Physiological and biochemical impacts of dietary *Nigella sativa* levels and feeding system on does performance at first parity under Egyptian condition

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Abstract: One hundred and twenty New Zealand White (NZW) doe rabbits at first parity were used in the present study. The does were randomly divided into 12 treatment groups (10 does in each), in order to study the reproductive traits as affected by season of the year (mild and hot), feeding system (*ad libitum* and fed at night only) and *Nigella sativa* dietary supplementation (0, 0.5 and 1% seeds / kg diet) and their interaction, under Egyptian conditions. All groups were nearly similar in average initial body weights. The traits studied were some performance traits (feed intake, feed conversion and water intake), thermoregulation parameters (rectal temperature and respiration rate), some blood components (serum total proteins, albumin, globulin, urea and creatinine) and doe traits (gestation length, litter size, weight at birth, 21 days and weaning, milk yield and pre-weaning mortality). The results showed that temperature–humidity index (THI) values estimated were 18.9 and 24.7 at mild and hot periods, respectively, indicating absence of heat stress during the mild period (less than 22.2) and exposure to severe heat stress during the hot period (23.3-25.5 ^{0}C)..The hot period of the year affected adversely (*P*<0.01 or 0.05) feed intake, water intake, rectal temperature, respiratory rate, litter size and weight at birth, 21 days and weaning, pre-weaning mortality and milk yield, while the effects were not significant on feed conversion, serum total proteins, albumin, globulin, urea and creatinine and gestation period. Feeding only at night, improved significantly (*P*<0.01 or 0.05) feed intake, litter size and weight at birth, 21 days and weaning and milk yield than in *ad libitum* feeding system. Meanwhile, water consumption decreased (13.2 %) significantly (*P*<0.05) with *ad libitum* than with the restricted feeding. Feed conversion, rectal temperature, respiratory rate, serum total proteins, albumin, globulin, urea and creatinine, gestation period and pre-weaning mortality, were not significantly affected by feeding system. Dietary supplementation of the doe rabbits with *Nigella sativa* seeds (1% / kg diet) improved significantly feed intake (*P*<0.05), litter size and weight at birth (*P*<0.01 and 0.05), 21 days and at weaning (*P*<0.05), while the effects were not significant on feed conversion, water consumption, rectal temperature, respiratory rate, serum total protein, albumin, globulin, urea and creatinine, gestation period and pre-weaning mortality. Interaction effects of season of the year x feeding system were significant (*P*<0.05) on litter size at birth and 21 days and milk yield / doe. Feeding at night only showed the best values during the two periods (hot and mild), as well as, within the hot period. All the other interaction effects were not significant.

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Key words: feeding system, heat stress, Nigella sativa dietary supplementation, rabbit doe traits.

1. Introduction

Economic intensive rabbit production is affected by many factors, particularly environment and nutrition. However, under the sub-tropical conditions, the combined effect of such factors may be more substantial due to the negative effect of elevation of ambient temperature on appetite and accordingly on the feed intake that ends with slowing growth and impairment of reproduction in rabbits (Abdel–Monem 2001 and Marai *et al.,* 2002, 2006).

Such phenomenon may suggest feed rabbits at the mildest period of the day during the hot season of the year, under the sub-tropical conditions.

Nigella sativa seeds and their meal are becoming commonly used for many purposes as feed additives and for medical purposes. Nutritionally, *Nigella sativa* seeds are considered a good source of each of crude protein, crude fat, crude fiber and the major minerals such as Ca, P, K, Mg, Fe and Na **(Abdel–Aal & Attia 1993, Al-Bataina *et al.,* 2003; Cheikh-Rouhou *et al.,* 2007 and Sultan *et al.,* 2009).** The unsaturated fatty acids, and oleic and linoleic (as two major fatty acids) are extracted from the black seed oil **(Abdel – Aal & Attia, 1993; Al-Naqeeb *et al.,* 2009; Matthaus and Ozcan, 2011).** Medically, *Nigella sativa* is emerging as a miracle herb with a rich historical and religious background *Nigella sativa* has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties**. (Ahmed *et al.,* 2013).** Most of the therapeutic properties of this plant are due to the presence of thymoquinone which is a major active chemical component of the essential oil. **(Ahmed *et al.,* 2013).**

