

## Effect of Oral Supplementation of Vitamin C and Exercise on Plasma Vitamin C Status in Marwari Horses

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### Abstract

The objective of the present study was to evaluate the effect of the oral supplementation with ascorbic acid on plasma levels in Marwari horses. The experiment was planned to supplement ascorbic acid @ 5 mg/kg. Body weight orally for 4 days on plasma ascorbic acid levels in horses. There was no significant difference in plasma ascorbic acid levels between day 1<sup>st</sup> and day 5<sup>th</sup> in supplemented group and control group. After that, a second trial was undertaken with supplementation of sodium ascorbate @ 20 mg/Kg body weight for 30 days in exercising horses. Exercising horses were under gallop + canter exercise of 5 km per day in sandy track. Blood samples were taken on each 10<sup>th</sup> day of the study period. There was no significant difference found in plasma ascorbic acid levels between supplemented and non-supplemented group of exercising horses. It was also found that plasma ascorbic acid levels were also significantly low in exercising non-supplemented horses than the horses which were under rest. So it was concluded that continuous 5 km gallop+ canter exercise in sandy track for a month reduces plasma ascorbic acid levels in horses but it cannot be replenished by oral supplementation of ascorbic acid because bioavailability of ascorbic acid on oral supplementation in Marwari horses is very poor.

**Keywords:** Vitamin C; Ascorbic acid; Oxidative stress; Antioxidant; Exercise; Bioavailability; Horses

### Introduction

Vitamin C supplementation in horses is advocated [1-6] to reduce oxidative stress. It is involved in many cellular reactions like synthesis of collagen, immune functions and scavenging of free radicals. It protects tissues from harmful oxidative products and keep certain enzymes in their required reduced forms [1,7,8]. Except primates and guinea pig all the mammals can synthesize their ascorbic acid requirements [9]. In horses prolonged stress may results in low plasma vitamin C concentrations and resultant decrease in immune functions [2]. Present study was initiated with the objective to evaluate the bioavailability of vitamin C on oral supplementation in Marwari horses. Vitamin C is a water soluble vitamin and its half-life varies between 5 and 17 hrs in horses [10] so there should be increased plasma ascorbic acid status on oral supplementation. When after 5 days of supplementation there was no increase in plasma level of vitamin C than ascorbic acid was replaced with sodium ascorbate and dose was increased from 5 mg/kg to 20 mg/kg and trail was done for one month. In one month trial ascorbic acid levels were estimated on 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> day. There was no significant difference observed between supplemented and non-supplemented group.

### Material and Methods

#### Study area and environment

Study was carried out at Bikaner, which is located in center of subtropical desert of Rajasthan. This area has very low annual rainfall (<250 mm) and low humidity. There is wide seasonal variation in environmental temperature and day and night also. First bioavailability trial was carried out in winter season when minimum and maximum environmental temperature ranged between -2°C in night to 20°C in afternoon. Second bioavailability trial was conducted in summer season when minimum and maximum environmental temperature ranged between 26°C in night to 49°C in afternoon. All the animals were kept in well shaded and covered area during adverse environmental conditions. Riding exercise in summer trial was carried out at morning time between 6.30 AM to 7.30 AM when environment was cool and

ambient temperature remained 26°C to 30°C. Blood samples were taken just before of exercise.

#### Experimental protocol

All the horses selected for the study were given same feed and fodder before one month of the start of experiment to throughout the study period and no green fodder was offered during this period. Concentrate feed contained crushed mixture of 40% Oats, 30% Barley and 30% Wheat bran. Mineral mixture @ 3% was also added to concentrate mixture. Dry fodder contained dry sewan hay. Body weight of horses ranged between 340 to 389 Kg. Concentrate was offered @ 1% of body weight and dry fodder @ 2% of body weight to the horses. In supplemented group vitamin C (Ascorbic acid) was mixed with small amount of concentrate at morning time and was observed by the attendant person till its complete consumption. All the horse liked it and consumed ascorbic acid mixed concentrate within minutes after offering. In trial 1<sup>st</sup> 6 adult horses were supplemented with ascorbic acid @ 5 mg/Kg. orally once daily for 4 days and 4 other adult horses were kept as control. Bioavailability trial 2<sup>nd</sup> was carried out in exercising horses, which were already under regular exercise since last 6 months. Exercise was being given as 5 km. gallop ride in desert sandy track, 4 horses were supplemented with sodium ascorbate @ 20 mg/kg body weight orally once daily at morning time for 30 days and 4 other horses were kept in control exercise group, Three other horses were kept under resting control which were neither given exercise or supplement (Table 1).

