PHYSIOLOGICAL RESPONSE, SEMEN QUALITY AND BLOOD BIOCHEMICAL PARAMETERS OF RABBIT BUCKS SUPPLEMENTED WITH PHYTOGENIC COMPONENTS DURING SUMMER SEASON OF EGYPT

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SUMMARY

Seventy-two mature New Zealand White (NZW) rabbit's bucks were randomly and equally divided into eight groups each of nine bucks to evaluate the impact each of rocket seeds (RS), carrot seeds (CS), or bay laurel leaves (BLL) and their mixtures on semen quality, biochemical components and the physiological response of NZW rabbits bucks during hot summer conditions. The study started in June and lasted for 8 weeks. Eight experimental diets were formulated such that diet (D1) free additives as a control group. Diets 2, 3 and 4 contained 1.0% RS, 1.0% CS and 1.0% BLL, respectively. Diet 5, 6 and 7 contained a mix of 0.5% RS+0.5% CS, 0.5% CS+0.5% BLL and 0.5% RS+0.5% BLL, respectively, while diet 8 contained 0.33% RS+0.33% CS+0.33% BLL. Insignificant effect on buck's body weight, however, feed intake was significantly increased as compared to bucks group given the control diet. Supplementation with RS, CS, BLL, and their mixtures caused significant improvement in ejaculate volume, individual motility, total motile sperm, sperm concentration, live sperm%, total functional sperm fraction, total sperm output, and decreased abnormal sperm%. Seminal plasma initial fructose and globulin increased significantly with RS, CS, BLL, and their mixtures treatments. Seminal plasma alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) appeared reduction significantly with the RS, CS, BLL and their mixtures treatments. Seminal plasma total antioxidant capacity (TAC) increased, while, malondialdehyde (MDA) levels significantly decreased in all supplemented groups compared with the control bucks' value. Most of the supplemented groups showed a significant decrease in blood plasma glucose, cholesterol, triglycerides, and total lipids, low-density lipoproteins, AST and ALT as compared to the control group. However, blood plasma total protein and albumin were increased in comparison with the control group. Blood plasma TAC significantly increased due to the addition of RS, CS, BLL, and their mixtures as compared to the control group, but MDA levels decreased.

Keyword: *Semen, Rocket, Carrot, Bay laurel, Heat Stress, Total Antioxidant Capacity, Malondialdehyde.*

INTRODUCTION

In Egypt, summer temperature increases to more than 40 C^{o}. Some villagers believe that domestic rabbits as wild rabbit living outside can withstand extreme climatic conditions. This, in fact, is not as true as it occurs. Rabbits exhibit an inability to dissolve sufficient heat to maintain homeothermy. In addition, the relative humidity and metabolic heat are also, causative of heat stress. Most small - scale rabbit producers in the Egyptian villages confront numerous challenges, particularly stress of heat, related to the hot climate, that had a significant adverse effect on reproductive performance (Elnagar, 2010) and produces enormous economic losses (Ondruska *et al.* 2011, Mahrose *et al.*, 2010), cause a rise in oxidative stress (Lee, 2002) that may obstruct resistance to disease and weaken the status of antioxidants (Sabin *et al.*, 2001).

Recently, it was shown that in rabbit's fed natural feed additives (rocket seeds, carrot seeds or bay laurel leaves and their mixture) have increased antioxidant activities of rabbit females (Basyony and Azoz, 2017).

There is a growing interest in using natural sources of plants with medicinal properties, rocket (Eruca sativa) in Egypt. The strong demand for volatile oils for pharmaceutical goals has been steadily increasing. This plant has been found to personify the body condition to reduce disease stress (Eisenberg *et al.*, 1993).

Rocket seeds comprise vitamin C, flavonoids (luteoline and appiine), glucosinolates the ancestors of sulfaraphene and isothiocyanates, and carotenoids (Talalay and Fahey, 2001), volatile oils such as apiole β-phellandrene, myristicin (Leung and Foster, 1996, Bradley, 1992).

Glucosinolates have been found, which contain numerous antioxidant, antibacterial, anticarcinogenic and antifungal biological efficacy (Kim *et al.*, 2004). Additionally, have Fe, Mn, Cu, Mg, Zn and further elements (Abdo, 2003), that rise immune response of addition rocket seed, in rabbit fed diets significantly improved daily body gain and feed conversion and decrease daily feed intake (El-Nomeary *et al.*, 2016).

Carrot plants (Daucus Carota L) also contain a unique mixture of three flavonoids; quercetin, luteolin, and kaempferol (Horbowicz, *et al.*, 2008, Ching and Mohamed, 2001). Furthermore, they are opulent in other phenols along with several derivatives of cinnamic acid, containing β-hydroxybenzoic acids, caffeic and chlorogenic. Seeds of carrot caused to decrease in cholesterol (Da Silva Dias, 2014). Muralidharan *et al.*, (2008) observed that in carrot seed extract fed rats; levels of plasma lactate dehydrogenase, alanine transaminase, and aspartate transaminase were significantly declined. Moreover, Singh *et al.* (2010) observed that rats fed with carrot seeds showed a significant increase in antioxidant enzyme levels such as glutathione peroxidase, superoxide dismutase and, catalase as compared with the control group of rats.

Also, Bay laurel Nobilis L, which was presented as the high level of nutritional support due to the content of organic acids, polyunsaturated fatty acids, tocopherols and free sugars together with activity of antioxidant, like lipid peroxidation inhibition, scavenging effectiveness, and plummeting power (Dias *et al.* 2014). Plasma thiobarbituric acid reactive substances level was statistically lower in the rabbits receiving bay leaves in their diets, but plasma total antioxidant capacity was higher (Casamassima *et al.*, 2016).

The purpose of this investigation was hence to study the physiological response (body weight and feed intake), semen quality, antioxidant activities and blood biochemical parameters of rabbit bucks fed natural feed additives as rocket (Eruca Sativa) seeds, carrot seeds or Bay laurel leaves and their mixture during the hot climate periods.

