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SOME PHYSIOLOGICAL AND IMMUNOLOGICAL RESPONSES OF RABBITS FED DIETS CONTAINED *SALVADORA PERSICA*.

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A total number of sixty-four sexual mature Baladi Black (BB) rabbis (48 does and 16 bucks) of 8 months of age, was used in the present work. The study aimed to evaluate some physiological and immunological responses of rabbits as affected by Salvadora persica dietary supplementation*. The rabbits were divided into two comparable groups (24 does and 8 bucks in each group). The first group was kept untreated as a control and fed according to* ***NRC (1977)*** *recommendations, while the second group was fed the same diet, but supplemented with 0.2% Arak (Salvadora persica).*

The results showed that treatment with Arak (Salvadora persica) caused significant (P≤0.05) increase in blood picture and some blood constituents such as plasma total protein and its fractions, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes. Dietary supplementation of Salvadora persica (Arak) to BB rabbit does significantly (P≤0.05) improved their reproductivity (represented by litter size and weight at birth and at weaning and milk yield during the suckling period) and decreased significantly (P≤0.05) number of pre-weaning mortality. Feeding diets contained 0.2% Arak increased significantly (P≤0.05) ear thickness response to phytohemagglutinin.

Key words: Arak, fertility, immunity, performance, rabbits.

Arak (*Salvadora persica*) can be used as an alternative growth promoter as it enhances immune function in growing rabbits and improves productive and reproductive capabilities rabbit males (Ibrahim *et al.*, 2005 and El-Kholy *et al.*, 2008).

The Arak stick is obtained from a plant called *Salvadora persica* that grows around Mecca and the Middle East area in general. The Arak has immense medicinal value with manifold uses. Arak contained a number of

substances that possess antibacterial and bioantioxidant properties, some other components are astringents, detergents and abrasives (Almas and Stakiw, 2000 and Almas, 2002). Many researchers proved that the Arak contains more than 10 different natural chemical compounds. These are: fluoride, tannins, resins, alkaloids (Salvadoricine), volatile oils (sinigrin), sulfur, vitamin C, sodium bicarbonate, chlorides, calcium, benzylisothiocyanate (BIT) and others, including silica (salicylic acid), sterols, trimethylamine, saponins and flavenoid (Akhtar and Ajmal, 1981; Hattab, 1997 and Bahabri, 2000). Several chemical compounds contained in *Salvadora persica* have been suggested as being involved in the immunostimulatory power: flavenoids, polyphenolic compounds and certain alkaloids (Chang and Gershwin, 2000). Five flavonoid compounds, kaempferol, quercetin, quercetrin, rutin and quercetin glucoside, were isolated from the root of this plant (Islam *et al.*, 2000).

The objective of the present study was to evaluate the effects of Arak (*Salvadora persica*) dietary supplementation on some physiological, immunological and fertility traits of Baladi Black rabbit does, under Egyptian environmental conditions.

MATERIALS AND METHODS

The present work was carried out in Rabbitry near El-Hawamdia City, Giza Province, Egypt. Duration of the study was four months (the period from July until October 2007).

A number of 48 multiparous Baladi Black (BB) does aged 7-8 months and 16 bucks aged 8-9 months were used in this work to study the physiological reactions and biological performance of doe rabbits as affected by using *Salvadora persica* (Arak) as natural antioxidant and antibacterial. The does were selected in a herdbook (November 2005 to April 2006), had at least three litters and of similar reproductive performance. The animals were divided into two similar experimental groups (24 does and 8 bucks in two sequence parities in each group). The first group was fed a commercial diet *ad libitum* which covered the nutritional requirements according to NRC (1977) recommendations (16.5% crude protein, 13.4% crude fibre) and were kept untreated and served as a control, while the other group was fed with the same diet, but supplemented with 0.2% dried Arak powder (as recommended by El-Kholy *et al.*, 2008). Animals were allowed to become accustomed to treatment for a preliminary period of ten days during June month. The Arak sticks (root of the plant) were cut into small pieces and were kept for 2 days to dry at room temperature and were 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.

