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Article Effect of Marine Macroalga Enteromorpha sp. Enriched with Zn(II) and Cu(II) ions on the Digestibility, Meat Quality and Carcass Characteristics of Growing Pigs

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Abstract: In the present study, the effect of macroalga *Enteromorpha* sp. enriched with Zn(II) and Cu(II) ions on daily amounts of feces and urine excreted by growing pigs, apparent fecal nutrient digestibility and daily nitrogen balance and retention, meat quality and the slaughter value of carcasses was examined. The duration of feeding experiments was 87 days. In the control group, the requirement for zinc and copper was covered by inorganic salts, whereas in the experimental group algae enriched with these elements via biosorption were supplemented. No effect of *Enteromorpha* sp. on the increase in digestibility of dry matter, dry organic matter, crude protein, crude fat and nitrogen-free extractives was observed. Statistically significant differences concerned only the digestibility of crude ash. The daily amount of excreted feces and urine did not differ significantly between groups. Meat from pigs in the algal group was characterized by a lower water absorption and drip loss and contained less fat and more protein than meat from the control group. Furthermore, a slight darkening of the meat was observed. The weight of the liver was lower in pigs from the algal group. Enriched macroalga *Enteromorpha* sp. may be introduced into pig nutrition as a feed material as an alternative to inorganic salts.

Keywords: green macroalgae; microelements; feed additive; feeding experiment; growing pigs

1. Introduction

Seaweeds (called also macroalgae) have been used for millennia as a feed supplement in order to improve animal nutrition and productivity [1]. Macroalgae are recognized as a valuable raw material for the production of feed additives due to their enormous biodiversity, which can be exploited, and the fact that seaweeds are widely used as foods, fertilizers, components of pharmaceuticals, cosmetics, etc. [2,3]. Limited animal studies suggest that seaweeds may be also used in pig nutrition in order to ameliorate gut health, to boost the immune system and growth performance. In the present paper, the application of marine green macroalgae as a feed additive for pigs is proposed. In the review papers of Makkar et al. (2016) [4], Angell et al. (2016) [5], Corino et al. (2019) [6] and Øverland et al. (2019) [7], it was shown that seaweeds can serve as a source of active compounds for pigs, such as polysaccharides, proteins and amino acids (lysine, histidine, isoleucine, leucine, arginine, methionine, phenylalanine, threonine, tryptophan, valine, tyrosine, alanine, glutamine, asparagine), lipids including omega 3 and 6 fatty acids, vitamins (E, A, C, B₁, B₂, B₃), minerals (Ca, Mg, P, K, Na, Mn, Zn, Fe, Cu, I, Se, Co), phenolic compounds (e.g., phlorotannins) etc. [4–7]. These compounds demonstrate positive health effects, such as prebiotic, antibacterial, antioxidant, anti-inflammatory and immunostimulant

effects. [4,6]. Seaweeds in pigs' nutrition show a beneficial influence on the digestibility of feed (nitrogen, polysaccharides, fiber, dry matter, organic matter), health and welfare of pigs [6]. Due to the presence of sulfated polysaccharides, such as alginates, ulvans and fucoidans, seaweeds also play prebiotic functions and positively modulate the intestinal microbiota [6,8]. Seaweeds can be also considered as potential antibiotic replacers in pigs [4,6]. Healthy and valuable feed is responsible for animal health and thus the high quality of animal products such as meat.

Seaweeds in pig feed are used in different forms—as a dried biomass, as extracted compounds (mainly polysaccharides—alginates, laminarin and fucoidan), or seaweed extracts [6]. Among brown (Phaeophyceae), red (Rhodophyceae) and green seaweeds (Chlorophyceae), brown algae dominate in pig nutrition (e.g., Ascophyllum nodosum [4,9,10], Fucus vesiculosus [4], Laminaria japonica [11], Laminaria sp., as well as laminarin and fucoidan extracted from *Laminaria* [4,8]). In this work, we propose to utilize the valuable composition and properties of marine green macroalgae (*Enteromorpha* sp.) for the production of feed additives with microelements. Dry seaweeds are able to bind efficiently metal ions from the aqueous solutions to the functional groups that are present on the surface of biomass [12–14]. In the present study, we enriched the algal biomass with microelements—Cu(II) and Zn(II)—using a rapid and reversible process called biosorption [13,14]. These two elements were chosen, since they are crucial for animals and are known to exert positive influence on growth performance of young pigs [15]. On the other hand, there are some concerns associated with the increase in Cu and Zn load in the environment, derived mainly from piggery effluents, which could have adverse effects on the soil microbiota and potentially cause a decline in soil fertility and pasture, as well as on crop yields [16]. Therefore, there is a need to reduce the level of copper and zinc in the diet of growing pigs without detrimental effects on the production and mineral status. The solution is to replace traditionally used inorganic salts, which are not easily absorbed by organic form of minerals that can increase the mineral absorption and retention in pigs [17]. In our previous work, we evaluated the effect of the enriched with Zn(II) and Cu(II) ions macroalga Enteromorpha sp. on the mineral composition of blood, meat, liver, feces and urine of growing pigs, production parameters, as well as biochemical markers such as crude protein, albumins, glucose, urea, liver enzyme: aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, total cholesterol and its fractions: high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides. It was found that the bioavailability of microelements to pigs from algae was higher than from inorganic salts that were supplemented in the control group [18]. Moreover, Dierick et al. (2009) suggested that brown seaweed—Ascophyllum nodosum—may be introduced into pigs' diets as a feed material with a double role: the improvement of pig gut health and performance, and the iodine enrichment of porcine tissues [9].

The aim of the present study was to examine the effect of enriched with Cu(II) and Zn(II) ions *Enteromorpha* sp. on the daily amount of feces and urine excreted by growing pigs, apparent fecal nutrient digestibility, daily nitrogen balance and retention, meat quality and slaughter value of carcasses.

