Total Antioxidant Capacity of Feces of Mammalian Herbivores and Carnivores

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The total antioxidant capacities (TAC) of feces of mammalian herbivores and carnivores were compared. TAC were estimated using three different methods: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS$^{\bullet}$) reduction, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) reduction, and ferric reducing antioxidant power (FRAP). TAC of 18 herbivorous species were generally higher with respect to 16 carnivorous species [(14.21 ± 6.72) *vs.* (9.45 ± 7.32) mmol Trolox equivalents/kg feces; $P < 0.05$] in the FRAP assay. The ABTS$^{\bullet}$ reduction assay indicated that the TAC originating from "fast" reacting antioxidants were higher in the herbivores than in carnivores [(17.92 ± 7.18) *vs.* (12.22 ± 8.5) mmol Trolox equivalents/kg feces; $P < 0.05$], while a reverse trend was observed for TAC originating from "slowly" reacting antioxidants [(20.68 ± 4.85) *vs.* (24.68 ± 6.87) mmol Trolox equivalents/kg feces].

Key words: Antioxidant, Feces, Total Antioxidant Capacity

Introduction

Plant-derived food is the source of antioxidant vitamins for humans and many animals. Polyphenols are abundant components of plant-derived food and are present in higher amounts than antioxidant vitamins. The significance of plant-derived antioxidants for human health has been the subject of extensive research. Numerous *in vitro* studies have demonstrated antioxidant and anticancerogenic properties of plant-derived polyphenols, but the *in vivo* relevance of such results has been questioned due to the low bioavailability of these polyphenols (Halliwell, 2007; Sies, 2010; Visioli *et al*., 2011). Nevertheless, even though plant polyphenols are generally poorly absorbed and subject to metabolism, their concentration is quite high in the intestine and bowel, and they may be important for the protection of the digestive tract against oxidants (Jenner *et al*., 2005; Halliwell, 2007). Plant-based foods such as fruits and vegetables have been suggested to reduce the risk of developing colorectal cancer (Kyle *et al*., 2010; Araújo *et al*., 2011) although the epidemiologic data are contradictory (Boehm *et al.*, 2009). Lower incidence of colorectal cancer in vegetarians has been reported though not confirmed by all available data (Fraser, 2009). Even though the exact mechanism by which these foods exert a protective effect is unclear and may involve several factors, one of the most popular hypotheses links this effect to the high content of antioxidants (Araújo *et al*., 2011; Lofano *et al*., 2013).

In recent years, many studies have shown that components of the aqueous phase of human feces (fecal water) are able to alter the growth characteristics of colonocytes more effectively than components of the solid phase. It is generally considered that fecal water interacts much more with the colonic epithelium than the solid phase and has more influence on the development of colon disease (Jenner *et al.*, 2005). In human subjects, it is estimated that appoximately

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400 – 570 mg/day of phenolic acids, polyphenols, and tannins (such as aglycones) enter the colon (Clifford, 2004), and significant concentrations of them have been quantified in human fecal water (Jenner *et al.*, 2005). A human intervention study with carrot and tomato juice was conducted to show whether a diet rich in carotenoids, especially high in β-carotene and lycopene, can modify luminal processes relevant to colon carcinogenesis (Schnäbele *et al.*, 2008).

Eating plant-derived food should result in an increased content of antioxidants in the contents of the intestine and bowel; however, this expectation is not obvious since animal food also contains low-molecular weight antioxidants, especially glutathione, ascorbate, carnosine, and proteins as well as products of their digestion also have antioxidant properties (Kitts and Weiler, 2003; Hernández-Ledesma *et al*., 2008; Hipkiss, 2009).

The present study aimed at providing an answer to this question by comparing the total content of antioxidants in feces of carnivorous and herbivorous mammals. The parameter studied was the total antioxidant capacity (TAC) which measures the sum of antioxidant activities of all compounds present in the material studied (Bartosz, 2003a). TAC has been estimated by various methods, and the results of the assays were found to correlate to a limited extent only (Bartosz, 2003a, 2010); therefore, in this study we used three different methods to estimate TAC.

