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Effect on supplementation of *Spirulina maxima* enriched with Cu on production performance, metabolical and physiological parameters in fattening pigs

A. Saeid · K. Chojnacka · M. Korczyński · D. Korniewicz · Z. Dobrzański

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Abstract In this paper, the effect of addition of the biomass of *Spirulina maxima* enriched with copper (Sm-Cu) to the animal feed is discussed. The biomass was cultivated in the photobioreactor with the capacity of 10 m^3. After the biosorption process, the enriched biomass was investigated as the source of valuable nutrients. The feeding experiment was conducted for 87 days. The study was performed in individual rearing pens, with controlled microclimate, feed and water were available semi-ad libitum. Piglets (24) were divided into two groups (control and experimental). The experimental group was fed with addition of the biomass of Sm-Cu instead of inorganic salts. There were no statistically significant differences between the average daily and periodic weight gain, daily and periodic feed collection, as well as feed conversion ratio. There were no statistically significant differences between the amount of N excreted in faeces and urine, when considering the retention of N, both in relation to the consumed N, and relative N digested which was at a similar level. In the experimental group in comparison to the control group, the lower low-density lipoprotein cholesterol by 17.05 % ($P<0.05$) and total cholesterol by 9.43 % ($P<0.05$) were observed. Additionally, the increase of parameter a^* of 13 % ($P<0.05$) and the reduction of the natural leakage by 34 % ($P<0.05$) were found.

A. Saeid (✉) · K. Chojnacka
Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, I-26, ul. Smoluchowskiego 25, 50-372 Wrocław, Poland
e-mail: agnieszka.saeid@pwr.wroc.pl

M. Korczyński · D. Korniewicz · Z. Dobrzański
Department of Animal Hygiene and Environment, Wroclaw University of Environmental and Life Sciences, ul. Chełmońskiego 38 C, 51-630 Wrocław, Poland

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Introduction

Microalgae are prokaryotic and eukaryotic photosynthetic organisms with single or multicellular structure that can survive even in very difficult conditions. Examples of microorganisms are prokaryotic cyanobacteria (Cyanophyceae), a eukaryotic green algae (Chlorophyta) and diatoms (Bacillariophyta) (Li et al. 2008a, b).

Microalgae and their nutritional value have long been known. They are used in human and animal nutrition, in cosmetics and in the production of valuable substances (e.g. fatty acids, pigments) (Spolaore et al. 2006). There are alternative and unconventional sources of protein and many biologically active substances. Consequently, they can be used as dietary feed supplements for animals (Muller-Feuga 2000). Microalgae are a good source of nutrients. Examples of application of microalgae are as follows: food, feed, drugs, pigments, source of chemical constituents, fuels, hormones and others (Muller-Feuga 2000; Barclay 1986; Illman et al. 2000; Lipstein and Hurwitz 1980; Richmond 2004).

Microalgae have been used as food since about 2,000 years ago in China. Although microalgae are known as the source of nutrients for thousands of years (Borowitzka 1999), microalgal biotechnology began to develop only in the middle of the past century (Spolaore et al. 2006).

Regulation of the Polish Minister of Agriculture and Rural Development (2005) allows the use of algae as a feed material. The list of feed materials which have been authorized under the provisions of the European Union includes algae (Korol 2002).

The nutritional properties of microalgae are well-known and are thoroughly described in the literature (Brown et al. 1997; Navarro et al. 2001; Martínez-Fernández et al. 2006; Martínez-Fernández and Southgate 2007; Abril et al. 2003; Ponis and Parisi 2003). Table 1 presents the experiments on using the microalgae as feed additives. Several papers describe attempts of enrichment of *Spirulina platensis* biomass in selenium, iodine (Mosulishvili et al. 2002) and chromium (Zhi-Yong et al. 2003), which resulted in the production of pharmaceuticals that can be used as human dietary supplements. Such formulations provide the body with the ingredients, in which biomass was enriched in a more available form. Biosorption is one of methods of enrichment of biomass with microelements. It would be possible to elaborate a new generation of feed additives components with microelements bound with biological carrier (biomass of microalgae) by biosorption. Trend to enrich the biomass with nutrients through biosorption and bioaccumulation becomes a fact, and it is confirmed by literature reports. For example, copper-enriched yeasts were used in coping with the problem of micronutrient deficiency in the diet of humans and animals (Mrvcic et al. 2007).

