World Rabbit Sci. 2016, 24: 207-212
doi:10.4995/wrs.2016.4025

POMEGRANATE PEEL AS A NATURAL ANTIOXIDANT ENHANCED REPRODUCTIVE PERFORMANCE AND MILK YIELD OF FEMALE RABBITS

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Abstract: This study was designed to investigate the effects of dietary pomegranate peel (PP; 9.61% crude protein and 13.1% crude fibre) on reproductive performance and milk yield of heat-stressed rabbit does. Forty-eight V-line rabbit does with average initial live body weight of 3.59±0.11 kg (2nd parity; 9 mo old) were divided into 4 treatment groups (12 does per group). The first group was fed diet free of PP. The second, third and fourth groups were fed diets containing 0.75, 1.5 and 3.0% PP, respectively, included in substitution of clover hay from the basal diet. Feed of the 4 groups was free of added antioxidants. The studied traits were: kindling rate, gestation period, litter size, litter weight, kit weight at both birth and weaning (28 d of lactation) for the last 3 parameters, pre-weaning mortality and milk yield. Range of temperatures and relative humidity during the experimental period in the rabbitry varied between 27.5-33.5°C and 64-76%, respectively. The inclusion of PP quadratically affected litter size at birth ($P<0.001$) and weaning ($P<0.01$), as well as milk yield ($P<0.001$). However, it tended to reduce the kit milk intake in most lactation periods ($P=0.078$), leading to a linear reduction in individual kit weight at weaning ($P=0.060$). In conclusion, supplementations of PP in the diet of does during summer season in Egypt can improve their productivity.

Key Words: pomegranate peel, antioxidant, heat stress, milk yield, reproductive performance, rabbit.

INTRODUCTION

Heat stress is one of the most important stressors, especially in hot regions of the world like Egypt, with a detrimental effect on reproductive performance of does. The thermo-neutral zone temperature in rabbits is around 15-25°C (Gonzalez *et al.,* 1971), 15-20°C (Marai *et al.,* 1994). Thus, when rabbits are exposed to high ambient temperatures imbalances are induced in their body temperature, which adversely affects their reproductive traits (Marai *et al.,* 2002). Several studies have clearly demonstrated the stressful effect of high temperature, which can be explained by the drop in feed intake and milk yield (Szendrö *et al.,* 1999). Milk yield determines the growth rate of newborn rabbits (Di Meo *et al.,* 2003; Khalil *et al.,* 2005) and the litter size at weaning (Zerrouki *et al.,* 2005). A stressful condition like high environmental temperature also causes an increase in oxidative stress (Amici *et al.,* 2000; Pastorelli *et al.,* 2013; Fadila and Hekal, 2015), which can disturb normal cell functions, initiating chain reactions that can compromise cell integrity (Lykkesfeldt and Svendsen, 2007). Antioxidants, both enzymatic and non-enzymatic, provide necessary defences against oxidative stress as a result of thermal stress (Sunil Kumar *et al.,* 2011). Antioxidative activity has often been associated with a decreased risk of various diseases and mortality (Huxley and Neil, 2003) and enhances the blastocyst development rate in mouse (Wang *et al.,* 2002). Pomegranate peel (PP) is a good source of antioxidants (Singh *et al.,* 2001; Whitley *et al.,* 2003). Li *et al.,* (2006) reported that 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).

The aim of this study was to evaluate, under field conditions, whether PP may attenuate the negative effects of heat stress on reproductive status and milk yield and provide protection against heat stress.

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doi:10.4995/wrs.2016.4025

MATERIAL AND METHODS

Diets

Dried pomegranate peel meal was obtained from a local market in Alexandria Governorate, Egypt. Concentrations of total phenolics (TP) in pomegranate peel extracts were determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), Food Chemistry Department (Wageningen University, The Netherlands). Chemical analysis of the PP was performed according to AOAC, standard methods (AOAC, 2000; method 934.01 for dry matter, method 942.05 for ash, method 954.01 for CP, method 962.09 for crude fibre and method 920.39 for ether extract) in Table 1.

