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Effects of alternative dietary protein of *Nigella sativa* on some hematological, biochemical and immunological responses of pregnant Barki ewes

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Abstract

Thirty estrus synchronized pregnant Barki ewes (2.0-3.0 years old and 38.89 ± 1.02 kg average body weight) were used to investigate the effects of feeding *Nigella sativa* meal (NSM) as an alternative source of protein on some hematological, biochemical and immunological parameters during peripartum period. Animals were randomly assigned into three equal groups (10 each) after their conception. The first group (G1) served as control and was fed the basal diet containing 20% cotton seed meal and 6% soybean meal as a source of dietary protein, while the second group (G2) was fed diet containing13 % of NSM and 8% soybean meal. The third group (G3) was fed diet containing 25% of NSM. Blood samples were withdrawn during last month of pregnancy. Results indicated that, NSM groups had a slight higher concentration of total protein, albumin and globulin and lower concentrations of total lipids and low density lipoprotien (LDL) with non significant differences. However, cholesterol and high density lipoprotein (HDL) increased significantly in control group. ALT, AST, urea and creatinin were not affected by type of protein ration. Feeding NSM increased (P<0.05) significantly WBCs, RBCs, Ht and MCV values. Moreover, NSM supplementation improved blood plasma immunoglobulin (IgA and IgG) and total antioxidant. In conclusion, NSM could be used as a safe alternative source of dietary protein without any adverse effects on liver and kidney functions as well as improving some immunological parameters of Barki ewes under arid conditions.

Key words: *Blood picture*, *cholesterol*,peripartum period, *protein profile*.

INTRODUCTION

Nigella sativa (NS) is considered as one of the most important medicinal plants in the world due to its beneficial actions. It has been proposed as a natural alternative to antibiotics in order to improve the health status of animals, and to increase the production and quality of animal's products (Longato *et al.* 2015). Normally, the nutrients differ in concentrations according to differences in diets and ingredients or diet components or treatments may apply on diets via their interaction in metabolic pathways naturally occurring in different body tissues (Hassan and Saeed 2012).

Both hematological and biochemical parameters are considered as crucial indicators for the well-being's diagnoses (Nicoll *et al.* 2004 and Kaneko *et al.* 2008), revealing the metabolic status, and consequently evaluating, predicting, and preventing the incidence of several diseases during critical periods such as peripartum (Celi *et al.* 2008). Since peripartum is physiological stressor and critical period (Drackley 1999). Such critical period, may aggravate by different metabolic diseases (Iriadam 2007). Recently, it was reported that; soybean or soybean products can lead to some deleterious effects on the gastrointestinal tract. Such effects are related to abnormal cell cycle (Pan et al. 2017), cell apoptosis (Pan et al. 2018b), or altering the normal microbial composition of the gut (Pan et al. 2018a). Several studies have been conducted on sheep to evaluate the effects of *Nigella sativa* (NS) on different blood biochemical parameters in different physiological conditions. For example, Zanouny *et al.* (2013) and Abd El-Halim *et al.* (2014) studied the effects of NS on male sheep. Earlier, El-Harairy *et al.* (2006) reported the effects on NS on different physiological parameters (reproductive performance) in ewes. Whereas, the data for effect of NS on the peripartum period is limited. The objective of this study was to evaluate the effects of alternative dietary protein *Nigella sativa* meal (NSM) on some blood biochemical, hematological and immunological responses of Barki ewes instead of soybean and cotton seed meal during peripartum stage.

MATERIAL AND METHODS

The herein study was carried out in the Animal Production Unit in the Sustainable Development Center for Matrouh Resources, Matrouh Governorate, the unit belongs to the Desert Research Center (DRC) in the North Western Coast of Egypt. The trial involved a 2-week adaptation period to the diet followed by 5 months of feeding the experimental diets. All animals were reared according to Desert Research Center Guidelines for Animal Husbandry. Thirty estrus synchronized adult Barki ewes (2.0-3.0) years old and (38.89 ± 1.02 kg) average body weight, were assigned randomly into three equal groups (10 each) after conception. The first group (G1) served as control and was fed the basal diet contained 20% cotton seed meal and 6% soybean meal as a source of protein, while the second group (G2) was fed diet contained 13% of *Nigella sativa* meal (NSM) and 8% soybean meal. The third group (G3) was fed diet contained 25% of NSM. *Nigella sativa* meal was added in a dry form after extracting the oil and well mixing with the concentrate ration (Table 1).

