EFFECT OF DIETARY ASCORBIC ACID AND BETAINE SUPPLEMENTATION ON SEMEN CHARACTERISTICS OF RABBIT BUCKS UNDER HIGH AMBIENT TEMPERATURE

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ABSTRACT

The objective of this study was to evaluate the effects of dietary ascorbic acid and betaine supplementation on semen characteristics and some blood constituents of APRI line rabbit bucks under high ambient temperature. A total of 24 APRI line rabbit bucks were randomly divided into four experimental treatments (6 each). Animals were fed *ad libitum* the basal diet supplemented without (control), with 250 mg ascorbic acid /kg diet or betaine at 0.5 and 1 g/kg diet. Animals were provided with water freely. The average daily temperature and relative humidity inside the rabbitry were 32.5±4.5 °C and 75.5±3.5%, respectively. Under heat stress conditions, dietary ascorbic acid and betaine supplementation caused an increase ($P<0.05$) in ejaculation volume, where 1 g of betaine was more effective ($P<0.05$) than 0.5 g of betaine. Sperm concentration ranged from $180.7x10^6$ cell/ml to $214.8 x10^6$ cell/ml. Supplementing ascorbic acid and different levels of betaine in diets increased ($P<0.001$) sperm concentration, without differences ($P>0.05$) between ascorbic acid and 0.5 g betaine. Sperm motility was improved ($P<0.01$) with supplementing ascorbic acid and both doses of betaine in diets. The highest motility rate was recorded with rabbit bucks fed 1 g betaine/kg diet as 49.67% and the lowest motility was detected in those fed control diet as 37.83%. Ascorbic acid and different levels of betaine decreased ($P<0.001$) dead sperm, without differences between ascorbic acid and both doses of betaine. Fertility rate was increased with supplementing dietary ascorbic acid or betaine without any significance except for 1 g betaine, which recorded the highest value. With respect to the blood serum constituents in rabbit bucks serum total protein, albumin, globulin and total lipids significantly increased when ascorbic acid or betaine were supplemented. Betaine and ascorbic acid supplementation significantly decreased serum glucose concentration. Liver functions as judged by liver enzymes (e.g., GOT and GPT) were significantly decreased by dietary ascorbic acid or betaine supplementation. In this study, 1g betaine supplementation in rabbit bucks diet significantly improves semen quality and modify some blood constituents under high ambient temperature during summer in Egypt. If the results are confirmed on a larger scale, it could be therefore concluded that 1g betaine supplementation in the diet, could be advisable in hot climates.

Keywords: Rabbit, ascorbic acid, betaine, heat stress, semen characteristics.

INTRODUCTION

In the subtropics, heat stress is a major problem that adversely affects rabbit's performance and physiological traits. 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 (Fouad, 2005). Exposure of male rabbits to heat stress conditions in subtropical Egypt, showed significant adverse effects on most of the reproductive traits. The drastic changes that occur in rabbits' biological functions are depression in feed intake, feed efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Marai *et al.,* 2002b).

Betaine is a common term for trimethylglycine, substrate for Bet-homocysteine methyltransferase in the liver and kidney (Kettunen *et al.,* 2001). When the three methyl groups were transferred to homocysteine to produce methionine, betaine become the amino acid glycine then it is metabolized as normal (Graham, 2002). Betaine is often found in high concentrations in plants subjected to drought, and this is due to the water balance or osmoregulatory property (Kettunen *et al.,* 2001; Graham, 2002). However, betaine is not present in large quantities in animal feedstuffs (Wang *et al.,* 2004). Thus, betaine is a multi-nutritional agent that may help rabbit does to resist poor management and heat stress. Vitamin C (ascorbic acid) is one of the most widely studied vitamins used to alleviate heat stress in rabbits. Moreover, ascorbic acid promoted growth, reproduction and counteracted infections by pathogenic bacteria and viruses. Qota *et al.* (2008) found that supplementation of betaine at 1g/kg and ascorbic acid at 250 mg/kg diets was equally potent for partial relief of heat stress effects on the performance for slow-growing chicks.

