Influence of Using Pomegranate Peel Silage in Rations of Dairy Cows on Their Productive Performance

Ahmed Mahmoud Elmorsy (ahmedelmorsy55@yahoo.com)
Agricultural Research Center https://orcid.org/0000-0002-0292-7710
Mohsen Mahmoud Shoukry
National Research Center
Soliman Mohamed Soliman
Agricultural Research Center
Mahmoud Mohamed Soliman
Agricultural Research Centre

Research Article

Keywords: digestibility, milk, inoculant, enzymes, methane, rumen fermentation and silage.

Posted Date: February 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1325562/v1

Version of Record: A version of this preprint was published at International Journal of Plant, Animal and Environmental Sciences on June 22nd, 2022. See the published version at https://doi.org/10.26502/ijpaes.202133.

Abstract

This study was implemented to evaluate the effect of partial replacement of whole corn plant silage (WCS) by pomegranate peel silage (PPS) treated with enzymes mixed (ZYMOGEN) or lactic acid bacterial inoculants (Inoculant 1188) on nutrient digestibility and productive performance of dairy cows. The first experiment two stacks of PPS and WCS were prepared. WCS was replaced by PPS, at 25:75, 50:50 and 75:25, respectively used in forming three rations. The second experiment has been carried with twenty lactating crossbred Friesian cows in four similar groups (5 cows / group): the control group was fed a ration 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 the values of CP, NFE and lactic acid were highest, while values of NDF, ADF, pH, the concentration of NH3 -N and acetic acid were lowest in all groups treated PPS. The values of digestibility coefficients, nutritive values, ruminal fluid fermentation of TVFAs and acetic acid, milk yield, 4% FCM, milk composition, blood proteins were highest, while the values of ruminal fluid fermentation of pH and NH3 N were lowest in group 2 and 3 compared with other groups. It concluded that using of treated or untreated pomegranate peel is safe in dairy cows feeding at a level of 20%. The addition of ZYMOGEN or inoculant 1188 to PPS improved fermentation and nutritive quality of silage along with its nutritive values.

Introduction

Pomegranate is a scientific name *Punicagranatum* 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 **(Patil and Karade, 1996).** The world's pomegranate production amounts to approximately 8.1 million tons **(Pienaar, 2021).** Pomegranate production annually in Egypt could reach approximately 382587 tons **(Ministry of Agriculture, 2019).** The peels (pericarp, rind, or hull) amount to approximately 60 % of the weight of the pomegranate fruit **(Lansky and Newman, 2007).** The evolution of agro-industry has caused increased quantities of by-products such as peels and seeds of the pomegranate**.** Reducing the loss of non-ammonia nitrogen and essential amino acids in the small intestine may be attributed to the lowering of protein degradation in the animal rumen **(Atkinson *et al.,* 2007).** Recently, pomegranate by-products have attracted attention. Pomegranate peel is abundant in bioactive compounds, including 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, antibacterialand antiviral activities. High levels of tannins reduce palatability and protein and carbohydrate digestion, whereas low to moderate levels protect dietary protein from degradation **(Min *et al.,* 2003).** Tannins are two groups: hydrolysable and condensed tannins **(Li *et al.,* 2006).** So, we can use the pomegranate peel as a source of tannin in the diet to improve the process of rumen fermentation**.** Also, **Shabtay*et al.* (2008)** and **Sadq*et al.* (2016)** 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**.**

The pomegranate biomass is rich in moisture, but if it is not consumed in a short period, it gets mouldy and becomes useless **(Shabtay*et al.,* 2008).** Therefore, using the ensiling is an efficient way to preserve pomegranate peel for use in ruminant rations. High-quality silages are characterized by high concentrations of water-soluble carbohydrates (WSC) and dry matter content of 250–400g/kg (**Wilkinson, 2005**). The feed intake, nutrient utilization and milk production of ruminants are affected by the fermentation quality of silages (**Huhtanen*et al.,* 2003**). Employing several additives (bacterial inoculants, enzymes, etc.) during the ensiling process causes improved aerobic stability and enhances the nutritive value of silage (**Muck, 2010).** Lactic acid bacteria (LAB) inoculants are divided into two major groups: homofermentative LAB and heterofermentative LAB**.** They are used as biological additives in silage. Numerous studies have explained that the employment of homofermentative LAB inoculants in plant ensilage caused an excess of lactic acid and low values of acetic acid, butyric acid, ammonia nitrogrn(NH_3-N) and pH of the silage (**Aksu*et al.,* 2004 andNkosi*et al.,* 2011**). Moreover, the addition of enzymes to characterised ensiling caused the decay of cell walls and increased the availability of WSC that acts as a substrate to LAB **(Sheperd and Kung, 1996).** Recycling of the large quantities of by-products of industrial and agricultural in rations of livestock is met with great attention**.** This process contributes to transforming by-products that are misfit for human consumption into useful food for human consumption which contributes to meet the requirements of population growth**.** Also, it's lowering environmental pollution, health damage and costs of waste disposal **(Elkholy*et al.,* 2009).** Moreover, it's reduced the acute shortage of feedstuffs in developing countries, and reduces the feeding cost, thus increasing the economic efficiency of animal production **(Soliman*et al.,* 2020a).** This study's objective is to investigate the influence of thepartaily 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**.**

Materials And Methods

This study was designed forutilizing the pomegranate peel as silage (PPS) by replacement of the whole corn plant silage (WCS). Firstly, the laboratory study was done. It was prepared two stacks of PPS silage and WCS. PPS was tested at three levels of replacement of WCS, at 25:75, 50:50 and 75:25, respectively.The three different levels of silage were used in forming three rations in the laboratory to determine the optimum level of replacement WCS suitable for ruminant nutrition**.**The tannin concentrations in the three different rations were 3.49gm,6.92gm and 10.43gm/ kg diet that equivalent 0.69, 1.40 and 2.14% on DM basis,according to **Colombini*et al.*(2009) andHerremans*et al.*(2020)**,the level 50:50 (WCS: PPS) was chosen as the optimum level for dairy cows feeding experiment.This experiment was carried out at NoubariaExperimentalStation, Animal Production Research Institute. Evaluate the effect of adding the lactic acid bacterial inoculants (Inoculant 1188) or enzymes mixed (ZYMOGEN) toPPS 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 El-Marwa Company, 6 October Cityat the end of summer 2019**.** The whole corn plant was collected from the Noubaria region at the end of 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 has been carried out to prepare four silage stacks; the first silage stack was a WCS as a control group. The second silage stack was made of untreatedPPS.The third silage stacksPPS treated with bacterial inoculants (Pioneer brand 1188). The fourth silage stacksPPS treated with ZYMOGEN. Four stacks were covered separately by double-layered linoleum plastic and pressed with 30 cm of the soil layer. The ensiled materials were compressed by a heavy drum filled with sand to guarantee anaerobic conditions for ensiled for more than two months.

