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DETERMINATION OF TRACE ELEMENTS (FE, CU AND ZN) IN SERUM AND TAIL HAIR OF HEALTHY HORSES AS A FUNCTION OF NUTRITIONAL DIFFERENCES IN CERTAIN MONTHS

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SUMMARY

Strong and poor relationships between the levels of trace elements in blood, tissue and hair have been determined in various studies. The horses in this study have been fed with dry straw and artificial feed in winter months, with fresh grass in autumn and spring. The purpose was to investigate possible changes in the levels of trace elements such as Fe, Cu and Zn in different feeds (straw-fresh grass) supplied at different periods of the year. The levels of trace elements such as Fe, Cu and Zn in both serum and tail hair of horses from the Gemlik Military Veterinary School were determined by Atomic Absorption (Shimadzu AA-680 Spectrophotometer) in November (n=19), March (n=19) and May (n=19). According to the results of this study, the Fe levels in serum and hair samples was significantly ($p < 0.05$) increased in May as compared to November and March levels The Cu level in serum and hair samples examined in May was significantly ($p < 0.05$) decreased as compared to those in November and March. The zinc level in serum samples showed a significant ($p < 0.05$) decrease in March, while in May there was a significant ($p < 0.01$) increase when compared to November levels. The observed level of Zn in hair samples did not show statistically significant ($p < 0.05$) changes. In conclusion, changes in the levels of trace elements can occur with changes in nutrition programs without, causing any diseases. It is, however, advisable to provide a regular supplement of vitamins and trace element.

KEY WORDS: horse, hair, trace element, iron, copper, zinc

INTRODUCTION

Seasonal nutritional variations are a parameter which influences an organism. This effect is more obvious especially in horses. Horse are fed with dry straw and artificial feed during the period when no fresh grass is available. The nutrient composition of fresh grass, straw and artificial feed is different (Kızıler, 1997, Asano *et al.*, 2002). Nutritional differences affect the levels of trace element in organisms to different degrees (Prasad, 1978, Burnet, 1982, Kızıler, 1997).

Herbivorous animals show changes in trace elements in line with seasonal nutrition variations (Kossila *et al.*, 1972, Chavapil, 1976, Prasad, 1978, Burnet, 1982, Kızıler, 1997). Measurements of trace elements is becoming more important (Weigert *et al.*, 1981, Burnet, 1982, Barutcu, 1987, Steinbrecher *et al.*, 1990, Esenkaya, 1994, Asano *et.al.*, 2002).

The rate of change of the concentration of trace elements in serum samples is different from that in hair samples. While blood is a parameter used as a swift indicator of trace element levels in organisms, hair and tissue are considered as slow indicators. The rate of change is also different for different elements. One advantage of hair analysis for such changes is the relatively higher concentration of trace elements in hair than in other body organs and fluids. Another advantage is the ease of sampling, and storage of hair as compared to other biological materials (Kossila *et al.*, 1972, Prasad, 1978, Esenkaya, 1994, Asano *et al.*, 2002). However, the main parameter showing change of trace element levels in the organism is blood. Other body fluids or tissue are affected more slowly by those changes. Therefore, possible effects of differences in minerals at different times are shown by measurements of serum levels of the element (Prasad, 1978, Esenkaya, 1994).

Asano *et al.* (2002), have pointed out that studies on mineral analysis of hair samples have considerably developed over the last 10 years. However, there is still lack of reference data on the levels of most elements in tail hair, which they cited as the reason for their own study. Besides creatinine, hair of some animals contains up to 14% sulphur in addition to a host of other elements such as Mn, Al, Cl, Ca, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Cd, I, Hg and Au (Vir *et al.*, 1981, Steinbrecher *et al.*, 1990, Asano *et al.*, 2002).

The purpose of this study was to investigate possible changes in the levels of trace elements such as Fe, Cu and Zn as a consequence of seasonal nutritional differences (for example, straw instead of fresh grass).

MATERIALS AND METHODS

Serum and tail hair samples were collected from healthy horses of different breed, age and sex. The samples were collected in November (n=19), March (n=19) and May (n=19). The horses were treated regularly with anti-parasitic drugs. The horses were fed with dry straw and artificial feed in the winter months, and with fresh grass in autumn and spring. The tails were cleaned with a wet cloth before the samples were taken.

Hair samples were weighed and then placed into clean glass centrifuge tubes. Two millilitres of concentrated nitric acid was added to each tube and the tubes were put in a sterilizer at 100 ^{0}C until half of the total volume had evaporated. The samples were then taken out and cooled. Two millilitres of concentrated perchloric acid were added, and the tubes were again placed in the sterilizer until half of the total volume had evaporated. After this procedure, distilled water was added to give a total volume of 5 ml (Pomeroy *et al.*, 1975, Brown *et al.*, 1986, Esenkaya, 1994, Brown *et al.*, 1995).

