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EFFECT OF ASCORBIC ACID ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF DOES NEW ZEALAND WHITE RABBIT

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ABSTRACT

A total of 30 New Zealand White (NZW) doe rabbits 9~12 months old and weight 2.74±0.23 kg was divided in to three equal groups, according to their weights and age, ten animals in each. Rabbits of 1st group (G1) were fed commercial rabbit diet (control group). Rabbits of the 2nd group were received a dose of ascorbic acid (AA) 25 mg/rabbit/day in drinking water one week before service (G2) until the weaning age of bunnies while, those of the 3rd group (G3) were received a dose of ascorbic acid 50 mg/rabbit/day in drinking water one week before service until the age of weaning of bunnies. All rabbits were given diets to meet NRC (1977) requirements of doe rabbits for pregnancy and suckling. Results revealed that total dry matter intake (DMI), TDN and DCP were significantly (P<0.05) higher for G2 and G3 than those of control group. Digestibility coefficients of DM, CF and EE were significantly higher (P<0.05) by supplementation of ascorbic acid than in control group. Concentrations of total protein and globulin were significantly higher (P<0.05) in G3 and G2 than in G1. Urea-N concentration was lower (P<0.05) in G3 and G2 than in G1. Average daily milk yield (ADMY) of does was significantly (P<0.01) higher in G3 at 10 and 20 days of suckling. Fat percentage was significantly higher in does received ascorbic acid than the control group. However, lactose content was significantly (P<0.05) decreased in G3 compared to G2 and G1. Litter size at birth and weaning was significantly (P<0.05) higher in G3 than in G2 and G1. Litter weight at weaning was significantly higher in treated groups than the control group. Mortality rate during the suckling period was the highest in G1 than those in G2 and G3.
Keywords: Ascorbic acid, rabbits, milk yield and reproductive performance

INTRODUCTION

Ascorbic acid promotes antioxidant function, immune function, normal interferon levels, adrenal function and thyroid function (McCorkel *et al*., 1980; Andreson, 1981; Beisel, 1982 and Bendich, 1987). Ascorbic acid has three biological actions of particular relevance to reproduction, each dependent on its role as a reducing agent: it is required for the biosynthesis of collagen, for the biosynthesis of steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules. It is frequently involved in mixed-function oxidation, resulting in the incorporation of oxygen from molecular oxygen into a substrate (Sebrell and Harris (1967).

The immediate cause of these changes is not apparent, but authors have assumed that they reflect changes in the uptake of ascorbic acid by the periovulatory ovary. It has been suggested that changes in retention before ovulation facilitate luteal steroidogenesis (Mukerji *et al*., 1965), and that this relationship also explains its cycle-protective effects (Deb and Chatterjee, 1963). More recent studies with luteinizing granulosa cells show that ascorbate is stimulatory to progesterone and oxytocin secretion, consistent with its known roles in hormone biosynthesis, and synergizes with neurotransmitters in stimulating hormone secretion (Luck, 1990). Notwithstanding these effects, the concentration of ascorbic acid in the corpus luteum appears to be greatly in excess of that required to facilitate hormone production (Luck and Zhao, 1993).

The aim of this study was to evaluate effects of different levels of ascorbic acid (25 and 50 mg/rabbit/day) on digestibility trial, blood parameters, milk yield and composition and reproductive performance of does NZW rabbits.

MATERIALS AND METHODS

The present study was carried out on a flock of NZW rabbits belonging to Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture.

Experimental animals:

A total of 30 NZW doe rabbits 9~12 months old was divided into three equal groups, according to their weights and age, ten animals in each. All does were individually housed in 30 wire cages (60 x 50 x 40 cm).

All does rabbits were fed commercial rabbit diet without additives (G1, control group). Rabbits of the 2nd group were received a dose of ascorbic acid (El Nasr Pharmaceutical Chemicals Co. Abu Zaabal Egypt) 25 mg/rabbit/day in drinking water one week before service (G2) until the age of weaning of bunnies while, those of the 3rd group (G3) were received a dose of ascorbic acid 50 mg/rabbit/day in drinking water one week before service until the age of weaning of bunnies. All rabbits were given diets to meet NRC (1977) requirements of doe rabbits for pregnancy and suckling. The commercial diet was composed of different feedstuffs as shown in Table (1). The limited amounts of commercial diet for each rabbit were offered individually at morning.

