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HOT CLIMATE EFFECTS AND THEIR AMELIORATION ON SOME PRODUCTIVE AND REPRODUCTIVE TRAITS IN RABBIT DOES

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Abstract: This study aimed to improve productive and reproductive performance of female rabbit does during the summer season "hot climate" using vitamin C or cooled water in combination with equine chorionic gonadotropin (eCG) treatment. Sixty New Zealand White rabbit does were assigned to three groups, according to drinking-water treatment: 1) fresh tap water without any supplementation (control, C), 2) cooled drinking water (10-15ºC) (CW), and 3) fresh tap water supplemented daily with added ascorbic acid (1 g/L) (vitamin C). Twenty four hours before mating, does of each group were randomly divided into two subgroups; H does were intravenously injected with 40 IU/doe eCG, while NH does did not receive any hormonal treatment. Productive and reproductive performance were significantly (P<0.05) improved in the treated groups. Kit weights at kindling and weaning were greater (P<0.05) in both vitamin C and cooled water groups than in the control regardless of hormonal treatment. It is worthy noticed that conception rate and litter size at birth were adversely affected by eCG, especially in does drinking vitamin C. The percentage of mature oocytes was lower (P<0.01) in control than in treated groups. The oocyte maturation rate improved after treatment with vitamin C to reach 80% compared to 66% in control group. In conclusion, cool drinking water or vitamin C is recommended for alleviating heat stress during summer in rabbits.

Key words: rabbit, production, reproduction, oocyte, vitamin C, cooled water.

INTRODUCTION

Heat stress occurs whenever an animal has excess body heat that it cannot lose. The endocrine system plays an integral part in the animal's response to stress (Ayyat *et al.*, 2004). High temperatures, as encountered in Egypt and in many other countries during the summer, is a major constraint factor for rabbit production, as it negatively affects production due to heat stress (Fouad, 2005).

Many attempts have been done to overcome the adverse effects of heat stress by modifying environmental condition through nutritional, managerial, and physiological manipulation of rabbits (Selim *et al.*, 2003). Vitamin C (ascorbic acid) is one of the most widely studied vitamins used to alleviate heat stress in rabbits. Abdel-Hamid and El- Adawy (1999) confirmed the protective effect of vitamin C added to the diet of rabbits; vitamin C promoted growth, reproduction and counteracted infections by pathogenic bacteria and viruses. Amakye-Anim *et al.* (2000) and El-Ghaffar *et al.* (2000) showed that vitamin C has a role in lowering viral pathogenic actions and in protecting birds from heat stress as well as in the enhancement of the immune system of infected rabbits. Vitamin C is not considered a required dietary nutrient, but, under certain adverse environmental conditions, the metabolic need for this vitamin may exceed the inherent biosynthetic ability of ascorbic acid (Abou-Ashour *et al.*, 2004).

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The hypothalamus receives and monitors information about the environment and coordinates the responses through nerves and hormones (Conventry and Phillips, 2000). From this miniaturized center, the brain controls hormone secretions from the pituitary gland and other tissues such as the adrenal gland (Ayyat *et al.*, 2004). Moreover, it is well known the relationships between ACTH and ascorbic acid are also well known. In fact, ACTH regulates the transport of ascorbic acid from the adrenal cortex to the adrenal medulla and also causes dose-related depletion of vitamin C, through modification of the adrenaline metabolism and inhibition of corticosteroid biosynthesis (Brake, 1989).

The hot climate negatively affects the quality of oocytes and the process leading to the correct formation of meiotic chromosomes, as represented by the decreasing of telophase I and metaphase II (Hamam *et al.*, 2001). In addition, Al-Katanani *et al.* (2002) reported that the proportion of oocytes and cleaved embryos that developed to blastocysts was lower in the warm season than in the cool season. Also, short exposure of pre-implantation rabbit embryos to elevated temperatures (41.5ºC and 42.5ºC) *in vitro* reduced embryo development (Makarevich *et al.*, 2006).

Adding vitamin C (Al-Shanty, 2003) and providing cool water were found to be effective in alleviating the heat load of rabbits. In addition, eCG, having follicle stimulating and leuteinizing actions, is commonly used to improve reproductive performance of does, through maturation of ovarian follicles and sexual receptivity (Maertens *et al.*, 1995). Therefore, this study aimed to verify whether productive, reproductive and physiological performance of female rabbit does could be improved during summer season "hot climate" by using vitamin C and cool water, either alone and in combination with eCG treatment.

MATERIALS AND METHODS

Experimental animals

This work was carried out at the laboratory animal unit, Department of Animal Hygiene and Management, Faculty of Veterinary Medicine, Cairo University from June to August 2005 (period of hot summer months).

