RESPONSE OF DOE RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C DURING PREGNANCY AND LACTATION

Abdel-Khalek A.M.[1]*, Selim N.A.[1], El-Medany Sh.A.[2], Nada S.A.[2]

[1]Animal Production Research Institute, Dokki, ARC, Egypt
[2]Regional Center for Food and Feed, ARC, Egypt
*Corresponding author: aabdelkhalek_apri@hotmail.com

ABSTRACT

A seven-treatment experiment was carried out to evaluate the response to dietary supplementation with the antioxidant vitamins α-tocopheryl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in a combination, on some performance traits of 49 two-year old multiparous New Zealand White (NZW) doe rabbits during pregnancy and lactation. Does were equally assigned to one of the following supranutritional levels of the two vitamins: 1) Control of no supplement (40 mg Vit. E/kg diet); 2) 80 mg Vit. E/kg (E80); 3) 160 mg Vit. E/kg (E160); 4) 200 mg Vit. C/kg (C200); 5) 400 mg Vit. C/kg (C400); 6) 80 mg Vit.E+200 mg Vit. C/kg (E80C200); and 7) 160 mg Vit. E+400 mg Vit. C/kg (E160C400). Results indicate that vitamin C groups - C400, then, alternately C200 or E80C200 - with a few exceptions were ranked first, second and third as had the highest values for feed intake during pregnancy ($P<0.01$), and lactation ($P<0.05$), litter size at birth ($P<0.01$) and weaning ($P=0.08$), litter weight at birth ($P<0.01$) and weaning ($P<0.05$), and milk production at the 1st ($P<0.01$), 2nd ($P=0.06$), 3rd ($P<0.01$) and 4th ($P<0.01$) week of lactation. The eminent exception was that E80 group came second for litter weight at weaning. Conversely, the E160 and E160C400 treatments negatively affected these traits. Plasmatic concentrations of α-tocopherol and ascorbate, the total antioxidant capacity during lactation, and hematological parameters (PCV % and Hb concentration) were increased ($P<0.01$), especially as the vitamin level was increased either individually or in a combination. In conclusion, it is recommended to boost the diets of pregnant and lactating doe rabbits with extra doses of vitamin C either individually or combined to α-tocopherol at low doses to improve the performance traits during these critical periods.

Key words: Doe rabbit, Antioxidants, Vitamins E and C, Pregnancy, Lactation.

INTRODUCTION

Oxidants (reactive oxygen species; ROS) are normally generated during cell metabolism and are indispensable for the cellular redox regulation (Kobayashi *et al.*, 2001), supporting the phagocytosis of invading micro-organisms (Castellini *et al.*, 2000), and as a key signal molecules in physiological processes, such as oocyte maturation and fertilization, pregnancy and parturition (Kirschvink *et al.*, 2007). Exposure to metabolic, environmental, photo, drug-dependent or nutritional oxidative stress can disturb normal cell functions, initiating chain reactions that can compromise cell integrity (Lykkesfelt and Svendsen, 2007). To counteract, a series of defense mechanisms, one of which are the antioxidants defences, has been developed (Cheeseman and Slater, 1993). Vitamins E and C are the main natural antioxidants occurring in biological systems and feeds. Vitamin E is a chain breaking antioxidant, mainly works in cell membrane, while, vitamin C is an extra-cellular fluid antioxidant that can reduce ROS excessive generation, also it can regenerate the activity of tocopherol by reducing the tocopheroxyl radicals (Sies and Stahl, 1995). Pregnancy is a critical period at which different biochemical pathways eventually lead to oxidative stress (Krieger and Loch-Caruso, 2001), *e.g.* the synthesis of prostaglandins, involved in embryo implantation (Jenes and Harper, 1984) cause development of some free radicals species (Hope *et al*., 1975). Unlike otherwise indicated, the investigation on vitamin E and lipid peroxide status, carried out by Meżes and Pusztsi (1988) was the only study dealt with the relationship between an antioxidant and ROS generation in female rabbits during pregnancy, conversely plenty of studies (Ismail *et al.*, 1992; Saeed,1994; El-Medany, 1999; Abdel-Kafy, 2000; Meshreky and Shaheed, 2003) studied the relationship between vitamins E and C on the productive/reproductive performance of does on a theoretical basis that these vitamins have antioxidant actions with no identical measurements. The aim of this study was to evaluate, under field conditions, the relationships between oxidative stress, antioxidant nutrients (vitamin E or C and their combination) and the performance of multiparious doe rabbits during late pregnancy and lactation peak, since these critical stages present considerable challenge to homeostasis as a consequence to rapid changes in metabolic pathways.

