Izabela Michalak*, Katarzyna Chojnacka, Daniel Korniewicz

New feed supplement from macroalgae as the dietary source of microelements for pigs

Abstract: The aim of the study was to perform feeding experiments on growing pigs in order to assess the impact of macroalga Enteromorpha sp. enriched with Zn(II) and Cu(II) ions via the biosorption process on the mineral composition of blood, meat, liver, feces and urine. In the control group, microelements were supplemented as inorganic salts, whereas in the experimental groups they were replaced by enriched macroalga. After 3 months of the feeding experiment, it was found that the meat was biofortified with Cr, Mn, Fe, Cu and Zn. The average content of Zn in the blood from the pigs fed with algae was higher by 9.5%, compared to that in the blood from pigs in the control group. The liver of growing pigs from the experimental group contained 16% less Cu and 18% less Zn than the liver in the control group. Growing pigs fed with macroalgae excreted in feces 27% more Zn than growing pigs in the control group, but 3.5 times less Cu. It could be concluded that the bioavailability of microelements to pigs from algae was higher than from the inorganic salts. Baltic macroalgae enriched with microelement ions could be potentially used as a biological feed additive.

Keywords: macroalgae, microelements, biosorption, feed additive, growing pigs

1 Introduction

Dried macroalgae have been demonstrated to be a rich source of bioactive compounds in animal nutrition [1,2]. They have the potential to increase animal productivity, enhance resistance against diseases, provide a better digestibility of feed ingredients, and they can improve the gut flora, thus having a positive effect on the quality of animal products [3,4]. Seaweeds have been used as feed for domestic animals for a long time, if confined to the coastal regions. Hansen et al. (2003) found that macroalgae can be consumed by animals directly from the beach shore [5].

This paper investigates the use of green macroalga – Enteromorpha sp. in the pigs’ feed. Algae can play an important role as a feed supplement due to their rich composition of proteins [6-10], minerals [6,7,11-13], essential amino acids, including His, Arg, Thr, Val, Met, Lys, Iso, Leu, Phe, Try [6,8], essential fatty acids, including n-3 (ALA: alpha linolenic acid C18:3, EPA; eicosapentaenoic acid C20:5) and n-6 (AA: arachidonic acid C20:4; LN: linolenic acid C18:2) [6,8,9,10], fiber [6,8,9,10], vitamins, e.g., A, E [10] and carbohydrates [8-10].

In the present paper, the biomass of Enteromorpha sp., was additionally enriched with Zn(II) and Cu(II) ions via biosorption process, which was used to produce value-added products for agriculture i.e. biological feed additives with microelements [14]. These two elements were chosen because nowadays there were concerns related to their accretion from piggery effluents, which could have an adverse effect on the soil microbiota and might cause decline in pasture, soil fertility and crop yields. Because the use of additives with high concentrations of Cu and Zn in pig diets was banned, alternatives to inorganic forms of dietary Cu and Zn are searched for. On the other hand, in the composition of the daily ration for pigs, the greatest emphasis is put on providing optimal amounts of zinc and copper [15]. According to the Polish standards for swine feeding, the requirement for these two microelements is the highest (Cu: 20–165; Zn: 70–150; Fe 90–100; Mn 30–40; Co 0–0.5; Se 0.1–0.2; I 0.1–1.0 mg kg$^{-1}$ of feed) [16].
The concept of the application of the enriched biomass in animal feeding corresponds to the currently prevailing trend to seek alternatives to inorganic salts, commonly supplemented in animal diet. Previously, we hypothesized that biological materials enriched by biosorption would supply minerals in non-toxic and a highly bioavailable form to animals [17,18]. This assumption was later confirmed in the feeding experiments carried out on laying hens, where inorganic form of microelements was replaced with enriched macroalgae. It was found that supplementing biological feed additives to the diet resulted in higher microelement transfer to eggs and an enhanced color of yolks. It was also noticed that the presence of green macroalgae Enteromorpha prolifera and Cladophora sp. in laying hens’ diet influenced advantageously egg weight, eggshell thickness, as well as the body weight of hens [19]. Also Witkowska et al. (2014a) showed that the qualities of eggshell strength, eggshell thickness, albumen height and yolk color of the hens fed with soybean enriched via biosorption were enhanced. The biological form of microelements also improved the feed conversion rate, organoleptic parameters of eggs in comparison to the inorganic form and chelate microelements [20]. Soya-based preparations with Cu(II), Fe(II), Zn(II) and Mn(II) produced by biosorption were tested also on goats. It resulted in goats’ biofortified milk and cheese which could be used as designer milk to prevent micronutrient deficiencies [21]. The same enriched soybean meal was examined in the zootecchnical studies on fatteners. It was found that the application of the biological supplements with microelements increased the average daily gain of pigs, while reducing the consumption of feed. The content of iron increased in the meat by about 10% when the enriched biomass was supplemented in the recommended dosage [22]. Saeid et al. (2013a) examined the effect of the supplementation of macroalga Spirulina maxima enriched with Cu on production performance, metabolic and physiological parameters in fattening pigs. The results reported in this work showed that the application of micronutrients to the diet of animals, bound with the biological matrix in the form of S. maxima, had an advantageous effect on reared swine: improved profile of the liver, lowered LDL and total cholesterol [23]. Additionally, it was shown that by utilization of new biological feed supplements it was possible to reduce the excretion of copper in feces by 60% [24].

