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EFFECT OF *NIGELLA SATIVA* SEEDS SUPPLEMENTATION ON MILK YIELD AND MILK COMPOSITION IN SHEEP

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ABSTARCT

A total number of 20 Ossimi ewes were used in this experiment. Ewes were randomly assigned to four equal groups, two groups (G1& G2) suckled single and two groups (G3 & G4) suckled twins. Ewes in the first and second group were fed on basal diet (control-single) and on basal diet+100mg daily *Nigella Sativa* seeds/head for G2. Ewes in the third (G3) and fourth (G4) groups were fed on basal twin diet (control-twins) and on basal twin diet +100mg daily *Nigella Sativa* seeds/head for G4. Daily milk yield was measured for each ewe starting from the fifth day post lambing until weaning at 3 months. Milk samples were taken weekly throughout the suckling period from all tested ewes. The samples were taken in the morning and afternoon and then both samples were mixed together for chemical analysis. Milk energy values were calculated. The results of this experiment indicated that milk yield had insignificant differences at first and second weeks of the experiment but from the third to the twelfths week the difference among groups recorded highly significant ($P<0.01$) increase for G2 and G4 compared to G1 (control/single) and G3 (control/twins) groups. The results revealed that the ewes reached maximum yield (peak) at the third and fourth week of lactation and decreased after that. In addition, milk yield was significantly ($P<0.01$) higher for ewes suckling twins than those suckling single lambs.

Dietary supplementation of *Nigella Sativa* seeds significantly ($P<0.01$) increased milk fat percentage, protein percentage and milk energy. Also, fat percentage, protein percentage and milk energy gradually increased with the advance of lactation until the end of the lactation period.

Key words: Nigella Sativa seeds, Milk yield , Milk composition

INTRODUCTION

Improving productive and reproductive performance are the main goals of sheep producers. Increasing lambs production can be achieved through increasing the number of lambs per ewe and the number of lambing per year. There are large number of feed additives for inclusion in animal diet that can improve animal performance.

Generally, the use of chemical products may cause unfavorable side effects. The world health organization (WHO) encourages using medicinal herbs and plants to substitute or minimize the use of chemicals as a global trend to go back to natural nutrients. Using medicinal herbs and seeds as feed additives for ruminants seems to be a global trend **(Singh *et al*, 1993)**. Different studies showed a beneficial positive effect of using *Nigella Sativa* seeds as feed additives in diet of ruminants on most productive parameters. Several investigators illustrated that supplementation of *Nigella Sativa* seeds in the diet of ruminants had a beneficial effect on the efficiency of feed utilization as a result of improving digestion coefficient of different nutrients in the diet **(Abd El Gahni, 2003; Mohamed *et al*, 2003; Randa, 2007 and Zanouny *et al.*, 2012)**. In respect of buffaloes **(Sanad, 2010),** goat **(Mostafa, 1998 and Randa, 2007)** and sheep **(Sanad, 2000 and Safaa, 2005),** they reported that milk production was improved by adding *Nigella Sativa* seeds in the diet. There were wide variations in the literature about the suitable level of *Nigella Sativa* seeds, that can be supplemented to the diet of animals.

The objective of the present study was to evaluate effect of supplementation with *Nigella Sativa* seeds on milk yield and milk composition.

MATERIALS AND METHODS

Experimental design:

This experiment was carried out on lactating ewes. A total number of 20 Ossimi ewes of average body weight 46.40±1.40 kg were used. Ewes were randomly assigned to four equal experimental groups. First group G1 (control-single): ewes suckling single lambs and fed basal single diet. Second group G2 (NSS/single): ewes suckled single lambs and fed on basal single diet+100 mg *Nigella Sativa* seeds/kg/head/day. Third group G3 (control-twins): ewes suckled twin lambs and fed basal twin diet. Forth group G4 (NSS/twins): ewes suckled twin lambs and fed basal twin diet +100 mg *Nigella Sativa* seeds /kg/head/day. Ewes were offered their requirements of concentrate mixture (75%) and wheat straw (25%) as basal diet according to **APRI (1997)** in group feeding during the suckling period (12 weeks). The animals were housed in semi open pen.

Milk yield.

