Dept. of Animal Produced.,
Fac. of Agric., Assiut University
Head of Dept. Prof. Dr. M.N. Makled

EFFECT OF ASCORBIC ACID ON GROWTH PERFORMANCE AND SOME BLOOD CONSTITUENTS OF SUCKLING BUFFALO CALVES

(With One Fig. & 4 Tables)

By

M.A. KOBEISY and Th. S. ABD EL-ALL*

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تأثير حامض الاسكوربيك على النمو وبعض مكونات الدم في العجول الجاموسي الرضيعه

مصطفى قبيصى ، ثروت عبد العال

قسمت عشره من العجول الجاموس عمر اسبوع الى مجموعتين :
الاولى ضابطة والثانية اعطيت ٢ جرام حامض اسكوربيك لكل حيوان يوميا . غذيت العجول على اللبن الطبيعى لمدة ٦ اسابيع سجلت الظواهر الاكلينيكية يوميا وتم وزن الجسم اسبوعيا تم تقدير احدى عشر مكون من مكونات الدم فى ثلاث فترات من كل حيوان . ادى حامض الاسكوربيك الى تحسن معنوى فى وزن الجسم ($P < .01$) وقلل من الاعراض الاكلينيكية خاصة الاسهال . كان المتوسط العام لكرات الدم الحمراء اعلى قليلا فى الحيوانات المعاملة . ادى حامض الاسكوربيك الى زيادة معنوية فى تركيز الهيموجلوبين ($P < .01$) وفى حجم المكونات الخلوية فى الـــدم ($P < .04$) . زادت خلايا الدم البيضاء فى المجموعة المعاملة بحامض الاسكوربيك من ١٢ر١٢ X ١٠ عند الاسبوع الثانى الى ١٣ر٧ X ١٠ عند الاسبوع السادس من التجربة . ليس هناك تاثير معنوى لحامض الاسكوربيك على البروتين الكلى والالبيومين والجلوبيولين اما اليوريا - نيتروجين فقد كانت اقل قليلا فى الحيوانات المعاملة . الكولسترول فى السيرم قل بتقدم التجربة فى الحيوانات المعاملـــة (من ٢٢ر١١٨ عند الاسبوع الثانى الى ٦٤ر٩٤ ملليجرام/ ١٠٠ مل عند الاسبوع السادس) . الجلسريدات الثلاثية كانت قليلة فى الحيوانات المعاملة بحامض الاسكوربيك . اضافة حامض الاسكوربيك الى الغذاء ربما يحسن من نمو وصورة الدم فى العجول الرضيعة .

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*: Dept. of Animal Medicine., Fac. Vet. Med. Assiut University

SUMMARY

Ten buffalo calves at the age of one week were randomly assigned to two treatment groups, a control group with no L-ascorbic acid supplementation and a supplemented group by 2 gm of L-ascorbic acid per animal per day. Calves were fed raw milk during the 6 wk experimental period. Clinical inspection were examined daily and body weight was recorded weekly. Eleven hematological and serum constituents variables were measured at three sample periods. Dietary ascorbic acid improved (P<.01) daily gain and reduced the incidence of clinical illness, particularly diarrhoea. The overall mean of red blood cells was slightly higher in treated animals. Dietary L-ascorbic acid increased hemoglobin concentration (P<.01) and packed cell volum (P<.04). With ascorbic acid treatment, white blood cells counts increased towards the end of the experimental period, from 12.12 at wk 2 to 13.70 X 10^3 at wk 6. Ascorbic acid had no significant effect on total protein, albumin and globulin. Urea nitrogen was slightly lower in treated animals. Serum cholesterol decreased with advance of experimental period in treated group (from 118.22 at wk 2 to 94.64 mg/dl at wk 6) and triglycerides was lower in ascorbic acid treated animals. Dietary ascorbic acid may improve the performance and blood profile of suckling calves.

INTRODUCTION

The major metabolic roles of ascorbic acid are: 1, it participates as an oxidation reduction agents in numerous cellular oxidations processes and 2, it is essential in formation of skeletal and connective tissues, i.e bone, cartilage and skin *(JAFFE, 1984)*. Recently it has found many applications in poultry, particularly under stressful conditions. *SEED (1992)* reviewed that ascorbic acid was effective in reducing laying hen mortility due to either temperature and humidity or bacterial and viral diseases stressors. Moreover, it has been shown to improve egg, quality

and production and growth performance. However, in ruminants, particularly suckling calves, there is lack of information on the effect of dietary ascorbic acid on different phsiological functions. Dairy calves apparently do not produce endogenous ascorbic acid untill 4 months of age *(WEGGER and MOUSTGAARD, 1982)* and consequently, calves less than 4 months of age may have a marginal ascorbic acid deficiency. Therefore, the objective of this study was to observe the effect of dietary ascorbic acid on performance and some blood constituents of suckling calves.