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

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 followingVitamin 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.

2-1- Silage quality

Samples from the four stacks were taken after 60 d to determine silage quality and chemical composition. The same volume of water that was used to dissolve the silage additives was added to the first and second treatments to maintain equal moisture. For evaluating the silage quality, silage extract was prepared by homogenizing 30-gram fresh material with 270 ml distilled water, then blending for 10 minutes in a laboratory blender. The homogenized sample was filtered through 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**AOAC (1990).**Total volatile fatty acids (TVFA'S) concentration was determined according to **Warner (1964).**The molar proportion of TVFA'S (acetic, propionicand butyric) was measured according to **Bush *et al*. (1979)**using High-Performance Liquid Chromatography (HPLC). NH3-N concentration (ID 941.04) was determined by direct distillation according to the**AOAC (1990).**Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the procedure of **Van Soest*et al*.(1991).** The chemical composition and silage quality of WCS and untreated or treated PPSare shown in Table (2)**.**

2-2- Experimental dietsandlactationtrials

Twenty lactating crossbred Friesian 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, to cover their maintenance requirements according to **NRC (2001)** recommendationsand requirements for the production that were calculated from the preliminary period and also the milk yield previous according to **Barney Harris (1992).**The first group (control) was fed a ration that consisted of 50% CFM, 40% WCS and 10%. 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 bacterial inoculants ˝Pioneer brand 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.andwater was available all time. The residual diets were collected and calculated for the estimated feed intakefor each individual cow.Cows were machine milked twice daily at 06:00 and 18:00 pm, from 30 days to 90 day and samples were collected at each milking (1% from total milk of each period). A mixed sample of milk was taken daily. Milk composition (fat, total protein, lactose, and total solids) and somatic cell count (SCC) of milk samples were determined using MilkoScan FT 6000. Average yields of each milk component were calculated for individual cows by multiplying milk yield by the component content (g/kg) of milk. Fat corrected milk (4 %) was calculated according to **Gaines (1923)** 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 intake was recorded daily by weighing the offered rations and refusals from the previous dayfor each cow separately. 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 **Ferret *et al.*(1999)**.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 (1990)** official methods. NDFand ADF were determined by the procedure of **Van Soest*et al.*(1991)**.

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.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 NH_3-N determination. The concentration of NH_3-N in the ruminal contents was determined as described by **Al-Rabbat*et al.* (1971).** 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 **Warner (1964).** 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, SupelcoNukol; 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 **Chen and Gomes (1992).**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 × $BW^{0.75}$)} / 0.85, where 0.85 is the recovery of absorbed purines as urinary purine derivatives, and 0.385 * $BW^{0.75}$ is the endogenous contribution in the urinary excretion of PD **(Verbic*et al.*, 1990).**

In vitro methane production was determined as described by **Menke and Steingass, (1988).**

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. Blood serum was analyzed for total protein was determined according to **Armstrong and Carr (1964).**Plasma albumin was assayed according to **Doumas*et al.* (1971).**Globulin was calculated by subtracting the albumin value from totalprotein.Liver function was assessed by measuring the activities of aspartate transaminase (AST) and alanine transaminase (ALT) were measured with a colorimeter using commercial kits, according to **Reitman and Frankel (1957).**Kidney functionswere evaluated by measuring blood ureaaccording to the method of **Siest*et al.* (1981**) andcreatinine was measured with a colorimeter using commercial kits, according to the method of **Folin (1994).**

2-6- Statistical analysis

Data were analyzed as a completely randomizeddesign with repeated measures using the PROC MIXEDprocedure of SAS**(SAS, 2000).**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 means were separated using Duncan multiple range tests**(Duncan, 1955).**

Results

3-1- Silage quality

The results obtained from laboratory studies illustrate that the optimum level of replacement of PPStoWCSwas 50:50. This level contains an adequate amount of tannins suitable for ruminant nutrition. Data of the chemical composition and silage quality of untreated or treated PPS and WCS are shown in Table (2)**.**PPS treated with inoculant 1188 or ZYMOGEN led to significant increases ($P<0.05$) in the contents of CP, EE and NFE. While, the contents of CF, ash, NDF and ADF were significantly decreased ($P<0.05$) compared to WCS. Adding inoculant 1188 or ZYMOGEN to PPS improved CP by increasing it while NDF and ADF decreased. On the other hand, there were no significant changes in values of OM between all groups**.** PPS treated with inoculant had the lowest values($P<0.05$) of pH and concentrations of NH_3-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, 14.06and 17.77%, respectively) whilethe 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.

Table 2. Means for chemical composition and fermentation characteristics of treated or untreated silages (n = 5).

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 3.Chemical composition of the experimental rations.

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
Tanning/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.

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 treated PPS replacement by WCS led to an improvement in DM intake. The results were close to those fed a diet containing WCS and enhanced palatability compared to untreated PPS. Also, cows fed PPS treated with enzyme or inoculant had higher digestibility of DM, OM, CP, EE, NDF, TDN,ADF and DCP% compared to cows fed untreated PPS and WCS.

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

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.

3-3- Rumen fermentation

Rumen liquor parameters of lactating Friesian cows fed the experimental rations are presented in Table (5). Results revealed that addingZYMOGEN or lactic acid bacterial inoculant to PPS led to decreaseruminal pH values and NH_3-N concentrations, which were in the normal range for microorganism growth, while the values of TVFA's, acetic acid and acetic: propionic were significantly (P< 0.05) increased with diets G2 and G3, but the highest value was recorded with G3, including treated with ZYMOGEN. Despite the ZYMOGEN or lactic acid bacteriainoculants enhanced OM digestibility, the increase in microbial protein synthesis was not significant. Microbial protein synthesis was shown to have a numerical increase with diet G2 than with other diets. Replacement of WCS with PP 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 lactic acid bacteriaadditives had no effect on methane production when compared to G1.

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

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

$^{a,b \text{ and } c}$ 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 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.