MATERIAL AND METHODS

The experimental action was conducted at the Animal Production Research Station, El Nobaria (Behara Governorate), Animal Production Research Institute, Agricultural Research Center, Agriculture Ministry, Egypt. It was started in June and lasted for 8 weeks.

Experimental design

A total of 72 adults buck rabbits from New Zealand White (NZW) at age 7 months (mean initial body weight, 3095 g) were randomly assigned to eight experimental treatment groups each of nine bucks and assigned to a 2 X 4 factorial arrangement in a Completely Randomized Design. The buck rabbits were fed a diet in according to the following treatments order:

D1: Basal diets as a control group.
D2: + 1.0% rocket seeds.
D3: + 1.0% carrot seeds.
D4: + 1.0% bay laurel leaves.
D5: + 0.5% rocket seeds+0.5% carrot seed.
D6: + 0.5% carrot seeds+0.5% bay laurel leaves.
D7: + 0.5% rocket seeds+0.50% bay laurel leaves.
D8: + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves.

Experimental diets and housing

Rabbits were kept separately in galvanized wire cages (dimensions: 30 / 20 / 35 cm) under a light-dark cycle of 16:8 h. Pelleted feed ad libitum was fed to all rabbits. The experimental diet's chemical structure and feed ingredients are displayed in Table (1). Isocaloric and isonitrogenic were experimental diets. The experimental diets have been prepared to match to Lebas (2004) to converge the recommended nutrient

requirements of buck rabbits. A crushing machine (Thomas Wiley laboratory mill, model # 4, screen size-1 mm) was crushed in the laboratory plant seed material. Display to sunlight was averted to inhibit the damage of active constituents. Rocket seeds, carrot seeds, and bay laurel leaves with different levels as shown previous were mingled with one kg of every diet and mingled in the residual diet to achieve a homogeneous level of inclusion. Whole rabbits were retained in the same management, hygienic and environmental conditions. Rabbits were reared in a well-ventilated building; fresh water was automatically available all the time by stainless steel nipples fixed in each cage. During the experimental period (June−August), in the rabbitry, the minimum and maximum temperatures, the relative humidity, and the temperature-humidity index ranged 26.5– 33.5°C, 62–75% and 87.5–93.5, respectively. That means, during the whole experimental period rabbits were under harsh stress of heat as LPHSI (1990) describes.

Table (1). Composition and calculated chemical analyses (%) of the experimental diets.

Ingredient	D1	D2	D3	D4	D5	D6	D7	D8
Dried Egyptian clover	30.20	29.93	29.85	29.60	29.93	29.85	29.60	29.91
Barley	20.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
Rocket seed	0.00	1.00	0.00	0.00	0.50	0.00	0.50	0.33
Carrot seed	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.33
Bay laurel leaf	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.33
Yellow corn	14.80	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal (44% CP)	19.60	19.67	19.75	20.00	19.67	19.75	20.00	19.70
Molasses	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Di-Calcium phosphate	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit-Min premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100	100	100
Calculated chemical analysis (%, according to NRC, 1977)								
Dry matter	89.19	89.11	89.21	89.13	89.12	89.16	89.13	89.49
Organic matter	82.56	82.36	82.54	82.36	82.43	82.45	82.38	82.54
Crude protein	16.2	16.18	16.17	16.11	16.17	16.16	16.10	16.01
Crude fiber	12.34	12.39	12.41	12.51	12.40	12.80	12.11	12.69
Ether Extract	2.60	2.62	2.62	2.63	2.61	2.61	2.63	2.61
Ash	6.63	6.75	6.67	6.77	6.69	6.71	6.75	6.95
NFE**	51.42	51.17	51.34	51.11	51.25	50.88	51.54	51.23
NDF***	37.03	37.06	37.07	37.14	37.07	37.33	36.89	37.26
DE(kcal/kg)****	2542.0	2540.3	2539.70	2536.5	2540.0	2527.1	2549.4	2530.7

** Vit. and Min. mixture: Each kilogram of Vit. and Min. mixture contains: 150.000 IU Vit. D, 2000.000 IU Vit. A, 8.33 g Vit. E, 0.33 g Vit. B1, 0.33 g Vit. K, 1.0 g Vit. B2, 0.33g Vit. B6, 1.7 mg Vit. B 1, 8.33 g Vit.B 5, 23.33 g Pantothenic acid, 33 mg Biotin, 0.83g Folic acid, 11.7 g Zn, 12.5 g Fe, 16.6 mg Co, 66.7 g Mg16.6 mg Se, 5 g Mn. ,I 0.2mg and 200 g Choline chloride*
***NFE = Nitrogen free extract; ***NDF= Neutral detergent fibre; *** DE = Digestible energy of the experimental diets was calculated according to the equation described by Cheeke et al. (1986) as follows: DE (kcal/g) = 4.36-0.0491XNDF%, NDF = 28.924+0.657× CF%; D1= free additives supplementations (C), D2=C + 1.0% rocket seeds, D3= C+ 1.0% carrot seeds, D4= C+ 1.0% bay laurel leaves, D5=C + 0.5% rocket seeds+0.5% carrot seeds, D6=C + 0.5% carrot seeds+0.5% bay laurel leaves, D7= C+ 0.5% rocket seeds+0.50% bay laurel leaves, D8= CS+ 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves.*

Collection of data

For the whole experimental duration, feed intake and body weight were gauged. Utilizing an artificial vagina, semen samples were gathered weekly from each buck and chemical analysis of the samples was performed each week. Semen collection and handling were carried out and evaluated according to the international guidelines of (IRRG, 2005). Ejaculated volume was gauged to the nearest 0.01 mL. Weak eosin–formalin (10% formalin) solution was used for evaluation of sperm concentration by the improved

Neubauer hemocytometer slide method as described by Perumal *et al.* (2017). Total sperm output (TSO, 10^6/ejaculate) was calculated by multiplying semen ejaculate volume (mL) by sperm concentration (10^6/mL). Individual sperm motility was estimated at 400× magnification (Srivastava and Pande 2017a). Total motile sperm count (TMSC, 10^6/ejaculate), which is obtained by multiplying the sperm concentration (10^6/mL), the ejaculate volume (mL) and the percentage of individual motility. Total functional sperm fraction (TFSF, 10^6/ejaculate) as a product of TMSC by percentage of normal sperm morphology.