A total number of sixty non pregnant, non lactating, New Zealand White rabbit does were used, parturient since April 2005 as first parity, of 8-9 months age with an average initial weight of 2.5±0.31 kg. The does were assigned to three groups according to drinking water treatment as follow: 1) Fresh tap water without any supplementation (control, group C), 2) Cooled drinking water at 10-15ºC (Group CW) was provided between 10.00 a.m and 17.00 p.m., and 3) Fresh tap water supplemented daily with ascorbic acid (1 g/L) (group vitamin C) according to Al-Shanty (2003).

Twenty four hours before mating, does of each group were randomly divided into two subgroups (H and NH): H subgroup received an injection of eCG (40 IU/doe; Folligon, Intervet, The Netherlands), while NH received no hormonal treatment. Cooled drinking water and ascorbic acid treatments were initiated 10 days before the day of mating and continued throughout the experimental period till weaning of young (at 30-35 days).

The does were kept individually in flat-deck cages of galvanized wire net, equipped with automatic drinkers, feeding hoppers and movable next boxes. The ambient temperature and relative humidity ranged from 27 to 35ºC and 70-80%, respectively. All the groups were maintained under similar management and hygienic conditions and 16L:8D photoperiod throughout the experimental period.

Does were fed a balanced pelleted diet, formulated to meet the nutritional requirement for pregnancy and lactation according to the quantities recommended in NRC (1977). The diet contained 2660 (kcal/kg), 17.24% crude protein and 14.77% crude fiber. Does were bred naturally to adult fertile bucks in the same rabbitry with a 5:1 female: male ratio.

The following productive and reproductive parameters were recorded for all groups according to the recommendations of the IRRG (2005).

Productive parameters

- Live body weight, initial and final for non pregnant does (FLBW) and feed consumption were both recorded individually bi-weekly throughout the experiment period (12 weeks) regardless the stage of pregnancy and lactation.
- Performance index (PI) was calculated according to North (1981) as follows:

  PI= Final live body weight × 100/Feed conversion.
- Doe weight at mating and kindling.
- Litter weight and size of total kits born and individual kit weight at kindling and weaning (30-35 days).
- Total body weight gain for kit (from birth till weaning) and total pre-weaning deaths (%).

Reproductive parameters

- The conception rate was calculated as results of pregnancy diagnosis at first mating attempt for does that had accepted the male (Ahmed *et al.*, 2005).

Collection and classification of oocytes

At the end of the experiments, five non pregnant does from each group were slaughtered for collection of oocytes. The ovaries were rinsed several times in warm (30-38ºC) phosphate buffer saline (PBS, pH 7.2). Oocytes were harvested in aspiration media consisting of modified phosphate buffer saline supplemented with 3% heat-inactivated fetal calf serum or 3 mg/mL bovine serum albumin, fraction V, and 50 µg/mL gentamycin. Oocytes were collected by follicular puncture in which the visible follicles were punctured with a 22 gauge needle in sterile Petri-dishes containing the aspiration media. Oocytes were recovered under low power magnification (10×) by sterile Pasteur pipettes of suitable diameter. Oocytes were washed 2-3 times in TCM 199 with Earle`s salt (Gibco) and 10% fetal calf serum. The oocytes were evaluated morphologically according to the criteria of cumulus cell layers into four categories: class A, completely intact with cumulus; class B, partially intact with cumulus cells; class C, denuded oocytes, and class D, degenerated oocytes.

In vitro maturation of oocytes

The recovered oocytes with completed or partially intact cumulus cell layers were selected for maturation *in vitro*. The maturation media was TCM 199 (Earle`s salt) supplemented with 10% fetal calf serum and 50 µg/mL gentamycin. The oocytes were cultured in a 50 µL microdrop of TCM 199 covered with mineral oil (Sigma, M-8410, USA) for 26 h at 39ºC in an atmosphere containing 5% CO_2 and 95% relative humidity.

Evaluation of nuclear maturation

At the end of the culture period, the oocytes were incubated for 30-60 seconds in a solution of 0.25% trypsin and 0.02 M EDTA to remove the cumulus cells by pipetting. Chromosome slides were prepared according to the procedure described by Tarkowski (1966). After removing the cumulus cells, oocytes were transferred to 1% sodium citrate and fixed in a (3:1 v/v) solution of methanol:glacial acetic acid. Slides were stained with 1% acetic orcein. The rate of nuclear maturation was determined according to Di Berardino *et al.*, (1999). Oocytes that had reached haploid and diploid metaphase II were considered in the stage of nuclear maturation.