The present study was conducted to investigate the effects of season of the year (mild and hot), feeding system (*ad libitum* and feeding at night only), *Nigella sativa* seeds dietary supplementation (0, 0.5 and 1% seeds / kg diet) and their interaction on reproductive performance traits of NZW doe rabbits at first parity, under Egyptian sub-tropical conditions.

2. Materials and Methods

The study was carried out at the Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The practical work was conducted in a private farm in Zagazig city during two periods of the year: hot from May to September, 2013 and mild from October, 2013, to April, 2014.

The number of one hundred and twenty New Zealand White (NZW) doe rabbits at first parity were randomly divided into 12 treatment groups (10 does in each), in order to study the reproductive traits as affected by season of the year (mild and hot), feeding system (*ad libitum* and fed at night only), *Nigella sativa* dietary supplementation (0, 0.5 and 1% seeds / kg diet) and their interaction, under Egyptian conditions. All groups were nearly similar in average initial body weights. Averages of ambient temperature and relative humidity values at mid-day inside the rabbitry building during the experimental period were 20.0^{o}C and 70.3%, respectively in the mild period and 27.5^{o}C and 75.3%, respectively in the hot period. Feeding was available from 20.00 to 8 00 h in feeding at night only system.

The basal diet consisted of 28% alfalfa hay, 18% barley, 18% soybean meal (44%), 25% wheat bran, 6% yellow corn, 3 % molasses, 1.1% limestone, 0.3% sodium chloride and 0.6 % vitamin and mineral premix. Each kilogram of vitamin and minerals premix contained: 10.000 IU Vit. A, 900 IU Vit. D3, 2mg Vit. K, 50 mg Vit. E, 2mg Vit. B1, 6mg Vit. B2, 2 mg Vit. B6, 0.01 mg Vit. B12, 20 mg Panathonic acid, 50 mg Niacin, 5mg Folic acid, 1.2 mg Biotin, 12000 mg Choline, 3 mg Copper, 0.2 mg Iodine, 75 mg Iron, 30 mg Manganese, 70 mg Zinc, 0.1 mg Selenium, 0.1 mg Cobalt and 0.04 mg Magnesium. The basal diet contained of 18.2 % crude protein, 13.4% crude fibre, 2.3% ether extract, 2656 kcal/kg digestible energy.

All rabbits were kept under the same managerial, hygienic and environmental conditions and were maintained and treated in adherence to accepted standards for the humane treatment of animals.

Does were individually reared in wire cages and their offspring were collectively raised in cages, in the same batteries, in a well ventilated building. Fresh water was available all the time by stainless steel nipples. All cages were equipped with feeders and automatic nipples. During the experimental period, the total artificial light was about 16 hours/day.

At mating, rabbits were individually transferred to the buck cages and were returned to their own hatches after copulation. Each doe was palpated 10 days post-mating and was rebred until pregnancy was established. Litter kits were recorded within 12 hours after kindling. Weaning was carried out at 30 days of kits age.

The doe traits studied were 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 from birth to 30 days of age. Doe milk consumed by the pups from birth to 21 days of age was estimated by the following equation:

Y = Litter weight gain during the period 0-21days (kg) / 0.56.

Where Y was the milk consumed by pups during the period 0-21days of age and 0.56 was the 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. The feed conversion ratio (FCR) was calculated during the whole suckling period according to the following formula:

FCR= (feed intake during gestation period + feed intake during the suckling period) / total litter gain.

Rectal temperature and respiration rate were measured in does once every two weeks at 9.00-11.00 h. Respiration rate was recorded by counting the frequency of the flank movement per minute, using a hand counter. Internal body temperature was taken by medical thermometer inserted into the rectum for 2 minutes at depth of 2 cm.

