EFFECT OF DIETARY POMEGRANATE PEEL (*PUNICA GRANATUM*) SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE AND IMMUNE STATUS OF FRIESIAN DAIRY COWS. 29

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SUMMARY

This study was conducted to investigate the effect of adding different levels of pomegranate peel (2,3 and 4%) to dairy cows diets on milk production, chemical composition, digestion coefficients, decomposition rate of dry matter, rumen parameters and some blood serum measurements parameters and immune bodies of dairy cows. Sixteen crossbreed Friesian cows in the first week of lactation were randomly chosen used in the third or fourth season and divided into four groups (each group 4 cows) with the Latin square design (4x4) (28 days for each period) . The groups were fed the following rations (1)- control ration (40% concentrate feed mixture + 40% corn silage + 20% rice straw. (2) control ration + 2% pomegranate peel . (3) - control ration + 3% pomegranate peel . (4)- control ration + 4% pomegranate peel . The rations were formed in according to NRC, (2001) . Three Barki rams were used for digestion tests and three Barki ewes with rumen fistula were used to measure rumen activity and estimate the rate of decomposition of dry matter and rumen protein. The results showed that the ration containing 2% of pomegranate peel Significant ($P<0.05$) improved digestion coefficients and nutritive value with an increase in the concentration of ammonia and rumen volatile fatty acids with an increase in the rate of decomposition of both dry matter and rumen protein compared to the levels of other pomegranate peel . The diet containing 2% of pomegranate peel increased ($P<0.05$) the milk 4%FCM production . The results of blood serum measurements were within the normal levels and without any health impact negative on animals. The ration containing 2%, 3% and 4% pomegranate peels showed an increase in the antioxidant activity and immune bodies of the blood serum compared to the control ration. This study concluded that pomegranate peels at the level of 2% can be used for dairy cow rations to increase digestibility coefficients , nutritive values and increasing milk yield of dairy cows without any harm to the productivity and health of animals. Further long-term studies on lactating animals are recommended to confirm the results obtained in the current study.

Keywords :*pomegranate peel; acetic acid digestibility; degradability; milk production; lactating cows.*

INTRODUCTION

Natural increase in population undoubtedly associates with increase demand for animal products. This case prompts serious search for alternative feeds and improves utilization of the available sources in order to diminish the expected gap between ruminant requirements and currently used diets, taking in consideration that depending on concentrates should be reduced. Santos MBGA , et al.,(2010) However, healthy diets with high nutritive value are very important to produce quality and economic animal products Sakhawat (2011). referred to the importance of post ruminal degradability of dietary protein. Protection dietary protein from ruminal degradation may reduce loss of via rumen wall as ammonia produced as end product of amino acid metabolism (Atkinson , et al., 2007) .

Pomegranate characterized with high tannin content, especially the peel which, content is about 25-28% Li Y, C Guo (2006) . Accordingly, tannin may help to improve ruminant productivity by increasing flow of true protein from the rumen and enhance feed digestibility due to protected effect of ruminal degradability of dietary protein (Mirzaei-Aghsaghali A, et al., 2011) . Also, most of these compounds are potent free radical scavengers that could favorably a ect the antioxidant status of the animal (Liu, H.,et al., 2016).

In particular, phenolic compounds which have high correlation with disease-fighting compounds and natural antioxidant capacity, help to maintain human health to preventing diseases (Akhtar, et al., , 2015) . Protein tolerance in rumen has been reduced through the use of tannins where tannin types and forms are

low concentrations improve rumen fermentation of carbohydrate art as well as microbial protein synthesis and can increase dietary protein intake in rumen (Makkar et al., 1995).

Different pomegranate components appear to have different effects on the intake, digestibility, and milking performance of cows. It should be noted that although polyphenolic compounds might improve animal health, they can also decrease proteolytic activity and thus compromise protein digestion (Broderick et al., 1991). Therefore, potential benefits of added pomegranate components on cow health and production should be considered relative to the potential decline in nutrient digestion and milk production. This conflict dictates a need to measure optimal dosages of CPE inclusion in the TMR of lactating cows.

Pomegranate peel (PP) is a good source of antioxidants (Singh et al., 2001; Whitley et al., 2003). Li et al., (2006) reported that Pomegranate peel (PP) offers higher yields of phenolics, flavonoids and proanthocyanidins than the pulp. Flavonoid content was greater in the peel than in the pulp (59 vs. 17 mg/g), as was that of proanthocyanidins (11 vs. 5 mg/g). Liz et al., (2013), reported that the pomegranate peel could be used as antioxidants is effective in inhibiting lipid oxidation and does not significantly affect the overall sensory attributes of the finished product.

The objective of this study is to investigate the effect of addition of pomegranate peel at different levels on lactating Friesian cows on milk production, some blood and rumen parameters , nutritive values, digestibility coefficients, degradation kinetics, feed utilization and economic efficiency .