MATERIALS AND METHODS

Animals and diets

Forty nine-about two year-at service multiparous NZW doe rabbits were mated and equally allocated to study their response to supra-nutritional levels of vitamins E and C, as follows/kg diet; E40: Control of no supplement (40 mg Vit. E; NRC, 1977), E80: 80 mg Vit. E, E160: 160 mg Vit. E, C200: 200 mg Vit. C, C400: 400 mg Vit. C, E80C200: 80 mg Vit.E + 200 mg Vit. C and E160C400: 160 mg Vit. E + 400 mg Vit. C. Diets were formulated to meet the NRC (1977) requirements during pregnancy and lactation (40 mg vitamin E/kg diet), except for the studied vitamins. Ingredients and calculated chemical analyses are provided in Table 1. To avoid vitamin C oxidation during pelleting process the suggested level of the vitamin at each treatment was dissolved in about 20-30 ml water, and then sprayed over the pellets, in every other day intervals. Feed intake, litter size and weight at birth and weaning during pregnancy and lactation were studied. Milk production was estimated by using doe-suckle-weigh method (Lukefahr *et al,* 1983).

Table 1: Ingredients and diet chemical composition of the experimental diet

Ingredients:	Barley, 30.0%; Wheat bran, 23.0; soybean meal (44%), 8.50%; clover straw, 16.0%; corn gluten, 60%; Yellow corn, 9.50%; limestone, 1.5%; di calcium phosphate, 0.50%;, NaCl 0.30%, vitamin& mineral premix* 0.30%, Dl-Methionine 0.20%; anti-coccidial 0.10%, and anti-fungal 0.10 %; Total 100.0%
Chemical composition:	Moisture, 11.0%; CP, 18.0%; DE (kcal/kg) 2651; CF, 11.60%; Ca, 0.95%; P, 0.64%; Lysine, 0.60%; Methionine + cystene 0.72%

**Each 1 kg contain contains: 12000 IU vit.A; 2200 IU vit. D3; 13.4 mg vit. E (determined); 2.0 mg vit. K_3; 1.0 mg vit. B_1 4.0 mg vit. B_2; 1.5 mg vit. B_6; 0.0010 mg vit. B_{12}; 6.7 mg vit. PP; 6.67 mg vit. B_5; 0.07 mg B_8; 1.67 mg B_9; 400 mg choline chloride 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn;1 .67 mg Cu; 0.25 mg I and 0.033 mg Se

Chemical analyses on milk, feed and plasma and statistical analyses

Fatty acid profile of milk at a peak (16-21 d of lactation), three does of each treatment, each was injected with 3-5 IU of oxytocin. The composition was undertaken according to AOAC (2000). Vitamin E (dl- α-tocopheryl) in the mineral-vitamin premix added to feed formula, also in the pure supplement and in plasma of vitamin E and E+C groups was assayed using HPLC, according to Leth and Sondergaro (1983). Vitamin C in the pure supplement and ascorbate in the plasma of vitamin C and E+C was assayed using HPLC, ascribed by Danish Official (1996). Plasma was drawn in late pregnancy (20-25 d) and milk production peak (61-21 d), using three does of each treatment. As the most proper measure to describe the power of an antioxidant to scavenge the oxidants generated, plasma antioxidant status as total peroxyl radical-trapping antioxidant parameter (TRAP) was determined, blood samples from three does were withdrawn from the ear vein in late pregnancy (20-25 d) and milk production peak (61-21 d) over Na_2ETDA (1-2 mg/ml blood) and centrifuged (10000 X g for 10 minutes at 4°C). The supernatant was used immediately for determining TRAP according to Koracevic and Koracevic (2001). In late pregnancy (20-25 d) and milk production peak (61-21 d), using 3 does of each treatment, fresh blood samples of three does/group were assigned for pecked cell volume (PCV, %) and hemoglobin concentration (Hb, g/dl) determination. Data were subjected to a one-way analysis using SAS (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Vitamin E as dl- α-tocopheryl acetate was 13.4/kg premix (40.2 mg/kg diet in the control) and 49.8% in pure supplement, while ascorbic acid concentration in the supplement was 99.8%.