The aim of the present work was to assess the influence of addition of the microalgae *Spirulina maxima* enriched by biosorption biomass to the animal feed. Feeding experiments on swine were conducted to investigate their effect on production performance, metabolical and physiological parameters in fattening pigs.

Material and methods

Spirulina maxima obtained from the Culture Collection of Algal Laboratory Institute of Botany, Academy of Sciences of the Czech Republic was cultivated in Schlösser (1982) medium, prepared with technical grade reagents in a stirred tank reactor (dimensions 1.12 m×3.6 m) with a capacity of 10 m^3, covered by a glasshouse, equipped with the biomass separation system (six bag filters, average pore size 6 μm, Desjoyaux Co, Ltd.), mixing system (pumps) and six lamps (300W Astral Pool, Poland).

Biosorption experiments

The biomass of *S. maxima* was enriched with copper (II) ions via biosorption. The enrichment process was performed in containers containing 45 L of metal ions solution at ambient temperature in tap water. The solutions were prepared by dissolving appropriate amounts of $CuSO_4 \cdot 5H_2O$ admitted for using as a source of Cu(II), Fe(II) and Zn(II) in animal diets (from POCH, Gliwice, Poland) (Feeding Standards for Poultry and Swine 2005). The contact time was 2 h as determined previously in kinetic experiments (Michalak et al. 2007). After this time, enriched biomass was separated on a 6-μm pore size filter, dried at 50 °C and ground. Initial concentration of metal ions in the solution was C_0 300 mg L^{-1}. pH of the solution was adjusted to pH 5 with NaOH/HCl. The biomass concentration was 1 g of dry mass L^{-1}.

Feeding experiments

Feed

The enriched biomass of *S. maxima* via biosorption process was investigated as the source of microelements—Cu(II), Fe(II) and Zn(II). Because biomass enriched with copper also contains other microelements (Fe and Zn), and its content was taken into consideration during planning the experiment. Two experimental groups were distinguished:

Group I The microelement requirement was covered by inorganic salts, control (C)

Group II The requirement for Cu(II) was covered by *S. maxima* biomass enriched with copper (Sm-Cu)
The requirement for Fe was covered by *S. maxima* biomass enriched with iron (Sm-Fe) (25.5 %) and 74.5 % with inorganic salt
The requirement for Zn was covered by *S. maxima* biomass enriched with zinc (Sm-Zn) (17.3 %) and by inorganic salt (82.7 %)

The standard feed was composed of wheat, *Hordeum*, canola oil and soybean meal and specially prepared for each stage of the experiments (starter, grower and finisher); similar feed was used elsewhere (Korniewicz et al. 2012) (Tables 1 and 2). The content of nutrients and feed additives is presented in Table 2. The source of vitamins and microelements was a commercially available premix produced by LNB Poland. The experimental group was fed with the same feed, but microelements were supplemented by *S. maxima* enriched with microelements by biosorption.

Animals, housing

Dewormed (Dectomax® or Ivomec®) piglets (Big White Polish/Polish White Zwisloucha, dams × Hampshire/Pietrain) (24 heads, 20.9±2.2 kg) were randomly divided into two groups: 12 heads in the control group and 12 in the experimental group. The three different feed compositions according to the different nutritional requirements for growth of the animals were used. Piglets in rearing phase (20–40 kg) were fed with the standard starter feed mixture, porkers during the first period of fattening (40–65 kg) were fed with standard grower feed mixture and porkers in the second period of fattening (65–105 kg) were fed with the standard finisher feed mixture. Nutritional value of fodder in specific periods of

