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Effect of Different Medicinal Plant Seeds Residues on the Nutritional and Reproductive Performance of Adult Male Rabbits

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ABSTRACT

A total number of 50 male growing New Zealand rabbits were allotted and randomly divided into 5 equal groups, to study the effects of using radish, rocket and black cumin meal (at a level of 50%, respectively) and mixture of these meals at a level of 17% approximately for each as a replacement of soybean meal, on growth performance, semen characteristics, hemogram and serum biochemical parameters. Each group received diets containing nearly equal ratio of C/P under the same managerial conditions. Daily body weight gain showed a significant increment by 19.3, 19.4 and 14.2% and reduced by 20.0% for radish, rocket, mixture and black cumin diets, respectively compared to the control diet. In addition, daily feed intake for rabbit showed a significant increment by 4.9, 10.9 and 4.3% and declined by 9.4% for radish, rocket, mixture and black cumin diets, respectively compared to the control diet. Best feed to gain ratio was recorded for radish, mixture and rocket meals compared to the control diet. The semen characteristics revealed that the black cumin and the mixture diets gave the best results in case of reaction time, latency period, volume, motile sperm percentage, sperm concentration per mL, total sperm per ejaculate, total motile sperm and total function sperm fraction. On the other hand, radish showed good results concerning motile sperm percentage, motility percentage after one hour and the resazurin reduction activity. Feeding radish or mixture meals significantly decreased free radicals production in the seminal plasma. Addition of tested materials in the experimental diets of adult rabbits improved glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities and urea with no changes were observed for creatinine. To conclude, the inclusion of a mixture of equal quantities from radish, rocket and black cumin meals on the expense of 50% soybean meal protein caused improving in semen characteristics and nutritional performance with reduction of free radicals in male rabbits.

Key Words: Radish; Rocket; Black cumin; Rabbit semen characteristics; Nutritional performance

INTRODUCTION

The least cost diet formula is considered a target to rabbit nutritionists for achieving the best efficiency of utilization and economy in case of animal health and production. Soybean meal is the main plant protein source in rabbit diets. It is expensive in comparison to other non-traditional plant protein sources. The rabbit industry, especially in Egypt, needs new cheap non-traditional protein sources, locally available and enough to overcome the issue. These sources must have the privilege to enhance animal health and fertility. Few studies were carried out on rabbit non-conventional protein meal and literatures available are not enough to overcome the nutritive issue and its effects on animal health and reproduction.

Production of radish (Raphanus sativus), rocket (Eruca sativa) and black cumin (Nigella sativa) meals in Egypt has been steadily increased for the strong demand to volatile oils for pharmaceutical purpose. These plants were found to incarnate natural substances that promote health and ameliorate the body condition to counteract the stress of illness (Eisenberg *et al*., 1993).

Radish seeds were found to contain alkaloid like coumarins, saponins, flavonoids and anthocyanins (Sanaa, 2001). They decrease uric acid level in the serum, which related to circulating markers of inflammation and free radical reactions (Zaman, 2004). The anthocyanins are important group of dietary antioxidants that have many physiological functions. They protect living cells from oxidative damage resulting in the prevention of diseases (Matsufuji *et al*., 2003). Besides, radish seeds contain isothiocyanate that has antimicrobial activity, antimutagenic, anticarcinogenic and antiatherosclerosis activity (Suh *et al*., 2006).

The rocket seeds contain carotenoids, vitamin C, flavonoids such as appiin and luteolin and glucosinolates the precursors of isothiocyanates and sulfaraphene (Talalay & Fahey, 2001), volatile oils like myristicin, apiole and β-phellandrene (Bradley, 1992; Leung & Foster, 1996). Glucosinolates were found to have several biological activities including anticarcinogenic, antifungal, antibacterial plus their antioxidant action (Kim *et al*., 2004).

The major glucosinolate in seeds is Erucin, which is potentially capable of protecting cells against oxidative stress via three mechanisms: (i) induction of phase II enzymes, (ii) scavenging hydrogen peroxide and alkyl hydroperoxides accumulated in cells and peripheral blood and (iii) acting as a precursor of sulforaphene, a potent inducers detoxifying electrophiles and increase cellular antioxidant defenses (Barillari *et al*., 2005). They also contain Zn, Cu, Fe, Mg, Mn and other elements (Abdo, 2003), which increase immune response and the reproductive performance. Carotenoids can protect phagocytic cells from antioxidative damage, enhance T and B lymphocyte proliferative responses and increase the production of certain interleukins (Bendich, 1989). Also, they increase plasma I gG concentration (Chew *et al*., 2000).

