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## Inclusions of natural bioactive mixture in drinking water 2: Nutritional impact on productive, physiological and reproductive performance of Rex rabbits breed

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A total number of two hundred and sixteen Rex rabbits (180 females and 36 males) aged 12 weeks approximately ( $2452 \pm 44.6$  g) were divided into four equal comparable experimental groups (45 females and 9 males in each) to study the impact of adding natural bioactive mixture composed of lemon, onion and garlic (LOG) juice at portions (1.00: 1.00: 0.125/ liter clean water), respectively on productive, reproductive and physiological performance of Rex rabbits. The first experimental group rabbits received the basal diet that formulated to cover the requirements of growing rabbits recommendations and considered as (control group), meanwhile, drinking water was offered *ad libitum* without containing any additives (Zero LOG). On the other hand, the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were also fed the basal diet; meanwhile drinking water was added at levels 10, 20 and 30 ml LOG/ liter of drinking water for the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively. The results showed that adding LOG at different levels in drinking water significantly ( $P < 0.05$ ) increased values of final weight, total body weight gain, average daily gain, dry matter intake and feed conversion was also significant improved compared to control. Values of rectal, skin and ear-lobe temperatures; respiration and pulse rates were significantly ( $P < 0.05$ ) decreased. The age at first mating was significantly ( $P < 0.05$ ) decreased gradually with increasing the level of LOG supplementation in drinking water. Testes, epididymis, sexual-accessory glands and pituitary gland weights; in addition to scrotal circumference, testicular index were significantly ( $P < 0.05$ ) increased. Values of semen ejaculate volume; mass motility; advanced sperm motility percentages; sperm-cell concentration and total sperm output significantly ( $P < 0.05$ ) increased. Meanwhile, libido-sexual desire (second); Dead spermatozoa percentages; sperm abnormalities percentages and acrosomal damages percentages were significantly ( $P < 0.05$ ) decreased. The values of GOT, GPT, acid phosphatase and alkaline phosphatase of semen were significantly ( $P < 0.05$ ) decreased. Dietary treatments significantly ( $P < 0.05$ ) increased the values of mating activity (frequency of mating/ 15 minutes) and sexual hormones includes (testosterone concentration, estradiol 17  $\alpha$  and progesterone). An improving in both total numbers of pregnant does and kindled does comparing was noticed, abortion rate percentages were decreased, conception rate and kindling rate percentages were gradually increased with increasing levels of LOG supplementation. Litter size numbers at birth, litter weight at birth, bunny weight at birth, litter size numbers at weaning; litter weight at weaning and bunny weight at weaning were significantly ( $P < 0.05$ ) increased. Milk yield was significantly ( $P < 0.05$ ) increased and mortality rate was significantly

( $P < 0.05$ ) decreased by 29.81, 46.01 and 57.99% with adding LOG at 10, 20 and 30 ml/ liter drinking water, respectively compared to control. It can be mentioned that incorporation nature bioactive mixture composed of lemon, onion and garlic (LOG) juice in drinking water of Rex rabbits caused an improving in their average daily gain and feed conversion. Furthermore, it improves their reproductive performance through out increasing live body weight at first mating with decreasing the age at first mating and decreasing the mortality rate.

**Keywords:** Bioactive mixture, rabbits, growth performance, enzymatic semen activity, artificial insemination; fertility, milk yield, mortality rate

## INTRODUCTION

Rabbits are very susceptible to heat stress, since they have few functional sweat glands and have difficulty in eliminating excess body heat, when the environmental temperature is high. Exposure of growing and adult male and female rabbits to, 30 temperature humidity index (THI) units as severe heat stress during summer adversely affects their growth and reproductive traits and reduces the resistance to diseases. In female rabbits, conception rate, embryonic development, litter size, litter weight and milk production decrease and age at puberty and pre & post weaning mortality increase by exposure to heat stress. In males, testosterone concentration, spermatogenesis, temporary sterility, sexual desire, ejaculate volume, motility, sperm concentration and total number of spermatozoa in an ejaculate decrease and sperm abnormalities and dead sperm increase by exposure to the same factor. The drastic changes that occur in rabbits' biological functions are depression in feed intake and feed efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Marai et al., 2002)

Feed additives are a group of nutrient that helps in improving the efficiency of feed utilization and thus reducing the high cost of feed. In the past, antibiotics were the most routinely used feed additives. However, nowadays use of antibiotics is not only limited but their use in livestock and poultry industry also have been banned in many countries due to the reasons like alteration of natural gut microbiota and drug resistance in bacteria and humans. As a result, to replace them without adversely affecting the performance of birds, natural growth promoters such as prebiotics, probiotics, synbiotics, enzymes, plant extracts, etc., can be used to feed the broilers (Borazjanizadeh et al., 2011).

Through out the recent years, noticed that using bioactive feed additives become important materials used to improve the efficiency of feed

utilization and growth performance as noted by (Aiad et al., 2008; Ahmed et al., 2009) in feeding calves.

Medicinal herbs such as garlic, onion and have been reported to possess antibacterial, antiseptic, anti-inflammatory, antiparasitic and immunomodulatory properties (Muhammad et al., 2009 and Thomson et al., 2007).

On the other hand, natural additives such as lemon, onion or garlic to food will increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes (Wangensteen et al., 2004).

Most of the plant parts contain compounds with proven antibacterial, antiviral, antiparasitic, antifungal properties have antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory and antioxidant activity (Lampe 1999).

Garlic rich in sulphur compound such as cysteine sulfoxides (Lancaster and Shaw 1989). Also, it contained steroidal glycosides, lectins, prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and amino acids (Matsuura et al., 1988; Kaku et al., 1992).

Garlic as natural growth promoters can be potential alternatives for common artificial growth promoters like antibiotics (Demir et al., 2003).

Noticed that adding garlic to food encouraged stimulation of immune function, enhanced foreign compound detoxification and resistance various stresses (Amagase et al., 2001).

Onion bulbs possess numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, spropylcysteine sulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur compounds (Melvin et al., 2009).

Lemon is a good source of potassium (145

mg per 100 g fruit), bioflavonoid, and vitamin C (40 to 50 mg per 100 g), this equal twice time as much as oranges (Fenwick and Hanley 1985; Chevallier 1996; Hassan and Abdel-Raheem, 2013).

The optimization of reproductive performance is one of the main facts that assure high productivity on rabbit farms. This requires that the management practices take into account the physiology and behavior of the animals since environmental, managerial and sanitary aspects interfere with fertility and can impair it. Indeed, reproduction could be considered a "luxury" function and the female appears able to feel whether the conditions are too severe and risky for a successful reproductive cycle (Friggens, 2003).

So, this work was carried out to study the influence of adding bioactive natural mixture composed of lemon, onion and garlic juice (LOG) at different levels in drinking water on productive reproductive and physiological performance of Rex Rabbits.

