

Original Research Article

Hematological Values in Sheep Fed a Diet containing Black Cumin (*Nigella sativa*) Seed Oil

Abstract: The aim of this study was to investigate ~~about~~ changes in hematological values after feeding diet mixed with oil pressed from seeds of *Nigella sativa* (*N. sativa*) in sheep. The results indicated that there was no effect on the total (RBCs) count due to treatment when compared to the control group, but the treated group showed significant ($P \leq 0.05$) increase in the total RBCs count after six weeks compared to the transient decrease at week two. ~~→~~ Hemoglobin concentration showed similar pattern to what was observed in the RBC count in both the treated and control groups. PCV, MCV, MCH, and MCHC weren't affected by feeding *N. sativa* oil to sheep throughout the experiment. ~~But~~ However, there were significantly ($P \leq 0.05$) lower values in total mean of (WBCs), lymphocytes, and granulocytes of the treated group compared to the control. The levels of WBCs in the treated group were ~~significantly affected with time, as they significantly ($P \leq 0.05$) increased, then significantly ($P \leq 0.05$) decreased and again significantly ($P \leq 0.05$) increased by the end of the experimental period not time dependent~~. The Monocytes total mean was similar in both groups, but the cells were significantly ($P \leq 0.05$) lower in the *N. sativa* oil treated group compared to the control at week four. ~~Since lipid digestion pattern in ruminants is different from in mono gastric animals, then~~ The hematological blood cells changes ~~in sheep here~~, could be mainly due to the effects of *N. sativa* volatile oils incorporated in the sheep diet.

Keywords: Sheep, *Nigella sativa*, RBCs, WBCs and Monocytes.

1.0 Introduction

Black Seed, Black Cumin, Kalunji, Nutmeg Flower, Kalajira, and Roman Coriander are all commonly used names for the herbaceous plant (*Nigella sativa*). This plant is widely distributed, belonging to the botanical family *Ranunculaceae*. The genus *Nigella* is native to the Mediterranean region and Western Asia, the name being derived from the Latin word *niger*, meaning black, in reference to the colour of the seeds [1]. The seed has been used as a spice in cooking, and as a preservative for cheese products [2]. Raw seeds, seed oil, or seed extract have been used alone or in combination with other ingredients, as a traditional medicine in the treatment of various health conditions, such as eczema, cough, headache, diabetes, asthma, infection, and hypertension [1]. Pharmacological activities of seed extracts have been documented, and including activities against human and animal diseases, and against pests [3]. The

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crude extract of *N. sativa* has also been shown to cause relaxation of carbachol, histamine and K⁺ induced contractions of isolated guinea-pig trachea [4]. An oral dose of 0.6 ml/kg/day of *N. sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats comparable to that of 0.5 mg/kg/day of oral nifedipine [5]. In Unani medicine, *N. sativa* is promoted for the treatment of oligomenorrhoea, to induce menstruation and to treat infertility [6]. In Unani medicine, It is also used for stomachache and as a digestive, carminative, laxative and anti-jaundice [7], The antitumor activity study of a methanolic extract of the *N. sativa* seed against Ehrlich Ascites Carcinoma *in vivo* and demonstrated that the development of malignancy was completely inhibited at a dose of 2 mg/mouse per day for 10 days [8], The isolation of the antimicrobial agent nigellone, of the *N. sativa* volatile oil was achieved by the refrigeration of the oil at 4 C° for 3 days, during which time white crystalline needles separated out. The chemical structure of the compound was deduced from its physical and chemical properties, as well as its ultraviolet, infrared, proton magnetic resonance, and mass spectral data. The volatile oil of *N. sativa* possesses anti-microbial property against gram +ve microorganisms [9]. *Nigellone* was very effective in inhibiting histamine released from rat's peritoneal mast cells [10]. The volatile oil of *N. sativa* alone also produced a significant hypoglycemic effect on normal and alloxan-induced diabetic rabbits without changes in insulin levels [11]. *N. sativa* extract prevented the decreases in hemoglobin level and leucocytes count caused by cisplatin in mice [12],

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All previous studies detect the biochemical and hematological changes induced by the *N. sativa* in mono gastric animals (???) but no work was done in ruminants. Since *N. sativa* is a promising feed additive due to its known nutritive and protective effects, the main objective of this study is to determine the effect of the *N. sativa* oil on the following important blood parameters in sheep: White blood count (WBC) and deferential, Red blood cells (RBC), Hemoglobin (Hb) concentration, packed cell volume (PCV), Mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).

