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In Vitro Antioxidant Activities of Barley, Husked Oat, Naked Oat, Triticale, and Buckwheat Wastes and Their Influence on the Growth and Biomarkers of Antioxidant Status in Rats

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The study was aimed at verification of the following hypothesis: differences in antioxidant capacity of diets consisting of different cereals and byproducts affect the antioxidant status of the consumers of these diets. To validate that hypothesis this study investigated the contents of polyphenols and α-tocopherol as well as the total antioxidant capacity (TAC) in vitro of cereals and their fractions (barley, husked and naked oat, oat bran, and triticale); the nutritional and antioxidant properties of diets containing these cereals, applied in a 4-week feeding experiment on rats, were also assessed. Among the cereals examined, the highest TAC was reported for barley (13.16 μmol of Trolox/g) and the lowest for naked oat (3.84 μmol of Trolox/g). Compared with cereals, the TAC of buckwheat waste was 2−3 times higher (25.2 μmol of Trolox/g). The antioxidant capacity of diets, calculated in vitro, ranged from 6.35 μmol of Trolox/g for naked oat type diet to 10.51 μmol of Trolox/g for barley type diet. Results of an in vitro study were confirmed in changes of glutathione peroxidase (GPx) activities and the level of thiobarbituric acid-reactive substances (TBARS) in the serum of rats fed diets with the highest and lowest antioxidant capacities in vitro; the barley diet increased the activity of GPx (37.63 units/mL) and decreased the level of TBARS (4.82 μg/g), whereas the naked oat diet had an opposite effect (31.16 units/mL and 5.91 μg/g, respectively).

KEYWORDS: Polyphenols; α-tocopherol; total antioxidant capacity; biomarkers; enzyme activity; thiobarbituric acid-reactive substances; rats

INTRODUCTION

Epidemiological evidence has supported the hypothesis that the consumption of food rich in natural antioxidant compounds plays an important role in the prevention of several chronic diseases associated with oxidative stress, such as cardiovascular disease, cancer, and diabetes (*1*, *2*). Polyphenols, commonly referred to as antioxidants, are receiving increasing interest from consumers and food manufacturers because together with other dietary antioxidants, such as vitamin C, vitamin E, and carotenoids, they may protect the body's tissues against oxidative stress (*2*, *3*).

The main dietary sources of polyphenols are fruits, vegetables, and whole grain cereals. The total dietary intake accounts for ≈1 g/day (*4*). It is much higher than that of all other known dietary antioxidants, ≈10 times higher than that of vitamin C, and ≈100 times higher than those of vitamin E and carotenoids (*5*). Plant phenolics enhance the resistance of serum and body cell lipids to oxidation (*6*, *7*) and can be capable of sparing vitamin E in the organism (*8*).

Cereals are a very important source of polyphenols in human and animal diets. As in other plants, in cereals the phenolic compounds are numerous and chemically differentiated. This group includes mainly free and wall-bound phenolic acids and small amounts of other polyphenols, some of which are cereal grain specific (*9*−*12*). Due to different compositions of phenolic compounds, the antioxidant activities of cereal grains can be differentiated as well. In vitro studies have indicated that the antioxidant activity of an 80% methanolic extract originated from whole grains can be arranged as follows: buckwheat > barley > oat > wheat (*13*). However, it is known that the antioxidant capacity of plants depends also on other phytochemicals such as tocopherols, carotenoids, vitamin C, sterols, and phytic acids. For this reason determination of phenolic compounds is not sufficient for characterizing the antioxidant activity of plant materials. Thus, the total antioxidant capacity determined via in vitro studies has been accepted as a better indicator.

Actually, total antioxidant capacity (TAC) is a measure of the overall effect and potential synergistic interactions of all

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antioxidants contained in the test solution. Then, the measure of the Trolox equivalent antioxidant capacity (TEAC) of dietary plant extracts considers the cumulative action of all the antioxidants present in the extract, including their chain-braking, scavenging, and chelating effects, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore determined, thus giving a tool for screening a wide range of dietary plants for their antioxidant properties (*14*).

A number of assays have been introduced to measure the TEAC of dietary plant extracts (*15*, *16*). These methods are all essentially inhibition methods: a free radical species is generated, there is an end point by which the presence of the radical is detected, and the antioxidant capacity of the added sample inhibits the end point by scavenging the free radical. Methods vary greatly as to the radical that is generated, the reproducibility of the generation process, and the end point that is used. The end points commonly include fluorescence inhibition, chemiluminescence, oxygen uptake, and absorbance change (*15*). One of the most often used methods in this matter is a spectrophotometric assay of the TEAC based on the relative abilities of antioxidants present in food extracts to scavenge the $ABTS^{\bullet+}$ radical cation in comparison with the antioxidant potency of Trolox (*14*, *18*).

