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Research Article

The Effect of Feeding Commercial Oil of Black Cumin in Sheep

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Abstract: This study investigated the effects of feeding commercial oil of the Black cumin (seeds of *Nigella sativa*) on the levels of total cholesterol and the body weights in sheep. Twelve male cross breed sheep were divided individually into two groups on the basis of their weights. Each cage was supplied with fattening ration prepared as pellets at the rate of one kilogram per day. Barseem and water were supplied ad libitum throughout the day. The sheep were fed the commercial ration for two weeks as an adaptation period and then they were subjected to a feeding programme for 6 weeks as follows: In group I (control group) sheep's received pellets. In group II (treated group) received pellets mixed with 4.7% (47 gram) of black cumin NS oil seeds. Black seed oil of *N. sativa* significantly increased serum cholesterol and the body weights at the end of experimental period (6 weeks) compared to time zero. The treated group showed significant elevation. Feeding of black seed oil to the diets of sheep resulted in significant elevation in Serum total cholesterol and the body weights.
Keywords: Black cumin; sheep; cholesterol; body weights.

INTRODUCTION

Nigella sativa (*N. sativa*) is an annual erect herbous plant that belongs to the family *Ranunculacae* or Batter-cup family. In most of Arab countries it takes the popular name "El-Habba El-Sawda". It is commonly known as: black seed, Habat- Elbaraka and in Sudan it is known as (Black cumin) and produced in the northern region and Darfur at Melit and Jebal Marra [1]. The center origin was thought to be the Mediterranean Sea region, Turkey, and the neighboring parts of Asia. It is native to a different country such as: India, Pakistan, Iran, Iraq, Syria, Egypt and the United States of America [2]. It is one of the very important medicinal plants, which used for centuries in Middle East as a healer of many complains and disease [3]. *N. sativa* is an important medicinal herb, in many Arabian, Asian and African countries. Black seed oil is used as a natural remedy for a wide range of diseases, including various allergies. The plants mechanism of action is still largely unknown, due to the lack of study data on its efficiency in allergies [4]. In addition to that, seeds are used for edible and medicinal purposes, in many countries. The average proximate analysis of black cumin seeds on dry matter basis are 216 g/kg crude protein, 406 g/kg fat, 45 g/kg ash, 84 g/kg crude fiber and 249 g/kg nitrogen free extract [5].

The oil content of black cumin seeds from three different regions is 29.4, 29.5 and 29.7. The major fatty acids in black cumin seeds oil are linoleic 60%, oleic 22% and plamitic 12% [6]. Previous studies reported the lowering effect of *N. sativa* on the serum cholesterol in various animals including rabbits, rats and poultry [7-10]. Also the effect of *N. sativa* on body weight was reported previously [11]. However, no sufficient information was found available in literature about the effect of black cumin oil on serum cholesterol and the body weight in sheep or other ruminants. The objectives of this study were to determine the effect of feeding black cumin oil seeds on cholesterol and Body weight.

MATERIALS AND METHODS

Animals

The present study was conducted on Twelve male crossbreed sheep, (Hamary and desert sheep) from University Farm and Abu Zeid market during summer from April to May. The age groups of these sheep were varied from 3 to 6 month of age. The animals were individually housed in 1 x 1.5 m separate cages at the same environmental conditions. The cage sides were of a wire mesh set over a meter brick side wall. Before the insertion of the animals, they got a bath with cypermethrin as antitickes and antimanges in a dilution

of 1 ml per liter of water (produced by Veterinary and Agricultural products Mfg. Co. Ltd, Jordan), and the cages were cleaned manually, disinfected with cypermethrin in dilution of 2 ml per liter of water. After that the animals were treated by ivermectin as antiparasitic (intermectin 1%, Holland) at a dose of 0.5 ml s/c, oxytetracyclin as antibacterial (Rasomycin-5, star laboratories (PVT) Ltd, Pakistan) at a dose rate of 1 ml per 10 kg 1/M for 3 days and multivitamins (Ultravit M, Avico, Jordan) at gram per liter of drinking water for 5 days. Then the animals were divided into two groups on the basis of their weights with ±113 kg for each group. The sheep were fed the commercial ration for two weeks as an adaptation period and then they were subjected to a feeding programme for 6 weeks as follows: group (B) received Kenana feed as control group, and group (A) received Kenana feed mixed with 4.7% (47 gram) of black cumin oil seeds as treated group. The percentage of black cumin oil is determined by the difference between the highest levels of fats in ruminants' diet (7%) [12] and the crude fat percentage in Kenana feed (2.3%).