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2.0 Material and methods

2.1 Experimental design

This experiment was conducted in the department of Biochemistry Faculty of Veterinary Medicine, University of Khartoum (U.K) to investigate the effect of feeding commercial oil of *N. sativa* to sheep on the hemogram and iron metabolism. The experimental period was extended for eight weeks starting from March 2007. Twelve young male sheep (Hammary × Desert), were used. Their ages range from 3 to 6 months and weights from 13 to 26 kg. All animals were weighted in the (U.K) animal farm and divided into two groups of similar weights. Kept for two weeks as adaptation period and fed commercial fattening diet plus (Barseem). Each sheep was kept in a single separate cage. Before getting into cages, sheep were washed by with cypermetherin solution (1 ml in 1 liter); used for external parasites in cages and farms;

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L060501, MEDMC, Jordon, International Ambala for Development Khartoum, Cages were sterilized by cypermetherin solution of concentration (2 ml in 1 liter), Sheep were divided into two groups (sample size, n = 6 animals) according to their weights, each group contain 6 animals. During the adaptation period (which was 2 weeks) all Ssheep were supplied with feed the kenana ration Table (1)

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Table 1 : Approximate analysis of kenana ration.

D.M. dry matter (%)	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Ash (%)
%87	%17.5	%11.9	%2.3	%11.2

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Group A received the commercial oil of the *N. sativa* as daily requirement mixed with the concentrates, while the second group B received the concentrates without *N. sativa* and kept as control group. According to the general recommendation that the total dietary fat should not exceed 6 to 7% of dietary dry matter and that higher levels may adversely affect rumen microbial fermentation [13], then 47 gm of the *N. sativa* oil were added to each 1 kg of the concentrate.

2.2 Sample collection

Blood samples were collected early in the morning from each animal from the jugular vein with minimal excitement. 2ml of the blood from each animal were collected in ethylene diamine tetracetate (EDTA) vacuolationers and transported to the laboratory for hematological analysis. The samples were analyzed immediately within two hours after collection. Another 5ml of the blood of each animal were collected in a plane tube, incubated over night in the refrigerator and then separated using the centrifuge (Hettich) at 4000 rpm for 5 minutes. Then the serum was preserved in the freezer at 22 °C.

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2.3 Hematological methods

The hematological estimations were done by using the cell counter (Mythic-18, ORPHEE company; Switzerland, Id: 102405-000639), this instrument can estimate 18 blood parameter within 15 seconds. The method based on the conversion of the hemoglobin by means of drabkin's solution (0.2g potassium cyanide, 0.2 potassium ferricyanide, and 1g sodium bicarbonate per one liter of distilled water) to cyanomethemoglobin concentration was measured in g/dl of blood [14]. The packed cell volume (PCV) can be were determined by the microhematocrit method [Reference ?], Erythrocytes and leucocytes can

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were determined manually and whereas haemoglobin (Hb) can be measured by using the cyanomethemoglobin method. The mean corpuscular hemoglobin concentration (MCHC), the mean cell volume (MCV) and the mean corpuscular hemoglobin were calculated by the formulas:

MCV (in cubic micros) = PCV (%) X10

MCH (pg) = Hb (g/dl) x 10

MCHC (%) = Hb (g/dl) x 100

2.4 Statistical design

The experiment was designed by the complete randomized design (CRD); factorial arrangement 2x4. Where 2 indicate two groups (A; treated and B; control), and 4 indicate times (0, 2, 4 and 6 weeks). Data generated from the experiment were statistically analyzed by analysis of variance (ANOVA) [15]. The means were separated by Duncan's multiple range tests to determine the significant at 0.05% level of probability [16].