Samples of NSM and concentrate rations were (biweekly) collected. Samples were grounded in a hammer mill provided with a 1-mm pore size screen and analysed in triplicate for their content in DM (forced-air oven at 65°C and dried to a constant weight), ash, CP (N × 6.25), crude fiber (CF), ether extract (EE). Results of proximate chemical analysis of different experimental rations are shown in Table (2), according to AOAC (1990). All groups were offered berssem

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(*Trifolium alexandrinum) ad libitum*, rations were biweekly adjusted to cover their requirements during different physiological status according to Kearl (1982). Animals had free access to fresh water throughout the experimental period. The housing was semi-open pens roofed with wood**.**

Table 1. Ingredients percentage of different experimental rations for the experimental groups

Item	G1	G1	G3
Yellow corn	50	54	53
Wheat bran	20.5	21.5	18.5
Cotton seed meal	20	0	0
Soybean	6	8	0
Nigella sativa	0	13	25
Limestone	1.5	1.5	1.5
Mineral mixture	1	1	1
Sodium chloride	1	1	1

Table 2. Chemical analysis of different experimental rations (on 100% DM basis)

Chemical composition (%)	Experimental rations			
	Diet 1	Diet 2	Diet3	NSM
Dry Matter (DM)	91.5	91.4	91.5	91.0
Crude Protein (CP)	12.6	12.1	11.6	7.54
Crude Fiber (CF)	12.2	15.8	19.3	19.9
Ether extract (EE)	7.46	7.50	7.53	7.43
Nitrogen free extract (NFE)	61.2	58.2	55.1	63.1
Ash	6.5	6.4	6.4	2.03

Diet1; the control concentrate ration (0% Nigella sativa), Diet 2; 50% Nigella sativa, Diet 3; 100% Nigella sativa as a partial replacer of source of protein.

Blood samples were collected twice (on day 130 and 140 of pregnancy) at 8 a.m. before the morning feeding through vein puncture, then were centrifuged at 4000 x *g* for 20 minutes for the separation of serum and kept at -20 °C until further analysis. Blood biochemical parameters were determined for total proteins (TP) albumin (Alb), cholesterol, high density lipoprotein cholesterol (HDLc) concentrations using commercial coloremitrickits supplied by Biodiagnostic Company for Laboratory Services. While, globulin (Glb) albumin/globulin ratio (A/G) and low density lipoprotein cholesterol (LDHc) were calculated. Aspartate and alanine aminotransferases (AST and ALT), total lipids (TL) and total antioxidant (TAO) concentrations were determined by coloremitrictest kits supplied by Diamond Diagnostic Company for Laboratory Services. Immunoglobulins (IgG and IgA) determined by commercial coloremitrickits supplied by Clinical Chemistry Company. All chimecal kits made in USA. An automatic spectrophotometer was used during the current study (Model 6405 Brand Jenway,City Chelmsford, Essex,., Country England). Red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured using blood cell counter (Hemavet 950 F, Drew Scientific, Dallas, TX, USA).

Statistical procedure

Data were subjected to analysis of variance utilizing GLM model of SAS (2000) for repeated measurements and means were compared using Duncan Multiple Range Test (Duncan 1955). The following model was adapted:

$Y_{ijk} = \mu + T_i + e_{ijk}$

Where:

Y_{ijk} = Trait

μ = Mean

T_i = Effect of treatment, i, 1-3 (1 = 0% *Nigella sativa*, 2 = 50% *Nigella sativa*, 3 = 100% *Nigella sativa*)

e_{ijk} = Experimental error.

