Global Veterinaria 13 (5): 926-937, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.05.912

Influence of Dietary Supplementation with Antioxidants on the Growth Performance, Hematological and Serum Biochemical Alterations in Goat Kids

[1]*Hala A.A. Abou-Zeina,* [1]*Soad M. Nasr,* [1]*Somia A, Nassar,*
[2]*Mohamed A.F. Genedy and* [3]*Mamdouh I. Mohamed*

[1]Department of Parasitology and Animal Diseases, National Research Center, Giza, Egypt
[2]Military Medical Academy, Cairo, Egypt
[3]Department of Animal Production, National Research Center, Giza, Egypt

Abstract: This study was performed to determine the effects of the use of natural antioxidant such as *Nigella sativa* or black cumin seeds (BCS) *VS* organic multi-nutrient antioxidants, as dietary supplements on growth rate, hematological and some serum biochemical parameters. Fifteen goat kids of Baladi breed were divided equally into three experimental groups. Group I served as control (without any supplementation), group II dietary supplemented with crushed BCS at ratio of 2% and group III supplemented with the organic multi-nutrient antioxidants; vitamin E with selenium enriched yeast (Vit E/Se) and zinc methionine (Zn-Met) at levels of 2g/kg of diet. Experimental diets were analyzed for nutritive values. Blood samples were collected from the jugular vein at zero- time and every 30 days along the experimental period (90 days) and analyzed for hematological and some biochemical parameters in serum. For evaluation of growth rate, daily weight gains and total body weights of all the kids were measured on day 0 of the experiment and the sampling times. The results showed that Se and Zn levels in the basal diet were on marginal border line of deficiency for goats that indicate the justification of adding both elements. Goat kids in both supplemented groups recorded higher (P<0.05) body weights and daily gains than those in the control group. Some of hematological parameters were positively changed as a result of administration of both forms of antioxidants. Erythrogram evaluation revealed significant improvement in both supplemented groups when compared to control group. There were significant (P<0.05) increases in lymphocyte count in both groups II and III during all periods of experiment post supplementation. The activities of liver enzymes (AST & ALT) as well as the levels of urea, creatinine and lipid profile didn't show significant differences among all groups. Total proteins and total and gamma globulins levels marked (P<0.05) increased in group II on day 90 while, in group III starting from day 30 of treatment. It was concluded that, inclusion of BCS and Vit E/Se with Zn-Met in goat kids' diets improved the growth performance and immune status. Both forms of antioxidants were safe to liver and kidney at the examined dose as reflected on their biomarkers.

Key words: Vit E · Selenium · Zinc · *Nigella sativa* · Growth performance · Hematology · Serum biochemistry · Goats

INTRODUCTION

Antioxidants have been defined as molecules that prevent cell damage against free radicals and are critical for maintaining optimum health in both animals and humans [1, 2].

Certain nutrients such as copper, zinc, selenium and vitamin E are common antioxidants normally included in animal diet. Though required in minute amounts, they are highly essential for health and immunity [3, 4]. They contribute to growth [5, 6], production [7] and reproduction [8]. They act as antioxidants [8] and prevent oxidative stress by neutralizing oxidants produced under different stress conditions [5]. These nutrients also contribute to health of animal through maintaining proper homeostatic mechanisms and playing a vital role in many physio-biochemical processes like protein, enzyme and hormone synthesis [1, 9]. They are also been involved in oxidation reduction reactions and immune functions [10].

Corresponding Author: Soad M. Nasr, Department of Parasitology and Animal Diseases, National Research Center, El-Behouse Street, Dokki, P.O. Box 12622, Giza, Egypt. E-mail: soadnasr@yahoo.com.

A deficiency in one or more of these nutrients can compromise immunocompetence of an animal [1, 11]. Zinc is an essential trace element that has a catalytic, coactive, or structural role in a wide variety of enzymes that regulate many physiological processes including keratinization and general protein metabolism as well as growth [12, 13]. Being components of the anti-oxidant system, [1] zinc and manganese, in addition to copper, are integral parts of superoxide dismutase (SOD) [14], while Se and vitamin E share a common biological function. Selenium is a component of enzyme glutathione peroxidase, which destroys free radicals in the cytoplasm, whereas, vitamin E is a non-enzyme scavenger of free radicals that functions as a specific lipid soluble antioxidant in cell membranes [6]**.** All these are important for neutralizing free radicals or oxidants and protecting the tissues against oxidative damage [1]. Previous research suggested that relatively high levels of supplemental vitamin E (mega dose) may improve immunity [15] and carcass quality [16] by reducing the oxidation of meat [17].

As animal antioxidant -status declines, immunity and enzyme functions are compromised first followed by a reduction in maximum growth and fertility and finally normal growth and fertility decrease prior to evidence of clinical deficiency [18, 19]**.** Combinations of antioxidants may be more effective than larger quantity of any single one [20, 21].

Nowadays, there is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs which may have adverse effects. One of the alternatives used as feed additives is black cumin seed (*Nigella sativa*) [22]. The range of chemical constituents of black cumin seeds (BCS) (w/w) are crude protein (20.9%-31.2%), ether extract (22.0%–40.4%), total ash (3.7%–4.7%), total carbohydrate (24.9%–40.0%) and moisture (3.8%-9%) [23, 24, 25]. Khan *et al*. [24] recorded macro-mineral (mg/100mg BCS) content were calcium (572 mg%); phosphorus (540 mg%), magnesium (264 mg%), sodium (17.8 mg%) and potassium (810 mg%) and micro-mineral (µg%) content were copper (2.7 µg%), zinc (6.2 µg%), iron (9.7 µg%) and manganese (8.5 µg%). Also, BCS contain more than 30% fixed oil and 0.40-0.45% volatile oil [26]. The main active ingredients of black seed include thymoquinone, dithymoquinone, thymohydroquinone, nigellone and thymol, which play important roles as pharmacologically active substances [27].

The seed of *Nigella sativa* has been reported to have many biological properties including antiparasitic [28, 29], antibacterial [4], antidiabetic [30] and diuretic effects [31]. *Nigella sativa* has immuno-potentiating activities as well as antioxidative effect [4]. Besides, it showed hepatoprotective effect in some animal models of liver toxicity [32, 33] due its role as antioxidant [34]**.** The seeds of *N. sativa* contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements and proteins, including eight of the nine essential amino acids [35].

Keeping in view that very little information is available on the requirement and role of antioxidants on growth rate, hemogram, blood metabolites and serum protein electrophoresis in goats, *the present research work* was planned to evaluate the effect of dietary supplementation of different forms of antioxidants (natural *VS* organic multi-nutrient antioxidants) on these aspects in male goat kids.

