Research Article

The Effect of Pomegranate Peel on Soybean Meal Protein Degradation and Milk Production in Dairy Cows

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Abstract | Soybean meal is the most important protein source used to fodder mixtures. However, more than 60% of the protein is degradable in the rumen. Several attempts have been made to reduce protein degradation. This experiment was carried out to investigate the effects of different supplementation levels of pomegranate peel (PP) on the *in situ* degradation of soybean meal (SBM) by using three ruminally cannulated. In order to determine the optimal levels of PP to reduce the degradation of SBM, for evaluated their effects on *in vitro* methane production, *in vivo* ruminal fermentation and milk production using eighteen lactating crossbred Friesian cows that were randomly assigned to three groups. The first group contains untreated SBM, while the second and third groups contain SBM treated with pomegranate peel (PP) at levels of 200 and 250 gm / kg of SBM, respectively. All levels of PP treated to SBM in the first experiment had a positive effect on SBM ruminal degradability after 72 h of incubation. But the optimal levels were 200 and 250 g pp/kg SBM (PP), which were significantly lower in degradation than the other levels. This reduction in the extent of dry matter (DM) and crude protein (CP) degradation was mainly due to a marked decrease in the immediately degradable fraction (a), the potential fraction degradation (b), and a lower rate of degradation. Diets containing SBM treated with 200 or 250 gm of PP reduced in vitro gas production, methane production, and TVFA and ammonia concentrations without effect on physiological rumen activity. Moreover, milk yield and milk composition were not affected. However, the concentration of milk urea nitrogen (MUN) was significantly decreased. Overall, these results indicate that treated SBM with levels of 200 or 250 PP reduced its degradability in the rumen more than untreated SBM. Also, recorded decreased gas and methane production had no effect on milk production.

Keywords | Soybean meal, Rumen degradability, Methane production, Milk yield

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INTRODUCTION

In ruminants, the amount of protein that beneficial utilization takes place from dietary protein and microbial protein synthesis in the rumen. The quality of dietary protein is determined by the extent to which it contains balanced amino acids and the quantity of protein escapes from rumen degradation without decreasing digestibility in the small intestine. Improvement in milk production requires more intakes of crude protein in the diet, and an increase of amino acids (AAs) delivered to the intestine in order to meet the animal's needs for milk and milk protein synthesis, Katongole and Yan (2020). According to Savari *et al.* (2017), increased rumen un-degradable protein (RUP)

dairy cow diets result in increased absorption of AA profiles and consistently improved lactation performance. Soybean meal (SBM) is the main source of protein used to feed farm animals. It's about two-thirds of the total protein feed in the world. The nutritive value is incomparable to any other plant protein source, and it is the standard to which other protein sources are compared due to its containing good amino acid balance (Tangendjaja, 2020). SBM is an important part of the diets of ruminants due to its high amount of protein 43-53%, while its high amount of protein is rumen-degradable (more than 60%), which may not supply enough un-degraded intake protein and AA to meet the demands of highly productive animals. Therefore, many methods have been tested to develop techniques that focus on increasing the rumen by-pass of soybean meal protein (Brown and Bradford, 2020; Prasetiyono *et al*., 2018; Bilal *et al*., 2020). Thermal processes such as heating or chemical treatments like formaldehyde, NaOH, xylose, tannins, alkalis, and encapsulation with zein or fat (Castro *et al*.,2008; Colmenero *et al*.,2006; Chen *et al*.,2002) Pomegranate peel (*Punica granatum*) is considered as an agro-industrial waste from pomegranate fruit. Pomegranate peels contain a high concentration of phytochemicals with promising bioactive properties, including tannins, gallic acid, and others. These compounds have potent antioxidant, inflammatory, antimicrobial, and other biological effects, Shokoh *et al*. (2020). According to the Egyptian Agriculture Ministry (2017), the productivity of pomegranate fruit is approximately 91 thousand tons. The peel accounts for 40–60% of the fruit. Tannins are phenolic that have been shown to reduce ruminal degradation of protein because of their ability to form complexes with protein (Andrej and Alenka, 2021). They can form bonds with proteins at ruminal pH 6-7, preventing bacterial proteolysis, but the bonds dissociate at abomasum pH 3-4 and allow the amino acid release, resulting in increased protein utilization with lower rumen ammonia concentrations. Also, absorption of essential AA from the small intestine increased (Barry and McNabb, 1999). The possible effects of tannins have the ability to modify rumen fermentation and inhibit some of the rumen microorganisms Juan *et al*. (2020), to decrease emissions of enteric methane. Tannins are able to reduce methane production in the rumen directly or indirectly by either inhibiting methanogens or protozoal activity, respectively (Naumann *et al*., 2017). The natural origin of tannins as natural alternatives to improve animal performance is better accepted as feed additives than synthetic compounds. However, the balance between optimum levels of effective and potentially anti-nutritional doses is delicate. Therefore, the aim of this study was conducted in two trials. In the first trial, the effect of different levels of pomegranate peel on ruminal *in situ* degradability of SBM protein, and identified the optimum level for reducing degradability was investigated. The second trial selects the best two levels to determine their effect on the *in vitro* total gas production and *in vitro* methane production, as well as the determined parameters for rumen liquor fermentation *in vivo*, microbial protein synthesis and milk production in dairy cows.

