

## PROFESSIONAL PAPERS – STRUČNI RAD

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**COMPARISON OF SOME BLOOD PARAMETERS, SERUM  
VITAMIN E AND MINERAL CONCENTRATIONS OF  
ARABIAN AND ENGLISH THOROUGHBRED RACE HORSES\***  
*POREĐENJE NEKIH PARAMETARA U KRVI, SERUMSKIH  
KONCENTRACIJA VITAMINA E I MINERALA KOD ARAPSKIH I  
ENGLJSKIH ČISTOKRVNIH TRKAČKIH KONJA*

T. Bilal, E. Ercag, G. Demirel, T. Bilal\*\*

*The aim of this study was to determine some blood parameters, serum vitamin E and mineral concentrations of Arabian and English thoroughbred racehorses fed the same diets. The diet was formulated to provide 2.31 Mcal DE/kg, and 10.96% crude protein. Total protein, lactate, calcium, phosphorus, potassium, copper, cobalt and zinc were determined in serum obtained from 40 Arabian and 40 English healthy racing thoroughbred horses aged 2-3. The copper, cobalt and zinc concentrations were determined by atomic absorption, vitamin E by HPLC and the other biochemical parameters by a spectrophotometer. Mean values were 6.77 and 6.86 g/dl for total protein, 1.88 and 2.16 mg/dl for lactate, 13.18 and 12.80 mg/dl for calcium, 4.35 and 4.39 mmol/l for phosphorus, 2.64 and 3.14 mmol/l for potassium, 129 and 166 µg/dl for copper, 36 and 44 µg/dl for cobalt and, 160 and 58 µg/dl for zinc in Arabian and English horses, respectively, and Mean serum vitamin E levels were 2.65 and 2.81 µg/ml, respectively. This study did not demonstrate a significant effect of breed on serum total protein, lactate, calcium, phosphorus, copper, cobalt and vitamin E. However, breed may have an effect on potassium and zinc concentration in Arabian and English thoroughbred racehorses ( $p < 0.05$ ).*

*Keywords: Horse, vitamin E, serum, minerals, Arabian, English Thoroughbred*

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\*\* Tarik Bilal, Istanbul University, Veterinary Faculty, Department of Internal Disease, Istanbul, Turkey; Erol Ercag, Istanbul University, Engineering Faculty, Department of Chemistry, Istanbul, Turkey; Gulcan Demirel, Tanay Bilal, Istanbul University, Veterinary Faculty, Department of Animal Nutrition, Istanbul, Turkey, E-mail address: [tanbilal@istanbul.edu.tr](mailto:tanbilal@istanbul.edu.tr)

## **Introduction / Uvod**

All minerals play crucial roles in the physiological response to exercise, in energy metabolism and in tissue conservation during an exercise period [14]. Racehorses have needs for minerals as well as other nutrients in order to perform their task. Copper is particularly interesting for orthopaedic diseases in young horses. Zinc is also involved as a co-factor in many enzyme systems [9].

In cattle [6] and sheep [23], breed differences in ability to store copper were reported. This difference attributed to multiple genes causing maternal difference in copper absorption, retention or both. Unfortunately, there is limited information on mineral concentrations in the serum concerning horse breeds fed same diets. Grace *et al.* [7] reported differences in tissue copper concentration in horses that could not be explained by variation in dietary content possibly being due to the breed factor. Obviously, there is a need to establish reference values related to the breed.

Biochemical values are related to diet, sex, environmental agents and age and these parameters are used in diagnosis of diseases and assess the nutritional status of horses [2]. The establishment of reference values for biochemical parameters concerning breed could also be useful to assess nutritional status of horses and diagnosis of diseases. In addition, the effect of dietary vitamin E on serum vitamin E level and breed differences will also be determined.

This paper presents results of some blood parameters and vitamin E and mineral element analysis of serum collected from Arabian and English horses fed same diets.

## **Materials and methods / Materijal i metode rada**

### *Animals and Diets / Životinje i ishrana*

Forty Arabian and forty English thoroughbred racehorses (age from 2 to 3 years with an average initial weight of 415 kg, range 400 to 430; SE= 0.91) were used in this study. The diet was formulated to meet nutrient requirement for racehorses [16] and consisted of 63% grass hay and 37% mixed grain concentrate. Sample of feeds were analysed for dry matter (DM) and organic matter (OM) [1], and nitrogen (Kjeltec 1035 analyser, Tecator). The vitamin E content of the diet was 98.5 IU/kg DM. This level was around the NRC, recommendation [16]. Trace-mineralised salt (Equi-Choice, Wilson Enterprises, Disputanta, VA, Table 1) and water were available *ad libitum*. Housing and feeding were similar for all horses. Diet ingredients and analysed chemical composition of diet is presented in Table 1.