2. Materials and Methods

2.1. Raw Material

The alga *Enteromorpha* sp. was collected from the Baltic Sea (Niechorze—Poland) and identified in the Department of Botany and Plant Ecology of Wrocław University of Environmental and Life Sciences (Poland). This macroalga dominates in the macrophytobenthos on the Polish coast. Large quantities of seaweeds result from eutrophication. The touristic attractiveness of the seaside resorts nearby is therefore reduced [19]. On the other hand, this edible macroalga is characterized by a high nutritional value. It has been shown that *Enteromorpha* sp. is rich in lipids (in % of dry mass (DM)): from 3.47 ± 1.76 to 4.36 ± 2.17 [20], in proteins: from 9.42 ± 4.62 to 20.6 ± 5.0 [21], in carbohydrates: from 29.1 ± 6.44 to 39.8 ± 11.2 [21] and minerals [18,22]. The examined *Enteromorpha* sp. from the Baltic Sea contains microand macroelements in the amounts: Co 1.18 ± 0.18 mg/kg, Cu 2.17 ± 0.33 mg/kg, Fe 705 ± 106 mg/kg,

Mn 51.0 \pm 7.6 mg/kg, Zn 15.2 \pm 2.3 mg/kg, Ca 9040 \pm 1810 mg/kg, K 15,400 \pm 3100 mg/kg, Mg 20,500 \pm 4100 mg/kg, Na 19,400 \pm 3900 mg/kg [18].

2.2. Production of Algal Feed Additives

Baltic macroalga was enriched with Cu(II) and Zn(II) ions through biosorption. The solutions of microelements were prepared in 40 L of tap water (by dissolving appropriate amounts of CuSO₄·5H₂O and ZnSO₄·7H₂O (Avantor Performance Materials Poland S.A., Gliwice, Poland). Biosorption was carried out at room temperature for 4 h and the pH of solutions with a concentration of Cu(II) and Zn(II) equal to 300 mg/L was 5. The content of dry biomass was 1 g/L [18]. The best process parameters were established in our previous research [12]. After biosorption, the biomass of macroalgae was dried in air and then crushed in a blender. The content of Cu and Zn in the enriched algal biomass was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and was equal to 51.6 g of Cu/kg of dry mass (DM) and 56.4 g Zn/kg, respectively [18].

Three types of complete mixtures for growing pigs—"Starter"(S), "Grower" (G) and "Finisher" (F)—were produced and added to the standard feed, composed of ground wheat (S: 35%, G: 40% and F: 40%), ground barley (S: 41.7%, G: 43.4% and F: 47.9%), soybean meal (S: 15.5%. G: 11.5%, F: 8%), canola oil (S: 3.3%, G: 1.8%, F: 1.4%) and acidifier Lonacid Max (1017) (S: 0.5%, G: 0.3%, F: 0.2%), supplementary feed (S: 4.0%, G: 3.0%, F: 2.5%) [18,23]. The produced enriched algal biomass was sent to the company LNB Poland Ltd. (Poland), which was responsible for the preparation of premix—a source of trace elements and vitamins for growing pigs. The detailed chemical composition of the feed mixture is presented in Table 1.

Taking into account the content of Cu and Zn in the standard feed, the coverage of the requirement for these elements according to the Feeding Standards for Poultry and Swine (2005) [24] was calculated. The difference in the standard feed was supplemented by enriched algae (premix)—Table 2. The limiting factor was copper—the content of this microelement in the feed for growing pigs should not exceed 25 mg/kg feed.

The mineral composition of the feed used in the feeding experiments on growing pigs, both for the control group—microelements supplemented by inorganic salts (C), as well as for the experimental groups—microelements supplemented by enriched macroalga (MA) is presented in Table 3 [18].

2.3. Feeding Experiments on Growing Pigs

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. This work was carried out in accordance with EU Directive 2010/63/EU for animal experiments. These experiments were conducted at the Experimental Station of the Poznań University of Life Sciences in Gorzyń (Poland) and lasted for 87 days. The general scheme of these studies is shown in Figure 1.

The buildings where pigs were housed were cleaned and disinfected before experiments. The temperature inside the buildings was 16–18 °C. Natural and artificial lighting illuminated the whole area. Growing pigs originated from the following breeds: sow—the Polish Landrace/Polish Large White cross and boar—Hampshire/Pietrain cross. The study was conducted on two groups—the control and experimental. There were 12 piglets (eight barrows and four gilts) in each group. Both groups were fed with feed mixtures, which were characterized by the same content of nutrients, but in a different form. The control group received Zn and Cu as inorganic salts—CuSO₄·5H₂O and ZnSO₄·7H₂O—and the experimental group *Enteromorpha* sp. enriched with Cu and Zn (from the basic mixture, inorganic forms of Zn and Cu were removed and replaced by enriched algae). Before the start of the study, each pig was marked by ear tagging and also dewormed (Dectomax®or Ivomec®). Animals were kept in individual pens in order to control the feed mixture intake [18,23].

Table 1. The chemical composition of feed mixtures for growing pigs (Reproduced with	permission
from Saeid et al., J. Appl. Phycol.; published by Springer, 2013 [23]).	