Doe performance during pregnancy and lactation

In most studied variables, vitamin C groups; C400, then, alternately C200 or E80C200 were ranked first, second and third as had the highest values for feed intake during pregnancy ($P<0.01$), and lactation ($P<0.05$), litter size at birth ($P<0.01$) and weaning ($P=0.08$), litter weight at birth ($P<0.01$) and weaning ($P<0.05$), and milk production at the 1st ($P<0.01$), 2nd ($P=0.06$), 3rd ($P<0.01$) and 4th ($P<0.01$) weeks of lactation (Table 2 and 3). Exceptionally that E80 group came second for litter weight at weaning. Conversely, E160 and E160C400 groups negatively affected these traits. In our current study, E80 and E160 groups were close to the control. However a 9% increase in weaning weight of E80 kits was recorded. These results, partially, support those by Saeed (1994), Abdel-Kafy (2000) and Meshreky and Shaheed (2003) that weaning weight of kits was improved with extra

vitamin E inclusion in the does' diets, but not in conformity with Ismail *et al*. (1992) and El-Medany (1999), where they reported no further response to extra doses of vitamin E in performance of pregnant and lactating does, except the improve in litter size at birth in the study carried out by Ismail *et al.* (1992). The inconsistency in results with vitamin E supplementation could be partly attributed to the way of introduction (diet, oral or injection), the experimental conditions (normal vs. stress), the intervals of supplementation (daily or weekly), the state of does (primparous or multiparous) and more important is the level of supplementation, since NRC (1977) recommends a level of 40 mg Vit. E kg/diet to fulfill adequate performance during pregnancy and lactation, a rational supranutritio-nal doses rather than mega doses may be more influential. In this regard a daily oral supplementation with 30-50 mg vitamin E kg/doe (Saeed, 1994) or weekly injection with 56 mg Vit. E kg/doe (Abdel-Kafy, 2000) was more effective than a diet had an inordinate level of vitamin E (160 mg/kg; El-Medany, 1999). Concerning vitamin C, the studies on its effect on performance of does are scanty, in agreement with our findings; Ismail *et al.* (1992) reported that vitamin C at the rate of 50 mg/kg diet resulted in better litter size at birth. The clear improvement in performance of vitamin C groups might be due to its protective action against lipid oxidation in the cell membrane (Liebler, 1992). Also, it is important for newborns which exhibits a greater sensitivity to oxidative damage than adults, and for the development of the immune system in young animals (Debier *et al.*, 2005).Generally the current study provide *in vivo* evidence that when vitamin C, alone or combined with vitamin E, or vitamin E in low dosage could have a satisfactory effect on performance of rabbit does since they have a high total antioxidant effect against ROS as indicated in Table 5.

Table 2: Doe performance during pregnancy

	Mating weight (g)	Feed intake (g)	Litter size at birth	Litter weight at birth (g)
E40	3300±150	3875^{ab}±166	6.6^{b}±0.8	330^{bc}±23
E80	3278±150	3449^{b}±122	6.2^{b}±0.6	329^{bc}±12
E160	3283±71	4005^{a}±202	6.3^{b}±0.6	345^{bc}±23
C200	3188±121	4049^{a}±130	7.0^{b}±1.0	425^{ab}±44
C400	3300±131	4109^{a}±133	9.4^{a}±0.4	482^{a}±20
E80C200	3210±117	3703^{ab}±133	7.1^{b}±0.8	409^{ab}±40
E160C400	3274±64	2910^{c}±98	5.0^{b}±1.1	282^{c}±54
Probability	ns	**	**	**

Means differently superscripted are significantly different. ns: not significant, **: P<0.01

Table 3: Doe performance during lactation

	Feed intake (g)	Litter size at weaning	Litter weight at weaning (g)	Milk (g)			
				Week1	Week2	Week3	Week4
E40	5090^{a}±117	4.4±0.5	2377^{ab}±201	70^{b}±4	115±8	141^{bc}±8	82^{cd}±8
E80	5272^{a}±100	4.5±0.7	2640^{a}±136	86^{ab}±6	129±8	146^{bc}±7	108^{bc}±9
E160	5825^{a}±674	3.6±0.8	2034^{ab}±418	68^{b}±3	126±6	95^{d}±2	54^{d}±12
C200	5616^{a}±449	5.0±0.1	2685^{a}±468	101^{a}±6	149±10	194^{a}±5	148^{a}±18
C400	5859^{a}±558	6.4±1.1	2440^{ab}±389	101^{a}±10	158±8	149^{bc}±12	109^{bc}±5
E80C200	5312^{a}±300	5.5±0.6	2520^{ab}±95	91^{a}±7	121±13	168^{b}±6	128^{ab}±9
E160C400	3385^{b}±210	3.6±0.6	1656^{b}±238	72^{b}±7	99±25	124^{c}±19	83^{cd}±4
Probability	*	ns	*	**	ns	**	**

