

Nutritional properties of mussels *Mytilus galloprovincialis*

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Abstract The aim of this study was to determine the quality indices and assess the influence of diets supplemented with mussels from contaminated (MCont) and non-contaminated areas (MNCont) on some indices of protein and lipid metabolism in rats fed with cholesterol. A wide range of in vitro tests demonstrated that mussels

from contaminated area have a higher content of proteins, lipids and higher antioxidant capability. In the in vivo experiment, 28 male Wistar rats were divided into 4 diet groups each of 7 and named Control, Cholesterol (Chol), Chol/MCont and Chol/MNCont. During 30 days of the experiment rats of all four groups were fed basal diet (BD), supplemented with 1% of Chol, 1% of Chol and 5.6% of mussel's dry matter (DM) from contaminated and 1% Chol and 5.6% of mussel DM from non-contaminated areas for Chol/MCont and Chol/MNCont groups, respectively. At the end of the experiment, a high digestibility coefficient of DM and crude protein in the Chol/MCont and Chol/MNCont groups of rats was found: 93.56 and 93.87% and 91.64 and 91.36%, respectively, and the differences were not significant ($P > 0.05$). However, the protein efficiency ratio was significantly higher in the Chol/MCont and Chol/MNCont than in the Control and Chol diet groups. Also the level of nitrogen retention as shown by feces and urine examination was higher in the Chol/MCont and Chol/MNCont groups. Diet, supplemented with mussel dry matter from both contaminated and non-contaminated areas significantly hindered the rise in plasma lipids and also hindered the decrease in plasma antioxidant activity. Minor changes were fixed in the protein profile of rat's plasma after both mussels' diet. In conclusion, supplementation of diets, containing cholesterol with mussels' DM from both contaminated and non-contaminated areas improves animals' protein metabolism and positively affects plasma lipid profile and plasma antioxidant activity. Antioxidant tests can be used as an additional index for the quality of mussels.

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Introduction

Nowadays the seafood consumption as a source of protein is increasing, therefore the protein investigation of the seafood gains momentum [1, 2].

It was shown that the contents of bioactive compounds and the antioxidant capacity of mussels from contaminated and non-contaminated areas differ and in some cases significantly [3–6]. However, how the consumption of seafood influences plasma protein and lipid metabolism is less studied. Therefore, it was decided to conduct an investigation in vitro and in vivo to explore the influence of dry matter (DM) of mussels from both areas on some indices of protein and lipid metabolism and antioxidant activity in rats fed cholesterol.

For this purpose (1) the determination of the antioxidant capacity of the mussels from contaminated and non-contaminated areas by ABTS⁺ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] assay, proteins, carbohydrates and lipids [7, 8], (2) determination of digestibility of DM and crude protein (CP), the nitrogen retention, plasma lipid levels and plasma antioxidant activity in examination in vivo were used. The ABTS⁺ and DPPH tests were adopted for determination of the changes in rat plasma antioxidant activity [9, 10].

As far as we know there are no such published investigations.

Materials and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), BHA (butylated hydroxyanisole), ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], Folin–Ciocalteu reagent, cholesterol of analytical grade (USP), DPPH (1,1-diphenyl-2-picrylhydrazyl) and sodium dodecyl sulphate (SDS) were obtained from Sigma Chemical Co., St Louis, MO.

Animals and sites of collection

Mussels were collected in two regions of Mokpo coast, Mokpo bay (Republic of Korea): (1) an ecologically non-contaminated site (out of the port, 20 miles West-North from Mokpo bay) and in (2) contaminated site (the Mokpo port, in the bay of Halla Ship large scale construction company, which belongs to Hyundai groups) at sea depth of 3–4 m. The samples were collected on 20 March 2005.

