Research Article

Antioxidant and Antinematodal Effects of *Nigella Sativa* and *Zingiber Officinale* Supplementations in Ewes

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ABSTRACT

This study aimed to investigate the effect of *Nigella sativa* seeds as feed supplement and *Zingiber officinale* powder on antioxidant status and gastrointestinal nematodes in ewes. Therefore, fifteen pregnant crossbred ewes, aged 3-5 years old and weighing an average of 49.8 Kg and eight weeks before calving were allocated into three groups of five animals in each. The Group I as control which were fed on a basal diet only plus wheat straw which offered *ad lib*., Group II, which supplemented with *N. sativa* seeds in a dose of 3 g/animal/day plus wheat straw *ad lib.* and Group III, which supplemented with *Z. officinale* fine powder in a dose of 3 g/animal/day plus wheat straw *ad lib*. In addition, water supplementation was offered *ad lib.* The obtained data revealed that the supplementation of *N. sativa* and *Z. officinale* in late pregnancy and early after parturition were significantly decreased MDA, the product of oxidative stress and significantly increased the antioxidant activity in serum and erythrocytic hemolysates. Moreover, the efficacy of *N. sativa* seeds and *Z. officinale* powder used were judged by counting egg per gram (EPG) of the gastrointestinal nematodes. Fecal examination was revealed that the mean numbers of eggs per gram were significantly decreased till the last readings and in comparison with the control group.

Keywords: Anthelmintic, Antioxidant, Ewes, *Nigella Sativa*, *Zingiber officinale*.

INTRODUCTION

In Egypt, there are about 5.6 and 4.13 millions heads of sheep and goat, respectively. Sheep and goat serve as investment and insurance due to high fertility, short generation interval and adaptation to the harsh environment.[1,2] The productivity of sheep and goat was reported to be low due to a number of factors as feed shortage either in quality or quantity and health constraints.[1] The lack of sufficient feed to meet the nutritional requirements of existing animal population is one of the most critical problems of animal production in Egypt. Therefore, many attempts have been made for using of feed additives,[3] that are added to animal feed to improve their nutritive value, increasing growth rate and better feed conversion efficiency.[4]

Herbs could be expected to serve as feed additives due to their suitability and preference, reduced risk of toxicity and minimum health hazards.[5] The World Health Organization (WHO) encourages using medicinal herbs to minimize the use of chemicals through the global trend to go back to natural nutrients.[6] The medicinal herbs are desirable for improving digestion by increasing the bile secretion and pancreatic enzyme activity.[7] Indeed, they are accepted as feed additives to enhance the hepatic metabolism.[8] Different studies showed the effect of *N. sativa* (black seeds) supplementation or its products on the health performance of different animals,[9-13] and improves digestibility and rumen fermentation.[14,15] The supplementation of male lambs with *N. sativa* seeds by a level of 100 mg/kg has positive effect on blood metabolites as well as reproductive performance.[16]

The *N. sativa* seeds and their oil have been widely used for centuries in the treatment of various ailments throughout the world.[17] Among Muslims, *N. sativa* considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the remedy for all diseases except death in one of the Prophetic hadith.[18] The pharmacological studies of *N. sativa* indicated that, it has an analgesic[19], hypolipidemic[20], hepatoprotective[21], anthelmintic[22], antimicrobial [23,24] and anticancer.[25]

The use of *Z. officinale* (ginger) rhizome, has gained popularity among modern physicians in recent years.[26] *Z. officinale* contains several active compounds, including gingerol, shogaols, gingerdiol, and gingerdione and volatile oils, which are the medically active constituents of *Z. officinale.*[27,28] It has been reported to exert antioxidant and antiulcer, anti inflammatory, antitumor, carminative, diaphoretic and gastro-protective activities.[29,30] *Z. officinale* were used as feed additive to improve the health state, performance and productivity of many farm animals.[31-35] Moreover, many studies revealed that *Z. officinale* has anthelmintic activity such as Mostafa et al.,[36] who recorded that *Z. officinale* has antischistosomal activities and they recorded that *Z. officinale* has larvicidal effect against *Anisakis simplex.*[37]

Our study aimed to investigate the blood antioxidative status of ewes in late pregnancy and early after parturition and examine the anthelmintic effects of *N. sativa* and *Z. officinale.*

MATERIALS AND METHODS

Animals

The present study was carried out at the experimental farm of the Faculty of Agriculture; Damanhour University situated in Al-Bostan and lasted for 100 days. Fifteen pregnant crossbred ewes of 3-5 years old and weighing an average of 49.8 Kg at eight weeks before parturition were used in this study.

Group I a control negative, which were fed on a basal diet (free from medication) only plus wheat straw which offered *ad lib*.