3-4- Milk production and milk composition

The average daily milk yield and milk composition of lactating Friesian cows fed the experimental diets are presented 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 followed by those fed diets

containing WCS and PPS treated with inoculant. While, cows fed the diets containing untreated PPSwere 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 normal values, but the cows fed diets containing untreated or treated PPS recorded the lowest (P<0.05) values compared with control.

Table 6. Milk production and milk composition of crossbredcowsfed the experimental rations.

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 bmeans inthe same row with different superscriptsarediffersignificantly (P<0.05).

3-5- Plasma metabolites

Blood plasma constituents of lactating Friesian 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 controls. 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.

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

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).

3-6- Economic efficiency

The economic efficiency of lactating Friesian cows fed the experimental diets is shown in Table 8. The results showed that cows fed diets G2 and G3 were more efficient in producing higher milk yields with lower daily feed costs and, as a result, lower net revenue when compared to the control. In addition, when compared to the control, economic efficiency increased to 10.3, 10.5 and 12.2% in G1, G2and G3, respectively.

Table 8. Economic efficiency for lactating cows fed the experimental rations.

Item	Control	G1	G2	G3
Daily feed intake (kg/head /day)				
Concentrate feed mixture	9.23	9.22	9.25	9.24
Silage	21.98	21.41	21.85	21.92
Rice straw	1.80	1.79	1.81	1.80
Total feed intake	33.01	32.42	32.91	32.96
Economic efficiency				
Milk yield (kg/head/day)	18.02	17.76	18.20	18.44
Daily feed cost (LE /head/day)	61.12	56.50	57.70	57.90
Price of daily milk yield (LE)	180.2	177.6	182.0	184.4
Net revenue	119.1	121.2	124.3	126.5
Economic efficiency%	194.8	214.8	215.3	218.6

Free market prices (LE/ton) for the corn silage = 600 LE.

Free market prices (LE/ton) for the pomegranate peel silage untreated = 200 LE.

Free market prices (LE/ton) for the inoculants treated silage mixture = 440 LE.

Free market prices (LE/ton) for the enzymes treated silage mixture = 450 LE.

Free market prices (LE/ton) for Rice straw =1000E.

Free market prices (LE/ton) for CFM = 5000 LE.

Free market prices (LE/kg) for milk yield 4% fat = 10 LE.

According to the year 2020 market price.

Discussion

4-1- Silage quality

Since the pomegranate peel is containing tannin that considered as antinutrition factors that effect of animal performance.**Makkar(2003)** illustrated that tannin at higher levels than 50 g kg−1 DM had a negative effect on palatability of feed intake or animal performance**.**The purpose of silage is to preserve feed with many of the nutrients present in the original fresh forage as possible **(Wilkinson and Davies, 2012).** Therefore, biological additives were used during ensiling to speed the fermentation process and raise of lactic acid concentration led to speed reduction in silage pH and thus improving silage preservation **(McDonald *et al.* 2002).** A pH range of 3.7-4.2 is generally considered beneficial for whole-crop cereal preservation **(Kung and Shaver, 2001)** and in the presentstudy; pH was less than 3.93, indicative of well-preserved silage**.**The results obtained from PPS treated with inoculant 1188 or treated with ZYMOGEN are supported by the findings of various scientists **(Nkosi*et al.* 2012)** whoobserved that treating silage with inoculant led to a decrease in pH value**.** Also, **Kung and Muck (1997)** reported that pH was reduced when silages were treated with enzymes. In contrast **Ozduven*et al.*(2017)** reported an increase in pH when adding bacterial inoculants to sunflower silage**.** The addition of bacterial inoculants to corn silage had no effect on the pH value **(Reich and Kung, 2010).**The addition of additive inoculates 1188 to PPS silage led to an increase in the concentration of lactic acid while decreasing acetic acid. The same trend was observed with silage treated with ZYMOGEN. This may be because the silage additive improved the lactic: acetic ratio. These results supported the extent of pH decline and are consistent with **Nkosi*et al.* (2011).** In contrast, **Filya (2003)** found that lactic acid concentration decreased in inoculated maize silage 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 were in agreement with others **Nkosi*et al.* (2015)andKung(2014)**who explained that addition of enzymes or inoculant to silage led to the

enhancement degrade cell wall and increase the availability of WCS that serve as 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 **(Wilkinson, 2005).** NH_3–N as a percentage of DM should be less than 10% of total nitrogen (TN).In the present study, we had anNH_3–N concentration of less than 10% NH3-N/kg TN. This confirms that silage additives have a positive effect on rapid reduction of pH values, which leads to the desirable reduction of protein degradation in the silo **(McDonald *et al.* 2010 andNkosi*et al.* 2010).**The inclusion of PPS in replace about 50% of the WCS had a considerable effect on the reduction of NH_3–N concentration and increase in silage CPthat may be attributed to the presence of tannin in pomegranate peel. Tannin during the silage fermentation process protects forage proteins from degradation by forming complexes with proteins **(Kondo *et al.,* 2004).**Moreover, treating PPS with inoculant or ZYMOGEN led to improvement in the reduction of ammonia concentration and increased CP **(McDonald *et al.* 2002).**In addition, the use of inoculant or ZYMOGEN in PPS silage was enhanced to degrade the cell wall and reduce the fiber fraction (NDF and ADF). In particular, the addition of ZYMOGEN had more effect on the degradation of cell wall and the hydrolysis of cellulose and hemicellulose**(Addah*et al.* 2016a**nd **Lynch *et al.*2015).**While, inoculant had little ability to degrade plant cell wall**(Nkosi*et al.* 2012)**, but decreases in fiber fraction may be due to efficiency in the hydrolysis of hemicellulose**(Islam *et al.* 2001).** These results are supported by previous studies done by **Nkosi*et al.*(2011),Nkosi*et al.*(2015)and Dean*et al.*(2005).** It was suggested that the inoculants or enzyme additions caused degraded structural carbohydrates and improved fiber degradation during silage fermentation**(Dean *et al.* 2005).**