Table 1 The use of microalgae in the feeding of poultry and pigs

Microalgae	Animal	Effect
Haematococcus pluvialis	Broilers	Antibacterial activity of astaxanthin (Waldenstedt et al. 2003)
Chlorella sp.	Chicks and broilers	Addition of 60 and 150 g of algae per kg of feed had no adverse effect on the growth of animals (Lipstein and Hurwitz 1980)
Nannochloropsis oculata	Laying hens	Addition of microalgae (20 %) increased content of unsaturated fatty acids and carotenoids in the egg yolks (Fredriksson et al. 2006)
Crypthecodinium cohnii	Ducks	Addition of 5 g kg^{-1} of microalgae did not affect the weight gain and manure characteristics as well as chemical composition, colour, pH, shelf life, the aromatic characteristics of breast muscle (Lipstein and Hurwitz 1980)
Spirulina platensis	Broilers	Addition of 140 and 170 g kg^{-1} of microalgae was not adversely affected by the mass, composition and histopathology of organs. Meat quality did not change. More intense colour was observed (Crist 1981)
Chlorella sp.	Laying hens	Addition of 120 g of microalgae kg^{-1} feed did not affect the quality of eggs and feed utilization. High supplementation of algae in the feed caused a more intense yellow colour of egg yolks (Kadukowa and Vircikova 2005)
Schizochytrium sp.	Laying hens	Algae as a source of n-3 PUFAs administered for 8 weeks had no adverse effect on the organoleptic properties (Parpinello et al. 2006)
Schizochytrium sp.	Pigs	Addition of algae at 2.5 and 5 g kg^{-1}, did not affect negatively the pH, meat colour and composition of the loin (Sardi et al. 2006)

feeding was presented in Tables 3 (the nutrient content of the diets with amino acids) and 4 (the micro- and macroelemental composition of the control and the experimental feed). The study was performed in individual rearing pens, with controlled microclimate (16–18 °C). Feed and water were available semi-ad libitum. After the 21st day of feeding with grower mixture, six porkers from control and six porkers from experimental group were separated for 7 days in individual cages and fed with the same mixture as the rest of animals. After 3 days (treated as the preliminary step), urine and faeces were collected from each animal. Every morning, the amount of not consumed fodder was recorded. Collected samples were used to determine the nitrogen in faeces, urine and feed.

At the end of experiments, ten randomly chosen pigs were killed to obtain liver and meat. Slaughter procedure was carried out in the slaughterhouse with the required permits and according to Minister of Agriculture and Rural Development dated April 2, 2004 by persons entitled to professional slaughter and acceptable methods of slaughter and killing of animals (Polish Journal of Laws 2004.70.643). Approved procedure involves use of electronarcosis and exsanguination of pigs.

Sampling

The feeding experiment was conducted for 87 days and was divided into three series: starter (26 days), grower (31 days) and finisher (30 days), respectively. After each series, each animal was weighed. On 87th day, blood was collected. After separation, the concentration of microelements in serum was determined. Blood was sampled from the jugular vein. Before sampling blood, heparin was added to the samples in order to prevent blood coagulation. Muscle (*longissimus dorsi* muscle) and liver samples were homogenized. All samples with the exception of fodder were kept in the freezer for multi-elemental analysis.

Analytical methods

To determine the elemental content, the appropriate mass of biological sample (feed 0.5 g, microalgae

Table 2 Percent composition and feeding value of mixtures for fatteners

Ingredients	Units	Type of mixture		
		Starter	Grower	Finisher
Ground wheat	%	35.0	40.0	40.0
Ground barley	%	41.7	43.4	49.4
Soya bean oil meal	%	15.5	11.5	6.5
Soya oil	%	3.3	1.8	1.4
Acidifier	%	0.5	0.3	0.2
Supplementary feed: starter	%	4.0	–	–
Supplementary feed: grower	%	–	3.0	–
Supplementary feed: finisher	%	–	–	2.5
Total	%	100.00	100.00	100.00

Table 3 The nutrient content of the diets with amino acids of mixtures for fatteners