The commercial diets were fed to doe rabbits throughout the experimental period from one week before service to weaning of the litter. The diet weight was biweekly adjusted according to the physiological status of the rabbits (i. e. growth, pregnancy and suckling). Chemical composition of CFM was shown in Table 1.

Live body weight of does at parturition and throughout suckling period and LBW of their bunnies during the suckling period were weekly recorded. Thereafter, changes in LBW were calculated at various weeks studied. Reproductive performance of doe rabbits including number of service per conception and conception rate at the 1st service, litter size and gestation period were also recorded. At 10 and 20 days during suckling period, milk yield and composition were recorded. Milk yield was determined by the differences in LBW of does before and after suckling, while milk composition was estimated using milko-scan (Model 133 B).

Table 1: Ingredients and chemical analysis of pelleted commercial diet.

Ingredient	%	Composition	%
Yellow corn grain	18.00	DM	87.74
Barley grain	17.00	Composition of DM %	
Wheat bran	20.00	OM	92.34
Berseem hay	25.00	CP	17.56
Soybean meal	15.30	CF	12.41
Molasses	3.00	EE	4.77
Limestone	1.00	NFE	57.60
Common salt	0.25	Ash	7.66
Premix*	0.30		
DL-methionine	0.15		
Total	100.00		

*** One kg of premix contained 2000,000 IU vit. A; 10,000 mg vit. E; 400 mg vit. B1; 1200 mg vit. B2; 400 mg vit. B6; 20 mg B12; 400 mg K3; 180,000 IU vit. D; 240 mg choline chloride; 400 mg pantothenic acid; 1000 mg niacin; 1000 mg folic acid; 40 mg biotin; 1700 mg manganese; 14000 mg zinc; 1500 mg iron; 600 mg copper; 20 mg selenium; 40 mg iodine; 8000 magnesium and calcium carbonate carrier to 1 kg.**

Digestibility trial and nitrogen balance:

Digestibility trial was undertaken at the end of the experimental period on four animals (does) from each group. Rabbits were housed individually in metabolism cages (40 x 35 x 30 cm) which allowed feces and urine separation. The experimental diets were offered daily and fresh water was provided all the time. Feed intake was accurately determined and coprophagy was not prevented. Quantitative collection of urine and feces started 24 hours after offering the daily feed for 5 days as a collection period, then the feces was dried at 60^{o}C for 24 h. All collected urine or feces for each animal were mixed, then feces were ground for chemical analysis and urine was kept (4-5 ^{o}C) for analysis. Chemical analysis of different foodstuffs, feces and nitrogen in urine was determined according to A.O.A.C. (1980). Values of total digestible nutrients (TDN) were calculated according to the classic formula described by Cheeke *et al.* (1982). However, digestible energy (DE) was calculated according to the equation of Schiemann *et al*. (1972) as follows:

TDN (%) = DCP (%) + DNFF (%) + DCF (%) + 2.25 (DEE%).

DE (kcal/kg) = 5.28 (DCP, g/kg) + 9.51 (DEE, g/kg) + 4.2 (DCF + DNFE, g/kg).

where: DCP, DEE, DCF and DNFE =digestible CP, EE, CF and NFE, respectively.

Blood parameters:

Blood samples were collected biweekly in heparinized test tubes from the ear vein of five rabbits in each group. Thereafter, blood plasma were separated by centrifugation at 3000 rpm for 15 min and stored at -20^{o}C until chemical analyses. Concentration of total protein (Gornall *et al*., 1949), albumin (Weichselaum, 1946) and creatinine (Henry, 1965) as well as activity of aspartate (AST) and alanine (ALT) transaminases (Reitman and Frankal, 1957) in blood plasma were determined using commercial kits (Diagnostic System Laboratories, Inc USA). Plasma globulin concentration was calculated by subtracting concentration of albumin from total proteins.

Statistical analysis:

Results were statistically analyzed according to Snedecor and Cocharn (1982). However, the significant differences among treatments were tested using Duncan's Multiple Range Test (1955).

RESULTS AND DISCUSSION

Feed intake:

The present results in Table 2 show that total dry matter intake (DMI), TDN and DCP were significantly (P<0.05) higher in G2 and G3 than those in control group, which was in accordance with the designed feeding system for the experimental groups. Rabbits in all ascorbic acid groups showed significantly (P<0.05) higher total DMI than the control group to meet their energy and protein requirements, because of the increased milk yield in does received ascorbic acid than that in control group.