4-2- Dry matter intake and digestibility

The improvement in DMI of G2 and G3 may be related to silage treated with inoculant 1188 or ZYMOGEN, respectively, which caused improved silage characteristics, thus improving palatability. The improvement in DMI of G2 and G3 is in agreement with the results of other researchers **Nkosi*et al*,(2011)** and**Abedo*et al*,(2013)** who found that treated silage with inoculant led to a significant increase in feed consumption compared to untreated silage, while, **Romero*et al.* (2016)** recorded a significant increase when adding exogenous fibrolytic enzymesto corn silage.The study showed a considerable improvement in digestibility coefficients as a result of adding both inoculant 1188 and ZYMOGEN to the PPS,that may be attributed to adding the bacterial inoculants, or enzyme which contributed to improved digestibility of DM, OM and CP**Romero *et al.* (2016)** observed improvement in digestibility of DM, CP, NDF, ADF and ADL in silage treated with enzymes may be attributed to the effect of enzymes on the degraded cell wall during ensilage. The improvement in CP digestibility in treated or un-treated silage containing PPS could be due to an improvement in the fermentation quality of duringensilages by reducing proteolysis and nitrogen losses, which led to anincrease level of CP in the diets, thus improving the efficiency of protein utilization **(Wilkinson, 2005 and McDonald *et al.,* 2002).** Since the presence of tannin in pomegranate peel. Tannins have been shown to benefit from binding with diet proteins, reducing rumen degradability, increasing enzymes intestinal digestibility and improving nitrogen utilization efficiency **(Getachew*et al.,* 2000**

andMin*et al.,* 2003). Doce*et al.* (2007) reported that the presence of tannins in the rumen of animals at levels up to 1.5% of DM did not cause negative effects on digestibility**.** Moreover, **McSweeney*et al.* (1999)** and **Ott*et al.* (2005)** showed that the fermentation process decreases the levels of tannins in silages, thus animals have not been negatively affected by the tannins in silage. TDN and DCP values were higher in G2 and G3 than in other groups**.** The increase in TDN can be attributed to higher digestibility coefficients in rations containing treated PPS, while the increase in DCP can be attributed to better protein utilization efficiency by passing dietary protein from the rumen to the abomasum (**Patra and Saxena, 2011).** The current study's improvement in nutritional values and digestibility coefficients is consistent with other studies that have found adding that biological additives to agricultural or agro-industrial by-products during ensilage may beimproved nutritive values and digestibility coefficients for most nutrients **(Nkosi*et al.,* 2015 Soliman*et al.,* 2016 and El-Morsy*et al.,* 2018).**

4-3- Rumen fermentation

The results of ruminal pH ranged from 6.21 to 6.75. These levels are suitable for the normal function of cellulites bacteria and pH should be 6.4 to 7.0 according to **Wanapat and Cherdthong (2009).** The diets that contained untreated or treated pomegranate silage led to a reduction in NH_3-N that may be due to protection of dietary protein from ruminal degradation **(Jalč*et al.*, 2013 and Atkinson *et al.,* 2007).** A high level of tannins in pomegranate peel may play an important role in the reduction in rumen ammonia production because tannins have the ability to formed tannin-protein binding that is more stable in the rumen and resistant to degradation by rumen microorganisms at pH 5.0 to 7.0**.** However, it dissociates in gastric juice (abomasum pH 2-3) (**Oh and Hoff, 1987).**Also, **Soliman*et al.* (2020b)**reported that feeding ruminants with diets containing flavonoids led to a reduction in rumen ammonia production as a consequence of a decrease in protein degradation. Moreover, treated silage with inoculants or ZYMOGEN led to a greater reduction of NH3–N concentration compared to diets that contained untreated PPS or corn silage**.** According to **Makkar (2003),** this may be attributed to the improvement of fermentation quality of the silages by reducing proteolysis and nitrogen losses.Increased 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. These results are in agreement with**Jatkauskas and Vrotniakiene(2006).** The microbial protein synthesis in the current study showed insignificant improvement in G2 compared to other groups**.** This is in agreement with **Basso *et al.* (2014)** who showed that improvement of silage protein by LAB inoculant increased ruminal microbial protein synthesis. The microbial protein synthesis was not affected by pomegranate peel, which probably has an impact on reducing NH_3-N concentration as a result of containing tannin, but the NH_3-N concentration was still sufficient for microbial protein synthesis**.** These results are in agreement with those of **Wischer*et al.* (2013)** who found that adding tannin to silage did not affect microbial protein synthesis. Several studies have been done to reduce methane emissions by feed additives. Tannin is one of the additives that have been used as an important substrate for reducing methane **(Jayanegara*et al.,* 2015 andWei*et al.,* 2019).** In the present study, it was observed that CH_4 emissions decreased in cows fed diets containing treated or untreated PPS. This may be attributed to

PPS having an amount of tannin. These results are consistent with previous results (**Aboagye*et al.,* 2018**and**Stewart*et al.,* 2019)**who emphasis that tannins reduce CH_4 production and may therefore inhibit 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 while, the addition of ZYMOGEN (**Romero *et al.,* 2016)** or inoculants (**Soliman, 2014)** to the PPS had a positive effect on increased voluntary DMI**.** These results are in agreement with **Kung*et al.* (2018)** who observed that DMI increased with silage containing a lower percentage of acetic acid**.**Dairy cows fed diets that contained PPS treated with ZYMOGENor inoculantas well as those fed diet containing WCS had a positive effect on milk yield, 4% FCM and fatcomposition compared to fed diet containing un-treated PPS which may be attributed to the improvement in palatability and DMI**(Oliveira *et al.,* 2017)**, nutrients digestibility (**Morand-Fehr *et al.,* 2000**) and rumen fermentation especially VFAs **(Huhtanen*et al.,* 2003)** consequence to improve silage characteristics**.** Also, **Morand-Fehr *et al.* (2000**) showed that a higher level of energy intake leads to greater production of milk by the animal**.Abido*et al.* (2007)** reported that fed lactating buffaloes on maize silage treated with inoculated led toincreased daily milk yield, FCM (4%) and milk contents of fat and lactose**.**Moreover,**Peymanfar and Kermanshahi(2012)** reported that treating forages with the fibrolytic enzyme improvement the milk yield and fat composition compared to untreatedas result to increasing feed intake, fibre degradation and digestibility of nutrients.Furthermore, **Schmidely*et al.* (2005)**found that the increasing degradation of fiber fractions led to an increase in acetic acid that reflects an increase in fat milk composition**.Abedo*et al.* (2013)** showed that feeding the goats with biological inoculated corn silage led to increase milk protein and lactose synthesis may be attributed to an increase in the amino acid levels and ruminal microbial protein synthesis, those contributing to increases in overall production.The improvement of milk protein in cows fed diets containing PPS may be attributed to tannins of pomegranate peel is agree with several studies explained that inclusion of tannin in the diet of lactating ruminant led to increased milk protein and lactose percentages **(Liu, *et al.,* 2013 andAguerre*et al.,* 2016)**, this is attributed to tannin has a role in enhancing protein utilization during digestion and induce improvements in milk production **(Min *et al.,* 2005).**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 less than 200,000 cells /ml it indicates that animals are healthy and not infected with mastitis, reported by **Ruben (2003).**In the present study, each cow had a cell count less than 200,000 cells/ml. The cows fed PPS had the lowest significance, which could be due to the fact that pomegranate peel contains a lot of bioactive chemicals**(Mariana *et al.*, 2019).**

