The Effect of different *Nigella sativa L.* seed (cake) concentrations on leukocytes counts and some serum immunological parameters in calves

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Abstract

The present study aimed to evaluate the effects of a replacement of Soybean meal (SBM) of the control ration by *Nigella* Seed Meal (NSM) on leukocytes and some serum immunological parameters in calves. Fifteen calves were distributed randomly and equally into three groups, each of five animals, NSM was used at levels 0, 7 and 11% of the total food for a feeding period of 105 days. The results of immunological parameters: total serum protein, globulin, albumin and albumin:globulin ratio (A:G) of calves fed NSM were apparently higher than those fed with the control ration, but with non significant differences by the treatment; on the other hand, serum triglyceride, cholesterol, total leukocyte count and differential leukocyte count DLC were not affected significantly. It is recommended to use NSM diet as a non-traditional source of plant protein to be substituted with soybean meal protein for calves to improve the function of immunological body system against different diseases and to reduce the risk to illness.

Key words: *Nigella* Seed Meal (cake), globulin, albumin, triglyceride, cholesterol, leukocytes.

Introduction

Nigella sativa L. is a herbal plant which is popularly called black cumin, black seed, seed of blessing, black Caraway and kalonji, it belongs to the plant family of *Ranunculaceae*. The seeds have traditionally been used for thousands of years in the Middle East, Far East and Asia as a food additive and as a herbal health aid [1]. The chemical composition of *Nigella* seeds contain 5.7% moisture, 23.6% crude protein, 42% ether extract, 7.7% crude fiber, 5% ash, 16% nitrogen free extract also Ca, P, Na, Mg, K, Fe, Zn, Cu and vitamins [2]. For this, it has a number of pharmacological effects of profound therapeutic values like: analgesic, immune stimulation, anti-inflammatory, anti-allergic, anti-histaminic, anti-oxidant, anti-cancer, anti-asthmatic, hypoglycemic, hypotensive, anti-bacterial, anti-fungal, anti-viral and anti-parasitic effects [3-16]. Nowadays, [17] showed that solid lipid nanoparticle of *N. sativa* formulations are suitable carrier in pharmaceutical and cosmetic fields, so it serve frequently *Nigella* seeds as medical oil, second, *Nigella* Seed Meal (NSM) after oil removal can be used as protein-rich meal, crude protein about 33% of the essential amino acids that improve natural immune system activity [18]. It is considered as un-traditional feed proteins also crude fat and the major minerals such as Ca, P, K, Mg, Na and considerable amount of oil which rich in fatty acids (Oleic, Linoleic & Linolenic acid) and carotene which is inverted into vitamin A. NSM contains materials known as nigellon, thymoquinone and thymo-hydroquinone; but the recent reports suggest that thymoquinone present in the oil might be the active component [19-21].

For that reason, the NSM cake could be used as flexible ingredient to formulate balance rations, because of its relatively high crude protein and energy contents, which improves feed intake, digestibility coefficient and nutritive values [22], so NSM cake could be used safety and economically in ruminant feeding [23]. Even in veterinary medicine, besides the beneficial effects of *Nigella* seeds and its oil in many infectious diseases; there are reports mentioning that the addition of NSM cakes in the feed of buffalos and lambs improves their body weight and reproductively [24,25]. So, the objective of the present investigation is to evaluate the effect of using different amounts of NSM on leukocytes count and on some serum immunological parameters in calves.

Materials and Methods

** Animals and Experimental design*

The present study was carried out at the state of Board of Agriculture Research, Department of Agriculture Research in Nineveh. Fifteen male Sharabi calves with mean 180 Kg live body weight and aged between 10-13 months were divided into 3 equal groups of 5 calves each according to their live body weight randomly. The three groups were allotted randomly to three experimental rations. The first ration was a complete feed mixture (control) whereas the second and third rations were substituted with 60% and 100% of soybean meal protein of control ration by *Nigella* seed meal cake protein respectively. Three concentrate feed mixture were nearly iso-caloric and iso-nitrogenous (Table 1).

Table 1: The calculated chemical composition of concentrate rations (DM %) basis *

Items	Experimental rations			SBM	NSM**
	Control	NSM 7%	NSM 11%		
Dry M atter (DM) %	88.7	88.1	89.3	90.2	95.7
Organic Matter (OM) %	92.5	92.5	92.7	93.0	94.6
Crude Protein (CP) %	15.6	15.3	15.2	49.5	34.0
Crude Fiber (CF) %	8.0	8.3	8.2	6.3	9.4
Ether Extract (EE) %	2.9	4.5	5.4	2.0	26.2
Nitrogen Free Extract (NFE) %	66.0	64.4	63.8	35.2	25.0
Ash, %	7.5	7.5	7.4	7.0	5.4
Gross Energy (GE) (Mcal/Kg)	4.23	4.12	4.35	4.71	5.81

* calculated depending on [26]

** estimated in laboratory depending on [27]

All three groups were given 2% of concentrate rations of their body weight twice daily at 8 a.m and 5 p.m. Feed residues were collected and weighed once daily prior feeding to estimate daily feed intake. Fresh water and minerals blocks were freely available at all times. The experiment lasted for 105 days, calves weights were recorded at the beginning of the experiment (initial weight) and at the end of the experiment (Final weight). The feed mixture allowance was adjusted every two weeks according to body weight changes. Groups of animals were kept in separate semi-open pens.

