

EFFECT OF FEEDING SYSTEM AND DIETARY *Nigella sativa* SEED LEVEL ON PERFORMANCE OF RABBIT DOES AT FIRST PARITY DURING THE MILD AND HOT SEASONS OF EGYPT

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ABSTRACT

*One hundred and twenty New Zealand White doe rabbits at first parity were used to study the effects of period of the year (60 animals in the mild, and another 60 in the hot period), feeding system (30 ad libitum and 30 fed only at night per season) and *Nigella sativa* seed dietary supplementation (0, 0.5 and 1% seeds, 10 does feeding system / season).*

*The Temperature–humidity index (THI) estimated was 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). Exposure to severe heat stress decreased ($P<0.05$) feed intake, litter size at birth, 21 d and at weaning, litter weight at birth, 21 d and at weaning and milk yield than in the mild period. However, water intake, rectum temperature, respiration rate and pre-weaning mortality increased ($P < 0.05$) with heat stress. Feeding only during night improved ($P<0.05$) feed intake, litter size at birth, 21 days and at weaning, litter weight at birth, 21 days and weaning and milk yield than in ad libitum feeding system. While, water consumption decreased ($P<0.05$) in animals fed only during the night than with those fed ad libitum. Interaction effects of season of the year \times feeding system were significant ($P<0.05$) for litter size at birth and 21 days and milk yield indicated better results of feeding only at night especially during the mild season.. Dietary supplementation with 0.5% *N. sativa* seeds improved ($P<0.05$) feed intake, litter size at birth, at 21 d and at weaning, litter weight at birth, at 21 d and at weaning and milk yield than without supplementation. Comparison between the results of the two levels of *N. sativa* (0.5 and 1%) did not show any difference between them, except for milk yield that was improved with 1% *N. sativa* inclusion.*

Conclusively, it is recommended to fed rabbits only at night, under our warm sub-tropical environmental conditions and to supplement doe rabbits with 0.5% *N. sativa* seeds).

Key words: Feeding system, heat stress, *Nigella sativa* seed dietary supplementation, rabbit doe traits.

INTRODUCTION

Economic intensive rabbits 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 elevated ambient temperature on appetite and accordingly on the 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 to 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, the Egyptian *Nigella sativa* seeds composition was shown in Table 1.

Table 1: The Egyptian *Nigella sativa* seeds composition.

Chemical composition		Mineral contents ***	(Mg/kg)	Chemical content of oil extraction	%
Crude Protein* %	21-34	Calcium	2.99	<i>Saturated fatty acids</i>	11.8 ⁽¹⁾ -
Ether Extract* %	11-37	Phosphorus	9.3	<i>Unsaturated acids:</i>	
Crude Fiber* %	5.8- 16.4	Iron	4.6	Oleic acid	48.76 ⁽¹⁾ -24.0 ⁽²⁾
<i>Amino acids contents (g/100g):**</i>		Zinc	0.87	Linoleic acid	37.56 ⁽¹⁾ -56.0 ⁽²⁾
Aspartic acid	9.55	Manganese	0.29	Linolenic	1.88 ⁽¹⁾
Threonine	4.07	Copper	0.3	Palamatic acid	70.0 ⁽²⁾
Serine	4.12	Sodium	0.2	Stearic acid	3.0 ⁽²⁾
Glutamic acid	22.51	Potassium	0.39	Eicosadienoic acid	2.9 ⁽²⁾
Glycine	6.5	Magnesium	7.12	Myrestic acid	0.16 ⁽²⁾
Alanine	4.52				
Cysteine	1.07				
Valine	5.2				
Methionine	1.47				

* Abdel-Aal and Attia, 1993; Khalifah, 1995; Zeweil, 1996; Salah, 1997

** Khalifah, 1995; Zeweil 1996; Salah, 1997; Khalifah 1995; Zeweil, 1996; Salah, 1997

*** Nasr *et al.* , 1996; Khalifahm 1995, 1 - (Gad *et al.* 1963), 2 - Babayan *et al.* (1978)

Therefore, the present study was conducted to investigate the effects of period of the year (mild and hot), feeding system (*ad libitum* and feeding only at night), *Nigella sativa* seeds dietary supplementation (0, 0.5 and 1%) and their interactions on performance traits (feed intake, feed conversion and water intake, gestation length, litter size and weight at birth, 21 days and weaning, milk yield and pre-weaning mortality), thermoregulation parameters (rectum temperature and respiration rate), immunity (plasma total proteins, albumin and globulin), and kidney function (urea-N and creatinine)), under Egyptian sub-tropical conditions.