The objective of this study was to evaluate the effects of dietary ascorbic acid and betaine supplementation on semen characteristics and some blood constituents of APRI line rabbit bucks under high ambient temperature.

MATERIALS AND METHODS

Animals and experimental design

This study was carried out at the Rabbits Farm of Sakha Station, Animal Production Research Institute, Agriculture Research Center, Egypt, during the period from June 2011 to September 2011. Twenty four APRI line rabbit bucks (Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.,* 2010) of about 8-9 months with weights ranging from 3.150 to 3.275 Kg were randomly distributed into 4 groups (6 bucks/ each diet). Four experimental diets were used in this experiment. The basal diet composition (Table 1) was formulated to cover all essential nutrient requirements for adult rabbits according to De Blas and Mateos (1998). Chemical analyses of the basal diet were carried out according to AOAC (2000) for crude protein, crude fiber, organic matter and ether extract. Animals were fed *ad libitum* the basal diet without (control), with 250 mg ascorbic acid/kg diet (a heat stabilized product produced by Hoffmann-La Roche) or betaine at 0.5 and 1g/kg diet. Betaine was provided as Betafin®-BP (betaine anhydrous/pharmaceutical grade, Finnfeeds Finland Ltd.).

Table 1: Composition and chemical analysis of the basal diet.

Ingredient	%	Chemical analysis (% as DM):	%
Berseem hay (*Trifolium alexandrinum*)	30.05	Dry matter (DM)	85.81
Barley grain	24.60	Crude protein (CP)	17.36
Wheat brain	21.50	Organic matter (OM)	91.42
Soybean meal (44% CP)	17.50	Crude fiber (CF)	12.37
Molasses	3.00	Ether extract (EE)	2.229
Limestone	0.95	Metabolizable energy (ME, kcal/kg)(2)	2257
Di-calcium phosphate	1.60	Calcium(2)	1.243
Sodium chloride	0.30	Phosphorus(2)	0.808
Mineral-vitamin premix(1)	0.30	Methionine(2)	0.454
DL-Methionine	0.20	Lysine(2)	0.862

(1)Mineral–vitamin premix provided the following per kilogram of diet: vitamin A, 150,000 UI; vitamin E, 100 mg; vitamin K3, 21mg; vitamin B1, 10 mg; vitaminB2, 40mg; vitamin B6, 15mg; pantothenic acid, 100 mg; vitamin B12, 0.1mg; niacin, 200 mg; folic acid, 10mg; biotin, 0.5mg; choline chloride, 5000 mg; Fe, 0.3mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1mg; and Zn, 450mg.

(2)Calculated according to **De Blas and Mateos (1998)**

A cycle of 16 hours of light and 8 hours of dark were used throughout the experiment. All bucks were kept under the same managerial conditions. Animals were housed in individual cages provided with feeders, automatic nipple drinkers. The building was open air with electric exhaust fans on the sides.

During the experimental period, ambient temperatures and relative humidity were measured in the rabbitry twice a day at 06:00 h and 15:00 h. Means of ambient temperature, relative humidity and temperature humidity index (THI) inside the building were 32.5±4.5 °C, 75.5±3.5% and 31.1, respectively, which indicate severe heat stress. According to Marai *et al.* (2002a) there is very severe heat stress when THI is higher than 30.0. The THI was calculated according to Marai *et al.* (2001):

THI = db°C–[(0.31–0.031RH) × (db°C–14.4)]

Where, db°C is dry bulb temperature in Celsius degrees, and RH is the relative humidity as a percentage.

