Int J Plant Anim Environ Sci 2022; 12 (2): 074-095 *DOI: 10.26502/ijpaes.202133*

Research Article

Influence of using Pomegranate Peel Silage in Rations of Dairy Cows on their Productive Performance

El-Morsy AM[1]*, Shoukry MM[2], Soliman SM[1], Soliman MM[1]

[1]Regional Centre for Food and Feed, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt
[2]Animal Production Department, National Research Center, Dokki, Egypt

***Corresponding Author:** El-Morsy AM, Regional Centre for Food and Feed, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt

Received: 22 May 2022; **Accepted:** 03 June 2022; **Published:** 22 June 2022

Citation: El-Morsy AM, Shoukry MM, Soliman SM, Soliman MM. Influence of using Pomegranate Peel Silage in Rations of Dairy Cows on their Productive Performance. International Journal of Plant, Animal and Environmental Sciences 12 (2022): 074-095.

Abstract

This study was implemented to evaluate the effect of a partial replacement of the whole corn plant silage (WCS) with the pomegranate peel silage (PPS) treated with enzymes mixed (ZYMOGEN) or lactic acid bacterial inoculants (Inoculant 1188) on the nutrients digestibility and the productive performance of dairy cows. In the first experiment, two stacks from PPS and WCS prepared, and WCS replaced by PPS at 25:75, 50:50, and 75:25, respectively, for forming three rations. The second experiment was carried out with twenty lactating crossbred Friesian cows in four similar groups (5 cows/per group): the control group fed on as fed a ratio consisting of WCS. G1 (WCS replaced with untreated PPS at level 50:50), G2 (WCS replaced with PPS treated with bacterial inoculants at level 50:50), and G3 (WCS replaced with PPS treated with ZYMOGEN at level 50:50). The results showed CP, NFE, and lactic acid values were highest, while NDF, ADF, pH values, and the concentration of NH3-N and acetic acid were lowest in all groups of treated PPS. The digested coefficients, nutrient values, ruminal fluid fermentation of TVFAs and acetic acid, milk yield, 4% FCM, milk composition and blood protein values were higher, and rumen pH and NH3N values were lower in Groups 2 and 3 relative to the

other groups. It concluded that using treated or untreated pomegranate peel is safe in dairy cows feeding at a level of 20 %. Supplementation of ZYMOGEN or inoculant 1188 to PPS improved the fermentation and nutritive quality of silage.

Keywords: Digestibility; Milk; Inoculant; Enzymes; Methane; Rumen fermentation

1. Introduction

Pomegranate (Punica granatum L.) from the Punicaceae family; it has been used anciently for medicinal purposes. It is extensively cultivated in Iran, Spain, Egypt, Russia, France, Argentina, China, Japan, the USA, and India [1]. The world's pomegranate production amounts to approximately 8.1 million tons [2]. The annual production of pomegranate in Egypt is estimated at 649900 tons [3]. The amount of peels (pericarp, rind, or hull) is approximately 60 % of pomegranate fruit weight [4]. Increasing the evolution of agro-industry has contributed to the increase in the quantities of by-products such as peels and pomegranate seeds in the juice industry. Reduced loss of non-ammonia nitrogen and essential amino acids in the small intestine may be attributed to the decrease in protein degradation in the animal's rumen [5]. Recently, pomegranate by-products have attracted attention. Pomegranate peel is abundant with bioactive compounds like phenolic compounds, flavonoids, proanthocyanidins, various tannins and ascorbic acid, which are known as secondary plant metabolites. These compounds have several beneficial properties, like antioxidant, anti-inflammatory, antibacterial and antiviral activity. High levels of tannins reduce the palatability and digestion of proteins and carbohydrates, whereas low to moderate levels protect dietary protein from degradation [6]. Tannins are two groups: hydrolysable and condensed tannins [7]. Thus, we can use the pomegranate peel as a source of tannin in the diet to improve the ruminal fermentation process. Also, Shabtay et al. [8] and Sadq et al. [9] showed that the growth parameters were increased in animals that fed on rations containing pomegranate peels, which may be attributed to improving immune functions that can potentially affect an animal's health. Pomegranate biomass is rich in moisture, but if it is not consumed in a short period of time, it molds and becomes useless [8]. Consequently, silage is an effective way to preserve pomegranate peel for use in ruminant rations. High-quality silages are characterized by high water-soluble carbohydrate (WSC) concentrations and a dry matter content of 250-400 g/kg [10]. Dietary intake, nutrient utilization and milk production in ruminants are influenced by the quality of silage fermentation [11]. Employing of several additives (bacterial inoculants, enzymes, etc.) during the silage process improve the aerobic stability and enhances the nutritive value of silage [12]. Lactic acid bacteria (LAB) inoculants are divided into two major groups: the homofermentative LAB and the heterofermentative LAB. They are used as biological additives in silage. Numerous studies have explained that the employment of homofermentative LAB inoculants in plant ensilage rais the lactic acid concentrat and reduce the values of acetic acid, butyric acid, ammonia nitrogrn (NH3-N) of the silage [13, 14] and, rapid the fermentation producing and accelerating the reduction of the silage pH and thus improving the quality of the silage by preventing the

degradation of sugar and proteins [15]. Furthermore, the addition of enzymes to silage has led to in the decay of cell walls and increased the availability of WSC as a substrate for the LAB [16]. The recycling of the large quantities of industrial and agricultural by-products through its inclusion into livestock rations is met with great attention. This process contributes to the transformation of by-products that are unsuitable for human consumption into foods useful for human consumption, which contributes to meeting the requirements of population growth. It also reduces environmental pollution, health damage and costs to dispose of waste [17]. In addition, it reduces acute feed shortages in developing countries and feed costs, thus increasing the economic efficiency of animal production [18]. This study's objective is to investigate the influence of the partial replacement of the whole corn silage by pomegranate peel silage, and the effect of adding the LAB inoculants or enzymes to pomegranate peel silage on nutrient digestibility, rumen parameters, methane production and productivity of dairy cows.