4-5- Plasma metabolites

Increased concentrations of serum total protein, albumin, and globulin in cows fed diets containing PPS may be attributed to improving the utilization of CP and increasing the number of amino acids available for absorption in the intestine **(Min *et al.,* 2003).**Also, **Abido*et al.* (2007)** found that the addition

of lactic acid bacteria inoculants to silage resulted in enhanced silage quality with no deleterious effects on liver or kidney function. Thus, serum AST and ALT values were within normal limits in all the groups that corresponded with **Pithayanukul*et al.*(2009)**whofound that supplementing with 2000 mg/kg body weight tannin extracts from Areca catechu or nutgalls seeds had no effect on blood indices or liver and kidney function. The higher serum urea in diets G2 and G3 comp may be attributed to an increase in protein percentage in the diet as well as an increase in digestible CP intake **(Balikci*et al.,* 2007).** Increased urea values in cows fed PPS may be attributed to the low ruminal ammonia concentration, and greater intestinal absorption of amino acids, leading to increase serum urea content. This is consistent with **Min *et al.*(2003)** who reported that the presence of tannins in the rumen decreased protein degradation by ruminal microorganisms, which increases the dietary protein absorbed in the intestines. When compared to control cows, the liver and kidney functions of the PPS-fed cows did not reveal any negative impacts.It could be due to the high quantity of phenolic and flavonoids, in pomegranate peel **(Li *et al.,* 2006).**

4-6- Economic efficiency

Our results indicated an improvement in values of net revenue and economic efficiency % in G2 and G3 compared with G1 and control. This improvement may be attributed to increased milk production and lower daily feed costs.**Abido*et al.* (2007)** demonstrated that lactating buffaloes fed inoculated maize silage improved economic efficiency compared to the untreated silage. Numerous studies have found that adding biological additives to agricultural by-products and agro-industrial by-products in ruminant diets can reduce feed costs and, as a result, increase the economic efficiency of livestock production **(Soliman*et al.,* 2020a and Soliman*et al.,* 2016).**

Conclusions

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 lactic acid bacterial inoculants (Inoculant 1188) during ensilage of pomegranate peel caused improvements in fermentation and nutritive quality silage,and, it's nutritive values.It's contributed to increasing productive performance of cows and decreased feed cost, and subsequently leads to higher net revenue and economic efficiency.

Abbreviations

PP	pomegranate peel
PPS	pomegranate peel silage
CFM	concentrate feed mixture
RS	rice straw
WCS	whole corn plant silage
DM	dry matter
OM	organic matter
CP	crude protein
EE	ether extract
NDF	neutral detergent fiber
ADF	acid detergent fiber
ADL	acid Detergent Lignin
WSC	water-soluble carbohydrates
LAB	lactic acid bacteria
NH3-N	ammonia- nitrogen
AST	aspartate transaminase
ALT	alanine transaminase
ZYMOGEN	enzymes mixed
Inoculant 1188	lactic acid bacterial inoculants
TVFA's	total volatile fatty acid
4% FCM	fat corrected milk (4 %)
CFU	colony-forming units
TS	total solids percent
SNF	solid not fat
TDN	total digestible nutrients
DCP	digestible crude protein

Declarations

Acknowledgements

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

Funding:

The authors did not receive support from any organization for the submitted 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.

Ethics approval:

This experimental study was approved all procedures involving animals by the researchers committee of Regional Centre for Food and Feed, Agriculture Research Centre, Ministry of Agriculture, Egypt. Approval number: 00017/2019.

Consent to participate:

The sections are not relevant to our manuscript.

Consent for publication:

The sections are not relevant to our manuscript.

Availability of data and material:

All authors are support all journal requests.

Code availability:

All authors are support all journal requests.

Author Contribution:

Dr. Ahmed Mahmoud El-Morsy((first draft of the manuscripts designed the experimentation, writing the manuscripts, analysis computational and correspondence)).

Dr. Mohsen Mahmoud Shoukry((interpretation of data, reviewing and editing)).

Dr. Soliman Mohamed Soliman((designed the experimentation, interpretation of data, test in vivo, writing the manuscripts, analysis mathematical)).

Dr. Mahmoud Mohamed Soliman((writing the manuscripts, tests in vitro and statistical analysis)).All authors read and approved the final manuscript.

References

1. Abedo, A.A., Hafez, Y.H., Khalifa, E. I., Mohamed, Bahera, K., El-Zolak, O.A. 2013. Milk yield and composition of dairy Zaraibi goats fed microbial inoculated corn silage. Egyptian J. of Sheep and Goat Scie. 8 (1):141-151. DOI: 10.21608/EJSGS.2013.26780.
2. Abido, A.M., Soliman, H.S., Hamza, A. S., Kholif, S.M., Elsayed, H.M.,Abd-Elaziz, A.S. 2007. Effect of maize silage inoculated by bacteria on performance of lactating buffaloes. Int. J. of Dairy Scie, 2(4): 330-338. DOI: 10.3923/ijds.2007.330.338.
3. Aboagye, I.A., Oba, M., Castillo, A.R., Koenig, K.M., Iwaasa, A.D.,Beauchemin K.A. 2018. 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. 96:5276–5286.DOI: 10.1093/jas/sky352.
4. Addah, W., Baah, J., McAllister, T.A. 2016.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: 1-10.
5. Aguerre, M.J., Capozzolo, M.C., Lencioni, P., Cabral, C.,Wattiaux, M.A. 2016. Effect of 2-tier crude feed protein quebracho-chestnut tannin extracts on performance, ruminal fermentation and nitrogen distribution in dairy cows. J. Dairy Sci., 99:4476–4486. DOI.org/10.3168/jds.2015-10745.
6. Aksu, T., Baytok, E.,Bolat, D. 2004.Effects of a bacterial silage inoculant on corn silage fermentation and nutrient digestibility. Small Ruminant Research, 55: 249-252.DOI.org/10.1016/j.smallrumres.2003.12.012.
7. Al-Rabbat, M.F., Baldwin, R.L., Weir, W.C. 1971.*In vitro* nitrogen-treacer technique for some kinetic measures of rumen ammonia. J. Dairy Sci., 54(8): 1150-1161. DOI.org/10.3168/jds. S0022-0302(71)85993-3.
8. AOAC 1990.Official Methods of Analysis.15th ed. Association of Official Analytical Chemists, Washington D.C., USA.
9. Armstrong, W.D., Carr, C.W. 1964. PhysiologicalChemistry, 3rd ed. pp., 75 Burges Publishing CO., Minneapolis, Minnesota, USA.
10. Atkinson, R.L., Toone, C.D.,Ludden, P.A. 2007. Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminallyundegradable protein on site and extent of digestion and ruminal characteristics in lambs fed low- quality forage. J. Anim. Sci., 85: 3322-3330. DOI: 10.2527/jas.2006-417.
11. Balikci, E., Yildiz, A.,Gurdogan, F. 2007. Blood metabolite concentrations during pregnancy and post-partum in Akkaraman ewes.Small Rum. Res., 67: 247-251. DOI: 10.1016/J.SMALLRUMRES.2005.10.011.

