

## Effect of feeding time and vitamin C levels on performance of rabbit does during the mild and hot seasons in Egypt

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**Abstract:** One hundred and twenty New Zealand White (NZW) 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 time (30 *ad libitum* and 30 fed only at night per season), and vitamin C supplementation (0, 300 and 600 mg / L water intake 10 does per season and feeding time). Exposure to severe heat stress decreased ( $P < 0.05$ ) feed intake, litter size at birth, at 21 days and at weaning, litter weight at birth, at 21 days and at weaning and estimated 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 weaning, litter weight at birth, 21 days and weaning and milk yield than in *ad libitum* feeding time. While, water consumption decreased ( $P < 0.05$ ) in animals fed only during the night than with those fed *ad libitum*. Addition of vitamin C with 600 mg / L water intake improved ( $P < 0.05$ ) feed intake, litter size at birth, 21 days and at weaning, litter weight at birth, at 21 days and at weaning and milk yield than without supplementation. Comparison between the results of the two levels of Vitamin C (300 and 600 mg / L water intake) did not show any difference between them except for milk yield that was improved with vitamin C with 600 mg / L water intake.

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**Key words:** Feeding time, heat stress, vitamin C supplementation, rabbit doe traits.

### 1. 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 (Abdel-Monem, 2001 and Marai *et al.*, 2002 and 2006 and). 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.

Vitamin C, which is present in most animal cells, has numerous biochemical function. This metabolic factor is essential for growth and counteracting infections caused by pathogenic bacteria and viruses Verde and Piquer (1986) noted that the plasma vitamin C concentration was significantly reduced in animals exposed to stress. In other species, supplementary vitamin C has been shown beneficial in reducing the effects of stress. This means that the metabolic need for vitamin C increase at certain conditions. Therefore, the improve of animals performance effect of vitamin C may be vitamin C with the alleviation of retardation in the thyroid function, (Coates, 1984).

Abdel -Monem (2001) studied the effect of supplementation ascorbic acid at 250 and 500mg / kg of doe rabbits diet, on litter size, litter weight, pups gain, pre weaning mortality and gestation period.

The present study was conducted to investigate the effects of period of the year (mild and hot), feeding time (*ad libitum* and feeding only at night), vitamin C supplementation (0, 300 and 600 mg / L water intake) on performance traits, thermoregulation parameters, immunity, and kidney function of doe rabbits, under Egyptian sub-tropical conditions.

### 2. Materials and Methods

The present work was performed to study the effect of two periods of the year. Sixty New Zealand White (NZW) 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 time (*ad libitum* and fed only at night from 20.00 p.m to 8.00 a.m), *Vitamin C* supplementation (0, 300 and 600 mg / L water intake) under Egyptian conditions. 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. D<sub>3</sub> 900 IU, Vit. K 2 mg, Vit. E 50 mg, Vit. B<sub>1</sub> 2 mg, Vit. B<sub>2</sub> 6 mg, Vit. B<sub>6</sub> 2 mg, Vit. B<sub>12</sub> 0.01 mg, Panathonic 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 according to NRC, (1977). 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 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 kids 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 suggested formula:  $FCR = (\text{g feed intake during the suckling period doe} + \text{litter}) / \text{gI gained by litter during the same period}$ .

Rectal temperature and respiration rate of does were measured 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 according to the procedure outlined by the manufacturer. Globulin values were obtained by subtracting the values of albumin from 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  $db^{\circ}C$  = 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  $2 \times 2 \times 3$  factorial design according to Snedecor and Cochran (1982) by the following model:

$$X_{ijkl} = \mu + P_i + F_j + N_k + E_{ijk},$$

where  $X_{ijkl}$  = an observation,  $\mu$  = general mean,  $P_i$  = fixed effect of  $i_{th}$  period of the year (hot and mild periods),  $F_j$  = fixed effect of  $j$  feeding time (*ad libitum* and at night only),  $N_k$  = fixed effect of  $k$  Vitamin C supplementation (1,.....3) and  $E_{ijk}$  = random error. Differences among means were tested by Duncan's multiple range test (Duncan 1955).

### 3. Results and Discussion

#### Period of the year and feeding time

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 ( $P < 0.05$  and 0.01) feed intake (by 28%), litter size at birth, 21

days and weaning, litter weight at birth, at 21 days and at weaning and estimated milk yield compared to the mild period (Tables 1 and 3). However, heat stress increased ( $P < 0.05$  and  $0.01$ ) water intake, rectum temperature and respiration rate and pre-weaning mortality 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 1, 2 and 3).

Under heat stress conditions, depression in feed consumption is the most important reaction to exposure to elevated temperature (Marai *et al.*, 1994, 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  $38^{\circ}\text{C}$  compared to  $18.0^{\circ}\text{C}$ , 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.* (2004, 2007).

