

EFFECT OF DIETARY ADDITION OF ARAK (SALVADORA PERSICA) ON GROWTH AND REPRODUCTIVE PERFORMANCE IN BLACK BALADI RABBIT MALES

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Abstract: The study aimed to evaluate the effect of Arak (Salvadora persica) as feed additive on performance of pre and post-sexual maturity of rabbit males. A control diet was formulated with an estimated proportion of 18% crude protein and 14% crude fibre. Another three diets were formulated supplementing control diet with 0.1 0.2 or 0.3% Arak. The Arak used in this study contained (as % DM basis): 27.9 ash, 12.4 crude protein, 1.7 ether extract and 8.0 crude fibre. Ninety-six weaned Black Baladi (BB) male rabbits aged 30 d weighing 570±8.30 g (mean±standard error) were used (24 per diet). Daily weight gain and daily feed intake were recorded from weaning up to 70 d of age (slaughtering commercial age). At this time four rabbits from each group were slaughtered and genitalia were immediately taken and dissected. Blood samples were collected at 120 and 150 d of age from five bucks from each group. At the age of maturity several reproductive traits were also recorded. Final body weight at 70 d of growing BB rabbit males increased linearly and quadratically (P<0.001) with maximum Arak inclusion (0.23%), whereas feed efficiency improved linearly by 0.16±0.061 g/g (P=0.011) per increment of 1 unit of Arak inclusion. Weight of sexual-accessory glands at 70 d of age increased linearly and quadratically (P<0.01) as dietary concentrations of Arak increased, the highest values for genital organs weight being obtained by using 0.2% Arak. Plasma testosterone concentration, from 120 d to age of puberty, increased linearly by 5.87±1.69 ng/mL (P<0.001) with Arak inclusion. The minimum puberty age was obtained by 0.2% Arak inclusion (P=0.024). Sexual desire, mating activity, semen-ejaculate volume, advanced-sperm motility, sperm-cell concentration and total-sperm output were linearly and quadratically affected by Arak inclusion (P<0.05), being optimized for 0.2% Arak inclusion. In conclusion, the addition of 0.2% Arak (Salvadora persica) to the growing and mature BB male rabbit diets improved growth and reproductive capabilities.

Key words: rabbit males, Arak (Salvadora persica), growth performance, reproduction, semen quality.

INTRODUCTION

Several attempts have been made to improve rabbit production and reproduction using various commercial growth promoters (Metwally *et al.*, 2002; Ashour *et al.*, 2004; Osman Noha, 2005). Ibrahim *et al.* (2005) showed that Arak can be used as an alternative growth promoter as it enhanced immune function and favoured meat quality in growing rabbits. Arak stems are obtained from the roots of *Salvadora persica*, which grows in the area around Mecca and the Middle East area in general. It has medicinal value in manifold uses. Arak contains more than 10 different natural chemical compounds: fluoride, tannins, resins, alkaloids -Salvadoricine-, volatile oils -sinigrin-, sulfur, vitamin C, sodium bicarbonate, chlorides, calcium, benzylisothiocyanate and others, including silica -salicylic acid-, sterols, trimethylamine, saponins and flavonoid (Akhtar and Ajmal, 1981; Hattab, 1997). Five flavonoid compounds (kaempferol, quercetin, quercetrin, rutin and quercetin glucoside) were isolated from the root of this plant (Islam *et al.*, 2000). Flavonoids have antibacterial, astringent, detergent and abrasive properties (Almas and Stakiw,

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2000; Almas, 2002). Besides flavonoids, both polyphenolic compounds and certain alkaloids seem to stimulate immune function, reduce cholesterol level and play a role in the prevention of a number of chronic diseases such as cancer and cardiovascular disease in rabbits (Chang and Gershwin, 2000; Jeon *et al.*, 2001; Yousef *et al.*, 2004). The purpose of the present study was to evaluate how various productive and reproductive traits and the pre and post sexual maturity of Black Baladi rabbit males are affected by including Arak (*Salvadora persica*) as a dietary supplement. The study also aimed to establish the optimum level of Arak in rabbit diets.

MATERIAL AND METHODS

Diets and animals

Ninety-six weaned Black Baladi (BB) rabbit males aged 30 d and weighing 570±8.30 g (mean±standard error) were equally and randomly divided into four groups (24 in each). The first group was fed *ad libitum* a commercial pelleted diet according to NRC (1977) recommendations and was kept untreated to serve as a control, while the other groups (second, third and fourth) were fed the same diet, but supplemented with 0.1, 0.2 and 0.3% dried Arak powder, respectively. Chemical composition of Arak was determined in duplicate according to AOAC (2000), containing (% DM): 27.9 ash, 12.4 crude protein, 1.71 ether extract, 50.0 nitrogen free extract and 7.95 crude fibre. The ingredients and chemical composition of the experimental diets are shown in Table 1. The Arak stems (root of the plant) were cut into small pieces and allowed to dry at room temperature for 2 d and were then ground to powder in a ball mill. All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same management and hygienic conditions.