Materials and Methods

Sample collection and preparation

Stools of 3 – 12 individuals from 18 species of herbivores and 16 species of carnivores were collected thanks to the kindness of zoological gardens in Łódź and Zamość (Poland). All animals were adult. The samples were taken within 30 min after defecation to avoid significant changes in the fresh mass/dry mass ratio. To obtain representative fecal samples, the outer layer of dung balls was removed to avoid contamination of the sample as well as the effect of drying. The remaining material was thoroughly mixed, and a subsample representing 10% of the whole sample was taken and frozen at $-20\,^{\circ}$C. After thawing, all fecal samples were pooled per animal. Then, 1 g of the fecal sample was added to 9 ml of phosphate-buffered saline (PBS; 145 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4), and the mixture was homogenized and centrifuged. The supernatant was used for the TAC assays.

Assays of total antioxidant activity

ABTS$^{\cdot}$ reduction

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS$^{\cdot}$)-reducing capacity of the supernatants was estimated using a slightly modified (Bartosz, 2003b) procedure of Re *et al*. (1999). Briefly, ABTS$^{\cdot}$ was prepared by overnight (ca. 16 h) incubation of 41 mg of ABTS$^{\cdot}$ with 6.6 mg potassium persulfate in 10 ml of PBS in the dark. An appropriate volume of the respective material was added to the ABTS$^{\cdot}$ solution in PBS (absorbance of 1.0 at 414 nm). The decrease in the absorbance was measured 10 s after mixing the sample to determine the activity of "fast" antioxidants, and after a 3-min incubation to measure that of "slow" antioxidants.

DPPH$^{\cdot}$ reduction

Aliquots of the fecal supernatants were added to a 0.1-mM solution of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH$^{\cdot}$) in ethanol. After a 30-min incubation at room temperature, the absorbance of the samples was measured at 517 nm and compared with that of a sample to which the same amount of PBS had been added (Molyneux, 2004).

Ferric reducing ability

The measurement of the ferric reducing antioxidant power (FRAP) followed the method of Benzie and Strain (1996). Briefly, an aliquot of the fecal supernatant was added to a solution of ferric tripyridyltriazine [Fe(III)-TPTZ] in acetate buffer, pH 3.6. After 20 min, the absorbance of the reduced form of the complex was measured at 593 nm.

Expression of results

The results were expressed as mmol Trolox equivalents/kg feces on the basis of standard curves. We preferred, like most other authors, to express TAC per kg of fresh mass of feces to compare equivalents of total concentrations of antioxidants. Statistical significance of differences was estimated using the t test for independent samples for variables normally distributed. Significance level was set at $P = 0.05$. The statistical analysis of the data was performed using StatSoft Inc. (2011), STATISTICA, version 10 (www.statsoft.com).

Results and Discussion

It has been reported that human feces have a considerable antioxidant capacity of about 27 mmol Trolox equivalents/kg wet feces (Garsetti *et al.*, 2000), which is much higher than the TAC of physiological fluids including blood plasma (about 1 mmol Trolox equivalents/l) (Janaszewska and Bartosz, 2002; Bartosz, 2003a). This high antioxidant capacity of feces may be of importance for the antioxidant protection of cells of the intestine and bowel walls, as conditions of food digestion may involve production of significant amounts of reactive oxygen species (ROS), due to liberation of iron and other metal ions. A subsequent study confirmed the increase in TAC of feces after consumption of food rich in antioxidants (Bianchi *et al.*, 2010). However, lower values of TAC (ca. 1.2 mmol Trolox equivalents/kg fresh feces) were reported for feces of the black rhinoceros (*Diceros bicornis*) using the method of ABTS· decolourization. The study demonstrated that a diet rich in tannins increases the TAC of feces (Clauss *et al.*, 2006). These data point to the need of a broader interspecies comparison of the TAC of feces as was done here. Our data, obtained by different methods, confirm interspecies differences in the TAC of feces, in agreement with literature data

Table I. TAC of feces of the herbivorous (1 – 18) and carnivorous (19 – 34) mammalian species, expressed as mmol Trolox equivalents/kg feces (mean ± SD).

