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EFFECT OF VITAMIN-MINERAL PREMIX ON SOME RELATIONSHIPS BETWEEN SERUM PARAMETERS IN BREEDING HORSES

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ABSTRACT

Eight Arabian stallions were selected from clinically normal horses. After 10 day adaptation period the animals were divided into two equal groups: control and experimental. All horses were housed individually and allowed to walk on sandy paddock twice a day between meals. Both groups were fed the same diet which was composed of 6 kg hay and 4 kg barley. However, the experimental group was supplemented with 150 g of micronutrient premix. Premix was composed of 180 g of oats husk meal, 260 g dicalcium phosphate as carrier, 25 g lignosulphate, 20 g methionine, 20 g lysine, 200 g calcium, 40 g salt (NaCl), 30 g soy oil, 100 g yeast, 50 g mixture of vitamins, 75 g mixture of minerals and antioxidants per kg. The experiment was divided into adaptation and experimental period that lasted 10 and 30 days, respectively. The blood was drawn at the beginning of experimental period and thereafter in 10 days intervals. Serum samples were analysed for total serum protein, serum urea, serum lipids, serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). The results showed highly significant differences between both groups in serum protein, serum lipids, serum GOT and GPT. Significant difference has been found between both groups in serum urea ($P < 0.1$). These changes in serum parameters between groups could be due to the additive micronutrient premix fed to the experimental groups. The correlations between parameters: protein and urea was significant negative, between lipids and GOT highly significant negative, and between lipids and GPT non-significant.

Key words: horses / animal nutrition / micronutrients / animal physiology / serum parameters

VPLIV VITAMINSKO RUDNINSKE MEŠANICE NA NEKATERE PARAMETRE KRVNEGA SERUMA PLEMENSKIH KONJ

IZVLEČEK

V poskus smo vključili osem arabskih žrebcev brez kliničnih posebnosti. Po desetih dneh prilagajanja smo živali razdelili v kontrolno in poskusno skupino. Vse živali so bile vhlevljene individualno in so imele dvakrat na dan po krmljenju na voljo izpust v peščeno ograjo. Obe skupini smo krmili z istim osnovnim obrokom, ki je vseboval 6 kg sena in 4 kg ječmena na dan. Poskusna skupina je dobivala 150 g premiksa mikronutrientov. Premiks je bil sestavljen iz 180 g mletih ovsenih luščin in 260 g dikalcijevega fosfata kot nosilec ter 25 g lignosulfata, 20 g metionina, 20 g lizina, 200 g kalcija, 40 g soli (NaCl), 30 g sojinega olja, 100 g kvasovk, 50 g vitaminske mešanice, 75 g mineralne mešanice in antioksidante. Poskus je bil razdeljen v deset dnevno prilagoditveno obdobje in trideset dnevno poskusno obdobje. Živalim smo vzeli kri na

začetku prilagoditvenega obdobja in nato vsakih deset dni poskusa. V vzorcih seruma smo določali skupne serumske proteine, ureo, lipide v serumu, serumsko glutamat oksalacetat transaminazo (GOT) in glutamat piruvat transaminazo (GPT). Rezultati so pokazali statistično visoko značilne razlike med obema skupinama pri serumskih proteinih, lipidih v serumu, GOT in GPT. Značilne razlike med skupinama smo našli tudi pri vsebnosti uree v serumu ($p < 0,1$). Te spremembe v sestavi seruma bi bile lahko posledica dodajanja mikronutrientov v premiksu, ki smo ga krmili poskusni skupini. Korelacija med serumskimi proteini in vsebnostjo uree v serumu je bila negativna, med lipidi v serumu in GOT statistično značilno negativna, korelacija med lipidi in GPT pa ni bila značilna.

Ključne besede: konji / prehrana živali / mikronutrienti / fiziologija živali / serum / parametri

INTRODUCTION

Vitamins, minerals and other nutrients serve essential roles in cellular metabolism, maintenance and growth throughout life. The assessment of micronutrients status with horses of different catagories has been studied from different points of view. This may lead to an inadequate recommendation of some micronutrient diets suggested by the current NRC (1989), which has been found to be low, Donoughue *et al.* (1981); Putnam (1985). Due to the lack of information about the role and specific requirements for some micronutrients for horse diets sometimes relay on information based on experiments in other species. In this respect, considerable effort should be made to give the adequate recommendation for each nutrient for each horse category, i.e. growth, sport activity and breeding. On the other hand, a study of the influence of micronutrient supplement on the relationships between different metabolic parameters in the body, i.e. blood, urine, sweat and others should be done.