The aim of the present work was to investigate the applicability of Baltic macroalga – Enteromorpha sp. enriched with microelements by biosorption process as feed supplements for growing pigs. The effect of the preparation on the transfer of microelements to blood, meat, liver, urine and feces as well as the effect of biofortification of animal product were investigated.

2 Material and methods

2.1 Preparation of biological feed additives

The alga Enteromorpha sp. was collected from the Baltic Sea (Niechorze, Poland) and identified in the Department of Botany and Plant Ecology of the Wrocław University of Environmental and Life Sciences. The Baltic macroalga was enriched with Cu(II) and Zn(II) ions by biosorption process. The solutions of Cu(II) and Zn(II) were prepared in 40 L of tap water by dissolving appropriate amounts of CuSO$_4$·5H$_2$O and ZnSO$_4$·7H$_2$O (from POCh S.A. Gliwice, Poland). Both inorganic salts were approved for use as a source of Cu and Zn in animal diets (Feeding Standards for Poultry and Swine 2005). The efficient biosorption process requires its earlier optimization i.e. the selection of the best process conditions [25]. Biosorption was carried out at room temperature for 4 h; the pH of the 300 mg L$^{-1}$ concentration solutions of was 5. The amount of dry mass (DM) in the plastic container was 1.0 g L$^{-1}$. The best process parameters were established in our previous papers [17]. After biosorption, the biomass of macroalgae was allowed to air dry and then was crushed in a blender (Braun). The content of Cu and Zn in the enriched dry biomass of macroalgae was 51.6 g kg$^{-1}$ and 56.4 g kg$^{-1}$ respectively. The produced feed additives were sent to the company LNB Poland Ltd. (Poland), which prepared a premix – a source of trace elements and vitamins. Three types of complete mixtures for growing pigs: „Starter”, „Grower” and „Finisher” were produced.

2.2 Feeding experiments on growing pigs

2.2.1 Animals, housing

The feeding experiments on growing pigs were approved by the Second Local Ethical Committee on Animal Testing at Wrocław University of Environmental and Life Sciences. The work referred to has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Feeding experiments lasted for 87 days and were divided into three series: “Starter” (26 days), “Grower” (31 days) and “Finisher” (30 days). They were conducted at the Experimental Animal Feeding Plant in Gorzyń (Poznań University of Life Sciences, Poland). The temperature
inside the buildings was 16–18°C with natural and artificial lighting. Dewormed (Dectomax® or Ivomec®) piglets (Big White Polish/Polish White Zwisloucha, dams×Hampshire/Pietrain) (24 pigs, 20.9 ± 2.2 kg) were randomly divided into two groups: 12 pigs were assigned to the control group and 12 allotted to the experimental group. Each group consisted of 8 barrows and 4 gilts. Each pig was ear-tagged. Pigs were housed and fed individually. The amount of the feed mixture was the same for all animals and it was 2.5 kg per day.

### 2.2.2 Feed

The standard feed was composed of ground wheat, ground barley, soybean meal, canola oil and a mixed diet that was specially prepared for each stage of the experiments (“Starter”, “Grower” and “Finisher”). A similar feed was used in the work of Korniewicz et al. (2012) and Saeid et al. (2013a) (Table 1) [23,26]. The applied mixtures of “Starter”, “Grower” and “Finisher” type were characterized by a similar energy level, but various levels of total protein, slightly different amino acid composition and also macro- and microelement content that corresponds to standards of pig feeding (Table 2) [26].

Both groups were fed with the same feed mixtures, which were characterized by the same content of basic nutrients, while in the control group the source of Zn and Cu were inorganic salts (ZnSO₄·7H₂O and CuSO₄·5H₂O) and in the experimental group the biomass of the Baltic macroalgae – Enteromorpha sp. enriched with the same ions. Table 3 presents the mineral composition of the natural biomass of algae, used for the production of biological feed additives.

| Ingredient | Unit | Type of mixture „Starter” „Grower” „Finisher" |
|------------|------|-----------------------------------------------|
| Ground wheat | % | 35.0 | 40.0 | 40.0 |
| Ground barley | % | 41.7 | 43.4 | 47.9 |
| Soybean meal | % | 15.5 | 11.5 | 8.00 |
| Canola oil | % | 3.30 | 1.80 | 1.40 |
| Lonacid Max (1017)b | % | 0.500 | 0.300 | 0.200 |
| Supplementary feed Starter | % | 4.00 | - | - |
| Supplementary feed Grower | % | - | 3.00 | - |
| Supplementary feed Finisher | % | - | - | 2.50 |
| TOTAL | % | 100 | 100 | 100 |

*aThe composition of the standard mixture for growing pigs was given by the producer
bLonacid Max (1017) – acidifier produced by LNB Poland