The black cumin seeds contain thymoquinine that has antibacterial, diuretic, hypotensive and immunopotentiating activities via increasing neutrophil percentage and hence increasing the defense mechanism of the body against infection (Kanter *et al*., 2005). Black cumin oil and its derivatives inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation (El-Dakhakhny *et al*., 2002). The oil is also rich in fatty acid, (oleic, linoleic & linolenic acid) and carotene, which is converted into vitamin A (Al-Jassir, 1992). Besides, the seeds contain eight essential amino acid that improve natural immune system activity (Omar *et al*., 1999).

Reports about using radish, rocket, black cumin or mixture of these meals as non-conventional feed proteins and their effects on production and immunity in rabbit diets are not enough. On the other hand, no reports were found on their effect on the productive and reproductive performance of the rabbits. So, the present study aimed to monitor the effect of substitution a half part of soybean meal protein by inclusion each of radish, rocket, black cumin or mixture of these meals as cheap non-traditional sources of protein in rabbit bucks diet, on growth performance, semen characteristics and reactive species in seminal plasma and serum, the haemogram, liver and kidneys functions tests for male mature rabbits.

MATERIALS AND METHODS

The present study was carried out at the National Research Center Experimental Farm Station that located in Abou Rawash city, Giza Governorate, Egypt. Feed stuffs and tested materials (radish, rocket & black cumin meals) were obtained after extraction of oils at a commercial supplier. Meals were grounded to fine particles and chemically analyzed (Table I) for moisture, crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE) and ash according to the procedures of AOAC (1995).

Experimental design. A total number of fifty weaned white New Zealand male bunnies with average body weight of 611.7 g (6 weeks old including one week adaptation period) were randomly allotted into five equal groups (n = 10) in semi-automated horizontal steel batteries. Each bunny was caged separately. They were regimed on a photoperiod of 16 h light/day in the breeding season. Feed and water were provided ad libitum. Five experimental diets (Table II) was formulated and prepared in the form of pellets at Meladico Company to cover the requirements of the five experimental groups according to the NRC (1977). Individual live body weight, feed consumption and feed conversion ratio were recorded weekly during the experimental period.

Radish, rocket and black cumin meals were incorporated to the control diet on the expense of soybean meal protein at a level of 50% and a mixture of these meals at a level about 17% for each, composing 4 dietary treatments (Table II). Each experimental diet contained nearly equal ratio of calorie/protein (C/P) under the same managerial conditions. The experimental period lasted for 29 weeks.

Blood sampling and parameters. At the end of experimental period, blood was obtained from sacrificed rabbits in clean sterile tubes. Samples were let to coagulate and transferred to lab. on ice to be centrifuged at 3500 rpm for 15 min. at 4°C. Serum was separated and stored till assayed in ultrafreeze – 80°C. Heparinized blood samples were obtained at the end of the experiment for blood counts. Hemogram was carried out using automated cell counter (Model: Alsystem Al-Vet 300, Germany). Red blood cells count (RBCs x $10^6/mm^3$), haemoglobin (Hb, g/dL), haematocrit (Hct, %), with their indices [mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dL)], white blood cells count (WBCs x $10^3/mm^3$) and platelet cells counts ($10^3/mm^3$) were assessed.

Semen collection. Semen was collected twice weekly during the last 6 weeks of the experimental period. Bucks were trained to mount a teaser doe. Reaction time (the moment of subjecting a doe to the buck until the completion of erection estimated in seconds) was assessed using stopwatch. The latency period was assessed as the period between the dismount of the buck to the female and the second mount with complete erection (measured in seconds). Semen samples were collected twice weekly using an artificial vagina (IMV, France). Only the first ejaculate, white in color and gel-free was considered in the evaluation. Samples for reactive species and antioxidant assays were collected and centrifuged at 1000 rpm for 15 min. in cooling centrifuge at 4°C. The supernatant seminal plasma was aspirated into enumerated ependorff then stored at -80°C till assayed.

