The Beneficial Effects of *Nigella sativa*, *Raphanus sativus* and *Eruca sativa* Seed Cakes to Improve Male Rabbit Fertility, Immunity and Production

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Abstract: The present study provides a probable insight on the beneficial effects of medicinal plant in improving fertility and immunity of bucks. A total number of 50 male growing New Zealand rabbit 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, as a replacement of soybean meal) and mixture of these meals at a level of 17% approximately for each, on semen characteristics, and seminal plasma and serum biochemical parameters. Each group received experimental diets containing nearly equal ratio of C/P under the same managerial conditions. The semen parameters 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 (8.47 vs. 7.67; 100.22 vs. 102.00; 0.79 vs. 0.79; 7.11 vs. 6.44; 82.56 vs. 87.22; 565.56 vs. 510.22; 451.44 vs. 430.22; 377.52 vs. 378.09 and 320.28 vs. 323.41 respectively). On the other hand, radish showed good results concerning motile sperm percentage, motility percentage after one hour and the resazurin reduction activity (87.50, 70.83 and 4.18 respectively). Radish meal inclusion reduced significantly (P<0.0001) the production of free radicals in seminal plasma. *Nigella sativa* (NS), Raphanus sativus (RS) and *Eruca sativa (ES)* cakes contain different type of organic compound and antioxidant. The immunogenic results for the pervious cakes improve that, the RS giving the best results form the immunity point of view followed by the mixed cake and ES cake. While the NS cake giving just higher results than control. From the present study, we concluded that the inclusion of a mixture of equal quantities from radish, rocket and black cumin meals on the expense of approximately 50% soybean meal protein improved the semen characteristics and reduced free radicals in the seminal plasma.

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Keywords: fertility; immunity; buck; *Nigella sativa* (NS); Raphanus sativus (RS); *Eruca sativa (ES)*

1. Introduction:

The rabbit industry, especially in Egypt, needs new non traditional protein surceases cheap and enough in amount to overcome the expensiveness of traditional protein sources in the rabbit diet. These non traditional sources must have the privilege to ameliorate the animal health represented in supporting a good immunity, and animal production represented by the meat productivity and the animal fertility. Few studies were carried out on rabbit non-conventional protein feed and literature 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 increasing for the strong demand to volatile oils for pharmaceutical purpose. Those plants were found to incarnate natural substances that ameliorate health and promote 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. 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 and Fahey, 2001), volatile oils like myristicin, apiole and □-phellandrene (Bradley, 1992, and Leung and Foster, 1996). Glucosinolates were found to have several biological activities including anticarcinogenic, antifungal, antibacterial plus its 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 IgG concentration (Chew et al., 2000).

The black cumin seeds contain thymoquinine that has antibacterial, diuretic, hypotensive and immuno-potentiating 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 and 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 reproductive performance of the animals. So, the present study aimed to monitor the effect of substitution a half part of soybean meal protein, by inclusion of radish, rocket, black cumin or mixture of these meals as cheap non-traditional sources of protein in rabbit bucks diet, on semen characteristics and reactive species in seminal plasma and serum, and carcass performance for male mature rabbits.

2. Materials and methods

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

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 for Egypt. Feed and water were provided ad libitum. Five experimental diets (Table 1) was formulated and prepared in the form of pellets at Meladico Company to cover the requirements of the five rabbits group according the NRC (1977).

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 1). Each experimental diet contained nearly equal ratio of calorie/protein (C/P) under the same managerial conditions. Experimental period lasted for 29 weeks.

Blood samples:

At the end of experimental period, blood was collected in clean sterile tubes from sacrificed rabbits. 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 ultra freeze – 80°C.

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.

Table (1). Composition and chemical analysis of tested diets.

Ingredients	Control diet	Radish diet	Rocket diet	Nigella 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.