No.	Species	*n*	ABTS· reduction		DPPH· reduction	FRAP
			"Fast" antioxidants	"Slow" antioxidants		
1	Bactrian camel (*Camelus bactrianus*)	3	23.1 ± 1.4	17.5 ± 0.2	3.60 ± 0.18	8.39 ± 0.01
2	Domestic goat (*Capra aegagrus hircus*)	9	10.1 ± 0.7	25.2 ± 2.4	3.07 ± 0.07	6.55 ± 0.03
3	Domestic pig (*Sus scrofa domesticus*)	9	6.4 ± 0.5	18.8 ± 1.8	1.58 ± 0.06	4.92 ± 0.02
4	European bison (*Bison bonasus bonasus*)	5	21.8 ± 0.4	21.6 ± 0.2	1.76 ± 0.08	10.98 ± 0.02
5	European hare (*Lepus europaeus*)	7	17.9 ± 0.5	24.3 ± 0.09	3.75 ± 0.11	9.66 ± 0.06
6	European rabbit (*Oryctolagus cuniculus*)	8	8.7 ± 0.5	15.3 ± 0.7	1.78 ± 0.03	6.13 ± 0.03
7	European roe deer (*Capreolus capreolus*)	4	11.4 ± 0.2	28.7 ± 0.7	0.83 ± 0.03	10.38 ± 0.02
8	Golden agouti (*Dasyprocta leporina*)	3	10.2 ± 0.05	21.7 ± 0.5	0.88 ± 0.02	7.43 ± 0.05
9	Grant's zebra (*Equus burchelli granti*)	3	21.4 ± 1.7	19.1 ± 0.5	3.66 ± 0.23	12.41 ± 0.07
10	Horse (*Equus caballus*)	9	11.0 ± 0.4	27.2 ± 0.7	3.86 ± 0.34	14.51 ± 0.30
11	Cattle (*Bos taurus*)	10	14.6 ± 1.4	23.7 ± 1.4	3.78 ± 0.35	17.28 ± 0.08
12	Indian elephant (*Elephas maximus indicus*)	3	23.3 ± 2.6	19.1 ± 0.6	1.46 ± 0.08	10.27 ± 0.02
13	Lesser kudu (*Tragelaphus imberbis*)	3	17.1 ± 1.3	26.0 ± 0.6	1.12 ± 0.05	10.63 ± 0.02
14	Llama (*Lama glama*)	3	25.2 ± 1.4	15.9 ± 0.3	3.90 ± 0.07	8.86 ± 0.10
15	Rothschild giraffe (*Giraffa camelopardalis rothschildi*)	3	20.6 ± 2.2	23.5 ± 0.6	2.95 ± 0.32	11.01 ± 0.02
16	Salt's dik-dik (*Madoqua saltiana*)	3	24.2 ± 1.7	18.2 ± 0.2	1.83 ± 0.11	12.14 ± 0.02
17	Scimitar-horned oryx (*Oryx dammah*)	3	33.2 ± 1.2	9.6 ± 0.01	4.34 ± 0.19	17.72 ± 0.03
18	South American tapir (*Tapirus terrestris*)	3	22.5 ± 1.8	17.0 ± 0.5	0.66 ± 0.03	9.52 ± 0.01
19	African lion (*Panthera leo*)	3	7.9 ± 0.4	23.7 ± 0.6	0.34 ± 0.01	5.47 ± 0.030
20	Asiatic lion (*Panthera leo persica*)	3	5.7 ± 0.1	31.9 ± 1.0	0.38 ± 0.01	6.16 ± 0.20
21	Cheetah (*Acinonyx jubatus*)	3	16.0 ± 1.2	26.7 ± 0.2	1.60 ± 0.15	14.23 ± 0.45
22	Common genet (*Genetta genetta*)	3	34.6 ± 2.1	8.8 ± 0.1	4.56 ± 0.14	15.30 ± 0.48
23	Domestic dog (*Canis lupus familiaris*)	12	5.3 ± 0.4	27.0 ± 1.5	3.97 ± 0.14	10.11 ± 0.17
24	Eurasian lynx (*Lynx lynx*)	3	8.2 ± 0.2	31.3 ± 0.4	0.65 ± 0.10	11.94 ± 0.18
25	Gray wolf (*Canis lupus lapus*)	3	7.3 ± 0.1	23.7 ± 0.7	1.48 ± 0.06	5.33 ± 0.03
26	North Chinese leopard (*Panthera pardus japonensis*)	3	14.5 ± 0.4	29.7 ± 0.3	1.41 ± 0.01	9.93 ± 0.31
27	Pallas's cat (*Otocolobus manul*)	3	3.4 ± 0.09	16.6 ± 1.2	0.07 ± 0.01	0.52 ± 0.02
28	Serval (*Leptailurus serval*)	3	19.4 ± 1.1	21.8 ± 0.4	5.31 ± 0.28	10.64 ± 0.09
29	Siberian tiger (*Panthera tigris altaica*)	3	11.2 ± 0.2	24.6 ± 1.3	1.96 ± 0.03	4.61 ± 0.02
30	Silver fox (*Vulpes vulpes*)	3	6.8 ± 0.2	29.1 ± 0.4	0.50 ± 0.01	2.52 ± 0.01
31	Sitatunga (*Tragelaphus spekei gratus*)	3	27.1 ± 2.5	13.3 ± 0.2	2.12 ± 0.136	1.60 ± 0.35
32	Sri Lankan leopard (*Panthera pardus kotiya*)	3	11.6 ± 0.4	26.6 ± 0.9	1.91 ± 0.10	4.03 ± 0.03
33	Striped hyena (*Hyaena hyaena*)	3	9.6 ± 0.9	25.6 ± 2.1	0.15 ± 0.002	2.17 ± 0.08
34	Wildcat (*Felis silvestris*)	3	7.0 ± 0.4	34.5 ± 1.5	0.40 ± 0.01	2.33 ± 0.07