A basal diet (PP-0) was formulated containing 20.1% crude protein and 12.4% crude fibre. The second (PP-0.75), third (PP-1.5) and fourth (PP-3) treatments contained 0.75, 1.5 and 3% PP included in substitution of clover hay, respectively. All the experimental diets were formulated so as to ensure they were both isonitrogenous and isocaloric and to meet the requirements during pregnancy and lactation in accordance with De Blas and Mateos, (1998), except for the crude fibre and crude protein, as in warm climates it is recommendable to overcome the expected poor intake by using higher levels of protein and lower level of fibre, according to Cervera and Fernández Carmona (2010). Calculated chemical composition was obtained according to NRC (1977), and the experimental diets are provided in Table 1. Feed and water were offered *ad libitum* throughout 2 cycles per doe.

Experimental procedure

Rabbit does (non-pregnant) were fed experimental diets 4 wk before mating with non-treated bucks until weaning of the litter of the 2nd parity, during the period from May to August. Conception was tested at 15 d post mating by

Table 1: Ingredient and chemical composition of experimental diets and chemical analysis of pomegranate peel (PP).

Items	PP	PP-0	PP-0.75	PP-1.5	PP-3
Ingredient (as fed basis):					
Yellow corn		7.5	7.5	7.5	7.5
Wheat bran		24	24	24	24
Barley		20	20	20	20
Clover hay		22	21.25	20.5	19
Soybean meal (44 %CP)		23.5	23.5	23.5	23.5
Pomegranate peel		0	0.75	1.5	3
Limestone		1.15	1.15	1.15	1.15
Di-calcium phosphate		0.5	0.5	0.5	0.5
Dl-Methionine		0.2	0.2	0.2	0.2
Anti-aflatoxin+anticoccidial[1]		0.5	0.5	0.5	0.5
Vitamin and minerals premix*		0.30	0.30	0.30	0.30
NaCl		0.35	0.35	0.35	0.35
Chemical composition					
As fed basis	Analysed		Calculated **		
Dry matter %	90.00	88.36	88.41	88.43	88.46
Organic matter	82.12	82.13	82.22	81.73	81.51
Crude protein	9.61	20.20	20.15	20.10	20.08
Ether extract	2.35	2.85	2.83	2.65	2.55
Crude fibre	13.07	12.62	12.46	12.31	12.09
Nitrogen free extract	57.09	46.46	46.78	46.67	46.79
Total phenolic content: mg GAE/g dry matter	272				

[1]Toxinal dry®+Saleco®. Egy-vet Company.

*Each kg of vitamin and mineral mixture (premix) contained: Vit A 2 000 000 IU; E: 10 mg; B_1 400 mg; B_2 1200 mg; B_6 400 mg; B_{12} 10 mg; D_3 180000 IU; Choline chloride 240 mg; Pantothenic acid 400 mg; Niacin 1000 mg; Folic acid 1000 mg; Biotin 40 g; Manganese 1700 mg; Zinc 1400 mg; Iron 15 mg; Copper 600 mg; Selenium 20 mg; Iodine 40 mg and Magnesium 8000 mg.

**Calculated according to NRC (1977). GAE=gallic acid equivalents.

abdominal palpation. Females with a negative palpation were mated the same day, recording sexual receptivity parameters again. Gestation period (days), kindling rate, litter size, litter and kit weight (g) at both birth and weaning (28 d of age) for the last 3 parameters and pre-weaning mortality were recorded. Milk yield was measured by weighing the does immediately before and after suckling. The total milk production was divided by the number of kits of each doe to determine the milk intake per kit at 7th, 14th, 21st and 28th day of lactation.

Animals and housing

Forty eight V-line does (defined by Ragab and Baselga, 2011) with average initial live body weight of 3.59±0.11 kg (2nd parity; 9 mo old) were divided into 4 treatments (12 does/treatment). Doe rabbits were kept under a continuous 16 h light/8 h dark photoperiod and the ambient temperature ranged from 27.5-33.5°C and relative humidity ranged from 64-76%. Animals were housed individually in flat-deck cages provided with feeders and automatic drinkers. Nest boxes prepared with rice straw were provided before 4 d of kindling.