2. Materials and Methods

This study was designed to use the pomegranate peel in silage form (PPS) as a replacement for whole corn plant silage (WCS) in lactating Frisian cows rations. First, the laboratory study was carried out. It has been prepared two stacks from PPS and WCS. The silage characteristics in two stacks were good. The PPS has been mixed with the WCS in three formulas, 25:75, 50:50 and 75:25, respectively. The three different silage mixture levels were used to form three lab rations to determine the optimal alternative level suitable for ruminant nutrition. The total tannin concentrations in the three different rations were 3.49 gm, 6.92 gm and 10.43 gm/ kg diet that equivalent 0.69, 1.40 and 2.14% on DM basis, according to tannin concentration by Colombini et al. [19] 1.3% of DM, and Herremans et al. [20], 13gm/kg DM. The 50:50 level (WCS: PPS) has been selected as the optimal level for the dairy cow feeding experience. This experiment was carried out at the Noubaria Experimental Station, Animal Production Research Institute, for evaluating the effect of adding the lactic acid bacterial inoculants (Inoculant 1188) or enzymes mixed (ZYMOGEN) to PPS on nutrient digestibility, in vivo rumen parameters, in vitro methane production and milk production in dairy cows. The fresh pomegranate peel was obtained from the private company El-Marwa, 6 October City at the end of summer 2019. Whole corn plant was harvested in the Noubaria region in late July 2019. According to the manufacturer's recommendation, the Inoculant, 1188 (Pioneer®, USA), which contains four strains of Lactobacillus plantarum and two strains of Enterococcus faecium, was applied at a rate of 10 ml /ton. In lactic acid bacteria (LAB), a total of 125 billion colony forming units (CFU) per gram are guaranteed. ZYMOGEN is a liquid mixture of digestive enzymes, such as amylase (1500000 Units), lipase (500000 Units), cellulase (1000000 Units), xylanase (1000000 Units), protease (2500000 Units) and pectinase (20000000 Units) from WISEMED INC – USA. The approximate chemical analysis of fresh pomegranate peel and whole corn plant before ensiling, the concentrate feed mixture (CFM) and rice straw (RS) used in this experiment are presented in Table (1). The second experiment involved preparing four silage piles; the first silage pile was a WCS as a

control group. The second silage pile was untreated PPS. The third silage pile was a treated PPS with bacterial inoculants (Pioneer brand 1188). The fourth silage pile was a treated PPS with ZYMOGEN. The four piles were covered separately with double-layer plastic linoleum and pressed with 30 cm of a soil layer. Silage materials have been compressed by a large sand-filled drum to ensure anaerobic silage conditions for more than two months.

Item	**CFM***	**RS**	**whole corn plant**	**PP**
DM	91.87	93.88	32 .76	30.32
CP	23.34	04.69	8.64	10.42
CF	9.17	32.25	27.39	18.11
EE	3.25	1.76	2.23	4.69
NFE	57.05	47.41	56.17	61.09
Ash	7.19	13.89	5.57	5.69
NDF	15.95	76.06	46.17	19.68
ADF	10.61	45.23	25.39	16.48
ADL	4.01	4.96	4.52	4.59
Tannin g/kg DM	--	--	--	92.8

* CFM of Composition: 30%yellow corn, 27%wheat bran, 25% soybean meal (47%), 10% undecorticated cottonseed meal (26%), 5% molasses, 2.5% salt Limestone and 0.5%premix. The vitamin and mineral premix per kg contained the following Vitamin A 12 000 000 IU, Vitamin D3 3 000 000 IU, Vitamin E 30 g, Mn 50 g, Fe 52 g, Zn 50 g, Cu 10 g, I 0.8 g, Co 0.1 g, Se 0.15 g and antioxidant 10 g.

Table 1: Chemical composition of fresh pomegranate peel, whole corn plant, rice straw and concentrate feed mixture on DM basis.

2.1 Silage quality

Samples from the four stacks (5 samples /each pile) were taken after 60 days to determine silage quality and chemical composition. The same volume of water used to dissolve the silage additives was added to the first and second treatments to keep the moisture level. To evaluate the quality of the silage, the silage extract was prepared by homogenizing 30 grams of fresh material with 270 ml of distilled water, then mixing for 10 minutes in a laboratory mixer. The homogenized sample was filtered using a Whatman No. 54 filter paper until it becomes clear. The pH value was directly determined using Orion 680 digital pH meter. The lactic acid concentration was measured according to [21]. Total volatile fatty acids (TVFA'S) concentration was determined according to [22]. The molar proportion of TVFA'S (acetic, propionic and butyric) was measured according to Bush et al. [23]

using High-Performance Liquid Chromatography (HPLC). NH3-N concentration was determined by direct distillation according to the [21]. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the procedure of [24]. The chemical composition and silage quality of WCS and untreated or treated PPS are shown in Table (2).

2.2 Experimental diets and lactation trials

Twenty multiparous lactating crossbred Frisian cows were assigned randomly to four treatments (5 cows / each treatment) stratified by milk yield and live body weight (548 ± 5.7 kg); each cow has the individual pen. Each group was fed CFM, silage and RS at 50:40:10 (%DM basis), respectively, for each group. The first 20 days were considered as a preliminary period followed by 10 day collection period. Maintenance requirement calculated according to NRC [25] and requirement for the production were calculated from preliminary period and also the milk yield previous according to [26]. The first group (control) was fed a ration that consisted of 50% CFM, 40% WCS and 10% RS. The second group (G1) was fed a ration that consisted of 50% CFM, 40% silage (WCS replaced with untreated PPS at level 50:50) and 10% RS. The third group (G2) was fed a ration that consisted of 50% CFM, 40% silage (WCS replaced with PPS treated with Inoculant 1188 at level 50:50) and 10% RS. The fourth group (G3) was fed a ratio that consisted of 50% CFM, 40% silage (WCS replaced with PPS treated with ZYMOGEN at level 50:50) and 10% RS. The animals were fed twice daily at 8.00 A.M. and 5.00 P.M. and water was available all time. The residual diets were collected and calculated for the estimated feed intake for each individual cow. Cows were machine milked twice during collection periods and milk samples (1% of milk yield/ period) were taken during the 10 days at 7.00 A.M. and 16.00 P.M. Actual milk yields were recorded daily and milk samples were taken and kept at 4 °C for analysis. Milk composition (fat, total protein, lactose, and total solids) and somatic cell count (SCC) in the milk samples were determined using MilkoScan FT 6000. Average yields of each milk component were calculated for each cow by multiplying the milk yield by the component content (g/kg) of milk. Fat corrected milk (4 %) was calculated according to Gaines [27] using the following equation:

$$FCM4\% = M\ (0.4+0.15\ F\ \%)$$

Where M= milk yield, F = fat percentage.

2.3 Digestibility trials

The digestibility trial lasted three weeks as a preliminary period followed by one week as a collection period. Feed consumption was recorded daily by weighing feeds offered to head and refused per each animal to calculate feed intake. A nutrient digestibility trial was carried out in which acid insoluble ash was used as an internal indigestibility marker and coefficients of digestion were calculated according to [28]. Faecal grab samples were collected from each cow twice daily and then dried at 60 °C in a forced-air oven for 48 h. Faecal samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and analyzed for DM, OM, ash, CP and EE according to AOAC [21] official methods. NDF and ADF were determined by the procedure of [24].