The results were expressed as the mean ± SEM, unless otherwise stated.

RESULTS AND DISCUSSION

Feed intake

As shown in Table (3), total dry matter intake and crud protein intake were not affected by type of ration protein with insignificantly increase in G2 compared to the other groups. Similar results were observed by Mahmoud and Ghoneem (2014) who found that, dry matter intake was not influenced by the partial replacement of soybean meal and cotton seed meal by NSM in lactating Egyptian buffaloes. Earlier, Mohamed (2007) also reported that, total dry matter intake seemed to be similar in growing Maghraby camel fed control or NSM ration.

Table 3. Voluntary feed intake of ewes during experimental period as affected by experimental rations (DM g.h.d^{-1}).

Items	**G1**	**G2**	**G3**	**±SEM**
Roughage gm/h/d	698.05	711.20	691.62	16.28
CFM gm/h/d	629.75	641.67	624.54	16.20
Total Intake gm/h/d	1327.75	1352.87	1316.16	34.54
Total CPI gm/h/d	117.76	120.09	117.19	3.05

CFM: Concentrate feed mixture, CPI: Crud protein intake

Total proteins and its fractions

The obtained results revealed that feeding NSM had no significant effects on total proteins (TP), albumin (Alb) and globulin (Glb) concentrations in pregnant Barki ewes (Table 4). However, values tended to be higher in treated groups as compared to the control one. These values indicated that the experimental groups were in good nutritional status.

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Table 4. Effects of feeding *Nigella sativa* meal on blood biochemical parameters

Item	Experimental groups				
	G1	G2	G3	±SEM	P value
TP (g/dL)	6.46	6.70	6.73	0.15	0.43
Alb (g/dL)	3.64	3.73	3.77	0.11	0.70
Glob (g/dL)	2.82	2.97	2.96	1.77	0.82
A/G %	1.37	1.62	1.50	0.19	0.07

G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein

The present results are in agreement with those reported by Mansour *et al.* (2013) as they reported that TP, Alb and Glb increased in calves fed diets contained 7 or 11% NSM. The slight increase in TP and its fractions might be due to several reasons among which improved organic matter (OM) and crude protein (CP) digestibility and increased of nitrogen intake which reflected on the nutritional status of the animals (Kumar *et al.* 1981), or may be due to that NS contain essential amino acids, and other active compounds like thymoquinine, nigllen and nigllon, that are essentially required for protein building (Amin and Hosseinzadeh 2016). Similarly, A/G ratio ranged from 1.37 to 1.62 with insignificant increase in NSM groups. These results demonstrated that both Alb and Glb increased by the same level due to the replacement of NSM in the ration. The present results are in harmony with those reported by Mansour *et al.* (2013) who found that A/G ratio was not affected by feeding ration contained 7 or 11% NSM. It is important to note that all values were higher than 1.0% (Saleh 2005),thus using of NSM did not change the total protein levels and its fraction than the normal group.

Liver enzymes and Kidney function

Alanine aminotransferase (ALT) is particularly useful in measuring hepatic necrosis and increased in serum when cellular degeneration or destruction occurs (Nicoll *et al.* 2004). Data presented in Table (5) showed that NSM did not affect ALT concentration among the experimental groups with non-significant values of 15.16,17.26 and 18.57 IU/L for G3, G2 and control group, respectively. These results are in agreement with those reported by Dollah *et al.* (2013) who found that the content of NSM level in the diet was in parallel with decreasing liver enzymes in rats. On the other hand, the present results showed that (G2) had the highest value of AST followed by (G3) then control group, but these differences were not significant (Table 5).

The obtained values for both enzymes were within the normal range according to Kahn *et al.* (2010). Results are in agreement with those reported by Mahmoud and Ghoneem (2014) who found insignificant differences between groups fed basal diet and diet contain 50% NSM. In the same line, Khattab *et al.* (2011) reported that AST and ALT enzymes for buffalo calves tended to be insignificantly lowered in supplemented with NS oil group than those of control. These results are also comparable with earlier findings of Al Suhimi *et al.* (2005) in goats, as liver enzymes showed insignificant changes in their activities in NS supplemented animals that proved no harmful effect of NS on liver.