Table II. Comparison of the TAC of feces of herbivores and carnivores (mmol Trolox equivalents/kg feces).

Species, pooled	ABTS· reduction		DPPH· reduction	FRAP
	"Fast" antioxidants	"Slow" antioxidants		
Herbivores ($n = 18$)	17.9 ± 7.2^a	20.7 ± 4.9	2.5 ± 1.3	14.2 ± 6.7^a
Carnivores ($n = 16$)	12.2 ± 8.5^a	24.7 ± 6.9	1.7 ± 1.6	9.5 ± 7.3^a

[a] $P < 0.05$.

(Bartosz, 2003a; Gülçin, 2012). The ABTS· reduction assay consistently yielded the highest values, in agreement with the known high reactivity of ABTS· (Table I).

The results indicate a generally higher TAC of feces of herbivorous as compared to carnivorous mammals, the differences being statistically significant for the FRAP assay and the "fast" antioxidants in the ABTS· reduction test. The same tendency was observed for the DPPH· reduction assay, but the difference did not reach statistical significance (Table II).

We have demonstrated previously that typical antioxidants, such as ascorbic acid, tocopherol and its derivatives, uric acid, and glutathione, react rapidly with ABTS·, the reaction being completed within seconds. On the other hand, other compounds, among them tyrosine and tryptophan, react at a much lower rate, the reaction occurring only within minutes or even tens of minutes. Therefore, we routinely determined the ABTS· reduction after two arbitrarily chosen reaction times, *i. e.* 10 s and 3 min after mixing the sample with ABTS·. Such a procedure does not allow for the complete separation of the activities of "fast" and "slow" antioxidants, since the latter also react within the initial 10 s to some extent, nevertheless it gives an estimate of the activities of these two classes of antioxidants. On the other side, the reaction of the slow antioxidants may not be completed within 3 min (Bartosz, 2003a, b).

In the ABTS· reduction test, the TAC resulting from "fast" antioxidants was higher in herbivores than in carnivores, while the tendency was reversed for the TAC resulting from "slow" antioxidants. Taking into account that the latter is conditioned mainly by the reactivity of proteins and products of their digestion, this result seems plausible. The beneficial effect of the reactivity of proteins with free radicals and other ROS is rather doubtful within the cells, as it may lead to protein inactivation. Within the digestive tract, however, this reactivity, which inevitably results in protein oxidation, may contribute to the scavenging of ROS. As oxidatively damaged proteins are more vulnerable to proteolytic enzymes (Shacter, 2000), this may even facilitate protein digestion.

In summary, the present comparison suggests a higher TAC of feces and bowel content of herbivorous as compared to carnivorous mammals. It would be interesting to correlate these data with the susceptibility of various mammalian species to colorectal cancer, although the latter is undoubtedly dependent on many other factors including the sources of ROS in the bowel content and the level of defensive mechanisms.

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