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Productive and Reproductive Performances of New Zealand White Doe Rabbits as Affected by *Nigella Sativa* Oil Supplementation under Hot and Mild Conditions in Egypt

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ABSTRACT

Aim of the current study was to evaluate the effect of *nigella sativa* oil (NSO) supplementation under hot and mild Egyptian conditions, on physiological thermoregulatory response, blood components, gestation length, feed and water consumption during gestation and suckling period, litter size and weight at each of birth, 21 d and 30 d (weaning) of age and mortality rate of pups from birth to 30 days of age. Thirty New Zealand White (NZW) adult doe rabbits (15 in hot season and 15 in mild season) aged 6 months and weighed 2935.4 g were used. Results showed that feed intake, water consumption, rectal temperature, respiration rate, litter size and weight at birth, 21 d, weaning, pre-weaning mortality rate were affected significantly ($P<0.01$), while the gestation period, serum total proteins , albumin, globulin, urea and creatinine were not affected significantly by season. Supplementation the doe rabbits diet with NSO (30 g oil / kg diet) significantly improved litter size and weight at birth ($P<0.01$ and $P<0.05$), at 21 d and at weaning ($P<0.05$), while pre-weaning mortality rate, gestation period, feed intake, water intake, rectal temperature, respiration rate, serum total protein, albumin, globulin, urea and creatinine concentrations in blood plasma of does were not affected.It could be concluded that, feeding doe rabbits during the hot season with dietary supplementation of 30 g *Nigella sativa* oil/kg diet is very effective on productive and reproductive performances of rabbits.

Keywords: Rabbit, Nigella sativa, reproduction, blood biochemicals.

INTRODUCTION

There are many factors affecting the economic intensive rabbit production such as environmental and nutritional conditions. The environmental condition plays important elements in production cycle. The domestic rabbits are homoeothermic mammals. It has a high metabolic rate under developed sweat glands and slow heat loss. The thermo neutral zone of growing rabbits (6-12 weeks of age) is 15-18 ^{o}C (Marai and Habeeb, 1994; Habeeb et al., 1998). The high temperature in hot climate conditions affects negatively growth reproductive performance and feed intake (Marai *et al*., 1999, 2000, 2006; Abdel-Monem, 2012).

Nigella sativa (NS) oil (NSO) and meal (NSM) are becoming commonly used for many purposes, as feed additives and for medical purposes. The NS is well known for its antibacterial, antifungal, antihelminthic, antineoplastic bronchodilator immune enhancing and antispasmodic effects (Rathee *et al.,* 1982; Mahdi, 1993; Khodary *et al*., 1996).

NSO is considered good source of each of fat and the major minerals such as Ca, P, K, Mg and Na (Abdel-Aal and Attia, 1993). The unsaturated fatty acids, oleic and linoleic, as two major fatty acids are extracted from the black seed oil (Abdel-Aal and Attia, 1993).

The present study was conducted to investigate the effects of *Nigella sativa* oil supplementation on reproductive performance of adult NZW doe rabbits in hot and mild conditions in Egypt.

MATERIALS AND METHODS

This study was carried out at a Rabbit Farm, Faculty of Agriculture, Zagazig University during the period from July, 2015 to May, 2016.

Factorial design was conducted to study the effects of season (hot and mild), with or without NSO supplementation on productive and reproductive performances of New Zealand White doe rabbits during hot season from June to September, 2015 and mild climate period from October, 2015 to May, 2016.

Total number of 30 NZW does aged 6 months and weighed 2935.4 g, 15 does in hot season and 15 does in mild season were used in this study. During each season**,** does were randomly divided into three groups (5 does in each group).

Average of ambient temperature and relative humidity at midday inside building of rabbits during the experimental period were 20.31^{o}C and 73.6% in the mild season and 28.13^{o}C and 79.5% in the hot season, respectively.

Doe rabbits in all groups were fed a basal diet, but differed in dietary supplementation of NSO. Does in the 1st group (G1) were fed the basal diet without NSO and served as control group, while those in the 2nd (G2) and 3rd (G3) groups were fed the basal diet supplemented with 15 and 30 g NSO/kg diet, respectively.

