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EFFECT OF USING POMEGRANATE (*PUNICA GRANATUM L.*) PEELS AND IT'S EXTRACT ON PRODUCTIVE PERFORMANCE AND MILK YIELD OF DOES RABBIT

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ABSTRACT :A total number of 25 V-line rabbit does aged 8-9 months and weighed 3381 g ±5.575 were used in this study. Animals were distributed randomly into five experimental groups of 5 does each. The first group fed the basal diet *ad libitum* and served as a control. The second and third groups were fed the basal diet supplemented with 1.0 and 1.5% pomegranate peel powder (PPP) in the diet, respectively. The fourth and fifth groups were fed the basal diet supplemented with 1.0 and 1.5% pomegranate peel extract (PPE) in the drinking water, respectively. The results revealed that does supplemented with PPE at different levels significantly consumed (P≤0.05) more feed compared with does supplemented with different levels of PPP or control group during experimental period. Pregnant rabbit does supplemented with 1% PPP recorded (P≤0.05) more litter size at day 28th (at weaning age) than control group. Using dietary PPP or drinking PPE at different levels for pregnant rabbit does showed significantly (P≤0.05) higher litter weights at birth and at 7th, 14th and 21st days of lactation period and at weaning compared to control does. Adding 1.5% PPP or 1% PPE for pregnant rabbits had significant (P≤0.05) lower pre- weaning mortality rate compared to other treatments and the control group. Adding PPP or PPE for pregnant rabbit had significant (P≤0.05) increasing milk yield at all studied ages compared to control does. From the present study, it could be concluded that using powder pomegranate peel in diet or it's extract in drinking water had a positive effect on V-line does performance which reflected in litter traits (litter size and litter weight), pre weaning mortality rate and milk yield.

Keywords: Pomegranate peel, rabbits, productive performance and milk yield.

INTRODUCTION

Pomegranate (*Punica granatum L.*) is an important fruit crop in Egypt, which cultivated 13021 hectares produced 64573 ton annually (According to the statistics of the Ministry of Agriculture, 2011). Increasing agro-industrial units for producing pomegranate juice has led to increase by-products including peels and seeds. These processes have led to production of high quantities of pomegranate by-product biomass. Fresh pomegranate biomass contains high levels of moisture and soluble sugars (Shabtay *et al.,* 2008). If it cannot use by farmers and industries as well as medical activities cause serious environmental problems. Pomegranate is one of the oldest known drugs. It is mentioned in the Ebers papyrus of Egypt written in about 1550 BC (Ross 1999). Moreover, it has been reported to have antimicrobial activity against a range of Gram positive and negative bacteria (McCarrell *et al.,* 2008).

Pomegranate peel (PP) had the highest antioxidant activity in the peel (Li *et al.,* 2006), especially, the most synthetic antioxidants that have been restricted recently, mainly because of their possible carcinogenetic effect (Mhdavi and Salunkhe, 1995). Pomegranate is an important source of bioactive compounds and has been used for folk medicine for many centuries. Pomegranate attracts attention due to its apparent wound-healing properties (Chidambara *et al.,* 2004), immunomodulatory activity (Gracious *et al.,* 2001), antibacterial activity (Navarro *et al.,* 1996) and antiatherosclerotic and antioxidative capacities (Tzulker *et al.,* 2007). Antioxidative activity has often been associated with decreased risk of various diseases and mortality (Huxley and Neil, 2003). Azoz and Basyony (2012) indicated that the addition of pomegranate dried waste at level of 5.0 or 1.0% to New Zealand white does diet during pregnancy and lactation periods improved their milk production and most of the reproductive performance. Zeweil and El-Gindy (2016) showed that the inclusion of PP quadratically affected litter size at birth and weaning age, as well as milk yield.

Therefore, the current study aimed to investigate the effect of using pomegranate peel extract via oral treatment or involving pomegranate peel powder in experimental diets on productive performance and milk yield of V-line does rabbit.

MATERIALS AND METHODS

This experiment was carried out in the rabbitry of El-Gemeza research station, El-Gharbia Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agricultural, Egypt.