Few in vivo experiments have been conducted, so little is known about the relationship between the in vitro antioxidant capacity activity of feed and its effect in biological systems. It is not clear whether the potential small difference in polyphenol content and antioxidant capacity of diets consisting of different cereals and byproducts can affect the antioxidant status of consumers. An attempt at explaining this question is the purpose of the presented work concerning in vitro and in vivo antioxidant properties of diets with various cereals (barley, husked oat, naked oat, triticale) and byproducts (oat bran, buckwheat waste), and different levels of α-tocopherol.

MATERIALS AND METHODS

Materials. Grain samples of cereals were purchased from a local farmer. Grain representing typical feed varieties of spring barley (Rodion), winter triticale (Tornado), husked oat (Hetman), and naked oat (Akt) were obtained in northeastern Poland in the 2004 crop year. Oat bran and buckwheat bran were purchased from a local producer of oat flakes and buckwheat groats. The bran containing husk and a part of meal contained ≈45% mass of whole seeds of oat and ≈35% mass of whole buckwheat seeds. Grain and bran sample were ground in a mill to pass through a 0.5 mm screen. Raw (not refined) rapeseed and linseed oils were purchased from the local oil industry, and cellulose was purchased from Sigma-Aldrich.

Chemicals. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox) were obtained from Sigma-Aldrich (St. Louis, MO). Trolox (2.5 mM) was prepared in 80% methanol for use as a stock standard. Potassium persulfate (dipotassium peroxidisulfate) was obtained from Sigma (Sigma Chemical Co., St. Louis, MO). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

Measurement of in Vitro TEAC. The total antioxidant assay based on the reduction of the ABTS radical cation by antioxidants present in diet extracts was used in this study. The respective 80% methanol extracts were prepared by the extraction of raw material with 80% aqueous methanol (1/10; g/mL), shaking for 2 h at room temperature, and finally centrifugation at 12000*g* at 4 °C. The ABTS radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. The antioxidant capacity was determined following a procedure described by Re et al. (*18*) with a minor modification described below. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol, respectively, to an absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of the extracts or Trolox standards were mixed and measured immediately after 6 min at 734 nm at 30 °C using a spectrophotometer (UV-160 1PC, Shimadzu, Tokyo, Japan), and appropriate solvent blanks were run in each assay (*19*). The TEAC of 80% methanol extracts was calculated, using a Trolox standard curve, on the basis of the percentage inhibition of absorbance at 734 nm.

Determination of Total Phenolic Contents (TPC). Total phenolic compounds from 80% methanol extracts were determined according to the method of Shahidi and Naczk (*20*). A 0.25 mL aliquot of the methanol extract solution was mixed with 0.25 mL of Folin−Ciocalteu reagent (previously diluted with water, 1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution, and 4 mL of water. The mixture was allowed to stand at room temperature for 25 min, and then it was centrifuged at 2100*g* for 10 min. Supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu). The results have been expressed as (±) ferulic acid equivalents.

Rats and Diets. The use of animals was conducted in compliance with European guidelines for the care and use of laboratory animals and was approved by the Ethical Committee for Animal Experiments in the northeastern Poland region. The experiment was performed on 80 male Wistar rats of ≈4 weeks of age (body weight = 69.2 ± 4.7 g). The experimental diets (**Table 2**) were administered for 4 weeks to eight rats per group housed individually in Plexiglas cages. Diets contained similar contents of the main nutrients (protein, fiber, and fat), amino acids (e.a. Lys, 9.13; Met + Cys, 8.53; Thr, 5.36; Try, 1.83 g/kg) and fatty acids (saturated, ≈14.7%; monounsaturated, ≈30.9%; and polyunsaturated, ≈53.5% of FA sum). Each diet contained triticale, soybean meal, and cellulose (37.44−48.80% of a diet), and the remaining grain-part of experimental diets consisted of different cereals and their fractions: the barley diet (40% of barley), the husked oat diet (45% of husked oat), the naked oat diet (45% of naked oat), the naked oat + oat bran diet (35 and 19% of naked oat and oat bran, respectively), and the naked oat + buckwheat waste diet (35 and 15% of naked oat and buckwheat waste, respectively). Each diet was prepared in two replications: without vitamin E and with 120 mg of *all-rac*-α-tocopheryl acetate/kg. The animals were maintained under standard conditions: temperature, 21−22 °C; relative air humidity, 50−70%; intensive ventilation of rooms (15×/h); and 12-h lighting. The rats had free access to experimental diets and tap water (ad libitum). Individual body weights and food intakes were recorded. After 4 weeks, the rats were anesthetized using sodium pentobarbitone. Blood samples were taken from caval tail vein. Serum was prepared by centrifugation at 1500*g* for 15 min at 4 °C and stored at −40 °C until analyzed. Livers were separated, cleaned with ice-cold saline, blotted on filter paper, weighed, frozen in liquid nitrogen, and stored at −40 °C for subsequent assays of vitamin E and thiobarbituric acid-reactive substances (TBARS).