MATERIALS AND METHODS

The experiments was carried out at El-Noubari Research Station. Animal Production Research Institute, Egypt. Sixteen lactating crossbred Friesian cows (536.38 kg LBW) with 3-4 parities. The first week of lactation were allocated into 4 similar experimental groups based on their average body weight and average daily milk production (8-10 kg). Duplicated Latin Squares design (4 cows x 4 diets) was used. The four cows of each experimental fed each tested diet 28 day (21 d a preliminary interval and 7 d collection interval). Cows were fed the four experimental rations formulated as the following: (1)- control ration (40% concentrated feed mixture + 40% corn silage + 20% rice straw without add pomegranate peel . (2) control ration + 2% pomegranate peel . (3) - control ration + 3% pomegranate peel . (4)- control ration + 4% pomegranate peel . Cows were individually fed according to NRC (2001). Milk yield was collected twice daily from each cow at evening and morning, recorded individually on two successive days. Milk samples (100 ml) were taken from two consecutive milking according to Galatov (1994). Milk samples were chemically analyzed for fat, protein, total solid (TS), and ash according to AOAC (1995). Fat corrected milk (4%) was calculated according to Gaines (1923) using the following equation: 4 % FCM was calculated as: 0.4 X milk yield (kg) + 15 X . Digestibility trials were conducted to determine the digestibility coefficients of all nutrients. Three male Barki sheep were individually allotted in metabolic cages for 3 weeks adaptation and one week for urine and feces collection. Feces and urine were collected quantitatively once/day pre-morning feeding (8.00 a.m.), and daily samples representing 20% of feces and urine for seven days were frozen (-20°C) till analyses. In the laboratory, samples of feces were partially dried (60°C) for 72 hours. Samples of feeds and feces were ground (through 1 mm screen) by Wiley mill grinder, then samples of 20 g/treatment/sheep were analyzed for crude protein , crude fiber, ether extract and ash according to A.O.A.C,(1995). Urine samples output/ sheep were analyzed for nitrogen. Metabolizable energy (ME, kcal/kg) was calculated according to the method of Pantha (1982). Total digestible nutrients (TDN) was determined on a dry matter basis (DM) by classic formula according to Maynard et al. (1978). At the end of the experiment, blood samples were taken from the Jugular vein of each cow before the morning feeding. Blood plasma was analyzed for glucose Trinder, (1969), total proteins (the biuret method of Henry et al., (1974), albumin Doumas et al., (1977) and urea Henry and Todd, (1974) aspartate amino-transferases (AST) and alanine amino transferases (ALT) activities Reitman and Frankel, (1957) using commercial kits. Rumen liquor samples were taken at 0, 1, 3 and 6 hrs after the morning meal from three fistulated female Barki ewes (42.00 kg LBW). The collected rumen liquor was directly tested for pH using Orian 680 digital pH meter. Samples were strained through four layers of chesses cloth for each sampling time, while ammonia nitrogen (NH3-N) was determined using magnesium oxide (MgO) as described by Al-Rabbat et al. (1971). Total volatile fatty acid (VFAs) concentration was estimated using steam distillation methods Warner, (1964). Evaluation of innate immune response, Detection of bovine immunoglobulin: Serum and milk whey IgG samples were measured using ELISA kit (Bethyl

laboratories) Cat.No. E11-118. Lysozyme assay obtained from logarithmic curve of standard lysozyme (Peeters and Vantrappen 1977).Nitric oxide assay according to Green et al., 1982 and Rajaraman et al., (1998).

Data were subjected to statistical analysis as one way design (ANOVA) using the General Linear Models procedures (SAS, 2000). The significant differences were tested by least significant difference test.

RESULTS AND DISCUSSION

Chemical composition of CFM , corn silage , rice straw and pomegranate peel (Table 1) revealed high CP content in CFM and corn silage Also, corn silage , rice straw and Pomegranate peel contained high CF . pomegranate peel contained high total phenolics and NFE contents. It could be noticed that the CP% for CFM showed more double value of corn silage .However , they were normally equal in EE, ash and NE.

Table (1): Chemical composition of experimental ingredient .

Ingredient	CFM	Corn silage	Rice straw	Pomegranate peel
Analyzed composition %				
DM	89.83	30.24	89.54	87.52
OM	94.59	94.56	92.45	94.34
CP	16.21	8.03	3.41	3.58
CF	6.84	23.52	30.63	12.16
EE	2.71	2.49	1.08	1.87
NFE	68.83	60.52	57.33	76.73
Ash	5.41	5.44	7.55	5.66
Net energy of lactation (M cal/kg DM)	0.59	0.62	0.58	0.58
Pomegranate-peel extract, mg TP/g DM				
Total phenolics (TP)				74.33
Saponins				33.76
Total tannins				52.19

Feed intake and digestibility (sheep digestibility trials).

Data presented in Table (2) showed that the differences in feed intake were significant (P<0.05) , being the highest values were recorded with animals fed ration containing 2% pomegranate , recording 414.10 ,237.28 and 1190.36 kg corn silage , rice straw and total feed intake , respectively. The corresponding values of intake were decreased as the level of pomegranate peel was increasing.