Statistical Analysis

Data were analysed by analysis of variance using the general linear model procedure (SAS Inst. Inc., Cary, NC). Polynomial contrasts were used to test the linear and quadratic effects of the increasing level of PP supplementation. Kindling rate and pre-weaning mortality were compared within and between each treatment group and parturition order by chi square analysis. The mixed linear model includes the fixed effects of treatments, parity and linear and quadratic effects of increasing level of PP supplementation and random effects of doe and error.

RESULTS AND DISCUSSION

Reproductive performance

Under the conditions of this research, PP inclusion tended to increase kindling rate (P=0.083; Figure 1) with no effect of parturition order (88.5% on av.; P=0.77). Dietary supplementation of PP had no effect on gestation period (Table 2). The inclusion of PP linearly and quadratically increased litter size at birth (P<0.001), throughout lactation (P=0.012) and at weaning (P=0.003). However, litter weight at birth was not affected by PP inclusion, leading to a reduction in kit weight at birth (P=0.006). This result may be due to a high number of foetuses in the uterus or the uterine horn (Szendrö *et al.,* 1996), or to the negative relationship between litter size and kit weights (Poigner *et al.,* 2000). The inclusion of PP increased total milk production linearly and quadratically (P<0.001; Table 3) and tended to increase litter weight quadratically at weaning (P=0.054; Table 2). However, it tended to reduce the kit milk intake in most periods of lactation ($P \leq 0.078$), leading to a linear reduction of individual kit weight at weaning (P=0.060). 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). The inclusion of PP had no effect on

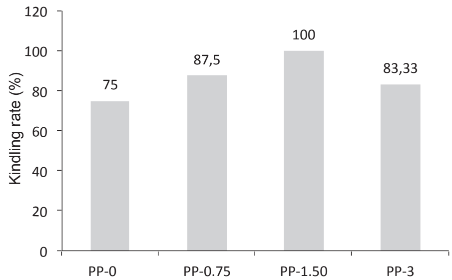

Figure 1: Kindling rate (%) of heat-stressed does treated with 0, 0.75, 1.5 and 3% pomegranate peel (PP). (n=12 animals/treatment); P-value=0.083.

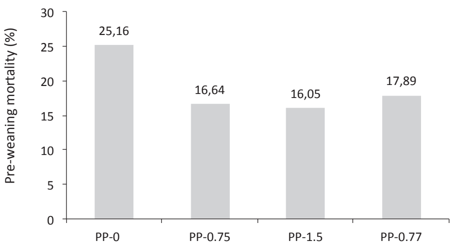

Figure 2: Pre-weaning mortality (%) of heat-stressed does treated with 0, 0.75, 1.5 and 3 % pomegranate peel (PP). (n=12 animals/treatment); P-value=0.33.

Table 2: Effect of pomegranate inclusion (PP) and parturition order on rabbit doe performance.

	Pomegranate peels level (%)					*P-value*		Parity order			
	0	0.75	1.5	3	SEM	Linear	Quadratic	1st	2nd	SEM	*P-value*
No. observations	18	21	24	20				42	41		
No. does	12	12	12	12				48	48		
Gestation period (d)	31.4	31.8	31.9	32.9	0.13	0.50	0.57	31.9	32.0	0.14	0.86
Litter weight (g)											
Birth	400	490	452	436	18.7	0.12	0.24	443	450	14.8	0.79
Weaning (28 d age)	1791	2393	1986	2130	123	0.089	0.054	1980	2166	92.8	0.32
Litter size											
Birth	5.66	8.00	6.83	6.93	0.46	<0.001	<0.001	6.74	7.06	0.17	0.33
7th d age	4.78	7.41	6.50	6.28	0.53	<0.001	0.006	5.71	6.91	0.59	0.002
14th d age	4.78	7.30	6.25	6.28	0.50	0.001	0.012	5.69	6.73	0.52	0.020
21st d age	4.56	7.30	5.75	5.90	0.55	<0.001	0.001	5.31	6.55	0.62	0.006
Weaning (28 d age)	4.25	6.83	5.75	5.83	0.51	<0.001	0.003	5.50	5.87	0.20	0.35
Kit weight/litter (g/kit)											
Birth	70.9	61.0	66.7	64.7	2.00	0.005	0.006	66.3	64.9	1.02	0.49
Weaning (28 d age)	410	352	345	355	14.0	0.060	0.56	353	371	9.00	0.23