Table 5. Effects of feeding *Nigella sativa* meal on liver and kidney parameters

Item	Experimental groups				
	G1	G2	G3	±SEM	P value
AST (IU/L)	105.5	112.1	109.0	3.54	0.59
ALT (IU/L)	18.57	17.26	15.16	0.32	0.76
Urea (mg/dL)	33.	35.41	34.44	1.60	0.75
Creatinine (mg/dL)	0.5	0.5	0.7	0.01	0.15

G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein

The levels of serum blood urea and creatinine are known to reflect the state of glomerular filtration rate of kidney function (Kaneko *et al.* 2008).In the present study, the effects of using NSM as a source of ration protein on kidney function are represented in Table (5). Data of urea concentration showed that there were no differences between the experimental groups with values being 34.44, 35.41 and 33.72 mg/dL for G3, G2 and control group (G1), respectively. Moreover, creatinine concentration showed approximate same trend among the different groups with values of 0.71, 0.52 and 0.50 mg/dL for G3, G2 and control group, respectively.

These results indicated that NSM had no negative effects on kidney function. In addition, values of the blood urea and creatinine reported here were within the normal values early reported by Khodary *et al.* (1997). The present results are in agreement with those reported by Awad-Aallah (2002) who reported that urea concentration was not significantly affected as a result of NSS supplementation at the rate of 100 mg/kg body weight in Friesian calves.

Lipid parameters

The effect of feeding NSM as a source of ration protein on serum lipid parameters; total lipid (TL), cholesterol (Cho), high density lipoprotein (HDLc) and low density lipoprotein (LDLc) of Barki ewes are presented in Table (6).

Serum total lipid (TL) insignificantly decreased in NSM groups as compared with the control group. These results are in agreement with those reported by Khattab *et al.* (2011) who found that TL was insignificantly decreased during pre and post-partum in dairy buffaloes fed ration supplemented with 10 $mL.h.d^{-1}$ NS oil as compared to control group. However, Shams Al-Dain *et al.* (2006) declared that TL concentration was increased when Awassi ewes fed diets contain 7 or 14% NSM. This line needs further studies.

Cholesterol plays an essential role in mammalian biochemistry, it is an essential component of animal cell membrane and precursor of the steroid hormones and there is a positive correlation between high level of serum cholesterol and cardiovascular diseases (Hill et al. 2012). In the current study, Cho concentration was significantly ($P<0.05$) lower in NSM groups compared to control group. The third group recorded the lowest value of Cho concentration (172.09 mg/dL), while control group recorded the highest value (187.17 mg/dL). These results are in accordance with those reported by several studies which indicated that NS has promising effects resembling to those drugs that reduce serum Cho and decrease its atherogenic pathological effects (El Bagir *et al.* 2016).The reduction in serum Cho might be due to the lower levels of thymoquinone and monounsaturated fatty acids on the synthesis of Cho by hepatocytes (Padhye et al. 2008), or fractional reabsorption from the small intestine (Brunton 1990). Also, it might be related to the stimulation effect of unsaturated fatty acids on the Cho excretion into the intestine and the oxidation of Cho to bile acids (Ibraheim 2002). Moreover, Morikawa *et al.* (2004) mentioned that Nigellamin has a crucial effects to decrease serum triglyceride since it could act as Clo fibrate.

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Table 6. Effects of feeding *Nigella sativa* meal on lipid parameters

Items	Experimental groups				
	G1	G2	G3	±SEM	P value
TL (mg/dL)	601.51[a]	556.34[a]	553[a].18	21.12	0.20
Col (mg/dL)	187.17[a]	182.85[ab]	172.09[b]	5.07	0.05
HDLc(mg/dL)	72.58[a]	68.34[a]	61.03[b]	2.47	0.005
LDLc (mg/dL)	114.58[a]	114.50[a]	111.64[a]	5.45	0.87

G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein, Means with different superscripts ([a], [b]) are significantly different within the same row.

