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Effect of Different Dietary Supplementation with Antioxidants on Gene Expression and Blood Antioxidant Markers as Well as Thyroid Hormones Status in Goat Kids

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Abstract: The present study aimed to determine the effects of the use of natural herbs antioxidant such as *N. sativa* or black cumin seeds (BCS) *vs.* nutrient antioxidants in the organic form, as dietary supplements on gene expression and blood antioxidant profile as well as thyroid hormones activities in goat kids. Fifteen male goat kids of Baladi breed were randomly assigned into three equal groups. Group I was fed the basal diet without any supplementation and served as control, group II received the basal diet supplemented with multi-nutrient antioxidants in the organic form; vitamin E with selenium enriched yeast (Vit E/Se) and zinc methionine (Zn-Met) at levels of 2g/kg of diet and group III was fed the basal diet supplemented with crushed BCS at ratio of 2%. Blood samples were collected from the jugular vein of all goats at zero- time and every 30 days along the experimental period (90 days). Gene expression of antioxidant enzymes, biomarkers of antioxidant status (E- GPx and SOD activities, serum-TAC, Zn and Cu) and thyroid hormones (T3 and T4) were assessed. At the end of the experiment, the results indicated that the animals fed either Vit E/Se and Zn-Met or BCS diet showed up-regulation in the expression of GPx mRNA and CuZn-SOD mRNA. The evaluation of antioxidant profile showed significant (P<0.01) elevation in the activities of E- GPx and SOD as well as serum TAC in both supplemented groups starting from 30th day and continued until the end of experiment in comparison to control. Moreover, the values of serum Zn significantly (P<0.05) increased in group II starting from day 30, while were significantly (P<0.05) higher in group III receiving BCS on 60th and 90th days in comparison to control. Serum Cu concentrations increased significantly (P<0.05) in goats of group III at the end of experiment, while was not significantly (P>0.05) affected in group II. Also, significant (P<0.01) elevation in serum concentrations of T3 were recorded in goat kids of both supplemented groups on day 30 and persisted throughout the experiment. However, T4 levels did not significantly differ among experimental groups. It was concluded that, inclusion of the both forms of antioxidants in goat kids' diets improved the gene expression of antioxidant enzymes and biomarkers of antioxidant status as well as thyroid hormones activities.

Key words: Antioxidants · Vit E · Selenium · Zinc · *Nigella sativa* · Gene Expression · Thyroid hormones · Goats

INTRODUCTION

Antioxidants have a vital role in the protection of cells against the damaging effects of lipid peroxides and free radicals produced during normal metabolism [1]. Endogenous antioxidant defenses include a network of compartmentalized antioxidant enzymatic and non enzymatic molecules that are usually distributed within the cytoplasm and various cell organelles [2]. The dietary and tissue balance of antioxidant nutrients is important in protecting tissues against oxidative damage of proteins, lipids, or DNA [3, 4].

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Certain micro nutrients such as vitamin E (Vit E), selenium (Se) and zinc (Zn) are common antioxidants normally included in animal diet that improve different immune functions and health condition [5-7]. These nutrients are playing a vital role in many physio-biochemical processes like protein, enzyme and hormone synthesis and involving in oxidation reduction reactions and gene expression [8, 9]. They are exhibiting an important role in growth [7, 10], production [11] and reproduction [12]. Combinations of antioxidants may be more effective than larger quantity of any single one [13, 14]. Interestingly, a high concentration of such nutrients in the diet above predicted requirements prevented protein and lipid oxidation and improve immune functions in ruminants [6, 7, 15-17].

Selenium in combination with Vit E serves as vital part of biological antioxidant system to maintain cellular integrity against lipid peroxidation [10]. Selenium as an essential component of glutathione peroxidase (GPx), acts to destroy peroxides before they attack cell membranes [18], while Vit E was reported to act within the membrane to prevent the formation of fatty acid hydro peroxides [19]. Likewise**,** Zn and manganese, in addition to copper (Cu), are integral parts of superoxide dismutase (SOD) which is considered the first line of defense against free radicals [20]. Zinc is also involved in gene expression at the transcription level [8, 21]. Selenium and Zn are important for thyroid hormones activation [22]. It has been reported that Zn in addition to its participation in protein synthesis, is involved in T3 bound to its nuclear receptor [23, 24]. Also, Se is a component of deiodinase enzyme, which transforms T4 into T3 beside its role as antioxidant in thyroid gland as a component of glutathione peroxidase [25]. The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body [26]. Thyroid hormones might be able to regulate the activities of SOD and GPx in the lymphoid organs and skeletal muscles [27].

