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TIME DEPENDENT FORMATION OF MARKERS OF OXIDATIVE STRESS INDUCED BY A HIGH FAT DIET SUPPLEMENTED OR UNSUPPLEMENTED WITH VITAMIN E IN PIGS

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Time dependent formation of markers of oxidative stress induced by a high fat diet supplemented or unsupplemented with vitamin E in pigs

The time dependent formation of oxidative damage induced by polyunsaturated fat in the diet was investigated in an experiment with pigs as a model for humans. The role of vitamin E in the prevention of oxidative stress was also studied. Twenty-four growing pigs were penned individually and after an adaptation period divided into three groups. All groups received isocaloric daily rations composed of a basal diet isocalorically supplemented with: starch, linseed oil or linseed oil and vitamin E. Oxidative stress was evaluated by measuring the degree of lymphocyte and granulocyte nuclear DNA damage, concentration of malondialdehyde (MDA) in blood plasma, 24-hour urine MDA excretion rate and concentration of vitamin E isomers in the blood at the beginning, after 24 hours, after 6 days and at the end of the 22 day experimental period. The results confirmed that a high proportion of polyunsaturated fat in the diet increased lymphocyte and granulocyte DNA damage only after 6 days. The lymphocytes appear to be more sensitive to this type of oxidative stress than granulocytes. The MDA concentration in the blood and urinary MDA excretion after 24 hours of oxidative stress seem to be more accurate indicators than the rate of lymphocyte and especially granulocyte DNA damage. Vitamin E supplementation effectively protects the blood cells against increased DNA damage during the whole course of the experiment, but failed to reduce MDA formation significantly 24 hours and 6 days after the beginning of oxidative load. The study further suggests that supplementation of vitamin E is able to completely prevent DNA damage of both types of investigated blood cells at any time, but is only able to reduce the formation of lipid peroxidation products after prolonged treatment.

Key words: pigs / animal nutrition / oxidative stress / DNA damage / polyunsaturated fatty acids / PUFAs / vitamin E / comet assay / malondialdehyde

Časovna odvisnost tvorbe kazalcev oksidacijskega stresa pri prašičih, povzročenega s prehrano, obogateno z maščobami ter z ali brez dodatka vitamina E

V raziskavi smo spremljali časovno odvisnost oksidacijskega stresa, povzročenega z dodatkom večkrat nenasičenih maščobnih kislin (VNMK) ter vlogo vitamina E pri njegovem zmanjšanju. Poskus smo izvedli na prašičih kot modelu za človeka. V individualne bilančne kletke smo uhlevili 24 mladih rastočih prašičev ter jih po obdobju prilagajanja razdelili v tri skupine. Vse tri skupine so dobivale enake osnovne izokalorične dnevne obroke z dodatki škroba, lanenega olja ali lanenega olja in vitamina E. Oksidacijski stres smo ovrednostili kot stopnjo poškodb jedrne DNK limfocitov in gralulocitov, koncentracijo malondialdehida (MDA) v krvni plazmi, 24-urno izločanje MDA s sečem in koncentracijo izomer vitamina E v krvni plazmi. Vrednosti navedenih parametrov so bile določene na začetku poskusa, po 24 urah, po 6 dneh in na koncu 22 dnevnega poskusa. Rezultati so pokazali, da je visoka vsebnost večkrat nenasičenih maščobnih kislin (VNMK) v prehrani povečala poškodbe jedrne DNK limfocitov in granulocitov že po 6 dneh. Limfociti so se, v primerjavi z granulociti, izkazali kot bolj občutljivi. Koncentracija MDA v krvni plazmi in v 24-tih urah izločena količina MDA s sečem se je v našem primeru izkazala kot boljši pokazatelj oksidacijskega stresa v primerjavi s stopnjo poškodb DNK limfocitov, še posebno granulocitov. Dodatek vitamina E je učinkovito zaščitil krvne celice pred povečanimi poškodbami DNK v celotnem poskusnem obdobju, medtem ko smo po 24 urah in tudi po 6 dneh ugotovili statistično značilno večjo koncentracijo MDA v primerjavi z začetnimi vrednostmi. Na osnovi dobljenih rezultatov lahko sklepamo, da dodatek vitamina E oba tipa preiskovanih krvnih celic v celoti zaščiti pred poškodbami DNK, nastajanje produktov lipidne peroksidacije pa se lahko zmanjša le po dolgotrajnejšem dodajanju.