The collected mussels (*Mytilus galloprovincialis*) from both contaminated and non-contaminated sites were characterized by a similar maximum length and size of analyzed

organisms (4.37 ± 0.5 cm): it was 75–85% of the maximum size reached within each population. This approach guaranteed that compared mussels had similar metabolic conditions and the influence of physiological differences between two populations was less pronounced [11]. The samples were designated as follows: MNCont, for non-contaminated, and MCont, for contaminated sites. Whole soft tissue from 30 specimens of each population were rapidly frozen in liquid nitrogen and stored at -80 °C. Then the samples were dried in glass flasks on Finn-Aqua, Lyovac GT-2 equipment for 36 h.

Extraction and determination of proteins from mussel samples

The whole soft tissue from MNCont and MCont mussels were separately subjected to extraction with 0.05 mol L^{-1} Na_2HPO_4 buffer (two portions of 20 mL each) at 4–6 °C for 48 h under constant stirring. Centrifugation (K-24 D centrifuge, Janetzki, Germany) at 15,000 rpm for 45 min at 4–6 °C was applied at the end of each extraction step and the supernatants were combined and freeze-dried. The obtained dry extracts were used for further analyses [12].

Determination of protein content was done according to the method of Bradford [13]. Carbohydrates were quantified as glucose by the phenolsulphuric acid method. Glycogen was also quantified as carbohydrates after precipitation with 100% ethanol [14]. Lipids were extracted and gravimetrically determined [15]. The data were calculated on the basis of μg of biochemical fraction per mg of total organic matter (OM).

Extraction of polyphenols

Defatted with acetone, lyophilized mussel samples were extracted from a 50 mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for 3 h for free hydrophilic polyphenols (FHPol) and under the same conditions with 5 mL of 1.2 M HCl in 50% methanol/water for total hydrophilic polyphenols (THPol). The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4,000g with a benchtop centrifuge to remove solids. FHPol and THPol were extracted and from the precipitates the lipophilic fractions such as total lipophilic polyphenols (TLPol) and free lipophilic polyphenols (FLPol) were extracted with 70% of acetone [16].

Polyphenol determination

The Folin–Ciocalteu method was used [17], and the measurements were performed at 765 nm with gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents (GAE)/g DM.

Antioxidant activities

The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was generated by the interaction of ABTS (250 μ M) and $K_2S_2O_8$ (40 μ M). The absorbance was monitored exactly 1 and 6 min at 734 nm after the addition of 990 μ L of ABTS solution to 10 μ L of mussel extracts or Trolox standards in methanol or phosphate buffered saline (pH 7.4). Trolox equivalent antioxidant capacity (TEAC) was expressed as micromolar trolox equivalents (TE)/g DM [8, 10]. ABTS antioxidant activities were determined in total and free polyphenol extracts as hydrophilic antioxidants: THABTS, total hydrophilic ABTS antioxidant activity and FHABTS, free hydrophilic ABTS antioxidant activity and in acetone extracts as lipophilic antioxidants: TLABTS, total lipophilic ABTS antioxidant activity and FLABTS, free lipophilic ABTS antioxidant activity.

Rats and diets

The Animal Care Committee of the Warsaw Agricultural University, Poland, had approved this study. The mean weight of the Wistar rats ($n = 28$) at the beginning of the experiment was 111 g. They were divided into four groups of seven and housed in the first part of the experiment (1–24 days) in plastic cages and then in metabolic cages (25–30 days). These groups were named Control, Chol, Chol/MCont and Chol/MNCont. During first 5 days all four groups were fed the basal diet (BD) only, which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group during the 30 days of the experiment received the BD only, and the diets of the other three groups was supplemented with 1% of cholesterol (Chol), 1% of Chol and 5.6% of mussel dry matter (DM) from contaminated and 1% of Chol and 5.6% of mussel DM from non-contaminated areas for the Chol, Chol/MCont and Chol/MNCont, respectively.

The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Our prior experiments on laboratory animals have shown that cellulose has no significant hypocholesterolemic effects [7]. Therefore, cellulose was used as a control fiber.