Rabbits were fed *ad libitum* basal diet consisting of 28% alfalfa hay, 18% barley, 18% soybean meal (44% CP), 25% wheat bran, 6% yellow corn, 3% molasses, 1.1% limestone, 0.3% sodium chloride, 0.6% vitamin and mineral premix. The basal diet contained 18.18% crude protein, 13.43% crude fiber, 2.29% ether extract and 2656.00 kcal digestible energy/kg diet. All rabbits were kept under the same managerial, hygienic and environmental conditions. Does were individually reared in wire cages and their offsprings were collectively raised in cages, in the same batteries, in a well-ventilated building. Fresh water was automatically available all the time by stainless steel nipples fixed in each cages. All doe cages were equipped with feeders

and nipples. During the experiment the total artificial light was about 16 hours/day. At mating, rabbits were individually transferred to the buck cages and returned to their own hatches copulation. Each mated doe was palpated 10 days post-mating to be rebred until pregnancy was established. Within 12 hours after kindling, litter kits were recorded and weaned at 30 days of age.

The traits studied for does included gestation length, feed and water consumption during gestation and suckling period, litter size and weight at each of birth, 21 and 30 days (weaning) of age, litter weight gain and mortality rate of pups from birth to 30 days of age.

Y = litter weight gain (kg) during the period from 0 to 21 days/0.56.

Where: 0.56 was standard figure given by Cowie (1969) for the NZW strain depending on the linear relationship between the litter weight gain (kg) and doe milk consumed.

Rectal temperature (RT) and respiration rate (RR) were measured in does once every two weeks at 1-2 p.m. RR was recorded by a hand counter, which counts the frequency of the flank movement per minute. Internal body temperature (RT) was taken by medicine thermometer inserted into the rectum for 2 minutes at depth of one cm.

Blood samples were monthly collected from the marginal ear vein of does after shaving and cleaning with alcohol in less than 2 minutes into dry clean centrifuge tubes containing some drops of heparin. Blood plasma was separated by centrifugation at 3000 rpm for 20 minutes and kept in a deep freezer at -20^{o}C until the time of analysis. Total proteins, albumin, creatinine and urea concentrations in plasma were estimated using commercial kits (Bio Merieux, France) according to the procedure outlined by the manufacturer. Globulin concentration was obtained by subtracting the values of albumin from the corresponding values of total proteins.

In order to study the combined effects of temperature and humidity, temperature humidity index (THI) was calculated according to the formula of Marai *et al*. (2001) as follows:

$$THI = db\ C^{o} - \{(0.31 - 0.31RH)(db\ C^{o} - 14)\}$$

Where: db ^{o}C = dry bulb temperature in Celsius and RH = relative humidity /100. The estimated values of THI were classified as follows: <22.2 = absence of heat stress, 22.2-<23.2= moderate heat stress, 23.3-<25.5 = severe heat stress and ≥25.5 = very severe heat stress. Data obtained from all does and their litters (offsprings) during hot and mild seasons were subjected to the statistical analysis using factorial design (2 seasons x 3 NSO levels) according to Snedecor and Cochran (1982) by the following model: $X_{ijkl} = \mu + P_i + N_k + PN_{ik} + E_{ikl}$.

where: μ = general mean, P_i = fixed effect of season (1, 2), N_k = fixed effect of NSO level (1,…..3), PN_{ik} = the interaction between season and NSO, and E_{ikl} = random error. The significant differences among means were tested by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Temperature–humidity index (THI):

Estimated THI values were 18.9 and 24.7 at mild and hot seasons, respectively. These results indicated absence of heat stress during the mild season (less than 22.2) and exposure to severe heat stress during the hot season (23.3-25.5). Marai *et al*. (1996) found that NZW does were under severe heat stress during the summer season in Egypt.

Feed intake and water consumption:

Feed intake which includes feed consumption significantly (P<0.01) decreased in hot compared with mild season (Table 1). This means that feed intake of does was affected by season. This may be explained on the basis that rabbits required more dietary energy during cold conditions, which was covered by increasing feed consumption. Conversely, rabbits which exposed to sever heat stress increases heat production, so the voluntary feed intake decreased. These results agreed with this obtained by Abdel-Monem (2000), Ayyat and Abdel-Monem (2002), and Marai *et al*. (2000, 2002, 2006), who stated that feed intake was decreased significantly in doe NZW rabbits under heat stress condition. Generally, feed intake covered rabbit requirements according to NRC (1977). The present results indicated that both NSO levels supplementation significantly (P<0.05) increased feed intake of does in G2 and G3 as compared to control group (Table 1).