Feeding rabbit does only during the 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 compared to animals with an *ad libitum* feeding time during all day (Tables 1 and 2). Water consumption decreased 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 time (Tables 1, 2 and 3). The present results were similar to those obtained by Mahrose (2000) and Abdel-Monem *et al.* (2009).

The 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 and 2007).

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. 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 (Abdel – Monem 2001 and Marai *et al.*, 2002, 2006).

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.

#### **Water supplementation of Vitamin C**

Water supplementation of *Vitamin C* with 300 and 600 mg / L to doe rabbits improved ( $P < 0.05$  and  $0.01$ ) feed intake, litter size at birth, at 21 days and at weaning, litter weight at birth, at 21 days and at weaning and milk yield than in the control group (Tables 1 and 2). Feed conversion, water consumption, rectum temperature, respiration rate, serum total proteins, albumin, globulin, urea N, creatinine, gestation period and pre-weaning mortality did not change with supplementation of *Vitamin C* (Tables 1, 2 and 3). It can be noted that inclusion of treated heat stressed doe rabbits with 600 mg / L of *Vitamin C* improved doe rabbits performance. Similar results were obtained by Abdel – Monem (2001) found that feeding doe rabbits on diets containing 250 and 500 mg of ascorbic acid improved feed intake, litter size at birth, 21 days and weaning, litter weight at birth, 21 days and weaning and milk yield. The effect of ascorbic acid may be due to reducing the effects of stress, increasing the immunological traits Coates (1984).

**Table 1. Means and standard errors ( $\bar{X} \pm$  S.E.) for feed intake, water consumption, rectum temperature, respiration rate and milk yield of NZW doe rabbits as affected by environmental conditions, feeding time and dietary supplementation with vitamin C.**

Items	Feed intake	Water intake	Rectum Temp.	Respiration rate	milk yield
Environmental conditions					
Hot	174.1 $\pm$ 1.9 <sup>b</sup>	384.6 <sup>a</sup> $\pm$ 5.9	39.8 <sup>a</sup> $\pm$ 0.39	126.9 <sup>a</sup> $\pm$ 5.7	2043.9 <sup>b</sup> $\pm$ 49.7
Mild	253.9 <sup>a</sup> $\pm$ 2.8	229.3 <sup>b</sup> $\pm$ 7.1	39.1 <sup>b</sup> $\pm$ 0.23	91.6 <sup>b</sup> $\pm$ 4.9	3699.1 <sup>a</sup> $\pm$ 65.3
Sig.	**	**	*	*	**
Feeding time					
Ad libitum	191.3 <sup>b</sup> $\pm$ 2.8	296.9 $\pm$ 5.2	39.7 $\pm$ 0.31	112.0 $\pm$ 4.0	2017.3 <sup>c</sup> $\pm$ 39.1
Feeding at night	202.6 <sup>a</sup> $\pm$ 3.1	301.5 $\pm$ 8.9	39.6 $\pm$ 0.35	107.6 $\pm$ 3.9	2122.9 <sup>b</sup> $\pm$ 34.8
Significance.	*	NS	NS	NS	NS
Vitamin C					
0 mg	194.7 <sup>b</sup> $\pm$ 3.9	291.4 $\pm$ 5.3	39.5 $\pm$ 0.37	106.1 $\pm$ 6.1	3120.6 <sup>b</sup> $\pm$ 55.9
300 mg	219.2 <sup>ab</sup> $\pm$ 4.0	313.2 $\pm$ 3.9	39.6 $\pm$ 0.33	101.9 $\pm$ 4.5	3271.4 <sup>a</sup> $\pm$ 45.4
600 mg	231.5 <sup>a</sup> $\pm$ 3.7	318.7 $\pm$ 8.8	39.4 $\pm$ 0.29	111.3 $\pm$ 5.8	3295.9 <sup>a</sup> $\pm$ 61.6
Significance.	*	NS	NS	NS	**

NS = Not significant, \* = P&lt;0.05 and \*\* = P&lt;0.01

Means bearing different letters in the same column within each factor differ significantly (P $\leq$ 0.05).**Table 2. Means and standard errors ( $\bar{X} \pm$  S.E.) for some NZW doe traits and their young as affected by environmental conditions, feeding time and dietary supplementation with vitamin C.**