SEM=standard error means.

pre-weaning mortality (birth to 28 d; *P*=0.33. Figure 2). The average pre-weaning mortality was 13.5% for the first parity and 19.6% for the 2nd parity (*P*=0.090). In general, kit mortality is higher in hot months (Abdel-Azeem *et al.,* 2007) which may be due to the general depression of metabolic activity in such conditions.

The improvement of reproductive performance in PP treatments might be due to protective action of antioxidants against lipid oxidation in the cell membrane (Liebler, 1992). Also, it is important for newborns, which exhibit a greater sensitivity to oxidative damage than adults, and for the development of the immune system in young animals (Debier *et al.,* 2005).

Heat stress can have negative effects on fertility of female rabbits, leading to a marked decline in ovulation rate (Hahn and Gabler, 1971), number of implantation sites per doe and number of viable embryos per doe (El-Fouly *et al.,* 1977). There is evidence to indicate that one of the causes of embryonic mortality in heat-stressed animals is the production of reactive oxygen species (ROS) by embryos developing at elevated body temperatures (Hansen, 2007).

Table 3: Effect of pomegranate inclusion (PP) and parturition order on rabbit milk yield.

	Pomegranate peels level (%)					*P-value*		Parity order			
	0	0.75	1.5	3	SEM	Linear	Quadratic	1st	2nd	SEM	*P-value*
No. observations	18	21	24	20				42	41		
No. does	12	12	12	12				48	48		
Daily milk yield (g/d)											
7th d lactation	73.5	90.3	87.7	87.3	3.64	0.080	0.240	86.5	83.8	2.20	0.55
14th d lactation	109	152	137	124	8.98	<0.001	0.020	129	134.1	3.35	0.47
21st d lactation	129	184	156	158	10.8	<0.001	<0.001	158	157.8	2.82	0.99
Weaning (28th d lactation)	112	142	129	130	5.79	0.033	0.064	129	128.6	3.58	0.96
Total milk yield (g)	2982	3976	3563	3495	198	<0.001	<0.001	3515	3536	54.6	0.85
Milk intake/kit (g/kit)											
7th d lactation	15.3	12.2	13.3	13.8	0.63	<0.001	0.018	14.5	12.7	0.92	<0.001
14th d lactation	22.8	20.8	22.0	19.7	0.65	0.391	0.176	22.5	20.1	1.22	0.026
21st d lactation	28.3	25.1	27.1	26.7	0.63	0.078	0.070	27.1	26.5	0.48	0.51
Weaning (28th d lactation)	26.4	20.7	22.4	22.3	1.17	0.006	0.044	23.3	22.4	0.54	0.44

SEM=standard error means.

In mice, administration of melatonin, an indoleamine with antioxidant properties reduced effects of heat stress on embryonic survival (Matsuzuka *et al.,* 2005).

Administration of antioxidants affects fertility in two ways. Firstly, a reduction in incidence of retained placenta or uterine infections caused by prepartum administration of antioxidants in dairy cows could increase fertility because pregnancy success after insemination is reduced with these disorders (López-Gatius *et al.,* 2006). Secondly, the oocyte and pre-implantation embryo are susceptible to damage by ROS (Schwarz *et al.,* 2008; Moss *et al.,* 2009) and increasing the antioxidant status of the reproductive tract in the postpartum period might improve competence of the oocyte or embryo for development).

Parity order effect was not a significant (P>0.05) source of variation in most studied traits except for litter size at 7th; 14th; 21st d, which were higher in 2nd parity compared to 1st parity (P=0.002; P=0.020 and P=0.006). These results agree with that obtained by Iraqi *et al.* (2007).

In conclusion, the supplementations of PP in the diet of does during summer season in Egypt improved their productivity.

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