Ključne besede: prašiči / prehrana živali / oksidacijski stres / poškodbe DNK / večkrat nenasičene maščobne kisline / vitamin E / kometni test / malondialdehid

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1 INTRODUCTION

Diet and nutritional-related lifestyle factors have a great influence on the formation of free radicals in humans and animals, and they are also important for protection against the harmful effects of radicals. Previous studies have shown that oxidative stress induced by too high an intake of dietary polyunsaturated fatty acids (PUFAs) enhances damage to DNA, while increased intake of antioxidants may have a protective function (RDA, 1989). However, to our knowledge no studies were performed to investigate the time dependent course of oxidative stress induced by a high dietary intake of polyunsaturated fat. At the same time, the protective effect of antioxidants in this process has also not been elucidated. Differences in the time dependent formation of various parameters of oxidative stress could be of interest not only from the theoretical point of view but also from practical considerations. Questions such as how fast does oxidative stress occur after increased oxidative load caused by high PUFA intake, how efficiently are antioxidants able to play a protective role, what is the response of different indicators of oxidative stress. The aim of the present study was to investigate time dependent changes of some markers of oxidative stress induced by a high intake of dietary polyunsaturated fat in pig, as a model for humans. At the same time, the potency of vitamin E in preventing these changes was studied.

The hypothesis of the study was that time dependent appearance of different markers of oxidative stress (malondialdehyde concentration in blood plasma and 24-hour urine MDA excretion rate, degree of leukocyte and granulocyte nuclear DNA damage, concentration of vitamin E isomers in the blood) is not the same, and that supplementation of feed with vitamin E provides protection against some of the damaging effects of PUFAs.

2 MATHERIAL AND METHODS

2.1 EXPERIMENTAL ANIMALS, DIETS, BLOOD AND URINE SAMPLES

Twenty-four young growing castrated male crossbreed pigs (live weight 11.9 kg ± 1.0) were included in the experiment. The animals were penned individually in balance cages that allowed separate collection of urine. The experiment was divided into adaptation and experi-

Table 1: *Composition and content of energy and nutrients in daily rations of experimental groups of pigs (estimated for a 12 kg pig)*
Preglednica 1: *Sestava ter energijska in hranilna vrednost dnevnega obroka posamezne poskusne skupine (preračunano na 12 kg prašiča)*

	Group		
	LowFat	HighFat	HighFat+Vit-E
Wheat starch, g/day	227.78	111.58	111.58
Linseed oil, g/day	0.0	53.12	53.12
Maize, g/day	59.80	59.80	59.80
Soybean meal, g/day	117.60	117.57	117.57
Skimmed milk powder, g/day	91.11	91.11	91.11
Mineral – vitamin supplement [1], g/day	2.53	2.53	2.53
Vitamin E, mg/day	0.0	0.0	57.64
Daily feed intake, g/day	498.82	435.71	435.71
Nutritive value:			
Metabolisable energy [2], KJ/day	7423.50	7423.50	7423.50
Proportion of energy from fat [3], %	5	30	30
Proportion of energy from PUFA[3], %	2.9	20.9	20.9
Protein, g/day	86.5	88.7	87.9
Fat, g/day	8.7	56.0	57.1
Total dietary fibre, g/day	44.0	45.2	45.9

[1] Calculated to meet nutritional requirements according to NRC (1998). Mineral-vitamin supplement provided daily: 2.0 g Ca, 3.4 g P, 0.15 g Na, 5500 IU vitamin A, 7.6 IU vitamin E.
[2] The energy value of feedstuffs and diets was estimated according to GEH (1988).
[3] Estimated.

mental periods that lasted for 14 and 22 days, respectively. The animals were fed 2.5 times the maintenance requirement (Prosky *et al.*, 1992). At the beginning of the experimental period, the animals were randomly assigned to three groups. All the groups received isocaloric daily rations composed of an equal amount of the basal diet which was supplemented according to the different dietary treatments: LowFat with starch, HighFat with linseed oil, HighFat+VitE with linseed oil and vitamin E (Table 1). The part of energy requirements which were met by fat in the LowFat group and both linseed oil supplemented groups was 5 and 30%, respectively. The amount of vitamin E in the HighFat+Vit-E diet should cover the increased needs for vitamin E because of the higher PUFA intake was calculated according to Muggli (1994).

