EFFECT OF DIETARY ASCORBIC ACID AND BETAINE SUPPLEMENTATION ON PRODUCTIVITY OF RABBIT DOES 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 productivity and milk production of APRI line rabbit does under high ambient temperature. A total of 24 APRI line rabbit does, 8-9 months old, were divided into four experimental treatments (6 each). Rabbit does 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. In another trial, twenty lactating rabbit does (five per diet) were used to measure milk production. Under heat stress conditions, pregnant rabbit does given diet containing 1 g betaine /kg diet showed the highest ($P<0.01$) weight gain than those fed other experimental diets during the pregnancy period. During the lactation period, the rabbit does irrespective of the experimental diets lost weight ranging from 93 to 113 g, which was about 3.60% of their initial live body weight with low losses for rabbit does fed diet containing 1 g betaine/kg diet. The rabbit does fed diets containing ascorbic acid or betaine weaned more rabbits than those fed control diet (5.73 *vs.* 3.71; $P<0.001$, as average). Rabbit does fed 1 g betaine/kg diet recorded the highest values of litters weights, during the first three weeks. The pre-weaning mortality rate was higher in the control diet compared to diets with 250 mg ascorbic acid/kg diet or 0.5 and 1 g betaine/kg diet (36.16% *vs.* 20.21, 20.39 and 13.81%; $P<0.001$, respectively). Fertility rate was increased by supplementing 1 g betaine /kg diet. On the other hand, daily feed intake of pregnant and lactating rabbit does increased ($P<0.01$) with supplementing 1 g betaine /kg diet. Supplementing dietary ascorbic acid and betaine increased ($P<0.001$) feed intake of kits in the last 9 days of the lactation period, where kits fed diet containing 1 g betaine /kg diet recorded the highest value. Rabbit does given diets with ascorbic acid or betaine showed a better ($P<0.01$) milk index. Moreover, feed conversion ratio was significantly improved ($P<0.001$) by supplementing ascorbic acid or betaine in diets during the first three weeks of the lactation period, where rabbit does fed 1 g betaine/kg diet recorded the best value. Also, the feed conversion ratio was better ($P<0.001$) for diets supplemented with ascorbic acid or betaine during the last 9 days of lactating period. Betaine at a level of 1g/kg caused an increase ($P<0.05$) in milk production whereas milk yield increased ($P<0.05$) by 26.2 and 42.8% in rabbit does fed 0.5 and 1 g betaine /kg diet, respectively. The same trend was observed for milk production in relation to week of lactation, which was increased only with 1g/kg betaine level throughout the lactation period. In this study, 1g betaine supplementation in rabbit does diet significantly improves productivity and milk production 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, Reproductive performance.

INTRODUCTION

Climatic heat is the major factor restricting animal productivity. Rabbits are very sensitive to high environmental temperature, where the dense fur and lack of sweat glands make heat loss very difficult above the zone of thermal neutrality. In female rabbits, conception rate, embryonic development, litter size, litter weight and milk production decrease and age at puberty and pre- and post-weaning mortality increase by exposure to heat stress. The drastic changes that occur in rabbits' biological functions are depression in feed intake and feed efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites (Marai *et al.,* 2002).

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 productivity and milk production of APRI line rabbit does 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. Twenty four multiparous lactating and non-lactating APRI line rabbit does (Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.* 2010) of about 8-9 months old with an average live body weight of 2913 g, were used during a period from 1st May 2011 to 30th September 2011. Four experimental diets (6 does per diet) were used in this experiment. The basal diet composition (Table 1) was formulated to cover all essential nutrient requirements for rabbit does 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. Rabbit does were fed *ad libitum* the basal diet without (control), with 250 mg ascorbic acid (a heat stabilized product produced by Hoffmann-La Roche) /kg diet or betaine at 0.5 and 1g/kg diet. Betaine was provided as Betafin®-BP (betaine anhydrous/pharmaceutical grade, Finnfeeds Finland Ltd.). Rabbit bucks received control diet without any supplementation.

Sex ratio was included to give a female: male ratio 3: 1 throughout the experiment. A cycle of 16 hours of light and 8 hours of dark were used throughout the experiment. All does were kept under the same managerial conditions and were presented to the males 7 days after parturition. Ten days after mating, the does were tested for pregnancy by abdominal palpation. Non-pregnant rabbit does were remated directly after abdominal palpation. The experimental period included three reproductive cycles. Feed intake of rabbit does was recorded daily and the weight of litters was measured weekly. Suckling kits were allowed to eat the same diet as their mother at the 21st day of lactation and were weaned at 30 days of age. During this period, solid feed intake of litters was recorded. Animals were housed in individual cages provided with feeders, automatic nipple drinkers and nest boxes. 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.