Animals were individually housed in wired battery cages supplied with feeders and stainless steel nipples. All batteries were located in a windowed Rabbitry with natural ventilation. Fresh tab water was automatically available all the time in each cage. The time of mating was in the morning between 8.00 and 9.00 h. Each doe was transferred to the buck's cage to be mated, and then returned back to its own cage. Does failing to conceive were immediately returned after palpation to the same mating buck for another service. On the 27th day of pregnancy, the nest boxes were supplied with wheat straw litter.

Pregnancy was diagnosed by abdominal palpation at the tenth day after mating.

Conception rate was estimated as number of does conceived / number of does mated. Litter size and weight at birth and at weaning values were recorded. Number of pre-weaning mortality and milk yield up to 28 days were estimated during the suckling period.

Air temperature (^{o}C) and relative humidity (%) inside the rabbitry building were measured four times each month. Averages of ambient temperature, relative humidity and temperature humidity index inside building were 31.0±0.8^{o}C, 70.5±2.3% and 29.5, respectively, which indicate severe heat stress during the experimental period. The temperature–humidity index (THI) was calculated using the equation modified by Marai *et al.* (2001):

$$THI = db\,^{o}C - [(0.31 - 0.31 \times RH) \times (db\,^{o}C - 14.4)],$$

Where db^{o}C = Dry bulb temperature in Celsius, RH= Relative humidity percentage/100. The THI values classified as follows: <27.8= absence of heat stress, 27.8 - < 28.9=moderate heat stress, 28.9 - <30.0 = severe heat stress and 30.0 and more = very severe heat stress (Marai *et al.*, 2001).

A cycle of 16 h light and 8 h dark was used throughout the experiment.

Five blood samples, about 3 ml, were collected biweekly at 08.00-09.00 h am from the marginal ear vein of five does from each group. Blood samples were collected into dry clean centrifuge tubes. Blood serum was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept in a deep freezer at -20 ^{0}C until biochemical analysis. Non-coagulated blood was tested shortly after collection for estimating blood picture. Red blood cells (RBCs), white blood cells (WBC's) and different subclasses of WBC's (lymphocyte, neutophils, monocytes, eosinophils and basophil percentages) were counted according to Feldman *et al.* (2000). Hemoglobin concentration and hematocrite percentages were measured according to Drew *et al.* (2004).

Total proteins level was estimated according to Armstrong and Corri (1960). Albumin level was estimated according to Doumas *et al.* (1971). Globulin level values were obtained by subtracting the values of albumin from the corresponding values of total protein. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to Reitman and Frankel (1957).

At the end of the experimental period, five representative rabbits from each group were randomly selected to determine cell mediated immunity (CMI). Fifty µg of PHA (Phytohemagglutinin-P; Difco, Detroit, MI) in 0.1 ml of sterile pyrogen-free physiologic saline or saline only was injected intradermally (ID) into the right and left ear, respectively. Central ear thickness of each rabbit was measured with a constant-tension dial micrometer (Mitutoyo Co., Tokyo, Japan) just before the injection and again every 3 hrs later (from 0-24 hrs). The response was recorded in millimeters as the difference between PHA response (right ear) and the saline response (left ear) after injection (Heba El-Lethey *et al.*, 2003).

Data were subjected to analysis of variance by using the General Linear Procedure Program of SAS (2001). Repeated measures were used to analyze blood determinations and cell mediated data over time (PROC MIXED) by using the compound symmetry (CS) model. Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed. All data are presented as least squares means. For all data analyses, each animal was considered as an experimental unit.

RESULTS AND DISCUSSION

1- Blood components :

Table 1 showed that, values of each of blood components including (red and white blood cells count, hemoglobin concentration and hematocrite percentage) were higher significantly ($P \leq 0.05$) in BB rabbit does dietary supplemented with 0.2% Arak (within normal range) than control. These increases were closed to those of that obtained by Ibrahim *et al.* (2005). In general, Arak diet causes an increase in total RBC's, which in turn caused an increase Hb and Ht. This is due to the positive relationships between RBC's, Hb and Ht (Sturkie, 1986). The Arak as feed additive may act as growth promoter through its effects by increasing activity of metabolic cycles (Ibrahim *et al.*, 2005 and El-Kholy *et al.*, 2008). This could explain the increase in blood components.

