

Basic nutritional investigation

# Efficiency of apples, strawberries, and tomatoes for reduction of oxidative stress in pigs as a model for humans

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## Abstract

**Objective:** The present study investigated the potency of apples, strawberries, and tomatoes in decreasing oxidative stress in pigs as a model for humans.

**Methods:** Forty-eight growing pigs were individually penned in metabolic cages and divided into six groups. All groups received isocaloric daily rations composed of a basal diet supplemented with starch, linseed oil, linseed oil and apples, linseed oil and strawberries, linseed oil and tomatoes, or linseed oil, apples, strawberries and tomatoes. Oxidative stress was evaluated at the beginning and at the end of the 22-d experimental period by measuring the degree of DNA damage in mononuclear blood cells, the concentration of malondialdehyde (MDA) in plasma, the MDA excretion rate, the concentration of tocopherols in plasma, plasma total antioxidant status, and erythrocyte glutathione peroxidase concentration.

**Results:** The results confirmed that the linseed oil diet significantly increased MDA formation in the body and DNA damage to mononuclear blood cells in comparison with the starch diet. Although the MDA concentration in plasma was significantly decreased in all fruit groups, urine MDA excretion rate was decreased only in the linseed oil/apple and linseed oil/apple/strawberry/tomato groups. Supplementation with fruit significantly decreased DNA damage, was more pronounced in the linseed oil/apple and linseed oil/apple/strawberry/tomato groups. Significantly lower glutathione peroxidase concentrations were observed only in the linseed oil/strawberry group.

**Conclusions:** Our findings support the hypothesis that supplementation with apples, strawberries, or tomatoes effectively decreases oxidative stress by decreasing MDA formation in the body and by protecting mononuclear blood cells against increased DNA damage. This effect was particularly pronounced in the group supplemented with a fruit mixture; among the single fruit supplements, the most beneficial effect was obtained with apples. © 2006 Elsevier Inc. All rights reserved.

## Keywords:

Oxidative stress; Fruit; DNA damage; Malondialdehyde; Vitamin E; Total antioxidant status; Glutathione peroxidase

## Introduction

A balance between oxidants and antioxidants sustains optimal physiologic conditions in the body. Overproduction of oxidants can cause an imbalance that leads to oxidative stress, which can result in cellular damage to cell constituents such as proteins, lipids, and DNA [1,2]. It has been

acknowledged that a high intake of fruits and vegetables, the main sources of water- and lipid-soluble antioxidants in the diet, could decrease the consequences of free radical attack on cellular DNA, which may lead to mutations [1,3]. Daily consumption of at least five servings of fruit and vegetables may therefore inhibit or slow down degenerative diseases, such as several common cancers, cardiovascular disease, and cell damage connected with ischemia and reperfusion [4–6].

In the literature various data on the comparative in vitro antioxidative capacity of different fruits and vegetables are available [7–13]. Some investigators have reported the beneficial effects of a high intake of fruit and vegetables in

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general [3,14,15], but only limited information is known about the protective effects in vivo of single fruits or vegetables on DNA damage and on peroxidation of body lipids [16–18]. To the best of our knowledge, almost no results of in vivo trials are available comparing mode of action and potency of different kinds of fruit, except that of Pool-Zobel et al. [19] who performed a human intervention trial with carotenoid-rich foods (tomato juice, carrot juice, and dried spinach powder).

The present research investigated and compared the antioxidant potentials of apples, strawberries, and tomatoes to decrease oxidative stress induced by a large proportion of polyunsaturated fatty acids (PUFAs) in the diet. The listed fruits were selected because, compared with other kinds of fruit in vitro, they exhibit higher antioxidative potential [12,13] and are frequently consumed. The hypothesis of the present research was that a high PUFA intake would increase oxidative stress and that dietary supplementation with fruit would provide protection against the damaging effect of PUFAs. We assumed that the ability of different fruits to lower oxidative stress, according to their different amounts of various antioxidants, would not be the same. To evaluate oxidative stress, the following parameters were measured: the degree of DNA damage in mononuclear blood cells (MNBC), the concentration of malondialdehyde (MDA) in blood plasma, 24-h urine MDA excretion rate, the concentration of tocopherols ( $\alpha$  and  $\beta + \gamma$ ) in blood plasma, plasma total antioxidant status (TAS), and erythrocyte glutathione peroxidase (GSH-Px) concentration. In the present study pigs were chosen as the experimental model for humans, not only because of similarities in digestion and metabolism but also because pigs as omnivorous animals may consume the same diet as humans.