Daily milk yield was measured for each ewe starting from the fifth day post lambing until weaning at three months post lambing. Daily milk yield was measured using lambs sucking technique as reported by **Ashmawy (1980), Morsy (2002) and Zanouny (2006).** Daily amounts of milk consumed by each lamb in the morning and afternoon were calculated by the difference between weight recorded before and after suckling. Hand-milked yield in the morning and afternoon and the daily milk consumed by suckling lambs were added to estimate milk production of ewe per 24-hr. Milk production per 24-hr of each ewe was multiplied by 7 (days) to give milk production per week. This was carried out at every week from lambing until 12 weeks post-lambing, when milking was terminated.

Milk composition.

Milk samples were taken weekly throughout the suckling period from all experimental ewes. The samples were taken in the morning and afternoon and then both samples were mixed together for chemical analysis. Milk composition was determined by using 50 ml milk sample. Milk samples were analyzed for fat% and protein% using Milk analyzer (Scope Electric :MODEL: Julie C8, , made in Europe)**.** Milk energy values were calculated using the following equation: Calorific value (MJ/kg) = 1.94+0.43× fat% **(Economides, 1986).**

Statistical analysis.

The results were statistically analyzed using the General Linear Model **(SAS, 1998)** for complete randomized design. Productive, reproductive and blood parameters were performed by methods of analysis of variance. All statements of significant difference are based on the 0.05 or 0.01 level probability. Significant differences among treatments means were analyzed using **Duncan (1955)**. The following model was used

$Y_{ij}k = \mu + Ti + M_j + (TM)_{ij} + e_{ij}k$

$Y_{ij}k$ = The trait of study.

μ = The overall mean.

Ti = The treatment effect, where:

i = 1, -----3 for lambs traits;

i = 1, -----4 for milk traits

M_j = The time (period) effect. Where:

J =1, -----5 for lambs traits;

J = 1, -----12----- for milk traits

$e_{ij}k$ = The random error.

RESULTS AND DISCUSSIONS

A-Milk yield:

Effect of supplementation with *Nigella Sativa* seeds on the average total milk yield of experimental Ossimi ewes during lactation periods are presented in Table (1). The results revealed that feeding *Nigella Sativa* seeds (G2 and G4) improved milk yield of lactating ewes suckling single or twin lambs compared to G1 and G3 groups. The present results are in agreement with those reported by **Mostafa (1998)** and **El-Saadany *et al.*, (2003)** working on different lactating animals.

Data of daily milk yield during the lactation periods (12 weeks) are presented in Table (2). Average daily milk yield of different treatments, during sulking period, were 508, 636, 649 and 719.29 gm/kg for G1, G2 ,G3 and G4 , respectively **.** There were insignificant increase in average milk yield at the first and

Table (1): Average total milk yield of Ossimi ewes as affected by *Nigella Sativa* seeds supplementation.

GROUPS	NO	TOTAL MILK YIELD (KG) MEANS ± SE
G1	5	42.66±1.5
G2	5	53.43±1.5
G3	5	54.55±1.5
G4	5	60.38±1.5

G1 Control/single = Basal diet 75% concentrate mixture+25%wheat straw (Single).
G2 = Basal diet + 100 mg/kg/body weight/daily *Nigella Sativa* seeds (Single).
G3 Control/twinning = Basal diet without *Nigella Sativa* seeds (Twinning).
G4 = Basal diet+100 mg/kg/body weight/daily *Nigella Sativa* seeds (Twinning).

second weeks of the experimental period, but from the third to the twelves week of the experimental period the differences among groups were highly significant (P<0.01). The present result is in agreement with several authors. **Mostafa (1998)** found that goats fed *Nigella Sativa* seeds at a level similar to the present experiment increased milk yield by 46%. In addition, **Saffa (2005)** studied the effect of *Nigella Sativa* seeds supplementation on dairy ewes and found that using a dose of 5 g daily/animal led to significant increase (P<0.05) in daily milk yield. **El-Saadany *et al.* (2008)** reported similar results on lactating Zaraibi goats as using 100 mg daily *Nigella Sativa* seeds /kg body weight increased milk production. **Sanad (2010)** found that dietary *Nigella Sativa* seeds significantly (P<0.05) increased buffaloes milk yield.