In gradiant (in 1 kg of Misturg)	TT */	Type of Mixture			
ingredient (in 1 kg of Mixture)	Unit –	"Starter"	"Grower"	"Finisher"	
Net energy	kcal	2340	2280	2281	
Metabolizable 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 fiber	%	3.00	2.80	3.50	
Crude fat	%	5.00	3.10	3.20	
Crude ash	%	5.10	4.30	3.70	
N-free extractives	%	56.8	61.3	62.2	
L-Lysine	%	1.17	0.93	0.85	
Methionine	%	0.39	0.29	0.26	
Methionine+Cysteine	%	0.71	0.60	0.55	
L-Threonine	%	0.75	0.59	0.54	
Tryptophan	%	0.23	0.20	0.16	
Isoleucine	%	0.66	0.59	0.51	
Calcium (Ca) total	%	0.73	0.68	0.60	
Phosphorus (P) total	%	0.55	0.50	0.43	
Mineral phosphorus (P)	%	0.16	0.15	0.13	
Digestible phosphorus (P)	%	0.34	0.30	0.25	
Phytase	FTU ^a	500	510	425	
Sodium (Na)	%	0.20	0.20	0.14	
Iron (Fe) ^b	mg	198	183	172	
Manganese (Mn) ^b	mg	91	82	73	
Copper (Cu) ^b	mg	167	25	22	
$Zinc (Zn)^b$	mg	157	148	126	
Iodine (I) ^b	mg	1.66	1.49	1.26	
Cobalt (Co) ^b	mg	0.88	0.81	0.68	
Selenium (Se) ^b	mg	0.49	0.48	0.44	
Vitamin A ^c	I.U.	16,000	12,000	10,000	
Vitamin D ₃ ^c	I.U.	2000	1998	1665	
Vitamin E ^c	mg	150	124	104	
Vitamin K ₃ ^c	mg	4.00	1.80	1.50	
Vitamin B_1^{c}	mg	2.40	1.80	1.50	
Vitamin B_2^{c}	mg	6.40	4.80	4.00	
Vitamin B_3 (Niacin) ^c	mg	32.0	24.0	20.0	
Vitamin B ₅ (Pantothenic acid) ^c	mg	16.0	12.0	10.0	
Vitamin B ₆ ^c	mg	4.8	3.6	3.0	
Vitamin B ₁₂ ^c	mcg	40.0	30.0	25.0	
Vitamin C ^c	mg	100	100	83.3	
Biotin ^c	mcg	160	120	100	
Folic acid ^c	mg	3.20	2.40	2.00	
Choline ^c	mg	350	250	208	

^a One "Phytase Unit" (FTU) 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; ^b Microelements supplemented: Fe as FeSO₄·H₂O 30%; Mn as MnO₂ 60%; Cu as CuSO₄·5H₂O 25%; Zn as ZnSO₄·H₂O 35%; I as Ca (IO₃)₂·H₂O 62%, Co as CoCO₃ 21%; Se as Na₂SeO₃ 5%; ^c Vitamins supplemented: retinyl acetate (A), cholecalciferol (D₃), DL α -tocopherol acetate (E), menadione sodium bisulfite (K), thiamine mononitrate (B₁), riboflavin (B₂), nicotinic acid; niacin (B₃), pantothenic acid; D-calcium pantothenate (B₅), pyridoxine hydrochloride (B₆), cyanocobalamin(B₁₂), ascorbic acid (C), D-biotin (biotin), folic acid, choline chloride (choline).

Microelement	Requirement	"Starter"	"Grower"	"Finisher"
Cu	Requirement for Cu in the standard feed that should be covered by the feed additive	25%	84%	84%
	The coverage of the requirement by enriched algae (as a premix)	26%	100%	100%
Zn	Requirement for Cu in the standard feed that should be covered by the feed additive	28%	15%	15%
	The coverage of the requirement by enriched algae (as a premix)	32%	18%	18%

Table 2. Enriched with microelements *Enteromorpha* sp. added to the mixtures in order to cover the requirement for Cu and Zn (%).

Table 3. The mineral composition of the feed for pigs in the control and experimental groups (Data from Michalak et al., Open Chem., De Gruyter Open, 2015 [18]).

	"Starter"		"Grower"		"Fini	sher″
Element	С	MA	С	MA	С	MA
	mean ± SD (mg/kg DM)					
Ca	4412 ± 1484	4476 ± 698	4038 ± 787	3819 ± 581	3611 ± 595	2872 ± 732
Cu	13.3 ± 7.6	4.08 ± 0.62	9.06 ± 2.16	5.54 ± 1.06	11.3 ± 3.7	6.16 ± 4.9
Fe	206 ± 141	166 ± 87	149 ± 79	193 ± 56	205 ± 9	91.2 ± 63
Κ	3550 ± 716	3216 ± 444	3459 ± 593	3461 ± 566	3721 ± 270	2854 ± 576
Mg	788 ± 156	728 ± 107	767 ± 125	750 ± 118	846 ± 72	674 ± 123
Mn	82.0 ± 29.7	61.3 ± 16.3	66.4 ± 21.0	77.0 ± 15.5	63.7 ± 12.8	52.0 ± 16.7
Na	999 ± 187	909 ± 111	866 ± 118	890 ± 136	841 ± 84	745 ± 222
Zn	91.4 ± 22.9	73.0 ± 9.8	78.1 ± 17.5	76.0 ± 14.3	86.5 ± 11.4	62.3 ± 14.8



Figure 1. General scheme of feeding experiment on growing pigs.

During the production test, 12 barrows (six in each group) with a body weight of approximately 50–60 kg, were tested in balance and digestibility trials. Exactly 31 days after of feeding with "Grower" mixture, barrows were placed in individual metabolism cages, where they were fed with the same mixtures as during the whole experiment. The amount of the feed mixture was the same for all animals and it was ca. 2.5 kg per day. Every morning, the uneaten remains of the mixture were determined by weighing. The period of the first 3 days was treated as preliminary—the preparatory period after changing the living conditions. Over a further 4 days (proper period), the amount of ingested mixture and the quantity of feces and urine was recorded each day. Pigs urine flew into a special plastic tanks

placed under the cages. Into these tanks, every day, 10 mL of 10% sulfuric acid was poured in order to bind the ammoniacal nitrogen. Pigs' feces were stopped on a grid placed under the grill of the pens. The daily collections of 10% pig feces and urine were collected in special jars with ground glass stoppers (urine) and in plastic bags (feces). The collected samples were stored in a refrigerator at 3–4 °C. Urine and feces collected during the period were thoroughly mixed.

