EFFECT OF SUPPLEMENTATION OF DIFFERENT POMEGRANAT PEELS LEVELS ON GROWING RABBITS.

Hala M. Gamal El-Dein[1]; Nematallah G. M. Ali[2] and Fatma, G. Ahmed[3]

[1]*Poultry Nutrition, Biological and Environmental Department, Faculty of Home Economic, Al- Azhar University, Cairo, Egypt.*
[2]*Poultry Production Department, Faculty of Agriculture, Ain Shams University, Shoubra Al-Khaymah, Cairo, Egypt.*
[3]*Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.*

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SUMMARY

This study was carried out to investigate the using different levels of pomegranate (*Punica granatum*) peels on the performance of growing rabbits. A total of 36 weaning New Zealand white rabbits, sex weeks old, were divided into four groups (nine rabbits in each). Rabbits were fed with four experimental diets: a basal diet as control and the three other diets supplemented with 0.5, 1.0 or 1.5% pomegranate peel, respectively. Digestibility trail was carried out using three animals for each group which were slaughtered for carcass characteristics, caecum microbial count and blood parameters. The results showed that adding 1.5% pomegranate peel decreased final body weight and daily weight gain. However, dietary 1.0% pomegranate peel level had the best effect on feed intake and feed conversion ratio. Apparent digestibility coefficients of CP, CF, EE and NFE decreased as well as TDN and DCP comparing with other treatments when rabbits fed on 1.5% pomegranate level, also dressing percentage value decreased under the same level. Blood plasma level of cholesterol, triglyceride and glucose decreased significantly by using 1.0% pomegranate peel level, but total protein and globulin levels were not affected with adding pomegranate levels. Different levels of pomegranate peel had no significant effect on ceacum microbial counts but tended to decrease total count of bacteria, cellulolytic bacteria and total yeast count with unstable trend between different experimental groups, and the best economic efficiency was for 1.0% pomegranate peel supplemented diet. The results indicated that dried pomegranate peel could be used up 1.0% in growing rabbit diets to improve growing rabbit performance under our local condition.

***Keywords**: pomegranate peel, rabbits, bacteria, plasma parameters*

INTRODUCTION

In Egypt, there is a serious problem of feed shortage for animal feeds and also a continuous increase in the prices of the conventional feed ingredients. Incorporation of cheap unconventional feedstuffs in the rabbit diets may participate in solving the problem of feed shortage, decrease the feeding cost and alleviate the pollution problems.

Many studies have shown that the pomegranate peel extract has wound healing properties (Chidambara *et. al.* 2004), possesses antioxidant (Chidambara *et. al.*, 2002), immunomodulatory (Gracious *et. al.*, 2001) and antibacterial activity (Prashanth *et. al.*, 2001), and has also gastro protective effect (Gharzouli *et. al.*,1999). Antifungal activity (Dutta *et. al.* 1998), antitumor (Mavhjanav *et. al.*, 1997), antimicrobial (Navarro et. al., 1996), antiviral (Zhang,1995) and hypoglycemic effects (Zafar and Singh, 1990).

Pomegranate (*Punica granatum L., Puicaceae*) has been known to have considerable pharmacological properties with antimicrobial, antiviral, anticancer, potent antioxidant and antimutagenic effects (Seeram *et. al.*,2005; Negi *et. al.*, 2003).

Pomegranate used in the markets in the preparation of tinctures, juice, cosmetics and therapeutic formulae (Kim *et. al.*,2002).

On the other hand pomegranate peel is a rich source of antioxidants, especially polyphenols, such as ellagic acid, quercetin, and punicalagin and other polyphenols, as well as their dietary sources have been shown to act as potent antimutagenic and anticarcinogenic agents (Bu-Abbas *et.al.*,1994).

The peel and seeds also contain polyphenols and tannins(Ignarro *et. al.*2006).