Roneus (1982) and Roneus *et al.* (1986) studied the relationship between serum selenium and serum glutathion peroxidase (GSH-px activity) in the age from 30 days to 12 years in standardbred horses, mares and their foals. In addition; relationship between serum vitamin E and two muscular enzymes, Aspartate aminotransferase (AspAT) and creatine-phosphokinase (CPK) were studied by Lindholm and Asheim (1973); and the relationship between selenium and gamma glutamyl transferase activity in the serum of throughbred horses by Blackmore *et al*. (1979).

The objective of present study is to assess the relationships between serum parameters of breeding horses when fed with additional micronutrients along with their traditional feeding regime, i.e. hay / grains ration. The relationship between serum protein and serum urea; and the relationship between serum lipids and either serum glutamic oxaloacetic transaminase (GOT) or glutamic pyruvic transaminase were studied.

MATERIALS AND METHODS

Eight mature stallions in the age of three to four years were selected from clinically normal horses of Arabian blood. The stallions had been equally divided into two groups: control and experimental. They were individually housed in stand boxes supplied with automatic trough, and all horses were allowed to walk in the paddock twice during the day time between meals as activity.

In 10 days adaptation period, all stallions were fed 6 kg hay in loose form and 4 kg barley grains daily. For 30 days of experimental period, the control group was continuously fed the same ration, while the stallions of experimental group were fed in addition to hay/barley ration, a quantity of 150 g of premix. The premix was offered to each horse individually with the morning meal after mixing with barley. The premix was prepared in pelleted form (5 mm diameter) and

the kilogram contained: 80 g of oats husk meal and 260 g dicalcium phosphate as carriers, and contained 25 g lignosulphate , 20 g methionine, 20 g lysine, 200 g calcium, 40 g salt (NaCl), 30g soy oil, 100 g yeast, 50 g mixture of vitamines, 75 g mixture of minerals and antioxidants were added i.e. 440 mg BHT and 105 mg Etoxyquin 66.6 %.

Vitamin mixture consisted of vit. A 500 000 IU, vit. E 2 000mg, vit. K 50 mg, vit B1 500 mg, vit. B2 500 mg, vit. B3 1000 mg, vit. B5 (PP) 1500 mg, vit. B6 100 mg, vit. B12 1 mg, vit. B15 375 mg, vit. C 500 mg, folic acid 200 mg, biotin 40 mg, choline 10 000 mg.

Mineral mixture consisted of copper (copper di- sulphate) 600mg, iron (iron di sulphate) 5 500 mg, iodine (calcium iodate) 30 mg, cobalt (cobalt sulphate) 30 mg, manganese (manganese dioxide) 2 400 mg, zinc (zinc di-oxide) 2 400 mg, and selenium (sodium selenite) 10 mg. Table 1 shows the total daily intake of vitamins and minerals from barley, hay and premix, and the recommended requirements for horses suggested by NRC (1989).

The blood samples were collected prior to the experiment after 10 days adaptation period, and three times every 10th day from each stallion during the experiment. The samples were drawn from jugular vein before the morning meal. Samples specified for serum analyses were allowed to chill down at room temperature and serums were alliquoted and kept at –20 °C untill analysis proceeded.

Table 1. Daily intake of micronutrients from barley (4 kg), hay (6 kg) and premix (150 g) comparing with the recommended daily requirements

Preglednica 1. Dnevni obrok mikronutrientov iz ječmena (4 kg), sena (6 kg) in premiksa (150 g) ter primerjava s priporočenimi dnevnimi potrebami