2.4 Rumen fermentation parameters, microbial nitrogen synthesized and measurement of methane production

Ruminal fluid contents were sampled at 0 times before feeding and at 3 and 6 h after the morning feeding using stomach tubing from cows, from day 27 to day 30 and then calculate overall mean. Approximately 100 ml of rumen fluid were collected from each cow and strained through a polyester screen (pore size of 355 μm). The supernatant was used for determining pH immediately using a glass electrode. Five millilitres of the filtered ruminal fluid were added to 1 ml of 1% sulfuric acid and samples were retained for NH3-N determination. The concentration of NH3-N in the ruminal contents was determined as described by [29]. The filtered rumen fluid was mixed with 0.2 ml of a solution containing 250 g of metaphosphoric acid/L for TVFA's analysis by titration according to the method of [22]. Samples were stored at −20 °C until analyses. Concentration and molar proportions of individual VFA were measured by gas-liquid chromatography (model 5890, HP, Little Falls, DE, USA). The separation process was carried out with a capillary column (30 m × 0.25 mm internal diameter, 1-m film thickness, Supelco Nukol; Sigma–Aldrich, ON, Canada) and with flame ionization detection. The column temperature was adjusted to 100 °C for 1 min, then increased by 20 °C/min to 140 °C, then by 8 °C/min to 200 °C and held at this temperature for 5 min. Helium was used as the carrier gas.

The microbial nitrogen (MN) synthesized was determined according to [30]. Equations used to calculate as follows: MN = (70 × AP) / (0.83 × 0.116 × 1000), where 70 represents the amount of N in the purines (mg N/mmol), 0.83 is the digestibility of the microbial purines, and 0.116 is the purine N: total N ratio in ruminal microorganisms. The absorbed microbial purines (AP, mmol/day) are calculated from the total excretion of purine derivatives (PD, mmol/day), using the equation:

AP = {PD – (0.385 × BW0.75)} / 0.85, where 0.85 is the recovery of absorbed purines as urinary purine derivatives, and 0.385 * BW0.75 is the endogenous contribution in the urinary excretion of PD [31].

In vitro methane production was determined as described by [32].

2.5 Plasma metabolites

At the end of the feeding trial, blood samples (10 ml) were taken by venipuncture from the jugular vein using heparinized vacuum tubes and were stored on ice. Then samples are centrifuged and the serum remains at the top of the tube immediately after the completion of the centrifuge we transfer the serum directly and prepared it for storage at − 20°C until analysis. The blood serum was analyzed for total protein in accordance with [33]. Plasma albumin was assayed according to [34]. Globulin was determined by subtracting the albumin value from the total protein. Liver function was estimated by measuring the activities of aspartate transaminase (AST) and alanine transaminase (ALT) using a colorimeter using commercial kits, according to [35]. Renal function was assessed by measuring urea in the blood according to Siest et al. [36] and creatinine was measured using a colorimeter using commercial kits, based on the [37].

2.6 Statistical analysis

Data were analyzed as a completely randomized design with repeated measures using the PROC MIXED procedure of SAS [38]. Statistical processes were carried out using the General Linear. The model describing each trait was assumed to be:

Yijkl = μ + Ti + a (T) IJ+ WK+ Eijkl.

Where: Yijkl= Parameter under analysis; μ = Overall mean; Ti = The fixed effect of treatment; a (T) IJ = The random effect of animal (j) nested within treatment (i); WK = The fixed effect of week when K = 1, 2,…., 8; Eijkl = random error. Significant differences among the means were compared by using Duncan multiple range tests [39].

3. Results

3.1 Silage quality

The results obtained from laboratory studies illustrate that the optimum level of replacement of WCS with PPS was 50:50. This level contains an adequate amount of tannins suitable for ruminant nutrition. The chemical composition and silage quality data for untreated or treated PPS and WCS are shown in Table (2). PPS treated with 1188 or ZYMOGEN resulted in a significant increase (P<0.05) in the content of CP, EE and NFE. While the content of CF, ash, NDF and ADF was significantly lower (P<0.05) than the WCS. The addition of 1188 or ZYMOGEN to the PPS improved the CP by increasing it, while the NDF and ADF decreased. On the other hand, there were no significant changes to the OM values among all groups. PPS treated with inoculant had the lowest values(P<0.05) of pH and concentrations of NH3-N, acetic acid, propionic acid, and butyric acid (11.74, 15.67, 14.6, 18.06, and 20.4%, respectively) compared to WCS, whereas concentrations of lactic acid were higher (P< 0.05) in PPS treated with inoculant 1188 or ZYMOGEN (22.22% and 11.77%, respectively) compared to WCS, On the other hand, the values of acetic, propionic and butyric acid were decreased (P<0.05) in PPS treated with inoculant (9.74, 14.06 and 17.77%, respectively) while the values of lactic acid were increased (15.51%) compared to untreated PPS. Data on the chemical composition of the experimental rations fed to cows is shown in Table (3). The chemical composition of rations fed to cows in G3 and G4 showed improvement and low tannin concentration compared to G1.

Item	Untreated WCS	Untreated PPS	PPS treated with inoculant 1188	PPS treated with ZYMOGEN	SEM	P-value
DM	30.41[a]	28.04[b]	29.62[a]	29.86[a]	0.248	0.021
OM	93.64	93.35	94.03	94.18	0.275	0.714
CP	7.86[c]	9.38[b]	10.07[a]	9.92[a]	0.222	0.001
CF	26.53[a]	17.61[b]	17.25[b]	16.91[b]	0.933	0.001
EE	2.58[b]	4.45[a]	4.41[a]	4.38[a]	0.189	0.001
NFE	56.67[b]	61.91[a]	62.30[a]	62.97[a]	0.678	0.018
Ash	6.36[a]	6.65[a]	5.97[b]	5.82[b]	0.110	0.019
NDF	43.65[a]	18.57[b]	17.25[c]	15.72[d]	2.650	0.001
ADF	26.02[a]	15.54[b]	14.48[c]	13.36[d]	1.187	0.001
Silage quality						
pH	3.93[a]	3.77[b]	3.47[c]	3.53[c]	0.076	0.001
Lactic acid % DM	8.99[d]	9.51[c]	10.99[a]	10.05[b]	0.300	0.001
Acetic acid % DM	1.56[a]	1.48[b]	1.33[c]	1.39[c]	0.085	0.001
Propionic acid %DM	0.107[a]	0.102[a]	0.088[c]	0.094[b]	0.005	0.001
Butyric acid% DM	0.050[a]	0.048[a]	0.040[c]	0.044[b]	0.005	0.001
NH_3-N % of TN	8.87[a]	7.94[b]	7.48[c]	7.86[b]	0.160	0.001

[a,b,c and, d] Means within the same rows with different superscripts are significantly different (P<0.05).

WCS: whole corn silage. PPS: pomegranate peel silage.

Table 2: Chemical composition and fermentation characteristics of treated or untreated silages (n = 5).