The chemical composition of feed mixtures for growing pigs.

| Ingredient | Unit | Type of mixture „Starter” „Grower” „Finisher" |
|------------|------|-----------------------------------------------|
| Net energy | kcal | 2.340 | 2.280 | 2.281 |
| Metabolisable energy | MJ | 13.6 | 13.2 | 13.2 |
| Dry mass | % | 87.3 | 87.2 | 87.1 |
| Crude protein | % | 17.4 | 15.7 | 14.5 |
| Crude fibre | % | 3.00 | 2.80 | 3.50 |
| Ether fat | % | 5.00 | 3.10 | 3.20 |
| Crude ash | % | 5.10 | 4.30 | 3.70 |
| Digestible nitrogen-free extract | % | 56.8 | 61.3 | 62.2 |
| Lysine | % | 1.17 | 0.930 | 0.850 |
| Methionine | % | 0.390 | 0.290 | 0.260 |
| Methionine+Cysteine | % | 0.710 | 0.600 | 0.550 |
| Threonine | % | 0.750 | 0.590 | 0.540 |
| Tryptophan | % | 0.230 | 0.200 | 0.160 |
| Isoleucine | % | 0.660 | 0.590 | 0.510 |
| Calcium total | % | 0.730 | 0.680 | 0.600 |
| Phosphorus total | % | 0.550 | 0.500 | 0.430 |
| Mineral phosphorus | % | 0.160 | 0.150 | 0.130 |
| Digestible phosphorus | % | 0.340 | 0.300 | 0.250 |
| Phytase | FTUa | 500 | 510 | 425 |
| Sodium total | % | 0.200 | 0.200 | 0.140 |
| Feb | mg | 198 | 183 | 172 |
| Mnb | mg | 91 | 82 | 73 |
| Cuc | mg | 167 | 25 | 22 |
| Znd | mg | 157 | 148 | 126 |
| Pd | mg | 1.66 | 1.49 | 1.26 |
| Cob | mg | 0.880 | 0.810 | 0.680 |
| Seb | mg | 0.490 | 0.480 | 0.440 |

*a1 FTU („Phytase Unit”) – is defined as that quantity of enzyme that will liberate inorganic phosphate at one micromole per minute from sodium phytate based on a 30-minute hydrolysis of sodium phytate at 37°C and pH 5.5.

Inorganic forms of Zn and Cu were removed from the basic mixture and replaced with enriched algae. The mineral composition of the feed for pigs in the control and experimental group is presented in Table 4. The level of toxic elements in the diet with macroalgae was below the permissible one. According to the Directive of Polish Ministry of Agriculture and Rural Development concerning the maximum acceptable levels of undesirable substances
in animal feed, the content should be no higher than 2.0 mg kg\textsuperscript{-1} for arsenic, 10 mg kg\textsuperscript{-1} for cadmium, and 1.0 mg kg\textsuperscript{-1} for lead.

### 2.2.3 Collection of samples

After 57 days of feeding, urine and feces were collected and weighed for 4 days. Urine was collected in plastic tanks under the pens with 10 mL of 10% sulfuric acid, which was added in order to bind ammonia nitrogen. Pigs’ feces were collected on a grid placed under the pens. Then they were thoroughly mixed and samples of 1 kg of feces and 1 L of urine were used for multielemental analysis. The 10% of daily collection of feces and urine was stored in special jars with ground glass stopper (urine) and in plastic bags (feces). The collected samples were stored in a refrigerator at 3–4°C.

After 85 days, in the morning, before feeding, the blood was collected from the jugular vein of pigs. Heparin was added to the samples in order to prevent blood coagulation. The blood serum was centrifuged in order to perform the biochemical analysis that aimed at the following biochemical markers: crude protein, albumins, glucose, urea, liver enzyme: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), total cholesterol and its fractions: HDL, LDL and triglycerides. They were determined by the analyzer: ABX Pentra 400; Horiba.

At 105 kg of body weight (BW), pigs were slaughtered according to meat industry standards and samples of liver and Longissimus dorsi muscle were collected. Slaughter procedure was carried out in a slaughterhouse with the required permits and according to Minister of Agriculture and Rural Development dated 02/04/2004 by the persons entitled to professional slaughter and acceptable methods of slaughter and killing of animals (Polish Journal of Laws 2004.70.643). Approved procedure involved the use of electronarcosis and exsanguination of pigs. After slaughter, samples of liver and meat were homogenized and kept in the freezer at a temperature of about -18°C before being subjected to chemical analysis.