In Vivo Nutritional and Antioxidant Properties of Diets. The concentrations of glucose, cholesterol, and triacylglycerol in the serum were determined enzymatically with commercial diagnostic kits (no. G6620, no. C6608, and no. T6630, respectively) from Alpha Diagnostics (Warsaw, Poland). Determination of glucose was based on the oxidation reaction described by Trinder (*21*). Total cholesterol concentration (TC) was assayed according to the method Allain et al. (*22*) as modified by Roeschlau et al. (*23*). Determination of triacylglycerol (TAG) was also based on Wako's method with modification by McGowan et al. (*24*). Serum lipid peroxidation was measured as TBARS using the method of Uchiyama and Mihara (*25*). Superoxide dismutase (EC 1.1.5.1; SOD) and glutathione peroxidase (EC 1.11.1.9; GPx) activities in erythrocyte lysates were assayed using kits from Randox Laboratories Ltd. (Crumlin, U.K.). The activity of SOD was measured using methods of Woolliams et al. (*26*) and the activity of Gpx using methods of Paglia and Valentine (*27*). For serum total antioxidant status (TAS) use was made of a Randox kit according to the procedure of Miller at al. (*28*). Tocopherols were measured in the diets and serum according to the method of McMurrey and Blanchflower (*29*) and in tissue samples with the method described by Rettenmaier and Schüep (*30*).

Statistical Analysis. The results were analyzed using two-way ANOVA, and significant differences between the groups were determined by using Duncan's multiple-range test. Standard deviation (SD) for each group was calculated. To analyze all results we included three

Table 1. Antioxidant Properties of Feeds Used in Diets of Rats

	total phenolic compounds[a] (mg/g)	α-tocopherol[b] (mg/kg)	Trolox equivalent antioxidant capacity[c] (μmol/g)
barley	1.78 ± 0.01	3.82 ± 0.14	13.16 ± 0.40
triticale	0.94 ± 0.01	4.80 ± 0.19	5.96 ± 0.44
husked oat	1.38 ± 0.06	2.12 ± 0.11	6.02 ± 0.14
naked oat	1.17 ± 0.03	6.32 ± 0.22	3.84 ± 0.34
oat bran with hulls	1.55 ± 0.03	2.14 ± 0.10	7.96 ± 0.28
buckwheat bran	4.06 ± 0.14	4.1 ± 0.17	24.24 ± 1.20
buckwheat hulls	4.48 ± 0.14	0.90 ± 0.04	26.15 ± 0.42
soybean meal	3.46 ± 0.05	4.22 ± 0.20	20.52 ± 1.14

[a] Determined in 80% methanol extracts according to the method of Shahidi and Naczk (*20*). [b] Determined according to the method of McMurrey and Blanchflower (*29*). [c] Determined in 80% methanol extracts according to procedure described by Re et al. (*18*).

main effects: α-tocopherol addition, group, and treatment, and corresponding two-way interactions. Differences were considered to be significant at $P \leq 0.05$.

RESULTS

Screening the TPC, TEAC, and α-T in Components and Diets. The antioxidant properties of the components of the diets investigated in this study are shown in **Table 1**. The cereal-based components of the diets contained different levels of phenolic compounds. The buckwheat bran and hulls and the soybean meal had the highest contents of TPC [4.06, 4.48, and 3.46 mg/g of dry matter (dm), respectively], whereas an ≈2-fold lower TPC level was found in barley and oat bran with hulls, and at least 3 times lower TPC contents were noted in triticale, husked oat, and naked oat.

The highest total antioxidant capacity, expressed in TEAC values, was found in buckwheat hulls and bran (26.15 and 24.24 μmol of Trolox/g of dm) and soybean meal (20.52 μmol of Trolox/g of dm) followed by a twice lower antioxidant capacity noted in barley and an ≈3−4 times lower one in the other feeds.

The content of TPC in feeds was one of the main factors responsible for the values of TEAC because the correlation coefficient between TPC and TEAC of the feeds was $r = 0.98$.

In this study also the α-tocopherol (α-T) content of feeds was assayed, showing the highest value in naked oat (6.32 mg/kg of dm), thus supporting the utility of this component in diet preparations. The level of α-T found in triticale, soybean meal, buckwheat bran, barley, both husked oat and oat bran with hulls, and buckwheat hulls was lowered by 24, 33, 35, 40, 66, 66, and 86%, respectively. In contrast to the previous observations made for TPC, α-T contents in feeds were not correlated with the respective TEAC value ($r = -0.37$) and respective TPC contents ($r = -0.39$).