MATERIALS AND METHODS

Animals, Feeding and Management: Fifteen clinically healthy male Baladi goat kids, aged 4-6 months and weighing 10-15 kg were used throughout the experimental periods from the 1st March 2011 to the end of May 2011). The kids were clinically healthy and kept under hyagenic condition and were routinely examined clinically according to the method of Radostitis *et al*. [36]. All animals were fed for 2 weeks (adaptation period) on constant basal diet formulated as 50% yellow corn; 17% wheat bran, 25% cotton seed cake meal, 5% molasses, 2% limestone and1% salt. Additionally, the animals were supplemented with seasonal green fodders essentially *Alfa Alfa* (Green Barseem) in winter. Bean straw was added at nights while fresh drinking water was offered *ad libitum*.

Experimental Design: Following a preliminary period of 2 weeks on the basal diet, the goat kids were randomly divided into three equal groups (n=5) depending on dietary treatments with antioxidants:

Group I: Kept as a control and was fed the basal diet only without any supplementation.

Group II: Received the basal diet- supplemented with crushed BCS at level of 2%.

Group III: Received the basal diet- supplemented with organic multi-antioxidants: zinc methionine (Zn-Met/10 Zinc chelated with methionine hydroxy analogue – IBEX International) at level of 2 g/kg of diet and Vit. E with Se

enriched yeast (E 60.000, Sanovet, Austria – Composition: Vit E 60 g, Se yeast 12g, L-lysine 0.08g and carrier: dextrose up to 1 kg at the same dose level.

Experimental animal groups were individually housed in separate semi-opened pens and were managed and kept at the same environmental and nutritional conditions throughout the trial which extended for 90 days. Feed ingredients of the experimental diets were well mixed manually to satisfy one week and individually offered daily at level of 2% of the body weight of the experimental animals, once at 10 a.m. Animals were weighed at the beginning and thereafter monthly intervals.

Sampling and Methods of Analysis: Feed Samples of experimental diets were collected and chemically analyzed for chemical composition and nutritive values according to the techniques carried out by A.O.A.C [37].

Blood Samples: Two blood samples were taken from all goats at zero-time and thereafter monthly intervals along the period of experiment by Jugular venipuncture using plain and EDTA containing vacutainer tubes. Blood on EDTA was used for hematological parameters, while blood on plain vacutainers was used for separation of serum which stored at -20°C until analysis.

Hematological Profile: Erythrogram; packed cell volume (PCV), Hemoglobin concentration (Hb), Red blood cell count (RBCs), calculated red blood indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] and leukogram; total leukocytic count (TLC) and its differential cells were investigated according to the methods of Weiss and Wardrop [38].

Assays of Serum Biochemical Metabolic Profile: The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as gamma glutamyl transferase (GGT) were assayed as described by Reitman and Frankel [39] and Persijn [40]. Serum creatinine was measured according to the method of Houot [41] and the enzymatic determination of urea was done according to Patton and Crouch [42]. Enzymatic determination of serum total cholesterol [43], triglycrides [44] and high density lipoprotein cholesterol (HDL-C) [45] were performed. Serum low denisity lipoprotein cholesterol (LDL-C) level was calculated according to the following equation of Friedewald *et al*. [46]:

Concentration of LDL-C in serum (mg/dl) = Total Cholesterol –HDL-C – (Triglycerides/5).

Serum Proteins Profile: Total serum proteins (TP) concentrations were determined by Biuret method [47]. Albumin and globulins were separated by cellulose acetate electrophoresis in barbital buffer, pH 8.6, at 180 V, 4 mA, for 15 min using Helena system (Helena France). The separated proteins were stained with Ponceau S for 15 min, then destained with 5% acetic acid and dehydrated in pure methanol and cleared with clearing solution (67% pure methanol, 29% glacial acetic acid and 4% clear aid). After plate drying at 50–60°C, the relative levels of proteins were scanned by densitometer at 525nm.

All test kits were supplied by BioMérieux, France. Measurements were performed using Spectrophotometer model T80, UV/Visible, double beam, UK.

Statistical Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error (mean±SE). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$ level [48] using SPSS version 16 computer program.

RESULTS

Diet Analysis: The experimental diets were formulated by the addition of substantial amounts of different forms of antioxidants to the basal diet. As presented in Table (1), this approach caused differences in the nutritive values of the experimental diets. When compared with the control diet, the experimental diets; II and III with BCS and multi-antioxidants (Zn-Met and Vit E with Se enriched yeast), respectively contained more protein and more macro- and micro minerals. Diet III supplemented with the multi-antioxidants had the highest concentrations of Zn and Se.

Growth Performance: Results presented in Table (2) demonstrated that initial body weights (Kg) of experimental animals fed Exp- diets did not differ significantly, indicating that all treatments were homogenous. However, at the end of experiment, goat kids in both groups II and III showed similarly a significant ($P<0.05$) higher body weights and daily gains when compared with the control group. There were non significant differences in body weights among experimental animals in groups II and III depending on dietary treatment with antioxidants.

Table 1: Chemical analysis and nutritive values of experimental diets on DM basis.

	Diet I*	Diet II **	Diet III***
Crude protein (CP) %	13.9	14.5	14.35
Macro- elements %			
Calcium (Ca)	0.7	1.15	0.81
Phosphorous (P)	0.39	0.46	0.42
Magnesium (Mg)	0.27	0.31	0.29
Sodium (Na)	0.54	0.66	0.74
Potassium (K)	0.92	1.2	0.83
Trace elements (ppm)			
Iron (Fe)	135	231	146
Copper (Cu)	8	10	8.4
Manganese (Mn)	27	31	29
Selenium (Se)	0.058	0.19	0.88
Zinc (Zn)	29	43	100

* Basal diet (control)

** Formulated diet (Basal diet supplemented with 2% crushed BCS)

***Formulated diet (Basal diet supplemented with multi-antioxidants; Zn-Met and Vit E with Se enriched yeast at levels of 2g/kg diet).

Table 2: Growth performance of goats in different groups of treatments

	Treatments		
Parameters	Control (basal diet only)	Basal diet + 2% BCS supplement	Basal diet +Multi-antioxidant supplement
Initial weight (Kg)	12.70 ± 1.00^{a}	13.60 ± 0.59^{a}	12.50 ± 0.53^{a}
Final weight (Kg)	19.30 ± 1.27^{a}	21.60 ± 0.78^{b}	23.40 ± 3.51b
Daily body gain (g)	71.04 ± 5.25^{a}	86.56 ± 5.76^{b}	90.00 ± 9.35^{b}

Means with different superscripts (a, b) between groups in the same row are significantly different at $P<0.05$.

