

## The effects of trace element supplements on blood levels of horses

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**Abstract.** This study concerns the effects of Se, Cu, Co, Mn and Zn supplements on blood levels of Finnish warm-blooded trotters. The results revealed that the blood Se content corresponding to the feed Se content of 0.1 mg/kg is  $0.17 \pm 0.03$  mg/kg in the horse. Blood Se level was directly related to the feed Se content and the other feed trace elements had a similar effect on blood levels.

### Introduction

The maximal performances demanded of race horses require that the various blood sub-factors fall within optimal, often very narrow, ranges (WILLIAMSON 1974). These sub-factors, macro- and microminerals, are influenced by the horses feed (KOSSILA et al. 1974, KÄÄNTEE and KURKELA 1978). In addition to the poor performances, deficiency conditions are reflected in diseases such as muscle dystrophy arising from lack of Se (LANNEK and LINDBERG 1975). Because the Se content of Finnish fodders is poor (KÄÄNTEE et al. 1978), particular attention has been paid to the Se supplement of horse feed. Selenium, together with vitamin E and biotin, acts as a cell membrane protective factor (WROGEMANN and PENA 1976).

The effect of different Se compounds on the body Se level varies (KÄÄNTEE and KURKELA 1980). Both the body and feed levels of Se affect the behaviour of Cd, Hg, Cu, and Zn in the organism because of the interaction between various trace elements (DAVIES 1974).

In the studies performed the effect of feed factors on macro- and micro-minerals in the horse was investigated, with main attention focused on selenium.

### Material and methods

Seven warm-blooded 4-8-year-old trotters in racing condition were used for the trials. The horses' weights varied between 340 and 410 kg and all were raced during the studies. The fodders and feeding periods are shown in Table 2. The feed

Se level was altered by replacing part of the oats with barley rich in Se (0.68 mg/kg) during trial period 2. This increased the feed proportion of grain Se. During trial periods 4 and 5 the horses received 0.9 mg and 1.5 mg daily, respectively, of selenium as sodium selenite in a trace element mixture. The daily dose of trace elements and vitamins was: iron 600 mg, copper 25 mg, zinc 150 mg, iodine 0.15 mg, cobalt 3.0 mg, manganese 70 mg, vitamin E 750 mg, vitamin B<sub>1</sub> 24 mg, vitamin B<sub>2</sub> 20 mg, vitamin B<sub>6</sub> 12 mg, vitamin B<sub>12</sub> 1 mg, folic acid 12 mg and vitamin C 500 mg.

The feed Ca, P and Se were determined at the Viljavuuspalvelu Oy company and the Oulu Research Laboratory of the Kemira Company (Table 1).

Hb was determined as cyanmethaemoglobin, serum alkaline phosphatase by the HUGGINS and TALALAY (1945) method, SGOT and SGPT by the REITMAN and FRANKEL (1957) method, serum total protein by the WECHSELBAUM (1946) biuret method, serum vitamin B<sub>12</sub> by isotope dilution analysis and folic acid by the DUNN and FOSTER (1973) method. Serum Ca, P, Mg, Na, K, Fe, creatinine and urate analyses were carried out with a SMA/60 Autoanalyzer in the Oulu Deaconess Institute Laboratory, and blood Se, Mn, Cu, Co and Zn determined by Perkin-Elmer atomic absorption spectrophotometer at Viljavuuspalvelu.

The blood samples were taken from the jugular vein during morning feeding on completion of each feeding period. On days prior to blood sample taking the horses did not take part in races or heats.

## Results

The results of the blood Se analysis are shown in Table 2. They show that as the feed Se content rose the blood Se concentration also increased. A feed Se content of 0.1 mg/kg corresponded to a blood Se concentration of  $0.17 \pm 0.03$  mg/kg.

Table 3 shows analysis results of samples taken on completion of feeding periods. The use of the trace element mixture clearly increased blood Cu, Mn and Zn concentrations. The freely available refined salt raised serum Na values.

It was established clinically that during the use of the trace element mixture the general condition of the horses improved and the quality of the hair became better. Perspiration due to strain decreased and recovery was quicker. Racing performances also improved during the trial.

Table 1. Ca, P and Se contents of the feeds given and daily amounts of feed used.