At the end of fattening (after about 105 kg of body weight), from each group, 10 pigs (seven barrows and three gilts) were selected for slaughter, according to the standards in the meat industry—Minister of Agriculture and Rural Development dated April 2, 2004 (Polish Journal of Laws 2004.70.643). The slaughter procedure was carried out in a slaughterhouse by persons entitled to professional slaughter and by using acceptable methods of slaughter and killing of animals. The approved procedure involved the use of electronarcosis and the exsanguination of the pigs [23]. The post slaughter evaluation was also performed, which concerned hot carcass weight, carcass yield, slaughter yield, loin eye area, backfat thickness and the weight of the liver.

2.4. Analytical Methods

Nitrogen was determined by Kjeldahl's method, according to PN-EN ISO 5983–1:2006/AC:2009 (animal feeding stuffs—determination of nitrogen content and calculation of crude protein content—part 1: Kjeldahl method). In the wet fecal samples, the content of dry matter and nitrogen was determined. In dried fecal samples, crude fat, crude fiber, crude ash was measured and in the urine samples, the concentration of nitrogen was measured.

The content of meat in the carcass was measured with the use of an optical needle device—CGM apparatus (France). The measurement of pH was performed 1 and 24 hours after slaughter with the use of a Radiometer Copenhagen PHM80 Portable pH Meter with combined electrode. Electrical conductivity in the muscles after 24 h was determined by conductometer MP–03. The color of the meat was measured by Minolta Chroma Mater CR 300 (Konica Minolta Sensing, Inc., Japan) to detect the $L^*a^*b^*$ values (where: *L*—color lightness, *a*—color value red, *b*—color value yellow).

The content of fat, water and protein in meat samples (*longissimus dorsi* muscle) was determined according to standard chemical methods [25]. The drip loss was calculated from the difference between the initial and final mass of the sample, and was placed in foil sack at a temperature of 4 °C for 48 h. IM-03 Pig Carcass Grading Apparatus was used to analyze the physical parameters of the meat such as the area of the loin eye and backfat thickness.

2.5. Statistical Methods

In our paper, two independent groups of pigs were compared: the first fed with inorganic salts as feed additive (the control group) and the second fed with enriched macroalgae (the experimental group). At first, we checked whether our dataset was well modeled by a normal distribution or not with the use of Shapiro–Wilk normality test. If the distribution of the dependent variables was non-normal, then the non-parametric Mann–Whitney U test was used. If the distribution of the dependent variables was normal, then for the two compared groups (of which the size was smaller than 30) the homogeneity of variance was checked with the use of the Brown–Forsyth test. If the variances were homogeneous, then the *t* test was chosen, if not, we used the Cochran–Cox test. The results were elaborated statistically by *Statistica* ver. 9.0. Results were considered significantly different when P < 0.05.

3. Results

The animals remained healthy throughout the experiment, as shown in our previous work, in which we examined the effects of enriched macroalgae on the growth performance of growing pigs—average feed intake, average weight gain and feed conversion ratio, as well as biochemical markers in the serum of examined pigs and mineral composition of blood, meat, liver, feces and urine of growing pigs. All the examined parameters were comparable in both examined groups and were not statistically significant [18].

3.1. Balance and Digestibility Trials

Balance and digestibility trials were conducted only in male individuals, which allowed for the separate collection of feces and urine. These trials usually start when the body weight reaches about 65 kg, which corresponds to the finishing feeding with "Grower" mixture. In this period, the best indicators, which concern protein, fat and mineral balance, are usually achieved. After this period of fattening, more intensive protein deposition and an increase in fat storage is observed. During the balance and digestibility trials, a "Grower" mixture in the control and experimental group contained the following components: dry weight (872 g/kg feed), total protein (157 g/kg), crude fat (31 g/kg), crude fiber (28 g/kg), crude ash (C—43 g/kg, MA—45 g/kg), nitrogen-free extractives (613 g/kg), minerals: Ca (68 g/kg), P total (50 g/kg), P digestible (30 g/kg), Cu (25 mg/kg), Zn (148 mg/kg), Mn (82 mg/kg), Fe (183 mg/kg), I (1.49 mg/kg), Co (0.81 mg/kg) and Se (0.48 mg/kg) [18].

3.2. Daily Amounts of Feces and Urine Excreted by Growing Pigs

Table 4 summarizes the results concerning the daily amount of feces and urine excreted by pigs during the collection period, which lasted 4 days. There were no statistically significant differences between the control and algal group, but the experimental group excreted 13% more feces and 20% less urine when compared with the control group. The nitrogen concentration in urine was 34% higher in the experimental than in the control group. Other differences were lower than 5%. These results are in agreement with data presented by Saeid et al. (2013) in analogous experiments carried out with microalga *Spirulina maxima* enriched with Cu(II), Zn(II) and Fe(II) ions as a feed additive [23].

Specification	С	MA	n Valuo	Chathathard Tract	
Specification	Mean ± SD		<i>p</i> value	Statistical lest	
Feces					
Feces excreted (g)	765 ± 74	865 ± 254	0.749	Mann-Whitney	
Dry matter (%)	32.7 ± 3.0	31.8 ± 5.9	0.750	Test t	
Excreted dry matter (g)	249 ± 11	264 ± 29	0.251	Test t	
Urine					
Urine excreted (g)	5095 ± 1035	$4\ 079 \pm 1\ 276$	0.161	Test t	
N (%)	0.427 ± 0.116	0.572 ± 0.154	0.0956	Test t	
N excreted in urine (g)	20.8 ± 1.9	21.8 ± 2.7	0.483	Test t	

Table 4. Daily amounts of feces and urine excreted by growing pigs in the collection period (4 days).