## Materials and methods

### *Experimental design and diets*

The experiment was divided into two phases: an initial 7-d adaptation period and a 22-d experimental period. During the adaptation period the animals adapted to the rearing system and all received the same diet (CONT). Thereafter, they were randomly assigned into six experimental groups ( $n = 8$ ). Animals from each group received isocaloric daily rations that provided 2.3 times the maintenance requirement [20]. The rations were composed of the basal diet, which was the same for all animals, supplemented according to the different dietary treatments with starch in the CONT group, linseed oil in the OIL group, linseed oil and apples in the APP group, linseed oil and strawberries in the STR group, linseed oil and tomatoes in the TOM group, and linseed oil, apples, strawberries, and tomatoes in the MIX group. All diets were kept isocaloric by using different amounts of starch.

Diets were constituted according to dietary guidance

proposed by the U.S. Department of Health and Human Services and U.S. Department of Agriculture [21], as specified in *The Food Guide Pyramid*. The composition of daily rations in the different groups, calculated energy values, and calculated composition of daily nutrients are presented in Table 1.

Rations were fed in the form of a feed mixture. All ingredients of the mixture, except linseed oil and fruit, were mixed together weekly. The linseed oil and fruit were added and mixed to the feed mixture of individual animals before every feeding. Unpeeled fruits were cut to approximately  $2 \times 2$ -cm pieces. Animals were fed twice daily. Water was provided ad libitum. At the beginning and at the end of the experimental period, the pigs were weighed.

### *Animals*

Animals were the offspring of Duroc and large white crossed with German Landrace pigs. Forty-eight 40-d-old pigs that weighed approximately 11 kg (lean body weight) were individually penned in metabolic cages to allow quantitative collection of urine. The animal ethics committee of the Veterinary Administration of the Republic of Slovenia approved the study protocol.

### *Collection and preparation of blood samples*

Fasting blood samples were collected at the beginning and at the end of the 22-d experimental period. Samples were collected by trained veterinary technicians from the jugular vein. Blood samples for determination of damage to MNBC (mostly represented by lymphocytes) were collected in tubes treated with ethylenediaminetetraacetic acid and  $K_3$  (367654, Brand, Wertheim, Germany). To prevent further DNA damage, blood samples were kept in the dark and on ice for a maximum of 1 h before the separation procedure. MNBC were isolated from the fresh blood samples according to a modified procedure described by Singh [23]. Histopaque-1077 (2.5 mL; H-8889, Sigma, Steinheim, Germany) was added to a 14-mL Eppendorfer centrifuge tube and overlaid with an 8-mL solution of blood and RPMI-1640 medium (R-8758, Sigma), which was previously gently mixed at a ratio of 1:1. Centrifugation (300g for 35 min at room temperature) followed. MNBC, as a white pellet approximately 3 mm under the red cell pellet, were transferred to another centrifuge tube and washed twice in 5 mL of sterile RPMI-1640 medium, followed by centrifugation (300g for 5 min at room temperature). The MNBC pellet were finally mixed with 0.5 mL of RPMI-1640 medium and used as isolate MNBC. The viability of the cells was measured by the dye-exclusion test with 0.6% trypan blue [24] and was found to be about 95%. The final concentration of the isolated MNBC was  $6.2 \times 10^5$  cells/mL.