The present results regard to feed intake are similar to those reported by Abu Shetiefa (1999); El-Hamd (2000) and Omara *et al*., (2005).

Feed consumption of rabbits depends basically on nutrient contents in accordance with the actual energy need of the animal, protein and fiber level of its ration (Fekete and Bokori, 1985).

Table 2: Average daily feed intake (g/day) by doe rabbits in different experimental groups

Item	Experimental groups		
	G1	G2	G3
Total DMI	256 ± 2.3^{b}	276 ± 2.4^{a}	281 ± 2.5^{a}
TDN	154 ± 6.4^{b}	178 ± 6.8^{a}	182 ± 7.1^{a}
DCP	31.49 ± 1.5^{b}	36.63 ± 1.6^{a}	38.13 ± 2.1^{a}

[a and b:] Group means denoted with the same superscripts within the same row are not significantly different at (P≥0.05).

Digestibility coefficients and nutritive values:

Results in Table 3 show that CP digestion was significantly (P<0.05) higher in rabbit received ascorbic acid (50 mg, G3 and 25 mg, G2) in drinking water than in the control group (67.83 and 67.58 vs. 64.87%). Also, EE digestion was significantly (P<0.05) higher in rabbit received ascorbic acid than in those the control (69.02 and 68.73 vs. 64.25%).

On the other hand, digestibility coefficients of DM, CF and EE were affected significantly by AA supplementation, although there were inconsistent effects of ascorbic acid on digestion of these nutrients. Digestibility coefficient of CF improved by about 8.56 and 7.60 % in doe rabbits treatments in G2 and G3 as compared to the control, respectively.

Table 3: Nutrients digestibility coefficients and nutritive values for the different experimental groups.

Items	Experimental groups		
	G1	G2	G3
Nutrients digestibility coefficients %:			
DM	65.24[b]	66.87[ab]	67.43[a]
OM	67.04	68.24	68.18
CP	64.87[b]	67.58[a]	67.83[a]
CF	43.59	46.21	45.87[a]
EE	64.25[b]	69.02[a]	68.73[a]
NFE	67.29	68.62	67.92
Nutritive values %:			
TDN	60.31[b]	64.39[a]	64.94[a]
DCP	12.30[b]	13.27[ab]	13.57[a]

[a and b]: **Means in the same row with different superscripts differ significantly (P<0.05).**

Blood biochemical parameters:

Data in Table 4 showed that total protein and globulin concentration in plasma were significantly (P>0.01) higher in G3 than in G2 and G1. But differences were not significance between rabbits in G2 and G1. Albumin concentration was similar in treated and control groups. Concentration of urea-N was significantly (P>0.01) lower in G2 and than in control group.

The normal function of liver in rabbits received ascorbic acid was indicated from the insignificant differences in creatinine concentration and activity of AST and ALT in plasma (Table 4). In addition, disappearance of significant differences in concentration of urea-N between treated groups and control may suggest normal function of kidney in all groups. So, inclusion different doses of ascorbic acid is in the save side without any harmful effects on liver and kidney function. The present results regard to biochemical concentrations are similar to those reported by Abu El-Hamd (2000).

Table 4: Average values of some biochemical concentrations and transaminases activity in blood plasma of rabbits at the end of the experimental period.

Item	Experimental groups		
	G1	G2	G3
Total protein (g/dl)	7.10±0.26[b]	7.73±0.21[a b]	7.96±0.22[a]
Albumin (g/dl)	3.46±0.1	3.73±0. 1	3.66±0.1
Globulin (g/dl)	3.64±0.1[b]	4.0±0.1[ab]	4.30±0.2[a]
Urea-N (mg/dl)	26.88±1.4[a]	21.35±1.1[b]	22.16±1.2[b]
Creatinine (mg/dl)	1.65±0.2	1.75±0.1	1.80±0.1
AST (U/l)	41.6±2.1	43.6±3.1	42.9±2.3
ALT (U/l)	26.3±1.4	26.9±1.2	27.6±1.2

[a and b:] **Group means denoted with different superscripts within the same row are significantly different at P<0.05.**

Milk yield and composition:

Data in Table 5 show that average daily milk yield (ADMY) of does was significantly (P<0.01) higher in G3 at 10 and 20 days of the suckling period.