3.0 Results and Discussion

3.1 Red blood cells (RBCs)

Figure (1) showed that *N. sativa* oil has no effect on the total mean (RBCs) count, since there was no significant difference between the treated group (A) and the control (B). This result agrees with previous findings [6], but the numerical decrease of the RBCs values observed in both groups in week two, explained as the well known fact that an increase over the normal RBCs value usually observed after handling due to excitement or strenuous exercise [17], as it leads to the release of adrenaline and hence spleen contracts which causes the release of more RBCs into circulation. In previous studies, only the use of psychological tranquillization reduced the splenic influence and the red blood cells count was increased gradually in all groups which received 2 and 10% w/w *N. sativa* and reached very high level after six weeks, then slightly increased in week seven and this level was maintained at week eight [17 and 18]. These variations could be due to differences in anatomical structure between rats and sheep. However in the present study the decrease seen in RBCs count after two weeks in the treated group, increased in week four and reached significantly ($P \leq 0.05$) higher level in week six compared to week two, this increase may be due to the iron content of the *N. sativa* reported [19].

3.2 Hemoglobin (Hb)

Figure (2) showed that *N. sativa* oil has no clear effect on Hb in sheep, whereas *N. sativa* oil in rats has was observed to increase the hematocrit and hemoglobin levels by 6.4 and 17.4 %, respectively [5]. The

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(1) present the results separately to highlight the unusual or marked observation before referring the reader to the appropriate chart or table.
(2) Discuss the results based on the presentation by highlighting the marked and significant observation and comparing same with previous studies. It is not acceptable to compare the results of this study with that of rats because that was not the aim of the study. And, the study was not designed to allow such wide comparison. Majority of the results were not significant, hence need not to be discussed.

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effect of feeding different doses of the seeds of *N. sativa* 2 and 10% w/w for considerably long periods in male and female rabbits showed an increase in Hb levels in all groups [18]. In the present study a similar pattern to what was observed in the RBCs count has been found in the level of Hb, but at week six the values were significantly ($P \leq 0.05$) higher than in time zero in both groups treated and the control one.

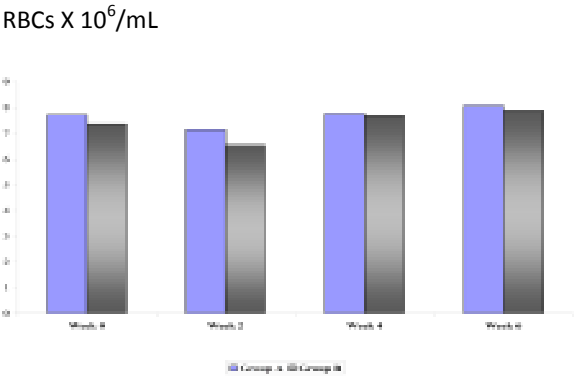


Figure (1) Effect of feeding *N. sativa* oil on red blood cells in sheep.

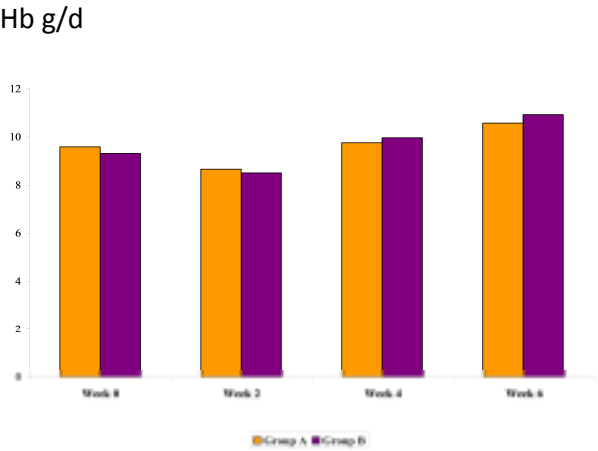


Figure (2) Effect of feeding *N. sativa* oil on hemoglobin in sheep.

PCV %.

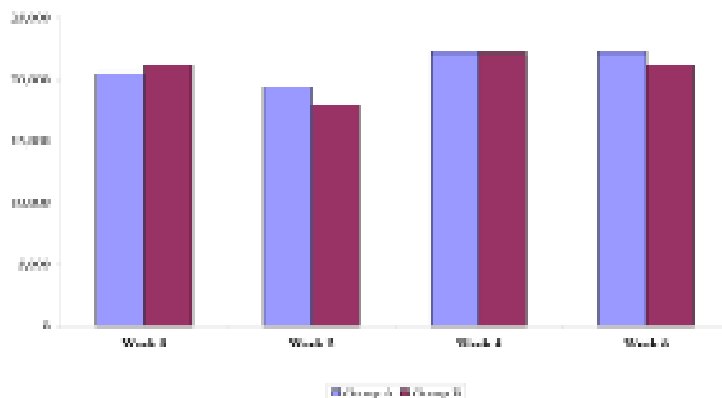


Figure (3) Effect of feeding *N. sativa* oil on PCV in sheep.

