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Taurine protects DNA of lymphocytes against oxidative alteration in riding horses

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The study aimed at evaluation the effect of dietary supplement of taurine on the oxidation-reduction status in riding horses, and especially on the extent of oxidative DNA degradation in lymphocytes. Ten Thoroughbred and half-bred geldings aged 6-13 years were classified according to breed and amount of work done into two groups – control (C, n=5) and experimental (E, n=5), the latter fed the diet with addition of 40 g taurine/horse/day. Blood samples were withdrawn from the horses' jugular vein before commencing the riding season and then after 30 days of working. In the blood some selected morphological and biochemical indicators were determined including the TBA-RS and 8-oxo-dG in lymphocyte DNA. It was found that physical effort of horses, being used for 30 days in recreation riding, affected homeostasis of redox status, and especially the rate of oxidative DNA degradation of lymphocytes. The addition of taurine to feed caused smaller oxidative stress, manifested by lower concentration of TBA-RS in plasma and of 8-oxo-dG in lymphocytes. The taurine lowered the lipid peroxidation intensity that occurred in horses due to the oxidative stress caused by physical effort. Furthermore, taurine revealed affinity to nucleic acids, counteracted the oxidative DNA degradation and decreased the damages of DNA in lymphocytes.

KEY WORDS: DNA / horses / lymphocytes / oxidative stress / taurine

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Approximately 95% of the oxygen consumed by organism is reduced to water during aerobic metabolism taking place in mitochondrial respiratory chain. The remaining amount may be converted to reactive oxygen species (ROS) that can damage lipids, protein and DNA. However, antioxidative system of defence against ROS is very efficient and protects cell's structures from these unusually active molecules. Increase in physical activity increases oxygen intake and rate of oxidation in the respiratory chain leading to ROS production larger than antioxidant potential of organism [Peake and Suzuki 2004]. ROS generation is a reason for oxidative stress, resulting in cells and tissues damage and causing decrease of vitality [Kanter *et al*. 1988, Devlin 2001, Dawson *et al*. 2002]. Oxidative stress has been suggested to contribute to several equine diseases, including the cartilage defect in osteochondrosis, the membrane damage in exertional rhabdomyolysis, the vascular defect in exercise-induced pulmonary haemorrhage, and degenerative motor neuron disease [Hoffman *et al*. 2001]. Dietary supplementation of antioxidants, especially vitamins E, C and lipoic acid has yielded some encouraging results in exercising horses [Avellini *et al*. 1995, Hoffman *et al*. 2001, Williams *et al*. 2004].

Taurine is synthesized as a final product of metabolism of sulphuric amino acids and plays an antioxidative function [Batt 2001, Kulasek *et al*. 2002, Ekremoglu *et al*. 2007]. The amine group of taurine has a high affinity to DNA and prevents it from damage, what seems to be very important in relation to active cells of immune system [Batt 2001, Zhang *et al*. 2004]. It has been demonstrated that taurine protects DNA against ROS generated from processes of respiratory burst in neutrophils [Redmond *et a*l. 1998].

The objective of the present investigation was to evaluate the effect(s) of supplementation the diet for recreation riding horses with taurine on the oxidative status of organism and particularly on DNA degradation in lymphocytes.

Material and methods

The study was carried out at the riding club of Warsaw Agricultural University on 10 Thoroughbred and half-bred geldings aged 6-13 years, clinically healthy and in good condition. By the method of analogues (breed, amount of work done) horses were divided into two groups – control (C, n=5) and experimental (E, n=5). In stable, the horses were kept in individual boxes with free access to water. The horse work consisted of 2-3 h of recreation riding daily and could be considered as moderate. Both groups were fed according to NRC [1989] with 4.0 kg oats, 3.5 kg meadow hay, and 1.5 wheat straw (789 g crude protein and 77.85 MJ digestible energy daily). The diet for E horses was supplemented with taurine introduced as 1% of daily dose of oats (*i.e.* 40 g taurine/horse/day) for 30 days. Taurine as pure powder was obtained from CORTEX Chemical Company Ltd, 14 Kruczkowski St., 33-101 Tarnów, Poland.