MATERIALS AND METHODS

The experiment took place from October 2020 to May 2021 at the Noubaria station farm in El-Beheira governorate.

In situ trials

This experiment was designed in two trials. The first trial was conducted to determine the effects of different levels of PP on soybean meal degradability. The second trial selected the optimal levels of PP that have affected on decreased SBM ruminal degradability to determine their effect on *in vitro* total gas production and methane production, as well as determined *in vivo* parameters of rumen liquor fermentation, microbial protein synthesis, and milk production in dairy cows. Pomegranate peel was prepared by drying in an incubator at room temperature and milling through a 2 mm mesh. Also, SBM was milled through a 2 mm mesh. Pomegranate peel powder was added to SBM at levels of 0, 50, 100, 150, 200, and 250 gm /1000 gm SBM. Table 1 showed chemical composition of SBM and PP.

First trial, three female sheep fitted with permanent rumen fistula (with an average of 52 kg ± 1.50 live body weight) were used to determine the rumen degradability SBM of DM and CP. Sheep were fed 60% of Egyptian clover hay and 40% of a commercial concentrate diet containing 14% CP.

Total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method as described by Singleton *et al*. (1999) total flavonoid content was determined using a colorimetric assay, as described by Dewi and Riska (2019), and tannins were determined according to Makkar (2003).

Two polyester bags (100 % Dacron polyester 7 X 15 cm) with a mean pore size of 45 µm were used at each incubation time. Dry the ground samples in an oven at 60–65˚C overnight to determine the DM, approximately 3 g of DM of sample in each nylon bag. Bags were then incubated in the rumen of each sheep and removed after 3, 6, 12, 24, 48 and 72 hrs. After the removal of the bags from the rumen, they were washed under a gently flowing tap water until the fluid was clear. Bags were drained, and dried at 60˚C for 48 hrs. DM and CP content were estimated according to the method of AOAC (2005). Two bags were washed in running water for 15 min. to determine the initial soluble fraction at 0 time. The kinetics of DM and CP disappearances was studied by fitting the individual values to the following model of equation proposed by Orskov and McDonald (1979).

Table 1: Chemical composition (%DM) of pomegranate peel (PP) and soybean meal (SBM) treated with PP.

Item	PP	PP-0	PP-50	PP-100	PP-150	PP-200	PP-250
OM %	91.58	94.60	94.65	94.55	94.55	94.60	49.55
CP %	8.51	46.50	45.70	44.30	43.40	42.10	40.20
CF %	16.94	3.80	4.40	4.83	5.31	5.70	6.30
NDF %	35.43	12.50	13.62	14.9	16.14	17.20	17.90
ADF %	22.10	6.42	7.51	8.24	8.7	9.43	10.20
Fat %	2.33	3.10	3.01	3.00	3.00	3.10	3.20
Total phenolic contents*	185.4	--	9.33	17. 72	24.31	30.76	37.13
Flavonoids **	2928	---	147.50	273.30	381.32	467.78	587.21
Total tannins ***	141.4	---	7.23	13.16	18.52	22.46	28.41

OM= organic matter; CP= crude protein; CF= crude fibre; NDF= neutral detergent fibre and ADF= acid detergent fibre. * Total phenolic contents mg gallic acid equivalents /g of dry weight PP. **Flavonoids mg quercetin equivalent /kg of dry weight PP. *** Total tannin mg/g dry weight.

$$Y = a + b\ (1-e^{-ct})$$

Y= degradability at time (t); a= water-soluble and rapidly degradable fraction; b= potentially degradable fraction; c= rate of degradation of b.