Natural additives such as herbs and edible plants can be used in therapy instead of using synthetic drugs which may have adverse effects. One of the alternatives used as feed additives is *Nigella sativa (N. sativa)* or Black cumin seeds (BCS) [6,7, 28]. The seed of *N. sativa* has been reported to have many biological properties including antibacterial [6, 29], antiviral [30] and anti-parasitic [31]. The oil fraction of *N. sativa* contains thymoquinone, which has immuno-potentiating activities and antioxidative effect [6, 32]. Moreover, *N. sativa* seeds provide relatively high amounts of some essential nutrients such as carbohydrates, fats, vitamins, minerals and proteins including eight of the nine essential amino acids that improve immunity and growth performance [33, 34].

No information on the effect of antioxidants on gene expression in goats, while few studies are available on their effects on blood antioxidants profile and thyroid hormones. Therefore, the present research work was planned to evaluate the effect of dietary supplementation of different forms of antioxidants (*N. sativa vs.* multi nutrient- antioxidants in organic form; Vit. E with Se enriched yeast and Zn-Met) on these aspects in male goat kids.

MATERIALS AND METHODS

Animals and Feeding: Fifteen clinically healthy male Baladi goat kids, aged 4-6 months and weighing 10-15 kg were used throughout. The kids were not exposed to either stressors or pathogens and were routinely clinically examined [35]. All animals were fed an adaptation period of 2 weeks on constant basal diet which consisted of: 50% yellow corn; 17% wheat bran, 25% cotton seed cake meal, 5% molasses, 2% limestone and1% sodium chloride. 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 lib*.

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

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

Group II: Fed the same basal diet supplemented with multi- nutrient antioxidants in organic form: Vit. E with Se enriched yeast (E 60.000, Sanovet, Austria – Composition: Vit E 60g, Se yeast 12g, L-lysine 0.08g and carrier: dextrose up to 1 kg) at level of 2 g / kg of diet and zinc methionine (Zn-Met/10 Zinc chelated with methionine hydroxy analogue – IBEX International) at the same dose level [6].

Group III: Fed the same basal diet- supplemented with crushed BCS at level of 2% [6].

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.

Blood Collection and Analysis: Blood samples were taken in the early morning from the jugular vein of all goats at the beginning of the study and thereafter monthly intervals throughout period of the experiment (90 days). For the determination of molecular markers in whole blood (Gene expression of antioxidants) and erythrocytes GPx &SOD, blood samples were collected into vacuum tubes containing EDTA as an anticoagulant. For the determination of serum total antioxidant capacity (TAC), Zn, Cu and thyroid hormones, blood samples were collected into plain vacuum tubes and sera were separated and stored at -20°C until analysis.

Studied Parameters and Methods

Gene Expression of Antioxidant Enzymes

RNA Isolation and cDNA Synthesis: Total RNA from EDTA-blood samples was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The concentration of isolated total RNA was quantified with UV spectrophotometer. Then, 1 µg of RNA was treated with RNase-Free DNase I(#EN0523, Fermentas Inc., Ontario, CA) to remove contaminating DNA. Thereafter, first-standard cDNA was synthesized using a RevertAidTM First Strand cDNA Synthesis Kit (K1622, Fermentas Inc.,Ontario, CA). The cDNA products were separated by agarose gel electrophoresis (1.5% agarose), visualized with UV light after ethidium bromide staining and then were stored at -20 °C for Semi-quantitative reverse transcription and PCR reaction. cDNAs were amplified using specific primers for goats GPx, Cu Zn- SOD and β-actin gene and were selected according to Zhong *et al*. [36]. The details of the oligonucleotide primer sequences and predicted amplified product lengths are listed in Table 1.