As a rule, the blood was withdrawn from the jugular vein of each horse before the meal, once before the beginning and once after a month of the riding period (day

0 and day 30, respectively) Blood sampled into heparinized tubes was cooled to 4°C and immediately examined for cell morphological indicators. Lymphocytes were separated, frozen to -70°C and identified. The remaining blood was centrifuged (3000 r.p.m. for 20 min to obtain serum, which was frozen to -70°C for further analyses.

Morphological parametres of blood; erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), leucocytes (WBC), neutrophiles and lymphocytes were determined using haematological counter Vet ABC 18. Albumins, glucose, asparagine transferase (ASP), alanine transferase (ALT), alkaline phosphatase (AP) and urea nitrogen (UN) were determined by dry chemistry methods using Vitros DT II and DTSC equipment (JOHNSON & JOHNSON, USA) according to 1996 Operator's Manual. In the serum, contents of substances reacting with thiobarbituric acid (TBA-RS) were measured using spectrophotometer UNICAM -5625 at 532 nm as described by Ohkawa *et al*. [1979].

To obtain lymphocytes, blood was sampled into heparinized tubes BD VACUTAINER CPT and after two h centrifuged at 3100 r.p.m. for 20 min. Lymphocytes were then washed out twice with PBS. Lymphocyte DNA was isolated according to Foksiński *et al* [2000]. Both 8-oxo-2-deoxyguanosine (8-oxo-dG) and 2-deoxyguanosine (dG) were determined using HPLC (Dionex 170S) with electrochemical detector (ESA Inc. CoulArray, Model 5600A) at 350 mV and UV detector at 254 nm, with column 250×4.6 mm Supelcosil LC-18-S (5µm grain) as described by Helbock *et al* [1998]. The amount of 8-oxo-dG in DNA was calculated as a number of 8-oxo-dG molecules/10^6 unmodified dG molecules.

Results were evaluated with two-factorial ANOVA, using the STATGRAPHIC 4.1 Plus software package. The differences at $P<0.05$ were considered significant.

Results and discussion

Physical effort increased the RBC, HCT and HGB content of blood in all horses. Those supplemented with taurine (group E) showed higher levels of RBC, HCT and HGB, but there was no interaction identified between the diet (taurine *vs*. no taurine) and work (Tab.1). WBC did not change after riding period and after receiving dietary taurine (Tab.1). Physical effort, however, affected the per cent of neutrophils. It also led to non-significant reduction of lymphocytes content, but taurine supplementation reduced this effect, what was indicated by significant interaction between work and diet.

Training, but not dietary taurine, increased the activity of alkaline phosphatase, alanine transferase and asparagine transferase (Tab. 1). Concentrations of blood glucose and urea nitrogen increased after riding period in both groups, but their levels were lower in group E (Tab. 1). Concentration of albumins in serum increased after 30 days of riding period, but there was no effect of supplementation with taurine.

Table 1. Blood morphology, biochemical serum indicators, serum concentration of TBA-RS and concentration of 8-oxo-2deoxyguanine in DNA of lymphocytes in saddle horses fed the experimental diet (with taurine) or control diet (with no taurine)

Indicator	Experimental group		Control group (no taurine)		SEM	P-value		
	day 0	day 30	day 0	day 30		work	diet	interaction work × diet
blood morphological indicators								
Haemoglobin (mmol/l)	7.75	8.60	6.96	7.98	0.88	0.0232	0.0045	ns
Haematocrit (l/l)	0.32	0.42	0.28	0.39	0.028	0.0000	0.0073	ns
Red blood cells (10^{12}/l)	8.18	11.37	7.43	9.59	0.42	0.0004	ns	ns
White blood cells (10^{9}/l)	6.94	6.35	6.26	6.70	0.78	ns	ns	ns
Neutrophils (%)	42.2	56.2	39.2	59.4	4.87	0.0029	ns	ns
Lymphocytes (%)	56.6	42.2	60.4	39.0	4.94	ns	ns	0.0244
serum biochemical indicators								
Alkaline phosphatase (U/l)	86.8	150.0	76.6	127.6	8.77	0.0000	ns	ns
Alkaline aminotransferase (U/l)	11.2	15.6	10.0	15.2	1.65	0.0090	ns	ns
Aspartate aminotransferase (U/l)	149.8	236.6	154.0	289.0	15.10	0.0000	ns	ns
Glucose (mmol/l)	2.56	4.66	3.12	5.05	0.22	0.0000	0.0092	ns
Blood urea nitrogen (mg/dl)	8.6	15.8	12.6	19.2	1.08	0.0000	0.0036	ns
Albumins (g/l)	21	26	22	27	1.33	0.0000	ns	ns
indicators of oxidative status								
TBA-RS (µmol/l)	1.92	2.38	1.67	4.10	0.19	0.0086	0.0155	ns
8-oxo-dG/10^{6}	3.113	4.690	3.802	7.252	0.46	0.0000	0.0121	ns

SEM – standard error of mean; ns – not significant.