### 2.3 Analytical methods

A microwave-assisted acid digestion Milestone MLS-1200 MEGA (Bergamo, Italy) apparatus was used for
biomass sample dissolution. Nitric acid, 65% trace-pure grade Merck (Darmstadt, Germany), was used as a reagent for the digestion of the samples. The following quantities of samples were used for the analysis: 0.5 g of biomass of macroalgae and feed, 4.0 g of blood, 1.0 g of meat samples taken from the *longissimus dorsi* muscle, 0.5 g of liver, 0.5 g of feces, 4.0 g of urine. Biological material was digested with 5 mL of nitric acid and after digestion filled with demineralized water to a volume of 50 mL. The concentration of mineral components was determined with the Inductively Coupled Plasma-Optical Emission Spectrometer coupled with with the ultrasonic nebulizer – Varian VISTA-MPX ICP-OES (Victoria, Australia). The accuracy of the method was checked by the regular use of certified reference materials: LGC 6010 “hard drinking water (UK) – metals” for the analysis of solutions and Oriental Tobacco Leaves (CTAOTL-I) from Institute of Nuclear Chemistry and Technology (Poland) for analysis of digested samples of the biomass. For the preparation of standard solutions (1.0, 10, 50, 100 mg L⁻¹) the multielemental standard (100 mg L⁻¹ Astasol®, Czech Republic) was used. The samples were analyzed in three repeats (the relative standard deviation of the measurement did not exceed 5%). The presented data are the arithmetic average from three measurements. The uncertainty of measurements by ICP-OES is as follows: < 0.1 mg kg⁻¹ ± 25%, from 0.1 to 1000 mg kg⁻¹ ± 15%, > 1000 mg kg⁻¹ ± 20% [18].

3. Results and Discussion

In the literature, the information about the application of macroalgae in pig feeding is rather insufficient. The effect of macroalgae on the composition of chemical elements in pigs is rarely studied. However, it was proved by He et al. (2002) that the application of brown macroalga, *Laminaria digitata*, in pig feeding caused a significant increase in iodine content in muscle, liver, kidney, and heart [27]. This is the first study that examines the chemical effect of Baltic macroalgae, enriched with Zn(II) and Cu(II) ions via biosorption, in the growing pigs.

3.1 Average feed intake, average weight gain and feed conversion ratio

In the present study it was shown that pigs’ performance and animal health were very good in both examined groups throughout the experiment. No pigs were excluded from the experiment. The production performance over all periods is summarized in Table 5. At the beginning of fattening, the animals had a similar body weight and health, and consumed the same amount of feed mixture. During feeding with the “Starter” mixture, the results in both groups were almost identical. Over the next 31 days (feeding with the “Grower” mixture), the daily weight gain of growing pigs and weight gain during this fattening period was only 5% lower in the algal group as compared to the control group. The same results were obtained during the third period, when the animals were fed the “Finisher” mixture and during the whole fattening period (87 days). No statistically significant differences were observed. This confirms the results obtained by Saeid et al. (2013a), who examined the effect of microalga *Spirulina* sp. enriched with Cu(II), Zn(II) and Fe(II) ions on the pig performance and the mineral composition of the samples (liver, meat, blood, etc.) [23].

3.2 The effect of macroalgae enriched with Cu and Zn on the mineral composition of blood, meat, liver, feces, and urine

Copper and zinc are two trace minerals involved in key metabolic reactions and are vital components of many enzymes and molecules in the organism [28]. Inorganic
salts are a commonly used form of supplementation of zinc and copper in feed, but they are characterized by a low absorption and bioavailability and therefore cause contamination of the environment [29]. From an environmental and economic point of view, it would be necessary to reduce trace mineral levels in diets close to requirements to minimize waste contamination [30]. The application of organic/biological forms of minerals can have a significant effect on reducing excretion of copper and zinc by pigs. Some experiments have shown that organic trace minerals might be added to the diet of pigs at levels considerably lower, compared to inorganic sources of trace minerals [31]. In our paper we compared the mineral content of blood, meat, liver, feces and urine in two groups of growing pigs: those fed with mixtures containing inorganic salts (CuSO₄·5H₂O and ZnSO₄·7H₂O) as feed additives and those fed with macroalgae enriched via biosorption process with Cu(II) and Zn(II) ions.

In the present work, we investigated the transfer of microelements from Baltic macroalga – Enteromorpha sp. enriched with microelements to blood, meat, liver, feces and urine as compared to inorganic salts. The biomass of macroalgae served as the carrier of a bioavailable

Table 5: The results of fattening of growing pigs during feeding with the „Starter”, „Grower” and „Finisher” mixtures and the average production results for the whole fattening period.