Item	Control	G1	G2	G3
DM	67.41	66.99	68.06	68.39
CP	15.44	15.74	16.06	15.90
EE	2.85	3.25	3.21	3.19
Ash	6.4	6.73	6.43	6.49
NDF	33.01	28.01	26.69	26.08
ADF	19.40	17.71	16.75	16.39
ADL	4.22	4.22	4.10	4.04
Tannin g/kg diet DM	0.23	13.48	12.98	12.31

Control: ration consisted of 50% CMF, 40% silage WCS and 10% RS.

G1: second group fed ration consisted of 50% CMF, 40% silage WCS replaced with untreated PPS at level (50:50) and 10%RS.

G2 third group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with bacteria inoculants PPS at level (50:50) and 10%RS.

G3 fourth group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with ZYMOGEN PPS at level (50:50) and 10%RS.

Table 3: Chemical composition of the experimental rations.

3.2 Dry matter intake and digestibility coefficients

Dry matter (DM) intake, digestibility coefficients and nutritive values are presented in Table (4). The results showed that the replacement of the WCS with the treated PPS resulted in an improved DM intake. The results were close to those fed with a diet containing WCS and enhanced palatability compared to untreated PPS. In addition, cows fed PPS that were enzyme-treated or inoculated had higher digestibility of DM, OM, CP, EE, NDF, TDN, ADF and DCP compared to cows fed untreated PPS and WCS.

Item	Control	G1	G2	G3	SEM	P-value
DMI kg/head/day	17.01[a]	16.87[b]	17.02[a]	17.09[a]	0.057	0.047
Digestibility coefficients						
DM	67.42[b]	66.12[b]	70.40[a]	71.10[a]	0.473	0.012
OM	68.86[b]	68.04[b]	72.13[a]	72.56[a]	0.445	0.023
CP	63.72[b]	64.03[b]	66.96[a]	67.82[a]	0.429	0.008
EE	71.93[b]	72.82[b]	74.34[ab]	75.77[a]	0.342	0.016
NDF	59.09[b]	58.87[b]	62.11[a]	62.85[a]	0.433	0.001
ADF	60.24[b]	59.59[b]	63.33[a]	63.56[a]	0.432	0.001
Nutritive value						
TDN	64.58[b]	65.75[b]	67.34[a]	68.96[a]	0.387	0.009
DCP	9.84[b]	10.08[b]	10.75[a]	10.78[a]	0.093	0.001

[a and b] Means within the same rows with different superscripts are significantly different (P<0.05).

Control: ration consisted of 50% CMF, 40% silage WCS and 10% RS.

G1: second group fed ration consisted of 50% CMF, 40% silage WCS replaced with untreated PPS at level (50:50) and 10%RS.

G2 third group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with bacteria inoculants PPS at level (50:50) and 10%RS.

G3 fourth group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with ZYMOGEN PPS at level (50:50) and 10%RS.

Table 4: Daily feed intake, digestibility coefficients (%) and nutritive values (%) of diets to cows.

3.3 Rumen fermentation

Parameters of the rumen fluid in lactating Frisian cows fed the experimental rations are presented in Table (5). Results revealed that adding ZYMOGEN or Inoculant 1188 to PPS led to decrease ruminal pH values and NH3-N concentrations, which were in the normal range for microorganism growth, while the values of TVFA's, acetic acid and acetic: propionic were significant (P< 0.05) increased with diets G2 and G3, whereas the highest value was recorded with G3. Although ZYMOGEN or Inoculant 1188 have improved OM digestibility but the increase in microbial protein synthesis was not significant. It has been shown that microbial protein synthesis has a numerical increase with the G2 diet compared with other diets. Replacement of WCS with PPS resulted in a significant (P < 0.05) decrease in methane emissions in G1, G2 and G3 (20.33, 17.72 and 16.24%, respectively), whereas ZYMOGEN or Inoculant 1188 additives had no effect on methane production when compared to G1.

Item	Control	G1	G2	G3	SEM	P-value
pH	6.75[a]	6.63[a]	6.21[b]	6.26[b]	0.102	0.012
NH3-N concentration (mg/100 mlR.L)	8.83[a]	8.11[b]	7.80[c]	8.06[b]	0.161	0.006
TVFA concentration (meq/100 mlR.L)	12.57[c]	12.98[c]	13.79[b]	14.21[a]	0.351	0.001
Acetic acid, %	58.99[b]	59.81[b]	63.71[a]	64.57[a]	1.217	0.002
Propionic acid, %	22.41	22.54	23.03	22.96	0.398	0.842
Acetic /propionic ratio	2.65[b]	2.66[b]	2.79[a]	2.83[a]	0.064	0.035
Methane production at 24h	9.45[a]	7.53[b]	7.78[b]	7.92[b]	0.166	0.001
Microbial protein synthesis (g/d)	55.84	55.15	56.18	55.73	0.222	0.876

[a,b and c] Means within the same rows with different superscripts are significantly different (P<0.05).

Overall mean values of 0, 3, 6 h after feeding.

Control: ration consisted of 50% CMF, 40% silage WCS and 10% RS.

G1: second group fed ration consisted of 50% CMF, 40% silage WCS replaced with untreated PPS at level (50:50)

and 10%RS.

G2 third group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with Inoculant 1188 PPS at level (50:50) and 10%RS.

G3 fourth group fed ration consisted of 50% CMF, 40% silage WCS replaced with treated with ZYMOGEN PPS at level (50:50) and 10%RS.

Table 5: The overall mean of rumen liquor parameters of lactating cows fed the experimental rations.

3.4 Milk production and milk composition

The average daily milk yield and milk composition of the lactating Frisian cows fed the experimental rations are present in Table (6). Cows fed on diets containing 50%WCS and50% PPS treated with ZYMOGEN have the highest daily milk yield, 4% FCM yield and milk composition of fat from the cows fed diets containing WCS and PPS treated with inoculant. While, cows fed on diets containing untreated PPS were recorded the lowest milk yield, 4% FCM yield and fat. Moreover, there were no significant differences in values of lactose, TS and SNF between all groups. The value of SCC recorded a normal value, but the cows fed on rations containing untreated or treated PPS had the lowest values ($P< 0.05$) compared to the control.

Item	**Control**	**G1**	**G2**	**G3**	**SEM**	***P-value***
Milk yield (kg/h/d)	18.02^{ab}	17.76^{b}	18.12^{ab}	18.34^{a}	0.139	0.031
4% FCM (kg/d/h)	17.05^{ab}	16.54^{b}	17.46^{a}	17.78^{a}	0.265	0.024
Fat (kg/d/h)	0.66^{ab}	0.63^{b}	0.68^{a}	0.69^{a}	0.015	0.001
Milk composition (%):						
Fat %	3.65^{a}	3.54^{b}	3.72^{a}	3.76^{a}	0.071	0.025
Protein %	3.43^{b}	3.49^{b}	3.68^{a}	3.71^{a}	0.097	0.012
Lactose	4.56^{b}	4.49^{b}	4.67^{a}	4.68^{a}	0.105	0.021
Total solids	12.80	12.77	12.91	12.95	0.194	0.649
Solid not fat	9.15	9.23	9.19	9.19	0.159	0.712
SCC×10^{3}/ml	88.54^{a}	81.26^{b}	80.80^{b}	81.07^{b}	0.825	0.001

a and b means in the same row with different superscripts are differ significantly ($P< 0.05$).