Blood samples for measuring MDA and vitamin E concentrations in blood plasma were collected in tubes contain-

Table 1  
Composition and calculated energy content of nutrients of daily rations

	Group					
	CONT	OIL	APP	STR	TOM	MIX
<b>Basal diet</b>						
Wheat flour (g/d)	60	60	60	60	60	60
Wheat starch (g/d)	30	30	30	30	30	30
Skimmed milk powder (g/d)	36	36	36	36	36	36
Fish meal (g/d)	32	32	32	32	32	32
Powdered eggs (g/d)	4.3	4.3	4.3	4.3	4.3	4.3
Soybean meal (g/d)	26	26	26	26	26	26
Sugar (g/d)	10	10	10	10	10	10
Rapeseed oil (g/d)	27	27	27	27	27	27
Mineral/vitamin supplement* (g/d)	5	5	5	5	5	5
<b>Supplements</b>						
Maize starch (g/d)	184	120	14	51	89	51
Linseed oil (g/d)	—	25	25	25	25	25
Apples (g/d)	—	—	690	—	—	230
Strawberries (g/d)	—	—	—	745	—	205
Tomatoes (g/d)	—	—	—	—	615	248
Total daily feed intake (g/d)	414	375	956	1048	957	986
<b>Dietary composition of nutrients and energy</b>						
Proteins (g/d)	58	58	60	63	63	62
Fats (g/d)	33	58	62	58	59	60
Carbohydrates (g/d)	245	189	177	171	179	175
Total dietary fiber (g/d)	24	24	38	36	30	35
Energy value <sup>†</sup> (kJ/d)	6418	6422	6418	6418	6418	6418
Energy from proteins (%)	15	15	16	17	17	16
Energy from fats <sup>‡</sup> (%)	20	35	37	37	36	37
Energy from PUFAs <sup>‡</sup> (%)	5	15	15	15	15	15
Energy from carbohydrates (%)	64	49	46	45	47	46

APP, diet rich in linseed oil and apples; CONT, basal diet without linseed oil and fruit; MIX, diet rich in linseed oil, apples, strawberries, and tomatoes; OIL, diet rich linseed oil; PUFA, polyunsaturated fatty acid; STR, diet rich in linseed oil and strawberries; TOM, diet rich in linseed oil and tomatoes

\* Calculated to meet nutritional requirements according to the National Research Council [20]. Mineral/vitamin supplement daily provided 2.0 g of calcium, 3.4 g of phosphorus, 0.15 g of sodium, 5500 IU of vitamin A, and 7.6 IU of vitamin E.

<sup>†</sup> The energy value of feedstuffs and diets was estimated according to the Gesellschaft für Ernährungsphysiologie [22].

<sup>‡</sup> Estimated.

ing ethylenediaminetetraacetic acid and K<sub>3</sub> (368457, Brand). Plasma was separated by centrifugation (400g for 10 min at 4°C), transferred to microcentrifuge tubes, and stored at -70°C.

Blood samples for TAS and GSH-Px concentration analysis were collected in evacuated tubes containing heparin anticoagulant (367685, Brand). Fifty microliters of whole blood for GSH-Px analysis was transferred to microcentrifuge tubes and stored at -70°C. Blood for TAS was centrifuged (3000g for 10 min at 4°C) and plasma was transferred to microcentrifuge tubes and stored at -70°C.

#### Collection and preparation of urine samples

To be more representative, urine was collected for 48 h at the beginning and at the end of the 22-d experimental period. Volumes of total urine were measured and aliquots were filtered into test tubes (10 to 15 mL) through filter paper (520 A, Schleicher & Schuell, Dassel, Germany), transferred to microcentrifuge tubes, and stored at -70°C.

#### Feed analysis

The content of protein, fat, and fiber was determined by standard procedures published by Neumann and Bassler [25]. The fatty acid composition of diets was analyzed by a gas chromatographic method after transesterification of lipids [26]. Daily ration composition analysis is presented in Table 2.