12. Barney Harris jr., 1992.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.
13. Basso, F.C., Adesogan, A.T., Lara, E.C., Rabelo, C.H.S., Berchielli, T.T., Teixeira, I.A.M.A., Siqueira, G.R., Reis, R.A.2014. Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield and growth performance of lambs. J. Anim. Sci., 92:5640–5650. DOI.org/10.2527/jas.2014-8258.
14. Bush, K.J., Russel, R.W., Young, J.W. 1979. Quantitative separation of volatile fatty acids by high performance liquid chromatography. J. Liquid Chromatography. DOI: 10.1080/01483917908060143.
15. Chen, X.B., Gomes, M.J. 1992. 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. 21p.
16. Colombini, S., Colombari, G., Crovetto, G.M., Galassi, G.,Rapetti, L. 2009. Tannin treated lucerne silage in dairy cow feeding. Ital. J. Anim. Sci., 8 (2): 289-291. Doi.org/10.4081/ijas. 2009. S2.289.
17. Dean, D.B., Adesogan, A.T., Krueger, N.,Littell, R.C. 2005.Effect of Fibrolytic Enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrasssilage.J.of Dairy Scie. 88(3): 994-1003. DOI: 10.3168/jds. S0022-0302(05)72767-3.
18. Doce, R.R., Hervas, G., Giraldez, F.J., López-Campos, O., Mantecón, A.R.,Frutos, P. 2007. Effect of immature oak (*Quercuspyrenaica*) leaves intake on ruminal fermentation and adaptation of rumen microorganisms in cattle.Journal of Animal and Feed Science, 16(2), 13- 18. DOI: 10.22358/jafs/74411/2007.
19. Doumas, B., Walson, W.,Blgga, H. 1971. Albumin standards and measurement of serum with bromocresol green. Clin. Chem. Acta, 31: 87.
20. Duncan, D.B. 1955. Multiple R and M F- T. Biometric, 11: 1-42.DOI: 10.2307/3001478.
21. Elkholy, M.El.H., Hassanein1, El.I., Edrees, N., Eleraky, W., Elgamel, M.F.A.,Ibraheim, D. 2009. Nutritional aspects of recycling plants by-products and crop residues (corn stalk) in sheep. Pakistan J. of Nutr., 8 (12): 1834-1841. DOI.org/10.3923/pjn.2009.1834.1841.
22. El-Morsy, A.M., Soliman, S.M.,Bakr, A.A. 2018. Influence of inclusion of fungal treated watermelon straw in the ration on nutrient utilisation and rumen fermentation in sheep. Indian J. Anim. Nutr., 35 (3): 251-259. DOI: 10.5958/2231-6744.2018.00039.7.
23. Ferret, A., Plaixats, J., Caja, G., Gasa, J.,Prio, P. 1999. 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: 145–152. DOI.org/10.1016/S0921-4488 (99)00015-2.
24. Filya, I. 2003. 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: 1080-1086. DOI: 10.1046/j.1365-2672.2003.02081. X.
25. Folin, O.Z. 1994. Colorimetric of determination of plasma cereatinine. Phys. Chem. 268: 228.

26. Gaines, W.L., Davidson, F.A. 1923. Relation between percentage fat content and yield of milk.Ill.Agr. Expt. Sta. Bull. 245.
27. Getachew, G., Makkar, H.P.S., Becker, K. 2000. 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: 73-83. DOI: DOI.org/10.1017/S0007114500001252.
28. Herremans, S., Decruyenaere, V., Cantalapiedra-Hijar, G., Beckers, Y.,Froidmont, E. 2020. Effects of hydrolysable tannin-treated grass silage on milk yield and composition, nitrogen partitioning and nitrogen isotopic discrimination in lactating dairy cows.Animal.14(4): 771–779. DOI:10.1017/S175173111900226X.
29. Huhtanen, P., Nousiainen, J.I., Khalili, H., Jaakkola, S.,Heikkila, T. 2003. Relationship between silage fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci., 81: 57–73. DOI.org/10.1016/S0301-6226 (02)00195-1.
30. Islam, M., Enishi, O., Purnomoadi, A., Higuchi, K., Takusari, N.,Terada, F. 2001. Energy and protein utilization by goats fed Italian ryegrass silage treated with molasses, urea, cellulase or cellulase lactic acid bacteria. Small Ruminant Research, 42(1): 49-60. DOI.org/10.1016/S0921-4488 (01)00235-8.
31. Jalč, D., Váradyová, Z., Mihaliková, K., Ledecký, V.,Kišidayová, S. 2013. Enterococci inoculated silages: Effect on rumen fermentation and lipid metabolism *in vitro*. African J. of Microbiology Research, 7(32): 4191-4199. DOI: 10.5897/AJMR2012.2456.
32. Jatkauskas, J.,Vrotniakiene, V. 2006. Effects of silage fermentation quality on ruminal fluid parameters.Biologija 4: 65–71.
33. Jayanegara, A., Goel, G., Makkar, H.P.S., Becker, K. 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. Anim. Feed Sci. Technol., 209:60–68. Doi:10.1111/jpn.12531.
34. Kondo, M., Kita, K., Yokota, H. 2004. Feeding value to goats of whole-crop oat ensiled with green tea waste. Anim. Feed Scie, and Tech. 113:71-81. DOI: 10.1016/j.anifeedsci.2003.10.018.
35. Kung, Jr. L. 2014. a review on silage additives and enzymes. http://ag.udel.edu/anfs/faculty/kung/articles/a_review_on_silage_additiv.
36. Kung, Jr. L., Shaver, R. 2001. Interpretation and use of silage fermentation analysis reports.University of Wisconsin, Madison, WI, USA. Focus on Forage 3 (13): 1-5.
37. Kung, Jr. L., Shaver, R.D., Grant, R.J., Schmidt, R.J. 2018. Silage review: Interpretation of chemical, microbial and organoleptic components of silages. J. of Dairy Scie., 101(5): 4020-4033. Doi.org/10.3168/jds.2017-13909.
38. Kung, Jr.L., Muck, R.E. 1997. Animal response to silage additives. Proc. from the Silage: Field to Feedbunk North American Conference. NRAES -99: 200-210.
39. Lansky, E.P., Newman, R.A. 2007. *Punicagranatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol., 109:177–206. DOI: 10.1016/j.jep.2006.09.006.

40. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem., 96:254-260. DOI.org/10.1016/j.foodchem.2005.02.033.
41. Liu, H.W., Zhou, D.W., Li, K. 2013. Effects of chestnut tannins on performance and antioxidative status of transition dairy cows. J. Dairy Sci., 96:5901–5907. DOI.org/ 10.3168/jds.2013-6904
42. Lynch,J.P., Baah, J.,Beauchemin K.A. 2015.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., 98:1214-1224.
43. Makkar, H.P.S. 2003.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: 241-256. DOI.org/10.1016/S0921-4488 (03)00142-1.
44. Mariana, P.S., Mariana, C.S., Beatriz, R.S.,Laise, C.da S., Diogo, T.C., Rosangela Maria, N.B.,Mauricio, A.R. 2019.Extraction of bioactive compounds from pomegranate peel (*Punicagranatum L.*) with pressurized liquids assisted by ultrasound combined with an expansion gas. J. UltrasonicsSonochemistry, 54: 11-17. https://doi.org/10.1016/j.ultsonch.2019.02.021.
45. McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A. 2002.Animal Nutrition.6th ed. Longman Scientific and Technical, Prentice Hall, New Jersey, USA.
46. McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G. 2010. Chapter 19: silage. In: Animal Nutrition, seventh ed., pp. 499-520.
47. McSweeney, C.S., Palmer, B., Bunch, R., Krause, D.O. 1999. Isolation and characterization of proteolyticruminal bacteria from sheep and goats fed tannin-containing shrub legume *Calliandracalothyrsus*. Applied and Environmental Microbiology 65:3075-3083. DOI. 10.1128/AEM.65.7.3075-3083.1999.
48. Menke, K.H.,Steingass, H. 1988. Estimation of the energeticfeed value obtained from chemical analysis and *in vitro* gasproduction using rumen fluid. Anim. Res. Dev., 28:7-55.
49. Min, B.R., Attwood, G.T., McNabb, W.C., Molan, A.L, Barry, T.N.2005.The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria.Anim. Feed Scie.andTechn. 121(1-2):45-58.DOI.org/10.1016/j.anifeedsci.2005.02.007.
50. Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Scie.andTechn. 106:3-19.DOI.org/10.1016/S0377-8401 (03)00041-5.
51. Ministry of Agriculture2019.Agriculture direction of governorate, Economic affairs sector, Ministry of Agriculture (Summer &Nili crops), P325.
52. Morand-Fehr, P., SanzSampelayo, M.R., Fedele, Y.V., Le Frileux, Y., Eknaes, M., Schmidely, P.H., GigerReverding, S., Bas, P., Rubino, R., Havrevoll, O.,Sauvant, D. 2000. Effect of feeding on the quality of goat milk and cheeses. In: Proceedings of the Seventh International Conference on Goats, Tour, France, Tome I, pp. 53–58.

53. Muck, R.E.2010.Silage additives and management issues.Proceedings of Idaho Alfalfa Forage Conference, Best Western Burley Inn, Burley, Idaho, USA. 16-17 February, pp. 49-55.
54. Nkosi, B.D., Meeske, R., Langa, T., Thomas, R.S. 2011.Effects of bacterial silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. South African J of Anim.Scie., 41(4): 351-359. DOI.org/10.4314/sajas. v41i4.5.
55. Nkosi, B.D., Meeske, R., Langa, T. Motiang, M.D.,Mutavhatsindi, T.F. 2015. 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: 28-35. DOI.org/10.1016/j.smallrumres.2015.04.013.
56. Nkosi, B.D., Meeske, R., Van der Merwe, H.J.,Groenewald, I.B. 2010. Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and digestibility in rams. Animal Feed Science and Technology, 157(3): 195–200. DOI: 10.1016/j.anifeedsci.2010.03.008.
57. Nkosi, B.D., Vadlani, P.V. Brijwani, K. Nanjunda, A.,Meeske, R. 2012. 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 (3): 332-240. DOI.org/10.4314/sajas. v42i3.4.
58. NRC 2001.Nutrient Requiremets of Dairy Animals.National Academy of Sciences, National Research Council. Washington, D C., USA.381p. DOI: 10.17226/9825.
59. Oh, H.I., Hoff, J.E. 1987. pH dependence of complex formation between condensed tannins and proteins. Journal of Food Science 52(5), 1267- 1269.
60. Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A.P., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Gonçalves, M.C.M., Vyas, D.,Adesogan, A.T. 2017.Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability and the performance of dairy cows. J. Dairy Sci., 100 (6):1–17.DOI.org/10.3168/jds.2016-11815.
61. Ott, E.M., Aragon, M., Gabel, M. 2005.Ensiling of tannin_containing sorghum grain.Silage production and utilisation.Prceedings of the XIVth International Silage Conference.pp. 178.
62. Ozduven, M.L., Okuyucu, B., BuyukkilicBeyzi, S.,Konca, Y.2017.The effects of lactic acid bacterial inoculants on the fermentation, aerobic stability and nutritive value of sunflower silages. Inter. J.of Current Research, 9 (7):54289-54295.
63. Patil, A.V.,Karade, A.R.1996. In T.K. Bose and S.K. Mitra (Eds.), Fruits: Tropical and subtropical. Calcutta, India: NayaPrakash.
64. Patra, A.K.,Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Sci. Food and Agri.,91:24-37. DOI: 10.1002/jsfa.4152.
65. Peymanfar, S., **Kermanshahi,** R.2012. The effect of bacteria, enzymes and inulin on fermentation and aerobic stability of corn silage.Iran Microbiol., 4(4):180-6.PMCID: PMC3507307.
66. Pienaar, L. 2021. The Economic Contribution of South Africa's Pomegranate Industry. Western Cape Department of Agriculture Division for Macro & Resource Economics.South African.|

www.sapomegranate.co.za .