Also, rabbits dietary supplemented with Arak were higher significantly ($P \leq 0.05$) in WBC's count and lymphocyte percentage than in

Table 1. Blood picture (Within normal range) of Baladi Black rabbit does as affected by *Salvadora persica* (Arak) treatment.

Items	Hematological responses			
	Control (C)	Treatment (T)	Difference (T- C)	Change (%)
Red blood cells (N×10^6 /mm^3)	5.2^b ± 0.4	6.7^a ± 0.6	+ 1.50	+ 28.85
White blood cells (N×10^3 /mm^3)	6.9^b ± 0.5	7.9^a ± 0.7	+ 1.00	+ 14.49
Hemoglobin (g/dl)	10.9^b ± 1.3	11.8^a ± 0.7	+ 0.90	+ 8.26
Hematocrite (%)	33.6 ± 3.7	37.1 ± 3.4	+ 3.50	+ 10.42
Lymphocyte (%)	65.0±0.7	75.3±0.7	+ 10.30	+ 15.85
Neutrophils (%)	29.0±0.6	20.3±0.6	- 8.70	- 30.00
Monocytes (%)	2.75±0.3	2.03±0.3	- 0.72	- 26.18
Eosinophils (%)	1.75±0.2	1.31±0.2	- 0.44	- 25.14
Basophils (%)	1.50±0.2	1.06±0.2	- 0.44	- 29.33

[a, b] Means within the same row bearing different superscripts, differ significantly (P≤0.05).

the control. The lymphocyte is considered the main type of white blood corpuscles and a good indicator of the increase in immune efficiency (Wieslaw *et al.*, 2006).

The improvements in the blood components as a result of treatment with Arak may be due to improvement in the immune response (minerals and flavonoids have a role in enhancing immune system). Inclusion of the minerals in Arak (calcium, 582 µg/ml; fluorine, 0.07 µg/ml; and sulphur, 34 g/ml; Hattab, 1997) and flavonoid compounds, (kaempferol, quercetin, quercetrin, rutin and quercetin glucoside; Islam *et al.*, 2000) may have a role in improvements of these hematological traits. Ingestion of Arak has a good effect on the composition of blood (Ibrahim *et al.*, 2005). Additionally, McDowell (2000) suggested that bioflavonoids are a special class of nutrients important in diminishing capillary fragility, preserving blood cell integrity, enhancing blood circulation and anti-bacterial activity. The body becomes more susceptible to bruising and hemorrhaging when capillaries lose their physical integrity and when tissues have become weakened, the body was vulnerable to allergies and immune system breakdown. The same author added that bioflavonoids and vitamin C (found in Arak) had worked synergistically to maintain blood capillary health and prevent capillary fragility. The increase of RBC's in treated rabbits can be discussed from the point that Arak as bioantioxidant may be covered the reduced of body antioxidant under heat stress (Chew, 1995). However, Bobyrev *et al.* (1988) found that free radical oxidation of lipids, lipoproteins and peroxide

Table 2. Some blood plasma constituents (Within normal range) of Baladi Black rabbit does as affected by *Salvadora persica* (Arak) treatment.

Items	Plasma metabolites			
	Control (C)	Treatment (T)	Difference (T- C)	Change (%)
Total protein (TP, g/100ml)	$5.90^{b} \pm 1.2^{b}$	$7.74^{a} \pm 0.7$	+ 1.84	+ 31.19
Albumin (Alb, g/100ml)	3.93 ± 0.3	4.18 ± 0.3	+ 0.25	+ 6.36
Globulin (Glb, mg/100ml)	$1.97^{b} \pm 0.6$	$3.55^{a} \pm 0.9$	+ 1.58	+ 80.20
Alb/Glb ratio	$2.05^{a} \pm 0.09$	$1.17^{b} \pm 0.07$	- 0.88	- 42.93
AST (U/ L)	22.5 ± 2.1^{b}	25.7 ± 1.1^{a}	+ 3.2	+ 14.22
ALT (U/ L)	14.2 ± 0.9^{b}	16.3 ± 0.7^{a}	+ 2.1	+ 14.79

[a, b] Means within the same row bearing different superscripts, differ significantly ($P \leq 0.05$).

hemolysis of erythrocytes increased in rabbits given diets devoid of antioxidants.