MATERIAL and METHODS

The study was conducted during the winter months (December through February) in Animal Production Experimental farm of the Faculty of Agriculture, Assiut University. Maximum and minimum air temperature and relative humidity were 29° C, 0° C, 96% and 15%, respectively. Ten buffalo calves at the age of one wk were randomly assigned to two treatment groups, similar in body weight, a control group and an ascorbic acid treatment group, receiving supplemental dietary L-ascorbic acid as 2 gm per animal per day. The supplementary ascorbic acid fed in two equal doses of 1.0 gm each at 08.00 and 16.00 h, it dissolved in whole raw milk just before feeding. Calves were fed raw milk according to *RAGAB and ASKAR (1968)* during the 6 wk experimental period.

Body weight was recorded, before morning feeding, weekly. Calves were examined daily for clinical illness, i.e. nasal discharge and diarrhoea. Blood samples were taken from each animal at wk 2, 4 and 6 of experimental period and immediatly transfered to two vials, one dry, clean and sterilized while the other EDTA containing vials. Serum was then separated by centrifugation at 3000 rpm for 15 min and stored at-20°C untill analysed. Serum glucose and triglycerides were determined using kits supplied by Stanbio Laboratory Inc. (Texas, USA). Total protein and urea nitrogen were determined using kits supplied by Diamond Diagnostics (Egypt). Serum albumin was determined using a kit supplied by bio Merieux (Bains, France) and serum cholesterol was determined using a kit supplied by Medical Marketing Service (Germany).

Red blood cells (RBCs, $10^6/mm^3$), white blood cells (WBCs, $10^3/mm^3$) and hemoglobin (Hb, g/dl) were determined by means of an electronic automatic cell counter (Cell Payne 300 Sequoia Turner). Packed cell volume (PCV), was estimated according to the standard methods of hematology *(SCHALM, 1986)*.

Data analysis was done according to *HARVEY (1987)*.

RESULTS

Results are presented in Tables 1, 2, 3 and 4, and Figure 1.

DISCUSSION

Growth performance:

Dietary ascorbic acid improved daily gain (table 1) and consequently, body weight (Fig. 1). Such improvement was found to be highly significant (P<.01) during the 4th week of age. The result of such an effect in this study was similar to those observed in chickens by *THAXTON* and *PARDUE (1984) and ESA (1992).* The high daily gain of treated animals probably due to, supplemental ascorbic acid may effective in incvreasing plasma thyroid hormones levels. *KAN* et al. *(1993)* found that dietary ascorbic acid increased plasma T3 and T4 levels of chickens. *TAKAHASHI* et al. *(1991)* stated that the growth-promoting effect of ascorbic acid probably associated with the alleviation of retardation in thyroid function, secretion of T3 and T4, by feeding propylthiouracil (PTU). Thyroid hormones (T3 & T4) improved protein, fat and carbohydrate metabolism and growth of the body *(KUTSKY, 1981).* Moreover, the absence of thyroid hormones caused severe growth retardation due to both arrest of bone elongation and retarded bone maturation *(HADLEY, 1984).* HOSHINO et al. *(1991)* found positive correlation between either T3 or T4 concentrations and daily gain in growing and fattening steers. Although, the improvement of thyroid hormone secretion due to ascorbic acid treatment, may be due to the increase of thyroid I uptake. *ABDEL-WAHAB* et al. *(1975)* found that dietary ascorbic acid (100 PPM) increased thyroid (^{125}I) uptake in chickens. Moreover, ascorbic acid has many metabolic roles particularly in formation of skeletal and connective tissues *(JAFFE, 1984)* consequently, it has to improve daily gain of ascorbic acid-treated calves.

Although, in the present study, calves fed ascorbic acid had lower incidence of clinical illness, particularly diarrhoea. Similar result was observed by *CUMMINS and BRUNNER (1989)* in suckling dairy calves Such effect could be involved in factors that responsible for improvement daily gain in ascorbic acid-treated animals.