The effective rumen degradability (ED) was estimated according to Orskov and McDonald (1979). Rumen out flow rate (k) was assumed to be 0.05 per hour for concentrate (McDonald, 1981a).

$$ED = a + bc/c+k$$

Feed intake, milk sampling and milk composition

Second trial, eighteen multiparous lactating crossbred Friesian cows were assigned randomly into three groups (6cows/each treatment) stratified by live body weight (535 ± 7.5 kg). Each group was fed one from three total mixed rations (TMR) designed to containing 55%:45% concentrate: roughage to meet their nutrient requirements according to NRC (2001) recommendations. Table 2 illustrated the ingredients and chemicals composition of three rations. Each concentrate diet containing 15% soybean meal treated with 0, 200, 250 gm PP/kg SBM.

Feed intake was recorded daily by weighing the offered rations and refusals from the previous day. Diets were offered twice a day at 07:00 and 19:00 pm. Samples of TMR were taken daily, dried at 60°C in a forced-air oven for 48 h (AOAC, 2005) and proximate analysis of the samples for ash, crude protein, fiber, fat and nitrogen free extract contents were determined as described by AOAC (2005). While, fiber fraction (NDF and ADF) were determined according to Van Soest *et al.* (1991).

The milk production experiment started after 6 weeks of calving, and the cows lasted for 4 weeks as a preliminary period followed by milk collection. Milk samples were collected from the eleventh week to the twentieth week. Cows were machine milked twice daily at 06:00 and 18:00 pm, 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

Table 2: Ingredients and chemical composition of the total mixed ration.

Item	Control	PP-200	PP-250
Ingredients (g kg^{-1} DM)			
Corn silage	400	400	400
Rice straw	50	50	50
Sunflower meal	90	90	90
Corn	127	127	127
Sugar beet pulp	45	15	7.5
Wheat bran	120	120	120
Soybean meal ground (46.5% CP)	150	150	150
Calcium carbonate	12	12	12
*Vitamin-mineral premix and salt	6	6	6
**Pomegranate peel	---	30	37.5
Chemical composition (% DM)			
Dry matter	67.10	67.10	67.10
Crude protein	16.17	16.17	16.17
Ether extract	2.8	2.8	2.8
Nitrogen free extract (NFE)	54.6	54.6	54.6
Neutral detergent fibre	32.44	32.38	32.30
Acid detergent fibre	19.31	19.28	19.25
Tannin mg/g DM	0.0140	0.468	0.585

* Supplied per kilogram of premix (Kav): Vitamin A 12 000 000 IU, Vitamin D3 3 000 000 IU, Vitamin E 30 mg, Mn 50 mg, Fe 50 mg, Zn 50 mg, Cu 10 mg, I 0.8 mg, Se 0.15 mg, antioxidant 10 mg. **The added of pomegranate peel to soybean meal has been taken from the sugar beet pulp content due to their closeness in terms of protein (8.5- 8.9) and metabolizable energy(301-298) for pomegranate peel and sugar beet pulp respectively.

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 and Davidson, (1923) using the following equation:

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

Where M= milk yield, F = fat percentage. Milk energy value (E) was calculated according to Kleiber *et al*. (1961):

$$E\ (kcal/kg) = (\%\ fat \times 92) + (\%\ protein \times 58.6) + (\%\ lactose \times 39.5).$$

Milk urea nitrogen (MUN) was determined using enzymatic methods described by İnal *et al* (2015), and sample absorbance at 625 nm was measured using a spectrophotometer.

Rumen parameters, and microbial nitrogen (MN) synthesized

Ruminal fluid contents were sampled at 0 time before feeding and at 3 and 6 h after the morning feeding using stomach tubing from cows that fed experimental diets after 20 d as adaptation period the samples collected for 3 days. Approximately 100 mL of rumen fluid were collected from each treatment (the same cows used in the lactation trials) and strained through 4 layers of cheesecloth. The supernatant was used for determination pH immediately using Orian 2 stars digital. Approximately 10 ml of the sample was preserved with 2-3 drops of formalin to prevent fermentation. Ammonia-N (NH_3-N) was determined according to method AOAC (2005). The concentration of total volatile fatty acids (TVFA) was determined according Anderson and Yang (1992). Concentration and molar proportions of individual VFA were measured by gas-liquid chromatography.

