

## Ascorbic acid (vitamin C) status in Icelandic horses in Iceland

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### SUMMARY

Ascorbic acid (vitamin C) status in Icelandic horses in Iceland was studied. Blood samples from 65 Icelandic horses were collected and analyzed for ascorbic acid. Horses evaluated were 7 horses used as blood donors, 13 hyperimmunized horses (*Clostridium Welchii* toxin), 12 horses used for occasional riding, 10 horses trained for competition, 12 pregnant mares in their last trimester and 11 yearlings. The average ascorbic acid concentration for all horses was  $1.34 \pm 0.69$  mg/100 ml. The lowest average concentration of ascorbic acid  $0.88 \pm 0.47$  mg/100ml was found in the pregnant mares in their last trimester and highest in horses used for occasional riding. Significantly lower mean ascorbic acid concentration was found in the horses trained for competition than in horses used for occasional riding ( $P < 0.05$ ),  $1.14 \pm 0.49$  and  $1.72 \pm 0.99$  mg/100 ml respectively. The lowest concentration was found in one of the yearlings 0.24 mg/100 ml.

The results seem to indicate that there is considerable variation in the ascorbic acid concentration in the serum of the Icelandic horses in Iceland. It is concluded that the ascorbic acid status is generally good, although low concentration of ascorbic acid is found in individual cases.

Key words: ascorbic acid, Icelandic horses.

### YFIRLIT

#### *Askorbínstyrkur (C vitamín) í blóði íslenskra hrossa*

Kannaður var askorbínstyrkur í blóði íslenskra hrossa hér á landi. Blóðsýnin voru tekin úr 65 hestum og askorbínsýra mæld. Tekin voru sýni úr eftirfarandi hestum: 7 hestum sem tekið var blóð úr reglulega, 13 hestum í bóluefnisframleiðslu (*Clostridium Welchii* toxín), 12 reiðhestum, 10 þjálfuðum keppnishestum, 12 fylfullum hryssum og 11 trippum. Meðalstyrkur askorbínsýru í blóði allra hestanna var  $1.34 \pm 0.69$  mg/100 ml. Minnsti meðalstyrkur fannst í blóði fylfullra hryssna,  $0.88 \pm 0.47$  mg/100 ml í síðasta hluta meðgöngu, og hæsti meðalstyrkur hjá reiðhestum. Marktaðt minni meðalstyrkur fannst í blóði þjálfara keppnishesta ( $P < 0.05$ ) en í reiðhestum, þ.e.  $1.14 \pm 0.49$  og  $1.71 \pm 0.99$  mg/100 ml. Lægsta gildið mældist í blóði trippis, 0,24 mg/100 ml.

Niðurstöðurnar virðast benda til töluverðs breytileika á styrk askorbínsýru í sermi hrossa hér á landi. Þá sýna niðurstöðurnar að styrkur askorbínsýru í blóði hrossa sé í góðu lagi, þó lág gildi finnist í einstaka tilfelli.

### INTRODUCTION

Most animals are capable of synthesizing ascorbic acid from simple sugars, where the L-gulonic acid is converted in an enzyme-

catalyzed reaction to L-ascorbic acid in those species which can synthesize their own vitamin C, i.e. all domestic animals. The en-

zyme is lacking in man, nonhuman primates and the guinea pig (Grollman and Lehninger, 1957) and these species are dependent on an exogenous supply of ascorbic acid. Research into the ascorbic acid status of the horse started around 1940 and led to the conclusion that the horse is not dependent on a supplementary exogenous supply of ascorbic acid. The view predominated that horses like other monogastric animals always could meet their demand for ascorbic acid by biosynthesis. Later research has shown that this is not always the case (Jaeschke and Keller, 1978a; Jaeschke, 1984) and physical stress situation such as intensive training programmes and competitions can draw upon the ascorbic acid pool of the organism and bacterial and viral infections will do the same and may cause deficiencies in the horse which can not always be compensated by endogenous biosynthesis. The symptoms which accompany a reduced ascorbic acid blood level include the "poor performance syndrome", anaemias, nose-bleedings (epistaxis). The growth and development phases of the young horses can be delayed due to the role of ascorbic acid in the collagen biosynthesis (Jaeschke, 1984; Naito, 1980).