The composition and analysis of daily rations in different groups is presented in Table 1. The feed was fed in the form of a feed mixture. All ingredients of the mixture, except linseed oil, were mixed together weekly. The linseed oil was added and mixed to the diet of individual animals before every feeding.

During the adaptation period the animals adapted to the rearing system and all of them received the same diet (LowFat). The animals were fed twice a day. Water was provided at libitum. At the beginning and at the end of the experiment, the pigs were weighted.

At the beginning, after 24 hours, after 6 days and at the end of the 22 days experimental period blood samples were taken from the jugular vein and 48-h urine was collected (except 24 hours after the beginning of the experiment when the collection time was 24 hours).

The content of protein, fat and fibre was determined by standard procedures published by Neumann and Bassler (1997). The fatty acid composition of diets was analyzed by a gas chromatographic method after transesterification of lipids as described previously (Fidler *et al.*, 2000).

2.2 LYMPHOCYTE AND GRANULOCYTE DNA DAMAGE – SINGLE-CELL GEL ELECTROPHORESIS – COMET ASSAY

Blood samples for single cell gel electrophoresis (Comet assay) were collected in 4.5 ml evacuated tubes containing $EDTAK_3$ anticoagulant. Blood samples were stored on ice for a maximum of 1 hour before the separation procedure. Lymphocytes and granulocytes were separated from the blood samples on a discontinuous Percoll gradient according to a modified procedure described by Hjorth *et al.* (1988) and Kjeldsen *et al.* (1999). A partially modified procedure of Singh *et al.* (1988) was implemented for the comet assay. Olympus CH 50 epifluorescent microscope at 200 × magnification was used for the examination of leukocyte nuclei in the microgels (100 W Hg lamp, excitation filter of 480–550 nm and barrier filter of 590 nm). The images were captured by Hamamatsu Orca 1 CCD camera, analyzed and the nuclear DNA damage estimated by a dedicated computer program Comet 4 (Single Cell Gel Electrophoresis, Kinetic Imaging Ltd., 2000). For each treatment, two slides were prepared and 50 cells (total 100 cells) were examined.

2.3 PLASMA AND URINE MALONDIALDEHYDE (MDA) CONCENTRATION

The blood samples for MDA concentration analysis and urine were collected and prepared as described previously (Pajk *et al.,* 2006). The methodology of Wong *et al.* (1987) modified by Chirico (1994) and Fukunaga *et al.* (1995) was used to measure the concentrations of malondialdehyde (MDA) in blood plasma and urine by HPLC using a Waters Symmetry C_{18} chromatography column (5 μm, 4.6 × 150 mm) and a Waters Symmetry C_{18} guard column (5 μm, 3.9 × 20 mm). A Waters Alliance 2690 apparatus equipped with a Waters Dual λ Absorbance Detector 2487 was applied. The results of the analysis were evaluated by the Millenium[32] Chromatography Manager program.

2.4 VITAMIN E CONCENTRATION IN PLASMA

Blood samples for vitamin E concentration were collected in 10 ml evacuated tubes containing $EDTAK_3$ anticoagulant. Plasma was prepared by centrifugation (400 × g for 10 min.) at 4 °C and transferred to micro centrifuge tubes. The samples were stored at −70 °C. According to Abidi (2000) and Aust *et al.* (2001) vitamin E (as α– and β+γ- tocopherols) was extracted from plasma by hexane, after precipitation of proteins with ethanol containing 0.3% (w/v) tert.-butyl-p-cresol (BTH) to prevent oxidation. Samples were analyzed by HPLC (Waters Alliance 2690), using a Waters Symmetry C_{18} chromatography column (5 μm, 4.6 × 150 mm) and an ODS C_{18} guard column (4 mm L × 3.0 mm ID). A Waters Dual λ Absorbance Detector 2487 and Waters Scanning Fluorescence Detector 474 were used.