3.4 PCV

Figure (3) showed that addition of *N. sativa* oil resulted in similar values in treated and control groups which indicated no significant effect of the oil on PCV value in sheep. This comes in line with the study [20] that found the treatment with *N. sativa* oil for two months to the anemic patients showed that the Hb concentration has increased by 10% for the initial level. PCV and MCV also have increased, but not significantly. This result also agrees [6], but contradicted [14].

3.5 MCH

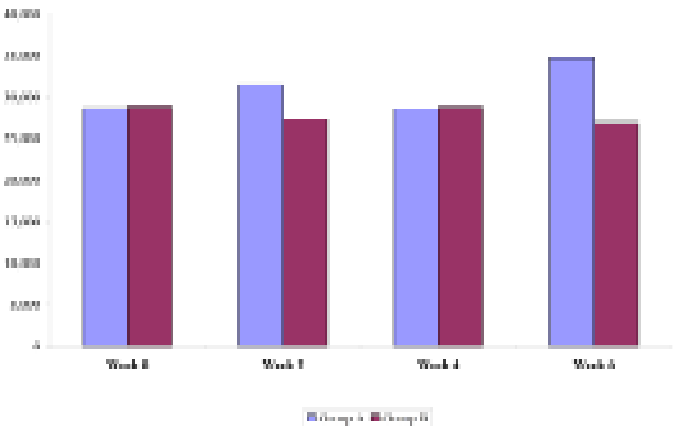
Figure (4) indicated the *N. sativa* oil treated group was similar to control group which indicates no effect of the oil on MCH value in sheep. The pattern observed in MCH was similar to what was observed in RBCs and changes were also not significant. This contradicted with previous findings [20].

3.5 MCV

Figure (5) indicated that the *N. sativa* oil treated group was similar to control group which indicates no effect of the oil on MCV value in sheep. The pattern observed in MCH, PCV and MCV was similar to what was observed in RBC count. This agrees with the finding [20] which showed MCV value between $96 \mu\text{m}^3$ and $85 \mu\text{m}^3$. This indicates that most of them were in normocytic condition. After 2 months treatment the MCV increased to the average value of 109.8, which indicate the lack of iron.

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MCV $10^3/\mu$



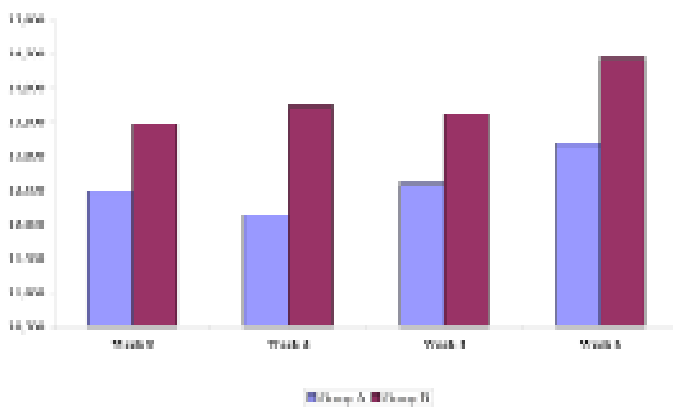
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Figure (4) Effect of feeding *N. sativa* oil on MCV in sheep.

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MCH Pg



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Figure (5) Effect of feeding *N. sativa* oil on MCHC in sheep