Finally the improvements in blood picture can be reflected positively on the immune system.

2- Blood constituents :

Table 2 shows that plasma total proteins and its fractions (albumin and globulin) concentrations; and some liver function enzymes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were significantly ($P \leq 0.05$) higher in BB rabbit does dietary supplemented with 0.2% Arak than in those fed free diet (control). This result was in a good agreement with those of Ibrahim *et al.* (2005). The increase in globulin concentration with Arak inclusion in the heat stressed rabbit's diet as observed in the present study may be an indication of increased immunity in the rabbits since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007). Increase of globulin concentration than albumin is important for immunologic responses (Tietz, 1986). Since Ismail *et al.* (2003) reported that albumin/globulin ratio is a good indicator of improved immunity. The protein synthesis associated with the immune response (Fortun-Lamothe and Drouet-Viard, 2002) indicated that treatment with Arak may result in the increase in the immune system.

Arak (*Salvadora persica*) includes some immunity stimulators such as flavenoids, polyphenolic compounds and certain alkaloids (Chang and Gershwin, 2000 and Fortun-Lamothe and Drouet-Viard, 2002). The important biochemical indices that can be used to assess the health status of

Table 3. Fertility traits of Baladi Black rabbit as affected by supplementing with *Salvadora persica* (Arak) to the diets.

Items	Biological responses	
	Control	Treatment
CR[1] (%)	$65.11^{b} \pm 4.11$	$78.52^{a} \pm 3.55$
Litter size at birth	$5.77^{b} \pm 0.39$	$7.28^{a} \pm 0.31$
Litter size at weaning	$3.00^{b} \pm 0.40$	$5.91^{a} \pm 0.42$
Litter weight at birth (g)	$322^{b} \pm 20.25$	$400^{a} \pm 12.12$
Litter weight at weaning (g)	$1652^{b} \pm 50.13$	$1992^{a} \pm 60.31$
No. of pre weaning mortality	$2.77^{a} \pm 0.41$	$1.37^{b} \pm 0.24$
Total milk yield up to 28 days (g)	$1695^{b} \pm 45.26$	$2011^{a} \pm 41.7$

[1]CR (%)=(No. of pregnant does / No. of copulated does) × 100.
[a, b] Means within the same row bearing different superscripts, differ significantly ($P \leq 0.05$).

the liver are the serum levels of albumin and globulin. Albumin, which was manufactured by the liver, is a major protein that circulates in the bloodstream (Tietz, 1986). Improved hepatic function is clearly observed throughout the low ratio of albumin/globulin (Ashour *et al.*, 2004). Also, increasing plasma total proteins and their fractions (Alb and Glb) within the normal range may reflect an increase in the hepatic function (El-Harairy *et al.*, 2003). This phenomenon was observed in treated groups compared to control group. As mentioned before, Arak increased the levels of aminotransferases (AST and ALT) but this increase was still within normal range as indicated by the non-sign of toxicity.

3- Reproductive traits :

Table 3 showed that, BB rabbit does dietary supplemented by 0.2% Arak naturally mated with bucks fed the same diet recorded significantly ($P \leq 0.05$) better values for conception rate, litter size and weight at birth and at weaning, number of pre-weaning mortality, and total milk yield up to 28 days than those of the control group. These results may be due to the improvement in libido and physical semen characteristics as a result of adding Arak to the buck diet (El-Kholy *et al.*, 2008). Similarly, present results were in conformity with the studies of Gad Alla *et al.* (2002) who found that supplementary vitamin C may largely help in improvement the nutritive value of feed, increasing the immunity of rabbits and consequently improve their conception rate, litter size, litter weight at birth and at weaning and pre-weaning mortality in rabbits. The increase in milk production may be due to the increase in litter size at birth where there was a positive correlation between the litter size and milk yield (Lebas *et al.*,