2.5 STATISTICAL ANALYSIS

The data were analyzed by the General Linear Model (GLM) procedures from SAS® software (SAS, 2000).

Comparisons between the different treatments were made by contrasts provided by the GLM procedure. The data were expressed as least square means ± standard error. A least significant difference of 0.05 was used to separate the treatment means.

3 RESULTS

During the experiment, the animals had no health or other problems, consumed feed without residues and normal body weight gain was observed in all groups (337 ± 61 g per day).

While at the beginning of the experimental period no statistical differences among groups in any of the measured parameters could be observed, already 24 hours after the nutritional intervention some very important differences among dietary treatments were found.

3.1 PLASMA AND URINE MALONDIALDEHYDE CONCENTRATION

The concentration of MDA in plasma and the urinary MDA excretion rate in the LowFat group remained at almost the same level during the whole experimental period (Table 2). In contrast, both MDA parameters increased in both linseed oil supplemented groups significantly already 24 hours after dietary intervention. Six days afterwards and at the end of the 22 day experimental period, the MDA concentration in plasma and the MDA excretion rate in urine in HighFat and HighFat+Vit-E groups were also significantly higher than in the LowFat group.

While 24 hours and six days after the beginning of the experiment increased MDA excretion rates in urine were at the same level in the HighFat and HighFat+Vit-E groups, on the 22nd day the value in the HighFat group

Table 2: *Effect of high polyunsaturated fat and vitamin E intake on plasma malondialdehyde concentration and malondialdehyde excretion in urine during the experiment*
Preglednica 2: *Vpliv zauživanja večkrat nenasičenih maščobnih kislin in vitamina E na koncentracijo malondialdehida v krvni plazmi in količino dnevno izločenega malondialdehida v obdobju poskusa*

	Group		
	LowFat	HighFat	HighFat+Vit-E
MDA in plasma, nmol/ml:			
At the beginning	0.25 ± 0.09	0.26 ± 0.09	0.28 ± 0.07
After 24 hours	0.22^{a} ± 0.10	0.64^{b} ± 0.28	0.47^{c} ± 0.18
After 6 days	0.24^{a} ± 0.14	0.67^{b} ± 0.35	0.69^{b} ± 0.25
After 22 days	0.24^{a} ± 0.12	0.66^{b} ± 0.31	0.48^{c} ± 0.12
MDA urine excretion, nmol/24 hour:			
At the beginning	2 225 ± 1 261	2 193 ± 1 089	2 315 ± 877
After 24 hours	$2\,115^{a}$ ± 652	$8\,589^{b}$ ± 1 679	$7\,363^{b}$ ± 1 519
After 6 days	$2\,519^{a}$ ± 1 126	$10\,067^{b}$ ± 5 342	$8\,659^{b}$ ± 5 279
After 22 days	3402^{a} ± 1421	$20\,588^{b}$ ± 11362	$10\,704^{ab}$ ± 5 100

[a, b] Means with different superscripts in the same line differ significantly; P≤0.05

Table 3: *The percentage of DNA in head of comets in lymphocytes during the experiment*
Preglednica 3: *Odstotek DNK v glavi kometov v limfocitih v obdobju poskusa*

	Group		
	LowFat	HighFat	HighFat+Vit-E
Percentage of DNA in head of comets in lymphocytes			
At the beginning	95.1 ± 0.83	94.9 ± 1.30	94.9 ± 0.66
After 24 hours	93.6^{a} ± 1.06	91.9^{b} ± 1.71	93.9^{a} ± 1.14
After 6 days	93.3^{a} ± 0.94	88.3^{b} ± 0.68	92.9^{a} ± 0.77
After 22 days	91.6^{a} ± 1.85	82.7^{b} ± 2.72	91.3^{a} ± 0.93

[a, b] Means with different superscripts in the same line differ significantly; P≤0.05

Table 4: *The percentage of DNA in head of comets in granulocytes during the experiment*
Preglednica 4: *Odstotek DNK v glavi kometov v granulocitih v obdobju poskusa*