MCHC g/dl

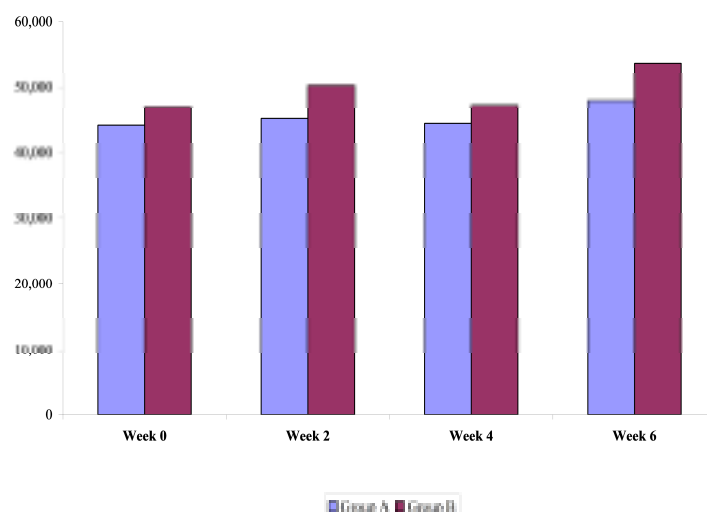


Figure (6) Effect of feeding *N. sativa* oil on MCH in sheep.

3.6 MCHC

The *N. sativa* oil treated group was similar to control group Figure (6). This was indicated no effect of the oil on MCHC value in sheep. The MCHC values are dependent upon RBC, Hb and PCV values. The result comes on line with the finding of [20]. The MCHC was always below the normal level and change was not significant.

3.7 White blood cells (WBCs)

The white blood cells (WBCs) are the soldiers of the body and their high counts may be due to increase or development of the immune systems of the animals at the early stage of life which they may not obtain from the colostrum of the dam [17]. Figure (7) showed that the general effect of *N. sativa* oil on white blood cells in sheep produced significantly ($P \leq 0.05$) lower value as total mean of WBCs of group A compared to the control. This result agree with [5]. and disagree with [6]., In the present study during the experimental period, week two showed increased WBCs values in both groups compared to other weeks, other increase was also observed in week six but it was not significant in the treated group compared to week four. This may be due to the stress during sample collection, which could be attributed to physiological phenomenon i.e. excitement or strenuous exercise during handling [20].

3.8 Lymphocytes

N. sativa oil affected lymphocytes of sheep in the present work by resulting in significant ($P \leq 0.05$) reduction of total mean value of lymphocytes in the *N. sativa* oil treated group compared to the control group Figure (8). This result disagrees with previous finding [6]. The lymphocytes were elevated in week two in both groups compared to other weeks, this could also be due to the excitement and stress during sampling. The lymphocytes constituted majority of the WBCs counts and the cells increase with age in early life of animals, this is favored as stated in the finding [20] and it might be attributed to stress and immune response to the environment [20].

3.9 Granulocytes

The total mean value of the granulocyte count in the *N. sativa* oil treated group was significantly ($P \leq 0.05$) lower compared to the control group Figure (9).. This result comes on line with previous study [5], but it is contradicted other work [21]. The pattern followed of the granulocytes count was similar to the behavior of the WBCs and contributed in the total count result.

3.10 Monocytes

Total mean of Monocyte count of the *N. sativa* oil for treated group was not significantly different compared to the control, but in week four the treated group showed significantly ($P \leq 0.05$) lower level compared to the control group Figure (10). This result contradicted with previous finding [6] but come in line with other work [18]. However a significant ($P \leq 0.05$) increase was observed in group A at week two, but followed by significant ($P \leq 0.05$) decrease in week four which decrease further in week six this effect was not observed in the control group

WBC ($\times 10^3/\mu$)

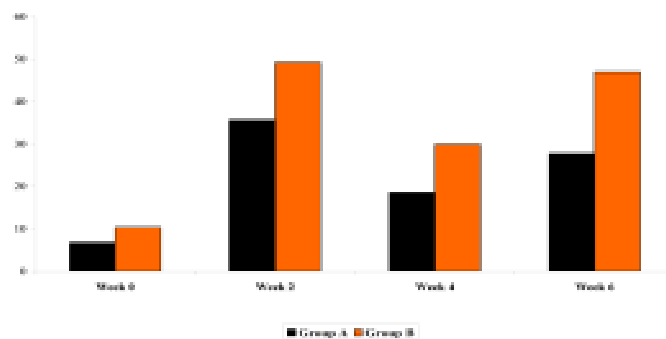


Figure (7) Effect of feeding *N. sativa* oil on white blood cells in sheep.