Gene expression was assayed using RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas Sigma, St. Louis, MO) according to the manufacturer's instruction. The PCR program cycles were set as follows: initial denaturing at 95°C for 20 s, followed by 40 cycles (95°C for 3 s, 60°C for 30 s). β -actin mRNA was used as an internal standard, GPx mRNA CuZn- SOD expressions were determined by quantitative reverse transcription-PCR and normalized against β-actin mRNA levels. The PCR product was run on a 2% agarose gel in Tris-borate-EDTA buffer and visualized over a UV Trans-illuminator. The ethidium bromide-stained gel bands were scanned and the signal intensities were quantified by the computerized Gel-Pro (version 3.1 for window 3). The ratio between the levels of the target gene amplification product and the β-actin (internal control) was calculated to normalize for initial variation in sample concentration as a control for reaction efficiency [37, 38].

Assays in Lysate of Erythrocytes: For the determination of GPx and SOD activities in the lysate of erythrocytes, erythrocytes were obtained by centrifuging 0.5 mL of blood for 15 min and were then washed four times with 3 mL of 0.9% Na Cl solution. After the final wash, the red blood cells were lysed by hypotonic shock using 2 mL of redistilled water. The lysate was mixed and stored at - 80C until analysis. The GPx was measured by the method of Paglia & Valentine [39] (Bio diagnostic Kit, Egypt). GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (Bio diagnostic Kit, Egypt) [40]. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4- iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form

Table 1: Sequence of Primers and PCR Products Size.

Genes	primers (50 --- 30)	Product size (bppp)
GPx	Forward - ACATTGAAACCCTGCTGTCC	216
	Reverse - TCATGAGGAGCTGTGGTCTG	
CuZn-SOD	Forward - TGCAGGCCCTCACTTTAATC	207
	Reverse - CTGCCCAAGTCATCTGGTTT	
β-actin	Forward - CCAACCGTGAGAAGATGACC	
	Reverse - CGCTCCGTGAGAATCTTCAT	201

a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. Measurements were performed using Spectrophotometer model T80, UV/Visible, double beam, UK.

Assays in Serum: Serum TAC was determined using a commercial kit according to the method of Koracevic *et al.* [41]. The sera were analyzed for Zn and Cu concentrations using Flame Atomic Absorption Spectrophotometer [42]. Thyroid hormones**;** triiodothyronine (T3) and total thyroxine (T4) were determined in sera using ELISA technique [DRG International Inc., USA] according to Walker [43] and Wistom [44]**,** respectively.

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 [45] using SPSS version 16 computer programmed.

RESULTS

All animals consumed the experimental rations were clinically normal during the course of the experiment.

Gene Expression of Antioxidant Enzymes: The ratio between GPx mRNA/β-actin indicate an up expression in GPx compared to the ratio between control/ β-actin (Fig. 1) which increased to reach 1.19 in goat kids supplemented with Vit E/Se and Zn-Met, while the goats fed on BCS the ratio of GPx mRNA reach to 0.97. Meanwhile, the ratio of CuZn-SOD/ β-actin was similarly increased compared to control/ β-actin ratio in goats fed on either Vit E/Se and Zn-Met or BCS which increased from 0.98 in control to 1.1, 1.2 respectively (Fig. 2).

Blood Antioxidant Markers: The natural antioxidant profile of the experimental animals at different periods for different trials is demonstrated in Table 2. The results pointed to high significant ($P<0.01$) increases in the activities of E- GPx and SOD in goat kids of both supplemented groups II and III, starting from day 30 and continued until the end of experiment in comparison to those in control group and the initial recorded values of the same groups. There were high significant ($P<0.01$) increases in concentration of serum TAC in both groups II and III on 30th and 90th days of experiment compared to the recorded initial values of same groups and the control group. The values of serum Zn increased significantly ($P<0.05$) in group II starting from day 30 and onward compared to initial values and the recorded values on days 0 and 30 of group III and control group. Meanwhile, the values of Zn were significantly ($P<0.05$) higher on days 60 and 90 of experiment in goats of group III which received BCS than their values on days 0 and 30 in the same group and control group. Serum Cu concentrations increased significantly ($P<0.05$) in goats of group III at the end of experiment compared to initial values of the same group and to those recorded in control group on days 30 and 90. Otherwise, no significant ($P>0.05$) changes were observed in the levels of serum Cu in group II throughout among experimental groups.