| Specification | C  | MA | P value  | Kind of statistical test |
|---------------|----|----|----------|--------------------------|
| „Starter” (26 days of fattening) |    |    |          |                          |
| Body weight (kg) | initial | 20.8±2.2 | 20.9±2.1 | 0.977 | Test t |
|                  | final   | 45.3±2.3 | 45.5±2.7 | 0.861 | Test t |
| Weight gain | in the period (kg) | 24.4±1.9 | 24.6±1.6 | 0.826 | Test t |
|                  | daily (g) | 942±73   | 943±61   | 0.959 | Test t |
| Feed intake in the „Starter” period (kg) | 48.9±1.1 | 48.7±1.6 | 0.954 | Mann-Whitney |
| Daily feed intake (kg) | 1.88±0.0404 | 1.87±0.0634 | 0.931 | Mann-Whitney |
| Feed conversion per kg of gain (kg) | 2.01±0.16 | 1.98±0.13 | 0.470 | Mann-Whitney |
| „Grower” (31 days of fattening) |    |    |          |                          |
| Body weight (kg) | initial | 45.3±2.3 | 45.5±2.7 | 0.861 | Test t |
|                  | final   | 76.0±2.2 | 74.5±5.2 | 0.368 | Cochrán-Cox |
| Weight gain | in the period (kg) | 30.7±1.8 | 29.0±3.2 | 0.149 | Mann-Whitney |
|                  | daily (g) | 987±60 | 935±104 | 0.184 | Mann-Whitney |
| Feed intake in the „Grower” period (kg) | 81.4±3.8 | 78.7±6.3 | 0.234 | Cochrán-Cox |
| Daily feed intake (kg) | 2.62±0.12 | 2.54±0.20 | 0.243 | Cochrán-Cox |
| Feed conversion per kg of gain (kg) | 2.65±0.068 | 2.74±0.31 | 0.350 | Cochrán-Cox |
| „Finisher” (30 days of fattening) |    |    |          |                          |
| Body weight (kg) | initial | 76.0±2.2 | 74.5±5.2 | 0.368 | Cochrán-Cox |
|                  | final   | 107±3 | 104±6 | 0.204 | Mann-Whitney |
| Weight gain | in the period (kg) | 31.1±2.0 | 29.5±4.0 | 0.249 | Cochrán-Cox |
|                  | daily (g) | 1036±68 | 985±132 | 0.251 | Cochrán-Cox |
| Feed intake in the „Finisher” period (kg) | 95.1±4.9 | 93.5±8.5 | 0.885 | Mann-Whitney |
| Daily feed intake (kg) | 3.17±0.16 | 3.12±0.28 | 0.817 | Mann-Whitney |
| Feed conversion per kg of gain (kg) | 3.06±0.21 | 3.20±0.43 | 0.525 | Mann-Whitney |
| Whole fattening period (87 days) |    |    |          |                          |
| Body weight (kg) | initial | 20.6±2.6 | 20.9±2.1 | 0.764 | Test t |
|                  | final   | 107±1 | 104±6 | 0.204 | Mann-Whitney |
| Weight gain | in the period (kg) | 86.5±3.9 | 83.2±6.1 | 0.157 | Mann-Whitney |
|                  | daily (g) | 992±44 | 956±70 | 0.204 | Mann-Whitney |
| Feed intake in the whole fattening period (kg) | 225±8 | 221±12 | 0.356 | Mann-Whitney |
| Daily feed intake (kg) | 2.59±0.086 | 2.52±0.15 | 0.248 | Mann-Whitney |
| Feed conversion per kg of gain (kg) | 2.61±0.10 | 2.66±0.13 | 0.328 | Test t |
form of microelements in the diet of animals. We based the development of new biological feed additives with micronutrients for animals on the similarity to the amino acid chelates available on the market. In both chelates and the biomass, cations (i.e. Cu(II), Zn(II)) are bound to the carboxyl group of biomass/chelate. Due to the similar chemistry of the process, it was hypothesized that the bioavailability of micronutrient cations from amino acids and the biomass was similar [3]. Therefore, in the discussion of the obtained results we compared the bioavailability of feed additives based on the biomass of macroalgae with organic forms – chelates. It should be noted that in our study the content of minerals in the mixture in the experimental group was lower than that in the control group.

### 3.2.1 The mineral composition of blood

In Table 6 presents the average content of minerals in the blood from examined growing pigs. The concentrations of macro- and microelements in the blood samples from both groups were comparable. There was no statistically significant difference in terms of the content of the analyzed elements between the control and experimental groups. In the case of the examined minerals, the average content of Zn in the blood from the growing pigs fed with enriched algae was by 9.5% higher than in the blood from the growing pigs in the control group, and in the case of Cu by only 0.5%. Results similar to ours were obtained by Revy et al. (2002), who examined the bioavailability of two sources of zinc in weanling pigs: ZnOrg as a zinc-methionine complex and Zn as sulfate (ZnSO$_4$·7H$_2$O). It was found that the source of supplemental Zn did not influence plasma Zn concentration [32]. Also Hernández et al. (2008) pointed that the concentration of Cu and Zn in the blood of pigs fed with the organic form of these microelements as proteinate amino acid chelate was higher by 4% and 11%, respectively, compared to the blood of pigs, the diet of which was supplemented with such inorganic form as sulfate after 77 days of the experiment (the total content of Cu and Zn in feed was 156 mg kg$^{-1}$ and 170 mg kg$^{-1}$, respectively) [33]. In the study of Wedekind et al. (1994), the concentration of Zn in the plasma of pigs was similar for all tested forms of zinc: zinc-methionine complex, zinc-lysine complex and ZnSO$_4$·H$_2$O. However, a slight decrease in plasma Zn concentration was observed in the case of the application of organic forms: by 4% for the zinc-methionine and 6% for the zinc-lysine complex when compared with ZnSO$_4$·H$_2$O [34].