Table 3: Hematological profile of different groups during the periods of experiment (Mean±SE)

	Treatments												
	Control				Black Cumin seed - supplement				Multi-antioxidants - Supplement				
Parameters	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	Sig.
RBCS ($\times 10^{6}$/µl)	11.56±0.70	11.06±0.52	10.64±0.56	11.04±0.43	10.80±0.41	12.10±0.62	11.80±0.85	13.20±0.75	10.68±0.38	10.86±0.39	11.90±0.53	12.00±0.61	NS
Hemoglobin (g/dl)	10.58^{ab}±0.99	11.13^{ab}±0.84	10.30^{ab}±0.64	10.25^{ab}±0.40	10.10^{b}±0.82	11.88^{ab}±0.67	10.82^{ab}±0.65	12.35^{a}±0.88	10.61^{ab}±1.07	10.89^{ab}±0.59	11.35^{ab}±0.43	11.24^{ab}±0.50	NS
PCV (%)	28.10^{a}±0.67	29.58^{ab}±0.59	29.69^{ab}±1.23	30.98^{b}±0.21	27.38^{a}±0.87	28.40^{ac}±1.38	31.09^{ab}±1.62	33.44^{b}±1.67	26.10^{a}±0.93	28.10^{ab}±0.80	29.84^{bc}±0.80	31.14^{b}±1.50	**
MCV (fl)	24.60±1.90	26.99±1.60	28.02±1.15	28.34±0.75	25.51±1.37	25.40±1.79	27.07±2.99	27.20±2.13	23.75±1.38	26.05±1.61	25.17±1.45	26.04±1.88	NS
MCH (pg)	9.98±0.31	9.30±0.28	9.60±0.25	9.42±0.46	9.97±0.34	10.30±0.86	9.92±0.77	9.32±0.51	10.01±0.58	10.16±0.63	9.42±0.26	9.42±0.47	NS
MCHC (g/dl)	37.0±3.34	36.72±2.45	34.6±1.29	32.97±1.70	36.88±3.36	39.24±2.60	35.14±3.00	37.31±3.83	34.74±4.90	38.19±1.25	37.35±1.31	36.53±3.14	NS
WBC (10^{3} /µl)	10.21^{bcd}±0.30	10.30^{bcd}±0.52	10.42^{abcd}±0.55	10.01^{cd}±0.35	9.80^{d}±0.39	11.48^{ab}±0.56	11.26^{abc}±0.51	11.25^{abc}±0.53	9.60^{d}±0.21	11.70^{a}±0.49	10.83^{abcd}±0.47	11.18^{abc}±0.34	**
Lymphocytes (10^{3} /µl)	4.86^{c}±0.28	5.03^{bc}±0.38	4.90^{c}±0.16	4.84^{c}±0.20	4.79^{c}±0.45	5.80^{ab}±0.19	5.83^{ab}±0.40	5.99^{a}±0.33	4.62^{c}±0.10	6.21^{a}±0.27	5.88^{a}±0.07	6.05^{a}±0.07	**
Neutrophils (10^{3} /µl)	4.57±0.15	4.48±0.11	4.82±0.37	4.39±0.31	4.32±0.30	4.93±0.46	4.78±0.22	4.50±0.41	4.18±0.30	4.18±0.43	4.33±0.45	4.66±0.22	NS
Eosinophils (10^{3} /µl)	0.27±0.06	0.31±0.05	0.30±0.04	0.34±0.05	0.29±0.04	0.34±0.06	0.23±0.03	0.36±0.07	0.33±0.04	0.30±0.05	0.26±0.05	0.22±0.07	NS
Monocytes (10^{3} /µl)	0.48±0.10	0.48±0.07	0.37±0.06	0.41±0.10	0.39±0.03	0.40±0.07	0.38±0.05	0.36±0.04	0.44±0.05	0.32±0.05	0.30±0.07	0.23±0.04	NS
Basophils (10^{3} /µl)	0.04±0.03	0.02±0.02	0.04±0.03	0.04±0.02	0.02±0.02	0.02±0.02	0.05±0.03	0.05±0.03	0.04±0.02	0.03±0.03	0.04±0.03	0.05±0.03	NS

Means with different superscripts (a, b, c) between groups in the same raw are significantly different at $P<0.05$

* = $P<0.05$., ** = $P<0.01$.

Table 4: Serum biochemical metabolic profile of different groups during the periods of experiment (Mean±SE)

	Treatments												
	Control				Black Cumin seed - supplement				Multi-antioxidants - Supplement				
Parameters	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	Sig.
AST (IU/L)	80.70±9.33	66.20±8.30	75.90±7.53	78.50±7.71	81.90±12.52	77.90±11.31	71.20±8.51	69.26±8.30	75.90±5.85	70.70±7.43	72.60±6.41	61.50±9.73	NS
ALT (IU/L)	26.30±4.38	27.50±4.81	32.00±6.58	25.60±4.11	26.20±3.06	24.00±3.20	25.06±2.22	22.50±3.00	29.20±4.50	28.30±3.02	22.20±2.44	23.60±2.14	NS
GGT (IU/L)	19.86±2.95	21.00±3.21	18.17±2.82	20.98±4.31	18.72±3.04	22.52±4.34	22.64±5.49	19.58±3.22	19.47±3.38	23.00±3.85	17.30±1.88	20.94±3.44	NS
Urea (mg/dl)	29.20±2.79	26.40±2.78	28.20±2.97	28.10±3.42	28.0±3.29	29.10±4.08	28.30±3.97	29.80±4.33	30.70±2.47	29.00±4.00	29.00±2.66	28.50±3.99	NS
Creatinine (mg/dl)	0.94±0.24	0.82±0.19	1.09±0.20	0.99±0.18	0.71±0.10	0.81±0.10	0.83±0.22	0.95±0.31	0.81±0.12	0.98±0.19	0.88±0.13	0.84±0.13	NS
Total cholesterol (mg/dl)	79.80±2.85	82.30±6.09	79.00±6.40	83.00±7.16	73.10±5.36	71.00±6.33	70.60±6.09	66.20±3.35	76.30±4.17	79.40±2.94	75.40±7.28	75.00±4.23	NS
Triglycerides (mg/dl)	26.80±3.12	27.60±3.31	24.00±3.00	25.40±2.99	28.00±3.83	30.80±6.30	28.00±3.41	25.00±2.74	23.30±3.10	27.20±4.19	27.00±3.45	26.40±3.20	NS
HDL-C	36.90±1.10	36.50±1.77	36.90±1.27	37.30±1.56	38.10±1.10	37.10±1.71	38.90±1.33	38.50±1.40	36.60±1.47	38.80±0.58	39.40±2.23	41.80±1.83	NS
LDL-C	37.54±1.99	40.28±6.87	37.30±7.48	34.66±3.96	29.46±6.22	27.74±6.20	26.10±5.89	22.70±2.27	35.04±3.41	36.56±2.33	30.60±5.22	27.92±2.46	NS

AST = Aspartate aminotransferase. ALT = Alanine aminotransferase. GGT = Gamma glutamyl transferase.
HDL-C = High density lipoprotein cholesterol. LDL-C = Low density lipoprotein cholesterol. NS = Non significant.