Average water consumption showed an opposite trend to that of feed intake during both seasons. Water consumption was significantly (P<0.01) higher in hot than in mild season (Table 1). These results agreed with Abdel-Monem (2000), who found that water intake was increased significantly (p<0.01) in doe NZW rabbits under heat stress condition. However, both NSO levels supplementation insignificantly increased water consumption as compared to control shown in table 1.

The effect of interaction between season and NSO supplementation was not significant on feed intake and water consumption ((Table 1).

Table 1. Feed intake and water consumption of New Zealand White doe rabbits as affected by season of the year, dietary supplementation with *Nigella sativa* oil and their interaction.

Item	Feed intake (g/day)	Water consumption (ml/day)
Season (s):		
Hot	200.4±3.1 [b]	500.3±11.7 [a]
Mild	296.9±1.7 [a]	287.5±6.4 [b]
Significance	**	**
Nigella sativa oil level :		
Without Nigella sativa oil	250.4±6.9 [b]	389.1±15.8
15 g oil / kg diet	277.9±8.1 [a]	400.8±18.0
30 g oil / kg diet	291.7±8.9 [a]	415.3±13.5
Significance	*	NS
Interaction:S x N	NS	NS

Means bearing different letters in the same column within each classification differ significantly at P<0.05 (*) and P<0.01 (**). NS: Not significant.

Physiological response:

Physiological response of doe rabbits was in terms of rectal temperature (RT) and respiration rate (RR). Hot climatic (THI= 24.7) showed significantly ($p<0.01$) higher values of RT and RR than mild conditions (THI = 18.9). These results may be due to exposure of rabbits to heat stress. These results agreed with those obtained by Ayyat and Abdel-Monem (2002), Marai *et al*. (2000, 2002, 2006) and Abdel-Monem (2015), who found that RT and RR of rabbits were significantly ($p<0.05$) higher in summer than in winter under Egyptian conditions. Both RT and RR were not affected significantly by NSO supplementation. These results agreed with Marai *et al.* (2009), Shewita and Taha (2011) and Rizwana *et al*. (2013), who found that RT and RR were not affected by *Nigella sativa* supplementation.

It is of interest to note that the effect of interaction between season and NSO supplementation was not significant on RT and RR (Table 2).

Blood constituents:

Results in Table 3 showed that all blood constituents studied including concentration of total proteins, albumin, globulin, urea and creatinines were not affected significantly by season, NSO supplementation and their interaction. The present results agreed with those reported by Ayyat and Abdel-Monem (2002) and Marai *et al*. (2006), Shewita and Taha (2011), Rizwana *et al*. (2013) and Abdel-Monem (2012)

Table 2. Rectal temperature and respiration rate of New Zealand White doe rabbits as affected by season of the year, dietary supplementation with *Nigella sativa* oil and their interaction.

Item	Rectal temperature (°C)	Respiration rate (Resp. /minute)
Season (S):		
Hot	39.7±0.08 [a]	136.3±1.12 [a]
Mild	39.0±0.03 [b]	101.9±0.96 [b]
Significance	**	**
Nigella sativa oil level :		
Without Nigella sativa oil	39.36±0.09	116.53±1.09
15 g oil / kg diet	39.39±0.06	114.68±1.11
30 g oil / kg diet	39.41±0.09	118.21±1.17
Significance	NS	NS
Interaction: S x N	NS	NS

Means bearing different letters in the same column within each classification differ significantly at P<0.01 (**). NS: Not significant.

Table 3. Concentration of some biochemicals in blood plasma of New Zealand White doe rabbits as affected by season of the year, dietary supplementation with *Nigella sativa* oil and their interaction.