Statistical analysis

The results were analyzed statistically by using two-way analysis of variance (water treatment and hormonal injection as main effects) according to the Statistical Analysis System, SAS (1996). The differences among means were partitioned using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive parameters

Results of the growth performance records are presented in Table 1. The results showed that supplementing with either vitamin C or cooled drinking water significantly improved FLBW and increased daily feed consumption when compared with the control group, but the Vitamin C feed additive did not have a significant improving effect on the performance index percentage. On the other hand, Yousef *et al.* (2003) have indicated that vitamin C supplementation in drinking water increased feed intake, but body weight gain was not significantly affected. These results tend to agree with those of Ismail *et al.* (1992b), who found that adding ascorbic acid had a favorable effect on FBW and TWG in rabbits. Similar results were reported by Hilton (1983) and Ismail *et al.* (1992a). Likewise, Lebas (2000) showed that vitamin C supplementation (25 to 30 mg per rabbit daily) can help the rabbit in stressful situations (e.g. heat stress).

Regarding hormonal treatment (Table 1), significant differences were observed between groups treated with hormones and those untreated concerning growth performance parameters, which did not improve due to hormonal treatment. However, a significant interaction between treatment groups and hormonal effect was noticed in all test performance parameters, indicating that using either vitamin C or cooled water succeeded in alleviating the heat load of rabbit does and subsequently improved productive performance parameters.

Reproductive parameters

Reproductive performance of rabbit does is shown in Table 2. The adverse effects of summer heat stress on conception rate could be attributed to the reduced development of mature ovarian follicles. These data coincide with the findings of the present work related to the decreased number and reduced percentages of developing oocytes in the control group. However, cooled drinking water as well as vitamin C supplementation significantly improved both overall mean receptivity and conception rate, as compared to control.

Cooled drinking water thus has beneficial effects on the homeostasis of the does, and probably improves physiological functions by bringing the animals closer to normal conditions (Habeeb *et al.*, 1992).The enhancement of reproductive performance obtained here in the vitamin C group is similar to that reported by Gadallah *et al.* (2004) in does fed a diet supplemented with 2 g of vitamin C/kg.

Hormonal treatment did not improve the conception rate (Table 2). This result may be attributed to the follicle stimulating effect of eCG (Seleem, 2003), which improves receptivity through elevated estrogen levels. Theau-Clément and Lebas (1994) also noticed increased estrus behavior in female rabbits after eCG treatment.

Compared to the control, both provision of cooled drinking water and vitamin C supplementation, regardless of hormonal treatment, enhanced ($P<0.05$) litter size and weight at both kindling and weaning (Table 3). Such improvements may be attributed to the perspective of the potential benefit derived by modulating the neuroendocrine system during early development (Theau-Clément, 2000; 2007).

Table 1: Growth performance parameters (mean±standar error) of rabbit does as influenced by Vit. C and cooled drinking water supplements.

	Initial weight (g)	Final weight (g)	Daily weigth gain (g/d)	Daily feed intake (g/d)	Feed conversion	Performance index (%)
Treatment Effect (T)[1]						
Vit.C	2505±33.7	3082^{b}±31.0	6.87^{b}±0.27	162.73^{b}±0.54	24.11^{ab}± 0.94	13.01^{b}±0.57
CW	2518±20.6	3167^{a}±21.7	7.73^{a}±0.21	166.50^{a}±0.56	21.72^{b}±0.57	14.71^{a}±0.45
C	2472±26.3	2995^{c}±16.2	6.23^{b}±0.26	157.47^{c}±0.55	25.76^{a} ±1.02	11.84^{b}±0.49
Hormonal Effect (H)[2]						
NH	2500±18.2	3093^{a}±23.2	7.06±0.25	162.58±0.98	23.51±0.83	13.46±0.52
H	2496±26.2	3069^{b}±27.4	6.81±0.24	161.89±1.02	24.22±0.77	12.91±0.46
T×H Effect						
Vit. C×NH	2492±31.4	3072^{bcd}± 37.4	6.90^{ab}±0.46	163.06^{b}±0.44	24.11^{ab}±1.50	13.04^{abc}±1.00
Vit. C×H	2518±52.3	3092^{abc}± 52.3	6.82^{ab}±0.34	162.39^{b}±1.03	24.11^{ab}±1.30	12.99^{abc}±0.66
CW×NH	2538±24.0	3192^{a} ±24.0	7.78^{a}±0.29	166.67^{a}±1.08	21.56^{b}±0.80	14.91^{a}±0.60
CW×H	2798±35.2	3142^{ab}±35.2	7.67^{a}±0.33	166.33^{a}±0.45	21.88^{b}±0.88	14.50 ^{ab}±0.70
C×NH	2470±61.7	3017^{cd}±61.7	6.51^{b}±0.44	158.00^{c}±0.90	24.85^{ab}±1.70	12.43 ^{bc}±0.85
C×H	2473±26.1	2973^{d} ±26.1	5.94^{b}±0.27	156.94^{c}±0.65	26.67^{a}±1.16	11.24 ^{c}±0.45