Table 6: Milk production and milk composition of crossbred cows fed the experimental rations.

3.5 Plasma metabolites

Blood plasma constituents of the lactating Frisian cows fed the experimental rations are shown in Table (7). Serum total protein, albumin and globulin concentrations were higher (P<0.05) in G3 (9.1, 7.0 and 12.1%, respectively) than in the control group. On the other hand, values of liver functions (AST and ALT) and kidney functions (urea and creatinine) were within normal values for all groups and had no significant effects between all groups.

Item	Control	G1	G2	G3	SEM	P-value
Total protein, g/dl	7.13[b]	7.27[b]	7.67[a]	7.78[a]	0.100	0.013
Albumin, g/dl	4.24[b]	4.32[b]	4.49 [a]	4.54[a]	0.083	0.019
Globulin, g/dl	2.89[b]	2.96[a]	3.18[a]	3.24[a]	0.044	0.001
AST, U/l	38.28	40.08	39.30	39.06	0.521	0.776
ALT, U/l	21.62	22.57	22.05	22.21	0.246	0.794
Urea, mg/dl	41.57	42.04	42.94	43.19	0.655	0.842
Creatinine, mg/dl	1.04	1.09	1.06	1.03	0.028	0.715

[a and b] means in the same row with different superscripts are differ significantly (P< 0.05).

Table 7: Blood parameters of crossbred cows fed the experimental rations.

4. Discussion

4.1 Silage quality

Since the pomegranate peel contains tannin that considered as anti-nutritional factors that has negative effects on animal performance. Makkar [40] illustrated that tannin level higher than 50 g kg−1 DM had a negative effect on palatability of feed intake or animal performance. The purpose of silage is to feed preserve and with as much as possible of the nutrient content of the original fresh feed [41]. Therefore, biological additives are used during ensiling for accelerate the fermentation process and raise the concentration of lactic acid, accelerating the reduction of silage pH and thus improve the quality and preservation of the silage [15], and this is consistent with the results of our study that explained add of inoculant 1188 or ZYMOGEN to PPS improved whoever the chemical composition and silage quality of PPS. A pH range of 3.7-4.2 is generally considered beneficial for whole-crop cereal silage preservation [42] and in the present study; pH was less than 3.93, indicative of well-preserved silage. In addition, the results indicated to treat SPP with either 1188 or ZYMOGEN were supported by the results of various scientists Nkosi et al. [43]. As well, Kung and Muck [44]. In contrast Ozduven et al. [45] reported a pH increase when bacterial inoculants were added to sunflower silage. While, Reich and Kung [46] demonstrated that the addition of bacterial inoculants to corn silage had no influence on pH. Adding 1188 inoculants to the PPS resulted in an increase in lactic acid concentration and a decrease in acetic acid. This

was also the case with silage treated with ZYMOGEN. This may be due to an improvement in the lactic-acetal ratio. These results supported the extent of pH decline and are consistent with [14]. On the other hand, Filya [47] found that the concentration of lactic acid in inoculated corn silage decreased compared to untreated silage. The reduction of pH values and increase of lactic acid values in PPS treated with inoculant or ZYMOGEN may be attributed to an increase in carbohydrate fermentation and hydrolysis of hemicellulose by lactic acid bacteria while ZYMOGEN could partially digest the plant cell walls (cellulose and hemicellulose). These results are similar to those of Nkosi et al. [48] and Kung [49] who explained that the addition of enzymes or inoculants to silage led to the enhanced degradation of cell walls and increase the availability of WCS that serves as the substrate for LAB and decreased the concentration of acetic, propionic and butyric acids. Ammonia-N concentration in silage is an indicator of the degree of protein degradation which impairs the nutritive value of forages and causes adverse effects on the utilization of nitrogen by ruminants [10]. The NH3-N as a percentage of DM should be less than 10% of total nitrogen (NT). That's in line with the current study, the concentration of NH3-N was less than 10% NH3-N/kg NT and increased CP in PPS treated with inoculant or ZYMOGEN, maybe an indicator of desirable reduction in protein degradation of silage [15, 50, 51]. Also, the reduction of NH3–N concentration and increase in silage CP may be attributed to the containing of pomegranate peel on tannin, which protects feed proteins against degradation by forming protein complexes [52]. In addition, the addition of inoculant or ZYMOGEN to the PPS enhanced cell wall degradation and reduced the fibre fraction (NDF and ADF). In particular, the addition of ZYMOGEN had a greater impact on the degradation of cell walls and the hydrolysis of cellulose and hemicellulose [53, 54]. While, Inoculants had the lowest capacity for degradation of plant cell walls [43], may be due to the reduced effectiveness in hemicellulosic hydrolysis [55]. These results are supported by previous studies done by Nkosi et al. [14], Nkosi et al. [48], and Dean et al. [56] who suggested that additions of inoculants or enzymes degraded the structural carbohydrates and enhanced fiber degradation during ensilage fermentation.

4.2 Dry matter intake and digestibility

Improvement of G2 and G3 DMI may be related to silage treatment with 1188 or ZYMOGEN inoculant, respectively, to improve silage characteristics, thus enhancing palatability. It's consistent with the results of other researchers Abedo et al, [57] and Romero et al. [58]. The improvement in the digestibility coefficients of PPS treated with inoculant 1188 or ZYMOGEN, is confirmed by Romero et al. [58] observed improvement in digestibility of DM, CP, NDF, ADF and ADL when animals fed on silage treated with enzymes may be attributed to the effect of enzymes on the degraded cell wall during ensilage. Improvement of CP digestibility in treated or un-treated PPS could be due to an improvement in the fermentation quality of silage and reduction in proteolysis, thus showing an increased level of CP in the rations and improving the efficiency of protein utilization [10, 15]. The presence of tannin in pomegranate peel has a beneficial role in binding to

diet proteins, reducing rumen degradability, increasing enzymes' intestinal digestibility and improving nitrogen utilization efficiency [59, 6]. Also, Doce et al. [60] reported that the presence of tannins in animal feed at levels as high as 1.5% DM had no adverse effects on digestibility. Moreover, McSweeney et al. [61] and Ott et al. [62] demonstrated that the fermentation process lowers the tannin levels in the silages, therefore the animals have not been negatively affected by the tannins in the silage. The increase of TDN and DCP values in G2 and G3 than in other groups can be attributed to higher digestibility coefficients of rations, while the increase in DCP can be attributed to more protein utilization efficiency by passing dietary protein from the rumen to the abomasum [63]. Improvement in nutritional values and digestibility coefficients in the current study is consistent with other studies that have indicated that the addition of the biological additives to agricultural or agro-industrial by-products in animal feed can be improved nutritive values and digestibility coefficients for most nutrients [48, 64, 65].