Increasing milk yield with *Nigella Sativa* seeds supplementation in the rations might attributed to:

1- *Nigella Sativa* seeds significantly improved digestibility coefficients of DM, OM, CP, CF and NFE and consequently nutritive values as TDN, ME and DCP compared to control ration **(Zanouny *et al.*, 2012)**. Also, it might stimulate the digestive enzyme and make the feeds more available for utilization, either positively by affecting the population of microflora or through slowing feed passage into digestive tract, which resulted in better utilization. **(Mostafa, 1998; Mohamed *et al.*, 2003** and **Randa, 2007).**

2- *Nigella Sativa* seeds stimulate thyroid hormones secretion (**Zanouny 2011**). The increase of thyroid hormones resulted in increased feed intake (**Vander Tuig*et al.*, 1979**). Thus, the great energy intake enhanced and activated metabolic rate for milk production. Thus, the increase of milk production in the present study may be due to the positive effect of *Nigella Sativa* seeds supplementation on secretion of thyroid hormones.

3- Nigella Sativa seeds stimulate prolactin hormone secretion. **Abo El-Makarim *et al.*, (1999)** found that, oral administration of 100 mg *Nigella Sativa* oil in the form of soft gelatin capsules before breakfast for 24 days produced significant increase in serum level of prolactin. Prolactin play important role in milk production.

Regardless of treatments effect, the results in Table (2) showed that weekly milk production recorded different amounts throughout the lactation period. The results revealed that ewes reached maximum yield production at the fourth week of lactation and decreased thereafter. The present results are in agreement with **Sanad (2000)** in Saidi ewes and **Randa (2007)** in Zaraibi goats**.** They reported that the peak of milk production was recorded at fourth week, then it gradually decreased till the end of lactation. Meanwhile, **Hayder (2004)** working on Ossimi crossbreed, **Hamdon (2005)** on Chios and Farafra sheep, **El-Medany (2005)** on Rahmany sheep and **Kassab (2007)** on Sohagi sheep all indicated that the highest milk yield during lactation period was recorded at the second week of suckling compared with other suckling weeks.

The effect of type of birth regardless of treatments on total milk yield is shown in Table (2). The results indicated that milk yield was significantly (P<0.01) higher for ewes suckling twins compared to those suckling single in control groups. Also, ewes suckling twins in groups G3 and G4 recorded significantly (P<0.01) higher milk production compared to those suckling single. The increase of milk

Table (2): Effect of supplementation with *Nigella Sativa* seeds on daily milk yield (gm) of Ossimi ewes during experimental period .

Items	Treatments (T):					
Lactation periods (P):	**G1** Control single LSM±SE	**G2** NSS single LSM±SE	**G3** Control twinning LSM±SE	**G4** NSS twinning LSM±SE	Sig. Among treatment:	Average
Week1	501±26	553±26	531±26	601±26	NS	546
Week2	584±27	636±27	644±27	667±27	NS	632
Week3	755±34^{b}	893±34^{a}	881±34 ^{a}	955±34^{a}	**	871
Week4	760±50^{c}	934±50^{b}	1009±50^{ab}	1102±50^{a}	**	951
Week5	718.20^{c}	901.00^{b}	964.00^{ab}	1070.40^{a}	**	913
Week6	661±45^{c}	824±45^{b}	928±45^{ab}	975±45^{a}	**	847
Week7	542±39^{c}	764±39^{b}	752±39^{b}	904±39^{a}	**	740
Week8	496±27^{c}	684±27^{ab}	630±27^{b}	764±27^{a}	**	643
Week9	474±36^{c}	547±36 ^{b}	572±36 ^{b}	612±36^{a}	**	551
Week10	328±24 ^{b}	390±24$^{a\ b}$	421±24^{a}	452±24^{a}	**	397
Week11	172±33^{b}	296±33^{a}	262±33^{ab}	326±33^{a}	**	264
Week12	104±24^{b}	212±24^{a}	199±24^{a}	199±24^{a}	**	178
Sig Among Periods:	**	**	**	**	Sig. of T×P **	
Average	508c	636b	649b	719a		

G1 Control-single = Basal single diet 75% concentrate mixture+25%wheat straw.
G2 = Basal single diet + 100 mg/kg/body weight/daily *Nigella Sativa* seeds.
G3 Control-twinning = Basal twin diet without *Nigella Sativa* seeds.
G4 = Basal twin diet+100 mg/kg/body weight/daily *Nigella Sativa* seeds.
a-c, Means in the same row under the same period followed by different superscript are significantly different. NS= Not significant** (P<0.01)=Highly significant.

yield for ewes suckling twins may be due to the fact that ewes rearing multiple fetuses or with higher weight of single fetuses have higher placental weight and higher serum progesterone and placental lactogen hormones during late pregnancy. These hormones stimulate greater development of the lobular alveolar structure during pregnancy and consequently greater milk production during lactation. The present results are in agreement with **Aboul-Naga *et al*. (1980)** and **Hamdon (2005)** who reported that the differences in milk production due to number of suckling lambs were highly significant for Rahmani ewes.