#### Plasma and urine MDA concentration

The methodology of Wong et al. [27] as modified by Chirico [28] and Fukunaga et al. [29] was used to measure concentrations of MDA in blood plasma and urine by high-performance liquid chromatography using a Waters Symmetry C<sub>18</sub> chromatographic column (5 μm, 4.6 × 150 mm) fitted with a Phenomenex Prodigy ODS C<sub>18</sub> guard column (Phenomenex, Milford, MA, USA) (4 mm long × 3.0 mm in inner diameter). A Waters Alliance 2690 apparatus equipped with a Waters 474 scanning fluorescence detector was applied. The results of the analysis were evaluated by

Table 2  
Analysis of the content of nutrients of daily rations

Nutritive value	Group					
	CONT	OIL	APP	STR	TOM	MIX
Dry matter (g/d)	370.99	316.88	343.34	312.95	325.55	327.09
Crude proteins (g/d)	60.43	58.26	63.01	64.65	64.65	63.50
Crude fats (g/d)	27.93	33.90	34.20	36.36	34.79	34.19
Crude fiber (g/d)	7.34	7.37	13.76	11.99	12.63	12.73
Total dietary fiber (g/d)	39.68	35.41	143.03	178.88	196.37	174.44
Soluble dietary fiber (g/d)	7.42	6.41	38.15	56.75	38.78	43.95
Insoluble dietary fiber (g/d)	32.27	29.00	104.88	122.13	157.59	130.49
Soluble dietary fiber in all dietary fiber (%)	18.6	18.08	26.67	31.72	19.75	25.19
Crude ash (g/d)	12.30	12.03	15.58	14.9	13.92	14.95
Nitrogen-free extract (g/d)	262.99	205.32	216.79	185.06	202.58	201.72
Vitamin E (mg/d)						
$\alpha$ -Tocopherol	29.66	30.27	40.54	39.23	48.25	40.99
$\beta$ + $\gamma$ -Tocopherol	22.21	23.96	38.87	24.47	25.91	28.51
$\delta$ -Tocopherol	5.51	4.20	4.13	3.84	3.91	3.73

APP, diet rich in linseed oil and apples; CONT, basal diet without linseed oil and fruit; MIX, diet rich in linseed oil, apples, strawberries, and tomatoes; OIL, diet rich linseed oil; STR, diet rich in linseed oil and strawberries; TOM, diet rich in linseed oil and tomatoes

the Millennium<sup>32</sup> Chromatography Manager program (Millennium, Torrance, CA, USA).

#### Mononuclear cell DNA damage—comet assay

A partly modified procedure of Singh et al. [30], as described previously [31], was implemented for the Comet assay. An Olympus CH 50 epifluorescent microscope (Olympus, Tokyo, Japan) at 200 $\times$  magnification was used for examination of mononuclear nuclei in microgels (100-W Hg lamp, excitation filter of 480–550 nm, and barrier filter of 590 nm). Images were captured by a digital camera (Hamamatsu Orca 1 CCD; Hamamatsu, Japan) and analyzed, and nuclear DNA damage was estimated by the Comet 5 dedicated computer program (Single Cell Gel Electrophoresis, Kinetic Imaging Ltd. (Liverpool, England), 2000). For each treatment, two slides were prepared and 50 cells (total 100 cells) were examined.

#### Determination of vitamin E in plasma

Concentration of plasma vitamin E was measured according to the methodology of Rupérez et al. [32] and Abidi and Mounts [33]. Samples were analyzed by high-performance liquid chromatography (Waters Alliance, composed of a Waters 2690 Separation module) using a Phenomenex Prodigy ODS2 column (250  $\times$  4.6 mm inner diameter, 5  $\mu$ m). A Waters Dual  $\lambda$  Absorbance Detector 2487 ( $\lambda$  = 298 nm) and a Waters Scanning Fluorescence Detector 474 (excitation  $\lambda$  = 280 nm, emission  $\lambda$  = 330 nm) were used. Methanol was used as mobile phase (2 mL/min).

#### TAS of blood plasma and erythrocyte GSH-Px concentration

GSH-Px was determined by using a commercially available GSH-Px kit (Randox, Crumlin, United Kingdom). TAS

of plasma was measured with the iron/metmyoglobin absorption method from Randox.

