuff ingredients is reported in Table 2. Cows were fed twice daily at 07:00 and 21:00 h. Dry matter intake was recorded during the last seven days of experimental period. Water was provided *ad libitum*. Cows were cared for and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching³² at a dairy farm Animal Production Research Institute, Ministry of Agriculture, Egypt and at the Laboratory of Dairy Animal Production, National Research Centre, Egypt.

Feed and fecal samples analysis: Samples of fecal and rations were analyzed for Dry Matter (DM), ash, Crude Fiber (CF),

Organic Matter (OM) and Ether Extract (EE) according to the method of AOAC³³. Nitrogen-Free Extract (NFE) was calculated by difference. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were determined using the methods of Van Soest *et al.*³⁴. The oxalate concentration was measured according to the method of AOAC³⁰. Calcium concentration was determined according to Chang and Bloom³⁵.

Determination of nutrients digestion coefficients: Grab sample method was applied to determine the nutritive value and digestibility in which Acid-insoluble ash (silica) was used as an internal marker for the calculation of coefficients of digestion. Feces grab samples were collected at 06.00 h for three successive days at the first three days of collection periods for each experiment. During these periods, DM Intake (DMI) was recorded daily by weighing feed offered. Representative feces samples were sprayed by 10% sulfuric acid solution and formalin, followed by drying in oven at 60°C for 48 h. The fresh feces samples for each animal were mixed by a representative ratio of daily fecal yield then grounded and stored in polyethylene bags for chemical analysis of DM, CP, CF, EE, NFE, NDF and ADF. The digestibility coefficient of nutrients was calculated according to Abd El Tawab *et al.*³⁶.

$$\text{Digestion coefficient} = 100 - \left[\frac{\text{AIA in feed (\%)} \times \text{Nutrient in feces (\%)}}{\text{AIA in feces (\%)} \times \text{Nutrient in feed (\%)}} \times 100 \right] \quad (1)$$

Sampling and analysis of rumen liquor: Rumen liquor samples were collected by stomach tube at the last day of

Table 2: Chemical composition of untreated or treated sugar beet leaves silage, corn silage, concentrate feed mixture and rice straw (% dry matter basis)

Parameters	USBT	TSBT	CS	CFM	RS
As g/100 g DM					
DM	27.86	27.48	29.33	89.46	90.11
OM	87.58	87.64	93.53	94.42	90.52
CP	6.83	6.76	8.64	15.48	3.64
CF	17.45	14.84	23.35	6.37	39.48
EE	1.16	1.22	2.74	3.11	1.06
NFE	62.14	64.82	58.80	69.46	46.34
Ash	12.42	12.36	6.47	5.58	9.48
NDF	44.29	41.58	61.63	32.74	69.98
ADF	29.85	27.33	36.86	21.31	43.73
ADL	5.15	5.03	5.22	4.85	10.66
Hemicellulose	14.44	14.25	24.77	11.43	26.25
Cellulose	24.70	22.30	31.64	16.46	33.07
Oxalate (mg/100 g FW)	967.00	431.00	-	-	-
Calcium (mg/100 g FW)	106.80	143.50	157.80	-	-
pH	4.36	4.02	3.96	-	-
Lactic acid (g kg ⁻¹)	14.66	18.58	20.52	-	-
Acetic acid (g kg ⁻¹)	8.74	11.36	14.19	-	-
Butyric acid (g kg ⁻¹)	3.59	3.01	2.68	-	-

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, NFE: Nitrogen free extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, USBT: Untreated sugar beet tops silage, TSBT: Treated sugar beet tops silage, CS: Corn silage, CFM: Concentrate feed mixture, RS: Rice straw, FW: Fresh weight, ADL: Acid detergent lignin

each experimental period for each animal. Samples were taken at 3 h after morning feeding to determine pH, ammonia-N and Volatile Fatty Acids (VFA). The rumen samples were filtered through two layers of cheese cloth. The pH of ruminal fluid was measured immediately with a pH meter (HI98127 pHep4 pH-temperature tester; Hanna Instrument) and NH₃-N was determined according to AOAC³³. Total Volatile Fatty Acids (TVFA's) were determined by steam distillation as described by Warner³⁷. Samples were stored at -20°C until further analyses. Concentration and molar proportions of individual VFA were measured by gas-liquid chromatography (model 5890; Hewlett-Packard, Little Falls, DE) according to Khattab *et al.*³⁸

Sampling and analysis of blood plasma: Blood samples were taken at the last three days of each experimental period. The samples were taken before morning feeding and at 4 h post feeding. Each blood sample was withdrawn from the jugular vein into a heparinized tube and centrifuged at 4000 rpm/15 min to separate blood plasma into clean dried glass vial and stored in at -18°C for further analysis. Plasma glucose, total protein, albumin, globulin, A/G ratio, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and calcium (Ca) were determined spectrophotometrically by kits using T80 UV/VIS Spectrometer, PG Instruments Ltd., UK; according to the standard protocols of the suppliers.