Seminal initial fructose assessment was conducted immediately after collection (Srivastava and Pande, 2017b). An eosin – aniline blue staining mixture was used to evaluate abnormal spermatozoa (Srivastava *et al.*, 2017). Seminal plasma was seceded for 20 minutes by centrifugation at 3000 rpm and stowed at -20 Co in Eppendorf tubes. Total protein, albumin, total lipids, cholesterol, total antioxidant capacity, malondialdehyde, alkaline phosphatase (ALP), ALT and AST were calorimetrically determined in seminal plasma using commercial kits obtained from (BIO- DIAGNOSTICS, Egypt) in accordance with the procedure outlined by the manufacturer.

Plasma was obtained by blood samples centrifugation for 20 min at 4000 rpm and stored at -20^{o}C in Eppendorf tubes until analysis. According to Fringes *et al.* (1972), Doumas *et al.* (1977), Armstrong and Carr (1964), respectively, the blood plasma total lipids, triglycerides, and glucose, were gauged using the spectrophotometer.

According to Johnson *et al.* (1999), albumin and total protein were measured; subtracting albumin values from the corresponding total protein values also obtained globulin values. Total cholesterol, lipoproteins of low density (LDL) and lipoproteins of high density (HDL) have been identified according to Burstein *et al.* (1970), Wieland and Seidel (1983), Bogin and Keller (1987), also very low-density lipoproteins (vLDL) was calculated as one-fifth of triglycerides. Plasma urea, AST and ALT were assayed according to Fawcett and Scott (1960), Reitman and Frankel (1975), respectively. Plasma total capacity of antioxidant (TAC) and lipid peroxidation; malondialdehyde (MDA) were gauged using Koracevic *et al.* (2001) method by calorimetric.

Statistical analysis

The experiment data were analyzed using one-way ANOVA from the SAS ® GLM procedure (SAS Institute, 2000), using the following model: $y_{ij}=\mu+T_i+e_{ij}$, Where: μ= Overall mean y_{ij}, T= treatment effect) and e_{ij}= experimental error. Using Duncan multi-range test (Duncan, 1955), the significant differences between means were detected.

RESULTS AND DISCUSSION

Effect of phytogenic feed additives on the physiological response (body weight and feed intake)

Dietary supplementation with different feed additives caused an insignificant effect on body weight of bucks, however, feed intake was significantly ($P \leq 0.05$) increased compared with the group of bucks given the control diet (Table 2).

Table (2): Overall means (±SE) of body weight and feed intake of rabbit bucks fed diets containing rocket seeds, carrot seeds, bay laurel leaves powder and their mixture during the experimental periods.

Treatment	Body weight (g)	Feed intake (g /kg/ day)*
*F*ree additives supplementations (control, C), D1	*3037±0.02*	*40.90±0.52*[b]
C+ 1.0% rocket seeds (D2)	*3090±0.05*	42.80±0.54[a]
C + 1.0% carrot seeds (D3)	*3120±0.04*	43.20±0.66[a]
C + 1.0% bay laurel leaves (D4)	3070±0.04	42.50±0.45[a]
C + 0.5% rocket seeds+0.5% carrot seeds (D5)	3045±0.04	43.00±0.53[a]
C + 0.5% carrot seeds+0.5% bay laurel leaves (D6)	3080±0.04	42.80±0.50[a]
C + 0.5% rocket seeds+0.50% bay laurel leaves (D7)	3060±0.05	42.90±0.53[a]
C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves (D8)	3050±0.04	42.70±0.63[a]

[a,b] *Means within a column having different superscripts are significantly different (P≤0.05).*

* *(g /kg/ day)= g for each 1 Kg of body weight in the day.*

Our results of feed intake were agreed with Ibrahim (2005), who found the basal diet supplemented by 1% rocket seed caused increase the feed intake when compared with the control group. The increase in feed intake of all groups treated compared to the control group this may be due to the stimulating effect of rocket seeds, carrot seeds or bay laurel leaves on the gastrointestinal system by enhancing dietary palatability and appetite.

Effect of phytogenic feed additives on Semen quality

Table (3) shows the overall means of ejaculate volume, individual motility, and sperm concentration. Ejaculate volume, individual motility and sperm concentration of rabbit bucks showed higher in D2, D5, D7, and D8 groups than D1, D3, D4, and D6 groups.

Table (3): Overall means (±SE) of semen ejaculate volume, individual motility and sperm concentration of rabbit bucks fed diets containing rocket seeds, carrot seeds, bay laurel leaves powder and their mixture during the experimental periods.

Treatment	Ejaculate volume (ml)	Individual motility (%)	Sperm concentration ($\times10^6$/ml)
*F*ree additives supplementations (control, C), D1	0.66±0.02^{c}	68.30±0.71^{c}	242.50±4.97^{c}
C+ 1.0% rocket seeds (D2)	0.91±0.03^{a}	84.10±0.91^{a}	269.10±3.97^{a}
C + 1.0% carrot seeds (D3)	0.68±0.02^{c}	66.90±0.73^{c}	233.00±4.54^{c}
C + 1.0% bay laurel leaves (D4)	0.70±0.02^{c}	67.90±1.26^{c}	241.30±3.30^{c}
C + 0.5% rocket seeds+0.5% carrot seeds (D5)	0.89±0.02^{b}	81.40±1.10^{ab}	262.90±3.34^{a}
C + 0.5% carrot seeds+0.5% bay laurel leaves (D6)	0.70±0.02^{c}	67.10±1.20^{c}	237.80±3.71^{c}
C + 0.5% rocket seeds+0.50% bay laurel leaves (D7)	0.88±0.02^{b}	80.40±0.80^{ab}	259.30±3.24^{a}
C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves (D8)	0.81±0.02^{b}	78.40±0.85^{b}	251.10±3.14^{b}

^{a,b,c} *Means within a column having different superscripts are significantly different (P≤0.05).*

Table (4) showed that D2, D5 and D7 treatment groups significantly ($P \leq 0.05$) increased in total motile sperm, total sperm output, and total functional sperm fraction due to different supplementations of feed additives as compared with the rest of treatment and control groups.