3.3. Apparent Fecal Nutrient Digestibility (%) and Daily Nitrogen Balance and Retention

Table 5 presents the apparent fecal nutrient digestibility (%) and daily nitrogen balance and retention. No effect of enriched macroalgae on the increase in digestibility of dry matter, dry organic matter, crude protein, crude fat and nitrogen-free extractives was observed. A statistically significant difference concerned the digestibility of crude ash, which, in the experimental group, was 15% lower than in the control group. In the case of nitrogen retention and retention in relation to N intake (%), these parameters were lower by 5% in the algal group compared to the control.

3.4. Meat Quality and Slaughter Value of Carcass

In Table 6, the results, which concern the meat quality and slaughter value of carcasses are presented. A statistically significant difference was observed only for the liver weight, which, in the experimental group, was 14.5% lighter than in the control group. Beside this, groups of pigs were not significantly different in the characteristics of meat quality and slaughter value of their carcasses.

Specification	С	MA	n Valuo				
Specification	Mean	Mean ± SD		Statistical Test			
Арр	Apparent Fecal Nutrient Digestibility (%)						
Dry matter	86.2 ± 1.3	84.9 ± 1.8	0.184	Test t			
Dry organic matter	88.1 ± 1.2	87.0 ± 1.6	0.208	Test t			
Total protein	87.4 ± 1.8	86.9 ± 3.7	0.689	Mann-Whitney			
Total fat	77.0 ± 3.4	78.9 ± 8.6	0.630	Test t			
Crude fiber	22.0 ± 8.2	18.8 ± 5.3	0.443	Test t			
Crude ash	50.2 ± 4.4	42.5 ± 6.3	0.0330	Test t			
Nitrogen-free extractives	91.9 ± 0.8	90.8 ± 1.1	0.0731	Test t			
Daily Nitrogen Balance and Nitrogen Retention							
Nitrogen taken in the feed (g)	50.2 ± 0.0	50.2 ± 0.0	-	-			
Nitrogen excreted (g) in:							
Feces	6.32 ± 0.90	6.53 ± 1.86	0.689	Mann-Whitney			
Urine	20.8 ± 1.9	21.8 ± 2.7	0.486	Test t			
Nitrogen retention (g)	23.1 ± 2.0	21.9 ± 2.0	0.331	Test t			
Retention in relation to N intake (%)—absorption	45.9 ± 3.9	43.5 ± 3.9	0.311	Test t			

Table 5. Apparent fecal nutrient digestibility (%) and daily nitrogen balance and nitrogen retention.

Statistically significant differences (p < 0.05) were written in Italics.

 Table 6. Assessment of slaughter value of carcass and meat quality.

Specification	С	MA	p Value	Statistical Test	
Mean ± SD		$1 \pm SD$,		
Assessment of Slaughter Value of Carcass					
Hot carcass weight (kg)	90.7 ± 2.8	88.3 ± 4.1	0.143	Test t	
Carcass yield (%)	54.7 ± 2.3	53.6 ± 3.5	0.408	Cochran-Cox	
Loin eye area (cm ²)	38.9 ± 4.8	37.5 ± 5.0	0.548	Test t	
Weight of liver (g)	1724 ± 227	1474 ± 200	0.0284	Mann-Whitney	
Average backfat thickness (mm)				-	
Over the shoulder	38.7 ± 6.7	37.7 ± 5.6	0.721	Test t	
On the midback	20.2 ± 5.1	21.9 ± 5.7	0.489	Test t	
On the rump I	20.2 ± 3.1	21.0 ± 5.3	0.650	Mann-Whitney	
On the rump II	13.8 ± 2.6	14.3 ± 4.4	0.762	Test t	
On the rump III	16.2 ± 3.6	16.2 ± 4.7	1.000	Test t	
Assessment of meat quality					
pH ₁ (after 45 minutes)	6.28 ± 0.24	6.26 ± 0.23	0.854	Test t	
pH ₂₄ (after 24 hours)	5.51 ± 0.0944	5.50 ± 0.0860	0.733	Test t	
Water absorption (%)	32.9 ± 0.9	30.7 ± 3.4	0.0686	Cochran-Cox	
Drip loss (%)	5.46 ± 2.13	5.05 ± 2.43	0.694	Test t	
Marbling (degrees)	1.75 ± 0.26	1.70 ± 0.42	1.000	Mann-Whitney	
Electrical conductivity (mS/cm ²)	3.97 ± 1.15	4.04 ± 1.08	0.890	Test t	
The content in muscles (%)					
Water	72.4 ± 1.1	72.5 ± 0.9	0.893	Test t	
Fat	3.24 ± 0.89	2.62 ± 0.65	0.0821	Mann-Whitney	
Protein	23.3 ± 0.7	23.8 ± 0.7	0.106	Test t	
Color					
L (color lightness)	50.8 ± 1.7	51.1 ± 3.0	0.838	Test t	
a (color value-red)	4.49 ± 0.72	4.52 ± 0.82	0.932	Test t	
<i>b</i> (color value-yellow)	0.265 ± 0.806	0.0340 ± 1.38	0.653	Test t	

Statistically significant differences (p < 0.05) are written in italics.