#### Table 6: The effect of enriched macroalgae on the average content of elements in blood of examined pigs (mg L$^{-1}$).

| Element | C (n = 12) | MA (n = 12) | P value |
|---------|------------|-------------|---------|
| Co      | 0.254±0.034| 0.229±0.048 | 0.379   |
| Cu      | 0.841±0.143| 0.845±0.190 | 0.422   |
| Fe      | 217±31    | 204±33.5    | 0.542   |
| Mn      | 0.0126±0.0033 | 0.0145±0.0038 | 0.443 |
| Zn      | 2.21±0.17 | 2.42±0.68   | 0.917   |
| Ca      | 50.2±7.8  | 56.9±17.2   | 0.754   |
| K       | 971±90   | 929±111     | 0.532   |
| Mg      | 22.8±2.8  | 22.5±4.5    | 0.899   |
| Na      | 866±115  | 863±153     | 0.754   |

*P values were determined with the use t test (for independent samples) for Co, Cu, Fe, K, Mg, Mn and Mann-Whitney Test for Ca, Na and Zn

#### 3.2.2 Biochemical markers in the serum of examined pigs

In the case of biochemical markers determined in the serum of pigs, there were no statistically significant differences ($P < 0.05$) between the control and experimental groups (Table 7). However, the algal group was characterized by a lower concentration in serum as opposed to the control group of the following markers: AST – about 10%, GGT – about 11%, creatine kinase – about 27%, creatinine – about 5%, lactic acid – about 16%. The lower content of AST and GGT (enzymes produced in the liver cell) in the serum of growing pigs from the experimental group could indicate better functions of the liver. Enriched biomass of algae contributed to the increase in the concentration of triglycerides in the serum in the experimental group (17% more than in the control group) and in glucose (about 7% more). The elevated level of triglycerides in the algal group was rather due to individual variation, since the total cholesterol and its fractions – HDL and LDL – in both groups were at similar levels. It may be assumed that the addition of algae did not affect the lipid balance. A higher concentration of glucose in the blood serum of the experimental group indicates a more energy-efficient metabolism. Glucose affects the balance of the protein and energy. Other differences were less than 5%. Similar results were obtained when feeding experiments were carried out on pigs, which aimed at the comparison of the effects of brown macroalga Laminaria digitata, rich in iodine and inorganic forms (KI), on the content of this
Table 7: The concentration of biochemical markers in the serum of growing pigs.

| Specification       | C (n = 12) | MA (n = 12) | P value |
|---------------------|------------|-------------|---------|
| Biochemical markers |            |             |         |
| Crude protein (g L$^{-1}$) | 65.6±6.5  | 63.1±2.9  | 0.284   |
| Albumins (g L$^{-1}$)  | 41.8±3.1  | 40.4±2.5  | 0.0821  |
| Globulins (g L$^{-1}$) | 23.8±6.1  | 22.7±3.2  | 0.880   |
| Urea (mmol L$^{-1}$)  | 5.7±0.75  | 5.4±0.85  | 0.433   |
| Glucose (mmol L$^{-1}$) | 5.13±0.85 | 5.51±0.77 | 0.310   |
| ALT (U L$^{-1}$)      | 33.1±4.2  | 34.4±10.0 | 0.215   |
| AST (U L$^{-1}$)      | 63.0±10.6 | 56.8±11.0 | 0.713   |
| GGT (U L$^{-1}$)      | 34.6±8.9  | 30.9±8.0  | 0.330   |
| Creatine kinase (U L$^{-1}$) | 1.48±1.25 | 1.08±0.86 | 0.151   |
| Lactate dehydrogenase (U L$^{-1}$) | 903±154 | 876±337 | 0.151   |
| LDH (U L$^{-1}$)      | 120±13    | 114±11    | 0.306   |
| Creatinine (µmol L$^{-1}$) | 4.49±2.00 | 3.78±1.83 | 0.199   |
| Lactic acid (mmol L$^{-1}$) | 2.40±0.28 | 2.44±0.21 | 0.717   |
| Cholesterol total (mmol L$^{-1}$) | 1.12±0.18 | 1.11±0.12 | 0.693   |
| HDL (mmol L$^{-1}$)   | 10.7±0.13 | 1.05±0.14 | 0.482   |
| LDL (mmol L$^{-1}$)   | 2.33±0.0650 | 2.62±0.0498 | 0.169 |

$*$ $P$ value was determined by the use of test t for independent samples in groups, besides: AST, (Cochran-Cox test for independent samples in groups) and LDH, creatine kinase, lactic acid, albumins, globulins (Mann-Whitney test for independent samples in groups).