Sampling and analysis of milk: The animals were milked twice daily at 6.00 am and 6.00 pm during the last three days of each experimental period. Milk samples were analyzed for

total solids, solids not fat, total protein, fat, ash and lactose using infrared spectroscopy (Bentley 150, Infrared Milk Analyzer, Bentley Instruments, USA). Energy Corrected Milk (ECM) was calculated according to Sjaunja *et al.*³⁹.

Statistical analysis: Data were statistically analyzed according to a completely randomized design using GLM procedure of SAS software (Version 9.2)⁴⁰. Level of significance between treatments was determined by Duncan test. Probability of <0.05 was considered as significantly different.

RESULTS

Silage treatments: Data in Table 2 show chemical composition of untreated or treated sugar beet leaves silage. Treated silage was higher in calcium content, lactic acid and acetic acid. While, it recorded the lowest values in pH, oxalate contents, cellulose, hemicellulose and butyric acid than that untreated silage.

Feed intake, nutrient digestibility and nutritive value: The dry matter feed intake, nutrient digestibility and nutritive value are shown in Table 3. Roughage dry matter intake decreased T2 compared with T1 and T3. Nutrients digestibility of DM, CP, CF, NFE, NDF, ADL and nutritive value of TDN and DCP significantly (p<0.05) decreased between T2 compared with T1 and T3. Also, EE digestibility significantly (p>0.05) increased for T1 compared with T2 and T3 and was not significant between T2 and T3.

Table 3: Effect of experimental diets on nutrients digestibility

Parameters	Treatments			p>F
	T1	T2	T3	
Dry matter intake (kg h⁻¹ day⁻¹)				
CFM	6	6	6	
Roughage	6.4	6.1	6.6	
Nutrients digestibility (%)				
DM	66.49±0.4388 ^a	61.74±0.527 ^c	64.88±0.260 ^b	0.0006
CP	62.67±0.4054 ^a	57.63±0.458 ^c	61.01±0.475 ^b	0.0006
CF	58.62±0.412 ^a	52.85±1.006 ^c	56.21±0.238 ^b	0.0021
EE	73.53±0.212 ^a	70.57±0.560 ^b	71.97±0.493 ^b	0.0101
NFE	72.20±0.487 ^a	68.63±0.365 ^c	70.38±0.175 ^b	0.0014
NDF	63.22±0.419 ^a	58.37±0.634 ^c	61.52±0.226 ^b	0.0008
ADF	60.74±0.508 ^a	55.41±0.172 ^c	57.72±0.413 ^b	0.0002
Nutritive value				
TDN	66.32±0.393 ^a	61.03±0.439 ^c	63.24±0.214 ^b	0.0001
DCP	6.93±0.043 ^a	6.06±0.045 ^c	6.40±0.048 ^b	0.0001

^{a-c}Means with different in the same row are significant (p<0.05), SEM: Standard error of the mean, T1 group was fed on 50% (CFM), 30% corn silage and rice straw 20%, T2 group was fed on 50% (CFM), 30% untreated sugar beet leaves silage, rice straw 20%, T3 group was fed on 50% (CFM), 30% treated sugar beet leaves silage, rice straw 20%

Table 4: Effect of experimental diets on rumen metabolizes

Parameters	Treatments			p>F
	T1	T2	T3	
Rumen pH	6.38±0.057	6.31±0.064	6.24±0.068	0.3944
NH ₃ -N (mg/100 mL)	13.41±0.084	13.97±0.433	13.68±0.155	0.4032
Total VFA (meq/100 mL)	11.99±0.092 ^a	9.92±0.179 ^c	11.26±0.173 ^b	0.0002
Acetate (mol/100 mol)	61.30±0.427 ^a	55.38±0.606 ^c	58.69±0.387 ^b	0.0004
Propionate (mol/100 mol)	24.52±0.632	26.00±0.199	25.04±0.386	0.1322
Butyrate (mol/100 mol)	7.41±0.123 ^b	9.70±0.184 ^a	8.54±0.640 ^{ab}	0.0175
CO ₂ (mmol)	47.89±0.133	48.74±0.473	48.42±1.220	0.7385
CH ₄ (mmol)	28.22±0.312 ^a	26.04±0.315 ^b	27.36±0.455 ^a	0.0157