[1] Treatment: Vit. C, group supplemented with ascorbic acid; CW, group receiving cooled drinking water; C, control group.
[2] Hormonal effect: NH, no hormonal treated group; H, eCG treated group.
Means in the same column and effect with different letters are significantly different ($P<0.05$).

Table 2: Doe weights at mating and kindling, and reproductive traits in control and treated groups during the hot season (mean±standard error), receiving eCG (H) or no hormonal injection (NH).

	Treatments			
	Control	Vitamin C	Cool water	Mean
Doe weight at mating, g				
NH	2586±66.7	2646±75.4	2690±56.4	2567±60.7
H	2630±58.9	2550±83.9	2520±64.7	2639±58.5
Mean	2600±45.9	2598±71.4	2610±48.1	
Doe weight at kindling, g				
NH	2860±44.0	3126±68.7	3090±82.6	3025±55.6
H	2890±55.3	3110±72.1	2890±52.2	2963±49.5
Mean	2875^{b}±46.7	3118^{a}±61.2	2990^{b}±50.3	
Conception rate %				
NH	37.5	70.0	80.0	58.5
H	50.0	40.0	70.0	53.6
Mean	43.7^{c}	55.0^{b}	75.0^{a}	

Means with different superscript in the same row for the same item differ significantly ($P<0.05$).

Kits showed the highest weight gain in the vitamin C group (287.5 g), followed by the cooled water group (253.0 g) and control (221.3 g), as shown in Table 3. Moreover, the pre-weaning death percentage was lower in both cooled water and vitamin C groups. It should be noted here that vitamin C scavenges free O_2 radicals, so preventing the oxidative stress of the cell membrane of the digestive system and restoring efficient feed utilization (Abou-Zeid *et al.*, 2000). This vitamin C action mechanism could partially explain the increased pre-weaning weight gain, as well as the heavier weaning weights and lower pre weaning deaths.

Oocyte meiotic chromosomes

The average numbers of collected oocytes per ovary in both control and treated groups are shown in Table 4. Neither treatments nor eCG injection had any influence on the number of rabbit oocytes. In the present study, the number of oocytes harvested was lower than that found by Mahmoud and Ezzo (2004) and Mahmoud *et al.* (2006). The lower number in our case can be attributed to the heat stress of the hot summer season. With regard to this, Datta and Goswami (1998) obtained poor recovery rates of oocytes in buffaloes in the hot months due to the relatively inactive status of ovaries during that time.

As shown in Table 4, the quality of oocytes was lower in the control group than in treated groups. The percentage of oocytes suitable for maturation significantly decreased and the percentage of denuded oocytes increased ($P<0.05$) in the control group, as compared to the other two treatments. These results are in agreement with those of Mahmoud (2001) and Mahmoud and Eashra (2004), who noticed that the quality of oocytes was significantly lower in the hot than in the cool season. In our study, regardless of eCG treatment, both vitamin C supplementation as well as provision of cooled drinking water significantly improved the quality of oocytes (Table 4).

The percentages of mature oocytes were significantly reduced ($P<0.05$) in the control group. Our *in vitro* results coincide with data demonstrating that the maturation rate in buffaloes was significantly decreased

Table 3: Litter and kit traits from birth till weaning in both control and treated groups during the hot season (mean±standar error)[1].