Table 1. *Diet ingredients and analysed chemical composition (concentrate + forage) / Tabela 1. Sastojci hrane i analizirani hemijski sastav (koncentrat + silaža)*

Formula, % as fed / Formula, procenat davanog	
Grass hay / <i>Seno</i>	63
Ground barley / <i>Mleveni ječam</i>	20
Ground corn / <i>Mleveni kukuruz</i>	10
Molasses / <i>Melasa</i>	5
Dicalciumphosphate / <i>Dikalcijumfosfat</i>	0.5
Salt / <i>So</i>	0.5
Vitamin Premix <sup>2</sup> / <i>Premiks vitamina</i>	0.25
Ground limestone / <i>Stočna kreda</i>	0.75
Analysis, DM basis / Analiza na bazi SM	
DM, % / <i>SM, %</i>	88,4
DE, Mcal/kg / <i>DE, Mkal/kg</i>	2.31
CP, % / <i>CP, %</i>	10.96
Ca, % / <i>Ca, %</i>	0.65
P, % / <i>P, %</i>	0.62
Cu, mg/kg / <i>Cu, mg/kg</i>	5.54
Zn, mg/kg / <i>Zn, mg/kg</i>	27.95
Co, mg/kg / <i>Co, mg/kg</i>	0.11
Vit E, IU/kg / <i>Vit E, IU/kg</i>	98.5

<sup>1</sup> Trace-mineralised salt was provided free choice. It contained (%) Ca, 7-9; P, 8; Mg, 1; S, 1; Zn, 5; Fe, 3; Cu, 12; Mn, 1; Co, 0.02; Na, 13.65; Cl, 21.35; Se, 0.01 /

*So sa mineralima u tragovima je davana u neograničenim količinama. Sadržavala je (%) Ca, 7-9; P, 8; Mg, 1; S, 1; Zn, 5; Fe, 3; Cu, 12; Mn, 1; Co, 0.02; Na, 13.65; Cl, 21.35; Se, 0.01*

<sup>2</sup> The premix provided the following 1 kg of diet: vitamin A, 1,380,000 IU, vitamin D<sub>3</sub>, 258,000 IU, vitamin E, 26,455 IU; riboflavin, 701 mg; niacin, 3,009 mg; folic acid, 66 mg; thiamine mononitrate, 1,521 mg; d-biotin, 42 mg; β-caroten, 3,527 mg. /

*Premiks je obezbeđivao u 1 kg obroka: vitamina A, 1,380.000 IU; vitamina D<sub>3</sub>, 258,000 IU; vitamina E, 26,455 IU; riboflavina, 701 mg; niacina, 3,009 mg; folne kiseline, 66 mg; tiamin mononitrata, 1,521 mg, d-biotina, 42 mg; beta-karotena, 3,527 mg*

#### Sampling / Uzorkovanje

At the end of the 4-week trial period, blood was collected from the jugular vein into evacuated collection tubes at rest in the horsebox. The blood sample was centrifuged and serum was then withdrawn and stored at -20°C until analyses could be performed.

#### Analytical procedures / Analitička procedura

Total protein (total protein liquicolor), lactate (lactate-PAP-Merieux), calcium (calcium liquicolor), phosphorus (phosphorus liquirapid), and potassium (potassium liquirapid) analyses were performed using the commercial kits (Human Geselschaf für Biochemica und Diagnostica mbH).

Serum copper, cobalt and zinc analysis were done according to Elmer [4] using atomic absorption (model 2380, Perkin-Elmer Cooperation). Feed analyses of copper, cobalt and zinc were performed according to Osborn and Voogt [18].

Serum samples were analyzed for  $\alpha$ -tocopherol using HPLC with a fluorescence detector according to the method of Hidioglou [15]. All samples were analyzed in duplicate.

#### Statistical analysis / Statistička analiza

Results are presented as means with their standard errors (Table 2). All data were compared by means of Student's t test by using SPSS [19] program.

### Results / Rezultati ispitivanja

Mean serum concentrations of total protein, lactate, calcium, phosphorus, potassium, copper, cobalt, zinc and vitamin E are presented in Table 2. Total protein values were similar in both Arabian and English horses. Lactate concentrations were higher in English horses but the difference was not significant. Similarly, serum concentrations of calcium and phosphorus and vitamin E were not statistically different between two breeds.