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 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). Total functional sperm fraction (TFSF) was calculated as the product of TSE x PMS x PAM (Correa and Zavos, 1996). The motility after 1 hour (PMS after 1 hour) 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) depends on 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 and Bordekar, 1999). The antioxidative capacity was performed according to the method assumed by Montgomery and Dymock (1961). Blood samples were collected for biochemical a ssays. Semen were collected for evaluation.Total Antioxidant Capacity (TAC), Nitric Oxide (NO) Assay and Lipid peroxidation (LPO) assay (Malondialdehyde). Lipid peroxidation (LPO) 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). Green et al. (1982). Blood samples and semen were collected for evaluation. Of Total Antioxidant Capacity (TAC), Nitric Oxide (NO) Assay and Lipid peroxidation (LPO) assay (Malondialdehyde). All chemicals used in the study were of analytical grade and obtained from commercial suppliers.

The immune response of the different groups was evaluated by: Estimate the antibody titre against Pasteurella vaccine by ELISA.and Detect nitric oxide and lysozyme levels in serum.
ELISA test: The antigen used for ELISA was prepared by boiling *Pasteurella multosida* local isolate strain from rabbit (Manning, 1984).

Statistical analysis:
Data were statistically analyzed adopting one way ANOVA in the general linear model (GLM) utilizing 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 and Cochran, 1980)**.**

3. Results and Discussion:
Chemical composition of tested meals:
The proximate chemical analysis of radish, rocket and black cumin meals (Table 1) 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 and 2697 kcal DE / kg, respectively) and protein (35.30, 32.20 and 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 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. Since, there is currently a lack of information on the composition of these experimental ingredients. 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 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 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 (P<0.0001) for rabbit received different diets showed an increment significantly affect feed consumption as compared to control diet group. However, rocket group consumed a significant highest feed than those of the other replacements. Similar results were observed by Ibrahim (2005) when the basal diet was supplemented with 1% rocket seeds to rabbits. Highest intake 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). 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 and Leung and Foster, 1996), while, black cumin diet gave the worst value, this agree with the results obtained by Amber et al. (2001).

Semen characters:
On the other hand, semen evaluation indicated an important tool for clarifying the effect of external and internal agents affecting male reproduction. Data in the present study (Table 2) 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 and RRT) when compared to the control diet. Although, they were non significantly different when compared to each other. Whereas, the radish gave a score of 4/14 followed by the control (3/14) and finally the rocket group (2/14). 3/14 parameters (pH, PLS and PAM) were non significant in the comparison between the five diets.

Table (2). The semen physical properties for male New Zealand bucks fed on diets including radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal.

Item	Control diet	Soybean substitution for 50% of its protein by			
		Radish meal	Rocket meal	Black cumin meal	Mixture meal
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
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 ± S.E.
Different superscript within rows are significantly different at $P<0.05$

Concerning the biochemical analysis in seminal plasma, 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 ($ONOO^{-}$) (Zaman, 2004). Since, semen of includes white cells, 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 and Takaya, et al., 2003) that reins in the reactive species production. This is interpreted via the highest results obtained in case of RRT, PMS and the PMS after one hour where the reactive species are impeded. Although, in case of the serum, the best results were obtained in case of 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 stander in a mid-way as it included the three meals. This is confirmed by approximately good performance in case of most of the semen character (main characters and calculated characters). Black cumin diet was the most effective in most semen parameters followed by radish while the worst was the rocket. 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 ameliorate semen characters. These results goes 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. Replacement of Soya been cake with NS cake lead to increase in the antibody titre and nitric oxide level than control but less than the effect of RS and ES cakes. These results may be explained by Islam, et al. (2004) who stated that, in contrast to its enhancing effect on the T cell mediated immune response, NS constituents have shown a tendency to down regulate B cell-mediated immunity.