Results obtained in Table (2) showed that the pomegranate peel with rate of 2% significantly (P<0.05) increased DM, OM, CP, CF , EE and NFE digestibilies . The digestibility of all nutrients . DM, OM, CP, CF, EE and NFE, being the highest with 2% of pomegranate peel supplementation compared with other levels of pomegranate peel supplementation then gradually decreased as the level of pomegranate peel was increase . Jami et al.(2012) showed that using 1% and 4% pomegranate peel extract improved DM, CP, and NDF digestibility of dairy cows. High concentrations of hydrolysable tannins might reduce the digestibility of nutrients particularly proteins (Abarghuei etal.,2010 and Reed,1995). Tannins in pomegranate peel extract have both adverse and beneficial effects in ruminants (Mueller-Harvey, 2006). High concentrations of hydrolyzable tannins could decrease feed intake, digestibility of CP , NDF, OM and animal performance through their negative effects on palatability and digestion (Broderick et al., 1991; Reed, 1995).

Oliveira et al.(2010) found that feeding a pomegranate extract to young calves for the first 70 d of life suppressed the intake of grain and the digestibility of fat and protein, likely because of the high tannin content. It should be noticed that although polyphenolic compounds might improve animal health, they can also decrease proteolytic activity and thus compromise protein digestion (Broderick et al., 1991). Therefore, potential benefits of added pomegranate components on cows health and production should be considered relative to the potential decline in nutrient digestion .

Table (2). Effect of different levels of pomegranate peel supplementation on feed intake , digestibility coefficient and nutritive values .

	Control	Pomegranate peel level				
		2 %	3 %	4 %	SEM	*P*-Value
		Feed intake, (g/h/d)				
CFM	538.98	538.98	538.98	538.98	0.0	0.0
Corn silage	350.16[b]	414.10[a]	359.29[b]	301.44[c]	21.16	0.059
Rice straw	223.85[ab]	237.28[a]	219.37[b]	196.99[c]	9.62	0.026
Total feed intake	1112.99[b]	1190.36[a]	1117.65[b]	1037.41[c]	32.93	0.018
		Digestibility (%)				
DM	59.42[b]	63.68[a]	57.69[b]	54.56[c]	1.69	0.001
OM	62.78[b]	66.48[a]	61.20[b]	58.45[c]	2.06	0.018
CP	65.84[b]	69.06[a]	63.79[b]	60.59[c]	2.47	0.004
CF	59.66[b]	65.40[a]	57.66[b]	52.41[c]	1.79	<0.001
EE	69.47[b]	73.88[a]	66.07[c]	62.27[d]	2.13	<0.001
NFE	58.11[c]	66.07[a]	61.51[b]	59.45[bc]	2.06	0.011
		Nutritive values, (%)				
TDN, %	61.12[b]	64.73[a]	59.54[b]	56.86[c]	1.51	0.005
DCP, %	6.83[b]	7.47[a]	7.06[b]	6.91[c]	0.07	<0.001

[a,b,c,d:] *means in the same row followed by different superscripts are significantly (P<0.05) different*

Feeding values expressed as TDN and DCP (Table 2) revealed that, ration containing 2% of pomegranate peel supplementation had significantly (P<0.05) higher feeding values as TDN and DCP , recording 64.73 and 7.47%, respectively compared with the other treatments..

Rumen parameters.

Ruminal pH , NH3-N concentration and Total VFA concentration were found lower values with increase levels of pomegranate peel compared with the control ration.(table 3).These results are agreement with those obtained by Williams and Coleman,(1991) who found that reduced ruminalNH3–N concentrations were typical when protozoa are inhibited presumably as a result of depressed bacteriallysis Hristov et al.,(1999). Belancheetal. et al. (2012) noted that the protozoa of Entodinium sp.were responsible for most ruminal bacterial breakdown Abarghuei et al (2013) found suppressing Entodinium by pomegranate peel extract addition may have led to the decrease ruminal NH3–N concentration. Also, concentration of NH3–N in the ruminal fluid is influenced by ammonia up take by ruminal microorganisms Agle et al.,(2010),suggested that another explanation for the reduced NH3–N concentration is an overall increase in microbial protein synthesis

Table (3). Effect of different levels of pomegranate peel supplementation on rumen parameters of sheep.

Item	Control	Pomegranate peel				
		2 %	3 %	4 %	SEM	*P*-Value
PH	6.55	6.40	6.31	6.25	0.28	0.527
NH3-N (mg/100mlR.L)	13.67 a	12.95b	12.41bc	11.85 c	0.49	0.023
Total VFA (meq/100 mlR.L)	11.33 a	10.10 b	9.99 b	9.84 b	0.33	0.046
Acetate (mol/100 mol)	54.64	54.02	53.97	53.79	1.36	0.617
Propionate (mol/100 mol)	25.67	25.54	25.42	25.36	0.63	0.788
Butyrate (mol/100 mol)	8.03	8.54	8.57	8.63	0.79	0.931

[a,b,c:] *means in the same row followed by different superscripts are significantly (P<0.05) different*

As VFAs are the end products of rumen microbial fermentation, and represent the main supply of energy for the ruminant (Van Soest, 1994), a reduction in their production would be nutritionally unfavorable for the animal. The addition of pomegranate peel extract had no effect on total and individual VFAs and acetate to propionate ratio, which is probably due to the lack of significant effect on DMI (Boudon et al., 2007).