4. Discussion

In the present study, green seaweeds—*Enteromorpha* sp.—were examined in terms of their potential application in pig feed. Several parameters such as the daily amount of feces and urine excreted, apparent fecal nutrient digestibility, daily nitrogen balance and retention, meat quality and the slaughter value of carcasses were evaluated. It was shown that the digestibility of crude ash in the experimental group was 15% lower than in the control group. This difference was statistically significant. This may result from the naturally high level of this component in the biomass of *Enteromorpha* sp. collected from the Baltic Sea, which ranges from 19% to 32% [21], while the ash content in the dry matter in cereals ranges from 1.4% in maize to 2.7% in oats [26]. Moreover, crude fiber digestibility was 14.5% lower. Similar results (the same trend) were obtained by Saeid et al. (2013), who used microalga Spirulina *maxima* enriched with Cu(II), Zn(II) and Fe(II) ions as a feed additive for growing pigs [23]. Moreover, Dierick et al. (2009) found that the overall digestibility of nutrients in the pigs' diet seemed not to be negatively affected by seaweed supplementation (10 and 20 g/kg of feed). However, the apparent fecal nutrient digestibility of dry matter, dry organic matter, crude protein and crude ash in the experimental group was slightly higher than in the control group (for seaweed content 20 g/kg of feed it was: 3.2%, 3.5%, 14.7% and 21%, respectively). Only the digestibility of crude fat was 8.4% lower in the algal group than in the control group [9]. In the paper of Lynch et al. (2010), the digestibility coefficient of dry matter and dry organic matter in the control group and in all experimental groups (the content of Laminaria hyperborea extract was 0.7 g/kg; 1.4 g/kg; 2.8 g/kg and 5.6 g/kg) was the same and equal 89% and 91%, respectively [8].

We have also found that nitrogen retention and retention in relation to N intake (%) was lower in the algal group when compared to the control. Increased nitrogen excretion in the urine (control group—20.8 g, experimental group—21.8 g), required much more metabolic effort on the part of the pigs, as was seen in slightly weaker daily gains (by about 4% lower in algal than in control group) and in the greater feed conversion per kg of gain (about 2% lower in algal than in control group) [18]. In the control group, pigs excreted slightly less nitrogen (6.32 g) than in the experimental group (6.53 g). This can result in improved protein digestibility in the control group, which was 87.4%, while in the experimental group the protein digestibility was 86.9%. Moreover, Gardiner et al. (2008) observed that the nitrogen retention was 31% lower in the experimental group (addition of 2.5 g of Ascophyllum nodosum extract) than in the control group [10]. In our study, in the urine from both groups, significantly higher amounts of nitrogen were excreted when compared with feces. These results were also confirmed in the paper of Gardiner et al. (2008) who noted that the nitrogen content in urine in the control group was 29 g per day and in the experimental group 37 g per day (addition of 2.5 g of Ascophyllum nodosum extract), and in feces 8.7 g per day and 9.4 g per day, respectively [10]. Moreover, Lynch et al. (2010) examined the effect of dietary Laminaria-derived laminarin and fucoidan on nitrogen utilization in pigs. There was a quadratic response to seaweed extract on urinary nitrogen excretion, total nitrogen excretion and nitrogen retention [8].

Since meat and meat products are considered vital components of a healthy diet, increased consumer demand for food with reduced fat levels and cholesterol and an enhanced fatty acid profile is observed [27]. In the present work, it was shown that the meat quality and slaughter value of the carcass was comparable in both tested groups—control and enriched macroalgae. The only difference concerned the liver weight, which in the experimental group was 14.5% lighter than the control group. These results also confirm the values of liver enzymes (aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT)), which, in the experimental group, had lower values than the control group [18]. Saeid et al. (2013) also noted 5% lighter liver in the experimental group, in which pigs were fed with *Spirulina maxima* enriched with Cu(II), Zn(II) and Fe(II), when compared to the control group—microelements supplemented as inorganic salts [23]. Moreover, Svoboda et al. (2009) did not observe a difference in the hot weight of carcass in the group of pigs, which were fed with the addition of inorganic sodium selenite (78.6 \pm 7.1 kg), and in the group fed with organic Se from Se-enriched alga—*Chlorella* spp. (78.5 \pm 5.0 kg) [28]. The same applied in the case of two other

microalgae, *Chlorella* and *Scenedesmus*, which were compared with pigs' diets containing fish meal. Carcass characteristics was comparable and the digestibility studies indicated that algae were low in digestible energy, but their protein was 70% digestible [29].

In our study, there was no statistically significant effect of algal additive on the average value of pH₁, pH₂₄ (acidity of meat), water absorption, drip loss (respectively lower by 7.0% and 7.5% in the experimental group than in the control group—the smaller the leakage, the better). A decrease in water absorption by 5% and drip loss by 34 % was also noted by Saeid et al. (2013) in the case of the application of *Spirulina maxima* in the experimental group of pigs [23]. In the research of Suzuki et al. (2002), the influence of dried seaweed on meat production and its quality was examined. The cooking loss in the first group—dried seaweeds and breadcrumbs mixed with feed at a rate of 0.3% and 5%—and the second group—dried seaweed as an additive—was significantly lower than in the third group—breadcrumbs used as an additive—and the fourth group, where neither additive was used. The obtained results showed that the addition of dried seaweeds improved the meat quality [30]. Our results also confirmed data obtained by Svoboda et al. (2009) [28]. The pH of the meat (24 h after slaughter) was 5.66 in the group with inorganic Se and 5.68 in the group with Se-enriched alga. However, the leakage in the experimental group was 12.5% higher than in the control group [28].