At the end of the experimental period, blood samples were collected from does. Blood was collected from the marginal ear vein after shaving and cleaning with alcohol in less than 2 minutes into dry clean centrifuge tubes containing some drops of heparin. Plasma was separated by centrifugation at 3000 rpm for 20 minutes and kept in a deep freezer at -20˚C until 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 values were 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^{o}C-{(0.31-0.31RH)(db^{o}C-14)},

where db^{o}C= dry bulb temperature in Celsius and RH = RH % /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 and more = very severe heat stress.

Statistically, the obtained data were analyzed by using (2x2x3) factorial design according to Snedecor and Cochran (1982) by the following model:

$$X^{ijkl} = \mu + P^i + F^j + N^k + PF^{ij} + FN^{jk} + PN^{ik} + PFN^{ijk} + E^{ijk},$$

where μ = general mean, P^i = fixed effect of i^{th} period of the year (hot and mild periods), F^j = fixed effect of j feeding system (*ad libitum* and at night only), N^k = fixed effect of k *Nigella sativa* supplementation (1,…..3), PF^{ij} interaction between period of the year and feeding system, FN^{jk} interaction between feeding system and *Nigella sativa*, PN^{ik} interaction between period of the year and *Nigella sativa*, PFN^{ijk} interaction between period of the year, feeding system and *Nigella sativa* and E^{ijk} = random error. Differences among means were tested by Duncan's multiple range test (Duncan 1955)

3. Results and Discussion

Doe reproductive performance traits as affected by:

Period of the year

Temperature – humidity index values (THI) estimated were 18.9 and 24.7 at mild and hot periods, respectively, indicating absence of heat stress during the mild period (less than 22.2) and exposure to severe heat stress during the hot period (23.3-25.5^{0}C). These results were similar to those of Marai *et al.* (1996) under the same Egyptian climatic conditions.

Exposure of NZW young doe rabbits to severe heat stress under the warm sub-tropical environmental conditions of Egypt, affected negatively (P<0.01 or 0.05) feed intake, water intake, rectal temperature, respiration rate, litter size and weight at birth, 21 days and weaning, pre-weaning mortality and milk yield, while the effects were not significant on Feed conversion, serum total proteins, albumin, globulin, urea and creatinine and gestation period (Tables 1–4).

Under heat stress conditions, depression in feed consumption is the most important reaction to exposure to elevated temperature (Marai *et al.,* 2002, 2004a). Such phenomenon is due to that environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulses to the appetite centre in the hypothalamus causing the decrease in feed consumption, i.e. dry matter intake and consequently fewer substrates become available for enzymatic activities, hormone synthesis and heat production (Marai *et al.,* 2002).

The high consumption of water in the hot period helps the animal to increase the heat loss through water respiratory vaporization. Stephan (1980) estimated the increase in water requirement by 50% at 38˚C than at 18.0˚C.

The highly significant increase in thermoregulatory parameters (rectal temperature and respiration) due to exposure to severe heat stress were similar to those reported by other workers (Rich & Alliston 1970; Shafie *et al.,* 1982 and Marai *et al.,* 2001). The increase in respiration frequency and evaporative water loss is linearly related to the increase in ambient temperature above the panting threshold (Richards, 1976) and thus enables the animals to dissipate heat by vaporizing high moisture through the respiratory air, which accounts to about 30% of total heat dissipation. Respiration becomes the main pathway for loss of the latent heat, since most sweat glands in rabbits are not functional and perspiration is not great due to the fur (Marai *et al.,* 2001). The increase in rectal temperature of the heat-stressed rabbits may be due to failure of the physiological mechanisms of the animals to balance the excessive heat load caused by exposure to high ambient temperature (Habeeb *et al.,* 1992).