The present results were in accordance with those reported by Shams Al-Dain *et al.* (2006) found that Cho level increased significantly in animals fed 7 or 14% NSM as a source of ration protein. In contrast, with El-Saadany *et al.* (2008) who found that value of Cho was significantly decreased in lactating Zaraibi does, fed on ration supplemented by 5 g NS.h.d^{-1} compared to control group. In consistency, Zanouny *et al.* (2013) noticed that value of Cho decreased in Ossimi lambs groups that fed ration supplemented with 100 or 200 mg NS /kg body weight /day.

On the other hand, LDLc is one of the four major groups of lipoproteins which are physiologically important in clinical diagnosis. The LDLc transports Cho from liver to the peripheral tissues, it is called (bad cholesterol) and its concentration in blood has positive correlation with incidence of cardiovascular diseases (Vasudevan *et al.* 2011). In the present study the level of LDLc followed the same trend of Cho in the NSM groups. The control group recorded the highest value (114.58 mg/dL) then G2 (114.50 mg/dL), while G3 recorded the lowest value (111.67mg/dL).

High density lipoprotein (HDLc) is the main transport form of cholesterol from peripheral tissue to liver which is later excreted through bile. The level of HDLc in serum is inversely related to the incidence of myocardial infraction. As it is anti atherogenic, HDLc is known as good cholesterol (Vasudevan *et al.* 2011). In this study, feeding ration contain NSM lead to a significant decrease in HDLc level **(Table 7)** compared to control group with values being 72.58 mg/dL followed by, 68.34 mg/dL for G2 and 61.03 mg/dL for G3, respectively.

Hematological parameters

Blood hematological profile is an important index of the physiological status of the animal (Rodgers and Young 2013). The mean values of hematological parameters are presented in Table (7).

Table 7. Effects of feeding *Nigella sativa* meal on hematological parameters

Items	Experimental groups				
	G1	G2	G3	±SEM	P value
WBCs(x10^3/mL)	8.61[c]	10.76[b]	11.44[a]	0.47	0.01
RBCs(x10^6)	11.56[b]	12.45[a]	12.51[a]	0.27	0.05
Hb(g/dL)	12.24	12.42	12.55	0.23	0.65
Ht (%)	25.76[c]	26.70[b]	27.37[a]	0.22	0.003
MCV (fl)	23.71[b]	24.20[ab]	25.14[a]	0.34	0.01
MCH (pgm)	10.99	10.99	10.72	0.16	0.39
MCHC (g/dL)	36.38	35.89	36.27	0.52	0.79

G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein,
Means with different superscripts ([a], [b], [c]) are significantly different within the same row.

White blood cells count

The major function of white blood cells (WBCs) and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infections, while those with high counts within the normal range are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al.* 2013), and enhance adaptability to local environmental and disease prevalent conditions (Isaac *et al.* 2013).

In the present study, WBCs count of the three experimental groups differed significantly (P>0.01) indicating that ewes fed on NSM had a highest value of WBCs compared to control one and all values were in the normal range. This finding showed that feeding ration containing NSM improved animal health and their nutrition. These results are in agreement with those reported by Mansour *et al.* (2013) who reported that WBCs count increased significantly with the increasing of NSM in the calves rations, whereas animals fed 11% NSM recorded the highest value followed by animals fed 7% NSM compared to control group. This increase in WBCs count might be attributed to the emergence of these cells from the sites of composition in the bone marrow into the circulatory system due to the effects of some hormonal factors, affected by NS (Mbassa and Poulsen 1991). On contrary, Abd El-Halim *et al.* (2014) demonstrated that the general effect of NS oil on WBCs in sheep was significantly lower compared to control group. Habeeb and El-Tarabany (2012) found that WBCs values were not affected by NSS additives to concentrate ration compared to basal ration.