In Vivo Nutritional and Antioxidant Properties of Diets. The chemical composition and antioxidant properties of the experimental diets are presented in **Table 2**. All diets contained the same compounds (soybean meal, triticale, linseed oil) and compared cereals and their fraction. To compare the antioxidant properties of cereals, the diet contained a similar content of nutrients. Crude fat was aligned by the addition of rapeseed and soybean oils, and crude fiber was aligned by the addition of cellulose. In all diets 3% of linseed oil was applied. Because the content of tocopherols is comparable with that of other oils, and the content of linolenic acid is substantially higher, linseed oil can be treated as a pro-oxidizing factor. Diets labeled αT− contained small amounts of α-tocopherols originated from feeds and diets, whereas those labeled αT+ were supplemented with 60 mg/kg of α-tocopherol.

The TPC of the composed diets reflected their contents noted in the main components. It was clearly seen with respect to the naked oat (1.29 mg/g of dm), which when enriched with oat bran with hulls or buckwheat bran and hulls (1:1; w/w), contained about 10 and 37% more phenolics, respectively (**Table 2**).

The highest TEAC was noted for the barley type diet (10.51 μmol of Trolox/g of dm); a high TEAC was also noted for naked oat supplemented with buckwheat waste (9.69 μmol of Trolox/g of dm) but decreased for husked oat and naked oat with oat

Table 2. Composition and Nutritive Value of Experimental Diets

	type of diet				
	barley	husked oat	naked oat	naked oat + oat bran	naked oat + buckwheat waste
feeds, %					
barley	40.0				
husked oat		45.0			
naked oat			45.0	35.0	35.0
oat bran				19.0	
buckwheat waste					15.0
triticale	27.8	29.04	27.25	21.54	26.02
soybean meal	17.5	15.6	14.6	15.0	14.7
linseed oil	3.0	3.0	3.0	3.0	3.0
rapeseed and soybean oils	2.6	1.9	0.2		0.6
cellulose	3.5		4.6	0.9	0.2
lysine (78%)	0.23	0.23	0.23	0.22	0.24
methionine (99%)	0.37	0.32	0.32	0.34	0.34
AIN-93G mineral mixture[a]	3.0	3.0	3.0	3.0	3.0
vitamin mixture[a]	2.0	2.0	2.0	2.0	2.0
nutrient content					
crude protein (g/kg)	154.4	154.3	154.4	154.3	154.6
crude fiber (g/kg)	68.6	68.6	68.2	68.2	68.7
crude fat	68.3	68.3	68.8	68.8	68.9
antioxidants					
TPC (mg/g)	1.58	1.43	1.29	1.43	1.81
α-tocopherol (mg/kg)	12.45	10.73	9.76	8.96	10.09
total tocopherols (mg/kg)	47.98	40.19	27.76	27.10	30.04
TEAC (μmol/g)	10.51	7.64	6.35	7.22	9.69

[a] Mineral and vitamin mixtures were prepared according to the method of Reeves (*49*). The vitamin mixture did not contain vitamin E.

Table 3. Diet Intake (DI), Final Body Weight of Rats (FBW), Body Weight Gain (BWG), and Feed Efficiency Ratio (FCR) during 28 Days of Experimental Feeding of Rats[a]

	DI (g)	FBW (g)	BWG (g)	FCR (g/g)
diet				
barley, αT–	483 ab ± 34	236 abc ± 19	167 ab ± 17	2.91 abc ± 0.19
barley, αT+	479 abc ± 33	235 abc ± 16	166 ab ± 14	2.88 abc ± 0.13
barley, average	481 a ± 33	236 b ± 17	166 b ± 15	2.90 a ± 0.15
husked oat, αT–	443 d ± 42	218 d ± 19	149 ab ± 17	2.99 a ± 0.17
husked oat, αT+	459 bc ± 46	225 cd ± 15	156 ab ± 13	2.96 ab ± 0.27
husked oat, average	451 b ± 43	221 b ± 17	152 c± 15	2.97 a ± 0.22
naked oat, αT–	490 ab ± 30	250 a ± 13	180 a ± 16	2.73 bc ± 0.20
naked oat, αT+	477 abc ± 16	246.1 a ± 10	177 a ± 13	2.71 c ± 0.24
naked oat, average	484 a ± 28	248 a ± 11	178 a ± 14	2.72 b ± 0.21
naked oat + oat bran, αT-	435 d ± 35	225 bcd ± 19	156 bc ± 17	2.80 abc ± 0.17
naked oat + oat bran, αT+	447 cd ± 19	224 cd ± 11	155 bc ± 14	2.89 abc ± 0.25
naked oat + oat bran, average	441 b ± 28	225 b ± 15	155 bc ± 15	2.85 ab ± 0.21
naked oat + buckwheat, αT-	487 ab ± 21	237 abc ± 16	169 ab ± 17	2.88 abc ± 0.24
naked oat + buckwheat, αT+	507 a ± 21	242 ab ± 11	173 a ± 13	2.94 abc ± 0.12
naked oat + buckwheat, average	497 a ± 23	240 a± 14	171 a ± 15	2.91 a ± 0.18
α-tocopherol				
–	468 ± 39	228 ± 20	159 ± 19	2.87 ± 0.21
+	474 ± 35	235 ± 15	165 ± 16	2.88 ± 0.22
P value				
diet	<0.001	<0.001	<0.001	0.080
α-tocopherol supplementation	0.473	0.729	0.739	0.862
group	<0.001	<0.001	<0.001	0.005
interaction	0.556	0.884	0.886	0.895