Experimental animals:

A total number of 25 V-line, nulliparous, rabbit does aged 8-9 months and weighed 3381 g±5.575 were divided and assigned randomly into five experimental groups of 5 doe rabbits in each. Five experimental diets were formulated to cover all essential nutrient requirements for rabbit does according to NRC (1994). The first group was fed the basal diet ad libitum and served as a control, while, the second and third groups were fed the basal diet supplemented with 1.0 and 1.5% pomegranate peel powder in the diet, respectively. Whereas, the fourth and fifth groups were fed the basal diet supplemented with 1.0 and 1.5% pomegranate peel extract in the drinking water, respectively. Table 1 shows the

formulation and nutrient composition of these diets.

Preparation of tested material

Powder of pomegranate peel (PP) used in this research was obtained from local market, then fresh peel was washed with water and cut into little pieces then sun dried and ground. While, an equal extract of the pomegranate peel prepared by mashing in a proportion of 1: 2: 2 (W peel/v water/v methanol) and left for about 48 hrs in refrigerator. The extract was filtered to remove the peel particles then concentrated under vacuum at 40: 50°C to get a concentrate, and stored at 3 to 4°C until used.

Managements:

Sex ratio was 5 female to 1 male throughout the experiment. A cycle of 16 hours of light and 8 hours of dark were used throughout the experiment. All does were kept under the same managerial conditions. Fourteen days after mating, the does were tested for pregnancy by abdominal palpation. Non-pregnant rabbit does were re-mated. Animals were housed in individual cages provided with feeders, automatic nipple drinkers and nest boxes. The building was open air with electric exhaust fans on the sides. All rabbits were kept under the same managerial, environmental and hygienic conditions.

Does studied traits:

Doe weight at mating, doe total feed intake from mating to palpation, doe total feed intake from palpation to parturition, total feed intake from mating to parturition, total litter size at birth, still birth (%), live litter size at birth, litter weight at birth (g), mean bunny weight at birth (g), litter size at weaning, litter weight at weaning (g), mean bunny weight at weaning (g) and pre weaning mortality (%) were recorded.

Milk yield evaluation:

Litters were standardized to eight pups on the day of birth and were separated from their mothers. They were allowed to enter the nest and suck once daily in the morning (10 min at maximum) (Parigi-Bini *et al*., 1996). Milk yield was recorded daily for the entire lactation period by weighing the doe and litter immediately before and after suckling. The increase in pups weight was indicated as the milk yield.

Statistical analysis:

Data were subjected to one-way analysis of variance using SAS (2000). Differences among means were detected using Duncan's multiple range test (Duncan, 1955). The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis, and then actual means are presented. The following model was used:

$Y_{ij} = G + T_i + e_{ij}$.

Where, Y_{ij} = observation for each dependent variable; G = General mean;

T_i = Treatment effects (i = 1, 2... and 5);

e_{ij} = Random error.

RESULTS AND DISCUSSION

Live body weight and feed intake of does:

Body weight and feed intake of rabbit does as influenced by supplementation of pomegranate peel (PP) as a powder (PPP) form or extract (PPE) form are illustrated in Table 2. Adding PPP or PPW for rabbit does had no significant influence on each of final body weight and change of body weight. Concerning total feed intake, Rabbit does fed diet supplemented with PPE at different levels consumed significant ($P \leq 0.05$) more feed compared with does fed diet supplemented with different levels of PPP or control group during experimental period. The highest

values were recorded by does supplied with 1% PPE, while, the lowest values were recorded by does fed 1% PPP. In this respect, Azoz and Basyony (2012) found that rabbit does fed 0.5, 1.0 and 1.5% of pomegranate dried waste showed increase in total feed intake in comparison with those does fed the control diet during pregnancy period. The significant increase in feed intake for does treated with pomegranate dried waste may be due to pomegranate dried waste having antioxidant properties (Ghasemain *et al*., 2006) which delays the start or slow the rate of oxidation reaction in animal cell (Little and Gladen, 1999). Phenolic substances present in these fruit wastes are believed to be behind this effect. They have been implicated in increasing the antioxidative systems, acting as enzyme modulators and metal chelators (Butera *et al*., 2002 and Edenharder and Grunhage, 2003). These agents inhibit peroxidation reactions and significantly reduce the oxidative stress (Fuhrman and Aviram 2001 and Pari and Saravanan, 2002).