Ingredients	Units	Type of mixture		
		Starter	Grower	Finisher
Chemical composition, analysed, per kg of mixture				
Net energy	Kcal	2,340	2,280	2,281
Metabolisable energy	MJ	13.60	13.25	13.25
Dry matter	%	87.3	87.2	87.1
Crude protein	%	17.4	15.7	14.5
Crude fibre	%	3.0	2.8	3.5
Crude fat	%	5.0	3.1	3.2
Crude ash	%	5.1	4.3	3.7
N-free extractives	%	56.8	61.3	62.2
L-Lysine	%	1.17	0.93	0.85
DL-Methionine	%	0.39	0.29	0.26
Methionine + cystine	%	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
Ca	%	0.73	0.68	0.60
P total	%	0.55	0.50	0.43
Mineral P	%	0.16	0.15	0.13
Digestible P	%	0.34	0.30	0.25
Phytase	FTU	500	510	425
Na	%	0.20	0.20	0.14
Fe[a]	mg	198	183	172
Mn[a]	mg	91	82	73
Cu[a]	mg	167	25	21.8
Zn[a]	mg	157	148	126
I[a]	mg	1.66	1.49	1.26
Co[a]	mg	0.88	0.81	0.68
Se[a]	mg	0.49	0.48	0.44
Vitamin A[b]	I.U.	16,000	12,000	10,000
Vitamin D3[b]	I.U.	2,000	1,998	1,665
Vitamin E[b]	mg	150.00	124.50	103.75
Vitamin K3[b]	mg	4.00	1.80	1.50
Vitamin B_1[b]	mg	2.40	1.80	1.50
Vitamin B_2[b]	mg	6.40	4.80	4.00
Niacin[b]	mg	32.00	24.00	20.00
Pantothenic acid[b]	mg	16.00	12.00	10.00
Vitamin B_6[b]	mg	4.80	3.60	3.00
Vitamin B_{12}[b]	mcg	40.00	30.00	25.00
Biotin[b]	mcg	160.00	120.00	100.00
Vitamin C[b]	mg	100.00	100.00	83.30
Folic acid[b]	mg	3.20	2.40	2.00
Choline[b]	mg	350.00	250.00	208.30

[a] Microelements supplemented: Fe as $FeSO_4 \cdot H_2O$ 30 %; Mn as MnO_2 60 %; Cu as $CuSO_4 \cdot 5H_2O$ 25 %; Zn as $ZnSO_4 \cdot H_2O$ 35 %; I as $Ca(IO_3)_2 \cdot H_2O$ 62 %, Co as $CoCO_3$ 21 %; Se as Na_2SeO_3 5 %

[b] Vitamins supplemented: vitamin A (retinyl acetate), vitamin D_3 (cholecalciferol), vitamin E (DL alpha tocopherol acetate), vitamin K (bisulphite menadione sodium), vitamin B_1 (thiamine mononitrate), vitamin B_2 (riboflavin), vitamin B_3 (nicotinic acid), vitamin B_5 (D-calcium pantothenate), vitamin B_6 (pyridoxine hydrochloride), vitamin B_{12} (cyanocobalamin), biotin (D-biotin), vitamin C (ascorbic acid), folic acid (folic acid), choline (choline chloride)

biomass 0.5 g) materials was digested with 5 mL concentrated—65 % HNO_3 suprapur grade from Merck in Teflon vessels (microwave oven Milestone MLS-1200). After mineralization, all samples were diluted to 50 mL. Inductively coupled plasma-optical emission spectrometer with ultrasonic nebulizer (Varian VISTA-MPX ICP-OES, Mulgrave, Victoria, Australia) was used to determine the concentration of elements in algae and in all digested and diluted biological samples in the Chemical Laboratory of Multielemental Analyses at Wroclaw University of Technology, which is accredited by the ILAC-MRA and the Polish Centre for Accreditation according to PN-EN ISO/IEC 17025. The total N content in the samples of faeces, urine and feed was determined by the Kjeldal method, according to PN-EN ISO 5983–1:2006/AC:2009.

The following biochemical parameters of the blood were determined: total protein, albumin, glucose, urea, liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total cholesterol (Chol t) and its fractions low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides. Analyses were performed at the Department of Animal Hygiene and Animal Welfare, at Wrocław University of Environmental and Life Sciences by Biochemical Analyzer Pentra 400 from Horiba ABX.