Semen evaluation and biochemistry. Immediately after semen collection, gel-free semen volume (using a graduated collection tube, IMV, France), pH (using a pH cooperative paper, ranged 5 - 9, Macherey-Nagel GmbH & Co, Düren, Germany), motility grade (0 - 9 score, according to the evaluation adopted by Petitjean, 1965) and percentage of motile sperm (PMS) were estimated (Boussit, 1989). Sperm cell concentration was estimated using an improved NeuBauer cell counter slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg, Germany). Total sperm per ejaculate (TSE) was obtained by multiplying the total gel-free volume by the sperm cell concentration per mL. The percentages of motile sperm and motility grade were estimated by visual examination under low-power magnification (10×) using a light binocular microscope. Total number of motile sperm (TMS) was calculated by multiplying PMS X TSE. Percentage of live sperm (PLS) and abnormal morphology percentage (PAM) were assessed using an eosin-nigrosin blue staining mixture (Blom, 1950) modified by the use of normal saline instead of sodium citrate. Total functional sperm fraction (TFSF) was calculated as the product of TSE X PMS X PAM (Correa & Zavos, 1996). The motility after 1 h (PMS after 1 h) of incubation at 37°C was estimated. All the measurements were made by the same expert researcher during the whole experiment. The resazurin reduction test (RRT) was used to detect the ability of metabolically active spermatozoa in the semen sample to reduce blue resazurin dye (20 mmol/L) at 37°C. A clear pink color (resorufin) is measured at two optical densities of 580 nm and 615 nm (Reddy & Bordekar, 1999). The antioxidative capacity was performed according to the procedure accepted by Koracevic *et al.* (2001). The nitric oxide was estimated according to the method assumed by Montgomery and Dymock (1961). Lipid peroxidation (LPO) assay was performed according to the procedure adopted by Ohkawa *et al*. (1979).

Liver and kidneys function. Liver function tests represented by serum glutamic oxalcetate transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) were estimated according to the method of Reitman and Frankel (1957). Kidneys function tests were represented in serum urea nitrogen [using a modified Berthelot reaction according to Tabacco (1979) and serum creatinine (using a modified Jaffe reaction adopted by Stanbio laboratory data)].

All chemicals used in the study were of analytical grade and obtained from commercial suppliers.

Statistical analysis. Data were statistically analyzed adopting one way ANOVA in the general linear model (GLM) using the statistical analysis system (SAS, guide ver. 6.04, 1988). The least significant difference test (LSD) was used for comparing means at a confidence limit of 95% in results (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

Chemical composition of tested meals. The proximate chemical analysis of radish, rocket and black cumin meals (Table I) showed that they contain reasonable amount of protein, NFE with little amount of CF. Concerning their feeding values, these nutrients may be considered as promising sources of energy (3345, 2988 & 2697 kcal DE/kg, respectively) and protein (35.30, 32.20 & 33.80%, respectively) in feeding rabbits. Osman *et al.* (2004) reported that radish meals contained 5.52% moisture, 24.90% CP, 6.71% EE, 10.07% CF, 50.40% NFE and 7.92% ash, while rocket meals contained 7.24% moisture, 36.03% CP, 7.64% EE, 7.69% CF, 36.81% NFE and 11.83% ash. The results of this study didn't vary from those reported by Flanders and Abdulkarim (1985), who reported that rocket seeds meal contained 4.1% moisture, 27.8% oil, 27.4% protein, 6.6% ash and 1186 Ca (mg/100 g). Also, Srinibas *et al.* (2001) mentioned that the EE content of taramira (*Eruca sativa*) full fat seeds was 24.87%, while the CP content was 30.24% on a dry matter basis. Concerning

Table I. Chemical analysis of feed stuffs for tested diets

Item	DM	Chemical analysis % (DM basis)						
	%	OM	CP	CF	EE	NFE	Ash	DE*
Feed stuffs:								
Yellow corn	89.00	98.50	8.47	2.19	4.37	83.47	1.50	3965
Soybean meal	98.00	94.50	44.00	7.10	0.70	42.70	5.50	3227
Wheat bran	89.00	88.70	14.00	11.00	3.00	60.70	11.30	2262
Clover hay	89.00	87.49	14.80	24.10	2.70	45.89	12.51	1660
Radish seed meal	94.80	94.80	35.30	4.80	10.67	44.03	5.20	3345
Rocket seed meal	94.30	92.20	32.20	4.24	10.85	44.91	7.80	2988
Black cumin meal	92.00	90.40	33.80	5.20	14.20	37.20	9.60	2697

DE*(Kcal/kg DM)= 4253 - 32.6 (CF %) - 144.4 (Ash %), according to Fekete amd Gippert (1986).