Table (4): Overall means (±SE) of total sperm output, total motile sperm and total functional sperm fraction of rabbit bucks fed diets containing rocket seeds**, carrot seeds, bay laurel leaves powder and their mixture during the experimental periods.**

Treatment	Total sperm output ($\times10^6$)	Total motile sperm count ($\times10^6$)	Total functional sperm fraction ($\times10^6$)
*F*ree additives supplementations (control, C) ,D1	160.05±6.77^{c}	109.31±3.99^{d}	90.84±3.47^{c}
C+ 1.0% rocket seeds (D2)	244.88±4.95^{a}	205.94±9.68^{a}	182.70±9.06^{a}
C + 1.0% carrot seeds (D3)	158.44±7.68^{c}	105.99±6.04^{d}	87.98±5.58^{c}
C + 1.0% bay laurel leaves (D4)	168.91±6.67^{c}	114.68±7.48^{c}	95.65±7.20^{c}
C + 0.5% rocket seeds+0.5% carrot seeds (D5)	233.98±6.15^{a}	190.46±7.60^{a}	168.56±7.24^{a}
C + 0.5% carrot seeds+0.5% bay laurel leaves (D6)	166.46±7.01^{c}	111.69±7.60^{c}	93.27±7.24^{c}
C + 0.5% rocket seeds+0.50% bay laurel leaves (D7)	228.18±7.35^{a}	183.46±7.60^{a}	158.69±7.24^{a}
C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves (D8)	203.39±7.22^{b}	159.45±7.60^{b}	139.53±7.24^{b}

^{a,b,c,d} *Means within a column having different superscripts are significantly different (P≤0.05).*

The results illustrated in Table (5) indicated that administration of (rocket seeds, carrot seeds, and bay laurel leaves) with different levels diet significantly ($P \leq 0.05$) raised initial fructose, conversely, they had significant decrease influence on abnormal sperm % as compared to the monitoring group. Additionally, significant ($P \leq 0.05$) improvement in live sperm % as compared to the control group.

Table (5): Overall means (±SE) of live sperm, abnormal sperm and initial fructose concentration of rabbit bucks fed diets containing rocket seeds**, carrot seeds and their mixture and bay laurel leaves powder during the experimental periods.**

Treatment	Live sperm (%)	Abnormal sperm (%)	Initial fructose (Mg/100 ml)
*F*ree additives supplementations (control, C), D1	71.00±0.41[c]	16.90±0.38[a]	235.60±5.27[b]
C+ 1.0% rocket seeds (D2)	80.30±0.55[a]	11.30±0.46[c]	287.20±7.53[a]
C + 1.0% carrot seeds (D3)	75.10±0.42[b]	17.00±0.31[a]	285.90±7.88[a]
C + 1.0% bay laurel leaves (D4)	76.80±0.60[b]	16.60±0.52[a]	291.10±10.54[a]
C + 0.5% rocket seeds+0.5% carrot seeds (D5)	79.80±0.61[a]	11.50±0.41[c]	292.00±6.19[a]
C + 0.5% carrot seeds+0.5% bay laurel leaves (D6)	74.80±0.56[b]	16.50±0.41[a]	297.00±6.12[a]
C + 0.5% rocket seeds+0.50% bay laurel leaves (D7)	78.10±0.66[a]	13.50±0.41[b]	291.50±6.72[a]
C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves (D8)	77.80±0.61[a]	12.50±0.41[b]	297.00±8.00[a]

[a,b,c] Means within a column having different superscripts are significantly different (P≤0.05)

Semen assessment pointed to a vital tool to elucidate the influences on male reproduction during summer conditions from internal and external agents. Eruca Sativa resulted in a significant augment in the concentration of sperm in the current report. This influence could be attributed to an enhancement in the synthesis of testosterone (hormone required to complete the spermatogenesis process; Walker, 2009) or to the activity of antioxidant in rocket seeds which can protect various stages of spermatozoa from apoptosis, leading to an increase in sperm production. In addition, using equal quantities of Eruca sativa, Raphanus sativus and Nigella sativa meals in place of 50% soybean protein meal, improved the semen characteristics (El-Tohamy *et al.*, 2010). The antioxidant effects of E. Sativa can be ascribed to this improvement, which is responsible for its stress - protective effect, thus protecting sperm by numerous antioxidants, belongs to the rocket seed phytochemical component and normalizes the concentration of sperm (Gharagozloo and Aitken, 2011, Ahlbom *et al.*, 2001). In addition, Hussein, 2013, Salem and Moustafa (2001) said that E. sativa improves healthy sperm characteristics and fertility. Ejaculate volume, percentages of motility and sperm concentration was significant (P<0.05) increase in the group of buck goat fed a diet containing 2 mg/Kg rocket seed oil as compared with control group (Hafez *et al.*, 2016).