Litter size:

Results in Table (3) shows that adding PPP or PPE for pregnant rabbit does had no significant influence on litter size at birth. Also, there were insignificant differences due to adding PP at different forms and levels on litter size at 7^{th}, 14^{th} and 21^{st} days of lactation period. On the other hand, pregnant rabbit does fed 1% PPP recorded significantly ($P \leq 0.05$) increased litter size at 28^{th} (at weaning age) compared to control group.

Pre- weaning mortality rate:

Data concerning pre- weaning mortality rate from birth to weaning age are presented in Table 3, it was noted that adding 1.5% PPP or 1% PPE for pregnant rabbit does had significant ($P \leq 0.05$) lower pre- weaning mortality rate compared with other treatments and control groups. However, lower values in this respect were recorded by does fed 1% PPE, followed by does fed 1.5% PPP, while, the highest ($P \leq 0.05$) values of pre-weaning mortality rate from birth to weaning age were recorded by does drank 1.5% PPE and control group, respectively. In this respect, Azoz and Basyony (2012) indicated that pomegranate dried waste supplementation reduced mortality rate from birth to weaning age and this reduction may be due to the increase in the defense mechanism system in these treatments. Doe rabbits milk are contain colostrums is the very first secretion of the mammary glands. It is very nutritious and contains high levels of protein, globulins, fats, milk solids and vitamin A. Most important, it contains antibodies against diseases to which the doe has immunity. It is critical to feed colostrums for the first three days for maximum protection against disease because the absorption of these antibodies disappears after three days. Immunoglobulin among factors absorbed from milk that have potential for regulating the immune responses of rabbit neonates.

Dietary polyphenols have been reported to possess potent antioxidant activity by endogenous and exogenous mechanisms. Li *et al*., (2003) they found that the extract of pomegranate leaves abundant with tannins was demonstrated to be a good gastric protective agent, increase the activity of pepsin, improve the secretion of bile, enhance the intestine peristalsis, inhibit the secretion of gastric acid and dispel intestinal parasite by continual intestinal tract concentration. Besides, pomegranate extract inclusion significantly enhanced the growth of *Bifidobacterium breve* and *Bifidobacterium inantis* which conceder a good probiotic essential for

good health (Viuda-Martos *et al*., 2010). However, Zeweil and El-Gindy (2016) found that the inclusion of pomegranate peel had no effect on pre-weaning mortality (from birth to 28 d).

Litter weight and litter weight gain:

Results in Table (4) shows that dietary PPP or drinking PPE at different levels for pregnant rabbit does had the significantly (P≤0.05) higher litter weight at birth, at 7th, 14th and 21st days of lactation period and at weaning compared to control does. The highest (P≤0.05) values at birth were recorded for does drank 1.5% PPE compared to does fed 1% PPP or does drank 1.5% PPE, while, pregnant rabbit does fed 1.5% PPP or does drank 1% PPE recorded significant (P≤0.05) higher litter weight at weaning compared with does fed 1% PPP. However, mean litter weight at weaning was significant (P≤0.05) higher due to adding 1.5% PPP to diet and drinking PPE at different levels compared to control does.