The above mentioned heat stress detrimental effects were reflected in significant (P<0.01 or 0.05) decrease in litter size and weight at birth, 21 days and weaning, pre-weaning mortality and milk yield, similar to that reported by Marai *et al.* (2004b, 2007).

In conclusion, exposure of rabbits to heat stress evokes a series of remarkable changes in their biological functions which ends with impairment of production and reproduction (Marai *et al*., 2002, 2004).

Feeding system

Feeding at night only, improved significantly (P<0.01 or 0.05) feed intake, litter size and weight at birth, 21 days and weaning and milk yield than in *ad libitum* feeding system (Tables 1 and 4). Meanwhile, water consumption decreased (13.2 %) significantly (P<0.05) with *ad libitum* than with feeding at night only. Feed conversion, rectal temperature, respiration rate, serum total proteins, albumin, globulin, urea and creatinine, gestation period and pre-weaning mortality, were not significantly affected by feeding system (Tables 1-4).

Feeding at night only, in addition to the nocturnal nature of rabbits may be the reason in the significant (P<0.05) increase in feed intake, which is reflected in improvement of litter size and weight (P<0.05) and milk yield (P<0.01). The present results were similar to those of Mahrose (2000).

The significant decrease in water consumption (13.2 %?) by feeding at night only than with *ad libitum* feeding, may be a reflection to the milder weather by night throughout the day.

Such phenomenon may suggest feeding rabbits at the mildest period of the day during the hot season of the year, under the sub-tropical conditions.

Table(1): Feed intake, feed conversion and water intake of New Zealand White doe rabbits at first parity as affected by season of the year, feeding system, dietary supplementation with *Nigella sativa* and their interactions

Items	Feed intake (g /day)	Feed conversion (kg feed/kg gain)	Water intake (ml /day)
Season of the year (S)			
Hot	195.27±2.84 ^{b}	2.18±0.13	474.68±9.83 a
Mild	271.33±1.46 ^{a}	2.24±0.18	311.15±4.50 b
Significance	**	NS	**
Feeding system (F)			
Ad libitum	234.79±5.52 ^{b}	1.98±0.17	426.65±18.16 a
Feeding at night only	252.34±3.87 ^{a}	2.19±0.20	370.26±11.76 b
Significance.	*	NS	*
Nigella sativa supplementation (Nig.S)			
Without Nig.S	231.55±4.61	1.99±0.12	436.30±11.17
1/2 % Nig.S	251.30±6.34 ^{a}	2.19±0.18	396.80±13.11
1 % Nig.S	254.45±3.46 ^{a}	2.12±0.14	399.00±17.36
Significance	*	NS	NS
Interactions			
S x F	NS	NS	NS
S x Nig.S	NS	NS	NS
F x Nig.S	NS	NS	NS
S x F x Nig.S	NS	NS	NS

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

***Nigella sativa* supplementation**

Dietary supplementation of the doe rabbits with *Nigella sativa* seeds (1% / kg diet) improved significantly feed intake (P<0.05), litter size and weight at birth (P<0.01 and 0.05), 21 days and at weaning (P<0.05), while the effects were not significant on feed conversion, water consumption, rectal temperature, respiration rate, serum total protein, albumin, globulin, urea and creatinine, gestation period and pre-weaning mortality.

The positive results obtained by dietary supplementation of the doe rabbits with *Nigella sativa* seeds (1% / kg diet) may be due to the nutritional importance of such seeds as a source of each of crude protein, crude fat, crude fibre and the major minerals such as Ca, p, K, Mg and Na (Abdel–Aal & Attia, 1993), as well as, the unsaturated fatty acids, and oleic and linoleic (as two major fatty acids) (Abdel–Aal & Attia, 1993). The medical properties of the same seeds as antibacterial antifungal antihelminthic antineoplastic bronchodilator immune and antispasmodic effects (Rathee *et al*., 1982; Mahdy, 1993; Khodary *et al.,* 1996), may be an additional reason, in this respect.