Item	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Season (S)					
Hot	6. 3±0.4	3.4±0.12	2.9±0.46	15. 6±0.09	1.10±0.07
Mild	6.5 ±0.3	3.9±0.15	2.6±0.33	15.9±0.07	1.04±0.02
Significance	NS	NS	NS	NS	NS
Nigella sativa oil level :					
Without Nigella sativa oil	6. 7±0.4	3.5±0.20	3.2±0.29	16.32±0.05	1.22±0.16
15 g oil / kg diet	6. 4±0.5	3.1±0.24	3.3±0.31	16.03±0.08	1.18±0.30
30 g oil / kg diet	6.8±0.3	3.4±0.19	3.4±0.39	15.11±0.08	1.20±0.40
Significance	NS	NS	NS	NS	NS
Interactions					
S x N	NS	NS	NS	NS	NS

NS = Not significant.

Productive and reproductive performance

Data presented in Table (4) showed insignificant effect due to season on gestation period length. However, litter size at birth, 21 days and weaning was significantly ($P<0.01$) higher for kids born in mild than in hot season. In addition, litter weight (Table 5) was significantly ($P<0.01$) higher for kits born in mild than in hot season.

Such difference may be a reflection of differences in seasonal climatic conditions. Also, the drop in litter size and weight in hot conditions may be attributed to the effect on mothering ability and milk production as a result of hyperthermia.

Similar trend was obtained by Shafie *et al.* (1984), Askar (1989), , Hassan *et al*. (1994), Bassuny (1999), Abdel-Monem (2000), Ayyat and Abdel-Monem (2002) and Marai *et al*. (2000, 2002, 2006). In respect with gestation period, Abd El- Moty *et al*. (1991), Shewita and Taha (2011) and Rizwana *et al*. (2013) reported similar results.

Data presented in Table (4) showed that pre-weaning mortality rate was significantly ($P<0.05$) higher in hot than in mild season. This result may be due to the direct effect of heat stress on the sensitive young offspring in addition to reduction of dam milk production as a result of heat exposure due to the general depression of metabolic activity in such conditions (Shafie *et al*., 1984). The present results agreed with those obtained by Abdel-Monem (2000), Ayyat and Abdel-Monem (2002) and Marai *et al*. (2000, 2002, 2006), who found that pre-weaning mortality rate was significantly ($P<0.05$) higher in summer than in winter under Egyptian conditions.

Data presented in Tables 4 and 5 showed that addition of NSO to diets of NZW rabbits significantly improved liter size ($P<0.05$) and litter weight ($P<0.01$) comparatively with control, while gestation period was

not affected by NSO supplementation. Generally, the supplementation of NSO at a level of 30 g /kg diet seemed to be an optimum level owing to the best performance of doe rabbits compared with 15 g/kg diet. These results coincide with those of Nasr and Attia (1998), Shewita and Taha (2011) and Rizwana *et al*. (2013). On the other side, the interaction effect between season and NSO supplementation was not significant on litter size and litter weight (Tables 4 and 5).

Table 4. Gestation period, litter size and pre-weaning mortality rate of New Zealand White rabbits as affected by season of the year and dietary supplementation with *Nigella sativa* oil and their interactions.

Item	Gestation Period (day)	Litter size Birth	Litter size 21 days	Litter size Weaning	Pre-weaning mortality (As number)
Season (S):					
Hot	31.0±03	3.8±0.4 [b]	2.3±0.1 [b]	1.5±0.4 [b]	2.3±0.08 [a]
Mild	31.2±0.1	5.9± 0.5 [a]	5.0±0.2 [a]	4.7±0.3 [a]	1.2±0.05 [b]
Significance	NS	**	***	***	*
Nigella sativa oil level:					
Without oil					
15 g oil / kg diet	31.1±0.2	4.8±0.6 [b]	2.9±0.3 [b]	2.0±0.4 [b]	2.80±0.11
30 g oil / kg diet	30.9±03	6.2±0.8 [a]	4.8±0.6 [a]	4.4±0.8 [a]	1.80±0.14
Significance	31.4±04	6.5±0.9 [a]	5.3±0.4 [a]	4.9±0.8 [a]	1.30±0.13
Interaction:	NS	**	**	**	NS
S x N	NS	NS	NS	NS	NS

Means bearing different letters in the same column within each classification differ significantly at P<0.05 (*), P<0.01 (**) and P<0.001 (***). NS: Not significant.

Table 5. Litter weight (g) of New Zealand White doe rabbits as affected by season of the year and dietary supplementation with *Nigella sativa* oil and their interaction.