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 farm in Zagazig city during two periods of the year: hot (from May to September, 2005), and mild (from October, 2005 to April, 2006).

Sixty New Zealand White doe rabbits at first parity during each period of the year (mild or hot) were randomly divided into 6 treatment groups (10 does/group), in order to study the reproductive traits as affected by season of the year (mild and hot), feeding system (*ad libitum* and fed only at night from 20.00 to 8.00 h), *Nigella sativa* dietary supplementation (0, 0.5 and 1% seeds) and their interactions, under Egyptian conditions. *Nigella sativa* seeds substituted in the ration. All groups were nearly similar in average initial body weights (3120 ± 29.9 g).

The basal diet contained: 28.0% alfalfa hay, 18.0% barley, 18.0% soybean meal (44.0% CP), 25.0% wheat bran, 6.0% yellow corn, 3.0 % molasses, 1.1% limestone, 0.3% sodium chloride and 0.6 % vitamin and mineral premix. Each kilogram of vitamin and minerals premix contained: Vit. A 10.000 IU, Vit. D₃ 900 IU, Vit. K 2 mg, Vit. E 50 mg, Vit. B₁ 2 mg, Vit. B₂ 6 mg, Vit. B₆ 2 mg, Vit. B₁₂ 0.01 mg, Pantothenic acid 20mg, Niacin 50 mg, Folic acid 5 mg, Biotin 1.2 mg, Choline 12000 mg, Copper 3 mg, Iodine 0.2 mg, Iron 75 mg, Manganese 30 mg, Zinc 70 mg, Selenium 0.1 mg, Cobalt 0.1 mg and Magnesium 0.04 mg. The basal diet contained of 18.2 % crude protein, 13.4% crude fibre, 2.3% ether extract, and 2656 kcal/kg digestible energy. The digestible energy value was estimated by calculation.

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

Does were individually reared in wire cages with their offspring until weaning, in a well ventilated building. Fresh water was available all the time by stainless steel nipples. Each cage was equipped with a feeder and a crock (container) containing drinking water. Feed or water consumption was estimated individually once a week by measuring the offered and residuals for each rabbit.

Means of ambient temperature and relative humidity at mid-day inside the rabbitry during the experimental period were 20.0°C and 70.3% in the mild period and 27.5°C and 75.3% in the hot period, respectively. 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 weight and number of kits were recorded within 12 hours after kindling. Kits were weaned at 30 days of age.

The traits studied were some performance traits (feed intake, feed conversion and water intake, gestation length, litter size and weight at birth, 21 days and weaning, milk yield and pre-weaning mortality), thermoregulation parameters (rectum temperature and respiration rate), immunity (plasma total proteins, albumin and globulin), and kidney function (urea-N and creatinine)).

Doe milk consumed by the kits from birth to 21 days of age was estimated according to Cowie (1969) using the modified formula:

Milk yield 0-21 d lactation (g/doe) = Litter weight gain [estimated for the live animals during the period 0-21days (g) + Gain weight (g) of each of the mortals from birth up to the day of its death, during the same period] / 0.56, Where 0.56 was the standard value 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= (g Feed intake during the suckling period doe + litter) / g l gained by litter during the same period).

Rectal temperature and respiration rate were measured in does every two weeks before 11.00 h (to avoid exerting more stress on the pregnant does during the peak of the ambient temperature). Respiration rate was recorded by counting the frequency of the flank movement per minute, using a hand counter. Internal body temperature was measured 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 the marginal ear vein into dry clean centrifuge tubes containing some drops of heparin in less than 2 minutes, after shaving and cleaning with alcohol. Plasma was separated by centrifugation at 3000 rpm for 20 minutes and kept in a deep freezer at -20°C until analysed. 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) modified from the formula of LPHSI (1990) as follows:

$$THI = db^{\circ}C - [(0.31 - 0.31RH)(db^{\circ}C - 14)],$$

Where db°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 as a 2 × 2 × 3 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 X_{ijkl} = An observation, 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* seed supplementation

(1,.....3), PF_{ij} = Interaction between period of the year and feeding system, FN_{jk} = Interaction between feeding system and *Nigella sativa* seed supplementation, PN_{ik} = Interaction period of the year and *Nigella sativa* seed supplementation, PFN_{ijk} = Interaction between period of the year, feeding system and *Nigella sativa* seed supplementation, and E_{ijk} = Random error.