The feed intake was monitored daily and body gains every week. For the determination of the nitrogen retention from the diets, feces and urine were collected in the last 5 days of the experiment. Samples were dried at the temperature of 80 °C for 3 days and then at the temperature of 105 °C for three additional days for the estimation of the dry matter content of diets and feces. The nitrogen was determined by Kjeldahl (Kjeltec-300 Tecator), according to AOAC, 1997 [18, 19]. Then the dry matter and crude protein's digestibility were assessed [20–24]. At the end of the

experiment after 24 h of starvation, the rats were anaesthetized using diethyl ether and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests, which included determination of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG) and plasma antioxidant activity. ABTS⁺ was done exactly as described above, but only with 10 μ L plasma [10]. The antioxidant activity of plasma by DPPH test was measured by mixing 25 μ L plasma, 75 μ L methanol and 0.8 mL 75 μ M-DPPH. The reaction mixture was maintained in dark at room temperature for 90 min, and the absorbance at 517 nm was then recorded [9]. The results of antioxidant activity were expressed in mM of trolox equivalents (TE)/L.

Plasma fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized. Plasma samples were dissolved in sample buffer: 2% sodium dodecyl sulphate (SDS), 10% glycerol, 2% mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris HCl, pH 6.8. Electrophoresis was performed with the Hoeffer SE 600 vertical unit (Hoeffer Pharmacia Biotech Inc., San Francisco, CA, USA) according to Laemmli method [25], using polyacrylamide gels (resolving gel T = 13.7%, C = 1.7%, stacking gel T = 3.8%, C = 1.8%) with gel size of 180 × 160 × 1.5 mm. Sample size was 5 μ L. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236 s Color image scanner).

Statistics

The reported values of the in vitro studies are mean \pm SD of five measurements. The analysis of variance and least significant difference tests were conducted to identify differences among means, while a Pearson correlation test was conducted to determine the correlation among means. Statistical significance was declared at $P < 0.05$.

Results

In vitro

Proteins were the dominated compounds in the mussel samples. In MNCont samples the amount of proteins was about 702.56 ± 6.45 and for MCont 722.32 ± 7.89 μ g protein/mg of organic matter (OM). The amount of proteins in mussels from contaminated area was higher than from the non-con-

taminated one. Our results were slightly lower than that reported by others [26]. During March, the amount of proteins was the highest in comparison with the other months of collection [26]. In our report, the samples were collected only during March. The variability in the content of proteins can be explained also by the geographical differences of the places, where the samples were collected. Carbohydrates (μg carbohydrates/mg OM) were about the same amount for MNCont (115.45 ± 10.25) and for MCont (116.32 ± 11.32) samples, respectively. Glycogen in the time of the collection of the samples showed about 32.3% for MNCont and 33.23% for MCont of total carbohydrates. The total lipids were 192.45 ± 16.32 and 191.54 ± 17.43 μg total lipids/mg OM for MNCont and MCont, respectively. The amount of carbohydrates and lipids in the samples of the two collected areas did not change significantly [27]. Our data can be compared with the recent reports [27], where mussels *Mytilus galloprovincialis* were analyzed for some quality parameters. The results obtained evidenced that many of the parameters studied, proximate composition, the lipid fatty acid composition and the microbiological indices, were subjected to seasonal fluctuations.

The amounts of total and free polyphenols such as THPol and FHPol for MCont and MNCont areas ranged from 28.48 to 23.56 mg GAE g^{-1} DM (Fig. 1a, b).

The polyphenols determined in lipophilic fractions after extraction of total (TLPol) and free (FLPol) polyphenols ranged from 0.104 to 0.056 mg GAE g^{-1} DM. The related antioxidant activities in total and free polyphenol extracts such as THABTS and FHABTS ranged from 66 to 56 $\mu\text{MTE g}^{-1}$ DM (Fig. 1a, b), as determined by ABTS assay, were significantly higher in Cont than in NonCont area ($P < 0.05$).