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Feed	Concentrate mixture	Sewan hay
Dry matter (%)	91.02 ± 0.05	93.34 ± 0.43
Crude protein (% of DM)	16.03 ± 0.09	4.43 ± 0.08
Ether extract (% of DM)	2.33 ± 0.06	0.73 ± 0.05
Neutral detergent fibre (% of DM)	23.54 ± 0.17	66.51 ± 0.99
Acid detergent fibre (% of DM)	17.61 ± 0.11	33.12 ± 0.84
Non fibrous carbohydrate (% of DM)	38.17 ± 0.40	17.22 ± 0.38
Ash (% of DM)	3.65 ± 0.07	5.93 ± 0.26
TDN	74.18%	
DCP	11.38%	

**Table 1:** Composition of feed and fodder given to the horses in the study.

### Blood sampling and estimation of ascorbic acid

Blood was collected in heparinized vacutainers from jugular vein at early morning between 6 to 7 AM in both the trials. In exercising group it was before feeding and exercise. Plasma was separated immediately after blood collection. Plasma ascorbic acid was estimated by the method of Denson & Bowers [11]. Optical density was taken on model UV-VIS 164 spectrophotometer of Elico India Ltd. One ml of plasma was taken in centrifuge tube and 2 ml of 5% TCA (Trichloro Acetic Acid) was added to it. Then centrifuged at 4000 rpm for 15 minutes and upper 1ml of clear supernatant was taken in other borosil glass tube of 10 ml. Reagent [100 ml (2.2% of 2,4 dinitrophenylhydrazine in 10 N H<sub>2</sub>SO<sub>4</sub>), 5 ml of 5% Thiourea, 5 ml of 0.6% CuSO<sub>4</sub>, 5 H<sub>2</sub>O] was prepared and 0.3 ml of the reagent was added to the supernatant. Then it was kept in incubation for 4 hours at 37°C. Samples were then cooled under ice water and 1.5 ml of 65% H<sub>2</sub>SO<sub>4</sub> was added to it. Optical density was measured at 520 nm against reagent blank.

### Statistical Analysis

Data obtained were subjected to Student's *t*-test and were expressed as mean ± standard error of the mean. Values of *P* < 0.05 were considered significant.

### Results

Table 2 shows the plasma ascorbic acid levels on supplementation of ascorbic acid @ 5 mg/Kg. body weight for 5 days in the resting group. Plasma ascorbic acid levels were not significantly different in both the supplemented and resting control group during 5 days supplementation period except day 3, when levels of ascorbic acid were significantly high.

Table 3 shows the plasma ascorbic acid levels on supplementation in exercising horses. No significant difference was observed between supplemented and un-supplemented group of exercising horses during 30 days of supplementation. Whereas plasma ascorbic acid levels in resting control group of horses were significantly higher than the exercising horses either supplemented or un-supplemented.

### Discussion

The study was planned to estimate requirement of ascorbic acid in exercising Marwari horses after reviewing previous studies, those supported beneficial effects of ascorbic acid supplementation [2-6]. Findings of present study suggested that there is almost nil or very poor bioavailability of ascorbic acid in horses on oral supplementation. Snow and Frigg [3] reported that there is significant increase in plasma and tissue ascorbic acid levels on daily oral administration of ascorbic acid @ 20 mg/Kg. Body weight. But they also found that on single dose there was no effect on plasma ascorbic acid concentration. In another trial Snow and Frigg [12] in thoroughbreds on 15 days of oral administration of ascorbic acid 20 g, marked differences in plasma ascorbic acid levels were observed between individuals and they warranted further investigation to assess significance of ascorbic acid supplementation. In 1<sup>st</sup> trial of present study there was significant high level increase in plasma ascorbic acid on 3<sup>rd</sup> day of study in compare to starting day of trial and then the control group. While on 5<sup>th</sup> day plasma levels become normalized again and there was no significant difference in compare to control group and pre supplementation levels even after continuous supplementation. This behaviour shows that there are fluctuations in plasma ascorbic acid levels in horses which are independent of supplementation. Due to these fluctuations, in some studies might have been increased plasma ascorbic acid levels on oral vitamin C supplementation observed. However such fluctuations were not observed in exercising horses and range of ascorbic acid was also narrow in exercising horses (Table 3). In an ascorbic acid supplementation trial Deaton and Marlin [8] reported significant increase in mean plasma ascorbic acid concentrations in compare to control after two weeks supplementation with ascorbyl palmitate, however supplementation with Calcium ascorbyl-2-monophosphate another more stable form of ascorbic acid supplementation did not significantly increase plasma ascorbic acid levels. In an another supplementation study Williams et al. [6] reported that addition of vitamin C increases plasma ascorbate status in horses but have no effect on the oxidative stress variables in horses. Ascorbic acid is a well-known