^{a-c}Means with different in the same row are significant (p<0.05), SEM: Standard error of mean, T1 group was fed on 50% (CFM), 30% corn silage and rice straw 20%, T2 group was fed on 50% (CFM), 30% untreated sugar beet leaves silage, rice straw 20%, T3 group was fed on 50 % (CFM), 30 % treated sugar beet leaves silage, rice straw 20%

Table 5: Effect of experimental diets on blood metabolizes

Parameters	Treatments			p>F
	T1	T2	T3	
Glucose (mg dL ⁻¹)	70.32±0.364 ^a	65.93±0.837 ^b	72.73±0.713 ^a	0.0011
Total protein (g dL ⁻¹)	8.14±0.130 ^a	6.38±0.191 ^c	7.47±0.212 ^b	0.0014
Albumin (g dL ⁻¹)	4.15±0.060 ^a	3.50±0.153 ^b	3.64±0.262 ^{ab}	0.0098
Globulin (g dL ⁻¹)	3.99±0.070 ^a	2.88±0.069 ^b	3.83±0.326 ^a	0.0149
A/G ratio	1.04±0.003	1.21±0.052	0.95±0.155	0.2670
Urea (mg dL ⁻¹)	39.62±0.324 ^a	44.13±0.206 ^a	40.86±0.613 ^b	0.0007
Creatinine (mg dL ⁻¹)	0.85±0.047 ^b	1.08±0.040 ^a	0.96±0.044 ^{ab}	0.0303
AST (U L ⁻¹)	31.24±0.554 ^b	34.96±0.498 ^a	32.60±0.389 ^b	0.0046
ALT (U L ⁻¹)	18.28±0.136 ^b	20.81±0.425 ^a	19.82±0.462 ^a	0.0083
Ca (mg dL ⁻¹)	8.12±0.153 ^a	5.77±0.263 ^b	7.36±0.336 ^a	0.0020

^{a-c}Means with different in the same row are significant (p<0.05), SEM: Standard error of mean, T1 group was fed on 50% (CFM), 30% corn silage and rice straw 20%, T2 group was fed on 50% (CFM), 30% untreated sugar beet leaves silage, rice straw 20%, T3 group was fed on 50% (CFM), 30% treated sugar beet leaves silage, rice, straw 20%, A: Albumin, G: Globulin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Ca: Calcium

Rumen fermentation: The effect of experimental diets on rumen metabolizes is shown in Table 4. Ruminal pH, ammonia nitrogen, propionate and CO₂ were not significant among the groups, while the total VFA's and acetate significantly decreased (p<0.05) in T2 compared with T1 and T3. However, butyrate recorded the highest (p<0.05) value for T2 compared with T1. On the other hand, methane production (CH₄) significantly (p<0.05) decreased between T2 compared with other groups.

Blood metabolites: The effect of experimental diets on blood metabolites is shown in Table, 5. Plasma glucose concentration, total protein, globulin and calcium were lowest in T2 compared with T1 and T3. Also, Plasma albumin significantly decreased (p<0.05) between T2 compared with T1 and was not significant between T1 and T3. There was no significant difference for A/G ratio among three treatments groups. The urea and AST significantly increased in T2 group compared with the other two groups. Plasma creatinine and ALT significantly increased (p<0.05) between T2 compared with T1.

Milk yield and composition: Data in Table 6 show milk yield and milk composition. T2 recorded the lowest values for milk yield and energy corrected milk compared with other groups. The increases in milk yield and energy corrected milk were 11.5 and 19.58 %, respectively for T3 compared with T2 and decreased by 9.37 and 20.32%, respectively for T2 compared with T1. Percentage of total solids, fat, lactose and solids not fat significantly (p<0.05) decreased in T2 compared with the other groups. There were no significant differences for protein percent among the groups. Ash percent significantly increased (p<0.05) between T2 compared with other the groups.