In TLABTS and FLABTS, the lipophilic extracts showed small amount of antioxidants with the activity, which ranged from 9 till 2 $\mu\text{MTE g}^{-1}$ DM.

The calculated correlation between the antioxidant activity determined by ABTS and their hydrophilic and lipophilic polyphenols was about 0.87 and 0.85 (Fig. 2a, b) for total and free polyphenols. Therefore a good correlation was observed between the antioxidant activity determined by ABTS in free and total polyphenol extracts.

In vivo

The results of the determination of the dry matter of diets, crude protein and their digestibility were summarized in the Table 1. As can be seen, the percentage of crude protein (CP) in diets of rats of Chol/MCont and to a lesser degree in Chol/MNCont groups was significantly higher than in the other two groups ($P < 0.05$).

The protein efficiency ratio in the Chol/MCont and Chol/MNCont groups was significantly higher in the other two

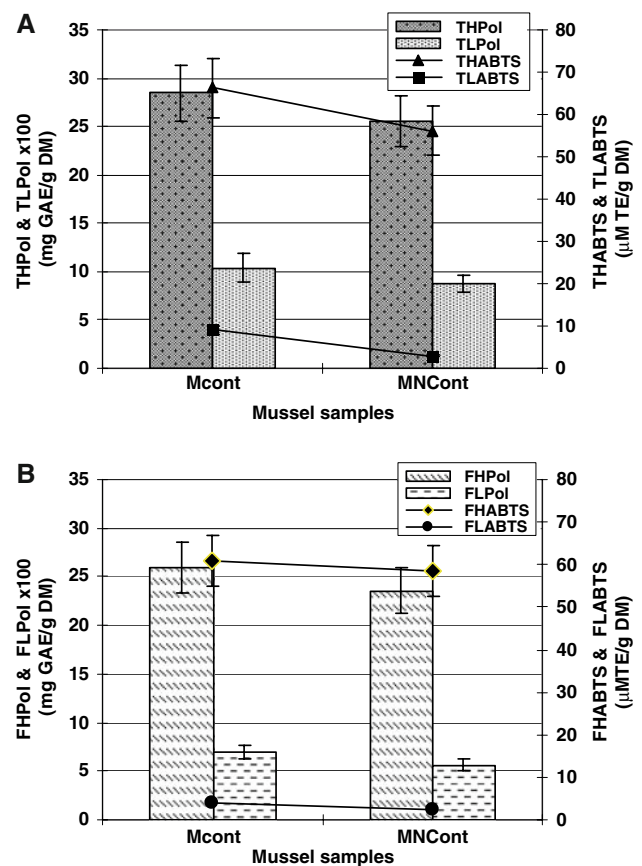


Fig. 1 a Total phenols and the antioxidant activities by ABTS in total and free polyphenol and b acetone extracts from mussel contaminated (Mcont) and mussel non-contaminated (MNCont) areas. THPol total hydrophilic polyphenols, FHPol free hydrophilic polyphenols, TLPol total lipophilic polyphenols, FLPol free lipophilic polyphenols, THABTS total hydrophilic ABTS⁺ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation antioxidant activity, TLABTS total lipophilic ABTS⁺ antioxidant activity, FHABTS free hydrophilic ABTS⁺ antioxidant activity, FLABTS free lipophilic ABTS⁺ antioxidant activity

groups (Table 2, $P < 0.05$). These results could be expected: the percentage of CP in diets of the rats of Chol/MCont and Chol/MNCont groups was significantly higher than in the other two groups.

Despite higher nitrogen excretion in feces of rats in the Chol/MCont group the nitrogen retention remains significantly high ($P < 0.05$). It can be explained by a higher intake of nitrogen from diet (Table 3, $P < 0.05$).