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; Vitamin B2, 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).

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. (2002) there is very severe heat stress when THI is higher than 30.0. The THI was calculated according to Marai et al. (2001):

$$THI = db°C - [(0.31 - 0.031RH) \times (db°C - 14.4)]$$

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

In another trial, twenty lactating rabbit does (five per diet) were used to measure milk production. Rabbit does were separated from their kits after parturition and controlled suckling was applied. To prevent free nursing, does were placed in cages next to the nest box. Suckling took place once a day, (around 09.00) in the nest box, for a short period (8 to 10 min.). Litter size of eight kits was kept constant throughout lactation and dead kits were replaced daily by kits of a similar weight and age provided from nurse does. Milk production was estimated daily from weight loss of rabbit does after suckling.

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

The effects of experimental diets on the productivity of rabbit does are shown in Table 2. It is clear that pregnant rabbit does given diet containing 1 g betaine /kg diet showed the highest ($P<0.01$) weight gain than those fed other experimental diets during the pregnancy period. The increase in the weight gain was due to the foetal growth as evident by almost similar body weight of rabbit does at the mating and partum (Parigi Bini *et al.,* 1991). During the lactation period, the rabbit does irrespective of the experimental diets lost weight ranging from 93 to 113 g, which was about 3.60% of their initial live body weight with low losses for rabbit does fed diet containing 1 g betaine/kg diet. The fat and energy balance are always negative in the lactating rabbit does (Xiccato, 1996).

Both size and weight of litters at the birth, 21 days and weaning (30 days) were improved by supplementing dietary ascorbic acid or betaine. The rabbit does fed diets containing ascorbic acid or betaine weaned more rabbits than those fed control diet (5.73 *vs.* 3.71; $P<0.001$, as average). Until 3 weeks the weight gain of young rabbits was affected by milk production, showing higher values of litter weight for diets supplemented with ascorbic acid or betaine than for control diet. Moreover, rabbit does fed 1 g betaine/kg diet recorded the highest values of litters weights, during the first three weeks. The same trend was obtained at weaning. These results confirmed by Hassan *et al.* (2011) who reported that dietary betaine supplementation help to overcome the negative effects of heat stress on growth performance. Moreover, Ramis *et al.* (2011) indicated that, in pigs, litter weight at weaning was greater for the betaine group than the control group (P<0.05).

Table 2: Effect of experimental diets on does and letters performance of APRI line rabbits.

Item	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 does	-	6	6	6	6	-	-
Does weight gain (g) at:							
Gestation	12	308^{B}	353^{B}	359^{B}	412^{A}	0.0019	17.11
Lactation	12	-113^{B}	-112^{B}	-102^{AB}	-93^{A}	0.0384	5.201
Litter size at:							
Birth (alive)	12	5.86^{B}	6.75^{A}	6.88^{A}	7.30^{A}	0.0118	0.288
21 days	12	4.57^{C}	5.63^{B}	5.88^{AB}	6.60^{A}	0.0005	0.298
Weaning (30day)	12	3.71^{B}	5.38^{A}	5.50^{A}	6.30^{A}	0.0001	0.323
Litter weight (g) at:							
Birth	12	287.9^{C}	352.5^{B}	358.8^{B}	416.0^{A}	0.0001	15.28
21st day	12	997.1^{C}	1380.6^{B}	1495.0^{B}	1774.0^{A}	0.0001	49.33
Weaning (30 day)	12	1402.9^{C}	2175.6^{B}	2276.9^{B}	2822.5^{A}	0.0001	90.29
Pre-weaning mortality rate (%)	12	36.16^{A}	20.21^{B}	20.39^{B}	13.81^{B}	0.0009	3.440
Fertility rate (%)	-	61.30^{B}	69.1^{AB}	70.2^{AB}	76.8^{A}	-	3.760
Daily feed intake (g/d) for:							
Pregnant does	12	132.6^{C}	139.5^{BC}	143.5^{BC}	161.1^{A}	0.0033	5.243
Lactating does	12	180.4^{B}	188.4^{B}	197.3^{AB}	214.1^{A}	0.0035	6.123
Kits (22-30 day)	12	88.41^{C}	148.87^{B}	155.88^{B}	169.10^{A}	0.0001	4.040
Milk index (1)	12	3.38^{B}	3.55^{A}	3.62^{A}	3.64^{A}	0.0031	0.047
Feed conversion rate (g/g):							
1 to 21 days of lactation (2)	12	5.44^{A}	3.88^{B}	3.68^{B}	3.35^{B}	0.0001	0.198
22 to 30 days of lactation (3)	12	6.03^{A}	4.37^{B}	4.39^{B}	3.35^{B}	0.0009	0.398