4.3 Rumen fermentation

The ruminal pH values were between 6.21 and 6.75. These levels are suitable for the normal function of cellulotic bacteria and pH should be between 6.4 and 7.0 according to [66]. Reduction of NH3–N concentration in the rumen of cows fed on PPS treated with inoculants or ZYMOGEN may be attributed to the improvement of the fermentation quality of the silages by reducing proteolysis and nitrogen losses [40], or may be due to the protection of dietary protein from ruminal degradation [5, 67], or maybe because tannins of pomegranate peel have the ability to link with protein to form a tannin-protein complex that is more stable in the rumen and resistant to degradation by rumen microorganisms at pH 5.0 to 7.0. However, this complex decomposed in the gastric juice (pH abomasum 2-3) [68]. Also, flavonoids of pomegranate peel contributed to a reduction of ammonia production in the rumen [69]. The increase in values of rumen TVFA's, acetic acid and acetic: propionic acid in diets G2 and G3 than in G1 and control diets may be due to improvement in DM or OM digestibility and increased degradation of cellulose and hemicellulose during ensilage. The results are consistent with those of [70]. The results of microbial protein synthesis in this study showed little improvement in G2 compared to other groups. That's consistent with Basso et al. [71] who suggested that the increase is a result of the improvement of silage protein. The microbial protein synthesis was not affected by pomegranate peel, which probably has a role in reducing the NH3-N concentration result to contain tannin, but NH3-N concentration was still sufficient for microbial protein synthesis. These results conform to those of [72] found that the addition of tannin to silage did not affect the synthesis of microbial proteins. Several studies have been conducted to reduce methane emissions through the use of feed additives. Where, tannin is one of these important in methane reduction [73, 74]. The reduction in CH4 emissions in cows fed rations containing treated or untreated PPS may be attributed to the presence of tannin. These results are consistent with Aboagye et al. [75] and Stewart et al. [76] explained that tannins reduce CH4 production and may be attributed to inhibiting methanogenesis.

4.4 Milk production and milk composition

Regardless effect of pomegranate peel on decreased palatability and DMI as a consequence of the presence of tannins, which bind with saliva proteins, the addition of ZYMOGEN or inoculants to the PPS had a positive effect on increased voluntary DMI that is consistent with findings from Romero et al. [58] and Soliman [77]. Kung et al. [78] observed an increase in DMI when animals fed on silage had lower acetic acid content. Dairy cows fed diets that contained PPS treated with ZYMOGEN or inoculant as well as those fed diet containing WCS had a positive effect on milk yield, 4% FCM and fat composition compared to fed diet containing un-treated PPS that agrees with findings Abido et al. [79], and Peymanfar and Kermanshahi [80] who indicated that improved the milk yield and fat composition may be attributed to increasing feed intake, fiber degradation and digestibility of nutrients, or may be because increased the concentration of acetic acid results increased degradation of fiber fractions in the rumen [81]. The improvement of milk protein in cows fed on rations containing PPS may be attributed to an increase in ruminal microbial protein synthesis and the amino acid levels [57] and may be attributed to tannins of pomegranate peel that have a role in enhancing protein utilization during digestion [82]. Somatic Cell Count (SCC) is secreted in milk during cows milking and is a general indicator of udder health and milk quality. Generally, when ranged an amount of SCC is less than 200,000 cells /ml it indicates that animals are healthy and not infected with mastitis, according to [83]. In the current study, the number of cells per cow was less than 200,000 cells /ml. The cows that feed on PPS had a low cell count relative to the control, which could be due to the pomegranate peel containing bioactive chemicals [84].

4.5 Plasma metabolites

Increased concentrations of serum total protein, albumin and globulin in cows fed on diets containing PPS may be because an improving the utilization of CP and increasing the number of amino acids available for absorption in the intestine [6]. The values of AST and ALT in this study were within normal limits in all the groups that correspond to Abido et al. [81]. Also, Pithayanukul et al. [85] found that supplementation with tannin extracts 2000 mg/kg of body weight from Areca catechu or nutgalls seeds did not affect blood indices or liver and renal functions. Higher serum urea in G2 and G3 may explain a reduction in the concentration of rumen ammonia and improved intestinal absorption of amino acids, in addition, to increased protein content in the diet and increased digestibility and utilization of CP intake [86]. Also, tannins' presence in the rumen reduced the degradation of proteins by ruminal microorganisms, thus increasing the dietary protein absorbed in the intestines [6]. No found any negative impacts on indicators of the liver and renal functions in cows that fed on PPS may be due to the high quantity of phenolic and flavonoids in pomegranate peel [7].

5. Conclusion

In conclusion, results obtained in this study showed that pomegranate peel silage can be used safely as good roughage during the feeding of dairy cows at a level 20% of dietary DM. The addition of enzymes mixed (ZYMOGEN) or Inoculant 1188 during

ensilage of pomegranate peel had improved the fermentation and nutritive quality silage, and, as well as nutritive values. It has also contributed to increased cow productivity.

Acknowledgements

We thank our colleagues who do not meet all of the authorship criteria for helping us in the experimental work and for doing this research. Also, I pass my special gratitude goes to the staff in the Noubaria Experimental Station of the Animal Production Research Institute - Agricultural Research Center - Dokki - Egypt, for their help in a facilitating this experimental work.

Conflicts of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

References

1. Patil AV, Karade AR. In Bose TK, Mitra SK (Eds.), Fruits: Tropical and subtropical. Calcutta, India: Naya Prakash (1996).
2. Pienaar L. The Economic Contribution of South Africa's Pomegranate Industry. Technical Report for Western Cape Department of Agriculture Division for Macro & Resource Economics. South African (2021).
3. Ministry of Agriculture. Agriculture direction of governorate, Economic affairs sector, Ministry of Agriculture (summer and Nili crops) (2019): 325.
4. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol 109 (2007): 177-206.
5. Atkinson RL, Toone CD, Ludden PA. Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on site and extent of digestion and ruminal characteristics in lambs fed low- quality forage. J. Anim. Sci 85 (2007): 3322-3330.
6. Min BR, Barry TN, Attwood GT, et al. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Science and Techn 106 (2003): 3-19.
7. Li Y, Guo C, Yang J, et al. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 96 (2006): 254-260.
8. Shabtay A, Eitam H, Tadmor Y, et al. Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as novel beef cattle feed. J. Agric. Food Chem 56 (2008): 10063-10070.
9. Sadq SM, Ramzi DOM, Hamasalim HJ, et al. Growth performance and digestibility in karadi lambs receiving different levels of pomegranate peels. Open J. of Anim. Scie 6 (2016): 16-23.
10. Wilkinson JM. In: Wilkinson JM. (Ed.), Silage. Part 6: Assessing Silage Quality.