#### Statistical analysis

One-way analysis of variance (effect of dietary treatment) was performed to compare multiple group means, followed by the Scheffé's test to determine statistically significant differences across treatment groups. Differences were considered statistically significant at  $P < 0.05$ . All data are expressed as means  $\pm$  standard deviations. All statistical analyses were made with SAS for Windows (SAS Institute, Cary, NC, USA).

## Results

The animals adapted well to the experimental conditions. During the experiment, the animals had no health or other problems, consumed feed without residues, and normal body weight gain for this level of feeding was observed in all groups (291  $\pm$  37 g/d). Some important statistical differences in the measured parameters (degree of DNA damage in MNBC, concentration of MDA in blood plasma, 24-h urine MDA excretion rate, concentration of  $\alpha$ -tocopherol in blood plasma, TAS of blood plasma, and erythrocyte GSH-Px concentration) were found at the end of the experimental period across dietary treatments.

#### Plasma and urine MDA concentration

Table 3 demonstrates that animals from the OIL group compared with the CONT group had a statistically significant higher concentration of MDA in blood plasma and a higher 24-h urine MDA excretion rate at the end of the

Table 3

Plasma MDA concentration and the amount of daily excreted MDA in urine at the beginning and at the end of the experiment\*

	At the beginning	At the end					
		Group					
		CONT	OIL	APP	STR	TOM	MIX
MDA in plasma (nmol/mL)	0.342 ± 0.101	0.340 <sup>a</sup> ± 0.151	0.577 <sup>b</sup> ± 0.137	0.397 <sup>a</sup> ± 0.171	0.378 <sup>a</sup> ± 0.207	0.381 <sup>a</sup> ± 0.198	0.375 <sup>a</sup> ± 0.163
MDA urine excretion (nmol/24 h)	3083 ± 1226	3924 <sup>abc</sup> ± 1338	5072 <sup>d</sup> ± 1325	3548 <sup>a</sup> ± 894	4526 <sup>bcd</sup> ± 1028	4905 <sup>bd</sup> ± 1129	3812 <sup>ac</sup> ± 791

APP, diet rich in linseed oil and apples; CONT, basal diet without linseed oil and fruit; MDA, malondialdehyde; MIX, diet rich in linseed oil, apples, strawberries, and tomatoes; OIL, diet rich linseed oil; STR, diet rich in linseed oil and strawberries; TOM, diet rich in linseed oil and tomatoes

\* Means with different superscripts in the same line differ significantly at  $P \leq 0.05$ . Values represent mean ± standard deviation ( $n = 8$ ).

experimental period. The intake of all types of fruit significantly decreased plasma MDA concentration to the same extent. In contrast, in comparison with the OIL group, a significant decrease in 24-h urine MDA excretion rate was observed only in the APP and MIX groups. In the latter, the 24-h urine MDA excretion rate was decreased to the level of the CONT group. The APP group even had a numerically lower MDA excretion rate than did the CONT group. The urinary MDA excretion rate in the STR or TOM groups did not differ significantly from that in the OIL group.

#### DNA damage to mononuclear cells

The DNA damage to MNBC found in the study is presented in Table 4 as a percentage of DNA in the head of the comet and as olive tail moment [34], defined as the product of the amount of DNA in the tail and the mean distance of migration in the tail (higher values represent higher rates of DNA damage). The large proportion of PUFAs in the OIL group significantly increased the rate of DNA damage by decreasing the percentage of DNA in the head and by increasing the olive tail moment when compared with the CONT group. The results showed that, in comparison with the OIL group, fruit supplementation in the APP, STR, TOM, and MIX groups significantly increased the percentage of DNA in the head of the comets and decreased the

olive tail moment after 22 d of the experiment. Supplementation with a mixture of apples, strawberries, and apples (MIX) totally prevented the damage induced by linseed oil.