Table 8: The effect of enriched macroalgae on the average content of elements in meat of examined pigs (mg kg$^{-1}$).

| Element     | C (n = 12) | MA (n = 12) | P value |
|-------------|------------|-------------|---------|
| $\bar{x}$±SD |            |             |         |
| Co          | <LLD       | <LLD        | -       |
| Cu          | 0.76±0.095 | 0.84±0.238  | 0.650   |
| Fe          | 7.83±1.72  | 8.86±2.33   | 0.290   |
| Mn          | 0.136±0.016 | 0.203±0.058 | 0.00250 |
| Zn          | 25.6±4.1   | 26.6±4.5    | 0.592   |
| Ca          | 214±33     | 341±223     | 0.112   |
| K           | 5989±630   | 5594±947    | 0.0821  |
| Mg          | 366±46     | 344±60.9    | 0.112   |
| Na          | 603±92     | 561±51.0    | 0.231   |

*statistically significant differences ($P<0.05$) were written in Italic.*

Microelements

Macronutrients

Table 8 shows the average mineral content in meat derived from the growing pigs in the control and experimental group. A statistically significant difference was observed only for Mn. However, the content of trace elements in the meat of the piglets fed with enriched algae was higher (Mn − 49% ($P < 0.05$), Fe − 13%, Cu − 12% and Zn by only 4.0%) than in the meat of the piglets from the control group. In the work by Witkowska et al. (2014b) it was found that the addition of soybean meal enriched via biosorption process with Cu(II), Mn(II), Fe(II) and Zn(II) to the feed of fatteners increased the level of iron in the meat by about 10% [22].

In the available literature it was also confirmed that the application of algae causes an increased level of elements in meat. Dierick et al. (2009) found a significant increase in iodine content in the muscles of pigs: from 15.5 µg kg$^{-1}$ in the control group to 59.5 µg kg$^{-1}$ in the seaweed group (for *M. psoas*) and from 19.9 µg kg$^{-1}$ in the control group to 55.4 µg kg$^{-1}$ in the seaweed group (for *M. longissimus dorsi*) [4]. In the work of Svoboda et al. (2009) it was also noticed that the pigs which were given selenium from Se-enriched alga (*Chlorella* sp.) had a significantly higher content of Se in muscle compared to the pigs that received the inorganic Se source [35]. Consumption of meat biofortified with microelements can contribute to the prevention of micronutrient deficiencies.

3.2.3 The mineral composition of meat

Table 9 presents the average content of elements accumulated in the liver of growing pigs. The liver of growing pigs from the experimental group contained 16% less Cu and 18% less Zn than the liver in the control group. Statistically significant differences between groups were observed in the quantity of accumulated Zn and Ca. Other authors also observed that the content of elements in the liver in the case of the supplementation of organic forms of elements was lower than in the case of the supplementation of inorganic mineral feed additives. Svoboda et al. (2009) found that Se content in the liver of pigs did not differ between groups of pigs fed with Se-enriched alga *Chlorella* sp. and the group fed with an
The effect of enriched macroalgae on the average content of elements in liver of examined pigs (mg kg⁻¹).

| Element | C (n = 12) | MA (n = 12) | P value |
|---------|------------|-------------|---------|
| Co      | 1.13±1.00  | 0.849±0.688 | 0.585   |
| Cu      | 34.0±13.2  | 28.7±8.8    | 0.564   |
| Fe      | 732±200    | 734±189     | 0.981   |
| Mn      | 9.44±1.42  | 10.3±1.1    | 0.102   |
| Zn      | 334±57     | 273±69.4    | 0.0347  |
| Ca      | 175±14     | 201±21      | 0.0089  |
| K       | 8.35±2.22  | 8.23±2.43   | 0.304   |
| Mg      | 503±14     | 493±25      | 0.321   |
| Na      | 1.95±2.15  | 1.93±2.97   | 0.760   |

*statistically significant differences (P<0.05) were written in Italics
* P values were determined with the use t test (for independent samples) for Ca, Zn, Fe, K, Mg, Na, Co and Mann-Whitney Test for Cu and Mn

The effect of enriched macroalgae on the average content of elements in feces of examined pigs (mg kg⁻¹).

| Element | C (n = 12) | MA (n = 12) | P value |
|---------|------------|-------------|---------|
| Co      | 7.18±0.71  | 7.01±1.08   | 0.873   |
| Cu      | 247±49     | 71.0±7.2    | 0.000260|
| Fe      | 4.274±934  | 3.085±371   | 0.0159  |
| Mn      | 64.2±51    | 645±78      | 0.950   |
| Zn      | 1.21±107   | 1.54±291    | 0.0374  |
| Ca      | 22354±2616 | 19226±3911  | 0.134   |
| K       | 8.19±1000  | 9.507±1553  | 0.112   |
| Mg      | 6.106±560  | 5.576±625   | 0.153   |
| Na      | 3.374±626  | 3.816±1727  | 0.462   |

*statistically significant differences (P<0.05) were written in Italics
* P values were determined with the use t test (for independent samples) beside for Co (Mann-Whitney Test)

The diet of which was supplemented with an inorganic form as sulfate, after 77 days of the experiment (the total content of Cu and Zn in feed was 156 mg kg⁻¹ and 170 mg kg⁻¹ respectively) [33]. However, Apgar et al. (1995) found that the supplementation of the organic form of Cu in the diet of weanling pigs in the form of the copper-lysine complex caused an increase in Cu content in liver by 102% when compared with CuSO₄ [36]. Also Schiavon et al. (2000) examined the effect of proteinate or sulphate mineral sources on trace elements content in the liver of piglets. The Cu content was 17% higher in the liver and Zn by 4.5% in the case of the supplementation of these microelements as proteinate in comparison with sulphates [37].