1997 and Rommers *et al.*, 1999). The increase in litter size in the treated groups may be related to calcium (one of the Arak contents), since, Homa *et al.* (1993) and Kaufman and Homa (1993) had reported calcium role in the development of female egg cells (oocytes). The beneficial effects of Arak on reduction number of pre-weaning mortality can be detected or it had been reflected on blood parameters and physiological reaction of their mothers (does). On the other side, the milk available per kit may also have a pronounced effect on the mortality of young rabbits (Rommers *et al*, 1999; Poigner *et al.*, 2000; Szendro *et al.*, 2002). The decreased of number of pre-weaning mortality in offspring's treated rabbit does may be discussed from the view, which was demonstrated by Fortun-Lamothe and Drouet-Viard (2002) who showed that in the rabbit, the immune system is established during foetal life and continues to mature during the first weeks of life. During gestation in the rabbit, the foetuses receive a generous supply of immunoglobulins via the amniotic fluid, which defends them from birth against environment (Fortun-Lamothe and Drouet-Viard, 2002). They added that after birth, the colostrum and then the milk continues to supply immuoglobulins to the young in significant quantities. The anti-infectious properties of milk originate from dissolved or cell-borne factors. The milk contains immunoglobulins in quite large amounts (mainly IgA in the rabbit, but also IgG and IgM), which protect the young from certain infectious diseases (Lönnerdal, 2000). Also, the milk contains numerous leukocytes, which are the most often activated. These cells not only contribute to local protection of the intestine, but also to general passive cell immunity as some of them can pass through the intestinal barrier (especially for the colostrum cells) (Fortun-Lamothe and Drouet-Viard, 2002).

4- Phytohemagglutinin-elicited skin reaction:

The present experiment demonstrates that *Salvadora persica* treatment impulses cell-mediated immunity in heat stressed rabbits (Table 4 and Figure 1). The increase in cell-mediated immunity in treated groups can be attributed to the antioxidant activity of Arak due to flavonoids inclusion. This result is in harmony with finding of Siegel and Morton (1977) who found that vitamin C (one of the Arak contents) as antioxidant stimulates either humoral or cell-mediated immunity of rabbits. Because of environmental stress causes an increase in the oxidative stress so, antioxidant nutrient supplementation protects against the oxidative DNA damage through its free radical scavenging activity (Lee, 2002). Also, antioxidants are very important to immune defense and animal health, consequently, to their production capacity (Chew, 1995). Flavonoids are chemical antioxidants that help to lower cholesterol levels. Besides

Table 4. Effect of *Salvadora persica* treatment on immune response of Baladi Black rabbit does at the end of the treatments (experimental period).

Time (hrs)	Control	Treatment
3	$0.81^{a} \pm 0.15$	$1.51^{b} \pm 0.15$
6	$1.74^{a} \pm 0.19$	$2.75^{b} \pm 0.19$
9	$2.85^{a} \pm 0.32$	$4.25^{b} \pm 0.32$
12	$3.97^{a} \pm 0.47$	$5.88^{b} \pm 0.47$
15	$4.77^{a} \pm 0.55$	$6.78^{b} \pm 0.55$
18	$3.64^{a} \pm 0.58$	$5.63^{b} \pm 0.58$
21	$2.35^{a} \pm 0.38$	$4.54^{b} \pm 0.38$
24	$2.18^{a} \pm 0.49$	$4.00^{b} \pm 0.49$

[a, b] Means within the same row bearing different superscripts, differ significantly ($P \leq 0.05$).

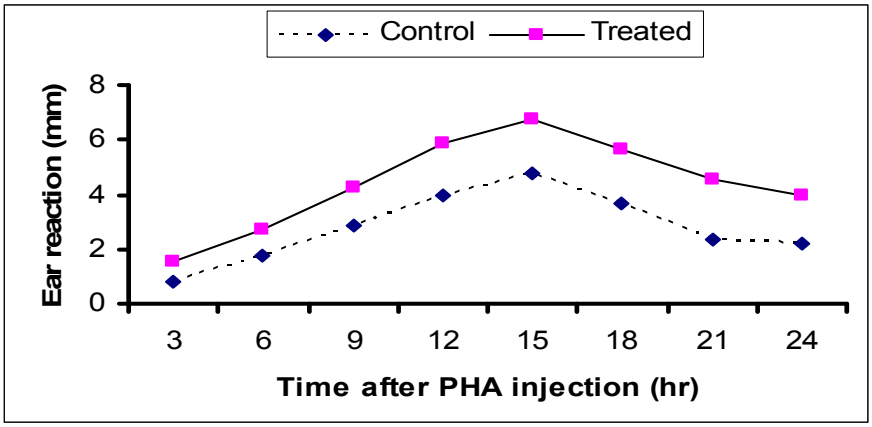

Figure 1. Cutaneous response of Baladi Black rabbit does as affected by *Salvadora persica* treatment to phytohemagglutinin-P (PHA) expressed as difference in ear thickness (mm).