Pomegranate not only protects low density of lipoproteins (LDL) against oxidation, but it also inhibits oxidation of toxic compounds within atheroslerotic lesions (Braga *et. al.*2005; Hidaka *et. al.* 2005). Additional research showed that pomegranate reduces abnormal platelet aggregation, lowers blood pressure (by inhibiting the angiotensin converting enzyme) and protects the arterial wall against cholesterol accumulation (Aviram,*et. al.* 2004). Pomegranate peels shows high antibacterial activity (Negi and Jayaprakasha, 2006).

This investigation aims to evaluate the role of different levels of pomegrante (*Punica granatum*) powder peels on the performance traits of rabbits, apparent digestibility of nutrients, carcass traits, some blood parameters, and antimicrobial activity.

MATERIAL AND METHODS

This study was carried out at Animal Production Research Institute, Agricultural Research Center. Thirty-six weaned New Zealand White rabbits were allotted to four experimental groups and the experimental period extended for eight weeks. Pomegranate air dried, finely ground and thoroughly homogenized before mixing with the other ingredients experimental rabbits. Four pelleted experimental diets were formulated to be approximately isocaloric and isonitrogenous in which dried pomegranate waste was incorporated at 0, 0.5, 1.0 and 1.5%. All experimental diets were formulated to meet the recommended nutrient requirements of rabbits according to NRC (1977) and Cheeke (1987). Ingredients and calculated nutrient content of the experimental diets are shown in Table (1).

Table (1): Ingredients and calculated nutrient content of the experimental diets.

Item	Experimental diets			
Ingredients	0.0	0.5%	1.0%	1.5%
Alfalfa hay	30.0	30.0	30.0	30.0
Yellow corn	22.7	22.7	22.7	23.7
Wheat bran	27.5	27.0	26.5	25.0
Soybean meal (44%)	15.0	15.0	15.0	15.0
Pomegranate peels*	-	0.5	1.0	1.5
Molasses	3.0	3.0	3.0	3.0
Salt	0.5	0.5	0.5	0.5
Limestone	1.0	1.0	1.0	1.0
Vitamin and mineral premix**	0.3	0.3	0.3	0.3
Total	100	100	100	100
Calculated nutrient content***				
Crude protein	16.50	16.51	16.51	16.60
Crude fibre	13.52	13.72	13.78	13.82
Ether extract	2.82	2.73	2.75	2.94
Crude ash	8.25	8.42	8.48	8.52
Nitrogen free extract	58.90	58.62	58.47	58.12

**Chemical analysis of pomegranate peels: 80.68, 95.14, 4.8, 4.32, 2.31, 76.71 and 4.86 % of DM, OM, CP, CF, EE, NFE, and ash, respectively.*

***Each 2.5 kg of vitamins and minerals mixture contain: 12.000.000 IU vitamin A acetate; 2000.000 IU vitamin D3; 10.000mg vitamin E acetate; 2000 mg vitamin k3; 100 mg vitamin B1; 4000 mg vitamin B2; 1500 mg vitamin B6; 10 mg vitamin B12 ; 10.000 mg Pantothenic acid ; 20.000 mg Nicotinic acid ; 1000 mg folic acid ; 50 mg biotin ; 500.000 mg Chorine ; 10.000 mg Iodine ; 300.000 mg Iron ; 55.000 mg Manganese ; 55.000 mg Zinc, and 100 mg Selenium.*