## MATERIALS AND METHODS

### Animals and feeds

This study was carried out in co-operation work among Animal Production Department, National Research Centre, 33 El-Bohouth Street, P.O: 12622, Dokki, Giza, Egypt; Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt and Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

The present work aimed to study the effect of adding natural bioactive mixture composed of lemon, onion and garlic (LOG) juice at portions (1.00: 1.00: 0.125/ liter clean water), respectively on productive, reproductive and physiological performance of Rex rabbits.

The natural bioactive mixture was prepared as the following:

- Fresh garlic bulbs, onion, and Limon were obtained from the local market and cut into small pieces.
- About 250 ml of distilled water per 100 g of peeled onion/ or garlic and limon were added and crushed in a mixing machine in the presence of some crushed ice.
- The resultant slurry of every one was squeezed and filtered 3 times through a fine cloth.

- The aqueous extract of 3 materials was stored in dark brown bottle with scrow cap at 4°C until use.

A total number of two hundred and sixteen Rex rabbits (180 females and 36 males) aged 12 weeks approximately (2452±44.6 g) were divided into four equal comparable experimental groups (45 females and 9 males in each). The first experimental group rabbits received the basal diet that formulated to cover the requirements of growing rabbits according to NRC (1977) recommendations and considered as (control group), meanwhile, drinking water was offered ad libitum without containing any additives (Zero LOG). On the other hand, the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were also fed the basal diet; meanwhile drinking water was added at levels 10, 20 and 30 ml LOG/ liter of drinking water for the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively.

The experimental work was carried out in private industrial rabbitry located near Skarah City, Giza Province, Egypt, during the period from July to October.

Rabbits were continuous feeding from 12 weeks to 24 weeks (168 days of age) or (adult age of male and female rabbits). Some physiological and reproductive parameters were estimated such as body-thermoregulation response; age and weight at first mating, some parameters indicated fertilizing ability, libido and physical semen characteristics, sexual hormones concentration and mating activity, fertility traits of does artificially inseminated, milk yield and pre-weaning mortality rate.

Semen samples were collected using an artificial vagina and samples were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to international guidelines (IRRG, 2005). Ejaculated volume was measured to the nearest 0.01 ml. A weak eosin-formalin (10% formalin) solution was used for evaluation of sperm concentration by the improved Neubauer hemocytometer slide, as described by Smith and Mayer (1955). Total sperm output was calculated by multiplying semen ejaculate volume by semen concentration. Semen mass motility was given an arbitrary score from 0 to 3 based on the following assessment and the following variables were estimated: 0=No current, (0.5)=Very few slow currents, 1=Few slow currents, 1.5=Many moderate waves, 2=Many sweeping waves, 2.5 = numerous vigorous waves, 3= numerous rapid and vigorous waves, as described by Moule (1965). Individual sperm motility was estimated at 400× magnification

(Kamar, 1960). Assessment of dead spermatozoa was performed using an eosin-aniline blue staining mixture (Shaffer and Almquist, 1948). Seminal plasma of experiment was separated by centrifugation at 3000 rpm for 20 min and was stored at  $-20^{\circ}\text{C}$  in Eppendorf tubes for further analysis. GOT, GPT, acid phosphatase and alkaline phosphatase were determined in seminal plasma calorimetrically using commercial kits obtained from Bio-Diagnostics Ltd., Egypt according to the procedure outlined by the manufacturer.

Both feed intake (FI) and live body weight (LBW) values were recorded through out the experimental period that lasted for 84 days to calculate the daily feed intake (DFI), average daily gain (ADG) and feed conversion (FC).

Both composition and calculated chemical analysis of the pelleted basal diet was presented in (Table 1).

**Table: 1. composition and calculated chemical analysis of the basal diet.**

Ingredients	Kg/ ton
Clover hay	405.00
Wheat bran	250.00
Yellow corn	140.00
Soybean meal (44%)	110.00
Molasses	30.00
Vinas	30.00
Bone meal	17.50
Lime stone	7.00
Sodium chloride	5.50
Vitamins & Mineral Premix*	3.50
DL-Methionine	1.50
<b>Calculated chemical composition **</b>	
Crude protein (CP)	18.00
Crude fiber (CF)%	14.00
Ether extract (EE)	3.00
Digestible energy (Kcal/Kg DM)	2720

\* Vitamins & Mineral Premix: Vitamins & Minerals premix per Kilogram contained Vit.A (10,000 IU), Vit.D<sub>3</sub> (9000 IU), Vit.E (10000 IU), Vit.K (3 IU), Vit.B<sub>1</sub> (2 IU), Vit.B<sub>2</sub> ( 6 IU), Vit.B<sub>6</sub> (2 IU), , Biotin (0.2 mg), Choline (1200 mg), Niacine (40 mg), Zinc. (60 mg), Cu. (0.1 mg), Mn. (85 mg), Fe. (75 mg), Folic acid (5 mg) and Pantothenic acid (20 mg).

\*\* Calculated chemical composition: calculated according to NRC (1977) for rabbits.

### Statistical analyses

Data collected were subjected to statistical analysis as one way analysis of variance according to SPSS (2008). Duncan's Multiple Range Test Duncan, (1955) was used to separate means when the dietary treatment effect was significant according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

W here:  $Y_{ij}$  = observation.  $\mu$  = overall mean.  $T_i$  = effect of treatment for  $i = 1-4$ , 1 = first group (control) fed commercial diet that no contained LOG in drinking water), 2 = second 2<sup>nd</sup> group fed commercial diet and adding 10 ml LOG/ litter drinking water), 3 = third 3<sup>rd</sup> group fed commercial diet and adding 20 ml LOG/ litter drinking water) and 4 = fourth 4<sup>th</sup> group fed commercial diet and adding 30 ml LOG / litter drinking water).  $e_{ij}$  = the experimental error.

Percentage values were transformed to Arc-Sin values to approximate normal distribution before being statistically analyzed. Duncan's new multiple range tests was used to test the significance of the differences among means (Duncan, 1955).

## RESULTS AND DISCUSSION

### Growth performance

Data presented in Table (2) showed that adding LOG at different levels in drinking water significantly ( $P < 0.05$ ) increased final weight (FW), total body weight gain (TBWG) and average daily gain (ADG) comparing to control. These results in agreement with those found by Ahmed *et al.* (2016) who noted that ADG of New Zealand white rabbits was improved by 20, 29, 36.1% and 19.3% for rabbits fed (5, 10, 15 and 20 ml LOG/ kg feed, respectively) compared to the control group (0 ml LOG/ kg feed). On the other hand, Onion and garlic caused an improvement in growth this reason related to increase the inflow of glucose into tissues and thyroid like activity (Habbak *et al.*, 1989). In addition to, improvement of live body weight gain by feeding animals on onion and/or garlic may be due to their contents of sulphar compounds that are considered as active antimicrobial agents and improve immunity (Dedi and Elssenuwenger 2000; Ibrahiem *et al.*, 2004)

Dry matter intake (DMI) significantly increased with adding LOG in drinking water. Meanwhile levels of adding was not affected on DMI (Table 2). These results might explain that adding bioactive mixture (LOG) at different experimental levels had no adverse effect on rabbit's palatability. These results were in harmony with those noted by Omer *et al.* (2015) and Ahmed *et al.* (2016) who reported that incorporation LOG at (0, 5, 10 15 and 20 ml/ kg feed) had no significant effect on DM intake and DM intake ranged from 106 to 112 g/head/day.