El-Nattat and El-Kady (2007) found that the total function sperm fraction, total sperm per ejaculate, and total mobile sperm of buck rabbits was augmented by supplementary black cumin, radish, and rocket meal (at a level of 50%, respectively) and a mix of these meals at a level of about 17% each as a substitute for soybean meal. The improvement in the quality of semen detected herein may be associated with the existence of polyunsaturated fatty acids and various components of antioxidant in rocket seeds, carrot seeds, and bay laurel leaves. Sperms are more susceptible to be damaged through free radicals that initiate a cascade of lipid peroxidation that may harm the structure of the sperm plasma membrane (Attia *et al.*, 2017, Castellini *et al.*, 2003). The findings herein are in line with the results by El - Desoky *et al.* (2017) in the combination with dietary polyunsaturated fatty acids and antioxidants. Eruca sativa (rocket) is therefore considered to be a medicinal plant with many stated characteristic, comprising its robust erogenous influences (Font *et al.*, 2003), the existence of alkaloids, saponins, and flavonoids in extract of rocket (Pasini *et al.*, 2012) led to augment spermatogenesis (Homady *et al.*, 2000), and a significant rise in activity of sperm (Barillari *et al.*, 2005). These findings suggest that androgenic testis activity and/or vesicular gland activity in bucks receiving rocket seeds, carrot seeds or bay laurel leaves and their mixture.

The initial concentration of fructose in the sample of semen provides a decent sign of androgenic testis activity and/or vesicular gland activity (Srivastava and Pande 2017c).

Effect of phytogenic feed additives on seminal plasma analysis

Data illustrated in Table (6) showed the effects of different feed additives on seminal plasma total protein, albumin and globulin concentrations. The results indicated that seminal plasma albumin and total protein concentrations were not significantly (P≥0.05) affected by adding different levels of feed additive in comparison with the control group. While seminal plasma globulin concentration increased significantly (P ≤ 0.05) in the group D2, D3 and D7 diet, however, bucks gave D4, D5, D6, and D8 groups showed an insignificant increase in comparison with the D1 group given feed additives free-diet. Table (6) shows the overall means of seminal plasma AST, ALT, and ALP in rabbits' bucks. Dietary supplementation significantly (P ≤ 0.05) decreased ALP, AST, and ALT compared with the control group. The obtained

results showed a positive correlation between decreasing AST, ALT and ALP activity of seminal plasma and with each volume of ejaculates, percentage of live cells, individual motility, and concentration (Tables 3 and 5). Seminal plasma total lipids (Table 6) significantly ($P \leq 0.05$) decreased with diets supplemented with addition rocket seeds, carrot seeds, and bay laurel leaves and their mixture, however, 0.1% rocket seeds showed an insignificant decrease on seminal plasma total lipids as compared to the control group or the other treatment groups. Malondialdehyde concentration results presented in Table (6) indicate a significant increase ($P \leq 0.05$) in the animals fed the D1 diet. The groups treated with rocket seeds, carrot seeds, and bay laurel leaves showed a significant reduction ($P \leq 0.05$) in plasma MDA concentration compared to the group that fed the control diet. However, addition different plant seeds or leaves resulted in improving (TAC) as compared to the D1 group. The best TAC was recorded in the group D2, D3, D4, and D8 supplements diet followed by those groups in D5, D6, and D7.

Table (6). Effect of rocket seeds, carrot seeds, bay laurel leaves and their mixture on seminal plasma and malondialdehyde (MDA) and total antioxidant capacity (TAC) of buck rabbits.

	D1	D2	D3	D4	D5	D6	D7	D8
				Seminal plasma				
Total protein (mg/dl)	3.58 ±0.10	4.37 ±0.08	4.20 ±0.16	3.82 ±0.05	3.87 ±0.09	3.79 ±0.11	3.99 ±0.08	3.80 ±0.13
Albumin (mg/dl)	1.67 ±0.04	1.58 ±0.02	1.78 ±0.03	1.72 ±0.03	1.84 ±0.04	1.67 ±0.02	1.58 ±0.03	1.78 ±0.05
Globulin (mg/dl)	1.91^{b} ±0.09	2.79^{a} ±0.09	2.42^{a} ±0.17	2.10^{b} ±0.07	2.03^{b} ±0.10	2.12^{b} ±0.09	2.41^{a} ±0.09	2.02^{b} ±0.17
Aspartate aminotransferase (IU)	33.28^{a} ±1.05	23.63^{b} ±0.46	24.66^{b} ±0.41	23.10^{b} ±0.74	23.25^{b} ±0.60	23.28^{b} ±1.05	23.63^{b} ±0.49	24.66^{b} ±0.40
Alanine aminotransferase (IU)	44.93^{a} ±0.66	37.10^{b} ±1.18	30.70^{c} ±0.68	31.65^{c} ±0.97	29.81^{c} ±0.74	34.93^{b} ±0.66	37.10^{b} ±1.18	30.70^{c} ±0.68
Alkaline phosphatase (IU)	13.71^{a} ±0.18	12.41^{b} ±0.27	12.10^{b} ±0.28	11.96^{b} ±0.41	12.00^{b} ±0.33	11.51^{b} ±0.18	12.40^{b} ±0.27	12.30^{b} ±0.28
Total lipids (mg/dl)	239.90^{a} ±3.16	201.10^{b} ±2.96	218.70^{b} ±5.17	211.70^{b} ±4.70	216.10^{b} ±4.18	208.90^{b} ±3.14	206.10^{b} ±2.97	210.70 ^{b}±5.07
Cholesterol (mg/dl)	77.10^{a} ±1.13	61.40^{b} ±1.35	71.40^{b} ±1.56	65.70^{b} ±1.93	69.70^{b} ±1.68	67.10^{b} ±1.11	68.40^{b} ±1.34	63.40^{b} ±1.06
MDA (mmol/L)	1.36^{a} ±0.41	1.05^{b} ±0.32	1.02^{b} ±0.30	1.06^{b} ±0.23	1.12^{b} ±0.30	1.15^{b} ±0.47	1.04^{b} ±0.33	1.06^{b} ±0.31
TAC (mmol/L)	1.79^{c} ±0.27	2.50^{a} ±0.25	2.42^{a} ±0.30	2.65^{a} ±0.28	2.11^{b} ±0.27	1.18^{b} ±0.27	2.20^{b} ±0.25	2.40^{a} ±0.30

^{a,b,c} *Means within a row having different superscripts are significantly different ($P \leq 0.05$).*

D1=Free additives supplementations (control, C)., D2= C + 1.0% rocket seeds, D3= C + 1.0% carrot seeds, D4= C + 1.0% bay laurel leaves, D5= C + 0.5% rocket seeds+0.5% carrot seeds, D6= C + 0.5% carrot seeds +0.5% bay laurel leaves, D7= C + 0.5% rocket seeds +0.50% bay laurel leaves, D8= C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves.