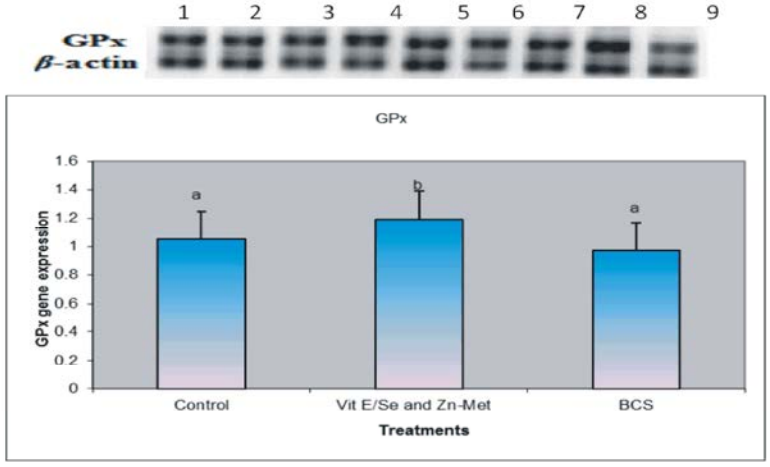

Fig. 1: The ratio between *GPx/ β*-actin in goats feed with either Vit E/Se and Zn-Met or BCS Lane 1, 2 &3 represented group I, lane 4, 5 &6 represented group II and lane 7, 8 &9 represented group III while, values represent mean ± S.E. for each group of goats.

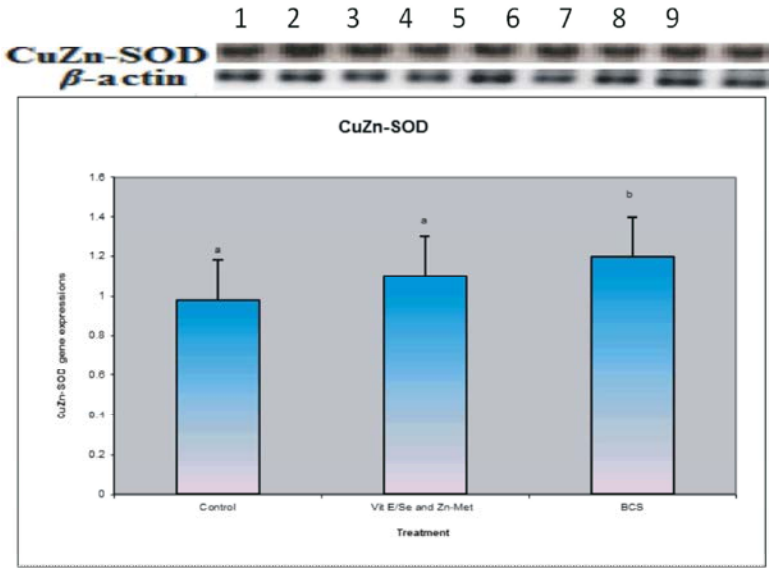

Fig. 2: The ratio between *CuZn-SOD/ β*-actin in goats feed with either Vit E/Se and Zn-Met-or BCS Lane 1, 2 &3 represented group I, lane 4, 5 &6 represented group II and lane 7, 8 &9 represented group III while, values represent mean ± S.E. for each group of goats.

Table 2: Antioxidant status for experimental groups in lysate of RBCs and serum during different periods of experiment (Mean ±SE)