Generally, average daily milk yield was significantly higher in rabbits resaved 50 mg ascorbic acid daily than the control group. No significant differences between G2 and G3 in milk yield.

The present results indicated the negative relationship between milk yield and LBW of does during different suckling period (Pascual *et al.,* 1996 and Rashed, 2002). Increasing milk yield of does resaved 50 mg ascorbic acid daily may be related to a tendency of increasing their litter size (LS) at different suckling days (Balley *et al.,* 1988; Yamani *et al.,* 1991 and Goerg, 2004). Also, Lukefahr *et al.* (1983) and Nasr (1994) found highly significant positive correlations between milk yield and litter size at different stages of lactation.

Table 5: Average daily yield and composition of milk produced by doe rabbits in different experimental groups at successive suckling.

Item	Experimental groups		
	G1	G2	G3
Average daily milk yield (g/day):			
At day 10	160.0±6.1[b]	175.0±9.0[ab]	189.3±8.2[a]
At day 20	120.0±8[b]	155.0 ±13[ab]	173.8±12[a]
Milk composition (%):			
Fat	9.63±0.03[b]	10.0±0.23[ab]	10.3±0.06[a]
Protein	6.8±1.1	7.0±1.1	7.4±0.14
Lactose	1.40±0.11[a]	1.40±0.10[a]	1.05±0.12[b]
Total solids	24.4±0.88	25.9±1.6	24.77±0.77
Solids not-fat	15.8±0.15	17.0±0.96	16.4±0.21

[a and b]: Means within the same row with different superscripts are significantly different at P<0.05.

Changes in MY might be related to the changes in the physiological efficiency of the doe especially those related to the capacity of mammary glands. Concerning the change in ADMY throughout suckling weeks, it was found gradual increase in ADMY of does in all groups at 10 day of suckling compared to at the 20 day of suckling period. This trend was obtained in NZW rabbits by Yamani *et al.* (1991) and Goerg (2004).

Milk composition including percentages of fat, protein, lactose, total solids and solids not fat presented in Table 5, revealed that all chemical contents in milk showed inconsistent trend of differences as affected by dietary treatments at different suckling. Fat percentage was higher significantly in does received ascorbic acid than the control group.

However, lactose content was significantly (P<0.05) decreased in doe received 50 mg ascorbic acid than the doe received 25 mg ascorbic acid and control groups.

The differences in total solids were attributed to the highest fat contents, which had negative relationship with milk yield. Pascual *et al.* (1996) reported that the highest values of DM content in milk of doe were mainly related to fat content.

Reproductive performance of does:

Data in Table 6 show that does in G2 and G1 showed more than one service to conceive (1.1 and 1.3 s/c, respectively). However, does in G3 required one service per conception. This reflected in 100%, 90% and 70% conception rate in G3, G2 and G1, respectively, which showed conception rate at the 1st service.

Inspit the nearly similar live body weight of dose in all groups at parturition and gestation period length was no significant. However, litter size at birth and weaning and litter weight at weaning were significantly ($P<0.05$) higher in G3 than in G2 and control groups, however, no significant differences between G2 and G3 (Table 6). On the other hand, litter weight at birth was significantly higher in G2 and G3 groups than the controls.

The overall mortality rate during the suckling period was the highest in control group, however, those in G2 and G3 groups showed the lowest mortality percentage (Table 6).

The present results indicated the beneficial effects of ascorbic acid treatment on reproductive performance of does and their bunnies as compared to the control diet. 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, Tawfeek and El-Gaafary (1991) and Rashwan and Gaafary (1992) found that average conception rate in natural mating ranged from 60 to 65.2%. The results of conception rate obtained on NZW rabbits ranged between 61.75 and 87.5% (El-Kerdawy and Rashwan, 1998 and Ibrahim, 1999). Recently, values of conception rate of NZW rabbits was 64.09% (Gadalla *et al.*, 2002) and 100% Goerg (2004). Values of litter size in NZW rabbits was found to range between 5.75 and 9.70 as reported by many authors under Egyptian condition (Tawfeek and EL-Gaafary, 1991; El-Kerdawy and Rashwan, 1998 and Ibrahim, 1999). Ibrahim, (1999) showed that litter size at day 21 ranged between 3.5 and 7.3 and litter size at weaning ranged between 2.5 and 7.3. Results of Gadalla *et al.* (2002) showed that values of litter size at birth (live and total), 21 and 28 days in the NZW rabbits were 7.03, 7.09, 6.40 and 6.13, respectively.