Milk production and its composition

The total daily intake with level 2% of pomegranate peel supplementation was significantly(P<0.05) higher than other levels of pomegranate peel and control ration as shown in Table (4). Also, differences in milk yield fat. 4% FCM , TS ,SNF and protein were significant higher with level 2% of pomegranate peel than other levels of pomegranate peel and control ration as shown in Table (4).

Table (4). Effect of different levels of pomegranate peel supplementation on feed intake, milk yield and its constituents of lactating cows.

Item	Control	Pomegranate peel				
		2 %	3 %	4 %	SEM	*P*-Value
Body weight of cows, kg	537.25	532.75	540	535.50	27.55	0.738
Daily feed intake (as DM),kg						
CFM	5.84	5.84	5.84	5.84		
Corn silage	5.83 ^{b}	6.17 ^{a}	5.86 ^{b}	5.62 ^{b}	0.18	0.037
Rice straw	2.91 ^{b}	3.18 ^{a}	2.82 ^{b}	2.46 ^{c}	0.07	0.031
Total daily feed intake, (kg/h/d)	14.58 ^{b}	15.19 ^{a}	14.16^{bc}	13.92 ^{c}	0.23	0.013
CFM:R Ratio	40:60	38.4:61.6	41.2:58.8	42:58		
Production (kg/ day) :						
Milk yield	13.61 ^{b}	14.18 ^{a}	13.50 ^{b}	12.23 ^{c}	0.41	0.001
4% FCM	11.65 ^{b}	12.78 ^{a}	11.54 ^{b}	10.38 ^{c}	0.37	0.001
Fat	0.414 ^{b}	0.474 ^{a}	0.409 ^{b}	0.366 ^{c}	0.01	0.017
Protein	0.412 ^{b}	0.462 ^{a}	0.402 ^{b}	0.353 ^{c}	0.02	0.021
Milk composition (%):						
Total solids	10.47	10.75	10.41	10.39	0.46	0.647
Solid not fat	7.43	7.41	7.38	7.40	0.09	0.908
Fat	3.04^{b}	3.34^{a}	3.03^{b}	2.99^{b}	0.11	0.039
Protein	3.03^{b}	3.26 ^{a}	2.98 ^{b}	2.89 ^{b}	0.10	0.021
Lactose	3.62 ^{a}	3.34^{b}	3.55 ^{a}	3.67 ^{a}	0.12	0.042
Ash	0.78	0.81	0.82	0.85	0.08	0.744

$^{a,b. c:}$ *means in the same row followed by different superscripts are significantly (P<0.05) different.*

Milk yield and 4% FCM yields were not changed by Cinnamaldehyde, queb- racho condensed tannin and Yucca schidigera saponin extracts (Benchaar etal.,2008). In contrast in another study Jami et al. (2012) reported that using 4% pomegranate peel extract as DM in dairy cows diet increased milk production.. The increased daily milk protein yield in cows fed pomegranate peel extract (particularlyPPE800) may be due to an increase in the flow of microbial protein to the intestine, benefiting the cows by increasing the amount of amino acids available for absorption (Makkar,2003).

Blood serum parameters:

Data presented in Table (5) illustrated that total protein, albumin , globulin and glucose were significantly (P<0.05) high increased with animal fed 2% level of pomegranate peel then other groups . The increase in blood serum total protein , albumin and globulin with addition pomegranate peel may be due to increase in protein synthesis and digestion of protein as shown in Table (2). The increase of blood serum globulin concentration with increased levels of pomegranate peel inclusion which was observed in the present study may be an indication of increased immunity in the dairy cows because the liver will be able to synthesize enough globulins for immunologic action. At the same time , significant reduction of AG ratio , total Lipids, cholesterol, triglyerides, HDL, LDL,AST,ALT, Urea and creatinine with increase levels of pomegranate peel were observed as shown in Table (5).

Data in Table (6) illustrated that total antioxidant capacity , CAT ,GPx and SOD were significantly (P<0.05) higher with increas levels of pomegranate peel than control ration as shown in table (6). Generally, using 2% pomegranate peel in ration formulation of lactating Friesian cows tended to higher digestibility coefficients , feeding values , milk either actual yield or 4% FCM yield .Moreover ,feeding pomegranate had no adverse effect on some rumen parameters and blood measurements.

Table (5). Effect of different levels of pomegranate peel supplementation on plasma biochemical parameters of lactating cows.