Red blood cells

There is relationship between the protein level of the diet, and red blood cells count (Al-Talib *et al.* 2010). Data in Table (7) indicated that *Nigella sativa* meal had significant effect on RBCs. The highest value of red blood cells (RBCs) was recorded for G3 then G2, while the lowest value was observed in control group. The corresponding values were 12.51, 12.45 and 11.56×10^6/mL for G3, G2 and control group, respectively. These results might be due to that those animals were increased in body weight or in phase of growth which demand the excessive quantity of erythrocyte cell count to do the activeness employments**,** or may they need more quantity of oxygen that transported by hemoglobin to stopping the need of activeness employ employments of the body which caused a positive reflect to increase the total RBCs, Hb and PCV% as lambs get older (Shams Al-Dain and Jarjeis 2015).These results are in harmony with those reported by Shams Al-Dain *et al.* (2006) who found that RBCs count was significantly increased in Awassi ewes fed ration contain 7 or 14% NSM as a source of protein diet. Also, According to the level of ration protein, Al-Talib *et al.* (2010) reported that high level of ration protein (15.07 %) showed significant improvement in RBCs count than low level of protein (12.38 %). This finding was supported our results.

In contrary, EL-Ghousein (2010) found that RBCs did not differ between animals fed NSM (10 g/ewe/d) compared with control group, while their lambs showed a significant increases of RBCs in NS group.

Hemoglobin concentration

The obtained results for hemoglobin concentration (Hb) demonstrated that no-significant differences among the different experimental groups. Data in Table (7) eleducated that Hb values were nearly similar in different groups (12.55, 12.42 and 12.24 g/dL for G3, G2 and control group, respectively). These findings matches with those reported by Abd El-Halim *et al.* (2014) who found that Hb values did not differ between sheep fed on diet supplemented with 47 g NS oil/kg CFM and control group. On contrary, Abou-Zeina *et al.* (2015) found that Hb value was significantly increased when crushed black seeds supplemented by about 2% to diet of goats compared to control group. Thus the relation between Hb and NS still need additional studies.

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Hematocrit ratio

In this study, animals fed ration contain 100% NSM as a main source of protein (G3) exhibited a highest value (P<0.05) of Ht followed by animal fed ration contain 50% NSM (G2) then control group with values of 25.76, 26.70 and 27.37% for control, G2 and G3, respectively (Table 7)**.** Such increase in Ht value might be related to either the essential mineral elements content of *Nigella sativa* on that used for building hemoglobin and thus increase the red blood cells and subsequently causing increase in Ht value (El-Shamaa 2002), or due to the direct effect of NS on haemopoietic tissue (Nair *et al.* 1991). These results are in agreement with those reported by Habeeb and El-Tarabany (2012)who found that Ht significantly increased when goats fed diet contain NS.

Wintrobe indices

Results of mean corpuscular volume (MCV) demonstrated that ewes fed on NSM have the highest values (25.14 and 24.20 Vs 23.71 fl) for G3 and G2, respectively compared with the control, however the differences were not significant. Contrariwise, Abd El-Halim *et al.* (2014) found that MCV was similar in animals fed basal ration and animals fed basal ration treated by 47 g NS oil/kg CFM.head.d^{-1}. Moreover, Galbat *et al.* (2014) found that lactating goats fed diet supplemented with or without supplement 25 g NS/kg body weight showed the similar values of MCV being 27.43 and 27.03 for treated and control group, respectively.

Owing the values of mean corpuscular hemoglobin (MCH) presented in Table (7). The obtained results indicated that MCH values were nearly similar in all treated groups with values 10.99, 10.99 and 10.72 pg for control, G2 and G3, respectively. These results are in harmony with those reported by Shams Al-Dain and Jarjeis (2015)who reported that MCH values were similar in Awassi lambs fed control diet or diet supplemented with 600 mg NS/kg body weight/d.