The protein and globulin are the part of immunity components that albumin-based antibodies are the main protein component of serum which synthesized in the hepatic tissues. The functions of albumin include regulation of the distribution of extracellular fluid, and transport agent of many substances as bilirubin, fatty acids, hormones, and vitamins (Attia *et al.*, 2015).

Juma (2000) reported increased activity of the AST enzyme in the seminal plasma in summer. In semen, activities of transaminase (ALT and AST) are a perfect semen quality index because it assesses the stability of the sperm membrane (Umar *et al.*, 2017, Attia and Kamel, 2012, Juyena and Stelletta, 2012). In addition, a positive correlation between sperm acrosomal damage and enzyme release was attained (Chauban *et al.*, 1993). Additionally, high activity of seminal alkaline phosphatase in buffalo was related to depressing sperm counts, declined percentage of live cells and motility, the lowered activity of dehydrogenase also, insignificant and a tenuous reduction in the fructolytic rate (Abdou *et al.*, 1978).

The decrease in seminal plasma total lipids correlated with decreasing level of seminal plasma cholesterol, which decreased significantly, compared with the heat stressed control through the summer

season. The lowest values of cholesterol and total lipids were recorded in the group of bucks fed diet containing 0.1% rocket seed.

The significant increase of MAD in the animals fed the D1 diet may indicate that rearing rabbits under hot summer condition stimulated lipid peroxidation production and deterioration of cell membrane. The endocrine and biochemical condition of male animals are also influenced by the stress of heat. Summer heat increased the level of thiobarbituric acid reactive substances, which is an oxidative marker and decreased level of glutathione peroxidase (Kowalowka *et al.*, 2008, Nichi *et al.*, 2006). The presence of these phytochemical compounds in rocket and carrot seeds or bay laurel leaves can facilitate animals ' ability to maintain their body homeostasis, comprising body temperature, through endogenous cell defense mechanisms to deal with heat stress-induced inflammation and oxidative stress (Attia *et al.*, 2017, Akbarian *et al.*, 2016). Under normal condition, the seminal fluid around the sperm comprises factors of antioxidant (such as ascorbate, glutathione, taurine, urate, α- tocopherol, etc.) that protect against oxidative harm (Kim and Parthasarathy, 1998). Whereas in heat stress, the seminal liquid can either lack sufficient protecting basics or the buck's body may be burdened with reactive oxygen species to devastate the normal anti-oxidant mechanisms inherent in it. In addition to leucocytes in seminal plasma, augmented levels of reactive oxygen species can be produced inside harmed or injured sperm (Tamura *et al.*, 1988).

Effect of phytogenic feed additives on blood plasma constituents

Results showed that bucks fed rocket seeds, carrot seeds and bay laurel leaves and their mixture containing diets reduced the plasma glucose as compared to the control group Table (7). Total protein, albumin, and globulin means as affected by summer heat stress and amelioration methods are presented in Table (7). Plasma concentration of total protein from (D1-D8) ranged between 6.76±0.06 - 7.73±0.06 mg/dl. It was obviously indicated that total protein concentration from D2-D8 groups was significantly ($P \leq 0.05$) higher than the D1 group. Regarding the present plasma albumin (mg/dl) levels in rabbit's bucks were higher ($P \leq 0.05$) in group fed diet containing different levels of rocket seeds, carrot seeds and bay laurel leaves and their mixture treated groups than that of the control group. The average plasma albumin values in these groups were ranged between 3.52±0.07 – 4.33±0.10 mg/dl. Concerning plasma globulin (mg/dl), D3, D6, and D8 treated bucks showed higher ($P \leq 0.05$) levels compared to control and the other experimental groups. Plasma AST and ALT concentration were not significant ($P \geq 0.05$) affected by the addition of different plant seeds or leaves. It was observed that the different supplementations had no significant ($P \geq 0.05$) effect on plasma urea concentration (Table 7). Mean of blood plasma total lipids, total cholesterol, HDL, LDL and vLDL concentrations of rabbit's bucks as affected by different levels of plant seeds or leaves are illustrated in Table (7). The current results indicated that treating bucks with rocket seeds, carrot seeds, and bay laurel leaves and their mixture caused a significant ($P \leq 0.05$) reduce in total cholesterol of plasma, vLDL and LDL concentrations compared to the control. Cholesterol levels showed nearly the same trend as noticed for total lipids in all treated bucks. The reduction of cholesterol, LDL and vLDL levels ranged from (16.9 to 24.2%), (34.34 to 42.02%) and (21.05 to 39.65%) in all treated groups compared to that in control group, respectively. Plasma HDL level was not significantly affected by supplementing different levels of rocket seeds, carrot seeds, and bay laurel leaves and their mixture. On the other hand, blood plasma levels of triglycerides concentrations decreased significantly ($P \leq 0.05$) in bucks treated with 0.1% rocket seeds, 0.1% carrot seeds and 0.1% bay laurel leaves in comparison with the control group. There were significant differences between individual feed additives and their mixture groups. In addition, mixtures of rocket seeds, carrot seeds, and bay laurel leaves was more effective than that on rocket seeds, carrot seeds and bay laurel leaves as a sole feed additive on this trait. Table (7) presents the effects of various levels of natural plant seeds and bay laurel leaves on rabbit blood antioxidant constituents. A significant, triple mixture-dependent (rocket seeds, carrot seeds, and bay laurel leaves) decrease ($p \leq 0.05$) plasma MDA compared to other groups.

Al–Daraji and Razuki (2012) reported that supplementation of the roosters ration with rocket salad seeds resulted in significant decrease concentrations of glucose in blood plasma during most months of the experimental diets when compared with the control group.