Table 5: Serum proteins profile of different groups during the periods of experiment (Mean±SE)

	Treatments												
	Control				Black Cumin seed - supplement				Multi-antioxidants - Supplement				
Parameters	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	0- day	30- day	60- day	90- day	Sig.
Total Proteins (g/dl)	6.74^{c}±0.18	6.68^{c}±0.15	6.64^{c}±0.10	6.70^{c}±0.09	6.78^{bc}0.17	6.90^{abc}±0.13	6.84^{bc}±0.16	7.28^{a}±0.08	6.78^{bc}±0.24	7.42^{a}±0.28	7.20^{abc}±0.13	7.34^{a}±0.12	*
Albumin (g/dl)	3.36±0.11	3.28±0.17	3.34±0.10	3.46±0.06	3.36±0.07	3.40±0.10	3.36±0.09	3.78±0.14	3.36±0.22	3.42±0.14	3.34±0.05	3.58±0.06	NS
Total globulins (g/dl)	3.38^{cd}±0.13	3.40^{cd}±0.09	3.30^{d}±0.06	3.24^{d}±0.05	3.42^{cd}±0.12	3.50^{bcd}±0.07	3.48^{bcd}±0.17	3.52^{ac}±0.07	3.42^{cd}±0.10	4.00^{a}±0.22	3.86^{ab}±0.13	3.76^{ab}±0.08	**
Alpha 1 globulin (g/dl)	0.40±0.04	0.36±0.02	0.38±0.04	0.38±0.04	0.38±0.04	0.38±0.04	0.38±0.05	0.34±0.03	0.40±0.03	0.42±0.04	0.44±0.04	0.32±0.02	NS
Alpha 2 globulin (g/dl)	0.90±0.03	0.96±0.02	0.88±0.04	0.90±0.03	0.92±0.06	1.00±0.03	0.98±0.04	0.88±0.02	0.98±0.04	1.02±0.04	0.96±0.02	0.94±0.04	NS
Beta globulin (g/dl)	0.34±0.02	0.34±0.02	0.36±0.02	0.36±0.04	0.36±0.02	0.38±0.04	0.38±0.04	0.34±0.02	0.38±0.04	0.40±0.03	0.42±0.04	0.38±0.02	NS
Gamma globulin (g/dl)	1.74^{bc}±0.17	1.74^{bc}±0.09	1.68^{bc}±0.08	1.60^{c}±0.03	1.76^{bc}±0.08	1.74^{bc}±0.11	1.74^{bc}±0.13	1.94^{a}±0.05	1.66^{c}±0.12	2.16^{a}±0.14	2.04^{ab}±0.12	2.12^{a}±0.12	**
A/G ratio	1.00^{abc}±0.03	0.97^{bc}±0.07	1.01^{abc}±0.04	1.07^{ab}±0.02	0.98^{bc}±0.03	0.97^{bc}±0.04	0.98^{bc}±0.05	1.08^{bc}±0.06	0.99^{abc}±0.07	0.86^{c}±0.03	0.87^{c}±0.05	0.96^{bc}±0.05	NS

Means with different superscripts (a, b, c, d) between groups in the same row are significantly different at $P<0.05$. A/G= Albumin/Globulins.
* = $P<0.05$., ** = $P<0.01$. NS = Non significant.

Hematological Profile: The results of hematological studies are presented in Table (3). Erythrogram (RBC, MCV, MCH and MCHC) did not show any significant ($P>0.05$) variations among control and /or within treated groups throughout the study. Meanwhile, Hb concentrations were significantly ($P<0.05$) higher on d- 90 in group II consuming BCS than the initial values of the same group. However, the values PCV% were significantly ($P<0.05$) elevated in both supplemented groups on d-90 in comparison with values recorded on 0-day in all group. As shown in Table (3), there was significant ($P<0.05$) increases in WBC counts in group II during all periods of experiment post supplementation compared to 0-day of the same group while, increased significantly ($P<0.05$) group III on days 30 and 90 in comparison with initial values of same group and control. Differential counting of WBC showed significant ($P<0.05$) increases in lymphocytes in both II and III groups during all periods of experiment compared to initial values in the same groups and those of control group.

Serum Biochemical Metabolic Profile: The tested biochemical parameters are presented in Table (4) at different periods for different treatments. The activities of AST, ALT and GGT as well as the concentrations of urea and creatinine didn't show significant ($P>0.05$) variations among control and/or within supplemented groups, except slight depression in levels of liver enzymes in test groups II and III (Table 4). No significant variances were recorded in the values of lipid profile among the experimental groups, except slight depression in concentrations of total cholesterol and LDL-C as well as slight elevation in HDL-C in both supplemented groups compared to initial values (Table 4).

Serum Proteins Profile: The results in Table (5) pointed to significant ($P<0.05$) increase in the levels of total serum proteins of goat kids in group III starting from d-30 until the end of experiment, while there were non significant ($P>0.05$) elevation in group II compared to initial values and control group. Otherwise, no significant changes

were recorded in serum albumin levels throughout the experimental period either within control and/or treated groups. Whereas, total serum globulins and gamma-globulin levels significantly (P<0.05) elevated in goat kids of group III after 30 days from initiation of treatment which persisted along the experimental period compared to the recorded initial values and control group. Electrophoresis of tested sera showed no significant variances in the values of alpha and beta –globulins among treated groups throughout. A/G ratio was significantly (P<0.05) higher in group II on d- 90 than on d- 0, 30 and 60 of the same group.

DISCUSSION

The increases of nutritive values of the experimental diets; II and III (Table 1) were due to the considerable protein and mineral contents of *N. sativa* [49]. Also, dry yeast makes a valuable source of micro elements and amino acids [50]. Based on the 0.1 and 45 ppm recommended for goats, respectively the Se and Zn levels in the basal diet were below the recommended values [51, 52], indicating that supplementation would be warranted. All animals consumed the experimental rations remained clinically normal during the course of the experiment.

Enhanced profitability of many animal production units is dependent upon optimum gain and efficient feed conversion of livestock. In the current study, dietary supplementation with different forms of antioxidants; BCS or Vit E/ Se and Zn met for 90 days significantly improved body weights and daily gains of goat kids (Table 2). Such improvement may indicate a high efficiency of feed utilization for treated-kids. Concerning BCS, our results are in agreement with many searches that recorded the positive effect of dietary supplementation with BCS on daily weight gains and feed conversion ratio for different farm animals including sheep [53] and goat kids [54]. The obtained results are attributed to that BCS is a good source of protein, energy and minerals [55]. Besides, Habeeb and El-Taraba [54] attributed the results to the higher digestibility that was recorded for goat kids supplemented with BCS which led to increase the absorbed nutrients from small intestine, consequently increased body weight gain.