**Table: 2. Growth performance of the experimental group rabbits drinking water containing different levels of LOG.**

Item	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Initial weight, g	2228 <sup>c</sup> ±42.6	2471 <sup>b</sup> ±41.8	2570 <sup>a</sup> ±45.5	2540 <sup>a</sup> ±48.4
Final weight, g (FW)	3320 <sup>d</sup> ±50.2	3731 <sup>c</sup> ±55.1	3998 <sup>a</sup> ±43.9	3884 <sup>b</sup> ±48.6
Total body weight gain, g (TBWG)	1092 <sup>d</sup> ±33.7	1260 <sup>c</sup> ±35.5	1428 <sup>a</sup> ±37.7	1344 <sup>b</sup> ±36.8
Duration period	84 days			
Average daily gain (ADG)	13.00 <sup>c</sup> ±0.88	15.00 <sup>b</sup> ±0.95	17.00 <sup>a</sup> ±0.87	16.00 <sup>ab</sup> ±0.49
Dry matter intake (DMI)	122 <sup>b</sup> ±3.55	127 <sup>a</sup> ±3.28	129 <sup>a</sup> ±2.98	127 <sup>a</sup> ±3.17
Feed conversion (g DMI/ g gain)	9.38 <sup>c</sup> ±2.26	8.47 <sup>b</sup> ±2.38	7.59 <sup>a</sup> ±2.15	7.94 <sup>ab</sup> ±2.31

a, b, and c: Means in the same row having different superscripts differ significantly ( $P < 0.05$ ).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water

The present results revealed that the corresponding values of average daily DMI ranged between 122 g to 129 g.

On the other hand feed conversion that expressed as (g DMI/ g gain) was significantly ( $P < 0.05$ ) improved. The best level of LOG adding was realized with adding 20 ml LOG/ liter drinking water (Table 2). These results might be due to the effective to improve immunity and decrease debility incidence, which agree with the findings of Aboul-Fotouh et al., (1999); Nadi (1999); Aboul-Fotouh et al., (2000); Ahmed et al., (2009) who reported that nutrition plays important role in diminishing growth rate. Also, Eid et al., (2010) noted that feeding growing Californian rabbits ration contained 0.50% green tea significantly ( $P < 0.05$ ) improved feed conversion compared to control ration, corresponding values were (3.07 vs. 3.21 g feed intake/g gain) for 0.50% green tea and control rations, respectively.

#### Body-thermoregulation response of the experimental group rabbits

Data of Table (3) cleared that adding 0, 10, 20 and 30 ml LOG/ liter drinking water of Rex rabbit significantly ( $P < 0.05$ ) decreased the mean values of rectal temperature that ranged from 38.04 to 38.38 °C; skin temperature that varied from 37.18 to 37.58 °C; ear-lobe temperature that ranged from 37.18 to 37.98 °C; respiration rate 111 to 139.8 (rpm) and pulse rate that varied from 203.2 to 234.9 (ppm) among the four experimental group rabbits.

These results cleared that the LOG as a natural bioactive mixture juice play an important role in depress the heat stress of rabbits. The comfort zone temperature for rabbits is around 21°C. At either higher or lower temperatures, the animal has to expend energy to maintain its body temperature. The heat load in rabbit's increases

by exposure to a high environmental temperature and animals try to sustain homeothermy by using internal physiological means to help re-establish thermal balance. Rabbits use general body position, breathing rate and peripheral temperature (especially ear temperature), as three means to increase heat loss. Respiration and the ear are the most important heat dissipation pathways, since most of the sweat glands in rabbits are unfunctional and perspiration (evacuation of water through the skin) is never great because of the fur (Marai et al., 2002). When the temperature is above 25-30°C, animals stretch out and erect their ears to loose as much heat as possible by radiation and convection (Lebas et al., 1986).

Rectal temperature increases with exposure to high environmental temperature as reported by (Shafie et al., 1982; Habeeb et al., 1993, 1997, 1998; Marai et al., 1994a & b, 1996; Tharwat et al., 1994a). However, Shafie et al. (1970) reported that the diurnal variation in body temperature varied within a very short range (0.2-0.3 °C) revealing a high efficiency of body heat regulation in rabbits.

Finzi et al., (1994) showed that the average body temperature goes up from morning till night, while environmental air temperature goes up from morning till noon then decreases at night, indicating that body temperature is not affected instantly by changes in air temperature during the day. However, Tharwat et al., (1994a) reported that both rectal and scrotal temperatures responded rapidly to elevated air temperature. Scrotal temperature was normally lower than rectal temperature by about 1.4 °C. Particularly, the increase value in rectal temperature was estimated to be about 1.0 8C, when the ambient temperature increased from 18.8 °C in winter to 30.7 °C in summer (Gad, 1996). The increase in

rectal temperature of the heat stressed rabbits may be due to either poor ability of the animals to prevent the rise in rectal temperature or to failure of the physiological mechanisms of animals to balance the excessive heat load caused by exposure to high ambient temperature (Habeeb et al., 1992, 1998).

Skin temperature directly affected by body temperature conduction through the blood and other body fluids, in addition to radiation (Shafie et al., 1970). Also, Gonzalez et al., (1971) estimated that the mean skin temperature increased by only about 3 °C, when environmental temperature was elevated from 20 to 30 °C. In addition to, Gad (1996) confirmed that skin temperatures of NZW and Cal rabbits were higher in summer than in winter and attributed that to the warmth effect of the coat. In this respect, it may be of interest to note that skin temperature was higher by 0.1-0.3 °C than rectal temperature in summer, while it was almost equal to rectal temperature in winter (Habeeb et al., 1998). Also, Shafie et al. (1970) reported that skin temperature becomes higher than body temperature after solar exposure for 10 and 30 min, although the air temperature in summer is two times larger than that of winter. Diurnally, it can be noticed that body and skin temperatures decrease slightly at night compared to noon during winter months due to the short time of sunshine and the weaker intensity of the thermal rays (Shafie et al., 1970). However, Brody (1945) indicated that skin temperature is nearly stable over all the year due to the efficient insulation provided by the hair coat, since that coat is denser and longer to conserve more heat during winter than during summer.

Between 0 and 30 °C, latent heat evacuation is only controlled by altering the breathing rate (Marai and Habeeb, 1994). Respiration rate in rabbits increased under heat stress conditions as noted by (Marai et al., 1994a & b, 1996; Gad, 1996; Habeeb et al., 1997 & 1999b). The increase respiration rate in summer estimated by Habeeb et al., (1998) was 42-59% higher than the winter average. Water loss by evaporation and respiratory frequency are linearly related and both increase with increases in ambient temperature above panting threshold (Richards, 1976). However, dissipation of heat through respiratory water vapour is decreased by the increase in ambient humidity (Lebas et al., 1986).