Item	Control	Pomegranatepeel			SEM	
		2 %	3 %	4 %	*P*-Value	
Total Protein, g/dl	7.23^{c}	8.33^{a}	8.12^{a}	7.83^{b}	0.22	0.001
Albumin, g/dl	4.17^{b}	4.48^{a}	4.43^{a}	4.18^{b}	0.08	0.004
Globulin conc., g/dl	3.06^{c}	3.85^{a}	3.69^{b}	3.65^{b}	0.03	0.001
AG ratio	1.36^{a}	1.18^{b}	1.20^{b}	1.15^{b}	0.07	0.016
Glucose, mg/dl	66.04^{b}	71.45^{a}	70.15^{a}	70.19^{a}	1.27	0.002
Total Lipids. mg/dl	274.70^{a}	226.78^{b}	210.58^{bc}	193.78^{c}	16.33	0.031
Cholesterol, mg/dl	182.47^{a}	146.67^{b}	127.38^{c}	114.23^{d}	9.37	<0.001
Triglyerides, mg/dl	90.03^{a}	72.74^{b}	70.00^{bc}	68.66^{c}	2.69	0.001
HDL-c, mg/dl	93.97^{a}	86.92^{b}	84.79^{b}	81.93^{c}	2.37	0.002
LDL-c, mg/dl	36.61^{a}	29.28^{b}	27.71^{b}	22.42^{c}	3.04	0.001
AST, IU/L	47.30	47.04	46.01	45.95	2.18	0.629
ALT IU/L	20.28	20.03	20.07	20.06	0.73	0.816
Urea mg/dl	23.13^{a}	20.09^{b}	20.38^{b}	20.39^{b}	0.47	0.015
Creatinine mg/dl	0.85	0.83	0.87	0.87	0.06	0.628

$^{a,b.c,d:}$ *means in the same row followed by different superscripts are significantly (P<0.05) different.*

Table (6). Effect of different levels of pomegranate peel supplementation on plasma antioxidant activity of lactating cows.

Item	Control	Pomegranate peel			SEM	*P*-Value
		2 %	3 %	4 %		
MDA mg/dl	25.79^{a}	20.41^{b}	18.85^{bc}	17.11^{c}	1.52	0.018
Total antioxidant capacity (U/mL)	90.04^{c}	113.90^{b}	118.41^{a}	120.38^{a}	2.61	0.005
CAT (U/L)	5.37^{b}	7.65^{a}	7.82^{a}	7.99^{a}	0.38	0.009
GPx (U/L)	2.72^{b}	3.95^{a}	4.05^{a}	4.12^{a}	0.19	0.016
SOD (U/L)	7.32^{b}	9.23^{a}	9.86^{a}	10.41^{a}	1.21	0.024

$^{a,b,c:}$ *means in the same row followed by different superscripts are significantly (P<0.05) different.*
CAT (Catalase enzyme). GPx (Glutathion peroxidase enzyme).SOD(Super oxide dismutase enzyme)

***Effect of humoral immune response**:*

Measurement of The IgG antibody titers in serum of cows fed on pomegranate peel extract was elevated significantly with cows fed on 2% and 3% pomegranate peel extract respectively in comparison with control . While there was no significant increase in igg antibody titers in milk whey samples of any of the pomegranate peel extract treated groups in comparison with control group (table 7). There was no significant change in nitric oxide values with 2% and 3% pomegranate peel extract comparing with control either in serum or milk whey samples whereas cows fed on 4% pomegranate peel extract showed significant increase in nitric oxide levels in both serum and milk whey samples comparing with the other groups table (7). Serum samples of Cows fed on 3% pomegranate peel extract showed significant increase in lysozyme levels in relation to group1 and 2% pomegranate peel extract . Milk whey samples showed negligible values of lysozyme contents in all tested groups table (7). The obtained results are supported by Yamasaki et al. (2006)who reported significant enhancement in IgG and IgM production in spleen lymphocytes of rats fed diet supplemented with pomegranate seed oils. Also Ahmed et al 2017 reported that dietary Punicagranatum L. By-products increased the weight of the spleen and bursa of Fabricius(the immune organs) as well as a significant increase in serum IgA and IgG concentration. Nitric oxide is generated during immune and inflammatory response, it is involved in innate immunity as a toxic agent towards infectious organisms and can induce or regulate death and function of host immune cells (coleman, 2001). our results of innate immune response showed that There was no significant change in nitric oxide values in 2% and 3% pomegranate peel extract comparing with control group1 either in serum or milk whey samples , these results were agree with those obtained by El-Sisi et al (2018) who found no significant change in nitric oxide values in rabbit tested groups fed on ppp1% and 1.5% or ppe 0.14% and 0.21%. Lysozyme was a member of innate humoral factors that elaborated from polymorph nuclear and mononuclear cells (moore et al., 2006).

Related to the results of lysozyme Serum samples of Cows fed on 3% pomegranate peel extract 3% showed significant increase in lysozyme levels in relation to control and 2% pomegranate peel extract . The obtained results are supported by Harikrishnan et al (2010) who suggested that intraperitoneal administration of the leaf extracts of P.granatum clearly enhance the immune responses. Also it was recorded that PPE supplementation increase the lysozyme values in broilers Kishawy et al (2016) and rabbits El-Sisi et al (2018). On the other side, Milk whey samples showed negligible values of lysozyme contents in all tested groups. these results are coincided with reported by yang et al (2011)who founded only trace amounts of lysozyme in cow milk . other studies estimated the lysozyme activity in bovine milk showing that lysozyme levels are definitely higher in colostrum and mastitis milk than in normal milk Goudswaard et al (1978).

Table (7): Effect of different levels of pomegranate peel supplementation of immune response .