Differences among means were tested by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Period of the year and feeding system

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). These results were similar to those of Marai *et al.* (1996) under the same Egyptian climatic conditions.

Exposure of young doe rabbits to severe heat stress under the warm sub-tropical environmental conditions of Egypt, decreased significantly ($P<0.05$) feed intake (by 28%), litter size at birth, 21 d and weaning (by 39, 54 and 68%, respectively), litter weight at birth, at 21 d and at weaning (by 12, 25 and 29%, respectively), and estimated milk yield (by 149%), compared to the mild period (Tables 2 and 4). However, heat stress increased ($P<0.05$) water intake (by 53%), rectum temperature and respiration rate (by 2 and 19%, respectively) and pre-weaning mortality (by 54%) compared to the mild period. Period of the year did not affect feed conversion, serum total proteins, albumin, globulin, urea-N, creatinine and gestation period (Tables 2, 3 and 4).

Under heat stress conditions, depression in feed consumption is the most important reaction to exposure to elevated temperature (Marai *et al.*, 1994a, 2002). 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, and consequently less 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 requirements by 50% at 38°C compared to 18.0°C, in growing rabbits.

The high increases in thermoregulatory parameters (rectum temperature and respiration rate) due to exposure to severe heat stress were similar to those reported by other workers (Rich and Alliston 1970; Shafie *et al.* 1984; 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 for around 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 detrimental effects of heat stress were reflected in a decrease of each of litter size and litter weight at birth, 21 days and at weaning and milk yield, and increase in pre-weaning mortality, 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 performances (Marai *et al.* 2002 & 2004).

Feeding rabbit does only during the night improved ($P < 0.05$) feed intake (by 7%), litter size at birth, 21 days and at weaning (by 31, 73 and 62%, respectively), litter weight at birth, 21 days and weaning (by 10, 17 and 21%, respectively) and milk yield (by 90%) compared to animals with an *ad libitum* feeding system during all day (Tables 2 and 4). Water consumption decreased (by 13%; $P < 0.05$) in animals fed only during the night compared to those with feed available all the day. Feed conversion rate, rectum temperature, respiration rate, serum total proteins, albumin, globulin, urea and creatinine, gestation period and pre-weaning mortality, were not affected by feeding system (Tables 2, 3 and 4). The present results were similar to those obtained by Mahrose (2000).

The favourable effects of feeding only during night may be due to the increase in feed consumption as a positive reflection to deprivation of the feed during the daylight, in addition to improvement of appetite during the mildest environment at night under the sub-tropical warm conditions. This is besides the stimulating effects of the nocturnal nature of rabbits. Improvement of the appetite by night is a result to stimulation of the peripheral thermal receptors by the mild environmental temperature to transmit suppressive nerve impulses to the appetite centre in the hypothalamus that causes the mentioned phenomenon (Marai *et al.*, 2002).

The significant decrease in water consumption when rabbit does were fed only at night compared with animals with available feed all the day, may be explained by the milder weather at night than in all day.

The interaction of season of the year \times feeding system were significant on litter size at birth ($P < 0.05$) and at 21 d ($P < 0.05$) ($P < 0.01$) and on milk yield ($P < 0.01$), while all the other interactions were not significant (Table 4). With regard to the significant effects, feeding only at night showed the highest values, during the two periods. This trend was more punctuated during the mild period. 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).

Table 2: Feed intake, feed conversion, water intake, rectum temperature and respiration rate of New Zealand White rabbit does at first parity as affected by season of the year, feeding system, and dietary supplementation with *Nigella sativa* seeds¹