MN synthesized was determined according to Chen and Gomes (1992). Equations used to calculate microbial nitrogen (MN) as follows:

$$MN = (70 \times AP) / (0.83 \times 0.116 \times 1000)$$

Where, 70 represent 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:

$$AP = \{PD - (0.385 \times BW^{0.75})\} / 0.85$$

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 measurement of gas and methane production

In vitro gas production was determined as described by (Menke and Steingass, 1988). Rumen fluid was collected before feeding in the morning using stomach tubing from cows fed a TMR. Samples of diets (200±10 mg) of the oven-dry feedstuffs and the respective mixtures were accurately weighed into 100-ml glass syringes fitted with plungers. *In-vitro* incubation was conducted in three run involving quintuplicate samples. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72, and 96 hours. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Gas production was fitted to the non-linear equation model of exponential (EXP0) by Schofield *et al*. (1994).

$$EXP\ V = VF\ (1-\exp(-kt))$$

Where: V: is the cumulative gas production (in ml) at different incubation times, VF: final asymptotic gas volume.

$$VF = V\ final - V0 - GP0$$

Where; V final = the final volume of gas recorded at the end of incubation time; V0 = the initial volume of gas recorded before incubation starts; GP0= the mean blank value. *K*= fractional rate of gas production, t= incubation time (h). The fractional rate (μ, h^{-1}). Where, μ= the point of inflection of the gas curve at time t.

Methane was measured by taking 1 ml from headspace gas from each syringe after incubation time at 24, 48, 72, and 96 hours by evacuated vials and injecting it into gas chromatography (GC) with flame ionisation detection. To collect gas samples from each syringe, the syringe (100 ml) used in trial gas production must be equipped with three-way taps (Luer-Lock) and pre-evacuated exetainers.

In-vitro dry matter and organic matter digestibility were calculated by a modified Tilley and Terry (1963) technique.

Statistical analysis

Data were subjected to analysis as a completely randomized design with repeated measures using the MIXED procedure of SAS 2002 (Version 9.2) Statistical processes were carried out using the General Linear. The model describing each trait was assumed to be:

$$Yijkl = \mu + Ti + a\ (T)\ IJ + WK + Eijkl$$

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 the least significance difference (LSD) Duncan's multiple range tests.

RESULTS AND DISCUSSION

Rumen degradation kinetics

Data in Table 3 showed the effects of adding pomegranate peel on the *in-situ* DM and CP soybean meal degradability. The results indicated that there was a linear inverse relationship between increasing levels of PP and decreased CP and DM degradation. The lowest (P<0.05) values of CP or DM degradability were recorded with treatment PP-250, followed by PP-200. The reduction of crude protein degradation was 26.30–29.50% for PP-200 and PP-250, respectively. At levels PP-200 and PP-250, the reduction in DM degradation was 20.40–24.60%, respectively. There is a significant correlation between increasing the addition of level PP and decreasing the degradability parameters. The water-soluble fraction (rapidly degradable fraction) 'a', potentially degradable fraction 'b', and the rate of degradation fraction 'c' showed a reduction effect by adding pomegranate peel to soybean meal. Moreover, the results showed that increasing levels of supplementation of pomegranate peel led to an increase in the escape CP and DM of SBM from degradation. The lowest (P<0.05) values of fractions "a", "b," and "c" were shown with SBM treated with pomegranate peel at levels of PP-250 and PP-200. In accordance with these degradation parameters, the reduction of degradation of the SBM was significantly (P<0.05) affected by the PP treatment, especially at levels PP-250 and PP-200.

Ruminal fermentation parameters

The results of ruminal fermentation parameters are shown in Table 4. Treatment of SBM with PP at levels of PP-200 and PP-250 led to a numerical increase in pH without significant differences (P<0.05). The results of total volatile fatty acid (TVFA) and ammonia nitrogen (NH3-N) concentration showed a significant (P<0.05) reduction with diets that included pomegranate peel. The addition of PP at levels of 200 or 250 to SBM contributed to reduced (P<0.05) TVFA concentration and molar proportions of acetate, propionate, and butyrate. The lowest value of VFA was shown with diet PP-250. However, the treatment of SBM with PP had no effect on the acetate to propionate ratio. Ammonia production was reduced (P<0.05) by increasing the level of PP (from PP-200 to PP-250) compared to the control. The diets containing SBM treatment with PP had a significant (P<0.05) decrease in microbial nitrogen production by 5.40 and 6.00% for diets including PP at levels 200 and 250, respectively. In although, the actual lower ammonia-N concentration was still sufficient for microbial nitrogen synthesis.

The effect of the addition of pomegranate peel to SBM on *in-vitro* gas production and methane production has been shown in Table 4. Significant differences between diets containing SBM treated with PP and untreated, but no differences between two levels of PP addition. Gas production was decreased by 20.9 and 22.8% by the addition of PP at levels of 200 and 250, respectively. Also, the addition of PP at level 250 or 200 significantly decreased (P<0.05) CH4 production by 30.3% and 26.5%, respectively.