In general, gestation period length ranged between 30.4 and 33.0 days as found in the literature on NZW rabbits (Tawfeek and El-Gaafary, 1991; Yamani *el al.*, 1994; Gadalla *et al.*, 2002 and Goerg, 2004), which indicated normal gestation period for all groups.

Table 6: Reproductive performance of doe rabbits in different dietary groups.

Item	Experimental groups		
	G1	G2	G3
Conception rate (%) at the 1st service	70	90	100
Conception rate at the 2nd service	30	10	-
LBW of doe at parturition (kg)	3.16±0.3	3.34±0.2	3.25±0.3
Gestation period length (d)	31.6±0.2	31.4±0.1	31.0±0.2
Litter size at birth	6.55±0.4[b]	6.75±0.3[b]	7.90±0.3[a]
Litter weight at birth (g)	417.0±21[b]	477.5±16[ab]	498.0±15[a]
Litter size at weaning	4.83±0.6[b]	5.63±0.7[ab]	6.88±0.4[a]
Litter weight at weaning (kg)	2.02±0.3[b]	2.52±0.4[ab]	3.22±0.3[a]
Mortality rate %	26.33	16.67	12.92

[a and b:] **Group means denoted with different superscripts are significantly different at P<0.05.**

Correlation between follicle volume and the serum ascorbate concentration, suggesting that follicle growth, particularly during the late stages of exogenous stimulation, may be limited by the availability of ascorbic acid in the circulation (Martin *et al*., 1995). The mechanism of uptake of ascorbate by the ovary has yet to be determined, but leukocytes are closely associated with the ovarian tissue cycle (Norman and Brannstrom, 1994) and may provide a locally concentrated source. As with males, vitamin C has been suggested as a regulator of female fertility.

Based on the obtained results, doe rabbits which received ascorbic acid in drinking water 50 mg/rabbits/day were improvement feed intake, concerning milk yield and composition and in turn good reproductive performance.

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تأثير اضافة حمض الأسكوبيك علي الأداء الإنتاجي والتناسلي لأمهات أرانب النوزلاندي الأبيض

محمد عوض أبو الحمد ، محمد عبد الغني شطيفة وأيات عبد المقصود رجب
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تهدف هذه الدراسة لمعرفة تأثير إضافة حمض الأسكوربيك في ماء الشرب على معدل الغذاء المأكول ومعاملات الهضم والكفاءة الإنتاجية والتناسلية للأرانب النوزلاندي الابيض. استخدم في هذه الدراسة ٣٠ أم متوسّط أوزانها ٢.٧٤ ±٠.٢٣ كجم وتتراوح أعمارها بين ٩ – ١٢ شهر. قسّمت الارانب إلى ثلاث مجموعات متماثلة في الوزن والعمر. كانت الارانب في المجموعة الأولى بدون معاملة (كنترول) بينما المجموعة الثانية و الثالثة تم اضافة ٢٥ و٥٠ ميكوجرام حمض السكوربيك علي الترتيب في ماء الشرب لكل ام قبل التلقيح باسبوع وحتي فطام المواليد التالية.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

- ازداد كل من معدل الغذاء المأكول اليومى والبروتين الخام المهضوم وتحسنت في القيمة الغذائية TDN في المجموعة الثانية والثالثة مقارنة بالكنترول.
- تحسنت القيمة الغذائية في مجموعات المعاملة نتيجة ارتفاع معاملات الهضم مقارنة بالكنترول.
- ارتفع معنويا تركيز كل من البروتين الكلي والجلوبيولين في سيرم الدم في المجموعات المعاملة مقارنا بالكنترول ، بينما انخفض تركيز يوريا الدم في المجموعات المعاملة مقارنة بالكنترول.
- ارتفع انتاج اللبن عند اليوم ١٠ و٢٠ من الرضاعة معنوية في المجموعات المعاملة مقارنة بالكنترول.
- ارتفع محصول الدهن في اللبن بينما انخفض اللاكتوز في اللبن معنويا في المجموعات المعاملة مقارنة بالكنترول.
- ارتفع عدد المواليد عند الولادة والفطام معنويا في المجموعات المعاملة مقارنة بالكنترول.
- تحسنت نسبه الحياتية في المجموعات المعاملة مقارنة بالكنترول.

قام بتحكيم البحث

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