For the measurement of trace element in serum and hair samples, standard solutions were prepared from standard stock solution Titrisol, 1000±0.002 mg (Merck). The concentration for zinc was 0.5 – 1 µg/ml ,

and for copper and iron 1-2 μg/ml (Barutcu, 1987, Brown *et al.*, 1995). Distilled water was used as blind. Hollow Cathode lamps (HCL) emitting light specific for each element under special wavelength and gas mixtures of air and acetylene convenient for each trace element were used for the measurement. Proper slit intervals and BGC (Background) modes were selected. Under similar conditions, blind and standard solutions were fed into device and the calibration graphs established.

Trace elements such as iron, copper and zinc were measured with a Shimadzu Atomic Absorption Spectrophotometer (AA-680). The mean values (x), and standard deviations (Sx) as well as multiple comparison were evaluated with the aid of the Duncan F test (1955).

RESULTS

During the periods of November/May and March/May a statistically significant ($p<0.01$ and $p<0.001$ respectively) increase in serum iron level was found. A statistically significant decrease in serum copper levels was confirmed for November/March, November/May and March/May ($p<0.01$, $p<0.001$, $p<0.001$ respectively). There was a significant ($p < 0.01$) decrease in serum Zinc levels between November/March, and a significant ($p < 0.001$) increase in November/May and March/May.

A significant ($p < 0.05$) increase was observed in the iron level of tail hairs of horses in November/May and March/May. Although no statistical significance ($p > 0.05$) was found for hair zinc levels over the months Nov. to March, a meaningful decrease was seen for the periods November/May and March/May ($p<0.01$ and $p<0.001$ respectively) (Table I).

TABLE I: Changes in the levels of trace elements in serum and hair samples of horses in different M\months (M±SD)

		November (n=19)	March (n=19)	May (n=19)
Fe	Serum (μg/dl)	140.8 ± 17.4	129.3 ± 11.8	$166.9 \pm 14.6^{**000}$
	Hair (μg/g)	105.3 ± 57.5	128.8 ± 70.5	$195.7 \pm 10.9^{*0}$
Cu	Serum (μg/dl)	144.7 ± 37.9	$87.9 \pm 27.27^{\phi\phi}$	$41.9 \pm 9.98^{***000}$
	Hair (μg/g)	14.5 ± 7.4	14.4 ± 4.4	$7.6 \pm 3^{**00}$
Zn	Serum (μg/dl)	58.5 ± 12.72	$37.8 \pm 9.27^{\phi\phi}$	$80.5 \pm 15.58^{***000}$
	Hair (μg/g)	77.1 ± 6.9	82.9 ± 11.4	69.2 ± 24.4

*(November/May), ^{0} (March / May), $^{\phi}$ (November/March)
*p<0.05, **p<0.01, ***p<0.001 (^{0}, $^{\phi}$ similar)

DISCUSSION

The levels of trace elements in tail hair of horses can be used in the diagnosis of various diseases and metabolic disorders; and the determination of the nutritional status of the horse. Examination of hair has the advantages of ease of sampling and storage as compared to other biological materials; and also the availability of reference data of trace elements in body hair of horses (Kossila *et al.*, 1972, Asano *et al.*, 2002, Dunnett and Lees, 2003). In Turkey no study on the correlation of trace element concentrations in tail hair of horses and serum as a function of various nutritional methods in the different months of the year has yet been carried out..

The levels of trace elements in serum are quickly affected by nutritional differences. However, it has been demonstrated that mineral nutritional differences have only little or no effect at all on the health of the animal (Weigert *et al.*, 1981, Burnet, 1982, Steinbrecher *et al.*, 1990). Steinbrecher *et al.* (1990) have reported that different nutrition has no effect on trace element levels even if there is no seasonal difference. They found, however, that different nutrition programs applied over a period of 9 months increased the levels of urea, free fatty acids and Ca but caused very little change in Fe, Cu, Zn, Mg, Ca, Na and K levels. Weigert *et al.* (1981) showed that trace element levels in different horse breeds are not affected at all by nutritional differences. Our study has shown that the levels of some elements are affected to a higher or lower degree by nutritional differences. In the present study, fresh grass in autumn and spring, dry straw and artificial feed during the winter months altered the serum tail and hail mineral levels. We are of the opinion that the increase in serum zinc levels is related to differences in the composition of straw, fresh grass and artificial feed, while the decrease in serum copper levels is due to competition between zinc and copper levels. For the same months (Nov - May), no significant ($p > 0.05$) change in the level of zinc in tail hair could be found. However, significant ($p < 0.01$) decrease in copper, and a significant ($p < 0.05$) increase inr iron were found. According to these results, hair zinc is not affected, copper is affected fast, iron is affected slowly by changes in nutrition.

It was found that changes occurred in the levels of trace elements in correlation with the different nutrition during different months of the year, especially in respect of iron and zinc, which decreased in march and increased in May, while the decrease in copper concentration, was even more pronounced.

It can be concluded that changes in the level of trace elements occur with different nutrition programs without being caused by diseases. As a precautionary measure, supplements of vitamin and trace elements would be beneficial.

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