****Calculated according to Cheeke (1987).*

The rabbits were housed in galvanized metal wire cages provided with feeders and automatic drinking system and were kept under the same managerial and hygienic conditions. Diets and fresh water were available all times *adlibitum*. Live body weight of rabbits and feed consumption were weekly recorded. Feed conversion ratio (FCR) was calculated as (g feed/g gain). Digestibility trial was carried out at the end of the growth experiment using 3 rabbit per diet to determine the apparent digestibility of nutrients and nutritive value of the experimental diets. Rabbits were housed individually in metabolic cages, which allowed separation of feces and urine. Feces were collected individually during 5 consecutive days. The chemical analysis of diets and feces for DM, EE, CP, CF, NFE, and ash were conducted according to AOAC (1996). The total digestible nutrients (TDN) were calculated according to the classic formula (Cheeke *et. al.* 1982) as following: TDN= DCP+DCF+DNFE+(DEE*2.25), where: DCP= Digestible Crude Protein, DEE=Digestible Ether Extract, DCF=Digestible Crude Fiber, DNFE=Digestible Nitrogen Free Extract. The chemical analysis of diets and feces for DM, EE, CP, CF, NFE, and ash were conducted according to AOAC (1996). At the end of the experimental period, three representative rabbits from each treatment were randomly chosen and fasted for 12 hours before slaughtering according to Blasco *et. al.* (1993), and also determining the carcass traits and plasma parameters. After complete bleeding of rabbits, pelt, viscera and tail were removed carcass and edible tissues (liver, heart, kidney) were weighed. Blood samples were collected at slaughtering into heparinized tubes. A drop of blood from each sample was used to make smears for the differential leukocyte count. Differential counts of 100 leukocytes were made using slides stained with Wrights' stain and neutrophils /lymphocytes ratio (N/L) was measured. Blood samples were centrifuged at 4000 r.p.m. for 20 minutes for preparation of blood plasma. The collected plasma was stored at -20° C until assay. Blood plasma contents of glucose, total protein, albumin, cholesterol and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercial kits (Vitro Scient Company). The total globulin values were calculated by subtracting the values of total albumin from the values of total protein for each sample. Total protein was determined according to Domas (1975), albumin according to Domas *et. al.* (1971), total cholesterol according to Pisani *et. al.* (1995), triglycerides according to Greiling and Gressner (1995), and activities of AST and ALT according to Harold (1975).

Microbiological analysis:

The microbial content was estimated in their selective media as described by Bryant and Robinson (1961) for total viable counts, Hungate (1957) for cellulolytic bacteria, Smith et al. (1952) for proteolytic bacteria, Hobson and Mann (1961) for lipolytic bacteria, and Kurihava, et al. (1976) for starch digesters. Method of Allen (1955) was used for measurement of fungi count. For yeast counts technique of colony forming unit (CFU) was incubation which took place at 30°C for 2-7 days.

Economic efficiency (%) of experimental diets was calculated according to the local market price of ingredients and rabbit live body weight as: Net revenue = Total revenue – Total feed cost.

Economical efficiency (%) = Net revenue / Total feed cost %.

Growth performance index (PI) was calculated according to the equation described by North (1981) as follows: PI = Live body weight (kg) / feed conversion X 100.

Statistical analyses:

The data obtained were subjected to analysis of variance according to SPSS (1997). Significant differences among individual means were analyzed by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISSCUSION

Growth Performance:

Body weight of weaning of rabbits is one of the important traits that influence rabbit production. Table (2) shows that the highest inclusion level of pomegranate peel (1.5%) caused a decrease in final body weight. However, rabbits fed at 1.0% inclusion level showed significant decrease in feed conversion. Feed intake during the rearing period seems to be an important factor influencing body development. The 1.0% inclusion level of pomegranate gave better results on feed intake and feed conversion, but showed no significant effect as compared to controls . The decrease of growth performance at the highest inclusion level (1.5%) may be due to the high level of tannins in pomegranate peel that affects palatability of the diet, and consequently feed intake and weight gain of rabbits (Ignarro, *et. al.*2006).

Table (2): Effects of dietary pomegranate peel levels on growth performance of growing rabbits.

Item	Experimental diets				Sign.
	Control	0.5%	1.0%	1.5%	
Initial body weight (g)	628.3 ±9.93	630.4 ± 5.39	622.8 ± 5.55	622.3 ± 5.63	NS
Final body weight (g)	1890.5 ±14.3	1631.3 ± 9.4	1833.5 ± 9.3	1577.7 ± 6.0	NS
Daily weight gain (g)	22.5[a] ±0.16	17.9[b] ±0.14	21.6[a] ±0.19	17.1[b] ±0.03	**
Feed intake g/d	67.8 ±7.4	66.9 ±2.5	59.7 ± 2.8	60.2 ± 0.35	NS
Feed conversion g,feed/ g, gain	3.01[ab] ± 0.33	3.8[a] ± 0.14	2.8[b] ± 0.13	3.5[a] ± 0.02	**