Table II. Composition and chemical analysis of tested diets

Ingredients	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixed diet
Clover hay	33.00	33.00	33.00	33.00	33.00
Yellow corn	21.00	19.00	19.00	19.00	19.00
Wheat bran	30.10	30.10	30.10	30.10	30.10
Soybean meal	14.00	7.00	7.00	7.00	7.00
Radish seed meal	--	9.00	--	--	3.00
Rocket seed meal	--	--	9.00	--	3.00
Nigella seed meal	--	--	--	9.00	3.00
Limestone	1.13	1.13	1.13	1.13	1.13
Vit+Min-Mix*	0.30	0.30	0.30	0.30	0.30
Common salts	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.17	0.17	0.17	0.17	0.17
Total	100.00	100.00	100.00	100.00	100.00
Chemical analysis determined (DM% basis)					
Dry matter (DM)	88.57	88.57	88.51	88.20	88.40
Organic matter (OM)	89.48	89.54	89.24	89.14	89.33
Crude protein (CP)	17.03	17.01	16.72	16.91	16.92
Crude fiber (CF)	12.73	12.70	12.60	12.80	12.70
Ether extract (EE)	2.79	3.80	3.70	3.90	3.80
Crude ash	10.53	10.46	10.76	10.86	10.67
Nitrogen free extract (NFE)	56.92	56.03	56.22	55.53	55.91
Calculated analysis DE** (Kcal/Kg)	2513	2508	2480	2450	2480
Calcium	0.92	0.87	0.87	0.87	0.87
Total phosphorus	0.50	0.45	0.45	0.45	0.45

* One kilogram of Premix provides: 2000000 IU vit.A, 150000 IU vit. D, 8.33 g vit. E, 0.33 g vit. K, 0.33 g vit. B_1, 1.00 g vit. B_2, 0.33 g vit. B_6, 8.33 g vit. B_5, 1.70 mg vit. B_{12}, 3.33 g Pantothenic acid, 33.00 mg Biotin, 0.83 g Folic acid, 200.00 g Choline chloride, 11.70 g Zinc, 12.50 g Iodine, 16.60 mg Selenium, 16.60 mg Cobalt, 66.70 g Magnesium and 5.00 g Manganese.

the black cumin, El-Adawy (2004) reported that black cumin seeds meal contained 6.53% moisture and the remained components % (on dry matter (DM) basis) were 94.58% OM, 34.21% CP, 3.07% CF, 8.81% EE, 48.49% NFE and 5.42% ash. A review of literature by Aherne and Kenelly (1982) and Ravindran and Blair (1992) have revealed that the differences between chemical compositions of oil seeds meals may be attributed to the variety of seeds, the processing method and the operators using the same techniques. This may have a pronounced reflection on the proportional content of different substances.

Rabbit performance. Final body weight and daily gain (Table III) for rabbit received different experimental diets showed a significant ($P < 0.0001$) increment by 19.3, 19.4 and 14.2% and significantly decreased by 20.0% for radish, rocket, mixed meals diet and black cumin diets, respectively compared to the control diet. While, daily feed intake for rabbits showed a significant ($P < 0.0001$) variation and rocket diet revealed an increment by 10.9% compared to the control diet. However, no significant differences were detected between radish or mixed meals or control diets in daily intake, while black cumin diet showed a significant decrease by 9.4% in daily intake compared to the control diet. Feed conversion ratio for rabbit received different diets showed a significant ($P < 0.05$) increment by 12.1, 7.1 and 8.7 for radish, rocket and mixed meals compared to the control diet. While, the worst value was 13.4% for black cumin compared to the control diet. Similar trend was observed by El-Alaily *et al.* (2001) and Ibrahim *et al.* (2005), who found that addition of radish extract to high fiber poultry diets contained artificial dried Miskawi Berseem or wheat bran as a source of fiber increased body weight gain. On the other hand, Abdo (2003) and Osman *et al.* (2004) found that radish or rocket seed meal at a level of 0 - 25% substitution for soybean protein showed no adverse effects on body weight and body weight gain values in broiler rations. However, Rajendra *et al.* (2000) reported that mustard cake, which belongs to the same family of *Eruca sativa* could be used up to 30% of the rabbit diet without an adverse effect on broiler rabbit performance.