Indicators of oxidative stress, *i.e* TBA-RS content of serum as well as 8-oxo-dG in lymphocytes increased significantly after 30 days of riding period. Both of them also increased in E, but to much lower extent than in C group (Tab. 1).

All morphological indicators of horse peripheral blood prior to and after 30 days of riding period did not exceed reference values [Kłopocki and Winnicka 1987, Szarska 2002]. Physical effort, however, increased parametres of oxygen capacity of horses – concentration of HGB, percentage of HCT and number of RBC – indicating

increased oxygen transport to muscles. Thirty days of riding period could stimulate the mechanism of adaptation to a higher level of oxygen requirement within the cells. However, obtained results may also suggest that applied work induced a small degree of dehydration.

Horses work in recreation riding had no effect on WBC, suggesting that moderate physical effort applied in this experiment did not depress immunity of animals. However, activity of non-specific immune response slightly raised contrary to the level of lymphocytes, being responsible for specific immune system. Taurine content of lymphocytes is relatively high, about 50% of free amino acid pool [Redmond *et al*. 1998], probably to protect DNA of these very active immune cells against oxidative stress damage and genetic code failure. It can be hypothesized that the interaction between applied work and taurine, observed in this experiment, can corroborate mechanism when lymphocytes enriched in taurine are more resistant to suppression caused by physical effort.

Activity of enzymes (Tab. 1) was within the reference values for horses used in recreation riding [Szarska 2002]. The levels of AP, ALT and AST were, however, elevated after 30 days of riding period in the horses from both groups. Exercise, even moderate, could cause physiological damage to muscle and liver cells as a consequence of high level of their activity. Furthermore, taurine content of muscles changes after exercise [King and Suleiman 1998]. Nevertheless, taurine did not alter this mechanism and did not show protective properties to these cells.

Recreation riding increased the level of glucose, albumins and urea nitrogen in blood serum probably as a result of increased metabolic rate. Supplementation with taurine decreased glucose and urea in blood serum, indicating a positive effect of taurine supplementation on glucose and protein metabolism during riding period. However, the mechanism is unknown. It has been concluded from experiments with mice that taurine maintains the level of glutaminate in cells [Lake *et al*. 1992], probably caused by taurine involvement not only in protein but also in energy metabolism. Moreover, taurine is employed to stabilize blood sugar levels by potentiating insulin, what is used in diabetes treatment [Militante *et al*. 2000].

Concentration of TBA-RS after 30 days of riding period was found increased. TBA-RS may indicate not only lipid peroxidation, but also synthesis of cytotoxic compounds originated from reaction of lipids as well as protein and carbohydrates oxidative products. Radak *et al.* [1997] showed that high altitude training increases the level of reactive carbonyl derivatives, but not lipid peroxidation in skeletal muscle of rats. In the current experiment, however, the horses receiving taurine (group E), showed the concentration of the end-product of lipid peroxidation somewhat lower than those of control (group C). In the study by Kanter *et al.* [1988] with exercising people, the 77% increase of TBA-RS concentration was found after terminating the exercise. Similar results with exercising man were obtained by Zhang *et al.* [2004] who demonstrated that seven-days administration of taurine lowered considerably the level of TBA-RS concentration. Also, Dawson *et al.* [2002] offered taurine and β-

alanine to rats in drinking water and found lower indicators of lipid peroxidation. They suggested that taurine showed protective action, being a scavenger of free radicals. Taurine, however, is not dissolved in lipid phase and rather protects water-soluble compounds and cell structures, but by scavenging free radicals may prevent peroxidation of lipids.