No differences among the experimental groups in mean corpuscular hemoglobin concentrations (MCHC), as all groups nearly scored the same values (36.27, 35.89 and 36.38 g/dL for G3, G2 and control group, respectively). These results are in the normal range that reported by R.A.R. (2009) which supported our results and indicated that NS has not adverse effects for type of ration protein on animal health or performance. MCHC values were within the normal range reported by R.A.R. (2009). Abd El-Halim *et al.* (2014) reported that MCHC was not affected by NS oil or NSS supplementation.

Immunological parameters

Our results revealed that NSM has been regarded responsible for the significantly improved blood plasma immunoglobulin concentration (IgA and IgG) Figure (1)**.** The third group recorded the highest value of IgG concentration (0.067 mg/mL) followed by G2 (0.052 mg/mL), while the control group recorded the lowest value (0.043 mg/mL). On the same trend, IgA concentration was significantly higher in G3 followed by G2 then control group with values being 0.065, 0.036 and 0.027 mg/mL, respectively.

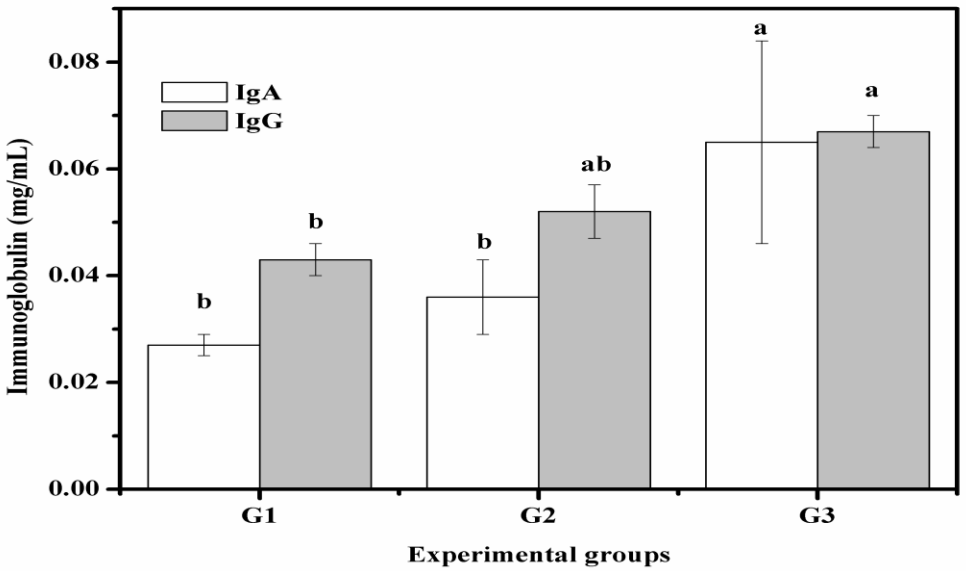

Figure 1. Effects of feeding *Nigella sativa* meal on Immunoglobulins concentration (mg/mL)
G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein., Means with different superscripts (^{a}, ^{b}) are significantly different within the same color.

These results might be attributed to that seed extract and seed oil or meal from NS exhibited immunostimulant properties (El-Sayed 2002), and exhibited immunopotentiating activities (Hailat et al. 1995). These results are in agreement with those reported by Khattab *et al.* (2011) who found that immunoglobulin concentration improved by addition of black seed oil to buffaloes rations. Earlier, El-Ekhnawy *et al.* (1999) reported that feeding rations supplemented with 150 and 250 g/d led to significantly increase in total globulins and their fractions (α, β and γ). The same results were obtained by El-Wafa *et al.* (2002) and Gaafar *et al.* (2014) in rabbits and El-Gaafarawy *et al.* (2003) in Friesian cows and their offspring. Hedaya (1995) reported that low doses of *Nigella sativa* seeds extract caused an increase in the immunity of the body through increasing the lymphocytes and globulins values. Supplementation of animal ration with NS improved animal performance, because NS can act as a free radical scavenger with other anti-oxidant vitamins and it can depress inflammatory responses (Al-Ghamdi 2001). Moreover, Gholamnezhad *et al.* (2015) showed that NSS have been widely used in traditional medicine and has been shown to have immunomodulatory properties.