67. Pithayanukul, P., Nithitanakool, S.,Bavovada, R. 2009.Hepatoprotective Potential of Extracts from Seeds of *Areca catechu* and Nutgalls of *Quercusinfectoria*. Molecules, 14: 4987-5000. DOI:10.3390/molecules14124987.
68. Reich, L.J., Kung Jr. L.2010. Effects of combining *Lactobacillus buchneri* 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage.Anim. Feed Scie.andTechn., 159:105– 109. DOI: 10.1016/j.anifeedsci.2010.06.002.
69. Reitman, S., Frankel, S. 1957. A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clinc. Path., 28: 56.
70. Romero, J.J., Macias, E.G., Ma, Z.X., Martins, R.M., Coy, B.Y., Martinez, F., Garbuio, D.H., Staples, C.R.,Adesogan, A.T. 2016. Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation. Journal of Dairy Science, 99(5):486–3496. DOI: 10.3168/jds.2015-10082.
71. Ruben, N. 2003.Monitoring udder health and milk quality using somatic cell counts.Veterinary Research. EDP Sciences, 34 (5): 579–596. DOI: 10.1051/vetres:2003028.
72. Sadq, S.M., Ramzi, D.O.M., Hamasalim, H.J., Ahmed, K.A. 2016.Growth performance and digestibility in karadilambs receiving different levels of pomegranate peels. Open J. of Anim.Scie., 6: 16-23. DOI: 10.4236/ojas.2016.61003.
73. SAS 2000. SAS users guide: Statistics, SAS Inst., Inc., Cary N.C., USA.
74. Schmidely, P.H., Morand-Fehr, P.,Sauvant, D. 2005. Influence of extruded soybeans with or without bicarbonate on milk performance and fatty acid composition of goat milk. J. Dairy Sci., 88: 757–765. DOI.org/10.3168/jds. S0022-0302(05)72739-9.
75. Shabtay, A., Eitam, H., Tadmor, Y., Orlov, A., Meir, A., Weinberg, P., Weinberg, Z.G., Chen, Y., Brosh, A., Izhaki, I.,Kerem, Z. 2008. Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as novel beef cattle feed. J. Agric. Food Chem., 56:10063–10070. DOI: 10.1021/jf8016095.
76. Sheperd, A.C., Kung Jr., L. 1996. Effects of an Enzyme Additive on Composition of Corn Silage Ensiled at Various Stages of Maturity. Journal of Dairy Science, 79, 1767-1773. http://dx.doi.org/10.3168/jds.S0022-0302(96)76544-X.
77. Siest, G., Henny, J.,Schiele, F. 1981.Interpretation des examens de laboratoire, K., P. 206-223.
78. Soliman, S.M., El-Morsy, A.M., El-Shinnawy, A.M., El-Ashry, G., Osman, M.A. 2020a.Utilization of biologically treated watermelon vine in rations for dairy cows. J. of Animal and Poultry Production, Mansoura Univ., 11 (3):117 – 123. DOI: 10.21608/jappmu.2020.87135.
79. Soliman, S.M., El-Shinnawy, A.M., El-Morsy, A.M. 2016.Effect of probiotic or prebiotic supplementation on the productive performance of Barkilambs. J. Animal and Poultry Prod., Mansoura Univ., 7(10): 369- 376.DOI: 10.21608/JAPPMU.2016.48743.
80. Soliman, S.M., Hassan, A.A., Bassuony, Neamat I., El-Morsy, A.M. 2020b.Effect of biological extract supplementation on milk yield and rumen fermentation in dairy cows.Intern.J. of Dairy Scie. 15 (2): 88-98. DOI: 10.3923/ijds.2020.88.98.

81. Soliman.M.S. 2014.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(5): 249-266.
82. Stewart, E.K., Beauchemin, K.A., Dai, X., MacAdam, J.W., Christensen, R.G.,Villalba, J.J. 2019. Effect of tannin-containing hays on enteric methane emissions and nitrogen partitioning in beef cattle. J. of Ani.l Sci., 97(8): 3286–3299.DOI: 10.1093/jas/skz206.
83. Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74:3583–3597. DOI.org/10.3168/jds. S0022-0302(91)78551-2.
84. Verbic, J., Chen, X.B., Macleod, N.A. 1990.Excretion of purine derivatives by ruminants.Effect of microbial nucleic acid infusion on purine derivative excretion by steers. J. of Agri. Sci. 114: 243-248.
85. Wanapat, M.,Cherdthong, A. 2009.Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr.Microbial., 58:294–299. DOI.org/10.1007/s00284-008-9322-6.
86. Warner, A.C.I. 1964. Production of volatile fatty acids in the rumen methods of measurements.Nutr.Abstr., and Rev., 34:339-352.
87. Wei, C., Guyader, J., Collazos, L., Beauchemin, K.A., Zhao, G.Y. 2019.Effects of gallic acid on in vitro rumen fermentation and methane production using rumen simulation (Rusitec) and batch-culture techniques. Anim. Prod. Sci., 59:277–287. Doi: 10.1071/AN17365.
88. Wilkinson, J. M., Davies, D.R. 2012. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci., 68: 1-19. DOI.org/10.1111/j.1365-2494.2012.00891.
89. Wilkinson, J.M. 2005. In: Wilkinson, J.M. (Ed.), Silage. Part 6: Assessing Silage Quality. Chapter 19: Analysis and clinical assessment of silage. Chalcombe Publications, UK, pp. 198–208.
90. Wischer, G., Boguhn, J., Steingaß, H., Schollenberger, M.,Rodehutscord, M. 2013.Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. Animal, 7(11):1796–1805.DOI: 10.1017/S1751731113001481.