Means (x 10³/μ)

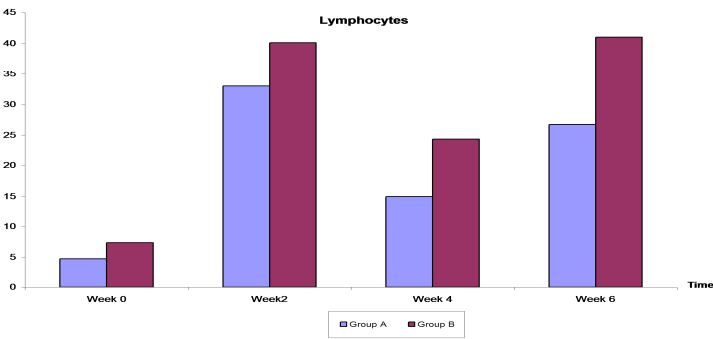


Figure (8) Effect of feeding *N. sativa* oil on Lymphocytes in sheep.

Means (x 10³/μ)

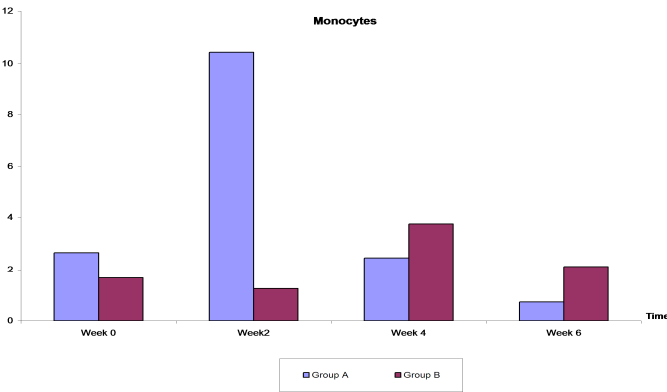
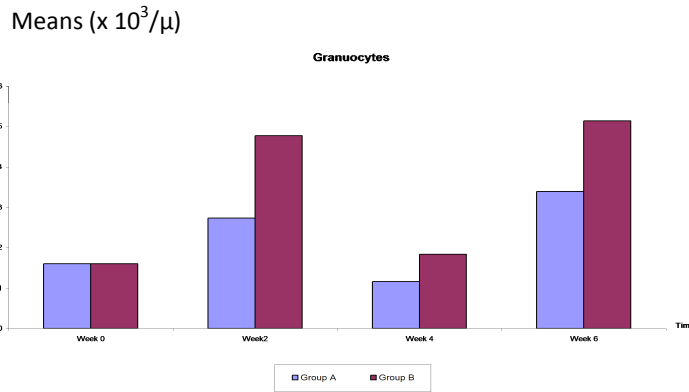


Figure (9) Effect of feeding *N. sativa* oil on Monocytes in sheep.



Figure

(10) Effect of feeding *N. sativa* oil on Granulocytes in sheep

Findings in the present study showed that Red Blood Cells, Hemoglobin, PCV, MCV, MCH, and MCHC total mean of the *N. sativa* oil treated group is similar to control group, only Red Blood Cells (RBCs) count of the *N. sativa* oil treated group showed significant ($P \leq 0.05$) increase at week six, whereas no significant change was observed in the control group. Hemoglobin concentration also showed similar pattern to what was observed in the RBC count in both treated and control groups. PCV, MCV, MCH, and MCHC weren't affected by feeding *N. sativa* oil to sheep throughout the experiment. The effect of *N. sativa* oil on white blood cells in sheep produced significantly lower values in, the total count of the (WBCs), the lymphocytes, and granulocytes of the treated group compared to the control and the count of these cells presented the same pattern in the two groups, that the levels were significantly ($P \leq 0.05$) increased, then significantly ($P \leq 0.05$) decreased and significantly ($P \leq 0.05$) increased by the end of the experimental period. The Monocytes total means was similar in both groups, but the cells were significantly ($P \leq 0.05$) lower in the *N. sativa* oil treated group compared to the control group at week four.

Conclusions

Findings in the present work showed some changes in the blood cell related to the use of the *Nigella sativa* oil and the changes in the white blood cells was were more pronounced. in the white cells, the different effect of this treatment in ruminants was expected, if compared to findings in mono gastric animals, due to the effect of the rumen atmosphere on the digestion of fats. The blood cells changes in sheep here, may be mainly due to the effects of *N. sativa* volatile oils

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