The average values of carcass yield of the growing pigs in both groups did not differ from the average carcass yield of pigs in the national population, which in 2011 was 55.4% [31]. This indicates that they meet the current standards for carcass yield and can be used in the meat industry. However, the carcass yield of pigs in the control group (55%) was slightly higher than in the experimental group (54%). The reason may be greater nitrogen excretion from the body (both in feces and urine) in the experimental group. The control group was characterized by a better utilization of nitrogen, which was used to build muscle tissue, hence the higher carcass yield. Moreover, Sardi et al. (2006) found that the lean meat (%) in the control group of pigs (a maize/soybean diet) was slightly higher (49.1%) than in the experimental groups: the first (macroalgae added at 2.5 g/kg over the last 8 weeks prior to slaughtering) was 48.1%, the second (5 g/kg over the last 4 weeks prior to slaughtering) was 48.6%, and the third (2.5 g/kg over the last 4 weeks prior to slaughtering) was 48.7% [32].

Loin eye area is also related to the carcass yield, and in the experimental group it was lower than in the control group. The content of fat in muscle was by 19% lower in the experimental group than in the control group, which may be approved by consumers who are looking for products low in fat. The reduced content of fat in pigs from the algal group is associated with marbling (increased fat tissue), which was also lower in the experimental group. In the group of growing pigs fed with algae, a positive change in terms of reducing fat content and the growth of the desired protein was observed. Moderate marbling (the amount and distribution of intramuscular fat in muscle cross section) that is uniformly distributed is a desirable property [33]. The backfat thickness on the midback of growing pigs from the algal group was 8.4% higher than in pigs from the control group. There is a tendency for a decrease in thickness towards the rear of pigs' bodies. The average backfat thickness from 5 measurements (over the shoulder, on the midback and on the rump I, II, III) was greater in the experimental group (22.2 mm) than in the control group (21.8 mm). Other differences were lower than 5%. Moreover, a slight darkening of meat from pigs in the experimental group was observed. Choi et al. (2012) also reported an improvement in the color and sensory characteristics of reduced-fat pork patties as a result of the supplementation of pig feed with Laminaria japonica powder extract [11]. Nowadays, the meat color is one of the most important properties taken into account by consumers [34].

5. Conclusions

In the present study, the effect of macroalga *Enteromorpha* sp. enriched with Zn(II) and Cu(II) ions via biosorption on the daily amounts of feces and urine excreted by growing pigs, apparent fecal nutrient digestibility and daily nitrogen balance and retention, meat quality and slaughter value of carcasses was examined. There were no statistically significant differences between the control and experimental group when taking into account the listed parameters. The average value of carcass yield

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of growing pigs in both groups did not differ from the average carcass yield of pigs from the national population. This indicates that the microalgae meet current standards for carcass yield and can be used by the meat industry. In the algal group, a positive change in terms of reducing fat content in meat, and the growth of the desired protein, was observed. The meat of pigs in the experimental group was characterized by lower water absorption and drip than in the control group. Furthermore, a slight darkening of the meat from pigs in the experimental group was observed. On the basis of conducted experiments, it was found that the enriched algal biomass had no negative effect on the examined parameters and therefore may be introduced in pig nutrition as a feed material as an alternative to inorganic salts.

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References

- 1. Craigie, J.S. Seaweed extract stimuli in plant science and agriculture. J. Appl. Phycol. 2011, 23, 371–393. [CrossRef]
- 2. Hurst, D. Marine functional foods and ingredients. A briefing document. Mar. Fores. Ser. 2006, 5, 1–72.
- 3. McHugh, D.J. *A Guide to the Seaweed Industry*; FAO Fisheries Technical Paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 2003; p. 441.
- 4. Makkar, H.P.S.; Tran, G.; Heuzé, V.; Giger-Reverdin, S.; Lessire, M.; Lebas, F.; Ankers, P. Seaweeds for livestock diets: A review. *Anim. Feed Sci. Technol.* **2016**, *212*, 1–17. [CrossRef]
- 5. Angell, A.R.; Angell, S.F.; de Nys, R.; Paul, N.A. Seaweed as a protein source for mono-gastric livestock. *Trends Food Sci. Technol.* **2016**, *54*, 74–84. [CrossRef]
- 6. Corino, C.; Modina, S.C.; Di Giancamillo, A.; Chiapparini, S.; Rossi, R. Seaweeds in pig nutrition. *Animals* **2019**, *9*, 1126. [CrossRef]
- 7. Øverland, M.; Mydland, L.T.; Skrede, A. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. *J. Sci. Food Agric.* **2019**, *99*, 13–24. [CrossRef]
- 8. Lynch, M.B.; Sweeney, T.; Callan, J.J.; O'Sullivan, J.T.; O'Doherty, J.V. The effect of dietary *Laminaria*-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *J. Sci. Food Agric.* **2010**, *90*, 430–437. [CrossRef]
- Dierick, N.; Ovyn, A.; De Smet, S. Effect of feeding intact brown seaweed *Ascophyllum nodosum* on some digestive parameters and on iodine content in edible tissues in pigs. *J. Sci. Food Agric.* 2009, *89*, 584–594. [CrossRef]
- Gardiner, G.E.; Campbell, A.J.; O'Doherty, J.V.; Pierce, E.; Lynch, P.B.; Leonard, F.C.; Stanton, C.; Ross, R.P.; Lawlor, P.G. Effect of *Ascophyllum nodosum* extract on growth performance, digestibility, carcass characteristics and selected intestinal microflora populations of grower–finisher pigs. *Anim. Feed Sci. Technol.* 2008, 141, 259–273. [CrossRef]
- Choi, Y.S.; Choi, J.H.; Han, D.J.; Kim, H.Y.; Kim, H.W.; Lee, M.A.; Chung, H.J.; Kim, C.J. Effects of *Laminaria japonica* on the physico-chemical and sensory characteristics of reduced-fat pork patties. *Meat Sci.* 2012, *91*, 1–7. [CrossRef]
- 12. Michalak, I.; Chojnacka, K. Edible macroalga *Ulva prolifera* as microelemental feed supplement for livestock: The fundamental assumptions of the production method. *World J. Microbiol. Biotechnol.* **2009**, *25*, 997–1005. [CrossRef]
- 13. Michalak, I.; Chojnacka, K.; Witek-Krowiak, A. State of the art for the biosorption process—A review. *Appl. Biochem. Biotechnol.* **2013**, *170*, 1389–1416. [CrossRef]
- 14. Michalak, I.; Witek-Krowiak, A.; Chojnacka, K.; Bhatnagar, A. Advances in biosorption of microelements—the starting point for the production of new agrochemicals. *Rev. Inorg. Chem.* **2015**, *35*, 115–133. [CrossRef]