The best apparent improvement in the growth performance of goats in the present experiment was achieved with the dietary supplement of VitE/Se and Zn-Met. Our results could be supported by the view of Shetaewi *et al*. [56] who reported that feed efficiency and daily weight gain could be improved by vitamin E supplementation to coarse-wool lambs. Recently, supplementation with vitamin E plus Se to ewes [57, 58] and buffaloes [6] during pregnancy was found to significantly improve the growth performance of their born lambs and calves, respectively. Vitamin E and Se supplementation were proved to improve immune competence and health status in sheep [18, 21] and subsequently the efficiency of growth and production [57]. As illustrated by McDowell [19], one of the first indicators of a marginal Zn deficiency is a depression in body weight gain and feed conversion that are often present prior to any change in blood or liver. In view of the role in growth, zinc serves as component in numerous enzyme systems associated with protein metabolism [1]. In consistence with our studies Hahn and Baker [59] and Mayland *et al.* [60] showed that Zn supplementation can cause significant increases in weight gain in young pigs and calves, respectively. On the other hand, Malcolm-Callis *et al.* [61] showed neither organic nor inorganic supplementation of Zn affected beef steer performance. It seems that this effect may depend on Zn status before supplementation.

On studying the effect of different forms of antioxidants on erythrogram of goat kids, the obtained data in table (3) show a significant improvement in Hb concentration and PCV% with slight improvement in RBC counts in the group II consuming BCS at the end of experiment. These increments may be attributed to the activation of haemopiotic tissues by BCS [62]. The increment of Hb concentration may be attributed either to increasing the synthesis of enzymes needed for biosynthesis of the heme [26] due to the presence of a large number of specific nutrients [Crude protein, fat carbohydrates, macro-mineral (calcium, phosphorus, magnesium and potassium) and micro-mineral (Copper, zinc, iron and manganese)] [24] and also the presence of thymoquinone (18.4–24%) of the essential oil of the seeds, has antioxidant, anti-inflammatory and analgesic properties [63] which lead to the lowered lipid peroxide in erythrocyte membrane, resulting in a decreased susceptibility of erythrocyte to haemolysis [64]. In the contrary, El-Sarha *et al*. [65] demonstrated lower haemopiotic values of BCS in goats. However, the conflict observed between the present results and the other mentioned findings may be attributed to different age and/or differences in dose and duration of the administered BCS.

The present findings indicated that supplementation of VitE /Se with Zn-Met for goat kids in group III could improve PCV% significantly while RBCs and Hb were

slightly affected. Similar results were reported earlier by Qureshi *et al*. [6] in buffaloes, Abou-Zeina and Hamam [18] in sheep and Mohri *et al*. [66] in dairy calves who recorded significant improvement of some of the hematological parameters as a result Vit E and Se supplementation. Likewise, they recorded unchanged MCV, MCH and MCHC. In contrary to the above results, supplementation of Se and vitamin E in the diet of buffalo calves had no significant effect on hematological parameters (Hb, PCV, RBCs and WBCs counts) as reported by Shinde *et al*. [67]. The hematological responses obtained in the current study could not be discussed away from the recent report of Jilani and Iqbal [68]. They stated that vitamin E supplementation led to increase the number of colony forming units of erythroid precursors, preventing the oxidation of polyunsaturated fatty acids in RBCs membrane, thus inhibiting the premature erythrocyte lysis, enhancing erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes. Thus, vitamin E may improve the post-supplemental blood Hb and PCV levels.

Moreover, the present work showed significant changes in TLC and some of its differential cells in the treated goat kids in both supplemented groups (Table 3). These findings confirm that administration of BCS and Vit E/ Se with Zn-Met could improve immune function in goats. Concerning the effect of BCS, similar results were obtained in rats [69] and rabbits [70]. The increase of TLC may be attributed to the activation of lymphoid tissues by BCS [62].

The effect of Vit E plus Se on increasing TLC agreed with similar response observed on sheep [18], Friesian heifers [71], adult buffaloes during late gestation [6], growing lambs [72] and dairy calves [66]. However, Shinde *et al.* [67] found that total count of leukocytes was not affected by Vit E and Se supplementation to buffalo calves. Among the circulating leukocytes, the lymphocytes are responsible for humoral and cellular immune responses and an increase in their number in blood may be a good indicator of an immunomodulatory response [6]. It could be noticed that changes in blood hematological parameters, in the present study, were within the normal physiological values of goats as previously documented [38].

Serum biochemical profiles have been used extensively by veterinarians to evaluate the nutritional, health and metabolic status of ruminants. In the present study, the slight depression in the serum activities of liver enzymes (Table 4) in goat kids in both groups consuming the different forms of antioxidants along the experimental periods could be supported by the view of the beneficial effects of the use of antioxidants as hepatoprotective. Many effects have been described for BCS and their constituents including its antioxidant role especially against hepatotoxicity in carbon tetrachloride (CCl_4)-induced toxicity, coinciding with improvement in serum lipid profile [32, 33], decreasing the elevated liver enzyme levels and increasing the reduced anti-oxidant enzyme levels [73]. Non-significant variation in the activities of AST and ALT were similar to earlier findings of Samanta and Dass [74] and Mudgal *et al*. [75] who did not find any effect of supplemental Vit E and Se on the activity of these enzymes in crossbred calves and buffalo calves, respectively. Contrary to present findings, Kursa and Kroupova [76] reported significantly higher activity of SGOT and SGPT in serum of calves given extra vitamin E (15-30 mg/kg) in milk replacer. The unchanged values of urea and creatinine observed in the present study in both supplemented groups indicated that both BCS and Vit E/Se with Zn-Met were safe to kidney at the recommended doses in goat kids. In consistence with our results, Abou-Zeina and Hamam [18] and Shinde *et al*. [67] reported that supplementation of sheep and buffalo- calves, respectively with Vit E and Se had no effect on serum urea and creatinine.

Concerning lipid profile (Table 4), the mean values of total cholesterol and LDL-C although numerically were lower in both supplemented groups, compared to their initial values and control, did not reveal any statistical differences. Similarly, Shinde *et al*. [67] reported that Vit E and Se supplementation had no effect on the cholesterol levels in buffalo-calves. Contrary to our findings, Nayyar *et al*. [77] who reported that supplementation of Vit E and Se to buffalo heifers caused a significant (P<0.01) increase in the levels of cholesterol. The mean values of HDL-C were found to be slightly elevated in the group III supplemented with Vit E/Se with Zn-Met. However, Shinde *et al*. [67] and Mudgal *et al*. [74] reported that Vit E and Se supplementation significantly increased HDL-C levels in buffalo- calves. The reason for an increase in the serum HDL cholesterol values due to Se and vitamin E supplementation may be due to the fact that Se is essential to maintain the integrity of pancreas and hence efficient digestion and absorption of fats, whereas regarding vitamin E, it is transported though circulation as components of lipoproteins and cattle maintain majority of their serum vitamin E in the HDL fraction. The overall mean triglycerides values though numerically were lower

in group II and higher in group III as compared to initial values, but on statistical comparison they were found to be similar. Similar to our findings, Shinde *et al*. [67] reported that supplementation of Vit E and Se to buffalo calves had no effect on the serum triglyceride concentration. Contrarily, Wojcicki *et al.* [78] reported that supplementation of Vit E partially reduced the serum triglycerides levels.