The Chol/MCont and Chol/MNCont groups were fed cholesterol, the supplementation of their diets with dry matter of mussels with high content of proteins and high antioxidant capacity prevented a significant increase in the plasma lipid levels and a significant decrease in plasma antioxidant activity (Table 4, $P > 0.05$ in both cases).

DM of mussels significantly hindered the rise of plasma lipids versus Chol diet group: TC [0.40 mmol L^{-1} (17.5.0%) and 0.31 mmol L^{-1} (14.9%)]; LDL-C [0.27 mmol L^{-1} (27.5%) and 0.19 mmol L^{-1} (17.9%)]; TG

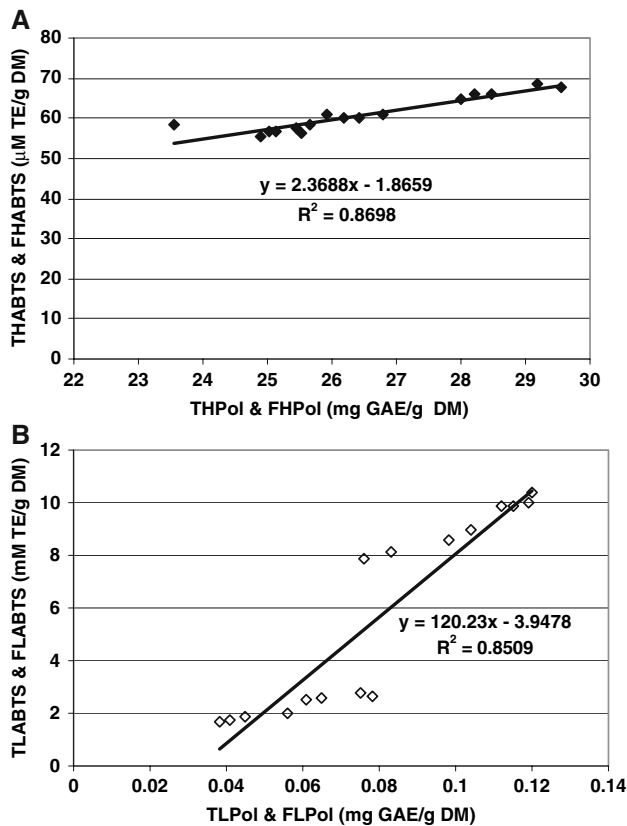


Fig. 2 Correlation coefficients (R^2) calculated by linear regression analysis for mussel extracts between polyphenols and antioxidants: **a** *THPol* and *FHPol* (mgGAE g^{-1} DM, X) and *THABTS* and *FHABTS* ($\mu\text{MTE g}^{-1}$ DM, Y) **b** *TLPol* and *FLPol* (mgGAE g^{-1} DM, X) and *TLABTS* and *FLABTS* ($\mu\text{MTE g}^{-1}$ DM, Y). *THPol* total hydrophilic polyphenols, *FHPol* free hydrophilic polyphenols, *TLPol* total lipophilic polyphenols, *FLPol* free lipophilic polyphenols, *THABTS* total hydrophilic ABTS^+ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation antioxidant activity, *TLABTS* total lipophilic ABTS^+ antioxidant activity, *FHABTS* free hydrophilic ABTS^+ antioxidant activity, *FLABTS* free lipophilic ABTS^+ antioxidant activity

[0.03 mmol L^{-1} (4.1%) and 0.02 mmol L^{-1} (2.7%)], in the Chol/Cont and Chol/MNCont diet groups, respectively. The decrease in the antioxidant activity determined by ABTS and DPPH was about 24.0 and 20.3% and 24.0 and 22.0%, respectively. The correlation between the most important fractions of lipid spectrum such as low-density

cholesterol and the antioxidant activity in plasma was as follows: LDL-Chol vs AA (ABTS), and AA (DPPH) were 0.99.