Measurements

After a period of 21 days of adaptation to each diet, semen was individually collected from the bucks 3 times weekly for a period of 6 weeks by using an artificial vagina. Ejaculate volume (ml), sperm motility (%), sperm concentration (x 10^6 /ml) and dead spermatozoa (%) were determined according to El-Gaafary (1987). Blood samples (3 ml from each rabbit) were collected at the end of experimental period from marginal ear vein in vacutainer tubes to determine blood components. Serum was separated by centrifugation at 5,900×g for 10 min and frozen at −20 °C until analysis. Blood serum total proteins, albumin, total lipids, total cholesterol, glucose and activity of serumglutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were colorimetrically determined using commercial kits (purchased from Bio-diagnostic, Egypt) according the manufacturers' instructions. Globulin was calculated as the difference. After semen evaluation, pools were stored at 18 ˚C for a maximum of 24 h prior to artificial insemination. A total of 180 inseminations (45 does per group) were carried out on multiparous lactating and non-lactating does with semen from 36 pools (9 pools each group) obtained from 216 ejaculates from 24 bucks. Does were fed control diet without any supplementation and treated with subcutaneous applications of 12–15 IU of eCG (FolligonR, Intervet, Holland) for oestrous induction 48 h before AI. The does were inseminated with 0.5 ml semen from pools with a concentration of 30_106 cells/ml. Ovulation of does was immediately induced after AI by an intramuscular injection of 0.8 mg Busereline acetate (SuprefactR, Hoechst-Roussel, Germany).

Statistical analysis:

Data were subjected to analysis of variance, using the general linear GLM procedure of SAS program (SAS, Institute, Inc., 1985). The application of the least significant ranges among the different treatment means was done according to Duncan (1955). The fertility rate was analysed by chi square test.

RESULTS AND DISCUSSION

Rabbit bucks semen characteristics are presented in Table 2. The results indicated that dietary ascorbic acid and 1g betaine supplementation caused an increase ($P<0.05$) in ejaculation volume. Sperm concentration ranged from 180.7x10^6 cell/ml to 214.8 x10^6 cell/ml. Supplementing ascorbic and different levels of betaine in diets increased ($P<0.001$) sperm concentration, without differences between ascorbic acid and 0.5 g betaine. The highest concentration was obtained for 1g betaine supplementation. Sperm motility was improved ($P<0.01$) with supplementing ascorbic acid and 1g betaine in diets. The highest motility rate was recorded with rabbit bucks fed 1 g betaine/kg diet as 49.67% and the lowest motility was detected with those fed control diet (37.83%). These results are in agreement with Johnson and Zeisel (2010) who indicated on mouse, that betaine (which quantity?)plays an important role in sperm cell motility apart from normalizing ATP concentration, perhaps through its role as an osmolyte. In the present study, ascorbic acid and different levels of betaine decreased ($P<0.001$) dead sperm, without differences between ascorbic acid and both doses of betaine. Fertility rate was increased with supplementing dietary ascorbic acid or betaine without any significance except for 1 g betaine, which recorded the highest value. Yousef *et al.* (2003) indicated that supplementation of 1.5 g ascorbic acid/l drinking water reduced the production of free radicals and can improve rabbit semen quality. Recently, Ezzat *at al.* (2011) found that supplementation of betaine or vitamin C in poultry diets, under hot summer condition, significantly ($P<0.05$) increased sperm

motility and decreased dead spermatozoa compared with the control group. The values obtained in the present study with control diet are within the normal ranges of the ejaculate volume, sperm cell concentration, sperm motility and dead sperm in rabbit bucks under heat stress (Marai *et al.,* 2002a). The adverse effect of heat stress on spermatogenic processes decreases in the total number of spermatozoa in the ejaculate, which is of great importance in fertility of the male (Marai *et al.,* 2002b).

Table 2: Effect of experimental diets on semen quality and some blood constituents of APRI line rabbit bucks.