	Treatments												
	Control				Multi-antioxidants - Supplement				Black Cumin seed - 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.
GPx (U/ml RBC lysate)	1.11^{a}±0.08	0.93^{a}±0.05	0.97^{a}±0.06	1.00^{a}±0.08	1.01^{a}±0.07	1.74^{b}±0.12	1.96^{b}±0.10	1.91^{b}±0.17	1.04^{a} ±0.08	1.80^{b}±0.10	1.89^{b}±0.13	1.58^{b}±7.33	**
SOD (U/ml RBC lysate)	4.81^{a}±0.42	4.57^{a}±0.36	4.60^{a}±0.33	4.95^{a}±0.25	4.54^{a}±0.32	7.80^{b}±0.33	7.72^{b}±0.43	7.83^{b}±0.50	4.84^{a} ±0.51	17.75^{b}±0.44	6.97^{b} ±0.56	7.55^{b}±0.57	**
TAC(mM/l)	0.25^{a}±0.02	0.27^{a}±0.05	0.27^{a}±0.02	0.27^{a}±0.02	0.28^{ab}±0.04	0.33^{ab}±0.05	0.42^{b}±0.05	0.49^{b}±0.05	0.32^{a} ±0.05	0.35^{ab}±0.05	0.49^{b}±0.04	0.44^{b}±0.03	**
Zinc (μg/dl)	71.90^{ac}±1.96	70.00^{ac}±3.76	70.74a±1.69	69.84^{a}±1.04	70.42^{ac}±1.39	75.82^{b}±1.53	74.60^{b}±2.65	78.30^{b}±1.93	66.30^{a} ±3.45	68.58^{a}±2.70	75.10^{b}±1.68	76.80^{b}±1.98	*
Copper (μg/dl)	73.50^{ab}±6.93	72.60^{ab}±3.99	71.90^{a}±1.83	69.60^{a}±4.48	68.90^{a}±4.86	71.40^{ab}±4.01	73.20^{ab}±3.36	72.20^{ab}±4.09	68.30^{a} ±4.38	75.60^{ab} ±7.19	76.10^{ab}±3.03	88.40^{b}±4.68	NS

Means with different superscripts (a, b, c) between groups in the same row are significantly different at $P<0.05$.* $P<0.05$. ** $P<0.01$.
NS= non- significant GPx= Glutathione peroxidase. SOD= Superoxide dismutase TAC= Total antioxidant capacity

Table 3: Triiodothyronine (T3) and thyroxine (T4) for experimental groups during different periods of experiment (Mean ±SE)

	Treatments												
	Control				Multi-antioxidants - supplement				Black Cumin seed- 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.
Triiodothyronine (ng/dl)	68.40^{b}±8.39	72.80^{b}±8.48	81.60^{b}±4.67	89.00^{b}±9.58	70.50^{b}±13.16	132.40^{a}±22.55	126.60^{a}±13.67	130.40^{a}±13.61	77.80^{b}±11.66	127.6^{a}±21.84	120.0^{a}±12.75	135.40^{a}±16.15	**
Thyroxine (μg/dl)	5.86^{ac}±0.67	8.36^{bc}±1.31	8.56^{bc}±1.88	10.20^{bc}±1.86	5.72^{ac}±0.94	8.24^{bc}±1.26	7.90^{bc}±1.12	7.64^{bc}±1.00	6.52^{bc}±1.00	10.54^{b}±2.13	9.74^{bc}±2.82	8.54^{bc}±1.38	NS

Means with different superscripts (a, b, c) between groups in the same row are significantly different at $P<0.05$.* $P<0.05$. ** $P<0.01$.
NS= non- significant

Thyroid Hormones Status: Table 3 showed thyroid hormones activities of the experimental animals at different periods for different trials. Serum T3 levels significantly ($P<0.01$) increased in both supplemented groups on day 30 of experiment and the elevation persisted along the experiment, compared to their initial values and control group at all periods. However, no significant ($P>0.05$) variations were recorded in the hormonal values of T4 throughout among experimental groups.

DISCUSSSION

The detection of antioxidant status gives complementary information about the metabolic status of the animal rather than metabolic parameters alone [46]. Previous studies by the same authors have included the determination of the effects of addition of different forms of antioxidants in the diets of goats on growth performance and blood metabolic profile [7]. Therefore, in the present study more investigations have to be

performed in aim to get overall information about metabolic path and to determine influence of dietary supplementation with antioxidants on gene expression and blood antioxidant biomarkers levels as well as thyroid hormones activities in goat kids.