Amber *et al.* (2001), Radwan *et al.* (2002) and El-Adawy (2004) found that live body weight, daily weight gain and feed conversion of rabbits fed control diet were significantly higher than those fed with substitution (25, 50, 75 & 100% black cumin meal) of CP supplied by soybean meal in control diet. The reduction may be due to lower feed intake and decreasing digestibility of almost nutrients and imbalance of essential amino acids profile with black cumin meal inclusion in diets.

Osman *et al.* (2004) indicated that replacing up to 15% of soybean meal by radish meal to broiler diets did not significantly affect feed consumption as compared to control diet group. However, rocket-fed group consumed a significant highest feed than those of the other replacements. Similar results were observed by Ibrahim (2005), who found that on supplementing rabbit basal diet with 1% rocket seeds, the highest intake observed may be due to its beneficial effect for stimulating and activating the digestive system by improving the diet palatability and enhancing appetite. Similar results in broiler were observed by Namur *et al.* (1988). In the present study, radish, rocket and mixed meals gave the best feed conversion values that may be attributed to the properties of these materials that act not only as antibacterial, antiprotozoal and antifungal but also as antioxidant (Bradley, 1992; Leung & Foster, 1996), while black cumin diet gave the worst value. These findings agree with the results obtained by Amber *et al.* (2001).

Semen characters and biochemistry. Semen evaluation indicated an important tool for clarifying the effect of external and internal agents affecting male reproduction. Data in the present study (Table IV) revealed that the black cumin and the mixture diets gave the best results in most of the semen parameters (9/14 parameters). They differed from each other in two parameters (motility grade & RRT) when compared to the control diet, although they were not significantly different when compared to each other. On the other hand, the radish gave a score of 4/14 followed by the control (3/14) and finally the rocket group (2/14). Moreover, 3/14 parameters (pH, PLS & PAM) were not significantly different among the five diets.

Concerning the biochemical analysis in seminal plasma (Table V), the present results showed that the radish fed group had the lowest free radicals production. This was indicated by the lowest concentration of malondialdehyde and the reactive nitrogen species, particularly the powerful oxidant molecule peroxynitrite (Zaman, 2004). Since, the semen includes white cells diffused from the male reproductive tract, so the production of reactive species is normal in certain limit due to cell activities. Thence, the lowering of production of these later products may be reasonable due to the presence of powerful antioxidant in radish (Matsufuji *et al*., 2003; Takaya *et al*., 2003) that reins in the reactive species production. This is interpreted via the results obtained in case of RRT, PMS and PMS after one hour, where the reactive species are impeded. Although, in case of the serum (Table V), the best results were obtained in the control group. The black cumin was good in case of lipid peroxides output in the seminal plasma, while it showed the highest reactive nitrogen species. The mixed diet standed in a mid-way as it included the three meals. This is confirmed by approximately good performance in case of most of the semen characters (main & calculated characters). Black cumin diet followed by radish diet, were the most effective in most semen parameters, while the worst was the rocket diet. Salem (2005) found that both the oil of black cumin and its active ingredients in particular thymoquinine, possess reproducible anti-oxidant effects through enhancing the oxidant scavenger system, which as a consequence lead to antitoxic effects induced by several insults. The presence of antioxidant and other stimulant materials in the meals of the three seeds had the power to

Table III. Growth performance of growing New Zealand rabbits fed on diets containing radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal protein