Interactions

Interaction effects of season of the year X feeding system were significant (P<0.05) on litter size at birth and 21 days and milk yield / doe. Feeding at night only showed the best values during the two periods (hot and mild), as well as, within the hot period. All the other interaction effects were not significant (Table 4).

Under the sub-tropical conditions, the combined effect of environment and nutrition is more substantial than in the other areas with milder climate, due to the negative effect of elevation ambient temperature on appetite and accordingly on feed intake that ends with slowing growth and impairment of reproduction in rabbits (Marai *et al.,* 2002, 2006; Abdel–Monem 2001).

Such phenomenon may suggest feeding rabbits at the mildest period of the day especially during the hot season of the year, under the sub-tropical conditions.

Table(2): Rectum temperature and respiration rate of New Zealand White doe rabbits at first parity as affected by season of the year, feeding system, dietary supplementation with *Nigella sativa* and their interactions

Items	Rectum temperature (^{o}C)	Respiration rate (Respirations/ minute)
Season of the year (S)		
Hot	39.79±0.07 ^{a}	124.29±0.43 ^{a}
Mild	38.96±0.04 ^{b}	104.25±0.84 ^{b}
Significance	**	**
Feeding system (F)		
Ad libitum	39.38±0.08	117.94±1.68
Feeding at night only	39.37±0.09	111.83±1.61
Significance	NS	NS
Nigella sativa supplementation (*Nig. S*)		
Without *N.S*	39.53±0.07	119.45±1.14
1/2 % *Nig.S*	39.42±0.08	112.15±1.21
1 % *Nig.S*	39.21±0.06	110.35±1.68
Significance	NS	NS
Interactions		
S x F	NS	NS
S x *Nig.S*	NS	NS
F x *Nig.S*	NS	NS
S x F x *Nig.S*	NS	NS

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

Table(3): Blood analysis of New Zealand White doe rabbits at first parity as affected by season of the year, feeding system, dietary supplementation with *Nigella sativa* and their interactions

Items	Total roteins(g/dl)	Albumin(g/dl)	Globulin(g/dl)	Urea(mg/dl)	Creatinine(mg/dl)
Season of the year (S)					
Hot	5.36±0.34	2.84±0.12	2.52±0.36	16.06±0.09	1.12±0.08
Mild	6.49±0.39	3.82±0.15	2.67±0.43	15.32±0.07	1.07±0.11
Significance	NS	NS	NS	NS	NS
Feeding system (F)					
Ad libitum	6.40±0.32	3.83±0.15	2.57±0.39	15.71±0.10	1.11±0.14
Feeding at night only	6.94±0.40	2.87±0.20	4.07±0.52	15.42±0.08	1.15±0.62
Significance	NS	NS	NS	NS	NS
Nigella Stiva supplementation (*Nig.S*)					
Without *Nig.S*	5.97±0.51	3.81±0.19	2.16±0.27	15.72±0.06	1.13±0.13
1/2 % *Nig.S*	6.43±0.60	2.99±0.18	3.44±0.41	16.03±0.09	1.09±0.32
1 % *Nig.S*	7.44±0.71	3.74±0.15	3.70±0.49	15.91±0.05	1.19±0.51
Significance	NS	NS	NS	NS	NS
Interactions					
S x F	NS	NS	NS	NS	NS
S x *Nig.S*	NS	NS	NS	NS	NS
F x *Nig.S*	NS	NS	NS	NS	NS
S x F x *Nig.S*	NS	NS	NS	NS	NS

NS = Not significant

Table (4): New Zealand White doe rabbits traits at first parity as affected by season of the year, feeding system, dietary supplementation with *Nigella sativa* and their interactions