	Treatments			
	Control	Vitamin C	Cool water	Mean
Litter size at kindling				
NH	4.70±0.36	6.25±0.41	5.95±0.32	5.62±0.31
H	5.20±0.25	5.00±0.21	5.80±0.42	5.33±0.23
Mean	4.93^{b}±0.21	5.65^{a}±0.28	5.88^{a}±0.36	
Litter size at weaning				
NH	3.16±0.19	5.90±0.36	5.50±0.52	4.85±0.51
H	4.00±0.28	4.67±0.50	5.80±0.44	4.82±0.36
Mean	3.58^{b}±0.21	5.29^{a}±0.32	6.65^{a}±0.46	
Litter weight at kindling, g				
NH	278±32.1	410±43.6	369±41.7	348±33.0
H	305±36.4	369±52.2	355±34.3	344±41.0
Mean	292^{b}±30.6	389^{a}±40.7	363^{a}±33.3	
Litter weight at weaning, g				
NH	878±44.2	1953±38.2	1788±48.2	1538±39.6
H	1132±58.7	1719±39.4	1750±65.4	1554±42.3
Mean	1005^{b}± 36.2	1845^{a}±25.4	1770^{a}±39.6	
Kit weight at kindling, g				
NH	59.40±0.41	65.60±0.48	62.17±0.68	62.39±0.39
H	56.92±0.44	73.80±0.67	61.33±0.72	64.02±0.45
Mean	58.90^{b}±0.36	69.70^{a}±0.42	61.70^{ab}±0.58	
Kit weight at weaning, g				
NH	276.8±12.56	345.9±25.10	325.3±23.76	312.0±16.00
H	283.0±22.00	368.0±39.10	301.6±20.16	317.3±24.60
Mean	280.0^{c}±15.30	356.5^{a}±25.10	313.0^{b}±16.81	
Kit weight gain, g				
NH	223.0±18.60	280.6±30.00	263.4±25.21	256.0±22.40
H	219.6±22.90	295.0±26.13	240.0±32.14	252.2±18.43
Mean	221.3^{c}±15.72	287.5^{a}±24.00	253.0^{b}±20.10	
Pre-weaning death (%)				
NH	32.48	5.60	0.00	15.60
H	23.08	6.60	7.56	9.89
Mean	27.80^{b}	6.61^{a}	3.78^{a}	

[1] Hormonal effect: NH, no hormonal treated group; H, eCG treated group.
Means with different superscript in the same row for the same item differ significantly ($P<0.05$).

Table 4: Recovery and quality of rabbit oocytes during the hot season.

Treatment effect	Hormonal effect[1]	No. oocytes	No. oocytes per ovary	Classification of oocytes			
				Class A+B		Class C+D	
				No	%	No	%
Control	NH	71	7.0±0.28	46	64.15±1.48[a]	25	35.83±1.48[a]
	H	74	7.2±0.35	57	62.97±2.25[a]	32	37.02±2.25[a]
Vitamin C	NH	83	8.2±0.52	60	73.07±2.79[b]	23	26.92±2.79[b]
	H	75	7.2±0.44	57	75.14±1.17[b]	18	24.85±1.86[b]
Cool water	NH	79	7.9±0.50	58	73.56±1.09[b]	21	26.42±1.09[b]
	H	76	7.5±0.29	58	74.89±1.97[b]	18	25.1±1.97[b]

[1] Hormonal effect: NH, no hormonal treated group; H, eCG treated group.
The results are expressed as Mean±SEM of three replicates. Numbers of ovaries=10 for each treatment. Values with different superscript within the same column differ significantly ($P<0.05$).

($P<0.01$) in hot versus cool seasons (Hamam *et al.*, 2001). On the contrary, Brück *et al.* (1996) in equine and Rivera *et al.* (2000) in bovine, did not find any effect of season on *in vitro* production of embryos in temperate or subtropical environments. The variation in response to the season could be attributed to the individual capability of different species or breeds for heat- tolerance.

Maturation rate was improved by vitamin C treatment (Table 5). In this study, vitamin C supplementation in combination with eCG induction of estrous was found to improve the quality and meiotic maturation of rabbit oocytes and to overcome the detrimental effect of the environment during the hot season. In another study, the negative effect of high altitude on ovine fertility was prevented by administration of antioxidant vitamins C and E (Parraguez *et al.*, 2006).

In this study, the hormonal treatment did not significantly improve the maturation rate of oocytes, either with vitamin C or with cooled drinking water (Table 5).

It can therefore be concluded that providing rabbit does with cooled drinking water or ascorbic acid is advisable in hot climates.

Table 5: Nuclear maturation of rabbit oocytes during the hot season.

Treatment effect	Hormone effect[1]	No. cultured oocytes	No. fixed oocytes	No. Metaphase II	Nuclear maturation
Control	NH	46	42	27	64.29± 1.49[a]
	H	57	41	27	66.00±0.65[a]
Vitamin C	NH	60	50	37	73.66±2.00[b]
	H	57	48	38	80.20±2.48[b]
Cool water	NH	52	40	26	65.41±2.47[a]
	H	55	45	31	67.73±3.92[ab]

[1] Hormonal effect: NH, no hormonal treated group; H, eCG treated group.
The results are expressed as Mean±SEM of three replicates. Values with different superscript within the same column differ significantly ($P<0.05$).

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