Experimental procedure

Daily weight gain and daily feed intake were recorded weekly for each rabbit during the growing period (from weaning up to slaughtering commercial age: 70 d of age).

At this age, four growing rabbit males from each experimental group were randomly slaughtered (by bleeding). Genitalia were taken immediately after slaughter and dissections were performed. Weights of pituitary gland, testes, epididymis and sexual accessory glands were recorded. Their relative weight was expressed with respect to body weight, as Samia *et al.* (2005) found a positive correlation between body weight and internal genitalia organ weight in male rabbits.

About 3 mL of blood samples were collected at 120 and 150 d of age between 08.00 and 09.00 h from the marginal ear vein of five bucks from each group. Plasma was separated by centrifugation at 3000 r.p.m. for 20 min and kept $-20\,^{\circ}$ C until hormonal assay. Blood serum testosterone (T) hormone concentration of the rabbit males was determined using RIA kits (Immunotech, A Coulter Co., Czech Republic) in accordance with the manufacturer's information. The minimum detectable limit was $0.20\,$ ng/mL and inter- and intra-assay coefficients of variation for T assay were $10.8\,$ and 5%, respectively. All samples were run in duplicate and assayed by the same operator, who was blind to the experimental situation.

On reaching maturity, the weight and age of fifteen rabbit bucks from each group at puberty (first mating) were recorded. Scrotal circumference (n=10 rabbits per group) was measured by the method described by Boiti *et al.* (2005). Testicular index (length×width×depth) (n=10 rabbits per group) was calculated in cubic centimeters as recorded by Castellini *et al.* (2006). At 6 months of age, semen was collected artificially twice a week from ten bucks from each group during the experimental period by means of an artificial vagina as described by Boiti *et al.* (2005). Immediately after collection, semen ejaculate volume (mL), advanced sperm motility (%), alive spermatozoa (%), morphological normal spermatozoa (%),

Table 1: Ingredients and calculated chemical composition of the experimental diets

Items	% as fed			
Ingredients				
Clover hay	40.5			
Wheat bran	25.0			
Yellow corn	14.0			
Soybean meal (44%)	11.0			
Molasses	3.00			
Vinasse	3.00			
Bone meal	1.75			
Calcium carbonate	0.70			
Sodium chloride	0.55			
Vitamins & Mineral Premix ¹	0.35			
DL-Methionine	0.15			
Calculated chemical composition, ²				
Ash	7.8			
Crude protein	18.0			
Ether extract	3.00			
Crude fiber	14.0			
Digestible energy, kcal/kg DM ³	2720			

¹ Vitamins and minerals premix per kilogram diet contains:

Vit. A, 6000 IU; Vit. D, 900 IU; Vit. E, 40 mg; Vit. K3, 2 mg; Vit. B1, 2 mg; Vit. B2, 4 mg; Vit. B6, 2 mg; Vit. B12, 10 µcg; Nicotinic acid, 50 mg; Biotin, 50 µcg; Folic acid, 10 mg; Choline chloride, 250 mg; Zinc, 50 mg; Manganese, 85 mg; Iron, 50 mg; Copper, 5 mg; Iodine, 0.2 mg; Selenium, 0.1 mg; Cobalt, 0.1 mg.

acrosomal damages (%), sperm-cell concentration (N×10⁶/mL) and total-sperm output (N×10⁶/ejaculate) were estimated according to Boiti *et al.* (2005) and Castellini *et al.* (2006). Libido (sexual desire) was assessed in terms of reaction time in seconds estimated from the time of introducing doe to the buck until the buck started to mount (Castellini *et al.*, 2006). Mating activity (frequency of mating within 15 minutes) of ten bucks was determined using sexually receptive does.

Housing

The study was carried out in an Industrial Rabbitry, near El-Hawamdia city, Giza Province, Egypt, from April to September, 2005. Rabbits were housed separately in individual cages (35×35×60 cm) of conventional universal galvanized wire batteries. All cages were equipped with feeding hoppers, which were made of galvanized steel sheets, and nipples for automatic drinking. The batteries were located in a well ventilated building. Averages of ambient temperature, relative humidity and temperature humidity index inside building were 30.3±0.9°C, 76.2±2.5% and 29.1, respectively, which indicate severe heat stress.

²According to NRC (1977) for rabbits.