SEM = Standard error of means,

A, B, C Means in the same row with different superscripts are significantly different (P<0.05).

(1) As [(Litter weight (g) at 21 days after birth - Litter weight (g) at 24 hours after birth)/ (21 X Litter weight (g) at 21 days after birth)] X 100 (Calculated according to Niedzwiadek, 1981).

(2) As feed intake of does from 1 to 21 days (g) per litter weight gain from 1 to 21 days (g).

(3) As feed intake of does from 22 to 30 days (g) per litters weight gain from 22 to 30 days (g).

The pre-weaning mortality rate was higher in the control diet compared to diets with 250 mg ascorbic acid/kg diet or 0.5 and 1 g betaine/kg diet (36.16% *vs.* 20.21, 20.39 and 13.81%; P<0.001, respectively).The recorded high values for pre-weaning mortality rate in summer may be attributed to the direct effect of heat stress on the sensitive offspring, in addition to a reduction of milk production (Ayyat *et al.*, 1995). As shown in Table 2, fertility rate was increased by supplementing 1 g betaine /kg diet.

On the other hand, daily feed intake of pregnant and lactating rabbit does increased (P<0.01) with supplementing 1 g betaine /kg diet. Supplementing dietary ascorbic acid and betaine increased (P<0.001) feed intake of kits in the last 9 days of the lactation period, where kits fed diet containing 1 g betaine /kg diet recorded the highest values. Daily feed intake of lactating rabbit does increased by 35.3% only for 1g/kg betaine as compared to that of pregnant rabbit does to compensate for the higher serve losses of body weight reverses. Rabbit does given diets with ascorbic acid or betaine showed a better (P<0.01) milk index. Moreover, feed conversion ratio was significantly improved (P<0.001) by supplementing ascorbic acid or betaine in diets during the first three weeks of the lactation period, where rabbit does fed 1 g betaine/kg diet recorded the best value. Also, the feed conversion ratio was better (P<0.001) for diets supplemented with ascorbic acid or betaine during the last 9 days of lactating period. During this stage, the differences were more evident than those observed during the first three weeks of lactation.

Results concerning the effect of dietary betaine supplementation on milk production of lactating rabbit does are presented in Table 3. Betaine at a level of 1g/kg caused an increase (P<0.05) in milk production whereas milk yield increased (P<0.05) by 26.2 and 42.8% in rabbit does fed 0.5 and 1 g betaine /kg diet, respectively. The same trend was observed for milk production in relation to week of lactation, which was increased only with 1g/kg betaine level throughout the lactation period.

Table 3: Effect of experimental diets on milk production of APRI line rabbit does.

Item	Control	Ascorbic acid 250 mg/kg	Betaine level		Number of observations	P-value	SEM
			0.5 g/kg	1 g/kg			
Number of does	5	5	5	5	-	-	-
Milk production (g/d) at:							
1st week	67.5^{B}	80.6^{AB}	87.5^{AB}	102.0^{A}	20	0.0911	9.105
2nd week	93.8^{B}	113.8^{AB}	121.3^{AB}	136.0^{A}	20	0.0436	9.245
3rd week	143.8^{B}	163.8^{AB}	170.8^{AB}	188.0^{A}	20	0.0188	8.553
4th week	72.5^{B}	90.0^{AB}	97.0^{AB}	113.0^{A}	20	0.0552	8.818
Milk yield (g/d)	94.4^{B}	112.0^{AB}	119.1^{A}	134.8^{A}	20	0.0121	6.945

SEM = Standard error of means,
$^{A, B, C}$ Means in the same row with different superscripts are significantly different (P<0.05).

CONCLUSIONS

In this study, 1g betaine supplementation in rabbit does diet significantly improves productivity and milk production 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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