flavonoids, both polyphenolic compounds and certain alkaloids seem to stimulate immune function, reduced 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)**.** There were good indications that cell-mediated immunity plays an important role in controlling and cleaning intracellular bacterium (Kougt *et al.,* 1995). On the other hand, many studies have demonstrated the antibacterial and anti-fungal properties of Arak (Al-Bagieh and Almas, 1997; Almas, 1999; Almas and Stakiw, 2000).

Presence of these properties in Arak may explain the increase (enhancement) of immune system activity.

The antioxidant function of Arak could, at least in part, enhance the immunity by maintaining the functional and structural integrity of important immune cells. The changes in the cell mediated immunity and lymphocyte percentage are due to Arak inclusion, provided evidence of improved doe rabbits adaptation to high environmental temperatures.

Generally, the necessity of adding Arak to diets of rabbits under Egyptian environmental conditions may aid the animal to enhance the activity of the immune system. Histopathological, hematological and endocrinology studies in the same respect may be needed. Further research studies must also be focus on the extract of *Salvadora persica* that may be therapeutically used.

Conclusion:

It can be concluded that supplementation of 0.2% Arak (Salvadora persica) to the diets of rabbit does showed a great role in enhanced blood picture, blood metabolites, reproductive performance and immune system.

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تأثير إضافة الأراك فى العليقة على بعض استجابات الأرانب الفسيولوجية والمناعية

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أستخدم فى هذه الدراسة 64 أرانب بلدى أسود ناضج جنسياً (48 أنثى + 16 ذكر) عمر 8 أشهر.صممت التجربة لدراسة تأثير إضافة نبات الأراك كمنشط نمو وكمضاد أكسدة طبيعى على بعض معدلات الأداء الفسيولوجية والتناسلية والمناعية لإناث الأرانب. قسمت تلك الأرانب إلى مجموعتين تجريبيتين (24 أم فى بطنين متتاليتين، 8 ذكور فى كل مجموعة). المجموعة الأولى مجموعة المقارنة غذيت على عليقة تجارية تبعاً لمقررات NRC (1977) بينما غذيت المجموعة الثانية (المجموعة المعاملة) على نفس العليقة ولكن مضاف إليها 0.2% نبات الأراك.

أوضحت النتائج أن قيم كل من الهيماتوكريت والهيموجلوبين وعدد كرات الدم الحمراء والبيضاء ونسبة الخلايا الليمفاوية ومستوى تركيز كلاً من البروتين الكلى والألبيومين والجلوبيولين أعلى (معنوية عند مستوى 5%) فى دم الإناث المغذاة على عليقة محتوية على نبات الأراك مقارنة بالغير معاملة. سجلت مستويات إنزيمي الـ ALT وAST الدالة على نشاط الكبد زيادة معنوية (عند مستوى 5%) كنتيجة للمعاملة بنبات الأراك مقارنة بمجموعة المقارنة. سجلت إناث أرانب البلدى الأسود المعاملة بنبات الأراك والملقحة طبيعياً من ذكور معاملة بنفس المعاملة قيم أفضل معنوياً (عند مستوى 5%) لكل من معدل ولادات، وعدد ووزن خلفات عند الميلاد وعند الفطام، ومحصول لبن لكل أم، وكذلك معدلات نفوق للخلفات قبل الفطام وذلك مقارنة بتلك التى سجلتها أرانب مجموعة المقارنة. وفى إختبار الكفاءة المناعية سجلت المجموعة المعاملة إرتفاع فى المناعة الخلوية مقارنة بمجموعة المقارنة.

و مما سبق فإن الدراسة أوضحت فى مجملها أن إضافة نبات الأراك إلى إناث الأرانب أعطى نتائج أفضل معنوياً (عند مستوى 5%) فى معدلات أدائها الفسيولوجية والتناسلية والمناعية على السواء.