[a] Values in one row with different letters are significantly different for $P \leq 0.05$ (a, b).

bran (by 27 and 31%, respectively) and for the naked oat diet by ≈40%. The TEAC values of basic components reported in **Table 1** reflected their contribution to the TEAC value of the diet, as seen for naked oat diet with oat bran and buckwheat components (**Table 2**). In this case, the TEAC of the enriched naked oat diets exhibited higher antioxidant capacities by 13 and 50% when compared to the oat bran and buckwheat bran and hulls, respectively. The TEAC values of the diets were correlated with their TPC contents ($r = 0.83$).

The α-T content in the diets did not reflect the level observed in feeds. Diets labeled as αT- contained small amounts of α-tocopherols originated from feeds and diets, whereas those labeled as αT+ were supplemented with 60 mg/kg of α-tocopherol. The α-T content in the barley diet was the highest (12.45 mg/kg) while that found in naked oat and oat bran diet was lower by 21% and 28%, respectively (**Table 2**). However, the contribution of α-T to TEAC of the diets ranged only from 0.24% to 0.35% while the supplementation with 60 mg of α-tocopherol acetate/kg of diet resulted in increase of its contribution to the TEAC by approximately 2.5%.

Diet intake and final body weight of rats after 4 weeks of experimental feeding were differentiated between the same treatment groups (**Table 3**). The intake of a diet containing naked oat supplemented with cellulose (484 g) or naked oat supplemented with buckwheat waste (497 g) was higher that than of the diet containing husked oat (451 g) or naked oat supplemented with oat bran (441 g). It affected the body weight gain (BWG) and, in consequence, the final body weight. The highest BWG was determined in rats fed the diet containing naked oat (178 g) and that with buckwheat waste (171 g). The group fed the diet containing husked oat or naked oat supplemented with oat bran was characterized by the lowest BWG of rats, that is, 152 and 155 g, respectively. The lowest feed efficiency ratio (FCR) was determined for the naked oat diet (2.72 g/g) and the highest FCR for the husked oat diet (2.97 g/g). Diet supplementation with α-tocopherol did not trigger any effect on animal performance.

The results of the measurements of biochemical and enzymatic indices of blood of rats are shown in **Table 4**. The contents of glucose, triglycerides, and total cholesterol in serum were statistically similar in all of the experimental treatments. The highest activity of GPx was reported for rats fed the diet with barley (37.63 units/mL) and the lowest GPx activity for rats fed the diet containing naked oat supplemented with cellulose or oat bran (31.16 and 31.79 units/mL, respectively). The rats fed the diet containing husked oat were characterized by the lowest activity of SOD (247 units/mL) and simultaneously the highest activity of TAS (0.97 mmol/L). In the other groups, the activities of SOD and TAS were alike. The highest serum concentration of TBARS was determined in rats consuming the diet with naked oat (6.27 μg/g), whereas the lowest was determined for those receiving the diets with barley (4.82 μg/g). These corresponded with the lowest and highest antioxidant capacities in vitro of the diet containing naked oat and barley (6.35 and 10.51 μmol of Trolox/g, respectively). Generally, diet supplementation with α-tocopherol did not increase the TBARS content of serum. A significant decrease in the content of TBARS upon α-tocopherol addition was observed only in rats receiving the barely diet. Supplementation of diets with 60 mg/kg of α-tocopherol was reported not to affect other biochemical and enzymatic indices of blood analyzed.

The composition of a diet has an influence on the vitamin E content of the serum and liver of rats (**Table 5**). In serum the highest vitamin E content was observed in rats fed the diet with barley (8.56 μg/mL) and the lowest in those fed diets with husked oat (5.97 μg/mL). In liver, the highest vitamin E content was determined in rats fed the diet with naked oat (10.82 μg/g), whereas the lowest was determined in rats fed diets with naked oat and oat bran (7.39 μg/g). Supplementation of diets with 60 mg/kg α-tocopherol increased significantly the level