Table 2. Plasma total protein, lactate, calcium, phosphate, potassium, copper, cobalt and zinc levels of Arabian and English thoroughbred horses /

Tabela 2. Plazma nivoi ukupnih proteina, laktata, kalcijuma, fosfata, kalijuma, bakra, kobalta i cinka kod arapskih i engleskih čistokrvnih konja

	Arabian / Arapski (n=40)	English / Engleski (n=40)
	X ± SE	X ± SE
Total protein / Ukupni proteini (g/l)	67.7 ± 1.60	68.6 ± 1.50
Lactate / laktat (mmol/l)	0.209 ± 0.109	0.239 ± 0.013
Calcium / kalcijum (mmol/l)	3.288 ± 0.095	3.194 ± 0.052
Phosphate / fosfat (mmol/l)	1.404 ± 0.005	1.417 ± 0.035
Potassium / kalijum (mmol/l)	2.64 ± 0.61	3.14 ± 0.75 <sup>a</sup>
Copper / bakar (μmol/l)	2.030 ± 0.126	2.613 ± 0.220
Cobalt / kobalt (μmol/l)	6.109 ± 0.509	7.467 ± 0.339
Zinc / cink (μmol/l)	24.480 ± 0.459 <sup>a</sup>	8.874 ± 0.499
Vitamin E / Vitamin E (μg/ml)	2.65 ± 0.72	2.81 ± 0.57

<sup>a</sup> Values for each mineral with superscripts are different (p<0.001) /  
Vrednosti za sve minerale sa indeksom su različite (p<0,001)

Potassium concentrations of serum in two breeds were different and this difference was statistically significant (p<0.05). Mean values for potassium

were 2.64 and 3.14 mmol/l for Arabian and English horses, respectively. The mean copper and cobalt concentrations of serum from both breeds were not found to be significantly different whereas zinc concentrations of Arabian horses were higher than those for English horses. Mean values were 160 and 58  $\mu\text{g/dl}$  for Arabian and English horses, respectively, and the difference was statistically significant ( $p < 0.05$ ) (Table 2).

### **Discussion / Diskusija**

All proteins and proteids in blood serum are called „total protein value”. Acute phase proteins can be affected by disease states such as acute inflammatory disease burnt, blood losses, trauma. It was reported that in the case of chronic inflammatory diseases, nephritic syndrome, chronic hepatopathy like liver cirrhosis, chronic enteropathy, malign tumour and dehydration this value could be changed [12]. In the study of Kraft [15] this value was reported between 55-75 g/l and 5.5-7.5 g/dl, respectively. Orsini and Divers [17] found the value between 5.2-7.9 g/dl. Harris [9] measured this value at  $58.2 \pm 3.4$  g/l before the race,  $71.8 \pm 8.36$  g/l at speed of 12 m/min and  $70.3 \pm 9.36$  g/l at the end of the race. Tyler-McGowen *et al.* [21] divided horses into two groups. The first group was control and the other one was a highly training (exercised) group. They measured the total protein value at 1, 15 and 32 weeks and they found the values  $68.0 \pm 1.0$  g/l and  $63.0 \pm 2.0$  g/l and  $69.0 \pm 2.2$  g/l in control group and  $68.0 \pm 0.8$  g/l,  $63 \pm 1.2$  and  $67.0 \pm 1.2$  g/l in exercised group, respectively. Heigl [12] reported this value between 5.8-8.0 g/dl. Our results support any of these reports discussed above and proved those horses, which were used in this study, had no sign of pathological disorders and our results could also prove that breed might not have an effect on total protein value.

Serum lactate concentration increases depending on exercise and has an effect on race performance and during the exercise, as a result of glycolysis, broken down glucose glycogen into lactic acid; large quantities of energy can be supplied. If the oxygen supply of the body is not enough, two pyruvate ions, which are the last product of glycolytic reaction, and hydrogen ions are reacted to produce lactic acid [22]. According to our observations values related to breed in serum lactate concentrations were not reported previously and our results showed that breed might not have an effect on serum lactate concentrations. It might be useful to indicate here reported lactate concentrations previously; Kraft [15] reported a maximum lactate concentration in horses of 9 mg/dl (0.999 mmol/l), Orsini and Divers [17] between 10-16 mg/dl. Harris and Snow [10] indicated that arterial lactate concentration ranged between 0.1-2.4 mg/dl (0.208-0.266 mmol/l) venous lactate concentrations between 1.0-1.36 mg/dl (0.111-0.150 mmol/l). Evans and Lorraine [5] used two different methods in their work and they observed that blood lactate concentration between  $1.5 \pm 0$  mmol/l and  $11.3 \pm 9.6$  mmol/l, and

plasma lactate concentration was between  $3.3 \pm 1.7$  mmol/l and  $20.7 \pm 6.6$  mmol/l depending on effort in exercise band.