The results of *in-vitro* dry matter digestibility (IVDMD) and *in-vitro* organic matter (IVOMD) digestibility are shown in Table 4. The results illustrated a slight increase without significant differences (P<0.05) with diets containing PP compared to control diets.

Dry matter intake, mike yield and milk composition

The effect of treating SBM with pomegranate peel on dry matter intake (DMI) is presented in Table 5. The addition of pomegranate peel at levels of 200 or 250 to the diets had no influence on dry matter intake (DMI). Cows fed a diet containing SBM treated with PP or those fed a control diet showed no significant differences (P<0.05).

The milk yield was increased in dairy cows fed diets containing SBM treated with PP at levels of 200 or 250, by 2.70% and 3.50%, respectively, but the differences were not significant (P<0.05). In the same trend, milk protein concentration was enhanced by 0.94% and by 1.25%, respectively. While milk fat content decreased by about 1.92%, with no statistically significant differences (P< 0.05). However, the milk lactose content was the same between cows fed diets containing PP or those fed a control diet.

Milk urea nitrogen reflects the amount of urea found in milk. Milk urea nitrogen is used as a management tool to optimize dairy herd nutrition and monitor the nutritional condition of lactation dairy cows. The addition of PP to dairy cow diets had a negative significant (P < 0.05) effect on milk urea nitrogen (MUN). It decreased by 9.85% and 10.40% in the diets PP-200 and PP-250, respectively. Also, cows fed a diet containing SBM treated with PP showed a significantly decreased SCC compared to those fed a control diet. Generally, despite the significant decrease in MUN and SCC, treating SBM with PP did not affect a significant increase in milk yield, milk fat, or milk protein concentration.

Table 3: *In situ* degradation for soybean meal treated with pomegranate peel.

Item	PP-0	PP-50	PP-100	PP-150	PP-200	PP-250	SEM	P-value
Dry matter degradability								
a	31.35[a]	30.84[a]	29.35[ab]	28.96[b]	27.24[c]	26.47[c]	0.77	0.009
b	67.08[a]	65.17[a]	62.60[ab]	56.41[b]	52.20[c]	50.06[c]	1.43	0.022
c	0.065[a]	0.064[a]	0.062[a]	0.059[b]	0.057[b]	0.053[c]	0.08	0.004
EDDM	69.26[a]	67.43[a]	63.95[ab]	59.49[b]	55.15[c]	52.23[c]	0.172	0.006
Crude protein degradability								
a	27.09[a]	26.34[a]	24.22[ab]	22.47[b]	20.86[b]	20.44[b]	0.61	0.006
b	75.05[a]	70. 49[a]	67.73[ab]	62.80[b]	57.41[c]	55.03[c]	4.83	0.022
c	0.060[a]	0.059[a]	0.057[a]	0.055[ab]	0.052[b]	0.05[b]	0.06	0.007
EDCP	68.02[a]	64.50[a]	60.30[b]	55.37[b]	50.13[c]	47.96[c]	2.25	0.038

EDDM: The effective rumen degradability of dry matter. EDCP: The effective rumen degradability of crude protein. SEM: standard error of the means; significant ($P < 0.05$) a, b and c: means in the same row with different superscripts are differ significantly. a: the water-soluble fraction; b: the potentially degradable fraction and c: the rate of degradation.

Table 4: Rumen fermentation, microbial nitrogen of lactating crossbred Friesian cows feed rations.

Item	Control	PP-200	PP-250	SEM	P-value
In vivo ruminal Mean pH	6.48	6.63	6.65	0.57	0.076
In vivo ruminal Mean NH_3-N (mg L^{-1})	13.66[a]	11.38[b]	10.41[b]	1.32	0.025
In vivo TVFA* (mmol L^{-1})	106.22[a]	102.71[b]	99.96[b]	4.73	0.027
Acetic, C_2 (ml/100ml)	65.33[a]	60.11[b]	59.87[b]	3.57	0.014
Propionic,C_3 (ml/100ml)	26.13[a]	24.83[b]	24.65[b]	0.69	0.017
Butyric, C_4 (ml/100ml[l])	13.53[a]	11.46[b]	11.28[b]	0.44	0.043
C2:C3 ratio	2.50	2.42	2.43	0.06	0.088
PD (mmol/day) **	136.17[a]	131.13[b]	130.60[b]	7.57	0.039
microbial nitrogen g/day	79.84[a]	75.53[b]	75.07[b]	0.57	0.023
IVDMD***	65.01	65.79	66.41	1.38	0.058
IVOMD****	62.01	62.35	62.88	2.66	0.078
Rates of gas production	0.0727[a]	0.0613[b]	0.0587[b]	0.054	0.0032
Total gas production (ml/200mg DM)	60.02[a]	47.46[b]	46.35[b]	5.54	0.021
Methane production at 24 h (ml/200mg DM)	10.16[a]	7.47[b]	7.08[b]	2.93	0.006