Data in Table (2) illustrated that the interaction between treatments and periods on daily milk yield was highly significant (P<0.01). This due to those differences in daily milk yield occurred between groups and also within groups.

B-Milk composition:

Data in Tables (3, 4 and 5) show the effect of *Nigella Sativa* seeds supplementation on milk fat, milk protein and milk energy of lactating Ossimi ewes during the experimental periods.

Generally, dietary *Nigella Sativa* seeds supplementation significantly (P<0.01) increased milk fat percentage (Table 3). The average of fat percentage for the different treatments (regardless of lactation periods) were 5.65, 6.06, 5.43 and 5.92 % for (G1) control-single, (G2), NSS-single, (G3) control-twinning and (G4) NSS-twinning, respectively. The present results are in-agreement with those reported by **Safaa (2005)**that using *Nigella Sativa* seeds at a dose of 5 g daily/animal led to significant (P<0.05) increase in milk fat concentration. In addition, **Mostafa (1998)** in Zaraibi goats and **Mohamed *et al.*, (2003)** in sheep**,** found that *Nigella Sativa* seeds supplementation at rate 100 mg/kg body weight

increased milk fat production. Also, **Sanad (2010)** in lactating buffalo found that using 50 or 100 mg/kg body weight significantly (P<0.01) increased milk fat production.

On the other hand **El-Saadany *et al.*, (2008)** found that the percentage of milk fat was not significantly increase by *Nigella Sativa* seeds supplementation to the ration of lactating dose.

Data in Table (3) showed that fat percentage gradually increased with the advancing of lactation until its end. The present results indicated that *Nigella Sativa* seeds supplementation in G2 (suckling single) or in G4 (suckling twinning) significantly increased fat percentage during lactation period. These results are in agreement with **Mousa *et al.*, (1997)** and **Kassab (2007).**

Data in Table (4) indicated that the mean values of protein percentages were not significantly different at the beginning of the experiment, but from the second week to the end of lactation the differences were significant (P<0.01). The average of protein percentages for different treatments during lactation period were 5.38, 5.59, 5.21 and 5.45 % for G1, G2, G3 and G4, respectively. The present result are in agreement with that reported by **Safaa (2005)** where using *Nigella Sativa* seeds at a dose of 5 g daily/animal led to significant (P<0.05) increase in milk protein concentration. Also, **Sanad (2010)** in lactating buffaloes found that supplementation with 50 or 100 mg/ Kg Body weight/day significantly (P<0.01) increase milk protein concentration. On the other hand, **El-Saadany *et al.*, (2008)** found that the percentage of protein in milk was not significantly affected by *Nigella Sativa* seeds supplementation to the ration of lactating dose.

Table(3): Effect of *Nigella Sativa* seeds supplementation on milk milk fat percentage (%) of Ossimi ewes during experimental period.