	Ca g/kg	P g/kg	Se mg/kg	Daily dose of feed kg
HAY .....	3.0	2.1	0.02	7
OATS .....	0.6	3.4	0.02	5 (3)
BARLEY .....	0.6	3.4	0.68	(2)
BRAN .....	1.2	13.6	0.13	0.75
PROMECA ....	10.0	10.5	0.08	1

Table 2. Feeds of the horses during different feeding periods. Horses' daily feed Se intake and blood Se content on completion of the feeding period. 7 horses

PERIOD 1 21 days	PERIOD 2 28 days	PERIOD 3 21 days	PERIOD 4 28 days	PERIOD 5 28 days
HAY 7 kg	HAY 7 kg	HAY 7 kg	HAY 7 kg	HAY 7 kg
OATS 5 kg	OATS 3 kg	OATS 5 kg	OATS 5 kg	OATS 5 kg
BRAN 0.75 kg	BARLEY 2 kg	BRAN 0.75 kg	BRAN 0.75 kg	BRAN 0.75 kg
PROMECA 1 kg	BRAN 0.75 kg PROMECA 1 kg	PROMECA 1 kg	PROMECA 1 kg TRACE ELEMENT MIXTURE	PROMECA 1 kg TRACE ELEMENT MIXTURE II
Se 0.03 mg/kg	Se 0.13 mg/kg	Se 0.03 mg/kg	Se 0.1 mg/kg	Se 0.14 mg/kg
Se 0.42 mg/day (0.42)	Se 1.79 mg/day (1.73)	Se 0.42 mg/day (0.42)	Se 1.32 mg/day (0.42 + 0.9)	Se 1.92 mg/day (0.42 + 1.5)
BLOOD Se 0.08 ± 0.04 mg/kg	BLOOD Se 0.14 ± 0.04 mg/kg	BLOOD Se 0.07 ± 0.02 mg/kg	BLOOD Se 0.17 ± 0.03 mg/kg	BLOOD Se 0.19 ± 0.04 mg/kg FREE ACCESS TO SALT

Table 3. Analysed serum and blood contents on completion of feeding periods (7 horses). The feeds given are in table 1.

- 1: Basic feed 2: Part of the oats replaced by barley  
 4: Trace element supplement during basic feeding  
 5: Trace element supplement + salt (NaCl) supplement during basic feeding

FEEDING PERIOD	1	2	4	5
Hb g/l	146 ± 14	144 ± 21	144 ± 12	155 ± 22
Serum GOT IU	321 ± 112	347 ± 216	291 ± 75	335 ± 385
Serum GPT IU		13 ± 8	12 ± 4	
Serum alkaline phosphatase IU	9.8 ± 2	11.5 ± 21	11 ± 2.8	10 ± 1.7
STP g/l	63 ± 2	62 ± 3	60 ± 3	63 ± 4
Serum Ca mmol/l		2.84 ± 0.07	2.78 ± 0.05	2.74 ± 0.09
" P " "		1.16 ± 0.16	1.12 ± 0.11	1.10 ± 0.21
" Mg " "		0.81 ± 0.14	0.75 ± 0.05	
" Na " "		135 ± 2	134 ± 2	141 ± 2
" K " "		4.1 ± 0.1	3.8 ± 0.2	4.0 ± 0.2
" Fe " "		36.4 ± 3.3	30.1 ± 6.8	
Blood Cu ug/kg	779 ± 395	749 ± 369	2044 ± 1613	
" Co " "	28.5 ± 13.5	38.3 ± 15.3	24.4 ± 6.4	
" Mn " "	38 ± 13.8	28.8 ± 123	79.8 ± 41.8	
" Zn mg/kg	2.67 ±	3.00 ± 0.63	3.19 ± 0.61	
Serum B <sub>12</sub> ng/ml		1842 ± 194	2010 ± 195	
" folic acid ng/ml		5.93 ± 1.2	7.33 ± 2.3	
" creatinine mmol/l		154 ± 9	153 ± 14	
" urate mmol/l		34 ± 6	30 ± 7	



## Discussion

### *Selenium*

The blood Se content of the horse depends on the feed Se content. The blood Se concentration of Finnish warm-blooded trotters has been measured as  $0.18 \pm 0.08$  mg/kg (KÄÄNTEE et al. 1978). Concepts of normal blood Se level differ greatly because of both variations in feed Se contents and inaccuracies in analytical methods employed. The Se requirement of animals is reported to be 0.1 mg/kg feed (NRC 1973).