Chapter 19: Analysis and clinical assessment of silage. Chalcombe Publications, UK (2005): 198-208.

11. Huhtanen P, Nousiainen JI, Khalili H, et al. Relationship between silage fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci 81 (2003): 57-73.
12. Muck RE. Silage additives and management issues. Proceedings of Idaho Alfalfa Forage Conference, Best Western Burley Inn, Burley, Idaho, USA. 16-17 February (2010): 49-55.
13. Aksu T, Baytok E, Bolat D. Effects of a bacterial silage inoculant on corn silage fermentation and nutrient digestibility. Small Ruminant Research 55 (2004): 249-252.
14. Nkosi BD, Meeske R, Langa T, et al. Effects of bacterial silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. South African J of Anim. Scie 41 (2011): 351-359.
15. McDonald P, Edwards RA, Greenhalgh JFD, et al. Animal Nutrition. 6th ed. Longman Scientific and Technical, Prentice Hall, New Jersey, USA (2002).
16. Sheperd AC, Kung Jr. L. Effects of an Enzyme Additive on Composition of Corn Silage Ensiled at Various Stages of Maturity. Journal of Dairy Science 79 (1996): 1767-1773.
17. Elkholy MEl H, Hassanein1 El I, Edrees N, et al. Nutritional aspects of recycling plants by-products and crop residues (corn stalk) in sheep. Pakistan J. of Nutr 8 (2009): 1834-1841.
18. Soliman SM, El-Morsy AM, El-Shinnawy AM, et al. Utilization of biologically treated watermelon vine in rations for dairy cows. J. of Animal and Poultry Production, Mansoura Univ 11 (2020a): 117-123.
19. Colombini S, Colombari G, Crovetto GM, et al. Tannin treated lucerne silage in dairy cow feeding. Ital. J. Anim. Sci 8 (2009): 289-291.
20. Herremans S, Decruyenaere V, Cantalapiedra-Hijar G, et al. Effects of hydrolysable tannin-treated grass silage on milk yield and composition, nitrogen partitioning and nitrogen isotopic discrimination in lactating dairy cows. Animal 14 (2020): 771-779.
21. AOAC. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington D.C., USA (1990).
22. Warner ACI. Production of volatile fatty acids in the rumen methods of measurements. Nutr. Abstr., and Rev 34 (1964): 339-352.
23. Bush KJ, Russel RW, Young JW. Quantitative separation of volatile fatty acids by high performance liquid chromatography. J. Liquid Chromatography (1979).
24. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci 74 (1991): 3583-3597.
25. NRC. Nutrient Requiremets of Dairy Animals. National Academy of Sciences, National Research Council. Washington, D

C., USA (2001): 381.

26. Barney Harris jr. Nutrient Requirements of Dairy Cattle. This document is Fact Sheet DS 38, of the Dairy Production Guide, originally published as part of Circular 594, Florida Cooperative Extension Service (1992).
27. Gaines WL, Davidson FA. Relation between percentage fat content and yield of milk.Ill. Agr. Expt. Sta. Bull (1923): 245.
28. Ferret A, Plaixats J, Caja G, et al. Using markers to estimate apparent dry matter digestibility, faecal output and dry matter intake in dairy ewes fed Italian ryegrass hay or alfalfa hay. Small Rumin Res 33 (1999): 145-152.
29. Al-Rabbat MF, Baldwin RL, Weir WC. In vitro nitrogen-treacer technique for some kinetic measures of rumen ammonia. J. Dairy Sci 54 (1971): 1150-1161.
30. Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of technical details. (Occasional publication) International Feed Research Unit. Bucksburnd, Aberdeen:Rowett Research Institute (1992): 21.
31. Verbic J, Chen XB, Macleod NA. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. J. of Agri. Sci 114 (1990): 243-248.
32. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev 28 (1988): 7-55.
33. Armstrong WD, Carr CW. Physiological Chemistry, 3rd ed. pp., 75 Burges Publishing CO., Minneapolis, Minnesota, USA (1964).
34. Doumas B, Walson W, Blgga H. Albumin standards and measurement of serum with bromocresol green. Clin. Chem. Acta 31 (1971): 87.
35. Reitman S, Frankel S. A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clinc. Path 28 (1957): 56.
36. Siest G, Henny J, Schiele F. Interpretation des examens de laboratoire KP (1981): 206-223.
37. Folin OZ. Colorimetric of determination of plasma cereatinine. Phys. Chem 268 (1994): 228.
38. SAS. SAS users guide: Statistics, SAS Inst., Inc., Cary N.C., USA (2000).
39. Duncan DB. Multiple R and M F- T. Biometric 11 (1955): 1-42.
40. Makkar HPS. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research 49 (2003): 241-256.
41. Wilkinson JM, Davies DR. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci 68 (2012): 1-19.
42. Kung Jr. L, Shaver R. Interpretation and use

of silage fermentation analysis reports. University of Wisconsin, Madison, WI, USA. Focus on Forage 3 (2001): 1-5.

43. Nkosi BD, Vadlani PV, Brijwani K, et al. Effects of bacterial inoculants and an enzyme on the fermentation quality and aerobic stability of ensiled whole-crop sweet sorghum. South African J. of Anim. Scie 42 (2012): 332-240.
44. Kung Jr. L, Muck RE. Animal response to silage additives. Proc. from the Silage: Field to Feedbunk North American Conference. NRAES 99 (1997): 200-210.
45. Ozduven ML, Okuyucu B, Buyukkilic Beyzi S, et al. The effects of lactic acid bacterial inoculants on the fermentation, aerobic stability and nutritive value of sunflower silages. Inter. J. of Current Research 9 (2017): 54289-54295.
46. Reich LJ, Kung Jr. L. Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. Anim. Feed Science and Techn 159 (2010): 105- 109.
47. Filya I. The effect of Lactobacillus buchneri with or without homofermentatitive lactic acid bacteria on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. J. Appl. Microbiol 95 (2003): 1080-1086.
48. Nkosi BD, Meeske R, Langa T, et al. The influence of ensiling potato hash waste with enzyme/bacterial inoculants mixtures on the fermentation characteristics, aerobic stability and nutrient digestion of the resultant silages by rams. Small Ruminant Research 127 (2015): 28-35.
49. Kung Jr. L. a review on silage additives and enzymes (2014).
50. McDonald P, Edwards RA, Greenhalgh JFD, et al. Chapter 19: silage. In: Animal Nutrition, seventh ed (2010): 499-520.
51. Nkosi BD, Meeske R, Van der Merwe HJ, et al. Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and digestibility in rams. Animal Feed Science and Technology 157 (2010): 195-200.
52. Kondo M, Kita K, Yokota H. Feeding value to goats of whole-crop oat ensiled with green tea waste. Anim. Feed Science and Tech 113 (2004): 71-81.
53. Addah W, Baah J, McAllister TA. Effects of an exogenous enzyme-containing inoculant on fermentation characteristics of barley silage and on growth performance of feedlot steers, Can. J. Anim. Sci 96 (2016): 1-10.
54. Lynch JP, Baah J, Beauchemin KA. Conservation, fiber digestibility and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. J. Dairy Sci 98 (2015): 1214-1224.
55. Islam M, Enishi O, Purnomoadi A, et al. Energy and protein utilization by goats fed Italian ryegrass silage treated with molasses, urea, cellulase or cellulase lactic acid bacteria. Small Ruminant Research 42 (2001): 49-60.