Items	Treatments (T) :					
Lactation Periods (P):	G1 Control single LSM±SE	G2 NSS single LSM±SE	G3 Control twinning LSM±SE	G4 NSS twinning LSM±SE	Sig. Among Treatment:	Average
Week1	4.34±0.01^{b}	4.48±0.01^{a}	4.11±0.01^{c}	4.35±0.01^{b}	**	4.32
Week2	4.60±0.02^{b}	4.81±0.02^{a}	4.49±0.02^{c}	4.66±0.02^{b}	**	4.64
Week3	4.82±0.03^{c}	5.22±0.03^{a}	4.64±0.03^{d}	5.05±0.03^{b}	**	4.93
Week4	5.07±0.02^{c}	5.35±0.02^{a}	4.73±0.02^{d}	5.20±0.02^{b}	**	5.09
Week5	5.25±0.03^{b}	5.51±0.03^{a}	4.94±0.03^{c}	5.42±0.03^{a}	**	5.28
Week6	5.50±0.02^{c}	5.80±0.02^{a}	5.32±0.02^{d}	5.63±0.02^{b}	**	5.56
Week7	6.09±0.03^{c}	6.42±0.03^{a}	5.81±0.03^{d}	6.22±0.03^{b}	**	6.13
Week8	6.20±0.02^{c}	6.75±0.02^{a}	6.11±0.02^{d}	6.62±0.02^{b}	**	6.42
Week9	6.42±0.03^{c}	6.98±0.03^{a}	6.29±0.03^{d}	6.82±0.03^{b}	**	6.61
Week10	6.63±0.02^{c}	7.21±0.02^{a}	6.39±0.02^{d}	7.03±0.02^{b}	**	6.81
Week11	6.58±0.02^{c}	7.16±0.02^{a}	6.22±0.02^{d}	7.07±0.02^{b}	**	6.76
Week12	6.39±0.02^{c}	7.08±0.02^{a}	6.14±0.02^{d}	6.92±0.02^{b}	**	6.63
Sig Among Periods:	**	**	**	**	Sig. of T×P NS	
Average	**5.65c**	**6.06a**	**5.43d**	**5.92b**		

G1 Control/single = Basal diet 75% concentrate mixture+25%wheat straw .
G2 = Basal diet + 100 mg/kg/body weight/daily *Nigella Sativa* seeds (Single).
G3 Control/twinning = Basal diet without *Nigella Sativa* seeds.
G4 = Basal diet+100 mg/kg/body weight/daily *Nigella Sativa* seeds (Twinning).
a-d, Means in the same row under the same period followed by different superscript are significantly different. NS= Not significant** (P<0.01)=Highly significant.

Table(4): Effect of *Nigella Sativa* seeds supplementation on milk protein percentage (%) in Ossimi ewes during experimental period.

Items	Treatments (T) :					
Lactation Periods (P):	G1 Control single LSM±SE	G2 NSS single LSM±SE	G3 Control twinning LSM±SE	G4 NSS twinning LSM±SE	Sig. Among Treatment:	Average
Week1	4.26±0.02	4.25±0.02	4.06±0.02	4.09±0.02	NS	4.16
Week2	4.38±0.03^{c}	4.65±0.03^{a}	4.21±0.03^{d}	4.51±0.03^{b}	**	4.43
Week3	4.41±0.01^{b}	4.68±0.01^{a}	4.24±0.01^{c}	4.55±0.01^{a}	**	4.47
Week4	4.71±0.02^{b}	4.90±0.02^{a}	4.60±0.02^{b}	4.83±0.02^{a}	**	4.76
Week5	4.82±0.1^{b}	5.00±0.1^{a}	4.63±0.1^{c}	4.88±0.1^{b}	**	4.83
Week6	5.04±0.02^{b}	5.33±0.02^{a}	4.97±0.02^{b}	5.25±0.02^{a}	**	5.14
Week7	5.23±0.02^{c}	5.59±0.02^{a}	5.13±0.02^{c}	5.47±0.02^{b}	**	5.35
Week8	5.47±0.02^{c}	5.83±0.02^{a}	5.37±0.02^{c}	5.64±0.02^{b}	**	5.57
Week9	5.86±0.04^{b}	6.09±0.04^{a}	5.53±0.04^{c}	5.95±0.04^{b}	**	5.85
Week10	6.61±0.03^{b}	6.75±0.03^{a}	6.50±0.03^{c}	6.55±0.03^{c}	**	6.60
Week11	6.81±0.02^{b}	6.94±0.02^{a}	6.62±0.02^{c}	6.84±0.02^{b}	**	6.80
Week12	6.99±0.02^{a}	7.10±0.02^{a}	6.69±0.02^{b}	6.94±0.02^{a}	**	6.93
Sig Among Periods:	**	**	**	**	Sig. of T×P NS	
Average	5.38c	5.59a	5.21d	5.45b		

G1 Control/single = Basal diet 75% concentrate mixture+25%wheat straw (Single).
G2 = Basal diet + 100 mg/kg/body weight/daily *Nigella Sativa* seeds (Single).
G3 Control/twinning = Basal diet without *Nigella Sativa* seeds (Twining).
G4 = Basal diet+100 mg/kg/body weight/daily *Nigella Sativa* seeds (Twinning).
a-d, Means in the same row under the same period followed by different superscript are significantly different. NS= Not significant** (P<0.01)=Highly significant.