Black cumin seeds

Black cumin seeds and/or *N. sativa* seed oil was obtained from commercial source in the local market. Before production of the oil, the viability of seeds was confirmed by germination test according to International seed testing agency, Ista (1976). The seeds were tested for germination rate and purity. The germination rate must be at least 85% in order for the seed to be certified by the agency. Hundred seeds selected randomly and distributed into 4 Petri- dishes (25 seeds in each), containing filter papers moisten with distilled water. The dishes were incubated at 20^{o}C for 7 days. On the 4th day, the normal seedling that developed was counted. At the end of the 7th day, the final count was done as follows: normal seedling, abnormal seedling and dead seedling. All the four sets of germinated seeds should have a germination rate of at least 85%. A germination rate of 85% is 85 normal seedlings for all sets of 100 seeds.

Feeding programmed

Each cage was supplied with fattening ration prepared as pellets (Product of Kenana Sugar Company Ltd) at the rate of one kilogram per day. Barseem and water were supplied *ad libitum* throughout the day. The components of Kenana ration are: sorghum, molasses, wheat bran, groundnut cake, bagasse, calcium salts, urea and sodium chloride. The proximate analysis of the ration is presented in Table 1.

Table 1: The proximate analysis of cattle feed pellets (According to Kenana Sugar Company Ltd, 2007, Sudan, Khartoum)

Ingredient	Percent
Dry matter	87.6*$^{.1}$
Crude protein	17.5
Crude fiber	11.9
Crude fat	2.3
Ash	11.2
Metabolizable energy	10.5*$^{.2}$

Note: *$^{.1}$On dry matter basis; *$^{.2}$Metabolizable energy per megajoul per kg dry mater MJ/Kg DM.

Blood samples collection

The first blood samples were collected as zero levels before the application of black cumin oil, and then the other blood samples were taken every two weeks for the rest of the experimental period. Fasting blood samples were taken in the morning before feeding as (5 ml of blood) from the jugular vein of each sheep into clean glass tubes. The blood was allowed to clot at 4^{o}C for overnight, and then centrifuged at 4000 rpm for 5 minutes; clear serum was then separated and stored at -20^{o}C till used for biochemical analysis.

Statistical analysis

Statistical software known as SPSS has been used to get the means and standard errors by utilizing the one sample t-test. The experimental design analysis and the mean separation were obtained using another computer package named SAS, (1982), software utilizing completely randomized design analysis and Duncan's multiple range lists for mean separation. Pre and post intervention mean ± standard deviation of each parameter was calculated for both groups. Paired T-test was applied to know the intergroup difference of each variable before and after intervention. Then unpaired t test was applied to know about intergroup difference between both groups. P -values < 0.05 were considered statistically significant.

RESULTS

Serum cholesterol

The effect of feeding the commercial oil of the black cumin seed on serum cholesterol concentration is represented in Table 2. At time zero there was no significant difference between the two groups, though control group (A) showed apparently higher value compared to group (B) at time zero and before feeding black cumin seeds oil to the treated group. Also, no significant differences were observed between the two groups until the end of the experiment. However, at week 2 and 4 group A showed a significant (P<0.05) reduction in the cholesterol level compared to time zero. A none- significant increased was also observed at

week 6 in group (A) compared to week 2; while in group B after 2 weeks of feeding black cumin oil, animals on this group showed a significant (P<0.05) elevation compared to time zero and week 4. However, at week 4 group B showed a significant (P<0.05) reduction in the cholesterol level compared to week 4 and 6, At the end of week 6, group B showed a significantly (P<0.05) higher total cholesterol level compared to week 4 and time zero, and group A showed a non- significantly lower value compared to time zero. There was no significant difference between the total means, but the treated group showed slightly higher total mean compared to the control group.

Body weights

The effect of feeding black cumin of *N. sativa* seed oil on the body weights is represented in Table (3). There was no significant difference between the two groups before and after the feeding of cumin seeds oil. This was also seen in the control group, but the treated group showed a significantly (P<0.05) higher weights at the end of the experiment compared to time zero. The total means showed nearly the same value, but was not significantly.

Table 2: The effect of feeding commercial oil of *N. sativa* on serum total cholesterol (mg/dl) of sheep

Groups	Parameters T. CC(mg/dl)				
	Time 0	2Wks	4 Wks	6Wks	T. mean
Groups A	90.80^{Aa} ± (12.48)	82.29^{Aa} ± (11.48)	65.39^{Aa} ± (8.65)	87.69^{Aa} ± (6.36)	81.54^{a} (9.74)
Group B	61.81^{Ca} ± (5.01)	99.99^{Aa} ± (5.26)	77.68^{Ca} ± (10.19)	93.90^{Aa} ± (3.64)	83.35^{a} (8.58)

Note: Means in the same row having different capital letters are significantly different at ($p < 0.05$). Means in the same column followed by different small letters are significant different at ($p < 0.05$). A = control group. B = treated group fed black cumin seeds oil of *N. sativa*. T.C= Serum total cholesterol. Means ± SE. (N = 6).