Hematology:

Ascorbic acid treatment had no significant effect on erythrocyte count (RBCs), but overall mean of RBCs was slightly higher in treated animals. Hemoglobin concentration (Hb) was

significantly (P<.01) higher in ascorbic acid-treated animals than controls. Accordingly, packed cell volum, erythrocyte mass, was significantly (P<.04) higher in ascorbic acid-treated calves than controls (Table 2). The results of this study were similar to those observed in growing chickens by *MISK I (1976)* who showed that dietary. 5% ascorbic acid increased the hemoglobin concentration and total body iron retention. *LEVANDER and CHENG (1980) and CALABRESE (1980)* found that L-ascorbic acid deficiency interferes with iron mobilization from spleen but not from the liver, L-ascorbic acid increases its mobilization from body stores during the treatment of iron overload. These properties of vitamin C are important in the prevention of anemia. Moreover, ascorbic acid, may increase the biological effectiveness of the red blood cell's normal oxygen dissociation of hemoglobin by increasing 2,3-diphosphoglycerate *(JAFFE, 1984)*. Such physiological role of ascorbic acid may also responsible for higher daily gain of treated animals (Table 1 & Fig. 1).

The overall mean of white blood cells (WBCs) was not significantly differ between treatments, control group had higher (P<. 03) value than treatment at wk 2 of the experimental period, but ascorbic acid-treated animals had higher value (13.70 v. 13.04 X 10^3) at wk 6 (Table 2). With ascorbic acid treatment, WBCs counts elevated towards the end of the experimental period, from 12.12 at wk 2 to 13.7o X 10^3 at wk 6 of the experimental period, opposite result was found in control animals. Such effect of ascorbic acid on increasing WBCs could be due to their protection of leukocyte membrane from autooxidation. Indeed, ascorbic acid is involved in the imunological and antibacterial functions of WBCs by several factors, First: increasing their mobility, stimulating the pnergy-producing monophosphate shunt within the cell *(ANDROSON, 1981)* and consequently coupled their phagocytic process *(REECE, 1991)*. Second: protecting leucocyte from autooxidation *(ANDERSON, 1981; JAFFE, 1984)*. Third: increasing serum immunoglobulin concentration *(CUMMINS and BRUNNER, 1989)* and antibody formation *(JAFFE, 1984)*. Such interpretation may confirm our presnt results, that calves fed ascorbic acid had lower incidence of clinical illness, particularly diarrhoea. However, the practical implication of these combined observation will only be clarified with further investigation of ascorbic acid-lymphocyte interactions and with specific infections disease chalenges of ascorbic acid-treated calves.

Serum constituents:

Blood constituents, means and standard errors, are in tables 3 and 4. Ascorbic acid had no significant effect on either total protien or their fractions, albumin and globulin. Ascorbic acid treated calves had slightly lower urea nitrogen concentration than controls, but not significant (Table 3). Glucose level had nearly similar values in both treatments (Table 4). Cholesterol concentration was found to be higher in treated-animals than controls, but not significant. In control animals, cholesterol level increased with advance of experimental period, at least until 4 wk, but it decreased in treated animals (from 118.22 at wk 2 to 94.64 mg/dl at wk 6). The low blood cholesterol level due to ascorbic acid treatment was found in rabbits and rats by *SOKOLFF et al. (1967),* In PTU-treated chickens by *TAKAHASHI et al. (1991)* and in chickens by *ESA (1992).*

Triglyceride concentration was lower in ascorbic acid treated animals than controls (22.7 vs. 28.3 mg/dl). Similar result was found in scorbutic monkeys by *BANERJEE and BANDYOPANDYAY (1963),* who showed that administration of ascorbic acid decreased plasma triglycerides and free fatty acids concentrations. Deficiency of L-ascorbic acid can reduce the formation of carnitine. Carnitine is synthesized from lysine and methionine by two hydroxlases, both containing ferrous iron and L-ascorbic acid. Carnitine, ∝-amino-B-hydroxybutric acid trimethyl betamine, stimulate the transport of fatty acids into mitochondria, in which they are oxidized to provide energy for the cell as well as the animal *(HUGHES, 1981).* Deficiency of L-ascorbic acid may account, therefore, for the accumulation of triglycerides in blood *(JAFFE, 1984).* Indeed, triglycerides are metabolized by lipolytic enzymes in the carnitine biosynthesis pathway, which lower their level in serum *(JAFFE, 1984). HANCK and WEISER (1977)* stated that the inverse relationship between L-ascorbic acid intake and human mortility rates migh be due in part to, ascorbic acid decreased both blood cholesterol and triglycerides, consequently decreased the occurance of arterial disease.

In conclusion, ascorbic acid may improve the performance of suckling calves. The study declared also the influence of L-ascorbic acid upon the haemogram picture and some constituents of blood sera of buffalo calves. However, more studies are needed to substantiate the role of supplementary ascorbic acid in the performance during environmental stress, i.e. cold and hot weather.