#### **Age and weight at first mating of the experimental group rabbits**

Data of Table (4) showed that the age at first mating of Rex rabbits was significantly ( $P<0.05$ )

decreased gradually with increasing the level of adding LOG in drinking water (0, 10, 20 and 30%). This improvement reach to 12.42, 16.99 and 19.61% for experimental group rabbits received 10, 20 and 30 ml LOG/ liter drinking wate, respectively in comparison with the control (zero LOG). On the othe hand, live body weight at first mating was significantly ( $P<0.05$ ) increased with increasing the level of adding LOG in drinking water (0, 10, 20 and 30%). The corresponding values of this improvement was recorded by 1.81, 3.32 and 4.43% for experimental group rabbits received 10, 20 and 30 ml LOG/ liter drinking wate, respectively comparing with the control (zero LOG). In the last 15 years noticed that, the profitability of rabbit farms has increased mainly due to improvements in management and genetic selection but several problems related to animal welfare have also occurred.

The replacement and the mortality rates of female per year are very high and the replaced does often show poor body condition and poor health status. The effect of kindling order, litter size, genetic strain, weaning age and reproductive rhythm (RR) on the reproductive performance and welfare of females (Castellini *et al.*, 2010). On the other hand, it seems that modern prolific strains of rabbit, particularly in the first parities, have many difficulties in balancing body condition and reproduction and require the use of different strategies to address the problem. Long-term it would seem that selection for strains with higher appetite is required along with the development of alternative nutritional strategies. Theoretically, when reproductive rhythms (RR) are shorter, the expected productivity (litter/year) is higher. However, the lower fertility rate obtainable in semi-intensive reduces the distance between expected and actual production (Castellini, 2007). Puberty within the same breed and the same sex is affected by several environmental factors such as ambient temperature, relative humidity, photoperiod and nutrition level, of which ambient temperature is the most important (Marai et al., 1991). Rabbit puberty was found to be delayed (Daader and Seleem, 1999) and, accordingly, age at first mating was increased (Daader et al., 1999a & 1999b) with increasing environmental temperature. Particularly, the Egyptian native rabbit breeds such as Baladi Red, Baladi White, Baladi Black and Giza White reached puberty at about 6 months of age (Hilmy, 1991). Age at first mating in both Californian (Cal) and NZW buck rabbits ranged between 140 and 150 days during the summer season in Egypt (Daader et al.,

1999a &amp; 1999b).

**Table 3. Body thermoregulation response pre mature (from 12 weeks of age up to adult age) of males and females Rex rabbits drinking water containing different levels of LOG.**

Item	Sex	Experimental diets			
		Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Rectal temperature (°C)	Males	38.36±0.04	38.15±0.03	38.06±0.02	38.03±0.03
	Females	38.39±0.05	38.17±0.04	38.04±0.03	38.04±0.04
Means ± SE		38.38 <sup>a</sup> ±0.07	38.16 <sup>b</sup> ±0.04	38.05 <sup>c</sup> ±0.04	38.04 <sup>c</sup> ±0.01
Skin temperature (°C)	Males	37.55±0.04	37.39±0.03	37.36±0.03	37.16±0.03
	Females	37.58±0.03	37.39±0.04	37.38±0.04	37.19±0.04
Means ± SE		37.57 <sup>a</sup> ±0.03	37.39 <sup>b</sup> ±0.03	37.38 <sup>c</sup> ±0.02	37.18 <sup>d</sup> ±0.01
Ear-lobe temperature (°C)	Males	37.93±0.06	37.60±0.04	37.39±0.04	37.17±0.05
	Females	38.02±0.03	37.63±0.03	37.42±0.04	37.19±0.03
Means ± SE		37.98 <sup>a</sup> ±0.05	37.62 <sup>b</sup> ±0.04	37.41 <sup>c</sup> ±0.04	37.18 <sup>d</sup> ±0.05
Respiration rate (rpm)	Males	135.9±6.71	122.7±5.92	116.3±5.48	105.7±4.73
	Females	143.6±6.86	131.7±6.27	120.9±5.37	116.2±5.03
Means ± SE		139.8 <sup>a</sup> ±5.09	127.2 <sup>b</sup> ±4.21	118.6 <sup>c</sup> ±3.36	111.0 <sup>d</sup> ±3.06
Pulse rate (ppm)	Males	231.9±9.01	211.8±7.61	205.3±7.49	201.2±5.99
	Females	237.9±9.11	222.5±6.96	212.1±5.99	205.1±7.01
Means ± SE		234.9 <sup>a</sup> ±8.31	217.2 <sup>b</sup> ±6.84	208.7 <sup>c</sup> ±5.27	203.2 <sup>d</sup> ±5.03

a, b, c and d: Means in the same row having different superscripts differ significantly (P&lt;0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

**Table 4. Age and weight at first mating from (weaning for up to adult age) of the experimental group rabbits drinking water containing different levels of LOG.**

Item	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Age at first mating (Days)	153 <sup>a</sup> ±7.31	134 <sup>b</sup> ±5.18	127 <sup>bc</sup> ±5.42	123 <sup>c</sup> ±4.73
Live body weight at first mating (g)	2980 <sup>c</sup> ±24.7	3034 <sup>b</sup> ±26.3	3079 <sup>ab</sup> ±25.3	3112 <sup>a</sup> ±27.9

a, b and c: Means in the same row having different superscripts differ significantly (P&lt;0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

**Table 5. Some parameters indicated fertilizing ability of Rex rabbit bucks drinking water containing different levels of LOG.**

Item		Experimental diets			
		Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Live body weight, g		2970.3 <sup>c</sup> ±22.9	3024.5 <sup>b</sup> ±27.5	3083.4 <sup>ab</sup> ±24.8	3102.5 <sup>a</sup> ±25.6
Testes weight	Absolute, g	5.572 <sup>c</sup> ±0.08	5.851 <sup>b</sup> ±0.11	5.960 <sup>b</sup> ±0.10	6.241 <sup>a</sup> ±0.12
	Relative (%)	0.188 <sup>c</sup> ±0.001	0.193 <sup>b</sup> ±0.002	0.193 <sup>b</sup> ±0.001	0.201 <sup>a</sup> ±0.004
Epididymis weight	Absolute, g	0.891 <sup>c</sup> ±0.001	0.963 <sup>c</sup> ±0.001	1.022 <sup>b</sup> ±0.001	1.042 <sup>a</sup> ±0.001
	Relative (%)	0.030±0.001	0.032±0.001	0.033±0.001	0.034±0.001
Sexual-accessory glands weight	Absolute, g	3.190 <sup>c</sup> ±0.140	3.473 <sup>b</sup> ±0.092	3.642 <sup>a</sup> ±0.121	3.701 <sup>a</sup> ±0.108
	Relative (%)	0.107 <sup>c</sup> ±0.004	0.115 <sup>b</sup> ±0.002	0.118 <sup>a</sup> ±0.001	0.119 <sup>a</sup> ±0.001
Scrotal circumference	Absolute, g	7.321 <sup>c</sup> ±0.132	8.110 <sup>b</sup> ±0.181	8.292 <sup>ab</sup> ±0.123	8.370 <sup>a</sup> ±0.151
	Relative (%)	0.246 <sup>c</sup> ±0.003	0.268 <sup>b</sup> ±0.000	0.269 <sup>a</sup> ±0.001	0.270 <sup>a</sup> ±0.000
Testicular index	Absolute, g	6.693 <sup>c</sup> ±0.101	6.964 <sup>b</sup> ±0.082	7.196 <sup>ab</sup> ±0.124	7.270 <sup>a</sup> ±0.093
	Relative (%)	0.223 <sup>c</sup> ±0.003	0.230 <sup>b</sup> ±0.002	0.233 <sup>a</sup> ±0.000	0.234 <sup>a</sup> ±0.001
Pituitary gland weight	Absolute, g	0.331 <sup>c</sup> ±0.010	0.353 <sup>b</sup> ±0.012	0.379 <sup>a</sup> ±0.007	0.386 <sup>a</sup> ±0.008
	Relative (%)	0.011±0.00	0.012±0.001	0.012±0.000	0.012±0.000