There is no information about the role of antioxidants on gene expression of antioxidant enzymes in goats. We hypothesized that feeding goat kids antioxidants either Vit E /Se enriched yeast and Zn- Met or BCS would improve their gene expression of antioxidant enzymes as indicated by higher GPx mRNA/β-actin and CuZn-SOD mRNA/β-actin compared to control/ β-actin ratio (Fig 1, 2). These improvements in the animals have been extended to identify the particular components in foods that account for the chemoprotective activity and a considerable proportion have been found to have antioxidant properties. In the current study, the increment in gene expression in goats received Vit E /Se enriched yeast and Zn- Met could not be discussed away from the recent report of Liao *et al*. [47] who found that Se-yeast supplementation of 3 mg Se/day increases mitochondrial gene expression in liver of Se-replete beef heifers and decreases expression of genes known to be up regulated during oxidative stress. In this regard, several hundreds of Zn-containing nucleoproteins are probably involved in gene expression of various proteins [8]. Also, it is a component of many transcription factors and proteins that control cell cycles. Zinc is also thought to have a critical role in the stabilization of biomembranes [48]. The multiple functions of these nutrients, at cellular and molecular levels, extend beyond antioxidant protection, as their inclusion in the diet at concentrations above requirements is associated with variable improvements in animal performance and immune function [6, 7, 15].

Among the herbs that are known to have antioxidant properties, the seeds of *N, sativa* which have been used traditionally for centuries for the treatment of different diseases [49]. Thymoquinone, the main compound in *N. sativa* oil, inhibits non-enzymatic lipid peroxidation in liposomes. In addition to thymoquinone, there are many other compounds in the oil possessing a strong antioxidative effect, such as *p*-cymene, *m*-cymene, □-thujene and carvacrol, which have been reported to possess antioxidant effects and radical scavenging properties [50]. From another point of view, the seeds of *N. sativa* contain large amounts of nutrient trace elements which increase antioxidant status, immunity and may help the animals to tolerate the stress conditions [6, 51].

Among different biomarkers of antioxidant profile, the natural antioxidants including metalloenzymes Se dependent GPx, CuZn-SOD and trace elements that are currently considered the most important markers [1]. The present study demonstrated that the supplementation with both forms of antioxidants was responsible for a lasting increase in the natural antioxidants biomarkers levels in the blood of experimental animals (Table 2).

The GPx has been used as an indicator of Se status in animals due to the high correlation found between dietary Se and the activity of this enzyme in plasma and red blood cells [52]. The present study approved significantly higher activity (P<0.01) of GPx in the goat kids blood from both supplemented groups in comparison with control group that is in agreement with results observed in lambs [53-55] and in sheep [14, 56, 57], supplemented with Se alone or combined with Vit E. The GPx increase appears to respond to Se supplementation. Benefits of supranutritional Se-yeast supplementation in ruminants include improvements in antioxidant status [58] and immune function [59, 60] and increases ruminal fermentation and nutrient digestibility [61]. Antunović *et al*. [62] and Antunović *et al*. [63] carried out trial with lambs fed different dietary supplementation of Se (Inorganic and organic sources) resulting in significantly higher activity of the blood GPx enzyme, compared to the control group.

In the current study, a similar trend of enzyme activity of SOD was found. The SOD play an important role in detoxification processes by catalyzing the conversion of free O^- 2 to O_2 and H_2O_2 and is associated with stress situations including zinc stress [64]. As a result of these reactions, hydrogen peroxide (H_2O_2) is produced. The antioxidant properties of Zn were first demonstrated *in vitro* and there is also clear evidence that zinc functions as an antioxidant in the body [48].

Interestingly, GPx and SOD activities were recorded to be increased with Zn-Met supplementation in blood of ewes [65]. According to some authors Se supplementation in the diet of animals increases the activity of SOD, which, together with GPx, represents main defense mechanism against free radicals [3, 66, 67]. Similarly, the supplementation of Se increases antioxidant capacity in sows (Production of GPx, SOD, GSH) [68, 69]. These conclusions were confirmed also in our results. On the contrary, in the experiments, which was carried out with ewes administered with Se alone or with Vit E [14] and rabbits [70] supplemented with Se in organic form

significantly increased GPx activity, while levels of SOD were not significantly affected in any of the experimental groups. However, another study describes that an absence of Se leads to the significant reduction of GPx activity ($P < 0.05$) and significant increase in SOD activity ($P < 0.05$) in the kidneys and blood serum of guinea pigs [71].

The measure of TAC considers the cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants [72]. Moreover, TAC could be used as a tool to evaluate the nutritional status of animals fed different diets or to evaluate the general nutritional status of animals throughout the year. The observation that serum TAC levels significantly increased in both supplemented groups during the periods of experiment (Table, 2), considered an indicator of the ability of both types of antioxidants to improve antioxidant condition of goats.