#### Vitamin E concentration in plasma

No significant differences in  $\alpha$ -tocopherol concentration in blood plasma between the OIL and other experimental groups were found (Table 5). Some significant differences, however, were observed among the single-fruit-supplemented groups. Animals from the TOM group had a significantly higher concentration of  $\alpha$ -tocopherol than did animals from the APP group. No statistically significant effect was observed on plasma  $\beta + \gamma$ -tocopherol concentration across experimental groups on day 22 of the experiment (Table 5).

#### Plasma TAS and erythrocyte GSH-Px concentration

As presented in Table 5, animals that were fed the strawberry supplement had a significantly higher plasma TAS than did animals in all other groups. The erythrocyte GSH-Px concentration in the STR group was significantly lower than that in all other groups.

Table 4

Rate of DNA damage in MNBC at the beginning and at the end of the experiment\*

	At the beginning	At the end					
		Group					
		CONT	OIL	APP	STR	TOM	MIX
DNA in head of comets in MNBC (%)	91.36 ± 1.53	93.37 <sup>a</sup> ± 0.46	84.41 <sup>b</sup> ± 1.01	91.33 <sup>c</sup> ± 0.34	87.93 <sup>d</sup> ± 1.46	88.31 <sup>d</sup> ± 1.35	93.12 <sup>a</sup> ± 0.87
OTM	1.54 ± 0.81	0.88 <sup>a</sup> ± 0.26	4.72 <sup>b</sup> ± 0.96	1.68 <sup>c</sup> ± 0.37	3.16 <sup>d</sup> ± 0.81	2.89 <sup>d</sup> ± 0.62	1.08 <sup>a</sup> ± 0.52

APP, diet rich in linseed oil and apples; CONT, basal diet without linseed oil and fruit; MIX, diet rich in linseed oil, apples, strawberries, and tomatoes; OIL, diet rich linseed oil; OTM, olive tail moment; MNBC, mononuclear blood cells; STR, diet rich in linseed oil and strawberries; TOM, diet rich in linseed oil and tomatoes

\* Means with different superscripts in the same line differ significantly at  $P \leq 0.05$ . Values represent the mean ± standard deviation ( $n = 8$ ).

Table 5  
Concentration of plasma tocopherols, TAS, and erythrocyte GSH-Px concentration at the beginning and at the end of the experiment\*

	At the beginning	At the end					
		Group					
		CONT	OIL	APP	STR	TOM	MIX
$\alpha$ -Tocopherol ( $\mu\text{g/mL}$ )	$1.78 \pm 0.36$	$2.58^a \pm 0.59$	$2.24^{ab} \pm 0.60$	$1.86^b \pm 0.59$	$2.20^{ab} \pm 0.44$	$2.47^a \pm 0.36$	$2.02^{ab} \pm 0.46$
$\beta + \gamma$ -Tocopherol ( $\mu\text{g/mL}$ )	$0.049 \pm 0.021$	$0.096 \pm 0.047$	$0.158 \pm 0.089$	$0.152 \pm 0.074$	$0.159 \pm 0.059$	$0.124 \pm 0.041$	$0.124 \pm 0.032$
TAS (mmol/L)	$0.419 \pm 0.050$	$0.466^a \pm 0.047$	$0.474^a \pm 0.089$	$0.485^a \pm 0.074$	$0.559^b \pm 0.059$	$0.487^a \pm 0.041$	$0.511^a \pm 0.032$
GSH-Px (U/L)	$17\,789 \pm 4052$	$27\,406^a \pm 4702$	$28\,144^a \pm 2314$	$27\,195^a \pm 2374$	$21\,654^b \pm 7189$	$28\,460^a \pm 8563$	$32\,520^a \pm 7155$

APP, diet rich in linseed oil and apples; CONT, basal diet without linseed oil and fruit; GSH-Px, glutathione peroxidase; MIX, diet rich in linseed oil, apples, strawberries, and tomatoes; OIL, diet rich linseed oil; STR, diet rich in linseed oil and strawberries; TAS, total antioxidant status; TOM, diet rich in linseed oil and tomatoes

\* Means with different superscripts in the same line differ significantly at  $P \leq 0.05$ . Values represent the mean  $\pm$  standard deviation ( $n = 8$ ).