[a,b] means the same row with different superscripts are significantly different (P<0.05).
± Standard error of the mean
Sig. = Significance NS = Not significant

Apparent digestibility of nutrients and Nutritive Value of feeds:

Different inclusion levels of pomegranate peel had no significant effect on apparent digestibility of DM, OM , CP and DCP (Table 3). The highest level of pomegranate peel (1.5%) resulted significant decrease on the apparent digestibility of CF, EE, NFE and TDN comparing to other treatments. Concerning the digestibility and nutritive value with adding 1.5% pomegranate peel may be due to tannins which have detrimental effect on rumen microbes, for instance depress fiber degrading bacteria (McSweeney, *et al.*, 1999), but with prolonged feeding some of the tannins caused proliferation of degrading bacteria. Tannins interfere in the determination of fiber components, probably by forming complexes with macromolecules such as protein and cellulose which are insoluble in the detergent solutions (Makkar and Singh, 1991).

Carcass Traits:

The data in Table (4) showed that carcass, kidney and heart percentages were not significantly affected by increasing the inclusion levels of pomegranate peel. Dressing percentage decreased significantly with increasing pomegranate peel levels as compared to the control. It may be due to the decrease of the apparent digestibility of different nutrients.

Table (3) : Effect of dietary pomegranate peel levels on apparent digestibility coefficient and nutritive values of growing rabbits.

Item	Experimental diets				Sig.
	Control	0.5%	1.0%	1.5%	
DM	71.33 ±0.34	70.54 ±0.39	71.57 ±0.27	72.2 ±1.7	NS
OM	78.66 ±0.19	76.21 ±7.26	79.94 ±1.51	74.51 ±2.41	NS
CP	73.7 ±0.43	73.9 ±0.88	74.9 ±0.85	73.2 ±0.19	NS
CF	57.48[a] ±0.35	33.67[b] ±1.81	32.95[b] ±1.54	31.45[b] ±0.36	**
E.E	88.1[a] ±0.44	83.51[a] ±6.12	84.08[a] ±1.39	71.73[b] ±1.06	**
NFE	75.76[ab] ±0.24	74.49[ab] ±0.67	76.12[a] ±0.32	71.44[b] ±0.53	**
DCP	12.15 ±0.03	12.19 ±1.15	12.48 ±0.05	12.13 ±0.03	NS
TDN	70.25[a] ±0.15	64.27[ab] ±1.2	66.7[ab] ±0.3	62.74[b] ±0.27	**

[a,b] means the same row with different superscripts are significantly different (P<0.05).
Sig. = Significance NS = Not significant.
± standard error of means.

Table (4): The effect of feeding the experimental diets on slaughter characteristics of growing rabbits.

Item	Experimental diets				Sig.
	Control	0.5%	1.0%	1.5%	
Carcass%	48.01 ±1.32	44.56 ±1.67	46.47 ±0.95	44.55 ±0.89	NS
Dressing%	52.92 [a] ±1.5	49.39 [ab] ±1.51	50.55 [a b] ±0.72	48.82 [b] ±0.86	**
Head%	5.57 [b] ±0.11	6.47 [a] ±0.21	6.43 [a] ±0.3	5.96 [ab] ±0.11	**
Liver%	3.73 [a] ±0.18	3.64 [ab] ±0.25	2.92 [b] ±0.19	3.17 [ab] ±0.29	**
Kidney%	0.83 ±0.007	0.68 ±0.002	0.81 ±0.11	0.79 ±0.1	NS
Heart%	0.35 ±0.007	0.51 ±0.009	0.35 ±0.002	0.31 ±0.004	NS

[a,b] means the same row with different superscripts are significantly different (P<0.05).
Sig. = Significance NS = Not significant.
± standard error of means.