From the present study, we concluded that the inclusion of a mixture of equal
quantities from radish, rocket and black cumin meals on the expense of 51% soybean protein for maintenance and improving the semen characteristics, reduction of free radicals and the carcass performance of male rabbits. *.The results showed that the NS 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.*The immunogenic results showed that, the RS giving the best results form the immunity point of view followed by the mixed cake and ES cake. While the NS cake giving just higher results than control In general, it could be recommended either to use Radish, Rocket, and mixture cakes at 50% replacement level of soybean meal in rabbit diets as cheap non-conventional by products in order to get higher economic efficiency without adverse effects on rabbits performance

Table (3). The reactive species and antioxidant indicators in serum and seminal plasma of male New Zealand bucks fed on diets including radish, rocket, black cumin meals or a mixture of them on the expense of soybean meal

Item	Control	Soybean substitution for 50% of its protein by			
		Radish meal	Rocket meal	Black cumin meal	Mixture meals
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 □mol/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

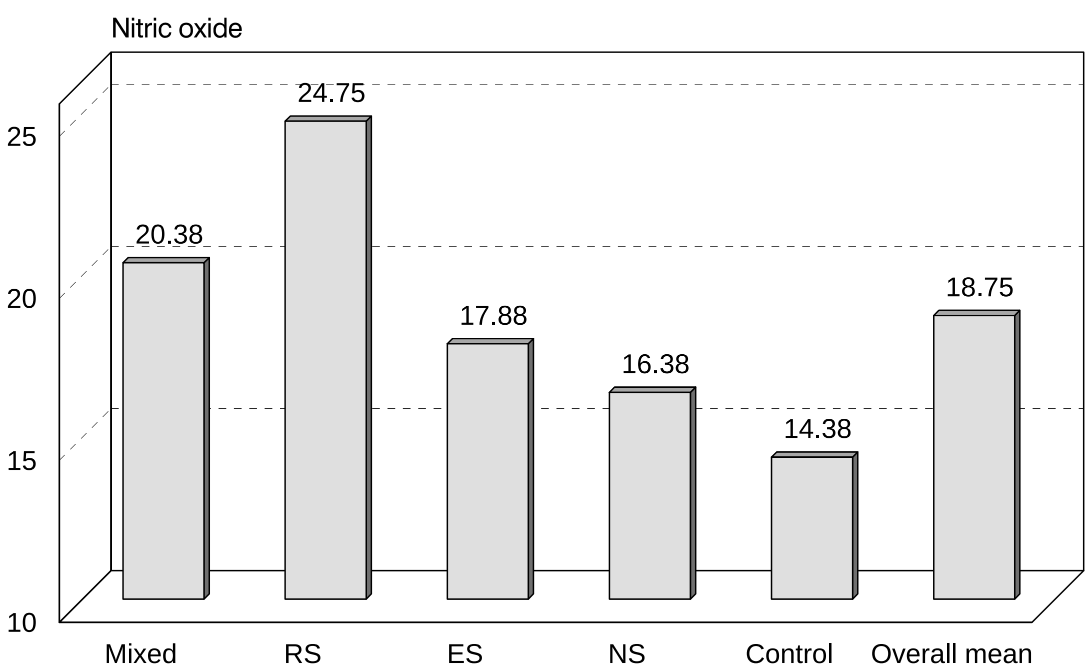
Data were recorded as Mean ± S.E.
Different superscript in rows are significantly different at $P<0.05$.

Table (4): Effect of different cakes on rabbit immune response to *Pasteruella* vaccine estimated by ELISA (optical density).

	First	Second	Third	Fourth	Total
Mixed	0.502±.0.066	0.804±0.047	0.856±.025	0.385±0.039	0.637±0.012
RS	0.731±0.092	0.819±0.100	0.897±.069	0.402±0.042	0.712±0.037
ES	0.4167±0.043	0.591±0.072	0.843±.109	0.446±0.105	0.574±0.052
NS	0.564±0.143	0.529±0.127	0.707±.109	0.413±0.047	0.553±0.035
Control	0.362±0.035	0.481±0.054	0.658±.061	0.297±0.039	0.450±0.033

RS= Raphanus sativus cake. ES = Eruca sativa cake. NS= Nigella sativa cake.

Figure (1) serum nitric oxide (mg/ml) in different rabbit groups.



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