## Discussion

The influence of various fruits or vegetables on oxidative stress formation is currently not well known. In particular, the absolute role of a particular fruit in relation to a high PUFA content in the diet has not yet been elucidated. As far as we know, this is the first study of the influence of individual fruits on lowering oxidative stress caused by a high intake of PUFAs. Our previous studies and a number of other published results demonstrated the harmful effect of PUFAs on oxidative stress [31,35]. In the present investigation, the *in vivo* comparative antioxidant potentials of apples, strawberries, and tomatoes were studied in relation to the oxidative stress induced by increasing the proportion of the energy supply from fats from 20% to 35%, which is the upper limit of recommended fat intake according to the World Health Organization (WHO) [36] and by selection of linseed oil, which contains 73 wt% of PUFAs [31]. Moreover, in comparison with the CONT group, the energy supply from PUFAs in the groups that were supplemented with linseed oil was increased from 5% to 15% (Table 1), which is appreciably higher than the 6% to 10% level proposed by the WHO [36] and the recommended dietary allowance [37].

According to our expectations based on previous studies [31,38], the present results provided additional confirmation that a high intake of PUFAs in the OIL group significantly increased peroxidation of body lipids as measured by the concentration of MDA in blood plasma and the urinary MDA excretion rate. According to Marnett [39] lipid peroxidation must be considered a significant endogenous source of DNA damage and mutations that contribute to human genetic disease.

The results of this study confirmed this finding because an increased degree of DNA damage in the MNBC was observed in the OIL group. 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 [40]. In the present study pigs were chosen as the experimental model for humans for several reasons. First, pigs as omnivorous animals have a very similar digestive tract [40] and may consume the same diet as humans. Second, comparison of dietary needs for humans [37] and pigs [20] shows a high correlation. Third, the similarities in the function and size of their organs make it possible for transgenic pigs to serve as donors in human organ transplantation (i.e., xenotransplantation) [41].

The *Dietary Guidelines for Americans* specifies that the daily diet for children 2 to 6 y old, women, and some older adults should include two to three servings of fruit and three to five servings of vegetables [21]. High fruit and vegetable intakes are recommended not only because of their high level of antioxidants but also because fruit and vegetables are low in fats, high in fiber, and good sources of vitamins and minerals [7]. There is increasing evidence that the consumption of fruit and vegetables is important in improving the antioxidant potential of the organism [17,42].

In the present study, as hypothesized, supplementation of the oil-supplemented diet with various kinds of fruit proved to be beneficial in the reduction of oxidative stress induced by the high fat intake in terms of peroxidation of body lipids and in terms of the genotoxicity of oxidative stress to MNBC. The efficacy of various fruits was somewhat different.

On day 22 of the experimental period, animals from the fruit-supplemented groups when compared with the OIL group had statistically significantly lower DNA damage to MNBC and a lower concentration of MDA in blood plasma, whereas the 24-h urine MDA excretion rate was statistically significantly lower only in the APP and MIX groups. However, there are still doubts about the specificity of the assay and an additional problem in the instability of MDA [43]. Data in the literature suggest that measurement of plasma MDA is a less reliable indicator of oxidative stress than daily excreted urinary MDA [27,31,44,45]. Among the groups with a single fruit supplement, the best results were obtained in the APP group. However, significantly better

results were observed in the MIX group, which was supplemented with a mixture of apples, strawberries, and tomatoes. Only in this group were the DNA damage to MNBC and the 24-h urine MDA excretion rate decreased to the level of the CONT group. A possible explanation for the most effective reduction of DNA damage to MNBC in the MIX group may be found in the synergistic activity of different water- and lipid-soluble natural antioxidants and in the good combination of their antioxidants and that therefore may be more effective than supplementation with any one type of fruit or vegetable. According to data in the literature apples contain more phenols, strawberries more vitamin C, and tomatoes more lycopene and other carotenoids. This finding is in accordance with that of previous studies in which different fruits and vegetables were found to decrease DNA damage [19,46]. For example, Pool-Zobel et al. [19] reported that consumption of tomato juice (rich in lycopene), carrot juice (rich in  $\alpha$ - and  $\beta$ -carotene), and dried spinach powder (rich in lutein) protect against endogenous DNA damage to lymphocytes in non-smoking males 27 to 40 y old.