a, b, c and d: Means in the same row having different superscripts differ significantly (P&lt;0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

The temporary sterility in buck rabbits may be produced by high temperature (Sandford, 1979; El-Gaafary, 1994; Daader et al., 1997; Zeidan et al., 1997; Daader and Seleem, 1999): Temperatures above 18 °C and incidence of infertility in male rabbits were found to be significantly correlated (Sammoggia, 1977). Reproductive ability of male rabbits was found to decrease during July, August and September due to the decrease in desire (Nalbandov, 1970).

#### **Some parameters indicated fertilizing ability of the experimental group rabbits**

Data of Table (5) showed that the parameters that indicated fertilizing ability of the rabbits received LOG at different levels related significantly ( $P < 0.05$ ) improving in their testes weight, epididymis weight, sexual-accessory glands weight, scrotal circumference, testicular index and pituitary gland weight that determined as Absolute (g) or relative (%). The knowledge of basic morphometric characteristics of the reproductive tract have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals (Ogbuwu et al., 2009).

Meanwhile, Gage and Freckleton (2003) noted that the mammalian testes as infallible predictors of spermatozoa production. The authors further asserted that knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. The paired testes weight in the study carried out by Ahemen *et al.* (2013) were not significantly influenced by dietary treatments. Also, Bitto and Gemade (2001) noticed non significant effect of pawpaw peel meal (PPM) up to 30% on testicular morphometry of male rabbits. In addition to (Ogunlade et al., 2006) observed non significant differences in testis weight among rabbits fed fumonisin contaminated diets.

On the other hand, (Bitto et al., 2000) observed a significant decrease in paired testes weight of cockerels fed cassava peel meal up to 30% in diet. The mean testicular length and width values ranged from 2.43 to 2.65 cm and 0.98 to 1.07 cm, respectively as noted by (Ahemen et al., 2013). Meanwhile, the values of testicular length and width obtained by Ajayi et al. (2009) in rabbits varied from 2.26 to 4.40 cm and 0.94 to 1.10 cm for testicular length and width, respectively.

Also, (Ajayi et al., 2009) reported a significant

effect of diets (Blood-wild sunflower leaf meal mixture diet) on testicular length of rabbits. Both Ezekwe (1998) and Perry and Petterson (2001) noted that testes size, length and width are good indicators of present and future sperm production.

#### **Libido and physical semen characteristics of the experimental group rabbits**

Data presented in Table (6) clearly that adding LOG at different levels in drinking water of Rex rabbits significantly ( $P < 0.05$ ) increased values of semen ejaculate volume; mass motility; advanced sperm motility percentages; sperm-cell concentration and total sperm output. Meanwhile, it significantly ( $P < 0.05$ ) decreased values of libido-sexual desire (second); Dead spermatozoa percentages; sperm abnormalities percentages and acrosomal damages percentages. Semen is a mixture of spermatozoa produced by testicles and seminal plasma secreted at different sites by accessory glands and by the epididymus, which are combined at the time of ejaculation. Seminal plasma also contains other particles of different size which affect the spermatozoa behavior during the transit along the female reproductive tract (Cesare Castellini, 2008).

Variation in the seminal characteristics is known to be affected by many factors (genetic strain, feeding, health status, rearing condition, season, age and collection frequency), thus contributing to the large variability in semen traits (Alvariño, 2000).

The present results in agreement with those noted by (El-Gaafary, 1994; Daader et al., 1997; Zeidan et al., 1997; Daader and Seleem, 1999) who noted that sexual desire or libido of male rabbits, as assessed by reaction time (in seconds) from the time of introducing the doe to the buck until the buck starts to mount and ejaculate, was found to decrease significantly with increasing ambient temperature. (El-Ashry et al., 1995) found that in Cal rabbit, overall mean of reaction time was 43.20 seconds. Meanwhile, Abd El-Azim and El-kamash (2010) recorded that reaction time per were 32.56, 31.51, 25.19 and 23.57 seconds for New Zealand White, Californian, Sinai and Balady, respectively. Reaction time of Balady and Sinai were faster than Cal and NZW. These results may be due to the effect of temperature on foreign breed of rabbits in the middle of Egypt.

On the other hand, (Tharwat et al., 1994b) found that libido was delayed by 11.9-18.5 second and by up to 40 min in NZW rabbit bucks exposed to 40°C with relative humidity 60-65%.



**Table: 6. Libido and physical semen characteristics of Rex rabbit bucks drinking water containing different levels of LOG.**

Item	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Libido-sexual desire-(Sec.)	43.56 <sup>a</sup> ±5.89	32.64 <sup>b</sup> ±4.18	25.17 <sup>bc</sup> ±3.46	22.31 <sup>c</sup> ±3.17
Semen ejaculate volume (ml)	0.44 <sup>c</sup> ±0.14	0.61 <sup>bc</sup> ±0.12	0.76 <sup>ab</sup> ±0.11	0.89 <sup>a</sup> ±0.13
Mass motility (Score)	2.98 <sup>c</sup> ±0.03	3.26 <sup>b</sup> ±0.06	4.41 <sup>a</sup> ±0.04	4.45 <sup>a</sup> ±0.06
Advanced sperm motility (%)	56.12 <sup>c</sup> ±1.47	68.52 <sup>b</sup> ±2.01	73.18 <sup>a</sup> ±1.98	76.24 <sup>a</sup> ±2.21
Dead spermatozoa (%)	23.24 <sup>a</sup> ±2.16	18.42 <sup>b</sup> ±1.19	15.05 <sup>c</sup> ±1.07	14.34 <sup>c</sup> ±1.11
Sperm abnormalities (%)	21.28 <sup>a</sup> ±1.98	17.89 <sup>b</sup> ±1.87	13.91 <sup>c</sup> ±1.00	11.82 <sup>d</sup> ±1.04
Acrosomal damages (%)	17.62 <sup>a</sup> ±1.72	14.71 <sup>b</sup> ±1.33	12.99 <sup>b</sup> ±1.43	10.15 <sup>c</sup> ±1.28
Sperm-cell concentration (N <sub>x</sub> 10 <sup>6</sup> / ml)	414.6 <sup>c</sup> ±31.4	482.4 <sup>b</sup> ±27.9	561.7 <sup>a</sup> ±38.1	597.6 <sup>a</sup> ±36.9
Total-sperm output ((N x 10 <sup>6</sup> / ejaculate)	182.4 <sup>d</sup> ±19.5	294.3 <sup>c</sup> ±22.7	426.9 <sup>b</sup> ±31.9	531.9 <sup>a</sup> ±34.0

a, b, c and d: Means in the same row having different superscripts differ significantly ( $P < 0.05$ ).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.