Concerning the litter weight gain, adding 1.5% PPP to diet and drinking PPE at different levels for pregnant rabbit does gave significant (P≤0.05) higher litter weight gain from birth to the 7th days of lactation period compared to control does. The highest (P≤0.05) values were recorded by does drank 1% PPE compared to does fed 1 or 1.5% PPP. Moreover, adding PPP or PPE for pregnant rabbit does had significant (P≤0.05) increase litter weight gain from 21st to 28th days of lactation period compared to control does. In this respect, the highest (P≤0.05) values were recorded by does drank 1.5% PPE compared to does drank 1% PPE or does fed 1% PPP. These results agree with the findings of Azoz and Basyony (2012) who showed that rabbit does fed 1.5% pomegranate dried waste in their diet caused clear to increase (P≤0.01) in litter size at birth by about 40.86 % as compared with those does fed control diet. On the other hand, does fed 0.5 or 1.0% pomegranate dried waste in their diet resulted in a significant increase in litter size at birth as compared to the control group. However, there were significant effects among treatment group on litter weight at birth with pomegranate dried waste supplementation, this increase due to the increase the number of bunny at birth. Moreover, litter size and weight at weaning generally affected (P≤0.05) by pomegranate dried waste supplementations compared to control group. The rabbit does fed 1.0 or 1.5% pomegranate dried waste in their diet increased (P≤0.05) litter size at weaning by about 47.3 % in comparison with those fed control diet. On the other hand, rabbits doe fed 0.5% pomegranate dried waste in their diet resulted increased in a significant litter size at weaning (by about 38.7%) in comparison with those group fed the control diet (Azoz and Basyony, 2012). The clear improvement in performance of polyphenol with pomegranate dried waste treatments might be due to its protective action against lipid oxidation in the cell membrane (Liebler, 1992). Also, it was important for newborns, which exhibits a greater sensitivity to oxidative damage than adults, and for the development of the immune system in young animals (Debier *et al.*, 2005).

However, Azoz and Basyony (2012) showed that litter weight at weaning age for the rabbit does fed 0.5, 1.0 or 1.5% pomegranate dried waste levels in their diet had been increased (P≤0.05) in comparison with those group fed the control diet. These improvement back to the number of pups per doe, but the reduction in pups weight tend to that young

rabbits reared in larger litters have access to less milk, which leads to reduce weight gain (Szendro, 1999).Vicenye and Garcia-Ximenez (1992), also established that both the ability of the doe for milk production and competition between suckling rabbits limits the maximum expression of genetically determined ability for growth. Moreover, Zeweil and El-Gindy (2016) showed that the inclusion of pomegranate peel linearly and quadratically increased litter size at birth, throughout lactation and at weaning. However, litter weight at birth was not affected by pomegranate peel inclusion, leading to a reduction in kit weight at birth. This result may be due to a high number of fetuses in the uterus or the uterine horn (Szendro *et al*., 1996), or to the negative relationship between litter size and kit weights (Poigner *et al*., 2000). The inclusion of pomegranate peel increased total milk production linearly and quadratically and tended to increase litter weight quadratically at weaning. However, it tended to reduce the kit milk intake in most periods of lactation, leading to a linear reduction of individual kit weight at weaning. These results agree with the correlation found between rabbit milk production and traits such as litter size at weaning (Ayyat *et al*., 1995 and Di Meo *et al*., 2004), litter weight at weaning or mortality in lactation (Khalil *et al*., 2005; Al- Sobayil *et al*., 2005).

Milk yield:

As shown in Table 5, adding PPP or PPE for pregnant rabbit does had significant ($P \leq 0.05$) increase in milk yield at all studied ages compared to control does. Moreover, pregnant rabbit does drinking PPE at different levels recorded the highest values compared to those fed PPP at different levels at all studied ages. However, pregnant rabbit does drank 1.5% PPE had significant ($P \leq 0.05$) increased milk yield at 28^{th} day (at weaning age) compared to other treatments and control groups. In this respect, Azoz and Basyony (2012) found that doe rabbits fed basal ration supplemented with 1.0 and 1.5 pomegranate dried waste showed the best value of milk yield. The differences were not significant between control and 0.5% pomegranate dried waste treatments. However, 1.0 and 1.5% of pomegranate dried waste supplementation gave significant increased ($P \leq 0.01$) in milk yield from birth to 21 and 28 days of lactation. These results were similar to those of Marai *et al*. (1996).

From the present study, it could be concluded that using pomegranate peel in powder form by diet or in extract form by drinking water of doe rabbits improved the litter traits (litter size and litter weight), pre weaning mortality and milk yield of V-line doe rabbits.

Table (1): Compositions and calculated analysis of the experimental diets.