Items	Litter Size at			Litter weight at		
	Birth	21 day	Weaning	Birth	21 day	Weaning
Environmental conditions						
Hot	3.2 <sup>b</sup> $\pm$ 0.19	2.9 <sup>b</sup> $\pm$ 0.21	2.4 <sup>b</sup> $\pm$ 0.16	118.7 <sup>b</sup> $\pm$ 8.0	812.5 <sup>b</sup> $\pm$ 74.0	982.0 <sup>b</sup> $\pm$ 111.2
Mild	6.4 <sup>a</sup> $\pm$ 0.23	5.0 <sup>a</sup> $\pm$ 0.28	4.1 <sup>a</sup> $\pm$ 0.21	249.7 <sup>a</sup> $\pm$ 11.1	1595.1 <sup>a</sup> $\pm$ 114.6	2678.5 <sup>a</sup> $\pm$ 171.3
Sig.	**	**	**	**	**	**
Feeding time						
Ad libitum	3.7 <sup>b</sup> $\pm$ 0.49	2.5 <sup>b</sup> $\pm$ 0.45	1.8 <sup>b</sup> $\pm$ 0.61	140.2 <sup>b</sup> $\pm$ 11.4	1497.8 <sup>b</sup> $\pm$ 112.7	1628.8 <sup>b</sup> $\pm$ 132.4
Feeding at night	4.0 <sup>a</sup> $\pm$ 0.56	3.4 <sup>a</sup> $\pm$ 0.53	2.3 <sup>a</sup> $\pm$ 0.44	173.0 <sup>a</sup> $\pm$ 11.5	1931.9 <sup>a</sup> $\pm$ 143.2	2518.3 <sup>a</sup> $\pm$ 189.7
Significance	*	**	**	*	*	*
Vitamin C:						
0 mg	4.0 <sup>c</sup> $\pm$ 0.58	3.9 <sup>c</sup> $\pm$ 0.61	3.1 <sup>c</sup> $\pm$ 0.70	136.2 <sup>c</sup> $\pm$ 16.1	1170.6 <sup>c</sup> $\pm$ 158.9	1550.1 <sup>c</sup> $\pm$ 218.2
300 mg	4.6 <sup>b</sup> $\pm$ 0.56	4.0 <sup>b</sup> $\pm$ 0.56	3.8 <sup>b</sup> $\pm$ 0.56	213.0 <sup>b</sup> $\pm$ 11.4	1320.4 <sup>b</sup> $\pm$ 111.4	2052.2 <sup>b</sup> $\pm$ 11.4
600 mg	5.2 <sup>a</sup> $\pm$ 0.62	4.5 <sup>a</sup> $\pm$ 0.75	4.1 <sup>a</sup> $\pm$ 0.69	251.3 <sup>a</sup> $\pm$ 11.5	1575.0 <sup>a</sup> $\pm$ 139.5	2419.0 <sup>a</sup> $\pm$ 11.5
Significance.	*	**	**	**	**	**

NS = Not significant, \* = P&lt;0.05 and \*\* = P&lt;0.01

Means bearing different letters in the same column within each factor differ significantly (P $\leq$ 0.05).**Table 3. Means and standard errors ( $\bar{X} \pm$  S.E.) for blood components of NZW doe rabbits as affected by environmental conditions, feeding time and dietary supplementation with vitamin C.**

Items	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	GOT	GPT
Environmental conditions					
Hot	5.8 <sup>b</sup> $\pm$ 0.07	2.9 <sup>b</sup> $\pm$ 0.02	2.4 <sup>b</sup> $\pm$ 0.02	23.9 <sup>b</sup> $\pm$ 1.3	40.2 <sup>b</sup> $\pm$ 2.1
Mild	7.3 <sup>a</sup> $\pm$ 0.06	4.1 <sup>a</sup> $\pm$ 0.03	3.2 <sup>a</sup> $\pm$ 0.03	25.6 <sup>a</sup> $\pm$ 1.1	43.8 <sup>a</sup> $\pm$ 1.9
Sig.	*	**	*	*	**
Feeding time					
Ad libitum	5.9 $\pm$ 0.04	3.1 <sup>b</sup> $\pm$ 0.03	2.8 <sup>a</sup> $\pm$ 0.03	26.5 $\pm$ 0.8	41.1 $\pm$ 1.7
Feeding at night	6.2 $\pm$ 0.05	3.5 <sup>a</sup> $\pm$ 0.03	2.7 <sup>b</sup> $\pm$ 0.02	26.9 $\pm$ 0.9	40.9 $\pm$ 2.3
Significance.	NS	*	*	NS	NS
Vitamin C					
0 mg	5.1 <sup>b</sup> $\pm$ 0.03	3.0 <sup>b</sup> $\pm$ 0.03	2.1 $\pm$ 0.02	25.7 <sup>b</sup> $\pm$ 0.89	39.4 <sup>c</sup> $\pm$ 1.6
300 mg	5.7 <sup>a</sup> $\pm$ 0.06	3.6 <sup>a</sup> $\pm$ 0.03	2.1 $\pm$ 0.02	29.4 <sup>a</sup> $\pm$ 1.1	41.7 <sup>b</sup> $\pm$ 1.9
600 mg	5.9 <sup>a</sup> $\pm$ 0.04	3.7 <sup>a</sup> $\pm$ 0.04	2.2 $\pm$ 0.02	30.1 <sup>a</sup> $\pm$ 1.3	42.5 <sup>a</sup> $\pm$ 2.0
Significance	*	*	NS	*	*

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

### 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 supplement the doe rabbits with *Vitamin C* with 600 mg / L of water intake under Egyptian conditions.

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