This delay may be due to the decrease in testosterone concentration, minimal spermatogenesis (Zeidan et al., 1997) and/or the low quality semen (El-Kelawy et. al., 1997), occurring in a hot climate.

Semen characteristics vary from one season to another. Such seasonal variations have been attributed to variations in atmospheric temperature and length of photoperiod (Hafez, 1987). Particularly, the increase in temperature adversely affects semen quality and quantity. The disorders caused by high ambient temperature become more pronounced when relative humidity is high (Hu et al., 1983; El-Sherbiny, 1994; Zeidan et al., 1997; Marai et al., 1998). Burfening and Ulberg (1968) reported that fertilization rate decreased from 98 to 96% and embryo survival rate decreased from 59 to 34% when rabbit spermatozoa were incubated at 38-40°C for 3 hrs, respectively.

Semen volume per ejaculate was found to be lowest in summer and highest during spring, winter and autumn, in NZW rabbits (El-Sherbiny 1987 and 1994). Similarly, Pingel and Abou El-Ezz (1981) observed that ejaculate volumes in NZW and Cal buck rabbits were lowest in summer and highest in autumn, winter and spring and Hu et al., (1983) reported that ejaculate volume was significantly lower in summer than in winter. Macirone and Walton (1983) confirmed such results. El-Sherbiny (1994), Marai et al., (1996,

1998) and Daader et al., (1999a,b) indicated that the lowest values of the semen ejaculate values of NZW and Cal rabbits were recorded in summer and the highest in winter season. However, Ibrahim (1994) found that semen ejaculate volume was higher in summer than in winter, and Blume et al., (1977) and Rastimeshin (1979) reported that season of the year had no significant effects on semen ejaculate volume of rabbit bucks. Also, Abd-El-Hakeam et al., 1992 reported a significant difference in semen volume between NZW (0.58 ml) and Cal (0.47 ml) bucks.

Generally, the changes in ejaculate volume may be due to a low sperm concentration, a decrease in the volume of seminal plasma (Macirone and Walton, 1983) or to hypoactivity of the accessory glands and the testes due to the adverse effect of high ambient temperature (El-Sherbiny 1994; Zeidan et al., 1997). Particularly, accessory gland secretion and spermatogenesis are controlled by testosterone hormone concentration which is lowest in the summer (Hammond et al., 1983).

Motility of rabbit spermatozoa was significantly reduced during the summer, but the differences among values obtained during autumn, winter and spring were not significant (El-Sherbiny, 1987). Similarly, Marai et al., (1996 and 1998) and Daader et al., (1997, 1999a and b) reported that the sperm motility percentage was lower in summer than in winter. However, Rastimeshin (1979) reported that sperm motility

was highest in summer in Soviet Chinchilla bucks. Other studies showed that sperm motility of NZW bucks was higher in spring than in autumn (Blume et al., 1977). Such seasonal variations may be due to the change in sperm cell concentration, which is influenced by variation in temperature or length of photoperiod (El-Sherbiny, 1987).

Sperm cell concentration was found lower in summer than in other seasons of year (El-Sherbiny, 1987; Ibrahim, 1994). Similarly, Kadlecik (1983) found that the lowest values of sperm cell concentration were recorded in summer and the highest ones in spring. However, Abou-Warda (1994) found insignificant effects of season of the year on sperm cell concentration.

Total number of spermatozoa in an ejaculate is a highly important factor in male fertility and depends on both semen ejaculate volume and sperm cell concentration. El-Sherbiny (1987) recorded a significantly lower number of spermatozoa in summer ejaculates than in the other seasons of the year. The same author estimated total sperm output of rabbits at 387.1, 362.9, 342.8 and 52.1 (310/ ejaculate) in autumn, winter, spring and summer, respectively. Similarly, Daader et al., (1997) and Marai et al., (1998) found that the lowest values of the total sperm-output (310/ ejaculate) of rabbits were recorded in the summer season. Such results may be due to the adverse effects of high temperature on spermatogenesis processes (Cosser et al., 1979).

Sperm abnormalities and dead sperm were highest in the summer (Pingel and Abou El-Ezz 1981; El-Sherbiny 1987; Finzi et al., 1995; Marai et al., 1996 and 1998; Daader et al., 1997), although (Virag et al., 1992) and (El-Masry et al., 1994) found that environmental conditions did not significantly modify the incidence of sperm abnormalities. The increase in percentage of sperm abnormalities in summer may be due to the adverse influence of high temperature on spermatogenesis, which could lead to high percentages of deformed spermatozoa (Zeidan, 1989).

The significant differences in dead sperm ratio during the different seasons of the year were confirmed by (El-Sherbiny 1987 and Ibrahim 1994). Also, El-Sheikh (1991) found that, the percentage of dead sperm was 21.85% and 23.75% in Cal and NZW, respectively.

The increase in percentage of dead spermatozoa after exposure to high temperature may be due to the adverse effect of heat stress on epididymal function, which is under the control of testosterone that is affected negatively by heat

stress (Marai and Habeeb, 1998).

### **Enzymatic semen activity of the experimental group rabbits**

As shown in Table (7) adding bioactive natural mixture (LOG) at 10, 20 and 30 ml LOG/ liter drinking water caused significantly ( $P<0.05$ ) decreasing in values of GOT, GPT, acid phosphatase and alkaline phosphatase of semen Rex rabbits. These results in harmony with those found by Zeweil et al., (2013) who noted that seminal AST showed a significant ( $P<0.003$ ) reduction that reaching about 94% with V-Line rabbit bucks fed 3% pomegranate peel (PP) containing diet compared to the control one. Mean while seminal alkaline phosphatase showed a non significant reduction that reach to 91, 89 and 83% for rabbits fed diets containing 1.5, 3 and 4.5% PP comparing to control bucks.

### **Mating activity and sexual hormones concentration**

Data presented in Table (8) cleared that dietary treatments significantly ( $P<0.05$ ) increased the values of mating activity (frequency of mating/ 15 minutes) and sexual hormones composed of (testosterone concentration, estradiol 17  $\alpha$  and progesterone) comparing to control. Values of the parameters mentioned above were increased with increasing the level addition of LOG in comparison with the control. These results in harmony with those reported by Gado *et al.* (2015) who noted that adding 1, 3 and 5 kg enzyme complex/ ton of diet significantly ( $P<0.05$ ) increased sexual hormones that includes (testosterone concentration, estradiol 17  $\alpha$  and progesterone).