As shown in table (5), most values of protein patterns (TP, total and gamma-globulins) were significantly changed in group III that received Vit E/Se and Zn-Met throughout the experimental period, while they were improved at the end of study in the group II consuming BCS. The increase in protein concentration in the supplemented goats when compared with the control is an indication of increase in protein synthesis that may include those of antibodies and enzymes. Concerning BCS, similar results were obtained in rats [79] and Fish [80] which demonstrated the positive effect of BCS on TP and globulins. The increment of TP and globulins perhaps explained either by the fact that, BCS contains high percentage of crude protein (20.5%) and free amino acids [81-82]. Such increase perhaps attributed also to the role of BCS [83] in protein biosynthesis as it is vitally concerned in the growth process. Moreover, the significant increase of serum globulins indicated the immunostimulant effect of BCS [4].

Due to rapid changes that can occur in an animal's plasma, electrophoresis can be used for evaluating the immune response through estimation of gamma globulin fraction. The immunoglobulins (Igs), being part of the gamma-globulin complex, naturally leads to an increase in this fraction [84]. Significant increase in gamma-globulins fraction observed in both supplemented groups, introduce another evidence of the immunostimulator effect of the antioxidants in concerned. Similarly, Abou-Zeina *et al.* [4] reported that dietary supplementation with either BCS or Vit E/Se with Zn-Met in goats could improve cellular immune function. At this point, Hamam and Abou-Zeina [21] suggested that injection of both vitamin E plus Se, in ewes, significantly increased the concentrations of natural antioxidants (*á*-tocopherol and glutathione peroxidase) in blood, hence ensures that they mount adequate immune globulins. Previous researches demonstrated the positive effect of Vit E/Se supplementation on TP, total and gamma-globulins in serum of sheep [21,57,58]**.** However, Reddy *et al.* [85] and Shinde *et al.* [67] did not observe any significant effect of supplemental Vit E and Se on serum TP and globulins values in calves.

CONCLUSION

The present study can conclude that, inclusion of BCS and Vit E/ Se with Zn-Met in goat kids' diets improved the growth performance and immune status, while slightly affect hematological and serum biochemical profile. Both forms of antioxidants were safe to liver and kidney at the examined doses as reflected on their undisturbed biomarkers.

ACKNWLEDGMENT

This work was financially supported by National Research Center as a part of a project: entitled "Improvement of general health condition and immune status of small ruminants using antioxidants" (The 9th research plan, No. 9040203) under the super vision of Prof. Dr. Hala A. Abou – Zeina.

REFERENCES

1. Yatoo, M.I., A. Saxena, P.M. Deepa, B.P. Habeab, S. Devi, R.S. Jatav and U. Dimri, 2013. Role of Trace elements in animals: a review, Veterinary World, 6(12): 963-967.
2. Rahal, A., A. Kumar, V. Singh, B. Yadav, R. Tiwari, S. Chakraborty and K. Dhama, 2014. *Review Article:* Oxidative Stress, Prooxidants and Antioxidants: The Interplay. BioMed Research International, Article ID 761264, pp: 19.
3. Spears, J.W., 2011. Role of mineral and vitamin status on health of cows and calves. Advances in Dairy Technology, 23: 287-297.
4. Abou-Zeina, H.A.A., A.A. Ghazy, M.K. EL-Bayoumy, S.M. Dorgham, E.A. Khairy and H.I. Twfik, 2013. Effects of dietary antioxidants supplementation on cellular immune response and evaluation of their antimicrobial activity against some enteric pathogens in goats. Global Veterinaria, 11(2): 145-154.
5. Gressley, T.F., 2009. Zinc, copper, manganese and selenium in dairy cows. Importance of trace in dairy cattle rations. Proceedings of the 7th Annual Mid-Atlantic Nutrition Conference.
6. Qureshi Z.I., M. Siddig, L.A. Lodhi, G. Muhammad and H. Jamil, 2010. Effect of vitamin E-selenium administration during late gestation on productive and reproductive performance in dairy buffaloes and on growth performance of their calves. Pakistan Veterinary Journal, 30: 83-86.

7. Spears, J.W. and W.P. Weiss, 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Veterinary Journal, 176: 70-76.
8. Andrieu, S., 2008. Is there a role for organic trace element supplements in transition cow health? Veterinary Journal, 176: 77-83.
9. Predieri, G., M. Tegoni, E. Cinti, G. Leonardi and S. Ferruzza, 2003. Metal chelates of 2-hydroxy-4-methylthiobutanoic acid in animal feeding: preliminary investigations on stability and bioavailability. Journal of Inorganic Biochemistry, 95: 221-224.
10. Nemec, L.M., J.D. Richards, C.A. Atwell, D.E. Diaz, G.I. Zanton and T.F. Gressley, 2012. Immune responses in lactating Holstein cows supplemented with Cu, Mn and Zn as sulfates or methionine hydroxy analogue chelates. Journal of Dairy Science. 95(8): 4568-4577.
11. Shinde, P.L., R.S. Dass, A.K. Garg and V.K. Chaturvedi, 2007. Immune response and plasma alpha tocopherol and selenium status of buffalo (*Bubalus bubalis)* calves supplemented with vitamin E and selenium. Asian-Australasian Journal of Animal Science, 20(10): 1539-1545.
12. Vallee, B.L. and K.H. Falchuk, 1993. The biochemical basis of zinc physiology. Physiological Reviews, 73: 79-118.
13. Wang, R.L., J.G. Liang, L. Lu, L.Y. Zhang, S.F. Li and X.G. Luo, 2013. Effect of zinc source on performance, zinc status, immune response and rumen fermentation of lactating cows. Biological Trace Element Research, 152(1): 16-24.
14. Markesbery, W.R., T.J. Montine and M.A. Lovell, 2001. Oxidative alterations in neurodegenerative diseases. In:Mattson, M.P. (Ed.), Pathogenesis Disorders. Humana Press, Totowa, NJ, USA.
15. McDowell, L.R., N. Wilkinson, R. Madison and T. Felix, 2007. Vitamins and minerals functioning as antioxidants with supplementation considerations. Florida Ruminant Nutrition Symposium. Best Western Gateway Grand. Gainesville, FL, 30-31 January. http:// dairy.ifas.ufl.edu/ files/ rns/ 2007/ Mcdowell.pdf.Google
16. McDowell, L.R., S.N., Williams, N. Hidiroglou, C.A. Njeru G.M. Hill, L. Ochoa and N.S. Wilkinson, 1996. Vitamin E supplementation for the ruminant. Animal Feed Science and Technology, 60(3-4): 273-296.
17. Webb, E.C., N. Casey and L. Simela, 2005. Goat meat quality. Small Ruminant Research, 60: 153-166.
18. Abou-Zeina, H.A.A. and A.M. Hamam, 2002. Effects of parenteral administration of vitamin E and/or selenium on health and reproductive function of ewes fed on marginal deficient diets. Egyptian Journal of Basic and Applied Physiology, 1: 205-223.
19. McDowell, L.R., 2002. Recent advances in minerals and vitamins on nutrition of lactating cows. Pakistan Journal Nutrition, 1: 8-19.
20. Young, A.J. and G.M. Lowe, 2001. Mini review: Antioxidant and prooxidant properties of carotenoids. Archives of Biochemistry and Biophysics, 385: 20- 27.
21. Hamam, A.M. and H.A.A. Abou-Zeina, 2007. Effect of vitamin E and selenium supplements on the antioxidant markers and immune status in sheep. Journal of Biological Sciences, 7: 870-878.
22. Goreja, W.G., 2003. Black Seed: Nature's Miracle Remedy. New York, NY7 Amazing Herbs Press.
23. Cheikh-Rouhou S., S. Besbes, B. Hentati, C. Blecker, C. Deroanne and H. Attia, 2007. Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. Food Chemistry, 101: 673-681.
24. Khan, S.H., M.A. Anjum, A.P.T. Khawaja and N.M. Ashraf, 2013. Effects of black cumin seed (*Nigella sativa* L.) on performance and immune system in newly evolved crossbred laying hens. Veterinary Quarterly, 33(1): 13-19.
25. Solati, Z., B.S. Baharin and H. Bagheri, 2014. Antioxidant property, thymoquinone content and chemical characteristics of different extracts from *Nigella sativa* L. seeds. Journal of the American Oil Chemists' Society, 91(2): 295-300.
26. El-Tahir, K.E., M.M. Ashour, and M.M. Al-Harbi, 1993. The respiratory effects of the volatile oil of the black seed *(Nigella sativa)* in guineapigs: elucidation of the mechanism(s) of action. General Pharmacology, 24: 1115-1122.
27. Al-Saleh, I.A., G. Billedo and I.E. Inam, 2006. Level of selenium, DL-a-tocopgerol, DL-c-tocopherol, all trans retinol, thymoquinone and thymol in different brands of Nigella sativa seeds. Journal of Food Composition and Analysis, 19: 167-175.
28. Mahmoud, M.R., H.S. El-Ahbar and S. Saleh, 2002. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. Journal of Ethnopharmacology, 79: 1-11.