Nutrient		6 kg hay	4 kg barly	150 g premix	Daily intake		Daily requirements NRC 1989!!!
					Control group	Experimental group	
Vit A	IU	36 000	15 000	75 000	51 000	126 000	100 000
Vit E	mg	240	144	300	384	684	600
Vit B1	mg	16	20.0	75	36.8	111.8	85
Vit B2	mg	52.2	8.0	75	60.2	135.2	110.0
Vit B3	mg	91.8	25.6	150.0	117.4	267.4	200.0
Vit B5	mg	211.8	228.8	225.0	440.6	665.6	360.0
Vit B6	mg	16	10	15	26	41	30
Vit B12	mg	0.03	Trace	0.15	0.03	0.18	0.18
Folacin	mg	7.5	1.5	30.0	9.3	39.3	35.0
Biotin	mg	0.6	1.8	6.0	2.4	8.4	25.0
Choline	mg	9 000	4 108	1 500	13 108	14 000	14 000
Cu	mg	58.8	30.4	90.0	89.2	179.2	120.0
Fe	mg	1380	200	825	1580	2405	500
I	mg	Trace	Trace	4.5	Trace	4.5	1.0
Co	mg	Trace	Trace	4.5	Trace	4.5	1.0
Mn	mg	162.0	65.2	360.0	227.2	587.2	400.0
Zn	mg	96.0	61.2	360.0	157.2	517.2	400.0

Serum protein was analysed colorimetrically based on the principle of Biuret reaction, where copper salts in alkaline medium was used, according to Weichsellaum (1946).

Serum urea was determined by the means of acidic diacetyl monoxime according to the instruction manual of Bio-Merieux reagent kit. Serum lipids were analysed colorimetrically using sulfophosphovanillic mixture according to Zollner and Kirsch (1962). Serum GOT and

GPT transaminase activities were determined according to the method of Reitman and Frankel (1957). Statistical analysis was done, where t-test using the difference between the means and their standard deviation was employed (Snedecor, 1956). The correlation coefficients and their signifficancies were calculated according to Bailey (1979).

RESULTS AND DISCUSSION

The veterinary staff recorded the respiratory rate, pulse and rectal temperature, and the obtained values were within the normal range. No gastrointestinal disturbances of nutritional origin were observed in horses during the experimental period (i.e. colic, diarrhea or obstruction). All horses chewed the pelleted premix, and the premix acceptability was good.

Table 2. The mean values and standard deviation of serum parameters during the experiment (n = 4)
Preglednica 2. Srednje vrednosti in standardni odklon serumskih parametrov med poskusom (n = 4)

Parameter	Control	±SD	Experimental	±SD
Total serum protein				
Day 0	5.525	±0.811	5.949	±0.507
Day 10	6.166	±0.465	7.135	±0.352
Day 20	5.964	±0.748	7.131	±0.599
Day 30	6.328	±0.541	7.771	±0.515
Serum urea				
Day 0	7.623	±0.207	7.230	±0.954
Day 10	6.533	±1.035	6.342	±0.500
Day 20	4.495	±1.852	2.649	±1.025
Day 30	6.276	±0.354	3.257	±0.428
Serum lipids				
Day 0	7.153	±1.343	6.807	±3.831
Day 10	3.846	±0.713	6.615	±2.518
Day 20	7.846	±1.514	8.807	±3.741
Day 30	1.999	±0.812	9.038	±4.845
Serum GOT				
Day 0	75.25	±4.02	68.75	±9.04
Day 10	82.50	±12.89	72.50	±9.01
Day 20	82.75	±5.49	67.75	±13.25
Day 30	68.25	±14.35	61.00	±7.28
Serum GPT				
Day 0	19.75	±4.26	23.00	±3.53
Day 10	28.00	±2.54	24.75	±1.47
Day 20	26.25	±4.81	23.50	±2.59
Day 30	34.00	±3.67	26.25	±5.40

Serum results

Feeds that supply the proper proportion and amount of the various essential amino acids supply a good quality protein, whereas those feeds that furnish an inadequate amount of any of the essential amino acids are considered poor quality protein sources (Hintz *et al*. 1971 and

Haready 1984). The most essential amino acids lacking in hay/grains ration are lysine and methionine. The micronutrient premix composition contains in the main formula described in the material, 20 g lysine and 20 g methionine per kg, this to supply each horse daily (in 150 g premix/horse) 3 g lysine and 3 g methionine. The enhancing of the protein quality by the addition of both essential amino acids in daily feed during the experimental period, appeared clearly in the results cited in table 2.