56. Dean DB, Adesogan AT, Krueger N, et al. Effect of Fibrolytic Enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. J.of Dairy Scie 88 (2005): 994-1003.
57. Abedo AA, Hafez YH, Khalifa Mohamed EI, et al. Milk yield and composition of dairy Zaraibi goats fed microbial inoculated corn silage. Egyptian J. of Sheep and Goat Scie 8 (2013): 141-151.
58. Romero JJ, Macias EG, Ma ZX, et al. Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation. Journal of Dairy Science 99 (2016): 486-3496.
59. Getachew G, Makkar HPS, Becker K. Effect of polyethylene glycol on in vitro degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. British J. of Nutr 84 (2000): 73-83.
60. Doce RR, Hervas G, Giraldez FJ, et al. Effect of immature oak (Quercus pyrenaica) leaves intake on ruminal fermentation and adaptation of rumen microorganisms in cattle. Journal of Animal and Feed Science 16 (2007): 13-18.
61. McSweeney CS, Palmer B, Bunch R, et al. Isolation and characterization of proteolytic ruminal bacteria from sheep and goats fed tannin-containing shrub legume Calliandra calothyrsus. Applied and Environmental Microbiology 65 (1999): 3075-3083.
62. Ott EM, Aragon M, Gabel M. Ensiling of tannin_containing sorghum grain. Silage production and utilisation. Prceedings of the XIVth International Silage Conference (2005): 178.
63. Patra AK, Saxena J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Sci. Food and Agri 91 (2011): 24-37.
64. Soliman SM, El-Shinnawy AM, El-Morsy AM. Effect of probiotic or prebiotic supplementation on the productive performance of Barki lambs. J. Animal and Poultry Prod., Mansoura Univ 7 (2016): 369-376.
65. El-Morsy AM, Soliman SM, Bakr AA. Influence of inclusion of fungal treated watermelon straw in the ration on nutrient utilisation and rumen fermentation in sheep. Indian J. Anim. Nutr 35 (2018): 251-259.
66. Wanapat M, Cherdthong A. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr. Microbial 58 (2009): 294-299.
67. Jalč D, Váradyová Z, Mihaliková K, Ledecký V, Kišidayová S. Enterococci inoculated silages: Effect on rumen fermentation and lipid metabolism in vitro. African J. of Microbiology Research 7 (2013): 4191-4199.
68. Oh HI, Hoff JE. pH dependence of complex formation between condensed tannins and proteins. Journal of Food Science 52 (1987): 1267-1269.
69. Soliman SM, Hassan AA, Neamat Bassuony I, et al. Effect of biological extract

supplementation on milk yield and rumen fermentation in dairy cows. Intern. J. of Dairy Scie 15 (2020b): 88-98.

70. Jatkauskas J, Vrotniakiene V. Effects of silage fermentation quality on ruminal fluid parameters. Biologija 4 (2006): 65-71.
71. Basso FC, Adesogan AT, Lara EC, et al. Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield and growth performance of lambs. J. Anim. Sci 92 (2014): 5640-5650.
72. Wischer G, Boguhn J, Steingaß H, et al. Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. Animal 7 (2013): 1796-1805.
73. Jayanegara A, Goel G, Makkar HPS, et al. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol 209 (2015): 60-68.
74. Wei C, Guyader J, Collazos L, et al. Effects of gallic acid on in vitro rumen fermentation and methane production using rumen simulation (Rusitec) and batch-culture techniques. Anim. Prod. Sci 59 (2019): 277-287.
75. Aboagye IA, Oba M, Castillo AR, et al. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use and performance of beef cattle fed a high-forage diet. J. Anim. Sci 96 (2018): 5276-5286.
76. Stewart EK, Beauchemin KA, Dai X, et al. Effect of tannin-containing hays on enteric methane emissions and nitrogen partitioning in beef cattle. J. of Ani.l Sci 97 (2019): 3286-3299.
77. Soliman MS. The Effects of addition microbial inoculants or formic aid to whole corn plant at ensiling on silage quality, nutritive value and rumen fermentation in sheep and milk yield and composition of lactation cows. J. of Animal and Poultry Production 5 (2014): 249-266.
78. Kung Jr. L, Shaver RD, Grant RJ, et al. Silage review: Interpretation of chemical, microbial and organoleptic components of silages. J. of Dairy Scie 101 (2018): 4020-4033.
79. Abido AM, Soliman HS, Hamza AS, et al. Effect of maize silage inoculated by bacteria on performance of lactating buffaloes. Int. J. of Dairy Scie 2 (2007): 330-338.
80. Peymanfar S, Kermanshahi R. The effect of bacteria, enzymes and inulin on fermentation and aerobic stability of corn silage. Iran Microbiol 4 (2012): 180-186.
81. Schmidely PH, Morand-Fehr P, Sauvant D. Influence of extruded soybeans with or without bicarbonate on milk performance and fatty acid composition of goat milk. J. Dairy Sci 88 (2005): 757-765.
82. Min BR, Attwood GT, McNabb WC, et al. The effect of condensed tannins from Lotus corniculatus on the proteolytic activities and growth of rumen bacteria. Anim. Feed Science and Techn 121 (2005): 45-58.

83. Ruben N. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research. EDP Sciences 34 (2003): 579-596.
84. Mariana PS, Mariana CS, Beatriz RS, et al. Extraction of bioactive compounds from pomegranate peel (Punica granatum L.) with pressurized liquids assisted by ultrasound combined with an expansion gas. J. Ultrasonics Sonochemistry 54 (2019): 11-17.
85. Pithayanukul P, Nithitanakool S, Bavovada R. Hepatoprotective Potential of Extracts from Seeds of Areca catechu and Nutgalls of Quercus infectoria. Molecules 14 (2009): 4987-5000.
86. Balikci E, Yildiz A, Gurdogan F. Blood metabolite concentrations during pregnancy and post-partum in Akkaraman ewes. Small Rum. Res 67 (2007): 247-251.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license 4.0