	Group		
	LowFat	HighFat	HighFat+Vit-E
Percentage of DNA in head of comets in granulocytes			
At the beginning	92.9 ± 1.2	92.6 ± 1.9	93.0 ± 2.2
After 24 hours	92.0 ±0.45	91.0 ± 2.15	91.7 ± 0.65
After 6 days	92.3^{a} ±1.0	88.6^{b} ± 0.4	92.0^{a} ± 1.1
After 22 days	92.3^{a} ± 1.8	87.6^{b} ± 2.0	91.7^{a} ± 1.2

$^{a, b}$ Means with different superscripts in the same line differ significantly; P≤0.05

was significantly higher. At this time MDA excretion with urine in the vitamin E supplemented HighFat+Vit-E group did not significantly differ from either the LowFat or the HighFat group. Also the value for plasma MDA concentration was in between that of the other two groups. In this case the difference from the LowFat and to HighFat groups was significant.

3.2 NUCLEAR DNA DAMAGE OF LYMPHOCYTES AND GRANULOCYTES

The results of DNA damage of lymphocytes and granulocytes are presented in Table 3 and 4 as a percentage of DNA in the head of the comet.

The experiment confirmed that a high proportion of polyunsaturated fat in the diet (group HighFat) increased lymphocyte and granulocyte DNA damage. An absolutely small, but significant decrease of degree of lymphocytes DNA damage was observed even after 24 hours. In both types of cells the effect was more strongly expressed after six days. While the decrease in the percentage of DNA in the head of lymphocyte and granulocyte on the 6th day was similar, on the 22nd day of the experimental period the percentage of lymphocyte DNA in the head was lower. In both types of cells degree of lymphocytes DNA damage in the HighFat+Vit-E group remained on the same level during the experiment as in the LowFat group.

3.3 VITAMIN E CONCENTRATION IN PLASMA

Plasma concentrations of α- and β+γ-tocopherol throughout the experiment are reported in Table 5. During the experimental period there was a significant effect of the type of diet consumed. The effect was observed even after 24 hours. While the plasma α–tocopherol concentration in the LowFat and HighFat groups was unchanged during the whole experimental period, the con-

Table 5: *Concentration of α- and β+γ-tocopherol in plasma during the experiment*
Preglednica 5: *Koncentracija α- in β+γ-tokoferola v plazmi v obdobju poskusa*

	Group		
	LowFat	HighFat	HighFat+Vit-E
α–tocopherol (ppm)			
At the beginning	2.46 ± 1.01	2.45 ± 1.03	2.17 ± 0.41
After 24 hours	2.21^{a} ± 1.12	2.02^{a} ±0.79	3.23^{b} ± 1.14
After 6 days	2.08^{a} ± 0.80	2.02^{a} ± 0.93	5.33^{b} ± 1.93
After 22 days	1.83^{a} ± 0.87	1.86^{a} ± 0.99	4.53^{b} ± 1.37
β+γ-tocopherol (ppm)			
At the beginning	0.034 ± 0.011	0.041 ± 0.019	0.051 ± 0.029
After 24 hours	0.032^{a} ± 0.013	0.249^{b} ± 0.0114	0.252^{b} ± 0.112
After 6 days	0.034^{a} ± 0.013	0.180^{b} ± 0.108	0.123^{c} ± 0.097
After 22 days	0.029^{a} ± 0.015	0.237^{b} ± 0.198	0.135^{c} ± 0.068

$^{a, b, c}$ Means with different superscripts in the same line differ significantly; P≤0.05

centration significantly increased in the HighFat+Vit-E group even 24 hours after the beginning of the experiment and remained so afterwards.

The concentration of β+γ-tocopherol in plasma in the LowFat group remained at the same level during the whole experimental period. β+γ-tocopherol concentration significantly increased in the HighFat and HighFat+Vit-E groups even 24 hours after dietary intervention and remained so also on the 6th and 22nd days of the experiment. At this time the concentration in the HighFat+Vit-E group was significantly lower than in the HighFat group.

4 DISCUSSION

The time dependent formation of oxidative stress induced by a high dietary intake of polyunsaturated fat is currently not well known. At the same time, the protective effect of vitamin E in this process has not yet been elucidated.