Group II supplemented with basal diet, *N. sativa* seeds in a dose of 3 g/animal/day and wheat straw *ad lib.*

Group III supplemented with basal diet, *Z. officinale* fine powder in a dose of 3 g/animal/day and wheat straw *ad lib*.

In addition, water supplementation was offered *ad lib.*

Blood and fresh fecal samples were collected at 40th, 70th and 100th days in which 40th day refers to the two weeks before parturition, 70th day refers to the 15th day after parturition and 100th day refers to the 45th day after parturition.

Chemical composition of ration

The basal diet consists of concentrate feed mixture (CFM) and wheat straws (WS). In which CFM was offered twice daily at a rate of 2.75% of animal weight while wheat straw was offered *ad lib.*

The basal concentrates feed mixture (CFM) composed of yellow corn grain (55 %), wheat bran (20%), soybean meal (10%), cottonseed meal (12.50%), sodium chloride (1%), limestone (1.3%) and avimix mineral mixture (0.2%). Whereas, each 1 kg of avimix mineral mixture (AGRI-VET) composed of manganese sulphate (16.66 g), iron sulphate (10 g), zinc sulphate (20 g), potassium iodide (0.83 g), cobalt chloride (0.17 g), sodium selinite (0.066 g) and calcium carbonate (952.27 g).

Wheat straw, concentrate feed mixture, *N. sativa* seeds and *Z. officinale* powders were analyzed for moisture, ash, crude protein, ether extract, crude fiber and urinary nitrogen according to the AOAC.[38]

Cell wall constituents were estimated according to the methods described by Van Soest et al.[39] The chemical composition of wheat straw and CFM are presented in Table 1.

Parasitological Examination

Fresh fecal samples were collected at 40th, 70th and 100th days of the experiment. All fecal samples were collected in plastic bags then all of them were checked immediately. Fecal samples were processed for qualitative examination, which was done by direct microscopic examination of flotation method using concentrated salt solution and the Mc Master Technique was done for quantitative examination.[41]

Biochemical analysis

Blood samples were collected at 40th, 70th and 100th days of the experiment from the jugular vein. Each blood samples were divided into two portions; the first portions were poured in tubes without anticoagulant for determination of serum malondialdehyde (MDA)[42], total antioxidant capacity (TAC)[43] and reduced glutathione (GSH).[44] The second portions were poured in tubes containing 20 IU heparin as anticoagulant in which 1 ml of whole blood used for preparation of erythrocytic hemolysed by digitonin.[45] These hemolysates were used for determination of erythrocytic, total superoxide dismutase (t.SOD) (EC 1.15.1.1)[46], catalase (CAT) (E.C. 1.11.1.6)[47], glutathione reductase (GR-ase) (EC: 1.6.4.2)[48], glutathione peroxidase (GSH-Px) (EC: 1.11.1.9)[49], glutathione S-transferase (GST) (EC: 2.5.1.18)[50] and hemoglobin content in the red blood cell lysate.[51]

Statistical analysis

Analysis of variance (one-way, ANOVA) was performed to compare between different groups at different weeks for the results of biochemical and parasitological studies.[52]

Table 1: Chemical composition and cell wall constituents of concentrate feed mixture, wheat straw, *N. sativa* and *Z. officinale* "on DM basis" used in this experiment

Items	Concentrate feed mixture	Wheat straw	*N. sativa*	*Z. officinale*
Chemical composition %				
Dry matter (DM)	87.89	92.11	91.23	89.98
Organic matter (OM)	93.89	92.66	96.55	91.89
Crude protein (CP)	15.51	2.72	28.94	7.65
Ether extract (EE)	2.43	1.61	11.45	6.54
Crude fiber (CF)	9.31	38.90	9.23	6.69
Nitrogen free extract (NFE)	66.64	49.43	46.93	71.01
Ash	6.11	7.34	3.45	8.11
Cell wall constituents				
Neutral detergent fiber (NDF)	30.23	71.44	–	–
Acid detergent fiber (ADF)	13.45	45.43	–	–
Hemicellulose	16.78	26.01	–	–
(NFC) *	45.72	16.89	–	–

*NFC: Non fibrous carbohydrates = {100 - % (CP + NDF +EE+ Ash)}[40]

RESULTS AND DISCUSSION

Ewes in all groups were clinically healthy throughout the experiment. There were no identifiable reactions following the administrations of *N. sativa* seeds and *Z. officinale* powder. The mean (±SE) antioxidant status of pregnant ewes were stated in Tables 2 and 3.