Means differently superscripted are significantly different. ns: not significant, *: P<0.05, and **: P<0.01

Fatty acid profile of milk

The fatty acid profile of milk at the peak (16-21 d) is shown in Table 4. Of the profile, palmitoleic ($C_{16:1}$; P<0.05) and linolenic ($C_{18:3}$; P<0.01) were the only affected by supplementation. Caprylic ($C_{8:0}$), capric ($C_{10:0}$), palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids are account for more than 85% of the total fatty acids (not included in the table below).

Table 4: Fatty acid profile of milk at lactation peak

	E40	E80	E160	C200	C400	E80C200	E160C400	Prob.
$C_{18:3}$	0.83^{ab}±0.09	0.73^{b}±0.03	0.73^{b}±0.03	0.60^{b}±0.11	0.83^{b}±0.08	1.03^{a}±0.03	1.07^{a}±0.06	**
MUS	15.53±3.53	14.10±0.49	13.30±0.90	17.13±2.07	14.07±1.29	17.16±1.16	16.63±1.16	ns
PUS	17.50±2.06	17.40±0.72	17.80±0.52	17.30±0.75	16.70±0.95	19.53±0.12	21.63±2.65	ns
TUFA	33.03±5.38	31.50±1.05	31.10±1.57	34.43±2.78	30.77±2.21	36.70±1.21	38.27±3.45	ns
TSFA	65.23±5.35	67.65±1.55	68.07±1.18	65.00±0.90	69.80±4.90	61.47±1.69	57.45±4.45	ns
Others	0.74	0.85	0.83	0.57	0.43	1.83	4.28	

Means within each factor differently superscripted are significantly different. ns: not significant, **: P<0.01

Generally, there is a positive trend in polyunsaturated fatty acid % in the combination groups, however, it did not reach a significant level. Lack of significance and reference data on the effect of antioxidant vitamins on fatty acid profile make it difficult to comment on.

Vitamin E and C content of plasma

Data in Table 5 indicate that plasma concentrations of α-tocopherol and ascorbate (mmol/l) were (P<0.01) ontinuously increased as the level of inclusion level of α-tocopheryl and ascorbic acid in the diet increased during pregnancy and lactation, such response was reported in sows (Hidiroglou *et al.*, 1993). The obvious decrease in plasma α-tocopherol and ascorbate during pregnancy compared to lactation, could be in one hand interpreted in light of the greater intake of these vitamins by the fetuses without a relationship with the oxidative stress depletion (Harma *et al.*, 2004), while on the other hand, kits on gradual change to solid feeding at 10 d after parturition, lead the does to re-establish higher plasma vitamin levels. It is worth notable in the current study is the elevation in α-tocopherol concentration in the plasma of the last two treatments (combination groups) compared with their matches in the individual group (treatment E80C200 *vs.* E80 and treatment E160C400 vs. E160). This condition supports the hypotheses reported by Reed (1992) and Sies and Stahl (1995) and the studies reported by Niki (1984) and Castellini *et al.* (2000) that ascorbate in biological systems is able to restore activity of α-tocopherol and increases its level substantially.

Total antioxidant capacity (TRAP)