Table 4. Glucose, Triglyceride (TAG), and Total Cholesterol (TC) Contents in Serum, Blood Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) Activities, Total Antioxidant Status (TAS), and Thiobarbituric Acid-Reactive Substances (TBARS) in Serum[a]

exptl treatment	glucose (mg/dL)	TAG (mg/dL)	TC (mg/dL)	GPx (units/mL)	SOD (units/mL)	TAS (mmol/L)	TBARS (μg/g)
diet							
barley, αT–	195 ± 46	132 ± 57	84.3 ± 10.7	37.63 a ± 5.44	272 ab ± 55	0.88 bc ± 0.06	5.52 ab ± 0.66
barley, αT+	207 ± 42	145 ± 48	85.7 ± 12.4	37.63 a ± 4.35	283 ab ± 50	0.88 bc ± 0.07	4.13 c ± 0.79
barley, average	201 ± 43	138 ± 51	85.0 ± 11.2	37.63 a ± 4.76	278 ab ± 51	0.88 b ± 0.06	4.82 c ± 1.01
husked oat, αT–	198 ± 34	155 ± 58	86.1 ± 12.9	36.10 a ± 5.50	249 ab ± 66	0.94 ab ± 0.12	5.41 ab ± 1.09
husked oat, αT+	197 ± 22	127 ± 61	86.5 ± 12.1	34.56 a ± 3.06	244 b ± 53	0.94 ab ± 0.09	5.52 ab ± 0.76
husked oat, average	197 ± 27	141 ± 59	86.3 ± 12.1	35.33 ab ± 4.37	247 b ± 58	0.97 a ± 0.11	5.46 b ± 0.91
naked oat, αT–	203 ± 50	184 ± 87	87.8 ± 11.0	30.98 b ± 4.80	292 ab ± 39	0.94 ab ± 0.10	6.18 ab ± 0.80
naked oat, αT+	203 ± 31	179 ± 67	90.4 ± 8.3	31.33 b± 5.21	303 a ± 47	0.81 c± ± 0.12	6.36 a ± 0.98
naked oat, average	203 ± 40	182 ± 75	89.1 ± 9.5	31.16 b ± 4.84	298 a± 42	0.87 b± 0.13	6.27 a ± 0.87
naked oat + oat bran, αT–	191 ± 39	141 ± 50	91.3 ± 15.7	31.07 b ± 4.55	294 ab ± 45	0.94 ab ± 0.04	5.35 bc ± 0.76
naked oat + oat bran, αT+	172 ± 28	142 ± 31	93.4 ± 8.0	32.52 ab ± 5.47	289 ab ± 30	0.88 bc ± 0.13	5.87 ab ± 0.94
naked oat + oat bran, average	181 ± 34	142 ± 40	92.4 ± 12.1	31.79 b ± 4.92	291 a± 37	0.87 b± 0.10	5.61 b± 0.87
naked oat + buckwheat waste, αT–	215 ± 39	147 ± 34	91.0 ± 10.9	33.97 ab ± 5.45	279 ab ± 44	0.87 bc ± 0.09	5.92 ab ± 0.94
naked oat + buckwheat waste, αT+	220 ± 33	131 ± 20	89.4 ± 13.7	35.41 ab ± 4.65	291 ab ± 37	0.91 abc ± 0.09	5.90 ab ± 0.60
naked oat + buckwheat, average	217 ± 35	139 ± 28	90.2 ± 12.0	34.69 ab ± 4.95	285 a ± 40	0.87 b± 0.10	5.91 ab ± 0.76
α-tocopherol							
–	200 ± 41	152 ± 59	88.1 ± 12.0	33.95 ± 5.58	277 ± 51	0.91 ± 0.09	5.68 ± 0.88
+	200 ± 34	145 ± 50	89.1 ± 10.9	34.29 ± 4.92	282 ± 47	0.88 ± 0.12	5.55 ± 1.10
P value							
diet	0.429	0.428	0.866	0.031	0.233	0.012	<0.001
α-tocopherol supplementation	0.953	0.545	0.708	0.757	0.646	0.145	0.578
group	0.099	0.108	0.369	0.002	0.032	0.025	<0.001
interaction	0.825	0.856	0.988	0.907	0.964	0.064	0.022

[a] Values in one row with different letters are significantly different for $P \leq 0.05$ (a, b).

Table 5. Vitamin E Concentration in Serum and Liver of Rats[a]