Item	Control	Pomegranate peel levels		
		2%	3%	4%
Bovine IgG				
Serum	608.25 ± 23.80^{a}	832.75 ± 31.38^{ab}	1017.00 ± 49.65^{abc}	677.50 ± 7.77^{bc}
Milk whey	224.75 ± 15.43	248.50 ± 30.69	290.75 ± 28.47	231.00 ± 20.55
Nitric oxide umol/ml				
Serum	4.70 ± 0.10 ^{a}	4.70 ± 0.10 ^{b}	5.00 ± 0.24	5.62 ± 0.33 ^{ab}
Milk whey	4.90 ± 0.25 ^{a}	5.50 ± 0.34 ^{b}	5.63 ± 0.13 ^{c}	6.85 ± 0.46 ^{abc}
Lysozyme activity				
Serum	39.27 ± 7.25^{a}	46.51 ± 7.25^{b}	88.78 ± 6.00^{ab}	65.86 ± 16.92
Milk whey	-	-	-	-

.$^{a,b,c:}$ *means in the same row followed by different superscripts are significantly (P<0.05) different*

CONCLUSION

Form those results, it can be use 2% pomegranate peel in ration formulation of Friesian cows to improve digestibility and feeding values beside increased milk yield with no adverse effect on animals.

REFERENCES

AOAC, (1995). Official Methods of analysis. 15th Ed. Association of Analytical Chemists, Washington, DC.

Abarghuei, M.J., Y. Rouzbehan and D. Alipour (2010). The influence of the grape pomace on the ruminal parameters of sheep. Livest.Sci.132, 73–79.

Abarghuei, M.J., Y. Rouzbehan,n, A.Z.M. Salem and M.J. Zamiri (2013) Nutrient digestion, ruminal fermentation and performance of dairy cows fed pomegranate peel extract LivestockScience157,452–461

Agle, M., A.N. Hristov, S. Zaman, C. Schneider, P. Ndegwa and V.K. Vaddella (2010). The effects of ruminally degraded proteinon rumen fermentation and ammonia losses from manure in dairy cows.J.Dairy Sci.93, 1625–1637.

Ahmed, S. T. and C. J. Yang (2017). Effects of dietary Punicagranatum L. By-products on performance, immunity, intestinal and fecal microbiology, and odorous gas emissions from excreta in broilers. The Journal of Poultry Science, 0160116.

Al-Rabbat, M.F.; R.L. Baldwin and W.C. Weir (1971). In vitro nitrogen-tracer technique for some kinetic measures of rumen ammonia. J. Dairy Sci., 54:150.

Akhtar, S., T. Ismail, D. Fraternale and P. Sestili,(2015) "Pomegranate peel and peel extracts: Chemistry and food features," Food Chemistry, 174: 417-425,

Atkinson, R.L., C.D. Toone and P.A. Ludden (2007). Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on site and extent of digestion and ruminal characteristics in lambs fed low-quality forage. J.Anim Sci. 85, 3322-3330.

Belanche, A.,DeLaFuente,G.,Moorby,J.M.,Newbold,C.J.,(2012).Bacterial protein degradation by different rumen protozoal groups. J. Anim. Sci., 90: 4495–4504.

Benchaar, C.,McAllister,T.A.,Chouinard,P.Y.,(2008).Digestion,ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin,or Yucca schidigera saponin extract.J.DairySci.91,4765–4777.

Boudon,A.,Faverdin,P.,Delagarde,R.,Lamberton,P.,Peyraud,J.L.,(2007). Effects of rumen or duodenal glucose infusions on intake in dairy cows fed fresh perennial Ryegrass in doors.J.DairySci.90,4397–4410.

Broderick, G. A., R. J. Wallace, and E. R. Orskov. (1991). Control of rate and extent of protein degradation. Pages 541–592 in Physiological Aspects of Digestion and Metabolism in Ruminants, Proc. 7th Int. Symp. Ruminant Physiol. T. Tsuda, Y. Sasaki, and R. Kawashima, ed. Academic Press Inc., San Diego, CA.

Caldas Neto, S.F., Zeoula, L.M., Prado, I.N.d., Branco, A.F., Kazama, R., Geron, L.J.V., Maeda, E.M., and Fereli, F. (2008). Proteína degradável no rúmen na dieta de bovinos: digestibilidades total e parcial dos nutrientes e parâmetros ruminais. Revista Brasileira de Zootecnia 37,1094-1102.

Coleman, J. W. (2001). Nitric oxide in immunity and inflammation. International immunopharmacology, 1(8), 1397-1406.

Doumas, B. T.; W. A. waston and H. G. Biggs (1977). Albumin standards and the measurements of serum albumin with bromocresol green. Clin.Chem. Acta. 31: 87-96.

El-Sisi , A.F., Bahakim,A.S.A.,Fatma,E.Saba and Osman,A.O. (2018). Assessment of dietary supplementation with Pomegranate Peel Powder or its extract on productive performance and immune status of rabbits. Animal health research journal vol.6,No.2, pp.51-62.

Jami, E.,Shabtay,A.,Nikbachat,M.,Yosef,E.,Miron,J.,Mizrahi,I.,(2012). Effects of adding a concentrated pomegranate-residue extract to the ration of lactating cows on in vivo digestibility and profile of rumen bacterial population. J.Dairy Sci.95,5996–6005.