DISCUSSION

Results in the current study refer that adding lactic acid bacteria to sugar beet leaves silage improve the quality of silage than untreated silage, this enhancing may be related to increases degradation of oxalate contents by lactic acid bacteria consequently, fermentation and total volatile fatty acid were increased and decrease pH values. These results

Table 6: Effect of experimental diets on milk production and composition

Parameters	Treatments			pr.>F
	T1	T2	T3	
Milk yield (kg h ⁻¹ day ⁻¹)	13.98±0.139 ^a	12.67±0.456 ^b	14.31±0.213 ^a	0.0016
ECM (kg h ⁻¹ day ⁻¹)	13.33±0.052 ^a	10.62±0.283 ^b	13.21±0.082 ^a	0.0001
Milk composition (%)				
Total solids	12.28±0.066 ^a	10.92±0.091 ^c	11.96±0.063 ^b	0.0001
Fat	3.70±0.101 ^a	3.24±0.052 ^b	3.59±0.092 ^a	0.0019
Protein	3.48±0.066	3.38±0.037	3.43±0.057	0.4377
Lactose	4.33±0.111 ^a	3.35±0.040 ^c	4.05±0.104 ^b	0.0001
Solids not fat	8.58±0.051 ^a	7.68±0.048 ^c	8.37±0.045 ^b	0.0001
Ash	0.77±0.005 ^c	0.95±0.018 ^a	0.89±0.013 ^b	0.0001

^{a-c}Means with different in the same row are significant (p<0.05), SEM: Standard error of mean, T1 group was fed on 50% (CFM), 30% corn silage and rice straw 20%, T2 group was fed on 50% (CFM), 30% untreated sugar beet leaves silage, rice straw 20%, T3 group was fed on 50% (CFM), 30% treated sugar beet leaves silage, rice straw 20%, ECM: Energy conservation measure

agree with Saleh⁴¹ who found that treated sugar beet tops silage with 1% probiotic has the lowest values of pH, oxalate and the highest values of total volatile fatty acid and acetic acid compared with untreated silage.

Dry matter intake was decreased in untreated sugar beet leaves group than the other groups, perhaps because of low palatability of animals. Oxalate contents have an acidic effect of feeds⁴². Also, calcium oxalate crystals may cause burning and irritation sensations in digestive tract of animals⁴³. These results are agreed with James and Butcher¹¹; Wang and Provenza⁴⁴, Kyriazakis *et al.*⁴⁵, Burritt and Provenza⁴⁶ and Rahman *et al.*¹⁴ who found that dry matter intake reduced when oxalate contents increased in the sheep diets. While, Saleh *et al.*⁴⁷ observed that feed intake significantly increased (p<0.05) in untreated sugar beet tops silages in Rahmany rams diets. However, Gowda and Prasad¹² found that dry matter intake had an insignificant difference in cows fed *Eleusine coracana* (high oxalate).

In this study, observed that nutrients digestibility and nutritive values for treated sugar beet leaves groups were enhanced. Results may be attributed to the reduction of oxalate contents by microbiological treatment for sugar beet leaves silage than untreated sugar beet leaves silage and increasing in rumen microorganism activity. James *et al.*¹ noted that the *in vitro* oxalate may lead to rumen dysfunction and inhibited cellulose fermentation which, has effect of microorganism in rumen⁴⁸. These results were in agreement with Saleh *et al.*⁴⁷ who found that NDF, ADF ADL, cellulose and hemicelluloses digestibilities significantly (p<0.05) decreased when Rahmany rams were fed untreated sugar beet tops than other all tested treatments. Also, Saleh⁴¹ reported that all nutrients digestibilities and nutritive value significantly (p<0.05) decreased when lambs were fed on untreated sugar beet tops silage than other all tested treatments. Gaafar *et al.*¹³ noted that digestibility coefficients and TDN

value increased (p<0.05) when replaced with sugar beet tops silage by corn silage but, DCP value decreased in lactating buffaloes diets. While, Bendary *et al.*¹⁰ suggested that TDN and DCP values increased with increasing sugar beet tops silage in animal's diet.