Plasma Parameters:

The effect of dietary treatments on blood plasma content of cholesterol, triglyceride, glucose, total protein, total albumin and total globulin, and activities of AST and ALT, are presented in Table (5). The data showed that rabbits fed high level of pomegranate peel (1.5%) resulted singificantly the highest level of cholesterol, triglyceride and ALT. However cholesterol content of plasma decreased significantly

Table (5): The effects of pomegranate peel levels on some plasma parameters of growing rabbits.

Item	Experimental diets				Sig.
	Control	0.5%	1.0%	1.5%	
Cholesterol mg/dl	40.2 [c] ± 3.0	42.1 [c] ±3.1	55.3 [b] ± 2.9	100.7 [a] ± 4.7	**
Triglyceride mg/dl	79.8 [c] ± 2.3	98.0 [b] ± 2.6	68.3 [d] ± 2.0	128.0 [a] ± 2.6	**
Glucose mg/dl	109.8 [a] ± 1.6	96.3 [b] ± 2.4	96.3 [b] ± 2.1	97.1 [b] ± 2.3	**
AST IU/dl	7.8 ± 1.2	7.3 ± 1.5	6.0 ± 2.1	5.3 ± 1.4	NS
ALT IU/dl	8.4 [b] ± 1.6	21.0 [a] ±1.7	13.0 [b] ± 1.3	19.0 [a] ± 1.2	**
Total Protein g/dl	5.7 ± 0.35	5.73 ± 0.37	5.5 ± 0.29	5.47 ± 0.29	NS
Total albumin g/dl (A)	2.43 [b] ± 0.03	2.33 [b] ± 0.1	2.73 [a] ± 0.03	2.23 [b] ± 0.04	**
Total globulin g/dl (G)	3.27 ± 0.38	3.4 ± 0.44	2.77 ± 0.26	3.2 ± 0.26	NS
A/G ratio	74.31 [b] ± 0.21	68.53 [b] ± 0.27	98.56 [a] ± 0.15	69.69 [b] ± 0.15	NS
N/L ratio	0.56 [a] ± 0.1	0.52 [a] ± 0.03	0.36 [b] ± 0.11	0.41 [b] ± 0.02	**

[a,b,c,d] means the same row with different superscripts are significantly different (P<0.05).
Sig. = Significance NS = Not significant.
± standard error of means.

at 1.0% and 0.5% pomegranate peel levels, respectively. Triglyceride content of blood plasma decreased significantly at 1.0% pomegranate peel level. Glucose content of blood plasma of the rabbits fed control diet showed the highest value ($P<0.05$) as compared to other treatments. These results agree with those of Khalil, (2004) who found that pomegranate possess strong antioxidant property and can act as free radical scavenger and protect β-cells and subsequently pancreas from damage, therefore increase insulin receptor and release it, consequently reduce glucose in plasma.

Nougueira and Pereira (1986) attributed the antihyperglycaemic action of the peel extract of *Punica granatum* to the inhibitory intestinal absorption in rats. No significant effect of dietary pomegranate peel levels on AST, total protein and total globulin was found. Whereas, rabbits fed 1.0% pomegranate peel level gave a significant increase on total albumin comparing with other treatments (Chidambara *et al*.2002)

Concerning A/G ratio, we noted that ratios were significantly affected by pomegranate peel treatment especially at 0.5 and 1.5 % inclusion level (Table 5). These low A/G ratios were found to be an indicator for immune function (March and Scanes, 1994). Therefore, the addition of pomegranate peel may enhance immune response of rabbits.

Table (5) showed the effect of dietary supplementation pomegranate peel on N/L ratios. Addition pomegranate peel to rabbits diets affect on N/L ratios compared with control. From these results we concluded that pomegranate peel added enhance immunity by increase lymphocyte numbers and consequently decreased the N/L ratio as showed in Table (5). It means that pomegranate peel may modulate the immune system function and therefore immune response of rabbits. These results are in agreement with those of Chidambara *et al.* (2002); Gracious, *et al.* (2001) and Negi and Jayaprokasha (2006), but disagree with those of Khalil (2004).