- 15. Jacela, J.Y.; DeRouchey, J.M.; Tokach, M.D.; Goodband, R.D.; Nelssen, J.L.; Renter, D.G.; Dritz, S.S. Feed additives for swine: Fact sheets—high dietary levels of copper and zinc for young pigs, and phytase. *J. Swine Health Prod.* **2010**, *18*, 87–91. [CrossRef]
- 16. McGrath, S.P.; Chaudri, A.M.; Giller, K.E. Long-term effect of metals in sewage sludge on soils microorganisms and plants. *J. Ind. Microbiol.* **1995**, *14*, 94–104. [CrossRef]
- Hernández, A.; Pluske, J.R.; D'Souza, D.N.; Mullan, B.P. Levels of copper and zinc in diets for growing and finishing pigs can be reduced without detrimental effects on production and mineral status. *Animal* 2008, 2, 1763–1771. [CrossRef]
- Michalak, I.; Chojnacka, K.; Korniewicz, A. New feed supplement from macroalgae as the dietary source of microelements for pigs. *Open Chem.* 2015, *13*, 1341–1352. [CrossRef]
- Dederen, L.H.T. Marine eutrophication in Europe: Similarities and regional differences in appearance. In Marine Coastal Eutrophication: Proceedings of an International Conference; Vollenweider, R.A., Marchetti, R., Viviani, R., Eds.; Elsevier: Bologna, Italy, 1992; pp. 21–24.
- 20. Haroon, A.M.; Szaniawska, A.; Normant, M.; Janas, U. The biochemical composition of *Enteromorpha* spp. from the Gulf of Gdańsk coast on the southern Baltic Sea. *Oceanologia* **2000**, *42*, 19–28.
- 21. Szefer, P.; Skwarzec, B. Concentration of elements in some seaweeds from coastal region of the southern Baltic and in the Żarnowiec Lake. *Oceanologia* **1988**, *25*, 87–98.
- 22. Żbikowski, R.; Szefer, P.; Latała, A. Distribution and relationships between selected chemical elements in green alga *Enteromorpha* sp. from the southern Baltic. *Environ. Poll.* **2006**, *143*, 435–448. [CrossRef]
- Saeid, A.; Chojnacka, K.; Korczyński, M.; Korniewicz, D.; Dobrzański, Z. Effect on supplementation of Spirulina maxima enriched with Cu on production performance, metabolical and physiological parameters in fattening pigs. J. Appl. Phycol. 2013, 25, 1607–1617. [CrossRef] [PubMed]
- 24. *Feeding Standards for Poultry and Swine*; Polish Academy of Sciences, Institute of Animals Physiology and Feeding: Warsaw, Poland, 2005.
- 25. Rak, L.; Morzyk, K. *Chemiczne Badania Mięsa*; Wydawnictwo Akademii Rolniczej we Wrocławiu: Wrocław, Poland, 2007. (In Polish)
- Rutkowski, A. Wartość pokarmowa zbóż dla kurcząt brojlerów. *Rocz. Akad. Rol. Poznaniu* 1996, 267, 22–25. (In Polish)
- 27. Toldrá, F.; Reig, M. Innovations for healthier processed meats. *Trends Food Sci. Technol.* **2011**, 22, 517–522. [CrossRef]
- Svoboda, M.; Saláková, A.; Fajt, Z.; Kotrbáček, V.; Ficek, R.; Drábek, J. Efficacy of Se-enriched alga *Chlorella* spp. and Se-enriched yeast on tissue selenium retention and carcass characteristics in finisher pigs. *Acta Vet. Brno* 2009, *78*, 579–587. [CrossRef]
- 29. Hintz, H.F.; Heitman, H. Sewage-grown algae as a protein supplement for swine. *Anim. Prod.* **1967**, *9*, 135–140. [CrossRef]
- 30. Suzuki, K.; Shimizu, Y.; Kadowaki, H. Influence of dried seaweed stalk and breadcrumbs in pig feed on meat production and quality. *Jpn. J. Swine Sci.* **2002**, *39*, 66–70. [CrossRef]
- 31. Ministry of Agriculture and Rural Development. *Analiza Sytuacji Rynkowej na Podstawowych Rynkach Rolnych w Grudniu 2011 Oraz w Całym 2011 Roku;* Ministry of Agriculture and Rural Development: Warsaw, Poland, 2012. Available online: www.minrol.gov.pl (accessed on 15 May 2012).
- 32. Sardi, L.; Martelli, G.; Lambertini, L.; Parisini, P.; Mordenti, A. Effects of a dietary supplement of DHA-rich marine algae on Italian heavy pig production parameters. *Livestock Sci.* **2006**, *103*, 95–103. [CrossRef]
- 33. Olszewski, A. Technologie Przetwórstwa Mięsa; WNT: Warsaw, Poland, 2007.
- 34. Przybylski, W.; Jaworska, D.; Czarniecka-Skubina, E.; Kajak-Siemaszko, K. Ocena możliwości wyodrębniania mięsa kulinarnego o wysokiej jakości z uwzględnieniem mięsności tuczników, pomiaru barwy i pH z zastosowaniem analizy skupień. Żywność Nauka Technologia Jakość 2008, 4, 43–51. (In Polish)



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