Table 3. Comparison of the mean values of some biochemical parmeters between blood serum of control group and blood serum of experimental group: total serum protein and serum urea

Preglednica 3. Primerjava srednjih vrednosti nekaterih biokemijskih parametrov krvnega seruma kontrolne in poskusne skupine: skupni proteini in urea v serumu

Sampling time	Protein (g/100ml)	Urea (mmol/l)
Pre – Experimental sample		
Control	5.525	7.623
Experimental	5.949	7.230
Difference	(–) 0.424	(+) 0.393
1. Experimental sample (After 10 days)		
Control	6.166	6.533
Experimental	7.135	6.342
Difference	(–) 0.969	(+) 0.191
2. Experimental sample (After 20 days)		
Control	5.964	4.495
Experimental	7.131	2.649
Difference	(–) 1.167	(+) 1.846
3. Experimental sample (After 30 days)		
Control	6.328	6.276
Experimental	7.771	3.257
Difference	(–) 1.443	(+) 3.019
Mean of difference	1.1930	1.6846
Stand. deviation of difference	0.1943	1.1600
Estimated t	10.6350**	2.5154*

*and ** = the value is significant and highly significant at ($p < 0.1$), respectively.

Total serum protein

The results of total serum protein showed that the highest value was 8.561 g/100ml and the lowest 4.473 g/100ml with an average of 6.496 g/100ml ($n = 32$). These values are within the normal range for breeding stallions established previously (Haready, 1984).

The significancy between the mean values of control and experimental groups in serum protein were highly segnificant ($P < 0.1$). The obtained results are confirming the results that have been described by Haready (1984), Habe *et al*. (1984) and Haready (1998).

Serum urea

The results for serum urea showed the values between 2.501 and 8.744 mmol/l, with an average of 5.551 mmol/l ($n = 32$). The obtained values are within the established normal range.

The differences between the mean values of control and experimental groups were significant ($P < 0.01$).

Relationship between serum protein and serum urea

The difference between the mean values of both groups may be attributed to the supplementation by feeding premix, which resulted in the increase of total serum protein and the decrease in serum urea concentrations. This inverse relationship between serum protein and serum urea could be explained as an increase in nitrogen utilization as already reported by Fonnesbeck and Symons (1969) with horses, Prior *et al.* (1974) with ponies and El-Ashry *et al*. (2002) with suckling buffalo calves.

In the present study, negative correlation between total serum protein and serum urea concentrations was found (i.e. correlation coefficients for control and experimental groups were 0.61 and 0.74, respectively). The obtained correlation was highly significant ($P < 0.001$). The difference between the obtained correlation coefficients (0.61 vs. 0.74) could be attributed to the better metabolism by the supplementation of micronutrients.

Table 4. Comparison of the mean values of some biochemical parameters between serum of control group and serum of experimental group; total serum lipids, serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT)

Preglednica 4. Primerjava srednjih vrednosti nekaterih biokemijskih parametrov v serumu kontrolne in poskusne skupine: skupni serumski proteini, lipidi v serumu, koncentracija glutamat oksalat transaminaze (GOT) in serumske glutamat piruvat transaminaze (GPT)

Sampling time	Lipids (g/l)	GOT (Units/ml)	GPT (Units/ml)
Pre experimental sample			
Control	7.153	75.33	19.75
Experimental	6.807	68.75	23.00
Difference	(+) 0.346	(+) 6.58	(–) 3.25
1. Experimental sample (After 10 days)			
Control	3.845	82.50	28.00
Experimental	6.615	72.50	24.75
Difference	(–) 2.770	(+) 10.00	(+) 3.25
2. Experimental sample (After 20 days)			
Control	9.571	82.67	26.25
Experimental	14.585	67.75	23.50
Difference	(–) 5.014	(+) 14.92	(+) 2.75
3. Experimental sample (After 30 days)			
Control	2.037	68.33	34.00
Experimental	9.038	61.00	26.25
Difference	(–) 7.001	(+) 7.33	(+) 7.75
Mean of difference	4.928	10.75	4.58
Stand. Deviation of difference	1.728	3.1436	2.2484
Estimated t	4.939**	5.9231**	3.5308**

*,** = the value is significant and high significant at ($P < 0.1$), respectively,
GOT = Glutamic Oxaloacetic Transaminase, GPT = Glutamic Pyruvic Transaminase.