	vitamin E (μg/mL)	
	serum	liver
diet		
barley, αT–	6.28 cd ± 3.45	3.45 d ± 1.57
barley, αT+	10.82 ab ± 3.59	17.63 a ± 5.61
barley, average	8.56 a ± 4.13	10.54 ab ± 8.33
husked oat, αT–	4.74 cd ± 2.23	4.97 d ± 2.11
husked oat, αT+	7.19 c ± 2.14	12.72 bc ± 6.26
husked oat, average	5.97 b ± 2.46	8.84 ab ± 6.03
naked oat, αT–	3.26 d ± 0.65	5.41 d± 2.20
naked oat, αT+	12.85 a ± 6.19	16.24 ab ± 5.10
naked oat, average	8.05 ab ± 6.52	10.82 a ± 6.76
naked oat + oat bran, αT–	8.34 bc ± 4.92	3.91 d ± 1.34
naked oat + oat bran, αT+	8.05 bc ± 2.37	10.86 c ± 6.19
naked oat + oat bran, average	8.19 ab ± 3.74	7.39 b ± 5.62
naked oat + buckwheat waste, αT–	6.76 cd ± 2.01	4.82 d ± 1.09
naked oat + buckwheat waste, αT+	6.02 cd ± 1.07	15.46 ab ± 6.44
naked oat + buckwheat waste, average	6.39 ab ± 1.60	10.14 ab ± 7.08
α-tocopherol supplementation		
–	5.88 b ± 3.63	4.51 b ± 1.78
+	8.99 a ± 4.20	14.58 a ± 6.15
P value		
diet	<0.001	<0.001
hνδ;1gα-tocopherol supplementation	<0.001	<0.001
group	0.266	0.595
interaction	<0.001	0.154

[a] Values in one row with different letters are significantly different for $P \leq 0.05$ (a, b).

of vitamin E in serum (from 5.55 to 8.99 μg/mL) and elevated the reserve of vitamin E in liver (from 4.51 to 14.58 μg/g). The addition of α-tocopherol increased the concentration of vitamin E in rats fed diets with barley or husked oat; however, it did not influence the vitamin level in the other group.

DISCUSSION

Screening the TPC, TEAC, and α-T in Components and Diets. The antioxidant capacity of cereal grains and bran fractions, used in this study as feeds, is formed by a number of bioactive substances with antioxidant properties and their interactions with other components of diet matrix (*31*−*34*). Cereals and their fractions are rich in phenolic acids; their total amounts may approach 500 mg/kg of edible cereals. They also contain small amounts of carotenoids, tocopherols, and tocotrienols, especially in the lipid-rich germ. In addition, cereals contribute to the supply of trace elements such as Se, Cu, Zn, and Mn, which are components of antioxidative enzymes (*35*). It should be emphasized that the total antioxidant activity of whole-grain foods is comparable to that of many fruits and vegetables (*36*). On the other hand, when a systematic screening of total antioxidants was performed in a dietary plant family, there was a >1000-fold difference among total antioxidants in various dietary plants. Whole-meal flours of barley, common millet, and oats were demonstrated to contain the highest amounts of antioxidants (0.59−1.09 mmol/100 g) among cereals (*37*), which supports our choice of barley and two kinds of oats as principal feeds in diet formulations. Moreover, a twice higher content of total antioxidants in barley than that in oats reported by Halvorsen et al. (*37*) confirms also 2-fold higher TEAC values of barley as compared to the TEAC value of husked oat reported in our study. Refining cereals results in a substantial loss of the antioxidant activity, and it can be the case of naked oat, which is devoid of bran, and this is probably why it exhibits the lowest value of TEAC.

In this study, no more than 4-fold differences were noted among antioxidant capacity and among total phenolics content, indicating that cereals and their bran or hull fractions used for diet formulations constitute a rather narrow group when compared to dietary plants. For this reason, very sensitive indices should be taken for in vivo study to demonstrate any influence

of diet on the antioxidant status of the serum in rats. This requirement is more important when the antioxidant capacity of the diets, calculated on the data related to the TEAC and TPC of the diet's components, is expressed at the same level of TPC. In this case, the barley diet showed the highest value, that is, 6.65 μmol of Trolox/mg of TPC, followed by the naked oat diet with buckwheat waste (5.40), the husked oat diet (5.34), the naked oat diet with oat bran (5.05), and the naked oat diet (4.92 μmol of Trolox/mg of TPC). Phenolic compounds present in barley grains include phenolic acids and their derivatives, small amounts of polyphenols, lignans, and substances related to lignin. Ferulic acid was reported as the predominant free phenolic acid, whereas the highest content of total insoluble bound phenolic acids (0.6−0.9%) was found in the husk, testa, and aleurone cells in barley grain. Among barley polyphenols a range of flavonols from monomeric, dimeric, and trimeric to higher molecular weight tannins was reported. These compounds collectively accounted for 58−68% of the polyphenolic content (*38*). All of them may contribute to the highest TEAC value of diets, in which the main component was barley.

In Vivo Nutritional and Antioxidant Properties of Diets. In the present experiment, animal performance was found to depend on the cereal that was used as the main component of the diet. The diet containing naked oat has yielded better results than those containing barley or husked oat. In part, it resulted from higher consumption of the diet with naked oat. A higher nutritive value of the diet with naked oat than of that with barley was demonstrated in an experiment on pigs (*39*). One of the factors that are likely to determine the higher nutritive value of naked oat is a more favorable composition of the nonstarch polysaccharide fraction. Compared with barley, naked oat contains a lower level of arabinoxylans (*40*).