29. Shalaby, H.A., N.M.T. Abu El Ezz, T.K. Farag and H.A.A. Abou-Zeina, 2012. *In vitro* efficacy of a combination of ivermectin and *Nigella sativa* oil against helminth parasites. Global Veterinaria, 9(4): 465-473.
30. Al-Hader, A., M. Aqel and Z.A. Hasan, 1993. Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds. International Journal of Pharmacognosy, 31: 96-100.
31. Zaoui, A., Y. Cherrah, M.A. Lacaille-Dubois, A. Settaf, H. Amarouch and M. Hassar. 2000. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapies, 55: 379-382.
32. El-Dakhakhny, M., N.I. Mady and M.A. Halim, 2000. *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung, 50: 832- 836.
33. Nagi, M.N., K. Alam, O.A. Badary, O.A. Al-Shabanah, H.A. Al-Sawaf and A.M. Al-Bekairi, 1999. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochemistry and molecular biology international, 47: 153-159.
34. Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research, 14: 323-328.
35. Chun, H., D.H. Shin, B.S. Hong, W.D. Cho, H.Y. Cho and H.C. Yang, 2002. Biochemical properties of polysaccharides from black pepper. Biological and Pharmaceutical Bulletin, 25: 1203-1208.
36. Radostitis, O.M., D.C. Blood, C.C. Gay and K.W. Hinchcliff, 2000. Veterinary Medicine: A textbook of the diseases of cattle, sheep, goats and horses. 9th edn. Bailliere Tindall, Philadelphia, USA, pp: 3-37.
37. A.O.A.C., 1990. Association of Official Analytical Chemists. Official Methods of Analysis. 15th edn. Washington DC, USA.
38. Weiss, D.J. and K.J. Wardrop, 2010. Schalm's Veterinary Hematology. 6th edn., Blackwell Publishing Ltd, Ames, Iowa, USA.
39. Reitman, S.M.D. and S. Frankel, 1957. A colorimeter method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic acid transferases. American Journal of Clinical Pathology, 28: 56-63.
40. Persijn, J.P., W. van der Slik and W.A. Zwart, 1971. Colorimetric assay for gamma-glutamyl transpeptidase. Clinica Chimica Acta, 35(1): 239-240.
41. Houot, O., 1985. Interpretation of Clinical Laboratory Tests. Ed. by G. Siest,; J. Henny, F. Schiele and D.S. Young, Biochemical publications, pp: 220-234.
42. Patton, C.J. and S.R. Crouch, 1977. Calorimetric determination of urea. Analytical Chemistry, 49: 464-469.
43. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 20: 470-475.
44. Gowenlock, A.H., 1988. Varely's Practical Clinical Biochemistry. 6th edn. Heinemann Medical Books, 22 Bedford square, London WCIB HH.
45. Lopes-Virella, M.F., P. Stone, S. Ellis and J.A. Coiweil, 1977. Cholesterol Determination in High-Density Lipoproteins Separated by Three Different Methods. Clinical Chemistry, 23(5): 882-884.
46. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 18: 499-502.
47. Henary, R.J., D.C. Cannon and J.W. Winkleman, 1974. Clinical Chemistry Principles and Techniques. 2nd edn. Harper and Roe, New York.
48. Snedecor, G.W. and Cochran W.G., 1989. Statistical Methods. 8th edn., Ames, Iowa State University Press.
49. Shomar, B., 2012. Major and trace elements in *Nigelle sativa* provide a potential mechanism for its healing effects. Journal of Medicinal Plants Research, 6(33): 4836-4843.
50. Dobrzański Z., B. Dolińska, H. Górecka, E. Bodak and F. Ryszka, 2002. The chemical composition of dietary yeast enriched with selenium, chromium and zinc. Folia Vet., 46(2)20: 36-37.
51. Kessler, J., 1991. Mineral Nutrition of Goats. (Ed.), Morand- Fehr, P Pudoc, Wageningen (Netherlands).
52. Mba, A.U., 1981. The mineral nutrition of goats in Nigeria. In: Nutrition et systèmes d'alimentation de la chèvre. Institut National de laRecherche Agronomique/Institut Technique de l'Elevage Ovin et Caprin, Paris, France.
53. Awadalla, I.M. and A.E. Gehad, 2003. Effect of supplementing growing sheep rations with black cumin seeds (*Nigella sativa*). Journal of Agriculture Science Mansoura University, 28: 185-194.
54. Habeeb A.A.M. and A.A. El-Tarabany, 2012. Effect of *Nigella sativa* or Curcumin on Daily Body Weight Gain, Feed Intake and some Physiological Functions in Growing Zaraibi Goats during Hot Summer Season. Arab Journal of Nuclear Science and Applications, 45(3): 238-249.