Days	Supplemented group (N=6)	Range	Control Group (N=4)	Range
1 <sup>st</sup> day (Mean ± SE), mg/L	5.09 ± 0.50	3.33-6.67	5.19 ± 0.54	4-6.55
2 <sup>nd</sup> day (Mean ± SE) mg/L	3.92 ± 1.12	2.00-9.18	2.39 ± 0.37	1.33-3.11
3 <sup>rd</sup> day (Mean ± SE) mg/L	6.00 ± 0.61*	3.66-7.55	3.78 ± 0.45	2.44-4.44
4 <sup>th</sup> day (Mean ± SE) mg/L	6.11 ± 0.65	4.44-9.11	4.11 ± 1.00	2.11-5.89
5 <sup>th</sup> day (Mean ± SE) mg/L	4.26 ± 0.65	2.44-6.11	4.00 ± 0.78	2.33-5.44

\*significantly high than the control group *P* value < .05.

**Table 2:** Plasma ascorbic acid levels on supplementation of Ascorbic acid 5 mg/kg body weight for 5 days.

Days	Supplemented Exercise group (N=4)	Range	Control Rest (N=3)	Range	Unsupplemented Exercise Group (N=4)	Range
1 <sup>st</sup> day (Mean ± SE) mg/L	3.35 ± 0.79	1.88-5.55	4.37 ± 0.22	4-4.7	2.49 ± 0.28	2.11-3.33
15 <sup>th</sup> day (Mean ± SE) mg/L	2.22 ± 0.43	1.33-3.33	3.11 ± 0.11	3-3.3	2.30 ± 0.44	1.22-3.22
30 <sup>th</sup> day (Mean ± SE) mg/L	2.24 ± 0.38	1.33-3.22	3.63 ± 0.20	3.22-3.89	2.58 ± 0.20	2.11-3.11

\* significantly height than the exercise group *P* value < .05.

**Table 3:** Plasma ascorbic acid levels in exercising horses on supplementation Sodium Ascorbate @ 20 mg Kg body weight for 30 days, (Summer season).

antioxidant and should have reduced oxidative stress to some extent; increased levels of plasma ascorbic acid as reported by Williams et al. [6] might have been found due to fluctuating behaviour of plasma ascorbic acid levels as observed in trial 1<sup>st</sup> of the present study. Results obtained in the present study are in agreement with the findings of Loscher et al. [10] that following oral administration, the systemic availability of ascorbic acid is very poor and plasma levels do not increase on oral supplementation. As compared to resting control group it was observed that vitamin C levels were low in both the exercise group with or without supplementation. Decrease in vitamin C levels in exercising horses indicates increased requirement of vitamin C in exercising horses. Exercise is associated with phenomena of oxidative stress [13-17] and in racing horse oxidative stress increases with extent of exercise [8,18,19]. Erythrocyte membrane fluidity significantly decreases in response to strenuous exercise and that have correlations with oxidative stress markers [20,21]. Oxidative stress is the phenomenon associated with exercise and it may cause damage to lipid, protein and DNA and consequently decreases the athletic performance [8]. Horses those have low level of oxidative stress parameters also performs better during long endurance race [22]. Present study is in agreement that under stress and performances demand for ascorbic acid increases and concentrations of ascorbic acid in blood decrease [4,23] but it cannot be replenished by oral supplementation [10].

## Conclusions

It is concluded that exercise induced oxidative stress reduces plasma ascorbic acid levels and bioavailability of oral supplementation of ascorbic acid is very poor in horses.

## Ethical Standard

All horses that served as subjects in this study were humanely cared. Study was approved by the Institute Research Committee and Institute Animal Ethics Committee of National Research Center on Equines Hisar as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

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