Negative non-significant correlations between serum protein and serum urea concentrations were found in mature stallions on breeding season fed diet prepared according to NRC (1978) recommendation, Haready (1984).

Serum lipids

The results for serum lipids (Table 3) showed that the highest, lowest and average value were 17.384, 1.384 and 6.620 g/, respectively (n = 32). There is a possibility to add fat to horse ration. This added fat usually ranges from 2 to 5 % of the total ration. In the trial cited in Cunha (1980), the blood serum cholesterol levels increased the most with adding the 5 % corn oil ration. In the present work, adding of soy oil in the main formula of micronutrients premix , may have contributed in the achieved increase in serum lipids of experimental group.

Serum transaminases

Several factors affect GOT and GPT enzymes; as feeding practices, environment, genetic control, response to stress, age, liver function and body weight (Boots *et al*., 1969). In the present study, the micronutreint supplementation was the main target.

SerumGOT

The results for serum GOT (Table 3) showed that the highest, the lowest and average values were 101, 45 and 72.3 Units/ml, respectively (n = 32). Highly significant was decrease in serum GOT values of experimental group ($P < 0.001$). This decrease may be due to the addition of micronutrients used in the experiment. El-Ashry *et al*. (2002) found that GOT activity was decreased significantly ($P < 0.05$) in calves due to the addition of live dried yeast.

Serum GPT

The results of serum GPT (Table 3) showed that the highest, lowest and average values were 40, 14 and 25.7 Units/ml, respectively (n = 32). Highly significant differences between both groups were in GPT activities ($P < 0.001$). The decrease in GPT activity in experimental group may be refer to the addition of micronutrients.

Relatioship between serum lipids and serum GOT

Significant negative correlation between serum lipids and serum GOT was found ($r = –0.79$; $P < 0.001$; $n = 4$). However, non-significant correlation was found in the for control group ($r = 0.4$). The difference between correlation coefficients for both groups may be a reflection of the improvement in metabolic processes by the supplementation with micronutrients.

Relationship between serum lipids and serum GPT

Non-significant positive correlation between serum lipids and serum GPT was found for experimental group ($r = 0.42$). However, highly significant negative correlation between both parameters was found in control group ($r = –0.82$; $P < 0.001$; $n = 2$). El-Ashry *et al*. (2002) found that GPT was not affected with feeding practices when live dried yeast were used in diets for calves.

CONCLUSION

From our results and from previous research in horses as well as in other animals concerning the addition of micronutrients, in case of single addition or some micronutrients, it can be concluded that attention should be paid to the study of micronutrients, their sources, requirements for each age and physiological stage of horses in order to avoid unbalanced supply. Comparative study based on the research data from horses from different areas was performed in order to unify the current NRC recommendations with the newer results. Preparation of advisory program for horse owners in order to inform them about the importance of micronutrients should be considered. Further studies on the influence of additional micronutrients on the relationships between different biochemical parameters should be done.

POVZETEK

Vitamini in minerali igrajo osrednjo vlogo v celičnem metabolizmu, vzdrževanju osnovnih življenjskih funkcij in uravnavanju rasti. Potrebe konj po mikronutrientih so proučevali z različnih aspektov za različne kategorije konj. Tak pristop lahko vodi do napačnih zaključkov in do neustreznih priporočil za prehrano konj. Zaradi pomanjkanja osnovnih informacij o prehranskih potrebah konj se v priporočilih pogosto zatekamo k prehranskim priporočilom, ki so bile oblikovane za druge vrste. Posebno pozornost moramo posvetiti izdelavi priporočil za prehrano posameznih kategorij konj, t.j. v obdobju rasti, športne aktivnosti in reprodukcije. Pomembno vlogo igra proučevanje vpliva dodatka mikro nutrientov na nekatere fiziološke funkcije, ki jih merimo z različnimi metabolnimi parametri, ki vplivajo na sestavo krvi in urina.