The fodder Se concentration 0.1 mg/kg fodder was consisted of the grain Se, 0.03 mg/kg, and of trace element mixture, 0.09 mg/kg. The blood Se concentration was established to be  $0.17 \pm 0.03$  mg/kg, which is of the same order as the normal value established in the previous studies (KÄÄNTEE et al. 1978).

The feed analyses carried out revealed the poor Se content of Finnish feeds. The Se content of basic feed was 0.03 mg/kg, which is clearly less than the critical Se level of 0.05 mg/kg below which not even large supplements of vitamin E can replace the lack of Se (JONES et al. 1977).

On basic feeding the blood Se content was  $0.08 \pm 0.04$  mg/kg. Muscular dystrophy has been established in horses with blood Se levels of 0.05 mg/kg or under (LINDHOLM et al. 1978). On basic feeds, however, the blood Se content was above this critical level. In horses fed on Swedish domestic feeds the blood Se level varied between 0.01 mg/kg and 0.08 mg/kg (values calculated on the basis of the GSH-Px activity values of the HENNICHS and PEHRSON 1979 trial). The values established in the present study were slightly higher because brans rich in Se and Promeca were used. In Sweden, a Se supplement of 4.4 mg has been added to domestic feeds 2–4 times monthly to satisfy the Se requirement (HENNICHS and PEHRSON 1979). In the present trial daily Se supplements of 0.09 mg and 1.5 mg were added to supplement the normal feed of Finnish trotters. The feed Se content then rose to the NRC (1973) animal requirement level and blood Se content to values of  $0.17 \pm 0.03$  mg/kg and  $0.19 \pm 0.04$  mg/kg, respectively.

It has been established in hens that grain Se raises body and blood Se contents considerably better than sodium selenite added to feed mixtures (KÄÄNTEE and KURKELA 1980). This was not found in the present material. The barley used was rich in selenium, containing 0.68 mg/kg grain. The selenium content of test feed 2 was 0.13 mg/kg with grain Se, but the blood Se content was  $0.14 \pm 0.04$  mg/kg. The selenium content of test feed 5 was almost the same at 0.14 mg/kg.

Most of the supplied selenium was sodium selenite from the trace element mixture; 0.03 mg/kg was grain Se and 0.11 mg/kg came from the trace element mixture. The blood Se content was  $0.19 \pm 0.04$  mg/kg. The differences in blood Se contents were probably due to factors caused by gastrointestinal bacteria influencing selenium absorption (the bacteria transform selenium compounds) as well as to the effects of vitamin C contained in the trace element mixture. (COMBS and PETO 1976)

### *Copper*

The Cu requirement of the horse is 5–8 mg/kg feed, according to OLSSON (1969). DREPPER (1972) recommends 10 mg Cu/100 kg bodyweight for racing horses. Low Cu contents have been found in the timothy grass of Northern Finland

(LAKANEN 1969). The Viljavuuspalvelu Company reports the mean Cu content of the hay of 1977–1978 as 4.4–4.6 mg/kg, of barley 5.5–5.1 mg/kg and of oats 3.9–5.3 mg/kg. The hay and oat Cu contents established by KOSSILA et al. (1972) were 6.7–7.4 mg/kg and 9.8–6.8 mg/kg, respectively, i.e. considerably higher than the values reported by Viljavuuspalvelu. The serum Cu content of horses in the material of KOSSILA et al. (1972) varied between 470 and 1140  $\mu\text{g}/\text{kg}$ . According to KOLB (1967), the normal value for horses is 1300  $\mu\text{g}/\text{kg}$ .

MÜLLER-REH (1972) established the plasma Cu content of the horse to be 820–1210  $\mu\text{g}/\text{kg}$  while the feed Cu content varied between 11 and 31 mg/kg dry feed. According to EKMAN et al. (1975) the normal plasma Cu content is  $1530 \pm 380$   $\mu\text{g}/\text{kg}$ .