The trace elements (TE) such as Se, Zn and Cu play an important catalytic role for the enzymatic activity of GPx (Se) and SOD (Zn, Cu). Dietary deficiency of these nutrients, lead to failure of the antioxidant- enzyme defense system [9, 14]. The marked increase of serum Zn and Cu concentrations in our results (Table, 2) confirm this relationship. The increase in the level of serum Zn was reported to be due to supplementation of Zn-Met. In the experiment, where ewes were supplemented with Se and Vit E, supplemented and unsupplemented groups of animals had no significant differences in the blood serum levels of Zn and Cu [73]. Also, Pechova *et al*. [74] and Cortinhas *et al*. [75] found no significant differences in the concentration of Cu and Zn in goats and cows, respectively supplemented with organic form of Se in the diet. In agreement to our study, Mudgal *et al*. [76] stated that at supplementation of Se and Zn in dairy cattle did not affect level of Cu in blood. The marked increase of serum Zn and Cu concentrations in goat kids received BCS was attributed to that *N. sativa* is a good source of mineral elements [34].

When evaluating the effect of antioxidants on the blood activities of thyroid hormones in goat kids, the present work (Table 3) demonstrated positive effects on the T3 hormone production in both supplemented groups comparable with the unsupplemented control ones. However, the mean concentrations of T4 kept constant through the course of the study. These findings confirm that supplementation of Vit E/ Se and Zn-Met and BCS could improve thyroid activity indicating their favourable metabolic role that reflected on higher growth performance [7]. The effect of Vit E/ Se with Zn- Met agreed with previous studies denoting that Zn and Se supplementation increase the total T3 in goats [77] and buffaloes [78]. Explanation may be due to that Se is essential part of type I iodothyronine-5´-deiodinase enzyme, which is responsible for the deiodination of T4 to biologically active thyroid hormone T3 and it protects the thyroid gland from peroxides produced during the synthesis of hormones as a component of glutathione peroxidase [25]. Also, it has been documented that Zn in addition to its participation in protein synthesis, is involved in T3 binding to its nuclear receptor [23]. Similar changes in thyroid hormone activity in sheep fed with addition of organic Se were determined by Antunović *et al*. [63] and Bik *et al.* [79]. In agreement with our results, lambs born from ewes received injection of Vit E plus Se exhibited higher concentration of plasma T_3 with no significant change in their T_4 levels [80]. Also, supplementation of ewes [73] and buffalo calves [81] with Se alone or Vit E plus Se significantly increased serum level of T_3, but did not affect T_4 levels and T_4/T_3 ratio. Similar T_3 response was reported by Pavlata *et al.* [82] with dairy cows and by Soliman *et al.* [80] with ewes parentally administered with vitamin E and Se, but they found significant increase in plasma T_4 levels. Contrary to our result, El-Shahat and Abd El-Monem [83] recorded significant increase in T_4 levels with insignificant increase in T_3 levels in Baladi ewes supplemented with vitamin E and Se. Moreover, the present results disagree with the study of Nazifi *et al.* [84] who revealed no significant correlation between Se and thyroid hormones in the serum of Iranian fat-tailed sheep. 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 antioxidants.

Concerning BCS, our results are in agreement with Habeeb and El-Tarabany [51] who recorded that *N. sativa* additive to the diet of Zaraibi kids increased significantly T_3 and T_4 (Contrary to obtained results) during months of the hot summer season. The obtained results are attributed to that BCS is a good source of protein, energy and minerals [34,85] and to the higher digestibility that was recorded for goat kids supplemented with BCS which led to increase the absorbed nutrients from small intestine [51]. Also the presence of thymoquinone (18.4–24%) of

the essential oil of the seeds, has antioxidant properties [32] which play a role in oxidative stress control at the thyroid gland during syntheses of hormones.

In conclusion, the present results declare that supplementation with either natural (BCS) or organically bound -nutrient antioxidants (Vit E/ Se enriched yeast and Zn-Met) in the diet of goat kids had beneficial effect on gene expression of antioxidant enzymes and biomarkers of antioxidants status as well as thyroid hormones activities. These results revealed that metabolic activity processes might be enhanced in these animals.

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