In the present study oxidative stress was induced by the selection of linseed oil which contains 73 wt. % of PUFA (Rezar *et al.*, 2003). The energy supply from PUFA was approximately 19% (Table 1). It is known that a high intake of PUFA increases the nutritive requirements for antioxidative vitamins (Muggli, 1994). The oxidative stress in both groups fed linseed oil was additionally increased by the fact that the supply of supplemented antioxidative vitamins was not increased.

As expected, feeding linseed oil in the HighFat group increased the oxidative stress by increasing not only the formation of products of lipid peroxidation but also the rate of blood cell DNA damage. The increased presence of MDA in plasma and urine reflects the products of lipid oxidation originating from diet and formed in the tissues (Guichardant *et al.*, 1994). Our previous studies (Rezar *et al.*, 2003) showed plasma MDA concentration and especially MDA excreted in the urine to be sensitive biochemical markers of the extent of lipid peroxidation.

A study by Marnett (2002) found that lipid peroxidation is one of the major sources of endogenous DNA damage in humans that may contribute to cancer and other chronic diseases linked to lifestyle and dietary factors. The results of the present experiment show that the high intake of PUFA in the HighFat group significantly increased not only the concentration of MDA in blood plasma and the urinary MDA excretion rate but also the degree of both lymphocyte and granulocyte DNA damage. The results obtained clearly demonstrate the harmful effects of polyunsaturated fat in the diet on the oxidative status of pigs, which in view of their metabolism and digestion may serve as a good model for humans (Darcy-Vrillon *et al.*, 1993).

While the effect of oxidative stress induced by a high fat intake on lipid peroxide formation has already been shown (Yang *et al.*, 1997; Rezar *et al.*, 2003), the present study is, to our knowledge, the first to demonstrate *in vivo* time dependent effects and the different effects on MDA formation and lymphocyte and granulocyte DNA integrity.

As assumed, the degree of lymphocyte and granulocyte DNA damage was not the same. According to their different physiological roles and life span, these two cell types can exhibit rather different sensitivities to chemical, physical or biological insults in DNA damage (Giovannelli *et al.*, 2003) and probably different DNA repair capacities (Šrám *et al.*, 1998). A difference between the DNA damage of lymphocyte and granulocyte was observed even after 24 hours and was even greater on the 22nd day of the experiment (Table 2, 3). In the present study, lymphocytes, which have a longer life-span, were found to respond faster and to accumulate DNA damage with prolonged PUFA exposure. The rate of DNA damage in lymphocytes increased with prolonged oxidative stress. Tice (1995) found that short-lived granulocytes may provide information only on current exposure whereas lymphocytes might also give information on past exposure. The results thus provide some evidence that lymphocytes are more sensitive to oxidative stress caused by PUFA than granulocytes, as a result of their DNA repair system and/or longer life span.

While the level of lymphocyte and granulocyte DNA damage did not change 24 hours after the beginning of the experiment, the plasma MDA concentration and urine MDA excretion rate already at that time showed a significantly higher rate of lipid oxidation (Table 4). This indicates that at least in the early stage of such a type of increased oxidative stress MDA measurements are more sensitive parameters of increased oxidative stress. During the experiment the MDA concentration in the blood and the rate of MDA excretion with urine increased. It is obvious that the increase in the latter was much more pronounced and correlates better with the rate of DNA damage of lymphocytes than granulocytes.

Determination of α- and β+γ-tocopherols in plasma may contribute information on the antioxidant status of an individual and may be useful for evaluation of nutritional status and risk of degenerative diseases (Aust *et al.*, 2001). It is known from other investigations (Mileva *et al.*, 2002) that as a consequence of increased oxidative load occurs a decrease in the concentration of antioxidative substances in the blood. On that account it was expected that the concentration of α-tocopherol in plasma would decrease in the HighFat group. But that was not

the case. The reason for this might be a low α-tocopherol concentration of the basal diet and might indicate that in the experiment the applied NRC (1998) recommendations are too low. Since linseed oil is a poor source of α-tocopherol (8.59 mg/100 g) and a good source of β+γ-tocopherol (106, 93 mg/100 g), an increase in plasma β+γ-tocopherol concentration in the HighFat group was expected and actually observed.