In the material studied the whole blood Cu content on feed 1 was  $779 \pm 395$   $\mu\text{g}/\text{kg}$  and on feed 2  $749 \pm 369$   $\mu\text{g}/\text{kg}$ . The content trebled following the giving of the trace mineral mixture to  $2044 \pm 1613$   $\mu\text{g}/\text{kg}$ . The daily Cu supplement was 25 mg, and thus the daily copper requirement of 60 mg was fully satisfied when feed Cu (5 mg/kg) was also taken into account.

### *Zinc*

The zinc requirement of horses is according DREPPER (1972) 40 mg/100 kg and that of Mn 20 mg/100 kg. KOSSILA et al. (1974) found Zn contents of 0.34–1.18 mg/kg in serum of horses that had received feed exceeding DREPPER's norms. According to SCHEUNEURT and TRAUTMANN (1965), there is less zinc in serum (2.0 mg/kg) than in erythrocytes (12.0 mg/kg). MÜLLER-REH (1972) established the plasma Zn concentration of the horse as 0.55–0.67 mg/kg while the feed Zn content was 0.22–0.44 mg/kg.

In the material studied, whole blood was used for determining the Zn content in order to avoid errors due to haemolysis. The blood Zn concentrations obtained were  $2.67 \pm 0.78$  and  $3.00 \pm 0.63$  mg/kg and after a daily supplement of 150 mg Zn  $3.16 \pm 0.61$  mg/kg. The Zn supplement used had no significant effect on the blood Zn value.

### *Manganese*

The blood Mn concentration doubled with a daily 70 mg supplement. According to the figures reported by Viljavuuspalvelu, the Mn contents of hay (71–84.7 mg/kg), oats (56.7–60.7 mg/kg) and barley (27.5–24.0 mg/kg) are sufficient to supply the 20 mg/100 kg requirement for a horse suggested by DREPPER (1972). MÜLLER-REH (1972) proposes 30–50 mg/kg as the recommended Mn content of horse feed.

In the material studied the horses' blood Mn concentration varied markedly because of feed factors. On feed 1 (hay + oats) the blood Mn content was  $38.0 \pm 13.8$   $\mu\text{g}/\text{kg}$ . On feed 2 where part of the oats was replaced by barley poorer in Mn, the blood Mn content fell clearly to  $28.8 \pm 12.3$   $\mu\text{g}/\text{kg}$ . The Mn content of barley, according to Viljavuuspalvelu, is about half that of oats. With the daily trace element supplement of 70 mg the blood Mn level rose to  $79.8 \pm 41.8$   $\mu\text{g}/\text{kg}$ .

## *Iron*

Both positive and negative interactions occurs between the various trace elements in the body. Certain interactions may generate diseases like anaemia, which may be due to iron, copper or cobalt deficiency, or excessive zinc or selenium intake (VUORI 1979). There is negative interaction between Cu and Zn as well as between Mn and Fe. Cu has positive interaction with Fe. A feed rich in Zn can give rise to conditional Cu deficiency, Which in turn affects Fe metabolism (DAVIES 1974).

In the present study, relatively large trace element supplements were used in association with feed 4. Clinically, the supplements were not established to be harmful. On the basis of analysis results, the greatest increases were found in blood Cu and Mn levels, but serum Fe fell slightly even though the trace element mixture contained Fe. This may have been due to the effects of Mn.

The Hb values and serum alkaline phosphatase, GPT, GOT, STP, Ca, P, Mg, K and Fe of the material studied were at normal levels for Finnish warm-blooded horses (KÄÄNTEE 1977). As can be seen in Table 3, these blood parameters remain within fairly narrow ranges in a healthy race horse.

## *Sodium*

Serum Na values at the lowest limits of the 'normal' range were established. The normal values for serum Na given in the literature material compiled by BEST (1979) range between 125 and 152 mmol/l, with most authors mentioning 139–141 mmol/l as the ideal concentration. The Na requirement of the body is strongly affected by perspiration as well as by secretion of gastric and intestinal juices. A horse engaged in heavy racing and training requires 85 g refined salt according to NRC (1973). Such a high daily requirement demands a salt supplement in the feed or free access to salt, as was the case with feed 5. Serum Na content rose to the level of 141 mmol/l demanded by maximal performance recommended by WILLIAMSON (1974).