Electrophoretic bands showed slight differences between the diets supplemented with mussels from contaminated and non-contaminated areas for full and methanol precipitated plasma (Fig. 3a, b). In total plasma and the methanol-precipitated fraction during electrophoretic separation intensity of proteins in the region of 45–66 kDa than in Control and Cholesterol groups was detected higher. The slight differences were detected at 66 kDa during comparison of lanes 1–3 (Control) and 4–10 (Chol) with lanes 11–16 (Chol/MCont). The lanes 11–16 were more intensive than lanes 1–3 and 4–10 (Fig. 3a).

Discussion

Proteins are an important component of human diets [28–30]. The growing population, mainly in the undeveloped countries, increases the demands for new sources of proteins and one of them is seafood [31, 32]. It was shown that the contents of bioactive compounds and the antioxidant capacity of mussels from contaminated and non-contaminated areas differ and in some cases significantly [3–6]. Therefore, it was decided to compare the possible changes in functional and antioxidant properties of mussels *Mytilus galloprovincialis*. It was found that the amount of proteins in mussels from non-contaminated areas is lower than in mussels from contaminated areas. These data are in agreement with other investigators [33].

The radical ABTS^+ has been widely used in model systems for investigation of the scavenging activities of phenolic compounds, anthocyanins, or crude mixtures such as methanol extracts of plants [34]. However, few studies have been reported on proteins, except antioxidative enzymes, with regard to the subject of direct antiradical effects [35–37]. Similar results were observed in our recent investigations using other methods [12, 38]: the antioxidant activity of the whole soft mussel tissue from the contaminated areas was significantly higher than from non-contaminated areas.

Table 1 Diet groups, dry matter (DM) and crude protein (CP) of the diets and their digestibility as determined at the end of the experiment (%)

Groups	DM	CP	Digestibility of DM	Digestibility of CP
Control	98.29 \pm 1.1a	11.62 \pm 0.3a	95.9 \pm 1.08a	91.22 \pm 2.54a
Chol	98.45 \pm 1.1a	11.25 \pm 0.3a	95.45 \pm 0.62a	91.86 \pm 1.17a
Chol/MCont	98.13 \pm 1.1a	16.07 \pm 0.4b	93.56 \pm 0.48a	91.64 \pm 0.49a
Chol/MNCont	97.91 \pm 1.0a	15.40 \pm 0.4b	93.87 \pm 0.60a	91.36 \pm 0.84a

Values are means \pm SD, $n = 7$

Values with different letters in columns are significantly different ($P < 0.05$)

Chol cholesterol diet group, *Chol/MCont* cholesterol/mussel contaminated diet group, *Chol/MNCont* cholesterol/mussel non-contaminated diet group

Table 2 Feed intake, body gains, FER and PER in all diet groups

Groups	Feed intake (g/day)	Body gains (g/day)	FER	PER
Control	13.77 ± 2.31a	4.57 ± 0.79a	0.353 ± 0.03a	0.350 ± 0.085a
Chol	14.58 ± 2.11a	4.50 ± 0.71a	0.308 ± 0.02a	0.361 ± 0.022a
Chol/MCont	14.98 ± 1.64a	4.71 ± 0.64a	0.305 ± 0.02a	0.519 ± 0.03b
Chol/MNCont	13.09 ± 1.12a	4.06 ± 0.74a	0.302 ± 0.06a	0.514 ± 0.094b

Values are means ± SD, $n = 7$

Values with different superscript letters in columns are significantly different ($P < 0.05$)

Chol cholesterol diet group, *Chol/MCont* cholesterol/mussel contaminated diet group *Chol/MNCont* cholesterol/mussel non-contaminated diet group *FER* feed efficiency ratio, *PER* protein efficiency ratio

Table 3 The influence of different diets on nitrogen retention (one day average)