The data illustrated in Table 2 showed a significant (P<0.05) decrease in serum MDA in both Group II and Group III in comparison to the control one with a highest decrease in the Group II at the 40th day (1.84±0.13) and 70th day (1.28±0.03) of the experiment. In addition, their levels were significantly (P<0.05) decreased in the Group III at 100th day (1.18±0.05) in comparison to the control ewes at each corresponding level.

On the other hand, serum TAC was significantly (P<0.05) increased in both Group II and Group III all over the experimental period in comparison to the control group at each period (3.52±0.23 and 3.40±0.10 vs. 1.52±0.05 at 40th day), (3.19±0.24 and 3.18±0.25 vs. 1.85±0.21 at 70th day) and (3.87±0.10 and 3.55±0.22 vs. 1.78±0.35 at 100th day), respectively. In the same context, reduced glutathione values were significantly (P<0.05) increased in both Groups (II & III) all over the experimental period in comparison to the control ewes at the corresponding level.

The data obtained in Table (3) revealed a significant (P<0.05) increasing in erythrocytic t.SOD in both Groups (II & III) in comparison to the control animals at 40th day and non-significantly (P>0.05) changed in both groups at 70th and 100th days of the experiment. Moreover, erythrocytic CAT activity was non-significantly (P>0.05) changed in the treated groups all over the experimental period. In the same Table time, the data revealed a significant (P<0.05) increase in erythrocytic GR-ase activity in the treated groups at 40th, 70th and 100th days of the experiment. By the same manner, erythocytic GSH-Px activities were significantly (P<0.05) increased in both Group II and Group III at 40th and 70th days and non-significantly (P>0.05) changed at 100th day when compared with its control. Finally, the erythrocytic GST activity was significant (P<0.05) in treated groups at 40th, 70th and 100th days of the experiment.

Good nutrition is the fundamental requirement for all farm animals and it is considered as one of the biggest contributors to the animal welfare. Specific feeding strategies have a significant influence in animal oxidative metabolism.[53] The increment in antioxidant status of animals improves their growth performance, production and reproduction.[12,54]

Supplementation of ewes with *N. sativa* seeds maintains their antioxidant status, especially during pregnancy and after parturition which considered stressful periods in their life. The antioxidant enhancement was evidenced by a significant decrease in MDA and significant increases in GSH, TAC, t.SOD, CAT, GR-ase, GSH-Px and GST.[55,56]

Table 2: The mean values of serum MDA, TAC and GSH in response to *N. sativa* and *Z. officinale* supplementation at 40th, 70th and 100th of experiment

Parameters	Periods	Experimental groups		
		Group I	Group II	Group III
MDA (mmol/L)	40th day	4.02±0.30^{a}	1.84±0.13^{b}	2.25±0.04^{b}
	70th day	2.72±0.04^{a}	1.28±0.03^{c}	1.55±0.02^{b}
	100th day	2.67±0.01^{a}	1.44±0.06^{b}	1.18±0.05^{c}
TAC (mmol/L)	40th day	1.52±0.05^{b}	3.52±0.23^{a}	3.40±0.10^{a}
	70th day	1.85±0.21^{b}	3.19±0.24^{a}	3.18±0.25^{a}
	100th day	1.78±0.35^{b}	3.87±0.10^{a}	3.55±0.22^{a}
GSH (mmol/L)	40th day	0.65±0.05^{b}	1.20±0.03^{a}	1.22±0.06^{a}
	70th day	0.59±0.01^{b}	1.33±0.05^{a}	1.65±0.24^{a}
	100th day	1.39±0.04^{b}	1.95±0.06^{a}	2.04±0.06^{a}

Means within the same row carrying different letters are significantly different at (P < 0.05); Values are expressed as means ±SE.

Table 3: The mean values of erythrocytic t.SOD, catalase, GR-ase, GSH-Px and GST in response to *N. sativa* and *Z. officinale* supplementation at 40th, 70th and 100th of experiment.