In our experiment, the content of oat bran decreased diet intake as compared with the diet containing naked oat and cellulose. Contrary results were obtained in research on broiler chickens (*41*). A study carried out on rats fed a diet with naked oat and buckwheat waste has indicated that diet supplementation with oat phenolics did not affect the diet intake (*42*). The results obtained indicate that the highest content of polyphenols in that diet did not lower the feed intake or the animal growth; however, it was found to increase the feed efficiency ratio as compared to the naked oat diet. It may be assumed, then, that not the phenolic compounds but other constituents of oat bran lowered the feed intake.

Results of some other works have indicated that diets rich in polyphenols had a greater, positive influence on plasma lipid level (*43*, *44*), but other experiments have failed to demonstrate any impact of dietary polyplenols (*8*, *45*). In the present study, the content of triglycerides and total cholesterol in serum of rats was similar in all of the experimental treatments, irrespective of the content of polyphenols and antioxidant capacity of diets. It has been shown that the differences between the contents of dietary polyphenols and antioxidant capacity of the diets were too small to induce any changes in lipid metabolism in rats.

Results of ample experiments have shown that dietary components rich in polyphenols and exhibiting high antioxidant capacity can influence biomarkers of antioxidants in rats, for example, increased SOD and GPx activities (*42*, *43*, *46*). However, some works indicate also that some polyphenols affect the antioxidant status in vitro but not in vivo (*47*). In the present study, a high antioxidant capacity of barley corresponded with a higher activity of blood plasma GPx and a lower level of TBARS, but did not influence the activity of serum SOD and TAS level. In the case of the diet with naked oats, a low antioxidant capacity in vitro corresponded with a low activity of GPx and a high level of TBARS, but did not affect the activity of SOD or the TAS level. Compared with naked oat, the diet with husked oat decreased the final body weight of rats, yet indices of their antioxidant status were beneficial, that is, a higher activity of GPx, a higher level of TAS, and a lower level of TBARS. In the diet with naked oats, the antioxidant properties of an additional source of fiber (oat bran or buckwheat waste) had no impact on the analyzed biomarkers of antioxidants in rats. Compared with the diet containing oat bran, a higher antioxidant capacity in vitro of the diet with buckwheat waste increased numerically, but not statistically, the activity of GPx and the level of TBARS. Some authors report that thiobarbituric acid reactivity does not have sufficient specificity in biological samples and has susceptibility to artificial ex vivo oxidation and for this reason the utility of the TBARS assay as a measure of lipid peroxidation in vivo is questionable (*48*). However, TBARS is commonly used as an indicator of changes occurring during lipid peroxidation process (*25*, *42*, *43*).

Results of numerous experiments, for example, Frank et al. (*8*), have indicated that dietary polyphenols seem to be capable of sparing vitamin E in the organism. Therefore, we expected higher reserves of vitamin E in rats fed a diet with a higher antioxidant capacity. In this respect the results of our study are vague. We observed a high reserve of vitamin E in the liver of rats fed the barley diet and the diet containing naked oat and buckwheat waste, both diets with a high antioxidant capacity in vitro, but high vitamin E reserves were also found in rats fed the naked oat diet, which has a low antioxidant capacity. Probably, the beneficial influence of polyphenols on vitamin E reserve evoked differences between rats fed the diet with naked oat and diets with the addition of oat bran or buckwheat waste. The reserve of vitamin E in the liver was higher in the case of rats fed the diet with a higher content of polyphenols, originated from buckwheat waste. An increasing reserve of vitamin E in the liver of rats fed diets supplemented with 60 mg/kg of α-tocopherol was consistent with our expectations.

In conclusion, the feeds investigated in this study contained different levels of phenolic compounds and α-tocopherol and demonstrated variable antioxidant capacities in vitro. Among the cereals examined, the highest antioxidant capacity was reported for barley and the lowest for naked oat. Compared with cereals, the antioxidant capacity of buckwheat waste was 2−3 times higher. In consequence, the calculated antioxidant capacity of the diet with a high content of barley was the highest and that of the diet containing naked oat the lowest. The application of buckwheat waste in the diet with naked oat decreased its antioxidant capacity to a level similar to that of the barley diet. Results of an in vitro study were, in part, confirmed in the in vivo experiment. It was clear in changes of GPx activities and the level of TBARS in serum of rats fed diets with the highest and lowest antioxidant capacities in vitro; the barley diet increased the activity of GPx and decreased the level of TBARS, whereas the naked oat diet had an opposite effect. Relatively small differences in the content of α-tocopherol in a diet did not have any influence on the reserve of vitamin E in the liver. Supplementation of diets with 60 mg of α-tocopherol was found to significantly increase the reserve of vitamin E in the liver of rats.

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