Item	Litter weight (g) Birth	21 days	Weaning
Season (S):	44.1±1.0 [b]	239.2±6.9 [b]	475.7±18 [b]
Hot	50.8±0.9 [a]	318.5±7.8 [a]	669.0±16 [a]
Mild	**	***	***
Significance			
Nigella sativa oil level:	46.1±1.1 [b]	245.5± 6.3 [b]	511.7±13 [b]
Without oil	50.3±1.6 [a]	303.0±8.9 [a]	605.9±10 [a]
15 g oil / kg diet	58.4±1.4 [a]	319.3±9.3 [a]	620.2±17 [a]
30 g oil / kg diet	*	**	**
Significance			
Interaction:S x N	NS	NS	NS

Means bearing different letters in the same column within each classification differ significantly at P<0.01 (**) and P<0.001 (***). NS: Not significant.

CONCLUSION

It could be concluded that, Nigella sativa oil supplementation to diet of New Zealand White adult doe rabbits increased feed intake and improved litter size and weight from birth to weaning of rabbits kept under hot conditions in Egypt.

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دراسه تاثير اضافه زيت حبه البركه فى علائق اناث الارانب النيوزيلندى الابيض على الاداء الانتاجى والتناسلى تحت ظروف المناخ الحار والمعتدل فى مصر.

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تهدف هذه الدراسه لتقييم تاثير اضافه زيت حبه البركه لعلائق اناث الارانب النيوزيلندى الابيض على المقاييس الفسيولوجيه وبعض مكونات الدم وطول فترة الحمل وكميه الغذاء والماء المستهلك اثناء الحمل وفترة الرضاعه وكذلك حجم البطن والوزن عند الميلاد وعند عمر ٢١ و٣٠ يوم (الفطام) ومعدل النفوق لصغار الارانب من الميلاد حتى ٣٠ يوم من العمر تحت ظروف الموسم الحار والمعتدل فى مصر خلال العام ابتداء من يوليه ٢٠١٥ حتى مايو ٢٠١٦.تمت الدراسه فى مزرعه كليه الزراعه جامعه الزقازيق بمدينه الزقازيق على ٣٠ ام من اناث الارانب النيوزيلندى الابيض البالغه عند عمر٦ شهورعند متوسط وزن ٢٩٣٥.٤ جم (١٥ ام فى كل فترة من الفترات المعتدله والحارة) والتى قسمت فى كل فترة الى ٣ مجموعات (٥ امهات فى كل مجموعه) والتى غذيت على علائق مضاف اليها ١٥ و٣٠ جم من زيت حبه البركه لكل كيلو جرام من العليقه مقارنه بعليقه مجموعه المقارنه بدون زيت حبه البركه.وكانت اهم النتائج المتحصل عليها ما يلى :١- تاثير الموسم من السنه كان عالى المعنويه ($P<0.01$) على الامهات فقد ارتفعت بها كلا من حرارة المستقيم ومعدل التنفس و معدل الماء المستهلك و وكذلك نسبه النفوق للمواليد حتى الفطام بينما نقص معدل الغذاء المستهلك و حجم البطن والوزن عند الميلاد وعند ٢١ يوم وعند الفطام للمواليد فى الموسم الحار من السنه فى حين ان طول فترة الحمل ومكونات الدم مثل البروتين الكلى والالبيومين والجلوبيولين واليوريا والكرياتينين لم تتاثر معنويا .٢- العلائق المضاف اليها زيت حبه البركه (٣٠ جرام لكل كيلو جرام عليقه) كان لها تاثير عالى المعنويه ($P<0.01$ and 0.05) على اناث الارانب الامهات فقد تسببت فى ارتفاع معدل الغذاء المتناول وحجم البطن ووزن مواليدها عند الميلاد وعند ٢١ يوم وعند الفطام فى حين ان حرارة المستقيم ونسبه التنفس والبروتين الكلى والالبيومين والجلوبيولين واليوريا والكرياتينين ونسبه النفوق وطول فترة الحمل لم تتاثر معنويا . الخلاصه: تغذيه اناث الارانب النيوزيلندى الابيض على عليقه مضاف اليها زيت حبه البركه بمعدل ٣٠ جرام لكل كيلو جرام عليقه أدى الى تحسن معنوى للاداء التناسلى والانتاجى تحت ظروف المناخ الحار من السنه.