### **Fertility traits of Rex rabbit does artificially inseminated**

Data of Table (9) cleared that Rex rabbit does that artificially inseminated with adding LOG in drinking water at 10, 20 and 30 ml/ liter water were realize an improving in both total numbers of pregnant does and kindled does comparing with the control. On the other hand abortion rate percentages was decreased with increasing level of adding from LOG in drinking water. Meanwhile, conception rate and kindling rate percentages were gradually increased with increasing additional levels of LOG in drinking water.

**Table: 7. Enzymatic semen activity Rex rabbit bucks drinking water containing different levels of LOG.**

Enzymatic semen activity	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/liter drinking water (D <sub>2</sub> )	20 ml LOG/liter drinking water (D <sub>3</sub> )	30 ml LOG/liter drinking water (D <sub>4</sub> )
GOT (U/ L)	30.91 <sup>a</sup> ±2.08	28.16 <sup>ab</sup> ±1.97	24.71 <sup>bc</sup> ±1.22	22.98 <sup>c</sup> ±0.65
GPT (U/ L)	21.37 <sup>a</sup> ±1.00	19.22 <sup>b</sup> ±1.04	18.02 <sup>bc</sup> ±0.61	16.99 <sup>c</sup> ±0.92
Acid phosphates (U/ L)	28.41 <sup>a</sup> ±1.11	25.32 <sup>b</sup> ±1.36	21.19 <sup>c</sup> ±1.21	20.37 <sup>c</sup> ±0.99
Alkaline phosphates (U/ L)	37.81 <sup>a</sup> ±1.67	33.14 <sup>b</sup> ±1.47	32.11 <sup>b</sup> ±1.14	28.91 <sup>c</sup> ±1.22

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.

**Table: 8. Mating activity and sexual hormones concentration of Rex rabbit bucks drinking water containing different levels of LOG.**

Item	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/liter drinking water (D <sub>2</sub> )	20 ml LOG/liter drinking water (D <sub>3</sub> )	30 ml LOG/liter drinking water (D <sub>4</sub> )
Mating activity (frequency of mating/ 15 minutes)	2.43 <sup>d</sup> ± 0.067	2.98 <sup>c</sup> ± 0.032	3.69 <sup>b</sup> ± 0.083	3.80 <sup>a</sup> ±0.014
Testosterone concentration (ng/ ml)	5.74 <sup>d</sup> ±0.006	5.86 <sup>c</sup> ±0.005	5.99 <sup>b</sup> ±0.004	6.15 <sup>a</sup> ±0.002
Estradiol 17 α (pg/ ml)	28.61 <sup>c</sup> ±1.87	32.54 <sup>b</sup> ±1.11	36.72 <sup>a</sup> ±1.52	38.65 <sup>a</sup> ±2.23
Progesterone (pg/ ml)	0.67 <sup>c</sup> ±0.009	0.74 <sup>b</sup> ±0.031	0.79 <sup>ab</sup> ±0.026	0.82 <sup>a</sup> ±0.028

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.

**Table: 9. Fertility traits of Rex rabbit does artificialy inseminated drinking water containing different levels of LOG.**

Item	Experimental diets			
	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/liter drinking water (D <sub>2</sub> )	20 ml LOG/liter drinking water (D <sub>3</sub> )	30 ml LOG/liter drinking water (D <sub>4</sub> )
No. of inseminated does	30	30	30	30
No. of pregnant does	17	21	26	27
No. of kindled does	16	20	26	27
Abortion rate (%)	5.88	4.76	0.00	0.00
Conception rate (%)	56.67	70.00	86.67	90.00
Kindling rate (%)	53.33	66.67	86.67	90.00
Litter size at birth (No.)	6.91 <sup>d</sup> ±0.87	7.88 <sup>c</sup> ±0.90	8.96 <sup>b</sup> ±0.82	9.79 <sup>a</sup> ±0.77
Litter weight at birth (g)	276.3 <sup>c</sup> ±18.5	318.4 <sup>b</sup> ±21.8	362.5 <sup>a</sup> ±20.6	395.9 <sup>a</sup> ±28.5
Bunny weight at birth (g)	39.99±2.75	40.41±1.59	40.46±1.77	40.44±2.16
Litter size at weaning (No.)	5.99± <sup>d</sup> 0.61	7.11±0.46	8.32 <sup>b</sup> ±0.41	9.24 <sup>a</sup> ±0.50
Litter weight at weaning (g)	4719 <sup>d</sup> ±101	5841 <sup>c</sup> ±84	7126 <sup>b</sup> ±149	8124 <sup>a</sup> ±158
Bunny weight at weaning (g)	787.9 <sup>c</sup> ±14.6	821.6 <sup>b</sup> ±16.2	856.5 <sup>a</sup> ±13.9	879.3 <sup>a</sup> ±17.4

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.

Also results obtained in Table (9) mentioned that dietary treatments significantly ( $P<0.05$ ) increased litter size numbers at birth, litter weight at birth, bunny weight at birth, Litter size numbers at weaning; litter weight at weaning and bunny weight at weaning. These results in agreement with those recorded by Kalaba (2012) who, reported that kindling rate was significantly ( $P<0.05$ ) higher in rabbit does fed diet supplemented by chromium yeast (Cr) group (85%) than in vitamin E plus selenium yeast (VE+Se) group (65%) and control group (60%), respectively.

Meanwhile, both Cr and VE+Se treatments significantly ( $P<0.05$ ) reduced gestation period length (GPL) of does as compared to those in the control group (30.90 and 31.37 vs. 32.5 days, respectively). Also, they noted that supplementation of Cr or VE+Se treatment of rabbit does significantly ( $P<0.05$ ) increased litter size in term of greater number of live and lesser number of dead kits, associating with higher viability rate and average weight of kits at birth as compared to the control group. This was reflected in significantly ( $P<0.05$ ) higher reproductive index in both treatment groups than in control group. It is of interest to observe that rabbit does treated with Cr showed better litter size characteristics than those of does treated with VE+Se, but the differences were not significant. Litter size of both Cr and VE+Se significantly increased (7.41 and 7.15/does) in term of greater number of live (7.07 and 6.21/does) and lesser number of dead kits (0.36 and 0.94/does) with higher viability rate (95.4 and 86.9%) and average weight of kits at birth (61.2 and 59.0 g) as compared to control (5.39, 3.17, 2.22/does, 58.8%, 55.9 g, respectively). reproductive index was higher ( $P<0.05$ ) in Cr and VE+Se (6 and 4) than in control (1.9).

#### **Milk yield and pre-weaning mortality rate**

As presented in Table (10) values of milk yield for Rex rabbits does were significantly ( $P<0.05$ ) increased with increasing level adding of LOG in drinking water at different times of determination (from birth to 7 days; 8 to 14 days; 15 to 21 days; 22 to weaning and over the entire period nursing from birth to weaning) in comparison with control one. Milk yield was significantly ( $P<0.05$ ) improved by 8.33, 17.29 and 24.67% for D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>, respectively compared to control (D<sub>1</sub>). Does' milk yield was found to be significantly lower in summer than in the other seasons of kindling (El-Sayiad, 1994 and Habeeb et al., 1999a). Particularly, Szendro et al., (1998)

indicated that milk yield was significantly lower at 30 °C (high ambient temperature) than at 5 °C (low ambient temperature) after the first 2 weeks of lactation.