Groups	Intake of nitrogen from diets (g)	Nitrogen excretion in feces (g)	Nitrogen excretion in urine (g)	Nitrogen retention (in g and %)
Control	0.292 ± 0.03a	0.036 ± 0.001a	0.108 ± 0.02a	0.148 ± 0.03a (50.6%)
Chol	0.302 ± 0.03a	0.034 ± 0.003a	0.12 ± 0.01a	0.148 ± 0.03a (49.0%)
Chol/MCont	0.356 ± 0.04b	0.052 ± 0.01c	0.116 ± 0.02a	0.188 ± 0.03b (52.8%)
Chol/MNCont	0.298 ± 0.03a	0.044 ± 0.01b	0.11 ± 0.01a	0.144 ± 0.01a (48.3%)

Values are mean ± SD, $n = 7$

Values with different letters in columns are significantly different ($P < 0.05$)

Chol cholesterol diet group, *Chol/MCont* cholesterol/mussel contaminated diet group, *Chol/MNCont* cholesterol/non-contaminated diet group

Table 4 The changes in the plasma lipids (mmol L⁻¹) and plasma antioxidant activity (mMTE L⁻¹) after the experiment

Indices	Control	Chol	Chol/Mcont	Chol/MNCont
TC	2.21 ± 0.11a	2.69 ± 0.12b	2.29 ± 0.11a	2.34 ± 0.11a
LDL-C	0.87 ± 0.04a	1.25 ± 0.06b	0.98 ± 0.05a	1.06 ± 0.05a
HDL-C	1.34 ± 0.07a	1.44 ± 0.07a	1.31 ± 0.06a	1.28 ± 0.06a
TG	0.69 ± 0.04a	0.76 ± 0.04a	0.73 ± 0.04a	0.74 ± 0.04a
AA (ABTS)	1.49 ± 0.07a	0.98 ± 0.05b	1.29 ± 0.06a	1.23 ± 0.06a
AA (DPPH)	0.83 ± 0.04a	0.57 ± 0.03b	0.77 ± 0.04a	0.73 ± 0.04a

Values are means ± SD, $n = 7$

Means in rows with letters in common differ significantly ($P < 0.05$)

AA antioxidant activity, *ABTS* [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], *Chol* cholesterol fed rats, *Chol/MCont* cholesterol and DM from mussels contaminated area fed rats, *Chol/MNCont* cholesterol and DM from mussels non-contaminated area fed rats *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *TC* total cholesterol, *TG* triglycerides

Antioxidant capacities determined by ABTS⁺ method and their correlations to the concentrations of the bioactive compounds were about 0.85. The radical scavenging capacity of individual mussel extracts against different testing radicals may be explained by the different mechanisms involved in the radical–antioxidant reactions [12, 38, 39]. In this study, the ABTS⁺ was generated by incubating ABTS with potassium persulfate [34, 40].

The nutritional values of mussels, and their influence on dry matter and protein digestibility are almost not known. Mussels in human diets have also been recommended for prevention of some health problems. The present investigation has shown, that there was no effect of mussels dry matter

on FER, PER and body gains. It can be concluded that mussels have no influence on performance, because their utilization in the body (evaluated on diet digestibility) was similar.

It was also found that the content of crude protein in the diets of the rats fed added mussels' DM was higher than in the two other groups. However, the digestibility of the DM and CP in all four groups was comparable. Also the feed intake, body gains and feed efficiency ratio were comparable. However, the protein efficiency ratio was significantly higher in the groups of rats, where diets were supplemented with mussels' DM from both studied areas.