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Fig. 1. Effect of dietary ascorbic acid on body weight of suckling calves

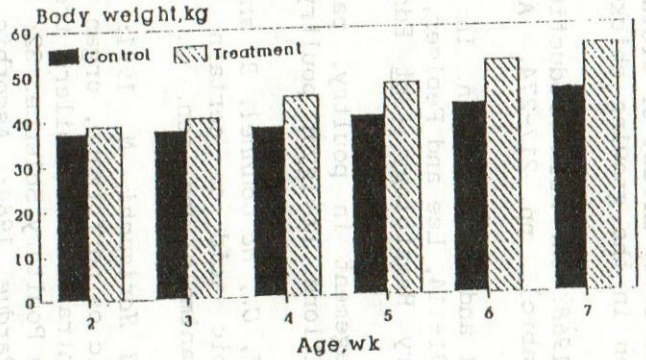

Table 1. Average daily gain (g/head/day) as influenced by ascorbic acid supplementation.

Age (days)	Control	Treatment	SE of LSM
14-21	51.6	226.4	131.28
22-28	91.4[a]	631.2[b]	131.28
29-35	297.0	354.2	131.28
36-42	317.0[c]	671.4[d]	131.28
43-49	428.6	492.8	131.28
Mean	237.12[a]	475.20[b]	58.71

a,b (P <.01)
c,d (P <.10)

Table 2. Red blood cell counts, hemoglobin, packed cell volume and white blood cell counts in buffalo calves as influenced by ascorbic acid supplementation.

	Red blood cells RBC (10^6/mm^3)			Hemoglobin Hb (g/dl)			Packed cell volume PCV, %			White blood cells WBC (10^3/mm^3)		
sampling week	Treatment A	B	SE	Treatment A	B	SE	Treatment A	B	SE	Treatment A	B	SE
2	5.61	5.67	.27	12.12	13.28	.50	33.65	36.00	1.45	13.86[a]	12.12[a]	.56
4	5.61	5.95	.27	11.50[c]	13.12[d]	.50	35.80	36.60	1.45	12.40	12.38	.56
6	5.78	5.73	.27	11.40[e]	13.56[f]	.50	32.20[g]	36.40[h]	1.45	13.04	13.70	.56
Mean	5.67	5.79	.15	11.73[c]	13.32[d]	.29	33.87[e]	36.33[f]	.84	13.10	12.73	.32

Values are least-squares means and SE = standard errors.
Treatments : A = control; B = vitamin C supplemented calves.
[c,d] (P<.01); [e,f] (P<.04); [g,h] (P<.05); [a,a] (P<.03).

Table 3. Serum total protein, albumin, globulin and urea nitrogen concentrations in buffalo calves as influenced by ascorbic acid supplementation.

sampling week	Total protein g/dl			Albumin g/dl			Globulin g/dl			Urea nitrogen mg/dl		
	Treatment			Treatment			Treatment			Treatment		
	A	B	SE	A	B	SE	A	B	SE	A	B	SE
2	7.95	7.20	.56	3.68	3.10	.46	4.28	4.09	.63	25.77	20.43	3.36
4	7.61	7.28	.56	2.99	3.50	.46	4.61	3.78	.63	28.16	21.95	3.36
6	7.43	7.98	.56	3.51	3.96	.46	3.87	4.04	.63	19.85	17.56	3.36
Mean	7.67	7.45	.32	3.39	3.32	.21	4.26	3.97	.36	24.59[c]	19.98[d]	1.94

Values are least-squares means and SE = standard errors.
Treatments: A = control; B = vitamin C supplemented calves.
[c,d] (P<.10)

Table 4. Serum glucose, cholestrol and triglycerides concentrations in buffalo calves as influenced by ascorbic acid supplementation.

Sampling week	Glucose mg/dl Treatment A	B	SE	Cholestrol mg/dl Treatment A	B	SE	Triglycerides mg/dl Treatment A	B	SE
2	121.69	118.16	9.34	74.49	118.22	19.09	30.18	20.00	4.71
4	119.13	121.22	9.34	89.71	108.82	19.09	28.83	26.50	4.71
6	131.68	130.10	9.34	81.19	94.64	19.09	25.86	21.59	4.71
Mean	124.15	123.67	5.83	81.80[c]	107.23[d]	11.02	28.30	22.70	2.72

Values are least-squares means and SE = standard errors.
Treatments : A = control; B = vitamin C supplemented calves.
[c,d] (P<.10).