From another point of view, milk yield was the highest in autumn and winter at all stages of lactation (Nasr, 1994), during autumn and winter at the third week of lactation (Ahmed et al., 1999), in winter and spring during the first 3 weeks of lactation (Ayyat et al., 1995) and during winter at 1<sup>st</sup>, 7<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days of lactation (Bassuny, 1999).

Daily milk yield was found to be lower by nearly 10% ( $P<0.05$ ) during the hot period of the day (Maertens and De Groote, 1990), by 7.7 g when the temperature rose above 20 °C (Rafai and Papp, 1984) and by 62.29% ( $P<0.01$ ) in summer (48.8 g) than in winter (Ayyat and Marai, 1998). Milk intake was higher for kits born in January (winter season) than for those born in July (summer season) during the 1<sup>st</sup> and 4<sup>th</sup> weeks of lactation. Milk efficiency (kg milk/kg meat) was also found to be affected by season of kindling, being significantly higher for kits born in January than for those born in July (Habeeb et al., 1999a). Also, mortality rate percentage during suckling period was significantly ( $P<0.05$ ) decreased at the same times mentioned above with adding LOG in drinking water compared to control (Table 13). Mortality rate was significantly ( $P<0.05$ ) decreased by 29.81, 46.01 and 57.99% for D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>, respectively comparing to control (D<sub>1</sub>). These results were in agreement with those noted by (Badr et al., 2013) who recorded that when rabbits received drinking water supplemented with 1, 2 and 3 ml of natural mixture juice (NMJ) of garlic, onion and lemon/ Liter filter drinking water caused significantly ( $P<0.05$ ) decreasing in total mortality rate (18.18, 12.73 and 10.91 %) in comparison with control group (21.82%). Also, ( Omer et al., 2016) reported that mortality rate was decreased with increasing the level of LOG that added to broiler chick rations through out over the entire period. Also, they noticed that the corresponding values of mortality rate were 5.77, 5.77, 3.48 and 1.92% for broiler chicks received diets containing 0, 10, 20 and 30 ml LOG/ kg feed, respectively. On the other hand, Tollba and Hassan (2003) reported that garlic as a natural feed additive decreased mortality rate of broilers. In addition, Rahimi et al., (2011) noted that adding 0.1% extract of garlic in the drinking water caused decreasing in mortality rate of broiler chickens compared to the control group (0.50% vs. 1.25%).



**Table: 10. Milk yield and pre-weaning mortality rate of Rex rabbit does artificially inseminated drinking water containing different levels of LOG.**

Item	Periods		Experimental diets			
	From	Up to	Control Zero LOG (D <sub>1</sub> )	10 ml LOG/ liter drinking water (D <sub>2</sub> )	20 ml LOG/ liter drinking water (D <sub>3</sub> )	30 ml LOG/ liter drinking water (D <sub>4</sub> )
Milk yield (g/ doe)	Birth	7 days	512 <sup>a</sup> ±21.2	552 <sup>b</sup> ±17.3	599 <sup>a</sup> ±20.9	622 <sup>a</sup> ±21.4
	8 days	14 days	706 <sup>c</sup> ±24.4	759 <sup>b</sup> ±26.2	829 <sup>a</sup> ±36.7	893 <sup>a</sup> ±34.5
	15 days	21 days	843 <sup>c</sup> ±41.9	931 <sup>b</sup> ±37.7	1023 <sup>a</sup> ±40.1	1102 <sup>a</sup> ±43.8
	22 days	Weaning	784 <sup>c</sup> ±28.3	841 <sup>b</sup> ±25.1	885 <sup>a</sup> ±24.7	931 <sup>a</sup> ±28.6
<b>Total milk yield, during nursing period (g/ doe)</b>			2845 <sup>c</sup> ±61.8	3082 <sup>c</sup> ±97.4	3337 <sup>b</sup> ±101.6	3547 <sup>a</sup> ±107.9
Pre-weaning mortality (%)	Birth	7 days	7.21 <sup>a</sup> ± 0.92	3.80 <sup>b</sup> ±0.575	3.00 <sup>c</sup> ±0.23	2.65 <sup>c</sup> ±0.27
	8 days	14 days	3.92 <sup>a</sup> ±0.23	2.63 <sup>b</sup> ±0.25	2.21 <sup>c</sup> ±0.083	1.56 <sup>d</sup> ±0.05
	15 days	21 days	2.04 <sup>a</sup> ±0.04	2.03 <sup>a</sup> ±0.05	1.36 <sup>b</sup> ±0.03	1.19 <sup>b</sup> ±0.03
	22 days	Weaning	1.04 <sup>a</sup> ±0.16	1.38 <sup>a</sup> ±0.14	0.92 <sup>b</sup> ±0.01	0.40 <sup>c</sup> ±0.01
<b>Total pre weaning mortality rate, during suckling period (%)</b>			13.52 <sup>a</sup> ±1.34	9.49 <sup>b</sup> ±1.02	7.30 <sup>c</sup> ±1.002	5.68 <sup>d</sup> ±0.162

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).

LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

However, Fayed et al., (2011); El-Tazi et al., (2014a & 2014b) noted that no mortalities were recorded among the different treatment broiler chicks groups that fed diets contained garlic or garlic essential oil throughout the experimental period; this may be due to the hygienic situation of the experiment.

During the post weaning phase, Habeeb *et al.* (1997) estimated the mortality rate at 18% in the summer, while no mortality was recorded during the winter. During 4–5 weeks of age, mortality rate percentage was significantly lower in spring than in winter and autumn (El-Desoki, 1991). During 5-9, 9-13 and 5-13 weeks of age intervals, mortality rate values were higher in summer and lower in spring than in autumn and winter (Shehata et al., 1998). However, El-Maghawry (1993) found that the maximum mortality was recorded during spring, while the minimum mortality was recorded in autumn.

## CONCLUSION

From the results obtained it can be mentioned that under condition as that available during carrying out of this work, incorporation nature bioactive mixture compose of lemon, onion and garlic (LOG) juice in drinking water of Rex rabbits caused an improving in their average daily gain and feed conversion. Also, it improves their reproductive performance through out increasing live body weight at first mating with decreasing the age at first mating with decreasing the age at first mating and decreasing the mortality rate.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

Hamed A.A.Omer cooperation in designed and performed the experiments, alculated the data, statistical analysis, wrote the manuscript and reveision the MS during the steps of publication.

Azza M.M. Badr cooperation in designed and performed the experiments, following the publication of the MS.

Neamat I. Bassuony cooperation in prepared bioactive mixture reviiion the MS.

Tarek S. Toufic cooperation in designed and performed the experiments and following up the field work

All authors read and approved the final version.

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