The higher values of plasma proteins as affected by administration of different medicinal plants may reflect the improvement in digestibility and metabolism as previously referred (Attia *et al.*, 2015, Saleh, 2005).

Table (7). Effect of rocket seeds, **carrot seeds, bay laurel leaves and their mixture on blood plasma biochemical components and plasma total antioxidant capacity (TAC) and malondialdehyde (MDA) of buck rabbits.**

	D1	D2	D3	D4	D5	D6	D7	D8
Blood plasma								
Glucose (mg/dl)	110.00^{a} ±2.44	92.50^{b} ±1.78	93.90^{b} ±1.14	95.50^{b} ±0.88	93.60^{b} ±1.34	92.10^{b} ±2.44	91.80^{b} ±1.78	91.90^{b} ±1.14
Total protein (mg/dl)	6.76^{b} ±0.06	7.14^{a} ±0.10	7.70^{a} ±0.11	7.52^{a} ±0.15	7.26^{a} ±0.06	7.73^{a} ±0.06	7.15^{a} ±0.10	7.71^{a} ±0.11
Albumin (mg/dl)	3.52^{b} ±0.07	4.14^{a} ±0.11	4.33^{a} ±0.10	4.25^{a} ±0.15	4.09^{a} ±0.13	4.31^{a} ±0.05	(	4.05^{a} ±0.14
Globulin (mg/dl)	3.24^{b} ±0.06	3.00^{b} ±0.10	3.47^{a} ±0.14	3.27^{b} ±0.16	3.07^{b} ±0.10	3.42^{a} ±0.06	3.01^{b} ±0.10	3.66^{a} ±0.14
AST (IU)	56.50 ±0.46	54.20 ±0.35	55.70 ±0.36	55.60 ±0.33	54.00 ±0.38	56.50 ±0.40	57.20 ±0.35	56.70 ±0.35
ALT (IU)	30.20 ±0.21	31.20 ±0.20	29.30 ±0.30	28.90 ±0.28	30.50 ±0.29	30.70 ±0.31	32.20 ±0.32	34.30 ±0.30
Urea (mg/dl)	40.34 ±0.28	38.65 ±0.51	37.89 ±1.03	37.79 ±0.71	40.33 ±0.52	41.37 ±0.38	36.65 ±0.50	37.86 ±1.03
Cholesterol (mg/dl)	127.74^{a} ±2.61	111.62^{b} ±4.03	104.66^{b} ±4.31	109.96^{b} ±2.70	104.22^{b} ±2.39	101.14^{b} ±2.61	101.78^{b} ±4.13	105.28^{b} ±3.34
HDL (mg/dl)	62.00 ±2.08	68.50 ±2.86	59.90 ±2.46	63.00 ±2.21	60.20 ±1.99	55.00 ±2.09	56.50 ±2.85	62.90 ±2.47
LDL (mg/dl)	49.50^{a} ±0.77	28.70^{c} ±1.09	30.30^{b} ±1.03	31.30^{b} ±0.81	30.80^{b} ±0.98	32.50^{b} ±0.77	31.70^{b} ±1.09	29.00^{b} ±1.03
vLDL (mg/dl)	16.24^{a} ±0.02	14.42^{b} ±0.10	14.46^{b} ±0.10	15.66^{ab} ±0.09	13.22^{c} ±0.07	13.64^{c} ± 0.02	*13.58^{c} ±0.10*	13.38^{c} ±0.10
Triglyceride (mg/dl)	81.20^{a} ±1.12	72.10^{b} ±0.94	72.30^{b} ±2.34	78.30^{ab} ±1.15	66.10^{c} ±1.12	68.20^{c} ±1.10	*67.90^{c} ±0.91*	66.80^{c} ±2.24
Total Lipid (mg/dl)	405.90^{a} ±4.33	321.10^{b} ±13.61	318.30^{b} ±13.12	301.80^{b} ±12.79	302.50^{b} ±11.51	304.90^{b} ±4.33	321.30^{b} ±13.62	328.20^{b} ±12.15
TAC (mmol/L)	0.72^{d}±0.02	1.42^{b} ±0.07	1.43^{b} ±0.10	1.45^{b} ±0.10	1.10^{c} ±0.10	1.11^{c} ±0.04	1.12^{c} ±0.08	1.64^{a} ±0.10
MDA (mmol/L)	1.49^{a} ±0.04	1.00^{b} ±0.06	1.00^{b} ±0.05	1.06^{b} ±0.05	0.88^{c} ±0.05	0.84^{c} ±0.03	0.80^{c} ±0.07	0.60^{d} ±0.04

^{a,b,c,d} *Means within a row having different superscripts are significantly different (P≤0.05).*
D1= Free additives supplementations (control, C)., D2= + 1.0% rocket seeds, D3= C + 1.0% carrot seeds, D4= C + 1.0% bay laurel leaves, D5= + 0.5% rocket seeds+0.5% carrot seeds, D6= C + 0.5% carrot seeds+0.5% bay laurel leaves, D7= C + 0.5% rocket seeds+0.50% bay laurel leaves, D8= C + 0.33% rocket seeds+0.33% carrot seeds+0.33% bay laurel leaves, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, HDL=High density lipoproteins, LDL=Low density lipoproteins, vLDL=Very low density lipoproteins.

The extract and volatile oil of Eruca sativa seeds work as diuretics and significantly augmented Cl-, Na+, and K+, extraction in animal urine (Mahran *et al.*, 1992). Nevertheless, Eruca sativa seeds are utilized as an antimicrobial, lactagogue, and a diuretic to break the calculi of the renal system and to stimulate vomiting (Boulos, 1983).