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixed diet
Initial live body weight (g)	612.5^{a} ± 50.19	586.25^{a} ± 60.05	632.5^{a} ± 60.84	618.75^{a} ± 72.12	608.5^{a} ± 36.40
Final live body weight (g)	2555^{b} ± 39.26	2902.50^{a} ± 115.14	2951.00^{a} ± 97.08	2171.25^{c} ± 74.90	2826.75^{a} ± 33.40
Daily feed intake (g)	123.78^{b} ± 1.18	129.85^{b} ± 1.53	137.37^{a} ± 2.97	112.17^{c} ± 2.26	129.11^{b} ± 2.46
Daily body weight gain (g)	12.07^{b} ± 0.21	14.39^{a} ± 0.79	14.40^{a} ± 0.61	9.64^{c} ± 0.30	13.78^{a} ± 0.39
Feed conversion ratio	10.27^{b} ± 0.10	9.03^{d} ± 0.14	9.54^{c} ± 0.12	11.63^{a} ± 0.08	9.37^{cd} ± 0.12

Data were recorded as mean ± SE.
Different superscripts in a row are significantly different at P<0.05

Table IV. The semen characteristics of New Zealand bucks fed on diets containing radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal protein

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixture diet
Reaction time (sec.)	17.44^{a} ± 1.63	10.61^{b} ± 0.78	16.64^{a} ± 1.92	8.47^{b} ± 0.62	7.67^{b} ± 1.03
Latency period (sec.)	80.67^{b} ± 7.72	134.14^{a} ± 14.94	153.80^{a} ± 15.20	100.22^{b} ± 10.63	102.00^{b} ± 12.10
Volume (ml)	0.58^{b} ± 0.05	0.68^{ab} ± 0.07	0.67^{ab} ± 0.06	0.79^{a} ± 0.08	0.79^{a} ± 0.07
pH	8.17^{a} ± 0.17	8.08^{a} ± 0.08	8.29^{a} ± 0.10	8.06^{a} ± 0.15	8.22^{a} ± 0.09
Motility grade (score 0-9)	7.11^{ab} ± 0.26	6.50^{b} ± 0.22	7.29^{a} ± 0.42	7.11^{ab} ± 0.31	6.44^{b} ± 0.24
Motile sperm % (PMS)	82.77^{ab} ± 3.55	87.50^{a} ± 1.71	81.43^{b} ± 2.61	82.56^{ab} ± 1.94	87.22^{ab} ± 0.88
Live sperm % (PLS)	90.33^{a} ± 2.01	92.33^{a} ± 0.84	90.29^{a} ± 1.96	92.44^{a} ± 0.93	89.33^{a} ± 2.25
Abnormal morphology % (PAM)	13.56^{a} ± 1.17	13.50^{a} ± 0.72	15.14^{a} ± 1.16	15.89^{a} ± 0.59	13.67^{a} ± 1.25
Motility after one hour (%)	57.22^{b} ± 4.80	70.83^{a} ± 0.83	50.00^{b} ± 2.44	37.78^{c} ± 3.34	49.44^{b} ± 2.12
Concentration (x 10^6/ml)	356.22^{bc} ± 36.65	253.33^{c} ± 37.92	482.00^{ab} ± 43.70	565.56^{a} ± 76.87	510.22^{a} ± 60.39
Total sperm per ejaculate (TSE x 10^6)	210.26^{b} ± 35.09	166.30^{b} ± 11.44	294.68^{ab} ± 20.45	451.44^{a} ± 96.88	430.22^{a} ± 81.22
Total motile sperm (TMS %)	178.72^{b} ± 34.21	145.40^{b} ± 10.05	239.90^{ab} ± 17.32	377.52^{a} ± 84.85	378.09^{a} ± 73.83
Total functional sperm fraction (TFSF)	154.72^{b} ± 29.99	125.52^{b} ± 8.37	204.28^{ab} ± 15.43	320.28^{a} ± 74.33	323.41^{a} ± 62.20
Resazurin reduction ratio (RRT)	2.54^{c} ± 0.34	4.18^{a} ± 0.11	1.75^{d} ± 0.12	3.38^{b} ± 0.07	3.69^{ab} ± 0.18

Data were recorded as mean ± SE.
Different superscripts in a row are significantly different at P<0.05.

ameliorate semen characters. These results go parallel to the investigation of Yousef (2005) on the *Acasia saligna* leaves fed to 8 weeks old white New Zealand male rabbits until maturity. He concluded that up to 40% Acacia leaves could be used successfully and safely in the diet of rabbits without adversely affecting their reproductive performance as their semen quality and characteristics were improved, besides the lowering of thiobarbituric acid reactive substances in seminal plasma.