Galatov, A.N. (1994).The effect of crossbreeding on milk production of fine wool ewes. Animal Breed Abst., 62: 211.

Gaines, W.L. (1923). Relation between percentage of fat content and yield of milk. 1. Correction of milk yield for fat content. Agric. Exo. Sta. Bull. 245 (C.F. Gaines, 1928).

Goudswaard, J., & Bakker-de, E. K. (1978). Lysozyme and its presence in bovine milk and serum. Tijdschriftvoordiergeneeskunde, 103 (8), 445-450.

Gracious RR, Selvasubramanian S and Jayasundar S. (2001). Immunomodulatory activity of Punicagranatum in rabbits- a preliminary study. Journal of Ethnopharmacology, 78: 85-87.

Green, L.C, Wagner, D.A, Glogowski, J, Skipper, P.L, Wishonk, J.S and Tannebaum, S.R. (1982); Anal. Biochem. 126 (1): 131-8.

Harikrishnan, R., Heo, J., Balasundaram, C., Kim, M. C., Kim, J. S., Han, Y. J., &Heo, M. S. (2010). Effect of Punicagranatum solvent extracts on immune system and disease resistance in Paralichthysolivaceus against lymphocystis disease virus (LDV). Fish & shellfish immunology, 29(4), 668-673.

Hristov,A.N.,McAllister,T.A.,VanHerk,F.H.,Cheng,K.J.,Newbold,C.J., Cheeke, P.R.,(1999).Effect of Yucca schidigera on ruminal fermentation and nutrient digestion in heifers.J.Anim.Sci.77,2554–2563.

Henry, J. B. and S. D. Todd (1974). Clinical Diagnosis and Measurement by Laboratory Methods. 16th Ed., W. B. Saunders and Co., Philadelphia., PA. P 260.

Henry, R. J.; D. C. Cannon and J, W. Winkelman (1974). Clinical chemistry: Principles and Techniques, 11th Ed., Happer and Row Publishers PP. 1629.

Kishawy, A. T., Omar, A. E., & Gomaa, A. M. (2016). Growth performance and immunity of broilers fed rancid oil diets that supplemented with pomegranate peel extract and sage oil. Japanese Journal of Veterinary Research, 64(Supplement 2), S31-S38.

Li Y, C Guo, Yang J, Wei J, Xu J, and Cheng S. (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96:254-260.

Liu, H.; Li, K.; Mingbin, L.; Zhao, J. and Xiong, B.(2016) Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs. Meat Sci., 116, 236–242

Li Y, Guo C, Yang J, Wei J, Xu J and Cheng S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry, 96: 254-260

Liz, K., Lopez, K. and J.K. Getty, 2013. Natural antioxidants in meat and poultry products. Meat Science, 94, 220–227

Manuel Viuda-Martos JA, Pérez-álvarez ES, and Fernández-López J. (2013) In vitro antioxidant properties of pomegranate (Punica granatum) peel powder extract obtained as co-product in the juice extraction process. J. Food Proc. Pres.; 37(5): 772-776.

Maynard, L. A.; U. K., Loosli; H. F. Hintz and R. G. Warner (1978).Animal Nutrition.(7th Ed.)Mcgraw. Hill Book Co., New York

Makkar, H.P.S.,2003.Effects and fate tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds.SmallRumin.Res.49,241–256.

Makkar, H.P.S., Barowy, N.K., Becker, K., and Degen, A. (1995). Some problems in fibre determination of a tanninrich forage (Acacia saligna leaves) and their implications in in vitro. Anim. Feed Sci. Technol. 55, 67-76.

Mirzaei-Aghsaghali A, Maheri-Sis N, Mansouri H, Razeghi ME, Mirza-Aghazadeh A, Cheraghi H, and Aghajanzadeh-Golshani A. (2011) Evaluating potential nutritive value of pomegranate processing by-products for ruminants using in vitro gas production technique. ARPN J. Agric. Biol. Sci. 6 (6): 45-51

Moore, M. N., Allen, J. I., &mcveigh, A. (2006). Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Marine Environmental Research, 61(3), 278-304.

Mueller-Harvey,I.,2006. Review,unraveling the conundrum of tannins in animal nutrition and health.J.Sci.FoodAgric.86,2010–2037.

Negi PS, and Jayaprakasha GK. (2003) Antioxidant and antibacterial activities of Punica granatum peel extracts. J. Food Sci. 68 (4), 1473-1477.

NRC,National Research Consul.(2001). Nutrient requirement of Domestic Animal. The Nutrition requirements of Dairy Cattle .7th rev .ed. National Academy press .Washington ,DC.

Oliveira, R. A., C. D. Narciso, R. S. Bisinotto, M. C. Perdomo, M. A. Ballou, M. Dreher, and J. E. P. Santos. (2010). Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immunocompetence of calves. J. Dairy Sci. 93:4280–4291

Pantha, B. (1982). The use of soybean in practical feeds for Tilapia nilotica. M. Sc., Thesis University of Sterling, UK

Patra AK, and Saxena J. (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric.; 91: 24-37.