SEM, Standard error of the mean. a and b: means in the same row with different superscripts are differ significantly ($P< 0.05$). *TVFA= Total volatile fatty acid; ** PD= Purine derivatives (allantoin and uric acid in urine); ***IVDMD= *In vitro* dry matter digestibility and ****IVOMD = *In vitro* organic matter digestibility.

Table 5: Dry matter intake, milk yield and milk composition of lactating crossbred Friesian cows feed rations (mean ± SE).

Item	Control	PP-200	PP-250	SEM	P-value
DMI, kg/d	19.15	18.92	18.87	3.39	0.058
Milk yield, kg/d	18.80	19.32	19.46	2.73	0.072
4 % FCM	17.67	18.10	18.23	0.19	0.117
Fat, kg/d	0.690	0.690	0.696	0.27	0.195
Milk composition (%)					
Total solids	11.79	11.73	11.76	0.45	0.720
Fat	3.65	3.58	3.58	0.78	0.068
Protein	3.20	3.23	3.24	0.26	0.082
Lactose	4.25	4.24	4.24	0.73	0.077
Ash	0.69	0.68	0.70	0.04	0.081
MUN mg/dl*	14.01[b]	12.63[a]	12.56[a]	1.38	0.034
SCC × 10^3/mL**	83.60[a]	75.70[b]	75.10[b]	5.31	0.041
Milk energy content (kcal/kg)	691.20	687.94	689.70	0.07	0.068

SEM, standard error of the mean. a and b: means in the same row with different superscripts are differ significantly ($P< 0.05$). *DMI= dry matter intake, ** 4%FCM= fat correct milk 4%, *** MUN=milk urea nitrogen and ****SCC= somatic cell count.

Rumen degradation kinetics

The effective degradability (ED) of DM and CP was calculated at k = 5% h-1 according to (NRC, 1985). The reduction of CP degradability was greater than the reduction of DM degradability at the same level of PP, due to the presence of tannins in PP that may have the ability to complex primarily with proteins, and to a lesser extent with polysaccharides and other organic compounds. (Makkar, 2003). The processes of tannin-protein binding are influenced by the relative concentration of both tannins and protein (Hagerman and Butler, 1981) and are dependent upon the pH value. Many studies (Andrej and Alenka, 2021; Makkar, 2003; Arisya *et al.*, 2019) found that tannin-protein binding is more stable in the rumen and resistant to rumen microorganism degradation at pH (5.0 to 7.0). However, it dissociates in gastric juice (abomasum pH 2-3), Oh and Hoff (1987); and Priolo *et al.* (2000). Therefore, an increase in the inclusion of pomegranate peel into SBM leads to decreased CP and DM rumen degradability. Our results support those of Basria *et al.* (2021), Heendeniya *et al.* (2012), and Alipour and Rouzbahan (2010), who observed that tannin-treated SBM decreased CP and DM degradability, whereas the escape degradation of CP was greater than DM. According to Hvelplund and Weisbjerg (2000), small particle loss, especially formed during sample preparation, affects fraction "a". The pomegranate peel was finely ground. Therefore, an increase in the pomegranate peel levels may be led to an increase in the loss of small particles, which caused a decrease in fraction "a", which agreed with previous studies by McDonnell *et al.* (2017) who suggested that the small particles might easily escape from a nylon bag. According to the findings, tannins in PP decreased the rate of degradation and fraction "b." That was probably due to the ability of phenolic groups of tannins establish hydrogen bonds with the NH groups of peptides or proteins, which are not broken by rumen microorganism, Basria *et al.* (2021), Alipour and Rouzbehan (2010), Furthermore, tannins can interfere with the action of extracellular microbial enzymes, inhibiting their activity, Panel *et al.* (2018).