V poskus smo vključili osem zdravih žrebcev arabske pasme, ki niso kazali nobenih kliničnih posebnosti. Poskus smo razdelili v začetno desetdnevno prilagoditveno obdobje in tridesetdnevno poskusno obdobje. Živali smo razdelili v kontrolno in poskusno skupino. Vse živali so bile vhlevljene individualno, krmili smo jih dvakrat na dan in po vsakem obroku so imele možnost izpusta v peščeno ograde. Obe skupini smo krmili z istim osnovnim obrokom, ki je vseboval 6 kg sena in 4 kg ječmena na dan. Poskusna skupina je ob osnovnem obroku dobivala še 150 g premiksa mikronutrientov. Premiks je bil sestavljen iz 180 g mletih ovsenih luščin in 260 g dikalcijevega fosfata kot nosilec ter 25 g lignosulfata, 20 g metionina, 20 g lizina, 200 g kalcija, 40 g soli (NaCl), 30 g sojinega olja, 100 g kvasovk, 50 g vitaminske mešanice, 75 g mineralne mešanice in antioksidante. Živalim smo vzeli kri na začetku prilagoditvenega obdobja in nato v desetdnevnih intervalih do konca poskusa. V vzorcih seruma smo določali skupne serumske proteine, ureo, lipide v serumu, serumsko glutamat oksalacetat transaminazo (GOT) in glutamat piruvat transaminazo (GPT). Rezulati so pokazali statistično visoko značilne razlike med obema skupinama pri serumskih proteinih, lipidih v serumu, GOT in GPT. Rezultati so predstavljeni v preglednici 4.

Značilne razlike med skupinama smo našli tudi pri vsebnosti uree v serumu ($p < 0.1$). Spremembe v parametrih sestave seruma utegnejo biti posledica dodajanja mikronutrientov v premiksu, ki smo ga krmili poskusni skupini. Korelacija med serumskimi proteini in vsebnostjo uree v serumu je bila negativna, med lipidi v serumu in GOT pa statistično značilno negativna. Med lipidi v serumu in GPT nismo našli statistično značilne korelacije.

Naši rezultati in rezultati študij, objavljeni v literaturi, kjer so proučevali vpliv posameznih mikronutrientov ali njihovih kombinacij, kažejo na njihov pomen v posameznih fizioloških stanjih. Pri določanju potreb posameznih kategorij konj po mikronutrientih je treba posvetiti posebno pozornost njihovi izkoristljivosti. Naše rezultate smo primerjali z rezultati podobnih študij pri drugih živalskih vrstah, da bi lahko izboljšali priporočila za prehrano različnih kategorij konj. Za oblikovanje novih priporočil za preskrbo konj z mikronutrienti pa bodo

potrebne še dodatne raziskave, ki bodo vključevale širši izbor fizioloških parametrov v odvisnosti od različnih ravni preskrbe.

Preglednica 4. Primerjava srednjih vrednosti nekaterih biokemijskih parametrov v serumu kontrolne in poskusne skupine: skupni serumski proteini, lipidi v serumu, koncentracija glutamat oksalat transaminaze (GOT) in serumske glutamat piruvat transaminaze (GPT)

Čas vzorčenja	Lipidi (g/l)	GOT (Enot/ml)	GPT (Enot/ml)
Predposkusno obdobje			
Kontrolna skupina	7,153	75,33	19,75
Poskusna skupina	6,807	68,75	23,00
Razlika	(+) 0,346	(+) 6,58	(–) 3,25
1. poskusni vzorec (po 10 dneh)			
Kontrolna skupina	3,845	82,50	28,00
Poskusna skupina	6,615	72,50	24,75
Razlika	(–) 2,770	(+) 10,00	(+) 3,25
2. poskusni vzorec (po 20 dneh)			
Kontrolna skupina	9,571	82,67	26,25
Poskusna skupina	14,585	67,75	23,50
Razlika	(–) 5,014	(+) 14,92	(+) 2,75
3. poskusni vzorec (po 30 dneh)			
Kontrolna skupina	2,037	68,33	34,00
Poskusna skupina	9,038	61,00	26,25
Razlika	(–) 7,001	(+) 7,33	(+) 7,75
Srednja razlika	4,928	10,75	4,58
Standardni odklon razlik	1,728	3,1436	2,2484
Ocena t	4,939**	5,9231**	3,5308**

* = vrednost je značilna oziroma ** = visoko značilna ($p < 0,1$).
GOT = glutamat oksalat transaminaza, GPT = glutamat piruvat transaminaza

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