As expected, the consumption of vitamin E in the form of α-tocopherol in the HighFat+Vit-E group also significantly increased the concentration of α-tocopherol in plasma during the whole course of the experiment (Table 5). The increase in plasma α-tocopherol was also positively associated with the observed parameters of oxidative stress (Tables 2, 3, 4). The degree of lymphocyte and granulocyte DNA damage in the HighFat+Vit-E group was significantly lower than in the HighFat group. Moreover, the degree of lymphocyte and granulocyte DNA damage in the HighFat+Vit-E group was at the same level as in the LowFat group and was not influenced by the high unsaturated fat intake during the whole experimental period. The positive effect of vitamin E on oxidative stress was also demonstrated as a reduction in MDA concentration in plasma and urine (HighFat+Vit-E group) (Table 4). But in contrast to the protective effect in blood cells, a significant protective effect was not observed earlier than at the 22nd day of the experiment. At this point the vitamin E supplementation was able to reduce MDA formation by approximately 50%. Some other investigators also found that supplementing the diet with vitamin E reduces the plasma or urinary MDA level as well as liver MDA concentration (Cadenas *et al.*, 1996; Naidoo *et al.*, 1998; Kirimlioglu *et al.*, 2006). Since the amount of MDA excreted in the urine correlates positively with its synthesis in the body (Siu and Draper, 1982), and the measurement of urinary-excreted MDA is a more precise indicator of the plasma MDA concentration (Guichardant *et al.*, 1994; Kosugui *et al.*, 1994), the reduced oxidative load in the vitamin E supplemented group could be regarded as even more important.

5 CONCLUSIONS

The results confirmed that a high proportion of polyunsaturated fat (PUFA) in the diet increased the measured parameters of oxidative stress. The lymphocytes proved to be a more sensitive indicator of this type of oxidative stress but the difference was observed only after longer exposure to this type of oxidative stress (22 days). The concentration of MDA in plasma and the rate of urine MDA excretion proved to be very sensitive indicators of oxidative stress, since they responded to an increased unsaturated fatty acid load even after 24 hours. The study further suggests that supplementation of vitamin E is able to completely prevent the formation of DNA damage of both types of investigated blood cells at any time, but is only able to reduce the formation of products of lipid peroxidation after prolonged treatment.

6 REFERENCES

Abidi S.L. 2000. Chromatographic analysis of tocol-derivated lipid antioxidants. Journal of Chromatography A, 881: 197–216

Aust O., Sies H., Stahl W., Polidori M.C. 2001. Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and carotenoids. Journal of Chromatography A, 936: 83–93

Cadenas S., Rojas C., Mèndez J., Herrero A., Barja G. 1996. Vitamin E decreases urine lipid peroxidation products in young healthy human volunteers under normal conditions. Pharmacology and Toxicology, 79: 247–253

Chirico S. 1994. High-performance liquid chromatography-based thiobarbituric acid tests. In: Oxygen radicals in biological systems. Packer L. (ed.). Methods in Enzymology. San Diego, Academic Press: 314–318

Darcy-Vrillon B., Morel M.T., Cherbuy C., Bernard F., Posho L., Blachier F., Meslin J.C., Duee P.H. 1993. Metabolic characteristic of pig colonocites after adaptation to a high fiber diet. Diet Journal of Nutrition, 123: 234–243

Fidler N., Salobir K., Stibilj V. 2000. Fatty acid composition of human milk in different regions of Slovenia. Annals of Nutrition and Metabolism, 44: 187–193

Fukunaga K., Takama K., Suzuki T. 1995. High-performance liquid chromatographic determination of plasma malondialdehyde level without a solvent extraction procedure. Analytical Biochemistry, 230: 20–23

GEH. 1988. Gesellschaft für Ernährungsphysiologie. Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere. Frankfurt am Main, DLG-Verlag: 23–24

Giovannelli L., Pitozzi V., Riolo S., Dolara P. 2003. Measurement of DNA break and oxidative damage in polymorphonuclear and mononuclear white blood cells: a novel approach using the comet assay. Mutation Research, 538: 71–80