Results of TRAP presented in Table 4 indicate that both vitamins E and C, alone or altogether**,** during lactation, but not during pregnancy, had (P<0.01) higher TRAP values. The superiority was for the combinations. These results run parallel with the results of plasmatic vitamins level, where working in a combination is better than separately. In fact, when the animal reaches peak lactation, the metabolic status is stabilized and used to monitor the health, reproductive and nutritional status, which in turn is reflected by TRAP status (Castillo *et al.*, 2006). The relationship between TRAP and α-tocopherol was investigated in the pioneer study of Meźes and Pusztsi (1988) who reported inconstant plasma α-tocopherol trend during pregnancy; it was high in the first two weeks, followed by a drastic decline in the third week and re-start to increase in the fourth week, but not to levels equivalent to the first half of pregnancy. In fact we cannot totally rely on the study of Meźes and Pusztsi (1988) to draw a precise relationship between plasma vitamin level and TRAP status, where their study was based on one level of vitamin E of the basal diet. The findings of the present study and of Meźes and Pusztsi (1988) imply two possibilities, the first is that no relationship between plasma levels of antioxidant vitamins and oxidants generation, is exist, since oxidative stress biomarker-TRAP was not affected by the change in plasma oxidant vitamin levels, and the second, which is more realistic that circulating plasma is not a proper compartment to assess the concentration of antioxidant vitamins during pregnancy. According to Harma *et al.* (2004) oxidative stress is higher in pregnant women that may attribute to a greater consumption of the vitamins by the fetuses, without any relationship with the oxidative stress depletion, while, Vannaucchi *et al.* (2007) working on dogs, suggested that lipid and protein oxidation may be restricted to specific tissues such as the fetal or placental tissues, which in general terms, may alter the blood concentrations of the major oxidative markers.

Hematological parameters

PCV % and Hb concentration (Table 5) were (P<0.01) increased, especially in the vitamin combination diets. Saeed (1994) reported higher PCV and Hb values in pregnant and lactating does administrated 30 or 50 mg vitamin E/doe/day. In opposite, El-Medany (1999) reported a significant decrease in these values when pregnant does administrated 10 mg Vit. E (10 mg/doe/day) compared to the control.

Table 5: Plasma TRAP and haematological study during pregnancy and lactation

	E40	E80	E160	C200	C400	E80C200	E160C400	Prob.
Plasma Vit.E pregnancy (mmol/l)	1.30^{e}±0.02	2.21^{d}±0.05	2.69^{b}±0.04	ND	ND	2.51^{c}±0.02	2.97^{a}±0.08	**
Plasma Vit.E lactation (mmol/l)	1.55^{d}±0.03	2.63^{c}±0.08	3.08^{a}±0.07	ND	ND	2.86^{b}±0.01	3.10^{a}±0.01	**
Plasma Vit.C pregnancy (mmol/l)	0.51^{d}±0.02	ND	ND	0.86^{c}±0.03	1.17^{a}±0.02	0.81^{c}±0.03	1.00^{b}±0.02	**
Plasma Vit.C lactation (mmol/l)	0.59^{d}±0.01	ND	ND	0.91^{c}±0.02	1.30^{a}±0.01	0.87^{c}±0.02	1.10^{b}±0.03	**
TRAP pregnancy (mmol/l)	2.54±0.29	2.47±0.55	2.42±0.11	2.50±0.02	2.58±0.02	2.50±0.08	2.33±0.19	n.s.
TRAP lactation (mmol/l)	2.01^{d}±0.46	2.34^{c}±0.07	2.54^{ab}±0.30	2.36^{c}±0.03	2.44^{bc}±0.02	2.56^{ab}±0.02	2.60^{a}±0.03	**
PCV pregnancy (%)	29.7^{bc}±5.2	32.2^{abc}±0.9	24.0^{c}±4.1	39.5^{ab}±2.5	31.7^{abc}±3.4	36.0^{ab}±3.1	41.0^{a}±1.22	**
PCV lactation (%)	31.7^{bc}±1.25	33.0^{ab}±1.08	28.2^{c}±2.78	36.0^{ab}±1.08	34.0^{ab}±1.29	37.0^{a}±1.29	37.0^{a}±0.70	**
Hb pregnancy (g/l)	11.2^{ab}±1.21	11.6^{ab}±0.42	10.3^{b}±1.00	11.2^{ab}±0.59	11.7^{ab}±0.98	13.8^{a}±1.69	13.2^{ab}±0.72	**
Hb lactation (g/l)	11.0^{d}±0.19	11.3^{cd}±0.15	11.3^{cd}±0.37	12.2^{bc}±0.25	12.3^{b}±0.39	12.5^{b}±0.32	13.5^{a}±0.28	**

Means differently superscripted are significantly different. ns: not significant, **: P<0.01. ND: not determined

CONCLUSIONS

This study introduces an *in vivo* evidence on the antioxidant properties of vitamins E and C, that reflected in better performance during gestation and lactation. It indicates that doe rabbits are tolerant to high doses of vitamin C and also to a moderate level of vitamin E plus C. Much work is needed on weanlings performance, especially at the low doses of vitamin E to explore the anti-oxidant actions.

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