Total antioxidant

Total antioxidant (TAO) could be used as a tool to evaluate the nutritional status of animals either fed different diets or throughout the year. Among the herbs that are known to have antioxidant properties NSS which have been used traditionally for centuries for the treatment of different diseases (Merfort et al. 1997). Thymoqunone, the main compound in NS, inhibits non-enzymatic lipid peroxidation in liposome. From another point of view, NS contain large amounts of trace elements which increase antioxidant status, immunity and may help the animals to tolerate the stress conditions (Habeeb and El-Tarabany 2012; Abo-Zeina et al. 2013). Data in Figure (2) indicated that total antioxidant increased insignificantly in NSM groups where G2 recorded the highest values (0.210 mm/L) then G3 (0.178 mm/L), while the control group recorded the lowest value (0.168 mm/L) but the differences were not significant. These results indicated that the experimental animals were in good health status and in good nutrition management. The present results were in agreement with those reported by Abou-Zeina *et al.* (2015) who found that antioxidant capacity showed significant elevation when Baladi kids fed diet supplemented with 2% NSS compared to control group. The

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same results were obtained by El-Far *et al.* (2014) who reported that TAO was significantly increased in cross-bred ewes fed diet supplemented with 3g of NS.h.d^{-1} compared to control group.

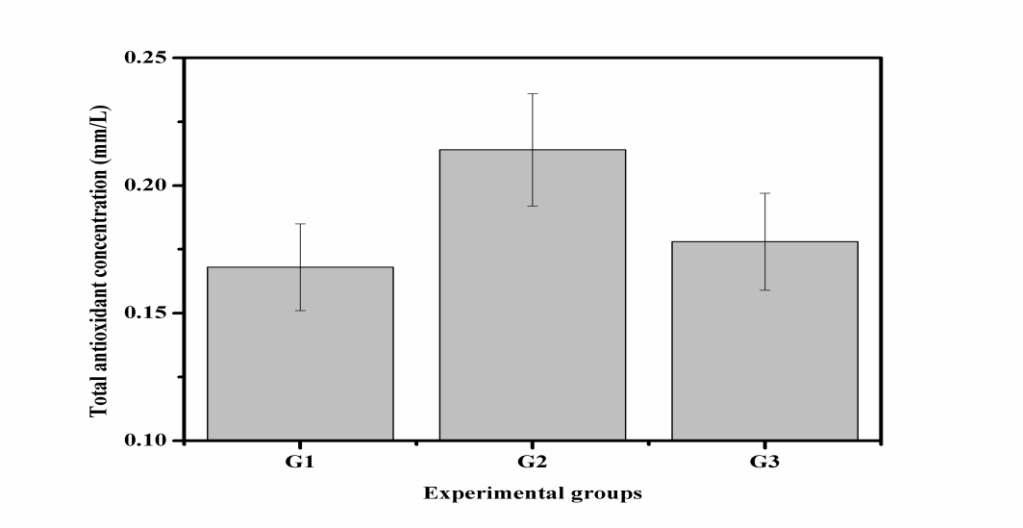

Figure 2. Effects of feeding *Nigella sativa* meal on Total antioxidant concentration (mm/L)
G1; the control concentrate ration (0% *Nigella sativa*), G2; 50% *Nigella sativa*, G3; 100% *Nigella sativa* as a partial replacer of source of protein

CONCLUSION

In the view of the current study, it could be concluded that NSM could be used as an alternative source of dietary protein without any adverse effects on liver and kidney function, as well as, improving some immunological parameters of Barki ewes. The effects of NS on some hematological and biochemical parameters are rather controversial and conflicting between studies and needs further investigations as well as the gut microbial modulation and the molecular impact of NSM supplementation should studied.

Conflict of interest
Non

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