Ruminal fermentation parameters, microbial nitrogen syntheses, *in-vitro* gas production and methane and *in-vitro* digestibility

The ruminal pH values were within the normal range of 6.48–6.65 for physiological rumen activity reported by Van Soest (1994). The addition of PP to SBM showed a slight increase in pH. That may be due to tannins, which have been shown to have an insignificant increase, Bhatta *et al.* (2015) in ruminal pH. This is consistent with findings in the decrease of total VFA concentrates in rumen but the increase in pH was limited, probably due to the buffering capacity of saliva reported by Bechir *et al.* (2021). The TVFA and ammonia concentrations were reduced due to the treated SBM with PP, Tan *et al.* (2011) reported that adding tannins to ruminant diets had a negative impact on rumen fermentation, decreasing NH_3-N and TVFA concentrations. The reduction of TVFA may be attributed to tannins having the potential to decrease the rumen digestion of carbohydrates and hemicellulose. Although this was countered by increased post-ruminal digestion, Hariadi *et al.* (2010) suggest that tannins form unfermentable complexes with substrates (carbohydrates, hemicelluloses) Kelln *et al.* (2020). Tannins also have the ability to decrease the activity of some species of rumen microorganisms Panel *et al.* (2018). This decreases the activity of rumen microorganisms, which may have an effect on organic matter fermentation. Therefore, a decrease in VFA concentrations may be linked to reduced organic matter fermentation. Also, the tannin-protein complex had a negative effect on proteolysis that may be responsible for the decrease in ammonia concentration, Dschaak *et al.* (2011). In terms of the acetate: propionate, it's indeed unaffected by adding pomegranate peel. Similarly, Hassanat and Chaouki (2012) observed that adding chestnut containing tannins at levels of 100, 150, and 200 g kg1 SBM had no effect on the acetate to propionate ratio when compared to the control. In addition, McSweeneye *et al.* (2001) found that avalonea, sumach, grape seed, and myrobalan enhanced acetate production when included in ruminant diets. This could be attributed to Ng *et al.* (2015) hypothesis that homoacetogens could use H_2 in combination with CO_2 to produce acetate. Thus, if less H_2 is converted to CH_4 by inhalation of methanogense, then more H2 is available for producing acetate. Previous *in-vitro* studies have indicated that supplemented tannins may reduce organic matter, and protein degradability in the rumen, consequently decreasing TVFA production, Bueno *et al.* (2020).

Since the PP treatment reduces the efficacy of microbial protein synthesis, these results are consistent with those of Gerlach *et al.* (2018) and Dickhoefer *et al.* (2016) who found a reduction in microbial protein synthesis when the cows fed diets containing tannin at 1.5% of DM. However, the decline was 16%, higher than found in our results. This could be due to higher levels of tannins in diets than those used in our studies. Our finding is considered compatible with the decrease in ammonia-N concentration but suggests that the reduction of rumen ammonia-N concentration was still sufficient for microbial protein synthesis.

The results of *in-vitro* dry matter digestibility (IVDMD) and *in-vitro* organic matter digestibility (IVOMD) are supported by the results shown by Gerlach *et al.* (2018) and Andrej and Alenka (2021), who found that there was no effect of tannins on the *in-vitro* digestibility. Tannin consistently promoted the duodenal flow of OM and non-

ammonia non-microbial N (NANMN) while reducing rumen OM and feed protein degradability. According to Fitriastuti *et al.* (2019), this may enhance the digestibility of OM post-rumen, implying that OM digestion has been compensated for and extended throughout the abomasum and intestines. This result contradicts results found by Ramos *et al.* (2020), who showed a significant decrease in the total digestibility and bioavailability of nutrients, while Basri *et al.* (2021) found a significant increase in total apparent digestibility.

A negative correlation exists between adding pomegranate peel to ruminat diets and CH_4 production. Tannins have the ability to reduce gas production due to their potential biological impact on rumen fermentation and effect on rumen microbiota, Bueno *et al.* (2008). According to Naumann *et al.* (2017), tannins have the ability to reduce methane production in the rumen either directly by inhibiting rumen methanogenic bacteria by adhesin of the microbial cell wall and inhibition their enzymes, or indirectly by reducing the availability of nutrients (i.e., carbohydrates, protein, and fiber) to rumen microorganisms, which may impair the rumen microbial population. Addition of pomegranate peel (PP) had a significant effect on modification in the rumen ecosystem via tannins, which have the potential to inhibit methanogens (Gunun *et al.*, 2018) and then reduce methane emissions and total gas production. All previous studies have confirmed that tannin plants were effective in reducing *in vitro* CH_4 production. The tannins have been proposed to directly inhibit some ruminal specialized bacteria, and methanogen population inhibition (up to 36%) has been reported by Costa *et al.* (2018). However, Bhatta *et al.* (2015) suggested that tannins directly reduce methanogen activity and, therefore, they might affect the abundance of methanogens through their effect on the decreased availability of hydrogen for rumen microorganisms. This may be achieved without significantly affecting the other rumen fermentation parameters.