** Samples*

Blood were individually collected before and after treatment by vein puncture from each calf into two tubes, the first in non-heparinized tubes to obtain serum which was separated by centrifugation at 3000 rpm for 15 minutes. The collected sera were stored at -18ºC until analysis; the other heparinized blood tubes were transferred quickly to the laboratory to perform TLC and to make blood films (leishman stain) for DLC [28]

** Chemical analysis*

Total protein, albumin, cholesterol and triglyceride values in serum were estimated using commercial kits (BioLabo, France) according to the procedure outlined by the manufacturer. Whereas globulin values beside the A:G ratios were estimated according to [28].

Analysis for feed DM, CP, EE, CF and ash were determined according to [27]. The NFE was calculated by subtracting CP, EE and CP out of organic matter OM.

** Statistical analysis*

Data of all experiments were expressed as mean ±SD. The data were compared by ANOVA (One-Way Analysis of Variance). All statistical analyses were performed by SigmaStat (Jandel scientific software V3.1). $P<0.05$ was considered as statistically significant.

Results and Discussion

The effects of different amounts of NSM on growth performance are included in table (2), the results showed that the three groups has no significant differences values of total weight gain.

Table 2: Effect of adding different amounts of NSM on body weight

Item	Feeding groups		
	Control	NSM 7%	NSM 11%
No. of animals	5	5	5
Initial body weight (Kg)	180±94.5	181±67.4	179.80±62.9
Final body weight (Kg)	265±115.8	250.8±47.8	265.40±54.5
Total body gain (Kg)	85±24.9	69±38.4	85.60±13.3

* The average of 5 calves± standard deviation (SD)

These results are in agreement with other study on camels, that there were no significant differences between the group of camels that fed on control ration and those fed on NSM ration 35% [29], but another similar study performed on rabbits, the results showed that the group of rabbits fed on 12% NSM had significantly the best values of total weight gain [30], our results disagree with a study performed by [31], in which the data of growth performance on crossbreed calves showed a significant increase in total body weight gain over the control group.

The results of immunological parameters: total protein, globulin and albumin showed that there were no significant differences between the groups, and the A:G ratio, the table (3) showed the results.

Table 3: Some immunological parameters and serum constitutes of calves fed on normal and experimental rations

Item	Feeding groups		
	Control	NSM 7%	NSM 11%
Total protein g/dL	8.1±0.5	9.2±1.1	8.6±1.1
Albumin g/dL	4.3±0.3	5.2±0.6	5.1±0.7
Globulin g/dL	3.8±.02	3.9±0.4	3.6±0.5
A:G ratio	1.1 to 1.2	1.1 to 1.5	1.3 to1.8
Triglyceride mg/dl	62.2 ± 7.6	59.5 ± 6.2	52.8 ± 3.3
Cholesterol mg/dl	110 ± 19.6	111.8 ± 13.2	111.6 ± 22.6

A wide discrepancy exists in normal values for the various components of the serum protein. This has occurred primarily because of the wide variation in techniques utilized for estimating normal values. Different techniques including salt precipitation, Biuret method, using of different kits, type of spectrophotometer, have all been utilized beside the person who performed the test may give different results on an identical specimen of sera. This disagreement in values that occurs with the various techniques is dependent upon the property of protein that is being determined.

The results showed that the immunological parameters: total serum proteins, globulin, albumin were not significantly affected by different treatments, on the other hand, in a study on rabbits showed that total serum protein and albumin of rabbits fed on 12% NSM were significantly higher than those fed on the control diet, while serum globulin were not significantly affected by the treatments [30]. Also another study on the effect of black seeds supplementation on dairy ewe's performance, the values of total serum proteins, albumin and globulin of treated group were significantly higher than these value of the control group, which may reflect the improvement in protein digestibility as well as lead to increase immune response in case of any infection may occur, while the A:G ratio in the same study ranged from 1.00 to 1.05 which was not affected by the treatment, like our results of A:G ratio which were ranged between 1.3 to 1.8 in the group that fed on 11% NSM so there was no significant difference, this may be due to that both albumin and globulin increased by the same level due to the supplemented NSM in diet. It is important to note that all values were higher than 1.0, which indicated that all animals did not suffer from any health problems [32]; in addition, high values of globulin in the treated group in that study may be due to the fact that the black seeds have potent effect for protection from decrease in hemoglobin and leukocyte counts [32]. On the other hand, another study on growing sheep fed on 1% and 2% on black cumin seeds; the blood total protein levels were significantly higher than the control group due to the significant increase in the globulin fraction since globulin is the main component of antibodies, an increase in the globulin levels indicates a good immune status of the animal [33].