3.2.5 The mineral composition of feces and urine

Table 10 shows the average content of elements in the feces excreted by growing pigs in the control and experimental group and the statistically significant differences in the level of mineral excretion between these two groups. Growing pigs fed with mixture with the addition of macroalgae excreted 27% more Zn than growing pigs in the control group, but 3.5 times less Cu and 28% less Fe. Statistically significant differences between the two groups were observed for these elements.

Lee et al. (2001) evaluated the efficacy of different metal-amino acid chelates and complexes of copper and zinc on fecal excretions by weanling pigs. It was found that in the group of pigs with the addition of 170 mg kg⁻¹ of Cu-amino acid, the chelate excretion of Cu with feces was 38% lower than in the group with CuSO₄, and in the group with the Cu-lysine complex – 34% lower. In the case of Zn supplemented to the feed at an amount of 120 mg kg⁻¹, the excretion of Zn in the group with Zn-amino acid chelate was 20% lower than in the group with ZnSO₄, but in the group with Zn-methionine complex slightly higher – 0.3% [38].

Table 11 presents the average content of microelements and light metals in the urine of growing pigs from the control and experimental groups. Statistically significant differences were observed between the following elements: Co (urine from the experimental group contained three times more Co than urine from the control group), Zn (4 times), Mg (2 times), K (about 78% more), Na (about 53% more), Fe (45% less). Revy et al. (2002) also reported higher concentrations of Zn in urine in the group of weanling pigs which were fed with organic form of Zn (zinc-methionine complex) compared to the group fed with addition of ZnSO₄·7H₂O (for the Zn level 10, 20 and 30 mg kg⁻¹ of feed the increase was by 34.7%, 63.3% and 1.1%, respectively).
Table 11: The effect of enriched macroalgae on the average content of elements in urine of examined pigs (mg L⁻¹).

| Element | C (n = 12) | MA (n = 12) | P value |
|---------|-----------|-----------|--------|
|        | ±S      | ±S      |
| Co      | 0.0093±0.00467 | 0.0215±0.0113 | 0.0352 |
| Cu      | 0.0812±0.0238 | 0.0861±0.0216 | 0.714  |
| Fe      | 1.92±0.72 | 1.05±0.38 | 0.0261 |
| Mn      | 0.12±0.034 | 0.205±0.088 | 0.0607 |
| Zn      | 1.32±0.49 | 4.88±2.25 | 0.00355 |
| Ca      | 146±39 | 265±150 | 0.0913 |
| K       | 1081±214 | 1921±746 | 0.0242 |
| Mg      | 87.8±24.4 | 186±68 | 0.00732 |
| Na      | 281±55 | 429±78 | 0.00349 |

* statistically significant differences (P<0.05) were written in Italics
* P values were determined with the use of t test (for independent samples)

Summarizing our results, it was found that in animals fed with feed with the addition of algae, the removal of Cu in the feces was greatly reduced (by 71%), while in the urine it was slightly increased (6%) as compared with the control group. In the case of Zn content, an increase in both the feces (27%) and urine (270%) was observed, in comparison with the control group. However, these results may demonstrate a higher absorption of biological feed additives than inorganic ones. Animal organisms fed with a fodder with an addition of enriched algae absorbed greater amounts of both microelements. Some of them were accumulated in the animals’ meat and liver, some were circulating with blood and unnecessary amounts were excreted.

4 Conclusions

In the present paper, Baltic macroalgae – Enteromorpha sp. enriched with Zn(II) and Cu(II) ions via biosorption was used in pig feeding. It was shown that the new, natural feed additive with microelements did not influence significantly the body weight (initial and final), the weight gain, the daily and periodic feed consumption and feed conversion per 1 kg of gain in both control and experimental groups. It was also noted that enriched macroalgae did not affect significantly the biochemical parameters in serum such as crude protein, albumins, glucose, urea, and liver enzymes: AST, ALT, GGT, total cholesterol and its fractions: HDL, LDL and triglycerides. However, the study confirmed that macroalgae enriched with microelements could be used to increase the mineral content of meat. Higher concentrations of elements were observed in the blood in the experimental group, which should be confirmed in other biological materials (i.e. liver, meat). It was demonstrated that the use of biological additives resulted in the enrichment of meat in trace elements: with manganese Mn – 49% (P < 0.05), iron (13%), copper (12%) and zinc (4.0%) when compared with the control group. Our results confirmed that the biological form of microelements was bioavailable for animals more than the inorganic form. This kind of new, biological feed additives could constitute an alternative to inorganic mineral salts, which are commonly used in feed industry, or to their supplementation. This approach will also allow to partially deal with the problem of excessive secretion of trace elements to the environment. As it was shown that the growing pigs fed with the preparation containing macroalgae excreted in feces 3.5 times less Cu than those in the control group.

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