Items	Gestat-ion period (Days)	Litter size at			Litter weight (g) at			Pre-weaning mortality (In number)	Milk yield (g/doe)
		Birth	21 days	Weaning	Birth	21 days	Weaning		
Season of the year (S)									
Hot	31.8±0.2	3.71±0.3^{b}	2.27±0.2^{b}	1.44±0.2^{b}	43.41±1.2^{b}	235.15±6.7^{b}	469.27±14^{b}	1.77±0.07^{a}	665.61±36^{b}
Mild	30.8±0.1	6.10±0.3^{a}	4.93±0.3^{a}	4.45±0.3^{a}	49.38±0.8^{a}	313.48±5.8^{a}	658.00±13^{a}	1.15±0.03^{b}	2221.86±12^{a}
Significance	NS	**	***	***	**	***	***	*	***
Feeding system (F)	30.7±0.2	4.25±0.3^{b}	2.64±0.3^{b}	2.25±0.3^{b}	44.26±1.5^{b}	252.63±10^{b}	509.31±24^{b}	1.45±0.10	1252.89±46^{b}
Ad libitum									
Feeding at night only	31.8±0.3	5.57±0.3^{a}	4.57±0.4^{a}	3.64±0.4^{a}	48.53±0.8^{a}	296.01± 9.8	617.97±15^{a}	1.47±0.19	1634.59±114^{a}
Significance	NS	*	*	*	*	*	*	NS	*
Nigella Stiva supplementation (Nig.S)									
Without Nig.S	31.4±0.1	3.80±0.5^{b}	2.90±0.2^{b}	2.00±0.7^{b}	44.65±1.4^{b}	240.50± 9^{b}	506.50±17^{b}	1.80±0.11	942.46±40^{e}
1/2 % Nig.S	31.3±0.2	5.90±0.8^{a}	4.45±0.7^{a}	4.20±0.5^{a}	49.05±1.9^{a}	293.00±10^{a}	599.63±15^{a}	1.70±0.14	1811.52±130^{a}
1 % Nig.S	31.3±0.1	6.15±0.6^{a}	4.80±0.6^{a}	4.50±0.8^{a}	48.24±1.5^{a}	307.25±13^{a}	588.41±16^{a}	1.65±0.13	1178.12±100^{b}
Significance	NS	**	**	**	*	**	**	NS	**
Interactions									
S x F									
Hot									
Ad libitum	31.3±0.4	3.50±0.3^{b}	1.27±0.2^{b}	1.00±0.2	2.41±1.3	215.05±7	418.1±12	1.90±0.16	565.31±78^{b}
Feeding at night only	32.2±0.2	3.93±0.3^{b}	3.27±04^{b}	1.88±0.3	44.41±1.7	255.25±6	520.44±14	1.64±0.13	765.91±49^{b}
Mild									
Ad libitum	30.1±0.3	5.00±0.3^{ab}	4.00±0.3^{a}	3.50±0.3	46.11±1.9	290.20±9	600.5±1.5	1.00±0.19	1940.46±91^{a}
Feeding at night only	31.5±0.1	7.20±0.3^{a}	5.86±0.2^{b}	5.40±0.4	52.62±1.6	336.76±11	715.5±18	1..30±0.17	2503.26±61^{a}
Significance	NS	*	*	NS	NS	NS	NS	NS	*
S x Nig.S	NS	NS	NS	NS	NS	NS	NS	NS	NS
F x Nig.S	NS	NS	NS	NS	NS	NS	NS	NS	NS
S x F x Nig.S	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means bearing different letters in the same column within each classification, differ significantly ($P \leq 0.05$).
** = $P<0.01$, * = $P<0.05$ and NS = Not significant

4. Conclusions

Exposure of NZW young doe rabbits to severe heat stress under the warm sub-tropical environmental conditions of Egypt, affected negatively most of the traits studied. This is due to that its exposure to heat stress evokes a series of remarkable changes in their biological functions which ends with impairment of production and reproduction. Such phenomenon may suggest feeding rabbits at the mildest period of the day especially during the hot season of the year, under the sub-tropical conditions. It is also recommended to dietary supplement the doe rabbits with *Nigella sativa* seeds (1% / kg diet), although further studies are needed to test inclusion of higher percentages of the same feedstuff for the same purpose.

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