Beside that a study was done on supplementing broad bean and corn proteins with *N. sativa* on rats diet, they found that the supplemented diet caused significant increases in total serum proteins with their two fractions albumin and globulin [34].

Cholesterol plays an essential role in mammalian biochemistry; it's an essential component of animal cell membrane and precursor of the steroid hormones and there is a correlation between a high level of serum cholesterol and cardiovascular diseases.

The serum triglyceride and cholesterol values were not affected significantly in this study. Our results are similar to [33] who found that cholesterol levels did not show differences among the three feeding groups in sheep; also [30] explained that diet with *Nigella* seeds showed non-significant effects on serum total cholesterol and triglycerides in a study performed on rabbits. Beside that [30] showed that the lowest values in serum cholesterol were recorded in the control group and followed by those different NSM feed diets in rabbits. While in another study performed on poultry fed on powdered *N. sativa* L. seeds showed serum triglycerides and cholesterol levels were reduced [35] and in a study performed on lactating ewes showed that serum cholesterol and triglyceride concentrations were markedly significantly lower in the treated group with NS than that on control group [32].

The results of this study showed that total leukocytes count, the percentage of neutrophils and lymphocytes in calves fed NSM at 11% level were higher than those fed the control diet, but the differences were non-significantly affected as shown in table (4).

Table 4: effect of different amounts of NSM on leukocytes in calves

Items	Feeding groups		
	Control	NSM 7%	NSM 11%
Total WBC /mm3*	8525 ± 2976	11453 ± 2682	12640 ± 1339
Neutrophil	68.9 ± 7.4	68.0 ± 6.2	69.2 ± 4.1
Lymphocyte	20.0 ± 1.9	23.4 ± 7.7	23.3 ± 4.1
Monocyte	7.8 ± 2.6	5.6 ± 1.5	6.2 ± 2.2
Eosinophil	3.2 ± 4.4	3.0± 2.3	1.4 ± 0.8
Basophil	0	0	0

* The group average of 5 calves ± SD

In a study performed on rats which infected with *Trypanosoma brucei* and the control group, there was no significant difference in infected oil-treated, infected untreated and uninfected oil-treated rats when compared together, while there were significant increases in the leukocytes count of the infected oil-treated in comparison with the infected untreated rats [33]. The increased leukocytes count is indicative for the increased host reaction in the presence of black seed oil against the infection as these will contribute in the development of phagocytes and antibodies against the recognizable antigens of parasitic origin.

In conclusion it is recommended to use NSM diet as a non- traditional source of plant protein to be substituted in place of soybean meal protein for calves to reduce the risk to illness and disease by strengthening immune system and protecting the body.

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تأثير تراكيز مختلفة من كسبة الحبة السوداء *Nigella sativa L.* على أعداد خلايا الدم البيض وبعض المعايير المناعية المصلية في العجول

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الملخص

استهدفت الدراسة تقييم لتأثير استبدال كسبة فول الصويا (SBM) لعليقة السيطرة بكسبة الحبة السوداء (NSM) على خلايا الدم البيض وبعض المعايير المناعية المصلية في العجول. شملت الدراسة خمسة عشر عجلا حيث وزعت بطريقة عشوائية لثلاث مجاميع بواقع خمسة عجول لكل مجموعة، وتم تغذية هذه العجول على علائق تحتوي كسبة الحبة السوداء بنسبة 0 ، 7 و 11% من العليقة لمدة 105 يوم. كانت نتائج المعايير المناعية: بروتينات المصل الكلي والكلوبيولين والالبومين ونسبة الالبومين الى الكلوبيولين للعجول التي تناولت علائق حاوية على كسبة الحبة السوداء أعلى من تلك التي بمجموعة السيطرة ولكن دون وجود فرق معنوي، وكذلك كانت الحالة بالنسبة للتراكليسيرايد والكوليسترول والعد الكلي والتفريقي لخلايا الدم البيض. لذا يوصى بامكانية استخدام كسبة الحبة السوداء كمصدربروتين نباتي غير تقليدي ليحل بدلا من كسبة فول الصويا في علائق العجول وذلك لتعزيز وظيفة الجهاز المناعي ضد الأمراض المختلفة وتقليل خطرها.