Degradation of DNA is an effect of oxidative stress, resulting from a high intensity of exercises [Kanter *et al*. 1988, Benzi 1993, Dawson *et al.* 2002]. The level of 8-oxo-2dG, marker of oxidative damage of DNA in lymphocytes increased after 30 days of riding period. However, the increase of 8-oxo-2dG in taurine group was relatively small, probably caused by a moderate intensity of work. It may be supposed that administration of taurine depresses the oxidative damage of DNA. Such suggestion was confirmed by Zhang *et al.* [2003] who reported that damages of the cells and tissues may occur as a result of oxidative stress, caused by physical effort, and the products of lipid peroxidation are potential mediators in DNA damage [Redmond 1998]. In their studies, the addition of taurine to the diets for sportsmen mediated the rate of DNA damages in leukocytes after physical effort. In addition, the studies of Batt [2001] and Devlin [2001], performed with racing dogs, demonstrated reduced DNA damage resulting from taurine administration.

It is concluded that physical effort of horses, used in recreation riding for 30 days, affected homeostasis of redox state, and especially increased oxidative DNA degradation of lymphocytes. Supplementing the diet with taurine caused lower oxidative stress manifested by lower TBA-RS concentration in plasma and 8-oxo-dG in DNA of lymphocytes. Taurine, being natural antioxidant lowered the process of lipid peroxidation occurring due to oxidative stress caused by physical effort. Furthermore, taurine showed an affinity to nucleic acids and by this it can counteract the oxidative generation of DNA and may reduce DNA damages in lymphocytes of horses.

REFERENCES

1. AVELLINI L., SILVESTRELLI M., GAITI A., 1995 – Training-induced modifications in some biochemical defences against free radicals in equine erythrocytes. ***Veterinary Research Communications*** 19,179-184.
2. BATT B., 2001 – Components of antioxidants developed by WALTHAM. International WALTHAM Symposium, Canada, 6 -7 August.
3. BENZI A., 1993 – Effect of intensity exercise on the volume damage DNA in leukocytes of various animals. ***European Radiology*** 23(9), 68-72.
4. DAWSON R., BIASETTI M., MESSINA S., DOMINY J., 2002 – The cytoprotective role of taurine in exercise-induced muscle injury. ***Amino acids*** 22, 309-324.
5. DEWLIN P., 2001 – Role of antioxidants in nutrition of accompanying animals. International WALTHAM Symposium, Canada, 6 -7 August.
6. EKREMOGLU M., TURKOZKAN N., ERDAMAR H., KURT Y., YAMAN H., 2007 – Protective effect of taurine on respiratory burst activity of polymorphonuclear leukocytes in endotoxemia. ***Amino Acids*** 32, 413-417.