Calcium is a component of bone and teeth and also plays an important role in muscle contraction, coagulation, enzyme and cellular defence system and secretion of some hormones. Total calcium concentration was found between 8.0-13.6 mg/dl by Kraft [15]. This value was around 11.2-13.6 mg/dl in the study of Orsini and Divers [17]. Haclechner [8] reported that calcium concentration in horses was between 2.5-3.3 mmol/l (10.0-13.22 mg/dl). In our case, this value was around  $13.18 \pm 0.32$  mg/dl ( $3.28 \pm 0.08$  mmol/l) in Arabian horses and  $12.80 \pm 0.21$  mg/dl ( $3.19 \pm 0.05$  mmol/l) in English horses as in normal range and no breed difference was found in calcium concentration in serum.

Levels of phosphorus, which is a component of bone and teeth and involved in most of the reactions in the body as a source of energy and also the main component of nucleic acids, important as a buffer in blood and cell and the main element of enzyme and proteins, were between 2.5-4.5 mg/dl in horses in the study of Kraft [15], between 3.61-5.6 mg/dl in the study of Orsini and Divers [17]. Haclechner [8] reported that the value of phosphorus concentration ranged between 2.2-6.6 mg/dl (0.71-2.13 mmol/l) in 95% of the horses and he claimed those numbers need to be taken as reference numbers. Our results are similar with those of these researchers and as discussed previously, no reported values are available for horse breeds for phosphorus concentration in serum.

Potassium is the main element of intracellular fluid, and plays a role in providing membrane potential, regulation of acid-alkali balance and enzyme activation. The values reported by Kraft [15] were 2.5-4.5 mmol/l (9.77-17.59 mg/dl). Orsini and Divers [17] and Haclechner [8] reported the values 2.4-4.7 mmol/l (9.38-18.38 mg/dl) and 2.2-4.8 mmol/l (8.60-18.77 mg/dl), respectively. In the study of Harris and Snow [10] effects of excessive exercise were observed. During the exercise, plasma potassium concentration was relatively increased, and at the end of the exercise this value reached a peak. Potassium concentration in some horses was around 10 mmol/l. In another work by these authors [11] in warming period, potassium concentration returned to the normal range. In our work, potassium concentration in Arabian horses was  $2.64 \pm 0.61$  mmol/l, and in English horses  $3.14 \pm 0.75$  mmol/l. This difference was statistically significant ( $p < 0.05$ ).

The results of the present study suggest that breed has no effect on serum vitamin E level since similar values were observed in both English and Arabian horses (Table 2).

After an examination of blood samples from Arabian and English horses it was found that Arabian horses had a higher mean value of zinc than English horses (Table 2). No significant breed-related difference was observed in plasma cobalt and copper levels (Table 2). Despite no data being available on breed differences in trace element content of the body or plasma of horses, some researchers attribute the differences in results to the genotype of horses. For ex-

ample, when draught cross Quarter horses or Quarter horse yearlings were fed diets containing 7.6 to 15.1 mg copper/kg DM, 22.5 to 52.7 mg manganese/kg DM and 25 to 52.2 zinc mg/kg DM [3], the increase of trace element intake caused no significant differences in copper, manganese and zinc concentrations in liver, kidney and plasma. Grace *et al.* [7] also concluded that the reported differences in the trace element content of the body between the various horse studies and their study with horses do not appear to be related to varying cobalt, manganese, iron and zinc intakes and could be explained by genotypic differences. However, genotypic differences reported in most cases and also in trace element concentrations. For example, Scottish Blackface ewes were less efficient in absorbing copper than Welsh Mountain ewes [23]. In our work, we did not observe differences in absorbing copper and cobalt in horses but differences were observed in absorbing zinc (Table 2), and therefore English horses had lower serum zinc concentration when both breeds are fed diets of identical zinc concentrations. On the other hand, Stark *et al.* [20] reported differences in zinc concentration between horses and they attributed this difference in zinc plasma levels to the location of housing. Our observations do not agree with this explanation since the same housing system and feed was used in our study.