The decrease in total cholesterol of plasma ($p \leq 0.05$) may be due to the highest binding capacity of natural plant seeds and leaves against glycodeoxycholic acid. It is slightly bound to taurocholic acid and taurodeoxycholic acid, which indicates that plant seeds and leaves may raise the excretion of fecal bile acid, resulting in lower cholesterol levels of plasma compared to control groups (Adisakwattana *et al.* 2010). In addition, Casamassima *et al.* (2016) showed that rabbits fed a diet containing one gram of dried bay leaves (Laurus nobilis) per one kilogram feed resulted in a significantly increased HDL cholesterol levels and reduction in AST and ALT levels, triglycerides, glucose, LDL cholesterol, and total cholesterol. The

capacity of total antioxidant may be a show for the accessibility of reduction factors in plasma, and therefore plasma's ability to clean free radicals created from processes of oxidation (Kambayashi *et al.*, 2009). So, the opposite influence was observed in regard to TAC, where the values were significantly ($p \leq 0.05$) augmented with an addition of additive-containing three types of plant products. These results were connected with Basyony and Azoz (2017) who reported that dietary supplementation of rocket (Eruca Sativa) seeds, carrot (Daucus Carota L) seeds or bay laurel leaves (Bay laurel Nobilis L), and their mixture improved significantly the antioxidant status of doe rabbits and their offspring's, during the pregnancy and lactation periods. In addition, Casamassima, *et al.* (2017) stated that serum levels of MDA were reduced in rabbits received a diet containing one g per kg of dried bay leaves (Laurus nobilis) in feed besides 2.5% pig fat than in rabbits fed 2.5% pig fat alone. This improvement tends to that phytogenic compound with antioxidant, antibacterial and anti-inflammatory properties (Attia *et al.*, 2017).

CONCLUSIONS

It is well demonstrated that the supplementation of rocket seeds, carrot seeds, bay laurel leaves, and their mixtures during the Egyptian summer season has positive effects on buck semen quality. In addition, it had a beneficial effect on blood lipid regulation which was demonstrated to be ascribed to their antioxidant activity and on lipid peroxide and the anti-oxidative status of rabbit reared under summer conditions. The phytochemical compounds in rocket and carrot seeds or bay laurel leaves can facilitate animals' ability to maintain their body homeostasis, comprising body temperature, through endogenous cell defense mechanisms to deal with heat stress-induced inflammation and oxidative stress.

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الاستجابة الفسيولوجية، وجودة السائل المنوي ومقاييس الدم البيوكيميائية في ذكور الأرانب المدعمة بإضافات علف نباتية خلال موسم الصيف في مصر

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تم تقسيم اثنان وسبعون ذكر أرانب نيوزيلندي الأبيض الناضج (NZW) بشكل عشوائي إلى ثماني مجموعات بكل منها تسعة ذكور لتقييم تأثير كل من بذور الجرجير أو بذور الجزر أو أوراق الاورا و مخاليطهم على جودة السائل المنوي، والمكونات الكيميائية الحيوية و الاستجابة الفسيولوجية لذكور الأرانب الـ NZW خلال ظروف الصيف الحارة. بدأت الدراسة في يونيو واستمرت لمدة 8 أسابيع. تمت عمل ثمانية اعلاف غذائية تجريبية كما يلي العلف الغذائي الأساسي(D1) بدون اي إضافات كمجموعة ضابطة (الكنترول). و تحتوي الوجبات الغذائية 2 و 3 و 4 على 1.0٪ بذور الجرجير و 1.0٪ بذور الجزر و 1.0٪ أوراق الاورا، على التوالي. يحتوي النظام الغذائي 5 و 6 و 7 على مزيج من (0.5٪ بذور الجزر+ 0.5٪ بذور الجرجير) و(0.5٪ بذور الجزر+ 0.5٪ أوراق الاورا و(0.5٪ بذور الجرجير+ 0.5٪ أوراق الاورا، على التوالي ، بينما يحتوي النظام الغذائي 8 على (0.33٪ بذور الجزر+ 0.33 % بذور الجرجير + 0.33٪ أوراق الاورا). تأثيرغير معنوي على وزن جسم الذكور، بالرغم من انه زاد تناول العلف بشكل معنوي بالمقارنة مع مجموعة ذكور مجموعة الكنترول. تسبب تدعيم العلف بـ بذور الجرجير أو بذور الجزر أو أوراق الاورا ومخاليطها في تحسن معنوي في حجم القذفة، الحركة الفردية التقدمية، تركيز الحيوانات المنوية، الناتج الإجمالي للحيوانات المنوية، إجمالي الحيوانات المنوية المتحركة، إجمالي عدد الحيوانات المنوية الوظيفية، الحيوانات المنوية الحية وانخفاض نسبة الحيوانات المنوية غير الطبيعية (الشاذة). زاد الفركتوز الاولي و الجلوبيولين في بلازما السائل المنوي بشكل معنوي مع المعاملات ببذور الجزر, بذور الجرجير, أوراق الاورا، ومخاليطهما. واظهرت النتائج انخفاضًا معنويا فى انزيمات AST، ALT، ALP في بلازما السائل المنوي مع المعاملات ببذور الجزر, بذور الجرجير, أوراق الاورا، ومخاليطهما. زادت القدرة الكلية لمضادات الأكسدة في البلازما المنوية، بينما انخفضت مستويات المالونالدهيذ (دليل اكسدة الدهون) بشكل معنوي في جميع المجموعات المدعمة مقارنةً بقيم ذكورالمجموعة الضابطة. أظهرت معظم المجموعات المدعمة انخفاضًا معنويًا في الجلوكوز والكوليسترول والدهون الثلاثية والدهون الكلية والكوليسترول منخفض الكثافة و AST و ALT في بلازما الدم مقارنةً بالمجموعة الضابطة. ومع ذلك، تم زيادة البروتين الكلي والألبومين في بلازما الدم مقارنة بمجموعة الكنترول. زادت القدرة الكلية لمضادات الأكسدة في بلازما الدم بشكل معنوي بسبب إضافة بذور الجزر, بذور الجرجير, أوراق الاورا، ومخاليطهما بالمقارنة مع مجموعة الكنترول, في حين انخفضت مستويات المالونالدهيد (دليل اكسدة الدهون).