Guichardant M., Valette-Talbi L., Canadini C., Crozier G., Berger G. 1994. Malondialdehyde measurement in urine. Journal of Chromatography, 655: 112–116

Kirimlioglu V., Kirimlioglu H., Yilmaz S., Ozgor D., Coban S., Karadag N., Yologlu S. 2006. Effect of fish oil, olive oil, and vitamin E on liver pathology, cell proliferation, and antioxidant defence system in rats subjected to partial hepatectomy. Transplantation Proceedings, 38: 564–567

Kjeldsen L., Sengelov H., Borregaard N. 1999. Subcellular fractionation of human neutrophils on Percoll density gradients. Journal of Immunological Methods, 232: 131–143

Kosugui H., Enomoto H., Ishizuka Y., Kikugawa K. 1994. Wariations in the level of thiobarbituric acid reactant in healthy humans under different conditions. Biological and Pharmaceutical Bulletin, 17: 645–1450

Muggli R. 1994. Physiological requirements of vitamin E as a function of the amount of type of polyunsaturated fatty acids. World Review of Nutrition and Dietetics, 75: 166–168

Marnett L.J. 2002. Oxy radicals, lipid peroxidation and DNA damage. Toxicology, 181–182: 2219–222

Mileva M., Bakalova R., Tancheva L., Galabov A., Ribarov S. 2002. Effect of vitamin E supplementation on lipid peroxidation in blood and lung of influenza virus infected mice. Comparative Immunology, Microbiology and Infectuous Diseases, 25: 1–11

Naidoo D., Lux O. 1998. The effect of vitamin C and E supplementation on lipid and urate oxidation products in plasma. Nutrition Research, 18: 953–961

Naumann C., Bassler R. 1997. Methodenbuch. Die chemische Untersuchung von Futtermitteln, 4. Darmstadt, Ergänzungslieferung, VDLUFA-Verlag: 20–54

NRC. 1998. National Research Council. Nutrient requirement of swine. Washington, National Academy Press

Pajk T., Rezar V., Levart A., Salobir J. 2006. Efficiency of apples, strawberries, and tomatoes for reduction of oxidative stress in pigs as a model for humans. Nutrition, 22: 376–384

Prosky L., Asp N.G., Schweizer T.F., DeVries J.W., Furda I. 1992. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. Journal-Association of Official Analytical Chemists, 75: 360–367

RDA. 1989. Recommended Dietary Allowance. Washington, National academy press: 99–105

Rezar V., Pajk T., Marinšek Logar R., Ješe-Janežič V., Salobir K., Orešnik A., Salobir J. 2003. Wheat and oat bran effectively reduce oxidative stress induced by high fat diets in pigs. Annals of Nutrition and Metabolism, 47: 78–84

SAS. 2000. Statistical Analysis Systems Institute. SAS/STAT User's Guide: statistics, release 8ed. Cary, SAS Institute Inc.

Share P.T. 1988. Methods of cell separation. In: Laboratory techniques in biochemistry and molecular biology. Burdon R.H., Van Knippenberg P.H. (eds.), Amsterdam, Elsevier Inc.: 33–66

Singh N.P., McCoy M.T., Tice R.R., Schneider E.L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175: 184–191

Siu G.M., Draper H.H. 1982. Metabolism of malonaldehyde *in vivo* and *in vitro*. Lipids, 17: 349–355

Šrám R.J., Podrazilová K., Dejmek J.G., Mračková Pilčík T. 1998. Single cell gel electrophoresis assay: sensitivity of peripheral white blood cells in human population studies. Mutagenesis, 13: 99–103

Tice R.R. 1995. The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cell. In: Environmental Mutagenesis. Phillips D.H., Venitt S. (eds.). Oxford, Biopub. Inc.: 315–339

Yang S., Huiyun W., Liping L., Jeusheng L. 1997. Effect of dietary fiber on antioxidation in rats. Journal of Hygiene Research, 26: 318–320

Wong S.H.Y., Knight J.A., Hopfer S.M., Zaharia O., Leach C.N., Sunderman F.W.J. 1987. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde – thiobarbituric acid adduct. Clinical Chemistry, 33: 214–220