Generally, the present results indicated that the average of fat and protein concentrations during lactation period, regardless of treatment, recorded lower values in twinning suckling compared to single suckling. Decreasing of fat and protein concentrations in milk of twinning suckling compared to single suckling may be due to the negative relationship between the milk yield, (which it was higher in twinning suckling compared to single suckling) and the concentrations of fat and protein in milk production.

The values of milk energy content recorded highly significant (P<0.01) differences during lactation periods Table (5). The average of milk energy (MJ/Kg) for different groups (regardless to lactation period) were 4.37, 4.54, 4.27 and 4.48 MJ/Kg for G1, G2, G3 and G4, respectively. The improvement of milk energy in the treated groups due to the increase in milk fat percentage (Table, 3).

The estimated values of milk energy in the present study, according to the equation of **Economides, 1986,** indicated that energy content of milk gradually increased with advance of lactation. Low values of energy content were recorded at first week of lactation, while the highest value were recorded at the end of lactation for the different groups. Increasing milk energy at the end of lactation period due to increasing fat concentration in milk by advance of lactation. Similar results were obtained by **Hamdon (2005) and Kassab (2007) who** found that energy content was increased gradually with increasing fat percentage as a result of advancing lactation period.

From the present results it can be concluded that supplementation of *Negilla Stavia* seeds at a level of 100 mg daily/head could led to positive effect on milk yield and milk composition of ewes either suckling single or twins lambs.

Table (5): Effect of *Nigella Sativa* seeds supplementation on milk energy (MJ/Kg) of Ossimi ewes during experimental period.

Items	Treatments (T) :					
Lactation Periods (P):	G1 Control single LSM±SE	G2 NSS single LSM±SE	G3 Control twinning LSM±SE	G4 NSS twinning LSM±SE	Sig. Among Treatment:	Average
Week1	3.80±0.008^{b}	3.87±0.008^{a}	3.70±0.008^{c}	3.81±0.008^{b}	**	3.79
Week2	3.92±0.008^{b}	4.01±0.008^{a}	3.87±0.008^{c}	3.94±0.008^{b}	**	3.93
Week3	4.01±0.013^{c}	4.18±0.013^{a}	3.93±0.013^{d}	4.11±0.013^{b}	**	4.06
Week4	4.12±0.012^{c}	4.24±0.012^{a}	3.97±0.012^{d}	4.17±0.012^{b}	**	4.12
Week5	4.20±0.013^{b}	4.31±0.013^{a}	4.06±0.013^{c}	4.27±0.013^{a}	**	4.21
Week6	4.30±0.012^{c}	4.43±0.012^{a}	4.23±0.012^{d}	4.36±0.012^{b}	**	4.33
Week7	4.56±0.014^{c}	4.70±0.014^{a}	4.44±0.014^{d}	4.61±0.014^{b}	**	4.57
Week8	4.61±0.012^{c}	4.84±0.012^{a}	4.56±0.012^{d}	4.79±0.012^{b}	**	4.70
Week9	4.70±0.012^{c}	4.94±0.012^{a}	4.64±0.012^{d}	4.87±0.012^{b}	**	4.78
Week10	4.79±0.013^{c}	5.04±0.013^{a}	4.68±0.013^{d}	4.96±0.013^{b}	**	4.86
Week11	4.77±0.009^{c}	5.01±0.009^{a}	4.61±0.009^{d}	4.98±0.009^{b}	**	4.84
Week12	4.68±0.012^{c}	4.98±0.012^{a}	4.58±0.012^{d}	4.91±0.012^{b}	**	4.79
Sig Among Periods:	**	**	**	**	Sig. of T×P NS	
Average	**4.37c**	**4.54a**	**4.27d**	**4.48b**		

G1 Control/single = Basal diet 75% concentrate mixture+25%wheat straw (Single).
G2 = Basal diet + 100 mg/kg/body weight/daily *Nigella Sativa* seeds (Single).
G3 Control/twinning = Basal diet without *Nigella Sativa* seeds (Twining).
G4 = Basal diet+100 mg/kg/body weight/daily *Nigella Sativa* seeds (Twinning).
a-d, Means in the same row under the same period followed by different superscript are significantly different. NS= Not significant** (P<0.01)=Highly significant

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