55. El-Faham, S.Y., 1994. Comparative studies on chemical composition of the Nigella sativa seeds and its cake (Defatted meal). J. Agric. Sci. Mansoura Univ., 19: 2283-2289.
56. Shetaewi, M.M., H.A. Daghas and T.S.A. Abd El-All, 1992. Growth performance, hematology and serum profiles of coarse-wool lambs as influenced by supplemental vitamin E. Assuit Veterinary Medical Journal, 54: 64-70.
57. El-Shahat, K.H. and U.M. Abdel Monem, 2011. Effects of dietary supplementation with vitamin E and /or selenium on metabolic and reproductive performance of Egyptian Baladi ewes under subtropical conditions. World Applied Sciences Journal, 12: 1492-1499.
58. Soliman E.B., A.K.I. Abd El-Moty and A.Y. Kassab, 2012. Combined effect of vitamin E and selenium on some productive and physiological characteristics of ewes and their lambs during suckling period. Egyptian Journal of Sheep & Goat Sciences, 7: 31- 42.
59. Hahn, J.D. and D.H. Baker, 1993. Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. Journal Animal Science, 11: 3020-3024.
60. Mayland, H.F., R.C. Rosenau and A.R. Florance, 1980. Grazing cow and calf responses to zinc supplement. Journal Animal Science, 51: 966-985.
61. Malcolm-Callis, K.J., G.C. Duff, S.A. Gunter, E.B. Kegley and D.A. Vermeire, 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics and serum values in finishing beef steers. Journal Animal Science, 78: 2801-2808.
62. Satish, N., M. Salomi, B. Panikkar and K. Pannikar, 1991. Modulatory effects of *Crocus sativa* and *Nigella sativa* extracts on clisplatin toxicity in mice. Journal of Ethnopharmacology, 31: 75-83.
63. Arslan, S.O., E. Gelir, F. Armutcu, O. Coskun, A. Gurel, H. Sayan and I.L. Celik, 2005. The protective effect of thymoquinone on ethanol-induced acute gastric damage in the rat. Nutrition Research, 25: 673-680.
64. Alimohamadi, K., K. Taherpour, H.A. Ghasemi and F. Fatahnia, 2014. Comparative effects of using black seed (Nigella sativa), cumin seed (Cuminum cyminum), probiotic or prebiotic on growth performance, blood haematology and serum biochemistry of broiler chicks. Journal of Animal Physiology and Animal Nutrition, 98(3): 538-546.
65. El-Sarha, A.L., H.Y. Hassan and K.M. Said, 1997. Haemato-biochemical changes induced by oral administration of Nigella sativa (Black seed) to goats. Egyptian Germany Society of Zoology, 22: 69-83.
66. Mohri, M., H.A. Seifi and J. Khodadadi, 2005. Effects of preweaning parenteral supplementation of vitamin E and selenium on hematology, serum protein and weight gain in dairy calves. Comparative Clinical Pathology, 14: 149-154.
67. Shinde, P.L., R.S. Dass and A.K. Garg, 2009. Effect of vitamin E and selenium supplementation on haematology, blood chemistry and thyroid hormones in male buffalo (*Bubalus bubalis*) calves. Journal of Animal and Feed Sciences, 18: 241-256.
68. Jilani, T. and M.P. Iqbal, 2011. Does vitamin E have a role in treatment and prevention of Anemia's? Pakistan Journal of Pharmaceutical Sciences, 24: 237-242.
69. Ali, B.H. and G. Blunden, 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytotherapy Research, 17: 299-305.
70. Meral, I., N. Donmez, B. Baydas, F. Belge and M. Kanter, 2004. Effect of Nigella sativa L. on heart rate and some haematological values of alloxan-induced diabetic rabbits. Scandinavian Journal of Laboratory Animal Science, 1: 49-50.
71. Suwanpanya, N., W. Wongpratoom, M. Wanapat, S. Aiumlamai, S. Wittayakun and C. Wachirapakorn, 2007. The influence of bovine neutrophils on *in vitro* phagocytosis and killing of *Staphylococcus aureus* in heifers supplemented with selenium and vitamin E. Songklanakarin Journal of Science and Technology, 29: 697-706.
72. Soliman, E.B., M.A.A. El-Barody and S.T.M. Fahmy, 2001. Some physiological reactions of male lambs subject to vitamin E and selenium injection during summer conditions. Assuit Veterinary Medical Journal, 46: 210-225.
73. Salem, M.L., 2005. Immunomodulatory and therapeutic properties of the *Nigella* sativa L. seed. International Immunopharmacology, 5: 1749-1770.
74. Samanta A.K. and R.S. Dass, 2007. Effect of dietary vitamin E supplementation on serum á-tocopherol and immune status of crossbred calves. Int. J. Cow Sci., 3: 34-43.
75. Mudgal, V., A.K. Garg, R.S. Dass and V.P. Varshney, 2008. Effect of selenium and copper supplementation on blood metabolic profi le in male buffalo (*Bubalus bubalis*) calves. Biological Trace Element Research, 121: 131-138.

76. Kursa J. and V. Kroupova, 1976. Osmotic and oxidative hemolysis of erythrocytes in calves with white muscle disease. Res. Vet. Sci., 20: 97-98.
77. Nayyar, S., V.K. Gill, V.S. Malik, K.S. Roy and R. Singh, 2003. Vitamin E and selenium improve the blood biochemical composition of anoestrous buffalo heifers. Indian Journal of Animal Science, 73: 654-656.
78. Wojcicki, J., L. Rozewicka, B.B. Wiszniewska, L. Samochowiec, S. Józwiak, D. Kalubowska, S.P. Tustanowski and Z. Juzyszyn, 1991. Effect of selenium and vitamin E on development of xperimental atherosclerosis in rabbits. Atherosclerosis, 87: 9-15.
79. Ekanem, J.T. and O.K. Yusuf, 2008. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *Trypanosoma brucei* infected rats. African Journal of Biotechnology, 7(2): 153-157.
80. Saad, T.T., E.N. Abou El-Geit, A.K.I. El-Hammady and M.S. Zaki, 2013. Effect of black cumin seeds (*Nigella Sativa*) and/or turmeric (*Curcumin*) on hematological, biochemical and immunological parameters of Sea Bass vaccinated with *Pseudomonas Fluorescence* Bacterin. Life Science Journal, 10(2): 1292-1303.
81. Babayan, V.K., D. Koottungal and A.G. Halaby, 1978. Proximate analysis, fatty acids and amino acid composition of Nigella sativa seeds. J. Food Sci., 43: 1314-1315.
82. Atta, M.B., 2003. Some characterisitics of nigella seed (*Nigella sativa* L.) cultivated in Egypt and its lipid profile. Food Chemistry, 83: 63-68.
83. Hedaya, S.A., 1995. Effect of *Nigella sativa* (black seeds) extract on some haematological and biochemical parameters in rats. Alexandria Journal of Veterinary Science, 11: 95-99.
84. Jain, N.C., 1993. Essentials of Veterinary Hematology. 1st edn., Lea & Febiger, Philadelphia PA.
85. Reddy, P.G., J.C. Morrill and R.A. Frey, 1987. Vitamin E requirements of dairy calves. Journal Dairy Science, 70: 123-129.