7. FOKSIŃSKI M., KOTZBACH R., SZYMAŃSKI W., OLIŃSKI R., 2000 – The level of typical biomarker of oxidative stress 8-hydroksy-2'deoxyguanosine is higher in uterine myomas than in control tissues and correlates with size of the tumor. ***Free Radical Biology and Medicine*** **29**, 597-601.
8. HELBOCK H.J., BECKMAN K.B., SHIGENAGA M.K., WALTER P.B., WOODALL A.A., YEO, H.C., AMES B.N., 1998 – DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. ***Proceedings of the National Academy of Sciences of the USA*** 95, 288-293.
9. HOFFMAN R.M., MORGAN K.L., PHILLIPS A., DINGER J.E., ZINN S.A., FAUSTMAN C., 2001 – Dietary vitamin E and ascorbic acid influence nutritional status of exercising polo ponies. Proceedings of 17th Equine Nutrition and Physiology Society, Lexington, USA, 129-130.
10. KANTER M., LESMES G., KAMINSKY L., HAM-SAEGER J., NEQUIN N., 1988 – Serum creatine kinase and lactate dehydrogenase changes following in eighty kilometer race. Relationship to lipid peroxidation. ***European Journal of Applied Physiology*** 57, 60-63.
11. KING N., SULEIMAN M.S., 1998 – Effect of regular training on the myocardial and plasma concentrations of taurine and alpha-amino acids in thoroughbred horses. ***Amino Acids*** 15, 241-51.
12. KŁOPOCKI T., WINNICKA A., 1987 – Wartości prawidłowe podstawowych badań w weterynarii (Reference values of basic tests in veterinary medicine). In Polish. Published by the Warsaw Agricultural University, Warsaw.
13. KULASEK G., LECHOWSKI R., SAWOSZ E., 2002 – Tauryna – biologiczne znaczenie u ssaków (Taurine – biological role in mammals). In Polish, summary in English. ***Medycyna Weterynaryjna*** **57**, 154-161.
14. LAKE N., WRIGHT E.D., LAPP W.S., 1992 – Effects of taurine deficiency on immune function in mice. ***Advances in Experimental Medicine and Biology*** 315, 241-243.
15. MILITANTE J.D., LOMBARDINI J.B., SCHAFFER S.W., 2000 – The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. ***Cardiovascular Research*** 46, 393-402.
16. NRC, 1989 – Nutrient Requirements of Horses, National Academy Press, Washington, DC.
17. OHKAWA H., OHISHI N., YAGI K., 1979 – Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. ***Analytical Biochemistry*** 95**,** 351-358.
18. Operator's Manual System Vitros DT II Chemistry 1996 – Johnson and Johnson. Clinical Diagnostics, New York, USA.
19. PEAKE J., SUZUKI K., 2004 – Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. ***Exercise Immunology Review*** 10, 129-141.
20. RADAK Z., ASANO K., LEE K.C., OHNO H., NAKAMURA A., NAKAMOTO H., GOTO S., 1997 – High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. ***Free Radical Biology and Medicine*** 22(6), 1109-1114.
21. REDMOND H.P., STAPLETON P.P., NEARY P., BOUCHIER-HAYES D., 1998 – Immunonutrition: The role of taurine. ***Nutrition*** 14, 599-604.
22. SZARSKA E., 2002 – Badania laboratoryjne w treningu koni (Laboratory tests in horse training). In Polish. Ed. Crex S.C., Warsaw.
23. WILLIAMS C.A., KRONFELD D.S., HESS T.M., SAKER K.E., WALDRON J.N., CRANDELL K.M., HOFFMAN R.M., HARRIS P.A., 2004 – Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race. ***Journal of Animal Science*** 82, 588-594.
24. ZHANG M., IZUMI I., KAGAMIMORI S., SOKEJIMA S., YAMAGAMI T., LIU Z., QI B., 2004 – Role of taurine supplementation to present exercise-induced oxidative stress in healthy young men. ***Amino acids*** 26**,** 203-207.

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Tauryna chroni DNA limfocytów koni użytkowanych rekreacyjnie przed oksydatywną degradacją

S t r e s z c z e n i e

Na koniach użytkowanych rekreacyjnie oceniano wpływ dodatku tauryny do paszy na stan oksydoredukcyjny, a zwłaszcza oksydatywną degradację DNA w limfocytach. Klinicznie zdrowe i w dobrej kondycji wałachy pełnej krwi i półkrwi w wieku 6-13 lat przydzielono metodą analogów do grupy kontrolnej (C, n=5) i grupy doświadczalnej (E, n=5), w której podawano paszę z dodatkiem tauryny. Konie żywiono według norm NRC, zgodnie z wymaganiami stawianymi rasie i zależnie od obciążenia wykonywaną pracą. W grupie E do dziennej dawki owsa wynoszącej 4 kg dodawano przez 30 dni 1% tauryny, Stąd średnia dawka tego aminokwasu wynosiła 40 g/konia dzienie. Materiał badawczy stanowiła krew pobrana jednorazowo z żyły szyjnej koni obu grup przed początkiem sezonu jeździeckiego i po 30 dniach od jego rozpoczęcia. W pobranej krwi oznaczono wybrane parametry morfologiczne i biochemiczne, w tym TBA-RS i 8-oxo-dG w DNA limfocytów.

Wysiłek fizyczny koni użytkowanych przez 30 dni w jeździectwie rekreacyjnym wpłynął na zaburzenie homeostazy stanu redoks, a zwłaszcza na poziom oksydatywnej degradacji DNA limfocytów. Dodatek tauryny do paszy E zmniejszył stres oksydacyjny koni w tej grupie, co objawiało się niższym stężeniem TBA-RS w osoczu i 8-oxo-dG w limfocytach w porównaniu z grupą C. Wykazano, że tauryna obniża proces peroksydacji lipidów, zachodzący na skutek stresu oksydacyjnego wywołanego wysiłkiem fizycznym. Ponadto aminokwas ten, wykazując powinowactwo do kwasów nukleinowych, przeciwdziała oksydatywnej degradacji DNA i zmniejsza uszkodzenia DNA w limfocytach koni.