Blood cell counts. With regard to the haemogram illustrated in (Table VI), RBCs count, Hb, MCH and MCHC were not significantly different among all diets. This agree with the finding of Osman *et al.* (2004), who reported that the addition of rocket and radish cakes to Hubbard broiler ckicks diets didn't alter the haemoglobin percentage, which indicated that there was no anaemic incidence and that it is safe to use these plants in animals diets. On the other hand, Hct showed a significant (P < 0.057) decrease for the rocket diet fed group compared to the control fed diet group (38.90 ± 0.88 vs 41.50 ± 0.27%). The later result saddled with her shaddows on MCV as the rocket diet fed group (67.00 ± 0.67 fl) was the lowest among the other groups (P < 0.0001). Ibrahim (2005) found that replacement of soybean with 0.5% or 1% rocket seeds in the diets of broiler rabbits significantly increased haematocrit percentage compared to the control fed diet. The disagree between our results and the later author may be due to the increase of soybean protein substitution with the rocket meals that may have adverse effect on RBCs volume. Concerning the total white blood cells count, rabbits given radish and mixture meal induced a significant (P < 0.0001) increase in WBCs (10.78 ± 0.41 and 10.43 ± 0.44 x 10^3/mm^3, respectively) compared to the other three groups. This response may be due to the high contents of direct and indirect antioxidants (Marsufuji *et al*., 2003; Takaya *et al*., 2003). On the other hand, the platelet count was significantly (P < 0.0003) increased in case of the control and black cumin diets (287.50 ± 2.81 &

Table V. The reactive species and antioxidant indicators in serum and seminal plasma of New Zealand bucks fed on diets containing radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal protein.

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixture diet
		Malondialdehyde (nmol/ml)			
in serum	10.84^{c} ± 0.31	13.85^{b} ± 0.27	14.87^{b} ± 0.27	14.85^{b} ± 0.47	19.14^{a} ± 1.59
in semen	10.95^{b} ± 0.78	9.24^{c} ± 0.24	14.48^{a} ± 0.53	10.03^{bc} ± 0.29	9.31^{c} ± 0.05
		Nitric oxide (μmol/L)			
in serum	117.89^{c} ± 4.12	158.58^{b} ± 4.06	149.76^{b} ± 4.35	132.11^{c} ± 0.96	202.21^{a} ± 9.04
in semen	103.39^{b} ± 1.49	90.59^{d} ± 0.41	98.31^{c} ± 0.82	109.04^{a} ± 3.39	97.18^{c} ± 0.16
		Total antioxidant capacity (mmol/L)			
in serum	0.487^{d} ± 0.006	0.533^{cd} ± 0.013	0.593^{bc} ± 0.045	0.647^{b} ± 0.024	0.740^{a} ± 0.031
in semen	1.183^{bc} ± 0.026	0.997^{c} ± 0.130	1.813^{a} ± 0.069	1.353^{b} ± 0.024	1.760^{a} ± 0.024

Data were recorded as mean ± SE.
Different superscripts in a row are significantly different at P<0.05.

Table VI. The hemogram of New Zealand bucks fed on diets containing radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal protein.