Peeters, T. L., &Vantrappen, G. R. (1977). Factors influencing lysozyme determinations by the lysoplate method. ClinicaChimica Acta, 74(3), 217-225.

Rajaraman, V., Nonnecke, B. J., Franklin, S. T., Hammell, D. C., & Horst, R. L. (1998). Effect of vitamins A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. Journal of dairy science, 81(12), 3278-3285.

Reed, J.D. (1995) nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73: 1516-1528.

Reitman, S. and S. A. Frankel (1957).Colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyrovic transaminase. Am. J. Clin. Pathol. 28: 56-63.

Santos MBGA, Naderc PH, Robinsona D, Kirand U, and Krishnamoorthy GMJ. (2010) Impact of simulated field drying on in vitro gas production and voluntary dry matter intake of rice straw, Anim. Feed Sci. and Technol., 159: 96-104.

SAS (2000).SAS/STAT. user's guide for personal computer.Release 6.12. Statistics, SAS Inst.,Inc.,Cary N.C., USA.

Sakhawat, I. (2011). The effect of silage quality on gross energy losses. Degree project 360, 30 Credit A2E-level. Swedish University of Agricultural Sciences.

Singh R.P., Jayaprakasha G.K., Sakariah K.K. 2001. A process for the extraction of antioxidants from pomegranate peels. Submitted for Indian Patent No. 392/Del/01, 29 March 2001.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, USA.

Williams, A.G., Coleman, G.S., 1991. The Rumen Protozoa. Springer-Verlag, New York Inc., New York

Warner, A. (1964). Production of volatile fatty acids in the rumen, methods of measurement. Nutr. Abst.And Rev., 34: 339.

Whitley A.C., Stoner G.D., Darby M.V., Walle T. 2003. Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid–extensive binding to protein and DNA. Biochem. Pharmacol., 66: 907-915. http://dx.doi.org/10.1016/S0006- 2952(03)00413-1

Yamasaki M, Kitagawab T, Koyanagi N, Chujo H, Maeda H, Kohno-Murase J, Imamura J, Tachibana H and Yamada K. (2006). Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. Nutrition, 22: 54-59.

Yang, B., Wang, J., Tang, B., Liu, Y., Guo, C., Yang, P., ... & Dai, Y. (2011). Characterization of bioactive recombinant human lysozyme expressed in milk of cloned transgenic cattle. Plos one, 6(3), e17593.

تأثير إضافة قشر الرمان (بونيكا جرانيتم) للعليقة على الآداء الإنتاجى والحالة المناعية للأبقار الحلابة.

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أجريت هذة الدراسة لمعرفة تأثير إضافة مستويات مختلفة من قشور الرمان (2،3، 4%) لعلائق الأبقار الحلابه على انتاج اللبن والتركيب الكيماوى ومعاملات الهضم ومعدل تحلل الماده الجافه والبروتين بالكرش و بعض مكونات الدم والمناعة للابقار الحلابة . وقد استخدم 16 بقرة فريزيان خليط حلابة فى الموسم الثالث أو الرابع بتصميم المربع اللاتينى وقسمت الى اربعة مجموعات كل مجموعة تحتوى على 4 بقرات وقد غذيت المجاميع على العلائق التالية 1- عليقة مقارنة (40% علف مركز + 40% سيلاج اذرة + 20% قش الارز بدون اضافة قشور رمان) 2- عليقة مقارنة +2% قشور رمان .3- عليقة مقارنة +3% قشور رمان.4 - عليقة مقارنة +4% قشور رمان. وقد أظهرت النتائج ما يلى:

ان العليقة المحتوية على مستوى 2% من قشور الرمان ادت الى تحسن فى معاملات الهضم والقيمة الغذائية وميزان الأزوت مع ارتفاع فى تركيز الأمونيا و الأحماض الدهنية الطيارة بالكرش مع زيادة فى معدل تحلل كلا من المادة الجافة والبروتين بالكرش مقارنة بنسب مستويات الاضافات الأخرى من قشور الرمان . وقد سجلت العليقة المحتوية على مستوى 2% من قشور الرمان زيادة فى معدل انتاج اللبن مع تحسين جودة اللبن مع الحصول على جودة واستساغة عالية عند تصنيع اللبنة وقد سجلت ايضا أعلى فى الكفاءة الأقتصادية وكانت نتائج قياسات الدم المتحصل عليها فى المعدلات الطبيعية ودون اى تأثير صحى سلبى على الحيوانات . وقد اظهرت العلائق المحتوية على مستوى 2و 3% من قشور الرمان الى زيادة فى الاجسام المناعية فى سيرم الدم بينما لم يلاحظ اى زيادة معنوية للاجسام المناعية فى عينات اللبن فى العلائق المختبرة مقارنة بعليقة الكنترول .

ونستخلص هذة الدراسة الى انة يمكن استخدام قشور الرمان بمستوى 2% لعلائق الأبقار الحلابة لتحسين القيمة الغذائية ة والاداء الانتاجى للابقار الحلابة دون حدوث أى ضرر على انتاجية وصحة الحيوانات على ان يوصى بمزيد من الدراسات على المدى الطويل على حيوانات اللبن لتأكيد النتائج المتحصل عليها فى الدراسة الحالية.