## *Folic acid*

In his studies ALLEN (1978) established that the requirement and use of folic acid by training and racing trotters was greater than that of inactive horses. Folic acid is particularly excreted from the body in the sweat. The serum folic acid content of training horses was  $3.3 \mu\text{g/l}$  ( $1.5\text{--}6.1 \mu\text{g/l}$ ) and in inactive animals  $10.6 \mu\text{g/l}$  ( $6.4\text{--}15.8 \mu\text{g/l}$ ).

In the present material, the serum folic acid content, or that of the basic form of tetrahydrofolic acid which acts as coenzyme in the transfer of hydroxymethyl groups, in training horses was  $5.93 \pm 1.2 \mu\text{g/l}$  at the beginning of the trial. This level approximates those measured by ALLEN. With the trace element mixture the horses received a 12 mg folic acid supplement daily. This raised the serum level to  $7.33 \pm 2.3 \mu\text{g/l}$ , which was clearly lower than those established by ALLEN in inactive horses. The folic acid supplement used in the material studied has to be regarded as recommendable because of increased consumption due to the heavy training of the horses.

## Vitamin B<sub>12</sub>

In the material studied the serum vitamin B<sub>12</sub> content was  $1.84 \pm 0.19$  ng/ml and after a daily vitamin B<sub>12</sub> supplement  $2.01 \pm 0.19$  ng/ml. Thus the vitamin supplement had no significant effect on the serum vitamin B<sub>12</sub> level of the horses. The values determined approximated those established by SALMINEN (1975) in Finnish horses, i.e.  $1.54 \pm 0.16$  ng/ml. SECKINGTON et al. (1967) found  $1.11 \pm 0.1$  ng/ml to be the mean serum vitamin B<sub>12</sub> content among 25 horses.

## Summary

In this study variations in blood parameters of 7 Finnish warmblooded trotters were investigated in association with changes in the feed trace element content. The latter were achieved by replacing part of the oats of the basic feed by barley rich in Se, and by giving a trace element mixture in association with the basic feed.

When the Se content of horse feed was 0.03 mg/kg the blood Se concentration was  $0.08 \pm 0.04$  mg/kg. With a feed value of 0.1 mg/kg the blood concentration rose to  $0.17 \pm 0.03$  mg/kg and with a feed content of 0.14 mg/kg to  $0.19 \pm 0.04$  mg/kg.

The blood Cu value ( $779 \pm 395$   $\mu$ g/kg) tripled when a daily 25 mg copper supplement was used. The Mn content  $38 \pm 13.8$   $\mu$ g/kg rose to  $79.8 \pm 41.8$   $\mu$ g/kg with a daily 70 mg Mn supplement. The blood Zn value of  $2.67 \pm 0.78$  mg/kg rose to  $3.19 \pm 0.61$  mg/kg when the horses received a daily 150 mg zinc supplement.

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## Hivenainelisen vaikutuksesta hevosten veriarvoihin

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Tutkimuksessa tarkastellaan seitsemän suomalaisen lämminverisen ravihevosen veriarvojen muutoksia rehun hivenainepitoisuuden muutosten yhteydessä. Hivenainepitoisuuden muutokset rehussa toteutettiin korvaamalla osa perusruokinnan kaurasta Se rikkaalla ohralla ja antamalla perusruokinnan yhteydessä hivenainescosta.

Hevosten rehun Se pitoisuuden ollessa 0.03 mg/kg oli veren Se pitoisuus  $0.08 \pm 0.04$  mg/kg. Rehun pitoisuutta 0.1 mg/kg vastasi veren pitoisuus  $0.17 \pm 0.03$  mg/kg ja rehun pitoisuutta 0.14 mg/kg veren pitoisuus  $0.19 \pm 0.04$  mg/kg.

Veren Cu pitoisuus  $779 \pm 395$  µg/l kolminkertaistui käytettäessä päivittäistä 25 mg:n kuparilisää. Mn pitoisuus  $38 \pm 13.8$  µg/kg kohosi  $79.8 \pm 41.8$  µg/kg 70 mk:n päivittäisellä Mn lisällä. Veren Zn-arvot  $2.67 \pm 0.78$  mk/kg kohosivat arvoon  $3.19 \pm 0.61$  mg/kg hevosten saadessa päivittäin 150 mg:n sinkkilisän.