The purpose of the present work was to study the ascorbic acid status of the Icelandic horse in Iceland. The growth and development phases of the Icelandic horses are slow and they are not fully grown until they are 4–6 years old which means they are not used for riding until then. The stress situation which may affect the ascorbic acid pool is not so serious as in racehorses. No contagious bacterial and viral infections are registered in Iceland such as strangles, rhino-pneumonia EHV-1 and influenza and other viral infections of the respiratory organs. The situation therefore is different from that of racehorses in other countries where viral infections of the respiratory organs are common so nearly all horses have been exposed to such infections (Jaeschke, 1984).

## MATERIALS AND METHODS

### *Animals*

Sixty-five Icelandic horses were evaluated. All horses were seemingly healthy and had not been given supplemental vitamins. Horses evaluated were: 7 horses used as blood donors, 13 hyperimmunized horses (Clostridium Welchii toxin), 12 horses used for occasional riding, 10 trained horses, 12 pregnant mares in their last trimester and 11 yearlings. The blood was collected during winter time when the horses were stabled, except for the pregnant mares. Blood samples from the mares were collected in May when they were in their last trimester and on pasture. In addition the mares received varying quantity of big bale silage. The yearlings were fed hay *ad libitum*.

### *Blood samples*

Blood samples were collected in siliconized tubes (Vacutainer, containing clot activator) and centrifuged within 2 hours and serum transferred to plastic tubes and stored at  $-20^{\circ}\text{C}$  until analyzed. Before analysis 1 ml of serum was precipitated by 2 ml of 0.6 N perchloric acid and centrifuged.

### *Analyses*

The ascorbic acid concentrations were assayed photometrically at 520 nm by the dinitrophenylhydrazine method of Denson and Bowers (1961) as modified by Jaeschke and Keller (1978a). No control system exists for ascorbic acid, but the precision of the method employed was calculated using the differences between duplicate determinations (interassay variation). The mean coefficient of variation for 15 determinations was 3.8%.

## RESULTS

Table 1 shows the mean, standard deviation and standard error of mean of the serum ascorbic acid concentrations in each group of horses. It also shows the minimum and the maximum concentrations in each group.

**Table 1.** Ascorbic acid concentrations in the serum of Icelandic horses.1. *tafla. Askorbínstyrkur í blóðvökva íslenskra hrossa.*

Horses evaluated <i>Metin hross</i>	Number <i>Fjöldi</i>	Mean <i>Meðaltal</i>	SD <i>Staðalfrávik</i>	SE <i>Staðalskekkja</i>	Min. <i>Lágmark</i>	Max. <i>Hámark</i>
Horses used as blood donors <i>Hross í reglulegri blóðgjöf</i>	7	1.19	0.36	0.14	0.74	1.80
Hyperimmunized horses <i>Hross í bóluefnisframleiðslu</i>	13	1.41	0.61	0.17	0.46	2.87
Riding horses <i>Reiðhross</i>	12	1.72	0.99	0.29	0.51	3.42
Trained horses <i>Keppnishross</i>	10	1.14	0.49	0.16	0.51	2.15
Pregnant mares <i>Fylfullar merar</i>	12	0.88	0.47	0.14	0.34	2.08
Yearlings <i>Trippi (veturgömul)</i>	11	1.62	0.62	0.19	0.24	2.27
All horses <i>Öll hross</i>	65	1.34	0.69	0.09	0.24	3.42

The average ascorbic acid concentrations for all horses was  $1.34 \pm 0.69$  mg/100 ml. According to this the reference range would be 0.65–2.03 mg/100 ml. The lowest average concentration of ascorbic acid  $0.88 \pm 0.47$  mg/100 ml was found in the pregnant mares in their last trimester and highest in horses used for occasional riding. Significantly lower mean ascorbic acid concentration was found in the horses trained for competition than in the horses used for occasional riding ( $P < 0.05$ ),  $1.14 \pm 0.49$  and  $1.72 \pm 0.99$  mg/100 ml respectively. A rather high average concentration was found in the blood of the yearlings,  $1.62 \pm 0.62$  mg/100 ml. The lowest concentration was found in one of the yearlings 0.24 mg/100 ml and the next lowest in one of the pregnant mares 0.34 mg/100 ml. Concentrations lower than 0.65 mg/100 ml were found in 7 of the 65 horses or in 10.8% of the cases. Very high concentrations were found in two of the riding horses 3.42 and 3.39 mg/100 ml and of the 65 horses concentrations higher than 2.03 mg/100 ml were registered in 12 cases (18.5%).