Microbiology results:

Data in Table (6): showed that the addition of pomegranate peels to the feed of rabbits did not affect significantly total counts of bacteria as compared to the control. Regarding the specific groups, the highest starch digester bacteria were isolated from treatment group fed with 0.5% pomegranate peels was (49 CFU/g) as compared to control (25 CFU/g), however, the cellulytic bacteria counts were lower than control for all treatments. This may caused by the tannin content of promenade peels, which known to has depressing effect on fiber-degrading bacteria (McSweeney, et. al. 1999). The addition of pomegranate peels to the diets has no effect on proteolytic bacteria.

Table (6): The effect of pomegranate peel levels on rabbit's caecum bacteria and fungi content.(CFU/g)

Item	Experimental diets				Sign.
	Control	0.5%	1.0%	1.5%	
Total count	348.75 ±20.45	398.33 ±45.31	305.0 ±35.0	301.67 ±85.36	NS
Starch digester bacteria	25.25 ±9.18	49.33 ±35.45	22.0 ±13.0	28.33 ±9.33	NS
Cellulolytic bacteria	115.25 ±51.24	51.67 ±19.65	27.0 ±16.0	49.33 ±17.33	NS
Proteoytic bacteria	30.5 ±11.32	38.67 ±33.69	26.5 ±22.5	39.67 ±6.33	NS
Lipolytic bacteria	29.25 ±12.46	24.0 ±13.8	42.5 ±6.5	54.0 ±17.9	NS
Total Fungi	9.75 ±6.22	10.0 ±3.46	11.5 ±1.5	20.0 ±10.0	NS
Total yeast	1055.25 ±261.84	643.0 ±180.0	720.0 ±110.0	734.33 ±255.38	NS

Sig. = Significance NS = Not significant.
± standard error of means.

On the other hand, treatment with (1.5%) pomegranate peels increased the total counts of lipolytic bacteria (54CFU/g) as compared to controls (29CFU/gram). The counts of fungi were higher with increasing the level of pomegranate peels, while, the yeast counts decreased in all treatment groups as compared to the control. This result supported by the results of Endo *et al.* (2010) who found that

separation of fruit peel of *Punica granatum* on a silica – gel column yielded a compound that exhibited strong antifungal activity against *Candida* spp.

Economical evaluation:

It is clear , from Table (7), that using pomegranate peel in rabbit diets decreased the total cost of diets as compared to the control diet. Using pomegranate peel at the level 1.0 % improved the economic efficiency and relative economic efficiency. This improvement based on the higher body weight and better feed conversion ratio. The results of performance index indicated that the 1.0 % pomegranate peel level gave better values compared to other treatment groups.

Table (7): Economical efficiency of growing rabbits as affected by feeding different levels of pomegranate peel.

Item	Experimental diets			
	Control	0.5%	1.0%	1.5%
Av. feed intake (kg/rabbit)a	4.34	4.28	3.82	3.85
Price/kg feed (PT)*b	195.0	194.0	193.0	192.0
Total feed cost (LE) aXb=c	8.46	8.3	7.37	7.39
Av. Body wt. gain (Kg/rabbit)d	1.26	1.001	1.21	0.955
Price/kg live wt. (LE)**e	21	21	21	21
Total revenue (LE) (dXe=f)	26.46	21.02	25.4	20.06
Net revenue (LE) (f-c=g)	18.0	12.7	18.03	12.67
Economic efficiency***(g/c)	2.13	1.53	2.45	1.71
Relative economic efficiency	100	71.83	115.02	80.28
Performance index	62.79	42.93	67.25	45.1

**According to the price of different in ingredients available in the market at the experimental time.*
***According to the local market price at the experimental period.*
****Net revenue per unit cost.*
*****Group fed control diet (1)=100%.*

CONCLUSION

Dried pomegranate peel could be used up 1.0% in growing rabbit diets without adverse effects on growing performance, apparent digestibility of nutrients, blood components and economic efficiency.

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