We only tested some biochemical parameters, macro, and some trace elements and just one vitamin as far as possible. But, the results emphasize the need to establish reference values related to the breed for whole biochemical parameters and minerals.

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SRPSKI

#### POREĐENJE NEKIH PARAMETARA U KRVI, SERUMSKIH KONCENTRACIJA VITAMINA E I MINERALA KOD ARAPSKIH I ENGLESKIH ČISTOKRVNIH TRKAČKIH KONJA

T. Bilal, E. Ercag, G. Demirel, T. Bilal

Cilj ovog rada bio je da se odrede neki parametri u krvi i serumske koncentracije vitamina E i minerala u arapskim i engleskim čistokrvnim trkačkim konjima hranjenim istom hranom. Ishrana je određena tako da obezbedi 2,31 Mcal DE/kg i 10,96% sirovih proteina. Ukupni proteini, laktat, kalcijum, fosfor, kalijum, bakar, kobalt i cink su određivani u serumu dobijenom iz 40 arapskih i 40 engleskih zdravih čistokrvnih trkačkih konja starih 2-3 godine. Koncentracije bakra, kobalta i cinka su određivane atomskom apsorpcijom, vitamin E putem HPLC, a ostali biohemijski parametri koristeći spektrofotometar. Srednje vrednosti bile su 6,77 i 6,86 g/dl za ukupne proteine, 1,88 i 2,16 mg/dl za laktat, 13,18 i 12,80 mg/dl za kalcijum, 4,35 i 4,39 mmol/l za fosfor, 2,64 i 3,14 mmol/l za kalijum, 129 i 166 µg/dl za bakar, 36 i 44 µg/dl za kobalt, i 160 i 58 µg/dl za cink kod arapskih i kod engleskih konja, a srednje vrednosti serumskih nivoa vitamina E bile su 2,65 i 2,81 µg/ml. Ova istraživanja nisu pokazala značajni uticaj rase na serumske vrednosti ukupnih proteina, laktata, kalcijuma, fosfora, bakra, kobalta i vitamina E. Međutim, rasa bi mogla da ima uticaja na koncentracije kalijuma i cinka kod arapskih i engleskih čistokrvnih trkačkih konja ( $p < 0.05$ ).

Ključne reči: konj, vitamin E, serum, minerali, arapski, engleski čistokrvni trkački konji



**СРАВНЕНИЕ НЕКОТОРЫХ ПАРАМЕТРОВ В КРОВИ, СЫВОРОТОЧНЫХ КОНЦЕНТРАЦИЯХ ВИТАМИНА Е И МИНЕРАЛОВ В АРАБСКИХ И АНГЛИЙСКИХ ЧИСТОКРОВНЫХ БЕГОВЫХ ЛОШАДЕЙ**

**T. Bilal, E. Ercag, G. Demirel, T. Bilal**

Цель этой работы была определить некоторые параметры в крови и сывороточной концентрации витамина Е и минералов в арабских и английских чистокровных беговых лошадей, кормленных таким же кормом. Кормление определено так, что обеспечить 2,31 Мкал ДЕ/кг и 10,96% сырых протеинов. Совокупные протеины, лактат, кальций, фосфор, калий, медь, кобальт и цинк определялись в сыворотке, полученной из 40 арабских и 40 английских здоровых чистокровных беговых лошадей им 2-3 года. Концентрации меди, кобальта и цинка определялись атомной абсорбцией, витамин Е путём ХПЛЦ, а остальные биохимические параметры, пользуясь спектрофотометр. Средние стоимости были, 6,77 и 6,86 г/дл для совокупных протеинов, 1,88 и 2,16 мг/дл для лактата, 13,18 и 12,80 мг/дл для кальция, 4,35 и 4,39 ммол/л для фосфора, 2,64 и 3,14 ммол/л для калия, 1,29 и 1,66 мг/дл для меди, 36 и 44 мг/дл для кобальта, и 160 и 58 мг/дл для цинка у арабских и у английских лошадей, а средние стоимости сывороточных уровней витамина Е были 2,65 и 2,81 мг/мл. Эти исследования не показали значительное влияние породы на сывороточные стоимости совокупных протеинов, лактата, кальция, фосфора, меди, кобальта и витамина Е. Между тем, порода бы могла иметь влияния на концентрации калия и цинка у арабских и английских чистокровных беговых лошадей ( $p < 0.05$ ).

Ключевые слова: лошадь, витамин Е, сыворотка, минералы, арабский, английский, чистокровны беговы лошадей