## DISCUSSION

The results seem to indicate that there is a considerable variation in the ascorbic acid concentration in the serum of the Icelandic horses in Iceland. A great individual variation is observed in other studies, although the individual variation is greater in this study. Many things seem to indicate that one can expect great differences between breeds and familial and individual variation in the capacity of the horses to synthesize ascorbic acid (Jaeschke and Keller, 1978a; Rasbech and Koefoed-Johnsen, 1987). The concentration of ascorbic acid in the serum is higher than reported in racehorses, trotters and thoroughbreds by Jaeschke and Keller (1978a). The reference range reported by Jaeschke and Keller (1978a) was 0.50–0.70 mg/100 ml and similar results were obtained by Rasbech og Koefoed-Johnsen (1987) in the same horse-breeds. Higher reference range is reported by Axt *et al.* (1968), 1.085–1.370 mg/100 ml in the serum of trotters and thoroughbreds. Baker *et al.* (1986) found that the reference range for ascorbic acid in a mixed

breeds of horses was  $2\pm1$  mg/100 ml. Jaeschke and Keller (1978a) found higher concentrations in the serum of heavy breeds of horses and reported that the ascorbic acid concentrations in the blood of these horses nearly always was higher than 1.0 mg/100 ml. The results in present work are similar to that of heavy breeds of horses, although concentrations found are in some cases much higher. In this study the highest average concentration of ascorbic acid was found in horses used for occasional riding and the concentration was significantly higher than that found in trained horses used for competition. It is known that stress situations can draw upon the ascorbic acid pool of the organism and cause a drop in the serum level (Jaeschke, 1984) and this may explain the lower average concentration found in horses used in competition. It should be born in mind that the stress situation of the Icelandic horses during training and competition are not so severe as in racehorses in other countries. It should also be born in mind that the Icelandic horses are older when tamed, 4–5 years, but the training of racehorses starts when they are 14–16 months old and racing when they are 2 years old. Under the increasing intensity of training which coincides with the rapid development and growth phase, these racehorses are exposed to an increased physical stress which must drain their ascorbic acid pool.

It is important to keep in mind that no contagious bacterial and viral infections like strangles, rhinopneumonia EHV-1, influenza and other viral infections of the respiratory organs have been registered in Iceland at the time of the blood collection. Virally induced stress situation causes a sustained drain on the ascorbic acid pool and the capacity of the liver to compensate for increased consumption by increasing endogenous synthesis is not unlimited. All racehorses are exposed to viral infections, but not the Icelandic horses in Iceland and this together with the slow growth of the Icelandic horse may explain

the higher serum level of ascorbic acid found in Icelandic horses in Iceland.

The lowest concentration was found in the pregnant mares in their last trimester of pregnancy. The pregnancy is a stressful condition and stresses the carbohydrate and lipid metabolism of the mare (Jeffcott and Field, 1985). This may explain the low ascorbic acid concentration found in the blood of the pregnant mares. Low concentrations have also been registered in lactating mares (Rasbech and Koefoed-Johnsen, 1987).

Very low concentrations were found in two cases, in a yearling and a pregnant mare. No obvious clinical signs were observed but the possibility remains that these horses may have been ill at least in the period before the samples were collected. Similar results have been demonstrated in horses which were ill or recently recovered (Jaeschke and Keller, 1978b; Rasbech and Koefoed-Johnsen, 1987). In the present study a lower concentrations than 0.65 mg/100 ml were found in 10.8% of the cases which indicates that one can find deficiencies in ascorbic acid concentration in the blood of Icelandic horses in Iceland, although in most cases ascorbic acid status is sufficient and in individual cases very high.

It is concluded that the results seem to indicate that the ascorbic acid status of the Icelandic horses in Iceland is generally good, although low concentration of ascorbic acid is found in individual cases probably without clinical signs.

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