³Digestible energy (kcal/kg DM)=4253 - 32.6 CF (% DM) - 114.4 Ash (% DM). According to Fekete and Gippert (1986).

Statistical analysis

Data were subjected to analysis of variance by using the General Linear Procedure Program of SAS (Statistical Analyse System, 1997). Polynomial contrasts were used to detect linear and quadratic relationships for the effect of the Arak dose on all parameters (PROC GLM). Initial weight was included as a covariate in a statistical model for growth traits (PROC GLM). Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed. All data are presented in least squares means. For all data analyses, each animal was considered as an experimental unit.

RESULTS AND DISCUSSION

Growth performance

Final body weight at 70 d (slaughtering commercial age) of growing BB rabbit males increased linearly and quadratically with Arak inclusion (P<0.001; Table 2) and was maximized at 0.2% Arak inclusion. The values of feed efficiency improved linearly by 0.16±0.06 g/g (P=0.011) per each increment of 1 unit of Arak inclusion. There was a linear trend of increasing daily weight gain values of growing BB (P=0.084) with increasing dietary inclusion of Arak. No effect was detected on daily feed intake, which on average was 94.0 g/d. Ibrahim *et al.* (2005) found that rabbits supplemented with Arak improved their crude protein digestibility. Therefore, increases in growth performance parameters for treated groups could be due to improved absorption of amino acids (Ibrahim *et al.*, 2005) and/or to the bactericidal, antimycotic or antifungal properties of Arak as reported by Al-Samh and Al-Bagieh (1996).

Genitalia organ weight and plasma testosterone concentration

The results given in Table 3 show that relative weights of testes and epididymis tended to increase linearly (P<0.09) with dietary Arak inclusion. However, relative sexual-accessory gland weight increased linearly and quadratically (P=0.006 and 0.003, respectively) as dietary Arak level increased, obtaining the highest value of sexual-accessory gland weight at 0.1% Arak. These results were similar to those observed in mice, in which the inclusion of 800 mg/kg of an extract of *Salvadora persica* increased the relative weight of the testes (Darmani, 2003).

The values of plasma testosterone concentrations increased linearly by 5.87 ± 1.69 ng/mL (P=0.009), per each increment of 1 unit of Arak inclusion (Table 4). The increase in plasma testosterone concentration in treated groups can be attributed mainly to the increase in sexual accessory gland activity with increasing Arak levels (Table 3). This can affect the secretion of testosterone from the interstitial tissues of the testes (Al-Sobayil and Khalil, 2002).

Table 2: Effect of Arak (*Salvadora persica*) inclusion on growth performance of growing BB rabbit males from 30 to 70 d of age (n=24 animals per treatment).

		ık		P-v	value		
	0 (control)	0.1	0.2	0.3	SEM	Linear	Quadratic
Initial body weight at 30 d, g	570	569	571	570	17	0.99	0.99
Daily weight gain, g	23.9	26.7	30.1	28.5	1.87	0.084	0.23
Final body weight at 70 d, g	1527	1639	1775	1711	20.8	0.001	0.001
Daily feed intake, g	92.2	94.1	95.1	94.7	4.08	0.69	0.78
Feed efficiency, g/g	0.256	0.282	0.314	0.299	0.014	0.011	0.14

Table 3: Effect of Arak (*Salvadora persica*) inclusion on body and internal genitalia organs weights of growing BB rabbit males at 70 d of age (n=4 per treatment).

	% Arak					<i>P</i> -value	
Items	0 (control)	0.1	0.2	0.3	SEM	Linear	Quadratic
Live body weight, g (BW)	1472	1531	1640	1619	54	0.19	0.47
Testes weight, % BW	0.277	0.304	0.315	0.313	0.012	0.077	0.221
Epididymis weight, % BW	0.044	0.046	0.051	0.049	0.002	0.090	0.263
Sexual-accessory glands weight, % BW	0.142	0.197	0.193	0.189	0.008	0.006	0.003
Pituitary gland weight, % BW	0.181	0.180	0.192	0.184	0.009	0.569	0.651