Tomatoes and strawberries were proved to be somewhat less effective in decreasing DNA damage to MNBC and in decreasing the degree of peroxidation of body lipids. The influence of tomatoes in lowering oxidative stress likely would be greater if they were given in the form of paste or sauce because lycopene, the main antioxidant in tomatoes, is better absorbed from heat-processed foods than from unprocessed sources [47,48].

In comparison with apples and tomatoes, strawberries contain more water-soluble antioxidants and have a higher antioxidant capacity *in vitro* [7–13]. In accordance with these observations, our *in vivo* study showed a significantly higher TAS of blood plasma from the STR animals than from animals in the other groups. The STR group also showed a significantly lower concentration of GSH-Px. However, other measured parameters did not demonstrate a significantly better effect of strawberries in lowering oxidative stress in comparison with apples, tomatoes, or their mixture. This is most likely due to the fact that analytical methods for measuring TAS *in vitro* are in most cases more appropriate for assessing water-soluble antioxidants than lipid-soluble ones [7,8,9,49,50]. The present results do not correlate with the study by Inal et al. [51] who found with respect to aging a positive correlation between MDA concentrations and GSH-Px. However, it is important to note that the results may not be totally comparable because our trial was performed in pigs and oxidative stress was induced by addition of PUFA-rich linseed oil. Pallegriani et al. [50] observed a significant increase in plasma lycopene and  $\beta$ -carotene concentrations in subjects ( $n = 11$ ) supplemented with 25 g of tomato puree for 14 d, whereas total plasma antioxidant capacity values did not change significantly. Netzel et al. [16] found that a single dose of 400 mL of antioxidant fruit juice (containing 30% white grape, 25% black grape, 15% elderberry, 10% sour cherry, and 10%

blackberry in 10% aronia) induced a significant decrease in plasma MDA *in vivo* and a significant increase in plasma antioxidant capacity.

Determination of  $\alpha$ - and  $\beta + \gamma$ -tocopherols in plasma may contribute information on the antioxidant status of an individual and may be useful for evaluation of their nutritional status and risk of degenerative diseases [52]. It is known from other investigations [53] that as a consequence of increased oxidative load a decrease in the concentration of antioxidative substances in the blood occurs. On that account it was expected that the concentration of  $\alpha$ -tocopherol in plasma would decrease in the OIL group, but only a non-significant decrease in  $\alpha$ -tocopherol was observed. The reason for this might be the low  $\alpha$ -tocopherol concentration of the basal diet calculated according to the National Research Council [20]. Animals from the groups supplemented with linseed oil, due to their high intake of PUFAs, would have to consume an extra 42 mg of vitamin E daily [54]. However, this extra dose would influence the reduction of oxidative stress parameters and the differences between the influences of fruit on oxidative stress would hardly be observable. Because linseed oil, according to our analysis, is a poor source of  $\alpha$ -tocopherol (8.6 mg/100 g) and a good source of  $\beta + \gamma$ -tocopherol (106.9 mg/100 g), an increase in plasma  $\beta + \gamma$ -tocopherol concentration in the OIL group was expected but was non-significant. The results of the present study showed that the OIL group did not differ in plasma  $\alpha$ -tocopherol concentration from the APP, TOM, and MIX groups. Animals from the TOM group had the highest plasma  $\alpha$ -tocopherol concentration but this was statistically different only from the APP group. This result may be due to the daily intake of  $\alpha$ -tocopherol from tomatoes (20.42 mg), which was the highest compared with daily intake from apples (8.76 mg), strawberries (8.21 mg), and the mixture of apples, strawberries, and tomatoes (13.59 mg).

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