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixed diet
Red blood cells count (RBCs x 10^6/mm^3)	6.00[a] ± 0.03	5.80[a] ± 0.07	5.60[a] ± 0.13	5.90[a] ± 0.14	5.91[a] ± 0.12
Haemoglobin (Hb, g/dl)	15.20[a] ± 0.03	15.28[a] ± 0.20	15.05[a] ± 0.09	15.27[a] ± 0.08	15.48[a] ± 0.25
Haematocrit (Hct, %)	41.50[a] ± 0.27	41.73[a] ± 0.54	38.90[b] ± 0.88	40.13[ab] ± 0.93	41.30[a] ± 0.87
Mean corpuscular volume (MCV, fl)	67.50[c] ± 0.40	71.50[a] ± 0.19	67.00[c] ± 0.53	67.67[c] ± 0.67	69.50[b] ± 0.63
Mean corpuscular haemoglobin (MCH, pg)	25.00[a] ± 0.05	26.30[a] ± 0.12	26.90[a] ± 0.43	25.93[a] ± 0.48	26.55[a] ± 0.90
Mean corpuscular haemoglobin concentration (MCHC, g/dl)	37.05[a] ± 0.31	36.75[a] ± 0.21	39.30[a] ± 0.72	38.63[a] ± 0.76	38.55[a] ± 1.29
White blood cells count (WBCs x 10^3/mm^3)	7.40[b] ± 0.40	10.78[a] ± 0.41	8.65[b] ± 0.79	7.57[b] ± 0.22	10.43[a] ± 0.44
Platelet cells count (x 10^3/mm^3)	287.50[a] ± 2.81	210.25[b] ± 6.76	124.50[c] ± 16.44	234.33[ab] ± 12.03	189.00[b] ± 45.15

Data were recorded as mean ± SE.
Different superscripts in a row are significantly different at P<0.05.

Table VII. The liver and kidneys functions tests of New Zealand bucks fed on diets containing radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal protein

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish diet	Rocket diet	Black cumin diet	Mixed diet
GOT (U/L)	85.66[a] ± 2.33	72.33[b] ± 1.45	75.66[b] ± 2.96	74.00[b] ± 2.08	75.66[b] ± 3.48
GPT (U/L)	25.33[a] ± 0.88	21.66[a] ± 0.88	22.33[a] ± 1.45	23.00[a] ± 0.58	22.00[a] ± 1.53
Urea nitrogen (mg/dl)	36.00[a] ± 2.08	29.66[b] ± 0.88	28.66[b] ± 1.86	29.70[b] ± 0.67	30.33[b] ± 0.88
Creatinine (mg/dl)	2.66[a] ± 0.38	1.76[a] ± 0.46	1.53[a] ± 0.23	2.66[a] ± 0.38	1.99[a] ± 0.40

Data were recorded as mean ± SE.
Different superscripts in a row are significantly different at P<0.05.

$234.33 \pm 12.03 \times 10^3$ mm^{-3} respectively) compared to the other groups. This designates that the bleeding and coagulation times are shorter than the other groups, especially the rabbits fed on diets containing rocket meal. In addition, the decrease in blood platelets count (thrombocytopenia) may decrease the coagulation factors produced by the platelets. This may specify that there are one or more contents in the rocket seeds meal that affect bone marrow to produce platelets in the blood stream.

Liver and kidney functions. The activities of SGOT and SGPT enzymes (Table VII) tended to decrease indicating improved liver function tests, in addition, urea decreased in the tested meals compared to that of the control-fed group. Such reduction of liver enzyme activities with no effect on relative weight of liver and also reduction of urea concentration exhibit healthy, non-pathological and non-toxic effect of dietary radish, rocket, black cumin or mixture diet. Similar results were obtained by Abdel-Malak *et al*. (1995) with biotonic as herbal feed additive, Afifi (2001) with black cumin seeds, Abdo (2003) with rocket meals, Osman *et al*. (2004) with rocket and radish meals and Ibrahim (2005) with rocket seeds. They reported that SGOT and SGPT activities were significantly decreased as the result of the studied treatments. As to radish or rocket, this decrease may be due to their antioxidant status as reported by Bradley (1992) and Leung and Foster (1996). In case of rocket inclusion, the decrease in blood urea may be due to the effective role of rocket isothiocyanates volatile oils as diuretic. Mahran *et al*. (1992) found that the ethanolic extract and volatile oil of *Eruca sativa* (rocket) seeds have been shown to act as diuretics in dogs and the oil significantly increased Na^+, K^+ and Cl^- extraction in urine. *Eruca sativa* seeds are used in folkloric medicine as a lactagogue, diuretic, antimicrobial, to disintegrate renal calculi and to induce vomiting (Boulos, 1983).

From the present study, we could conclude that the inclusion of a mixture of equal quantities from radish, rocket and black cumin meals on the expense of 50% soybean meal protein resulted in improving semen characteristics and nutritional performance with reduction of free radicals in male rabbits.

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