Items ²	Feed intake (g /d)	Feed conversion rate (kg feed/kg gain)	Water intake (ml /d)	Rectum temperature (°C)	Respiration rate (Respirations/minute)
Season of the year					
Hot	195±2.8	2.18±0.13	475±9.8	39.8±0.07	124.3±0.43
Mild	271±1.5	2.24±0.18	311±4.5	39.0±0.04	104.2±0.84
P-value	P<0.01	NS	P<0.01	P<0.01	P<0.01
Feeding system (FS)					
<i>Ad libitum</i>	235±5.52	1.98±0.17	427±186	39.4±0.08	118±1.68
Feeding only at night	252±3.87	2.19±0.20	370±12	39.4±0.09	112.34±1.61
P-value	P<0.05	NS	P<0.05	NS	NS
NSSI*:					
0% N. sativa	231±4.6 ^b	1.99±0.12	436±11	39.35±0.07	(113.01)
0.5 % N. sativa	251±6.3 ^a	2.19±0.18	397±13	39.42±0.08	119±1.14
1 % N. sativa	254±3.5 ^a	2.12±0.14	399±17	39.21±0.06	110±1.68
P-value	P<0.05	NS	NS	NS	NS
P-value interactions:					
Season × FS	NS	NS	NS	NS	NS
Season × NSSI	NS	NS	NS	NS	NS
FS × NSSI	NS	NS	NS	NS	NS
Season × FS × NSSI	NS	NS	NS	NS	NS

¹ n = 10 Rabbit does per each combination of season, feeding system and N. sativa level.

² Results are presented as Mean ±SE, NSSI* = *N. sativa* seed inclusion.

Means bearing different letters in the same column within each classification, differ significantly (P≤0.05).

These results may suggest to feed rabbits at the mildest period of the day specially during the hot season of the year, under the sub-tropical conditions.

Dietary supplementation with *Nigella sativa* seeds

Dietary supplementation the doe rabbits with 0.5% *Nigella sativa* seeds improved (P<0.05) feed intake (by 8%), litter size at birth, at 21 d and at weaning (by 55, 53 and 110%, respectively), litter weight at birth, at 21 d and at weaning (by 10, 22 and 18%, respectively) and milk yield (by 122%) compared to no supplementation. Dietary supplementation with 1% *Nigella sativa* seeds improved (P<0.05) feed intake (by 9.89%), litter size at birth, 21 d and weaning (by 61.84, 65.52 and 125%, respectively), litter weight at birth, 21 d and weaning (by 8.40, 27.75 and 16.17%, respectively) and milk yield (by 150.55%) than without supplementation. It can be

Table 3. Blood analysis of NZW doe rabbits at first parity as affected by season of the year, feeding system, and dietary supplementation with *Nigella sativa* seeds.

Items	Total proteins (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	17±0.11
Significance	NS	NS	NS	NS	NS
Feeding system (F):					
<i>Ad libitum</i>	6.40±0.32	3.83±0.15	2.57±0.39	15.71±0.10	1.11±0.14
Feeding only at night	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
<i>Nigella sativa</i> supplementation (NS):					
Without NS	5.97±0.51	3.81±0.19	2.16±0.27	15.72±0.06	1.13±0.13
1/2 % NS	6.43±0.60	2.99±0.18	3.44±0.41	16.03±0.09	19±0.32
1 % NS	7.44±0.71	3.74±0.15	3.70±0.49	15.91±0.05	1.19±0.5
Significance	NS	NS	NS	NS	NS
Interactions:					
S x F	NS	NS	NS	NS	NS
S x NS	NS	NS	NS	NS	NS
F x NS	NS	NS	NS	NS	NS
S x F x NS	NS	NS	NS	NS	NS

Mean ±SE and NS = Not significant

n = 10 rabbit does per each combination of season, feeding system and *N. sativa* level.

noted that inclusion of 0.5 and 1% of *Nigella sativa* in the diets did not show a definite trend regarding their effectiveness, which might suggest to carry out further studies with inclusion of different percentages of the same seeds on groups with more numbers of animals. Feed conversion, water consumption, rectum temperature, respiration rate, serum total proteins, albumin, globulin, urea and creatinine, gestation period, pre-weaning mortality did not change with dietary supplementation of *Nigella sativa* seeds.

The favourable effects of dietary supplementation of *Nigella sativa* seeds on doe rabbits might be due to its qualitative and medicinal properties.

CONCLUSIONS

The negative effects of exposure of young doe rabbits to severe heat stress under the warm sub-tropical environmental conditions of Egypt, may suggest to feed rabbits at night only during the mildest period of the day (during night) especially during the hot season of the year, under the sub-tropical conditions. This could minimize reproductive losses. It may be also recommended to dietary supplement the doe rabbits with *Nigella sativa* seeds (0.5%), although further studies are needed to test inclusion of different percentages of the same feedstuff for the same purpose.

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