Parameters	Periods	Experimental groups		
		Group I	Group II	Group III
t.SOD (U/mg Hb)	40th day	0.45±0.15^{b}	1.97±0.56^{a}	2.35±0.25^{a}
	70th day	1.17±0.23^{a}	2.01±0.54^{a}	2.24±0.54^{a}
	100th day	0.91±0.13^{a}	2.03±0.87^{a}	1.98±0.66^{a}
CAT (U/mg Hb)	40th day	6.39±0.29^{a}	7.74±0.61^{a}	8.01±1.14^{a}
	70th day	6.74±1.26^{a}	8.08±2.49^{a}	8.68±1.24^{a}
	100th day	8.12±1.14^{a}	8.75±0.87^{a}	10.39±2.19^{a}
GR-ase (U/mg Hb)	40th day	0.92±0.24^{b}	1.65±0.07^{a}	1.92±0.10^{a}
	70th day	1.41±0.04^{b}	2.01±0.13^{a}	2.07±0.08^{a}
	100th day	1.26±0.08^{b}	1.99±0.02^{a}	2.04±0.02^{a}
GSH-Px (U/mg Hb)	40th day	1.07±0.01^{b}	2.14±0.03^{a}	2.17±0.02^{a}
	70th day	1.00±0.01^{b}	2.11±0.01^{a}	2.13±0.01^{a}
	100th day	1.06±0.01^{a}	1.14±0.02^{a}	1.15±0.05^{a}
GST (U/mg Hb)	40th day	0.28±0.06^{b}	1.33±0.01^{a}	1.34±0.08^{a}
	70th day	0.31±0.02^{b}	1.29±0.01^{a}	1.37±0.08^{a}
	100th day	0.28±0.02^{b}	1.30±0.01^{a}	1.35±0.05^{a}

Means within the same row carrying different letters are significantly different at (P < 0.05); Values are expressed as means ±SE.

The significant decrease in plasma MDA in case of *N. sativa* supplementation is due to the significant increase in the activities of SOD and GSH-Px in erythrocytes.[57,58] SOD is an antioxidant enzyme and rapidly converts O_2 to less dangerous H_2O_2 which is further degraded by endogenous antioxidant enzymes GSH-Px and CAT to water.[59] Moreover, *N. sativa* oil is an effective free radical scavenger showing antioxidant activities and protecting against the damage caused by free radicals.[60] The pre-treatment with thymoquinone (TQ), the main active constituents in seed oil, and protected organs against oxidative damage induced by a variety of free radical generating agents.[61-63] All the tested compounds from *N. sativa* exerted strong antioxidant effects; thymol acted as

singlet oxygen quencher, while TQ and dithymoquinone showed SOD-like activity[64] and free radical scavenging activity.[65] Moreover, liver antioxidant enzymes are significantly increased with TQ medication.[66] Administration of TQ restored the activities SOD, CAT, GSH-Px, and GST activities as well as reduced the levels MDA.[67]

Z. officinale addition in ewes diets was significantly decreased MDA and significantly increased GSH, TAC, t.SOD, CAT, GR-ase, GSH-Px and GST.[68-71] *Z. officinale*, an aromatic but pungent food spice, is used for its medicinal value.[72] Its antioxidant properties contribute to its radical scavenging activities.[73,74] However, free polyphenols of this plant elicited better effect, possibly due to the fact that they are freely available and more readily absorbed and exert beneficial bioactivities in early digestion.[75]

Table 4 concerning the egg count/g feces. The egg count was significantly (P<0.05) high at the 40th day in treated groups, *N. sativa* (1200±1.02) and *Z. officinale* (1500±1.01). By the 70th day the egg count was decreased significantly (P<0.05) dropped to (300±0.01) and (200±0.71) in *N. sativa* and *Z. officinale* treated groups, respectively. The egg count was the lowest in (200±0.44) in *N. sativa* and (100±0.52) in *Z. officinale* treated group at the 100th day of the trial.

Table 4: The mean number of egg count/g feces in response to *N. sativa* and *Z. officinale* supplementation at 40th, 70th and 100th of experiment

Periods	Group I	Group II	Group III
40th day	1000±1.04^{a}	1200±1.02^{a}	1500±1.01^{a}
70th day	1400±0.81^{b}	300±0.01^{b}	200±0.71^{b}
100th day	2000±0.52^{c}	200±0.44^{c}	100±0.52^{c}

Means within the same row carrying different letters are significantly different at (P < 0.05); Values are expressed as means ±SE.

There was an obvious decrease in fecal egg count reading among both treated groups. The decrease was time dependent as with long period of food supplement of these materials, best results were obtained. Our results mostly agree with most researchers who use medicinal plants as a new trend to treat helminths such as concluded that the use of *N. sativa* seeds as the anti schistosomal drug may affect the adaptive capability of adult worms against the oxidative killing by the host effector cells and this may help in the elimination of the parasite.[76] Moreover, *Z. officinale* were used as larvicidal agents against *Angiostrongylus cantonensis.*[77] In addition, it exhibits the strongest activity overall used plant extracts against *canine dirofilariasis.*[78] *Z. officinale* possesses *in vivo* anthelmintic activity in sheep.[79]

CONCLUSION

The last of gestation and the early after parturition periods of animal life is the time of great stress on it which leads to increase the oxidative stress. Therefore, we suggest supplementing ewes in these stages by *N. sativa* and *Z. officinale* to increase their antioxidant and beside their anthelmintic powers.

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