Ingredients	**(%)**
Yellow corn	19.55
Soy bean meal 44%	21.10
Alfalfa hay	25.85
Wheat bran	27.50
Di calcium phosphate	1.50
Limestone	0.75
Molasses	3.00
NaCl	0.30
Premix*	0.30
DLMethionine	0.10
Anticoccidia (Diclazuril)	0.05
Total	100
Chemical composition (%)	
Crud protein	18.21
Digestible energy (kcal/kg)	2554
Crud fiber	12.77
Ether extract	2.70
Calcium	1.10
Available phosphorus	0.34
Total Phosphorus	0.79
Lysine	0.99
Methionine	0.39
Methionine +Cystene	0.70
Na	0.16

*Supplied per 1 kg diet: 6000 IU vit. A; 900 IU, vit. D_3; 40 mg, vit. E; 2.0 mg, vit. K_3; 2.0 mg vit., B_1; 4.0 mg , vit. B_2; 2.0 mg, vit. B_6; 0.010 mg, vit. B_{12}; 5.0 mg, vit. PP; 10.0 mg vit., B_5; 0.05 mg, B_8; 3.0 mg, B_9; 250 mg, choline; 50.0 mg, Fe;50.0 mg, Zn; 8.5 mg Mn; 5.0 mg Cu; 0.20 mg I, and 0.01 mg Se.

Table (2): Body weight (g) and total feed consumption of does rabbit in experimental groups throughout three consecutive reproductive cycles.

Item	Experimental groups					SEM
	G1	G2	G3	G4	G5	
Initial body weight (g)	3383.50	3384.17	3381.33	3380.83	3376.83	5.575
Final body weight (g)	3835.33	3786.67	3793.33	3801.67	3800.00	25.092
Change in body weight (g)	451.83	402.50	412.00	420.83	423.17	25.796
Total feed consumption (g)	40667 [ab]	40500 [b]	41033 [b]	42500 [a]	42310 [a]	411.608

[a and b….] Means within each row having no similar letter(s) are significantly different ($P \leq 0.05$)
G1: Control G2: pomegranate peel (1%) G3: pomegranate peel (1.5%) G4: pomegranate peel extract (1%) G5**:** pomegranate peel extract (1.5%)**.**

Table (3): Litter size/doe and mortality rate (%) in different groups during suckling period.

Age (day)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
Litter size:						
1	7.17	7.50	8.00	7.83	8.33	0.370
7	6.00	6.33	7.00	6.67	6.50	0.348
14	5.33	5.50	6.17	6.17	5.83	0.298
21	4.83	5.17	5.67	5.50	5.50	0.327
28	4.50 [b]	5.00 [ab]	5.50 [a]	5.33 [ab]	5.17 [ab]	0.273
Mortality rate (%):						
1-28	36.81 [a]	32.74 [ab]	31.28 [b]	31.02 [b]	38.16 [a]	2.279

[a and b….] Means within each row having no similar letter(s) are significantly different ($P \leq 0.05$)
G1: Control G2: pomegranate peel (1%) G3: pomegranate peel (1.5%) G4: pomegranate peel extract (1%) G5**:** pomegranate peel extract (1.5%)**.**

Table (4): Litter weight and weight gain in different groups during suckling period.

Age (day)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
Litter weight (g):						
1	405.50 ^{c}	430.00 ^{b}	434.50 ^{ab}	426.00 ^{b}	441.50 ^{a}	3.114
7	632.50 ^{d}	665.50 ^{c}	713.83 ^{b}	756.50 ^{a}	746.83 ^{a}	10.611
14	1099.33 ^{b}	1237.17 ^{a}	1303.83 ^{a}	1318.50 ^{a}	1268.50 ^{a}	29.095
21	1657.00 ^{c}	1737.67 ^{b}	1797.50 ^{a}	1773.17 ^{ab}	1790.00 ^{ab}	17.293
28	1999.67 ^{c}	2266.83 ^{b}	2699.17 ^{a}	2650.67 ^{a}	2418.00 ^{ab}	126.146
Mean bunny weight at weaning (g)	449.64 ^{b}	460.34 ^{ab}	503.20 ^{a}	492.63 ^{a}	477.34 ^{a}	23.163
Litter weight gain (g):						
1-7	227.00 ^{c}	235.50 ^{c}	279.33 ^{b}	330.50 ^{a}	305.33 ^{ab}	10.849
7-14	466.83 ^{b}	571.67 ^{a}	590.00 ^{a}	562.00 ^{ab}	521.67 ^{ab}	32.514
14-21	557.67 ^{a}	500.50 ^{ab}	493.97 ^{ab}	454.67 ^{b}	521.50 ^{ab}	27.612
21-28	342.67 ^{c}	529.17 ^{b}	568.33 ^{ab}	544.17 ^{b}	628.00 ^{a}	27.236