Dry matter intake, mike yield and milk composition

Dry matter intake (DMI) was unaffected by the addition of pomegranate peel to the diet. Our results were confirmed by other studies that reported a non-detrimental effect of tannin on ruminant intake. According to Benchaar *et al.* (2008), adding tannins to the diet at a concentration of 0.5 percent DM had no effect on feed consumption. Morover, Ramos *et al.* (2020) found tannins had no effect when added at 0.75%. On the contrary, when Ramos *et al.* (2020) added tannins at 2.2% to Nellore cows' diets, the DMI was reduced because it is known that tannins influence ruminant palatability. Also, Beauchemin *et al.* (2007) observed that adding 2% DM tannins to cow diets resulted in a decrease in dry matter consumption.

Dairy cow feeding regimes must provide adequate protein quality and quantity to produce dairy cows to meet the request for milk yield. Dairy cows need both high quality protein and high quantities of protein to produce milk, since the SBM treated with PP provides an adequate amount of high quality RUP (rumen un-degradable protein) and proteolysis to amino acids that meet the requirements of dairy cow production (Schwab and Broderick, 2017). About 25% of nitrogen intake is converted into milk by dairy cows (Calsamiglia *et al.*, 2010). The increase flow of un-degrdable feed protein to the intestine could then potentially improve nitrogen use efficiency and milk yield.

In terms of milk yield, the results observed that dairy cows fed diets including soybean meal treated with PP enhancement milk yield and FCM4% without any negative significant effect, which might be due to the effect of tannin inclusion in diets providing cows with the requirements of amino acids. These results are in agreement with previous studies, Benchaar *et al.* (2008) and Aguerre *et al.* (2010) reported that reducing tannin concentrations to less than 2% DM when incorporated into dairy cow diets had no effect on milk output and composition. On the other hand, milk yield (kg/d), was consistently lowered due to increasing levels of tannin more than 3% by Yanza *et al.* (2020). Adding pomegranate peel to dairy cow diets led to an in-significant decrease in fat milk concentration. These results are consistent with Panel *et al.* (2011) observed no effects of tannins when supplemented with up to 0.8% of DM on milk fat and milk lactose composition. In contrast, Wanapat et al. (2000) reported improved milk production, milk fat content, and 4% fat corrected milk yield in cows fed ration supplemented with tannin up to 1% DM. milk protein was increasing by adding PP to the dairy cow while, had a negative effect on milk urea nitrogen which, has potentially positive for the environment. The positive effect of protein milk may be attributed to a metabolic supply sufficient amount of energy and dietary un-degradable protein will increase milk protein concentrations according to Kaufman and Pierre (2001). Our results agree with previous studies done by Aguerre *et al.* (2010) found that milk protein concentration increased when tannins were supplemented at level of 0.45% of DM, whereas Panel *et al.* (2011) reported that supplementing tannins at a level of 1.8% of DM decreased milk protein concentration. There is a high positive correlation between milk urea nitrogen, and the concentration of ammonia in the rumen. As discussed previously, forming tannins-protein complexes decreased protein degradation and NH_3-N production in the rumen. That may have reflected in decreased MUN concentration according to Herremans *et al.* (2019) and Dschaak *et al.* (2011).

CONCLUSIONS AND RECOMMENDATIONS

This study investigated the inclusion of pomegranate peel in dairy cows' diets. The potential effect could be beneficial for decreasing rumen degradation of SBM and contributing to overcoming methane emissions from ruminants. Also, it promoted an decrease in milk urea content without having an effect on quality and quantity of milk production. Future work will be necessary to investigate further the role of pomegranate peel in its effect on animal performances.

NOVELTY STATEMENT

A number of goals were achieved, namely, the utilization of natural tannins found in pomegranate peel, and the peel considered an agricultural residue that may cause pollution to the environment. Natural tannins in dairy cow diets helped to reduce rumen degradation of SBM protein, and reduce ruminant methane emissions.

AUTHOR'S CONTRIBUTION

Soliman Mohammed Soliman: Experimental design, performed experiment and wrote the manuscript.
Mohsen Mahmoud Shoukry: Revision to experimental design, scientific content, and grammar.
Ahmed Mohammed El-Okazy: Provided access research component (field-equipment and apparatus for analysis).
Ahmed Mahmoud El-Morsy: Animal processing on the farm - collected samples of the first trail.
Mahmoud Mohammed Soliman: Data collection and data analysis- collected samples of the second trail.

Conflict of interest

The authors have declared no conflict of interest.

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