Results of rumen parameter refer that ruminal TVFA's and acetic acid concentration for treated sugar beet leaves groups were increased. These may be microbiological treatment had positive effects on oxalate and fibre degradations which showed as TVFA's and acetic acid concentration^{36,41}. This may indicate that oxalate contents in diets have inhibited microbial activity in the rumen as adverse effects on digestive nutrients and fermentations which was clear from decreased nutrients digestibility (Table 3) and reduction of gas production in T2 compared with T3 and T1⁴⁹. These results were in good agreements with Gaafar *et al.*¹³ who found that ruminal total VFA's concentration increased (p<0.05) with decreasing the level of sugar beet tops silage and increasing the level of corn silage in lactating buffaloes diets. Also, Baker⁵⁰ found that ruminal TVFA's production decreased with feeding untreated sugar beet tops silage. Gowda and Prasad⁸ reported that total VFA's decreased in cows fed roughage high level oxalate compared with other groups. Amira *et al.*¹⁵ found that *O. ficus indica* (high oxalate) has negative effects on rumen microbial fermentations as suggested by the reduced gas production, total VFA's and acetic acid *in vitro* study.

Negative influence for blood metabolites in untreated sugar beet leaves group compared with other groups could be related to the negative impact of oxalate on cows. Enhancing in glucose concentration with biological treatment (T3) may be due to the parallel increase in TVFA's of rumen fluid (Table 3) than the untreated groups (T2)⁵¹. Kholif⁵¹ found a positive relationship between ruminal TVFA's and blood plasma glucose concentration. Rise of differences in total protein and albumin for biological treatment group (T3)

related to the improvements occurred in metabolic process specific Digestible Crude Protein (DCP) and indicated that these cows cover their protein needs¹⁰. Lack of plasma calcium concentration in T2 may be attributed to deficiency of calcium absorption by small intestines as a response to oxalate contents in diet¹². These results were in agreement with James and Butcher⁷ who found that blood calcium decreases with increasing oxalate contents in the diet. Also, Rahman *et al.*¹⁴ reported that lower plasma calcium concentrations of sheep fed untreated guinea grass had higher ($p < 0.05$) calcium concentrations than sheep fed treated guinea grass with oxalic acid solution. Rise of values in kidney (urea and creatinine) and liver function (ALT and AST) for T2 related to oxalate contents increase in diet^{18,22,23}. But, all values of blood parameters are within the normal range.

Significantly, milk yield and FCM was increased in treated sugar beet leaves group compared with untreated sugar beet leaves group. These results are in agreement with Clark *et al.*⁵² and Kholif⁵¹ who found a positive relationship between blood glucose contents and milk yield (Table 5). These enchainings may be related to improving of nutrients digestibilities and nutritive values (TDN and DCP) of T1 and T3 (Table 3). Also, Rai *et al.*⁵³ reported that increase level of oxalate in animal diets may have a negative effect on milk yield and fat contents. In addition, milk production might be lower by increasing oxalate in diets and reducing blood calcium^{15,16}. Gaafar *et al.*¹³ found that milk production increased with decreasing the level of sugar beet tops silage and increasing the level of corn silage in lactating buffaloes diets. Also, Gowda and Prasad¹² found that milk production increased in cows fed on finger millet straw (low oxalate content) as compared to cows fed on rice straw (high oxalate content).

Milk compositions was affected by biological treatment. The increasing fat percentage with biological treatment may be related to increases digestible fiber and total VFA's especially acetic acid in rumen (Table 4). Also, increasing of lactose percent was trend to increasing DCP and TDN intake (Table 3). These results are in agreements with Gowda and Prasad⁸ and Gaafar *et al.*¹³.

CONCLUSION

In general, making sugar beet leaves silage inoculated with lactic acid bacteria reduced oxalate effect on lactating Frisian cow's diets and led to a significant increase ($p < 0.05$) in digestibility of nutrients, nutritive values, milk yield and improving animal's performance without adverse effect on cow healthy. Lactic acid bacteria have a positive effect on sugar beet leaves silage as increase in lactic acid and acetic

acid contents. Also, increased roughage DMI, dry matter digestibility, blood glucose, ruminal TVFA's, milk yield, energy corrected milk, milk fat and lactose.

SIGNIFICANCE STATEMENTS

- This study was carried out to investigate inoculating sugar beet leaves silage with lactic acid bacteria to reduce negative effect of its content of oxalates
- Milk production and nutrients digestibility were significantly ($p < 0.05$) impacted by ensiling sugar beet leaves treated with lactic acid bacteria compared with using untreated sugar beet leaves silage in the diets

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