$^{a, b....}$ Means within each row having no similar letter(s) are significantly different ($P \leq 0.05$) G1: Control G2: pomegranate peel (1%) G3: pomegranate peel (1.5%) G4: pomegranate peel extract (1%) G5**:** pomegranate peel extract (1.5%)**.**

Table (5): Average daily milk yield (g/day) of does rabbit at different suckling period.

Suckling period (day)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
7	96.67 ^{d}	112.72 ^{c}	123.56 ^{bc}	135.83 ^{ab}	144.39 ^{a}	4.539
14	137.11 ^{c}	159.22 ^{b}	172.61 ^{a}	179.94 ^{a}	182.83 ^{a}	4.375
21	145.83 ^{c}	167.78 ^{b}	182.56 ^{a}	188.72 ^{a}	197.17 ^{a}	5.217
28	93.06 ^{e}	108.56 ^{d}	117.78 ^{c}	127.33 ^{b}	139.00 ^{a}	2.563

$^{a, b....}$ Means within each row having no similar letter(s) are significantly different ($P \leq 0.05$) G1: Control G2: pomegranate peel (1%) G3: pomegranate peel (1.5%) G4: pomegranate peel extract (1%) G5**:** pomegranate peel extract (1.5%)**.**

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الملخص العربى

تأثير إستخدام مسحوق قشر الرمان أو مستخلصه على الأداء الإنتاجى ومحصول اللبن لأمهات الأرانب

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تم إستخدام ٢٥ أم من أرانب سلالة V – line عمر ٨-٩ أشهر متماثلة تقريبا فى متوسطات أوزانها (٣٣٨١ جم) حيث قسمت عشوائيا الى خمس مجاميع متساوية العدد (٥ أم/معاملة) وغذيت المجاميع على النحو التالى : المجـــموعة الاولى : تم تغذيتها على العليقة الأساسية (كنترول) دون أى إضافات، المجاميع الثانية والثالثة تم تغذيتها على العليقة الأساسية مضاف إليها ١.٠، ١.٥% مسحوق قشر الرمان على التوالى. المجاميع الرابع والخامسة تم تغذيتها على العليقة الأساسية وقدم إليها ١.٠، ١.٥% مستخلص قشر الرمان فى ماء الشرب على التوالى. أظهرت النتائج ان الأمهات التى تناولت مستخلص قشر الرمان فى ماء الشرب بالمستويات المختلفة سجلت زياده معنويه فى الغذاء المأكول مقارنة بالأمهات التى غذيت على مسحوق قشر الرمان بالمستويات المختلفة وكذلك مجموعة الكنترول. سجلت الأمهات المغذاه على ١.٠% مسحوق قشرالرمان زيادة معنوية فى عدد الخلفات عند الفطام مقارنة بمجموعة الكنترول. سجلت الأمهات المغذاه على المستويات المختلفة من قشر الرمان سواء فى صورة مسحوق فى العليقة أو فى صورة مستخلص فى ماء الشرب زيادة معنوية فى أوزان الخلفة عند ٧، ١٤ و ٢١ يوم من فترة الرضاعة وكذلك عند الفطام مقارنة بمحموعة الكنترول. إضافة ١.٥% مسحوق قشر الرمان أو ١.٠% مستخلص قشر الرمان أدى إلى إنخفاض معنوى فى معدلات النفوق للخلفة فى الفترة من الولادة حتى الفطام مقارنة بالمجاميع الأخرى ومجموعة الكنترول.
التوصية : يمكن إضافة مسحوق قشر الرمان لعلائق أمهات الارانب أو أضافة مستخلص قشر الرمان لمياه الشرب لتحسين صفات الخلفة (عدد ووزن الخلفة)، معدلات النفوق للخلفة فى فترة ماقبل الفطام وكذلك إنتاج اللبن لأمهات سلالة أرانب V-line.