Items	Number of observations (per group)	Control	Ascorbic acid 250 mg/kg	Betaine level		P-value	SEM
				0.5 g/kg	1 g/kg		
Number of animal (per group)	-	6	6	6	6	-	-
Ejaculation volume (ml)	108	0.44^{B}	0.57^{A}	0.56^{AB}	0.66^{A}	0.0134	0.042
Sperm concentration (X 10^6 /ml)	108	180.7^{C}	198.8^{B}	197.5^{B}	214.8^{A}	0.0003	4.086
Sperm motility (%)	108	37.83^{B}	45.17^{A}	43.83^{AB}	49.67^{A}	0.0067	2.035
Dead sperm (%)	108	28.33^{A}	22.33^{B}	22.67^{B}	19.67^{B}	0.0004	1.166
Fertility rate (%)(1)	45	68.50^{B}	73.33^{B}	72.67^{B}	80.33^{A}	-	2.320
Blood constituents:							
Total protein (g/dl)	6	5.23^{B}	5.70^{AB}	5.63^{AB}	6.18^{A}	0.0291	0.202
Albumen (g/dl)	6	2.90^{B}	3.10^{AB}	3.02^{AB}	3.30^{A}	0.0862	0.123
Globulin (g/dl)	6	2.33^{C}	2.60^{B}	2.61^{B}	2.88^{A}	0.0027	0.087
Total lipids (mg/dl)	6	2.88^{B}	3.28^{A}	3.30^{A}	3.55^{A}	0.0120	0.126
Cholesterol (mg/dl)	6	92.33	93.67	93.50	95.50	0.7872	2.143
Glucose (mg/dl)	6	134.67^{A}	123.50^{B}	124.00^{B}	118.17^{B}	0.0016	2.469
GOT (IU/l)	6	33.17^{A}	29.00^{AB}	28.83^{AB}	26.83^{B}	0.0419	1.461
GPT (IU/l)	6	26.33^{A}	24.83^{AB}	24.67^{AB}	23.67^{B}	0.0637	0.795

SEM = Standard error of means,
$^{A, B, C}$ Means in the same row with different superscripts are significantly different ($P<0.05$).
(1) As number of kindling /number of mating x 100

With respect to the effect of dietary ascorbic acid and betaine supplementation on blood serum constituents in rabbit bucks under high ambient temperature, serum total protein, albumin, globulin and total lipids were significantly increased with 1g betaine supplementation. The increase in serum total protein, albumin and globulin due to 1g betaine supplementation could be associated with its ability as a methyl group donor which is fairly consistent in protein metabolism (Kidd *et al.,* 1997). These results are in agreement with Hassan *et al.* (2011) who postulated that betaine had increased ($P<0.05$) average serum total protein and globulin of growing rabbits.

Betaine and ascorbic acid supplementation significantly decreased serum glucose concentration. It is well established that under stress conditions, glucose uptake of the cells is suppressed and the level of serum glucose is increased in order to provide adequate amounts of glucose to the sensitive organs such as heart and brain (Lillehoj *et al.,* 1992). This assumption agrees with Huang *et al.* (2006) who documented that dietary betaine reduced serum glucose concentration due to elevating of the circulating insulin concentration in pigs. In the present study, liver functions as judged by liver enzymes (e.g., GOT and GPT) were significantly decreased by 1g betaine supplementation. Yousef and Zeitoun (1998) found that there were negative correlation coefficients between sperm motility on one side and GOT and GPT release on the other side. They reported that the activities of these enzymes could be used as an indicator of sperm integrity. Moreover, Yousef *et al.* (2003) observed that vitamin C supplementation in drinking of mature male rabbits significantly decreased the activities of GOT and GPT. Recently, Hassan *et al.* (2011) postulated that, in growing rabbits, betaine treatment significantly decreased GOT levels. Therefore, it can be hypothesized that 1g betaine supplementation helped in keeping serum transaminase enzymes within the normal ranges during heat

stress conditions. The results obtained in blood serum constituents were in adequation with those of semen characteristics.

CONCLUSIONS

In this study, 1g betaine supplementation in rabbit bucks diet significantly improves semen quality and modify some blood constituents under high ambient temperature during summer in Egypt. If the results are confirmed on a larger scale, it could be therefore concluded that 1g betaine supplementation in the diet, could be advisable in hot climates.

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