Reproductive performance

Age at puberty decreased linearly and quadratically (P=0.008 and P=0.024, respectively) as dietary proportion of Arak increased (Table 4). The minimum puberty age occurred at 0.2% Arak inclusion. This result could be related to the maximum final body weight obtained with this Arak dietary level and also to the improving effect of Arak on testosterone concentration that leads to faster maturity. The decrease in age at puberty observed in our trial was similar to that observed in previous studies in rabbits with other growth promoters (Amin et al., 2002; Daader et al., 2002). Similar results were obtained by Samia et al. (2005), who found that age at puberty was related to testosterone concentration. Also, Castro et al. (2002) mentioned that testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this process in the adult. El-Sherbiny (1994) found that the onset of puberty involves the appearance of the first spermatozoa in the caudal epididymis of rabbit males. Moreover, the testicular index increased linearly and quadratically (P<0.002) as the dietary proportion of Arak increased (Table 4), obtaining a maximum value at 0.3% of Arak inclusion. Testicular size is the best primary assessment of spermatogenesis, since the tubules and germinal elements account for approximately 98% of the testicular mass (Sherines and Howards, 1978). The testicular index also reflects spermatogenesis and testosterone production (Eskes, 1983; ElMougy et al., 1991). Arak inclusion had no effect on weight at puberty or scrotal circumference, which were on average 2774 g and 7.1 cm, respectively.

Arak inclusion influenced linearly and quadratically semen-ejaculate volume, advanced-sperm motility, sperm-cell concentration and total-sperm output (P<0.05), which were maximized at 0.2% Arak inclusion (Table 5). Libido and mating activity was also linearly and quadratically affected by Arak inclusion (P<0.001), being optimized for 0.24 and 0.21% dietary Arak inclusion, respectively. The percentage values of live spermatozoa and morphological normal spermatozoa increased linearly by 17.1 ± 7.08 (P=0.016) and 19.5 ± 7.39 (P=0.009) percentage units, respectively per each increment of 1 unit of Arak inclusion.

Table 4: Effect of Arak (*Salvadora persica*) inclusion on some reproductive traits of BB rabbit bucks.

		rak		P-	value		
Items	0 (control)	0.1	0.2	0.3	SEM ¹	Linear	Quadratic
Testosterone, ng/mL ²	3.15	4.15	5.05	4.81	0.76	0.001	0.10
Weight at puberty, g	2755	2762	2771	2810	22.5	0.84	0.47
Age at puberty, d	159.9	154.5	151.7	152.2	1.26	0.008	0.024
Scrotal circumference, cm	6.93	7.10	7.20	7.25	0.09	0.16	0.49
Testicular index, cm ³	4.46	5.74	6.09	6.25	0.17	0.001	0.002

n=15 per treatment except for testosterone where n=5, and scrotal circumference and testicular index where n=10

² Average value of analysis recorded at 120 and 150 d.

Table 5: Effect of Arak (*Salvadora persica*) inclusion on libido, mating activity and physical semen characteristics of BB rabbit bucks at 6 months of age (n=10 per treatment).

	% Arak				P-value		
Items	0 (control)	0.1	0.2	0.3	SEM	Linear	Quadratic
Sexual desire –libido-, sec.	39.4	33.8	27.8	29.7	2.28	0.001	0.001
Mating activity ¹	3.59	4.98	5.21	5.10	0.23	0.001	0.001
Semen-ejaculate volume, mL	0.505	0.705	0.839	0.794	0.033	0.001	0.001
Advanced-sperm motility, %	65.2	72.1	76.9	76.1	4.50	0.001	0.038
Alive spermatozoa, %	72.9	75.5	79.7	77.2	2.55	0.016	0.10
Morphological normal spermatozoa, %	77.2	81.3	83.5	83.0	2.95	0.009	0.17
Sperm-cell concentration, $N\times 10^6/mL$	457.5	570.2	610.3	598.8	53.9	0.001	0.008
Total-sperm output, $N \times 10^6$ / ejaculate	233.4	404.7	512.9	473.1	52.1	0.001	0.001
Acrosomal damage, %	15.5	13.7	11.6	11.9	1.52	0.001	0.071

¹No. of mated during 30 minutes.

Acrosomal damage values decreased (P<0.001) by 12.6±2.54 percentage units per each increment of 1 unit of Arak inclusion. Daader *et al.* (2002) improved semen quality by using a diet supplemented with either 5% *Nigella sativa* L. or Fenugreek. The increase in sexual accessory gland weight in treated rabbits (Table 3) suggests that an increase in ejaculate volume occurred, since the accessory glands and spermatogenesis are controlled by the testosterone concentration, which was higher in treated rabbits. The effects on sperm concentrations and motility observed in our trial are in agreement with Fields *et al.* (1979), who observed that sperm concentration was positively correlated with motility and testicular size in young bulls. The decrease in acrosomal damage in the treated groups could be attributed to the antioxidant activity of Arak due to flavonoids, which can protect the plasma membrane that surrounds the acrosome and the tail. Accordingly, it seems that Arak may display an indirect role in rabbit spermatogenesis.

We concluded that the inclusion of 0.2-0.25 % of Arak (*Salvadora persica*) in rabbit diets improved their productive and reproductive performance. Further research studies should be focused on *Salvadora persica* extracts for therapeutic use.

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