Table 3: The effect of feeding commercial oil of *N. sativa* on body weights (kg) of sheep [Mean ± SE (N = 6)]

Group	Time zero	Week 6	Total mean
A	19.00^{Ba} ± (1.77)	24.75^{Aa} ± (0.89)	21.88^{a} (2.88)
B	18.83^{Aa} ± (2.06)	23.67^{Aa} ± (1.83)	21.25^{a} (2.42)

Note: Means in the same row having different capital letters are significantly different at ($p < 0.05$). Means in the same column followed by different small letters are significant different at ($p < 0.05$). A = control group. B = treated group fed black cumin seeds oil.

DISCUSSION

Serum total cholesterol

Cholesterol is an amphipathic lipid present in tissues and plasma. Cholesterol levels play a central role in the genesis of atherosclerosis and coronary heart disease [13-14]. Previous findings showed that *N. sativa* has promising effect resembling to those drugs that reduce serum cholesterol and decrease its atherogenic pathological effect [7-15]. In the current study the level of serum cholesterol showed fluctuating manner. Group B showed significant (P<0.05) increase compared to time zero after two weeks, and then significantly (P<0.05) reduced it week 4 and significant (P<0.05) increased at week 6. Control group A showed slight decline it week 2 and week 4, then slight increase at week 6. This finding agree with study which reported that, feeding 2.5% *N. sativa* with 15g egg yolk (as a source of cholesterol) to rabbits showed no significant effect on serum total cholesterol [16]. But, these results was in contrast with another finding [11], when another authors investigated the effect of fixed oil of *N. sativa* seeds in rats, on serum cholesterol, triglycerides, glucose, and the body weight and reported that serum cholesterol decreased significantly in rats. This result was completely in the same line with another previous report [17]. Also, there was another study suggested that, *N. sativa* seeds oil might improve hyperlipidemic nephropathy in rats [18]. From this study, one can conclude that, feeding 4.7% of *N. sativa* seed oil to sheep, result in a significant (P<0.05) elevation in the serum cholesterol level compared to time zero, and nonsignificant elevation compared to control group in contrast to findings in monogastric animals.

Body weight (BW)

There was no significant difference in the body weight between the two groups at the beginning of the experiment, but group B the treated group showed significant (P<0.05) increase in body weight at the end of experimental period compared to time zero. This

could be explained as a result of the increase in the serum lipids studied as total cholesterol. These results disagree with previous experiments' which reported that, the effect of fixed oil of black cumin seeds in rats resulted in a significant decrease in the body weight [11]. In the present work it was observed that, feed in take was increased in the treated group, this suggest that the commercial oil of *N. sativa* has a slight appetizer effect. In addition to that, the effect of dietary black cumin oil, lead to increase the levels of serum cholesterol and the body weights. This can be explained as that, the rumen atmosphere may destroyed the active constituents of black cumin oil, specially thymoquinone which is considered as active ingredient responsible for the pharmaceutical interest of the plant [19]. Also, it was clearly known in the previous literature that, the high ratio of polyunsaturated fatty acid in the diet is a major lowering plasma cholesterol concentration by dietary means and hence black cumin seeds contain unsaturated fatty acid it may be the cause of reduction of serum cholesterol concentration in monogastric animals [14]. But in the ruminant animals although they consume a diet that predominantly contains polyunsaturated fatty acids as part of plant triglycerols and glycolipids, bacteria in the rumen split off the FA (and sugar) from the glycerol backbone (Hydrolysis process), and the resulting free fatty acids are acted upon by microbial enzymes which convert them ultimately into saturated FA (stearic acid) (Biohydrogenation Process) [20]. This suggested that, supplementation of black cumin oil, to ruminants' diet, act as additional source of PUFA which later will be converted to saturated FA, pass to the small intestine and absorbed.

CONCLUSION

Feeding of 4.7% of black cumin seeds oil to the diets of sheep, resulted in significant ($P<0.05$) elevation in serum total cholesterol concentrations and the body weights. These results incontrast to previous findings in the monogastric animals, which reported a lowering effect of black cumin seeds on the serum cholesterol concentration with increase in the high density lipoprotein cholesterol levels. Also, different effects were observed for the body weights.

RECOMMENDATIONS

Future studies are recommended to: investigate about the fate of thymonquinon in the rumen and to confirm and identify the presence of appetizer component in the commercial oil of black cumin seeds. Although, to confirm the presence of the intermediate compounds (conjugated linoleic acid) in the body fat which having potent anti-cancer effect specially after the elevation in the serum lipids observed after feeding black cumin oil to sheep in this work, since black cumin oil is known to contain their origins (linoleic and linoleinic acid).

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