The intake of nitrogen from diets was higher in the group of rats, in which diet was supplemented with mussels' DM

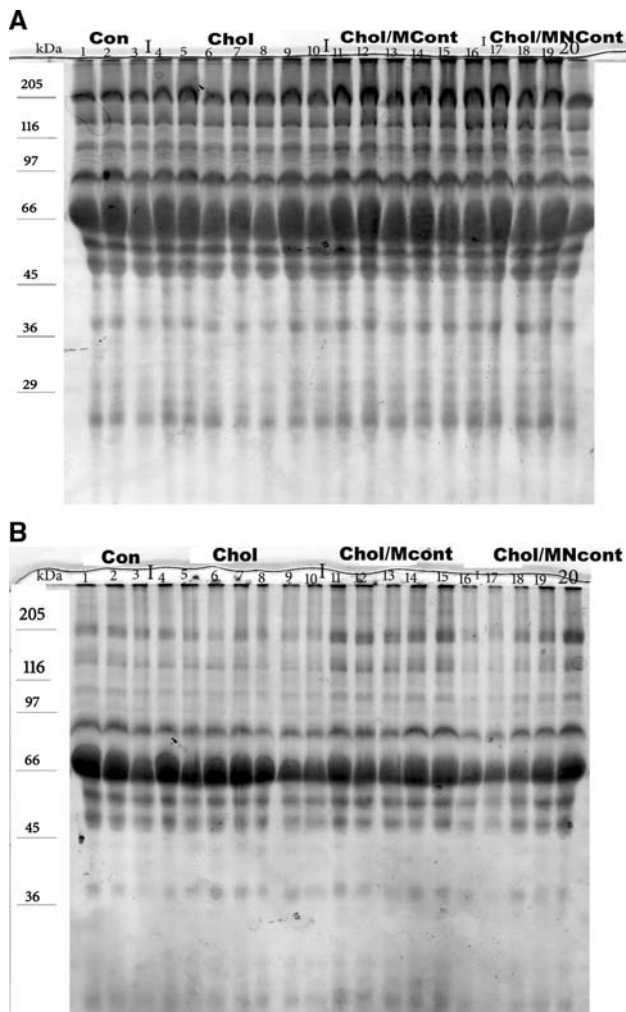


Fig. 3 Comparison of the band intensity of plasma proteins after different mussel's diets extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Molecular markers (kDa): 205 myosin, 116 β -galactosidase, 97 phosphorylase b, 66 albumin, 45 ovalbumin, 36 glyceralaldehyde-3-phosphate dehydrogenase, 29 carbonic anhydrase, 24 trypsinogen PMSF treated, loading 2 μ l: lanes 1–3 = *Con* control, lanes 4–10 = *Chol* cholesterol, lanes 11–16 = *Chol/MCont* mussel contaminated, 17–20 = *Chol/MNCont*

from the contaminated area. Also nitrogen excretion in feces and nitrogen retention were significantly higher in rats of this group. The above-mentioned results were expected: the DM of the mussels from the contaminated areas contains higher amount of proteins and other bioactive compounds. These data are in accordance with the data of others [21, 22].

The supplementation with mussels' DM containing high amounts of proteins and possessing high antioxidant capacity to the diets of rats of the Chol/Cont and Chol/NonCont groups prevented a significant increase in the plasma lipid levels and a significant decrease in plasma antioxidant activity. These results were expected. Also others found that cholesterol-rich diets have different effects on lipid per-

oxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits [41].

Protein profile of plasma samples showed that in fibrinogen fraction and the total plasma in Control sample less protein bands and lower intensity than in other groups were detected. The main patterns were located in the range of 45–66 kDa, showing that the amount of fibrinogen has decreased during such diet. Fibrinogen is one of the plasma proteins. Our findings indicate that one of the positive benefits of mussel consumption was to diminish the production of fibrinogen and its stability, which reduces the potential risk exerted by this protein. Therefore, from the health point these results are positive.

Conclusion

1. The dry matter of mussels from the contaminated areas contains higher amount of proteins and possesses higher antioxidant activity. This can be an additional index for mussels' characterization.
2. Supplementation of diets containing cholesterol with mussels' dry matter from both contaminated and non-contaminated areas improves animals' protein metabolism and positively affects plasma lipid profile and plasma antioxidant activity. In such way the quality of mussels can be compared.

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