Determination of carcass conformation (proportion of fat and muscle) was made by optical needle device—CGM (France). pH measurements in the *longissimus dorsi* muscle at the level of the last rib after 1 h and 24 h after slaughter were performed using a Radiometer PHM 80 Portable pH meter. Conductivity meter MP-03 was used to measure electrical conductivity in the same sample after 24 h. In meat, the content of water, fat and protein was determined in sample of *longissimus dorsi* muscle according to standard chemical methods (Rak and Morzyk 2007).

The leakage was calculated from the difference between the initial and final mass of the sample, which were placed in foil sacks in a temperature 4 °C for 48 h. Physical parameters of meat: meatiness, marbled, area of loin eye and back-fat thickness were analysed by IM-03—Pig Carcass Grading Apparatus. The colour of muscle carcass has been marked by Chroma Meter CR-400, Konica Minolta Sensing, Inc. (Japan) to detect the $L^*a^*b^*$ values. The L^* value represents lightness (negative towards black, positive towards white), the a^* value represents red–greenness (negative towards green, positive towards red) and b^* value represents the blue–yellow colour scale (negative towards blue, positive towards yellow). The instrument was calibrated using a Minolta calibration plate CR-22/CR-400 (Japan). To

Table 4 The content of the control and the experimental feed for pigs (mg kg^{-1} ± measurement uncertainty)

		Starter		Grower		Finisher	
		Control	Experimental	Control	Experimental	Control	Experimental
Microelements	Cu	13.3±2.0	6.57±1.64	9.06±2.27	7.85±1.96	13.7±2.1	7.25±1.81
	Fe	206±31	143.0±21.5	149±22	180±27	206±31	151±23
	Zn	56.9±8.5	49.3±7.4	48.0±7.2	59.9±9.0	53.2±8.0	46.5±7.0
	Co	0.576±0.144	0.491±0.123	0.457±0.114	0.568±0.142	0.488±0.122	0.434±0.109
	Mn	82.0±12.3	63.7±9.6	66.4±10.0	57.8±8.7	94.8±14.2	55.5±8.3
	Mo	2.96±0.74	0.760±0.190	0.670±0.168	0.742±0.186	0.677±0.169	0.534±0.134
	Cr	0.681±0.170	0.583±0.146	0.480±0.120	0.629±0.157	0.589±0.147	0.463±0.116
	Se	2.31±0.58	1.25±0.312	0.977±0.244	0.604±0.151	0.562±0.141	0.485±0.121
	B	167±25	95.1±14.3	75.8±11.4	75.6±11.3	70.2±10.5	43.0±6.44
Alkali and alkaline earth metal	K	3,550±532	3,289±493	3,459±519	3,720±558	3,722±558	2,968±445
	Ca	3,111±467	2,800±420	2,912±437	2,995±449	2,636±395	2,289±343
	Mg	788±118	697±105	767±115	805±121	846±127	711±107
	Na	848±127	823±123	862±129	918±138	836±125	747±112
	Ba	4.8±1.2	3.78±0.94	4.69±1.17	4.04±1.01	4.36±1.09	3.07±0.77
Toxic elements	As	3.0±0.6	1.25±0.25	0.788±0.158	0.621±0.124	0.546±0.109	0.318±0.064
	Cd	LLD	0.030±0.006	0.0137±0.0027	0.0167±0.0033	0.0172±0.0034	0.00798±0.00160
	Ni	0.758±0.152	0.568±0.114	0.607±0.121	0.626±0.125	0.747±0.149	0.439±0.088
	Pb	0.5±0.109	0.713±0.143	0.472±0.094	0.381±0.076	0.5228±0.1046	0.354±0.071
Other elements	Be	LLD	0.0122±0.0030	0.0090±0.0023	0.0110±0.0027	0.0106±0.0026	LLD
	Sr	8.8±2.2	8.13±2.03	8.52±2.13	9.13±2.28	8.35±2.09	7.34±1.84
	Ti	3.76±0.94	3.20±0.80	2.56±0.64	3.65±0.91	4.21±1.05	1.84±0.46
	Tl	0.137±0.034	0.303±0.076	0.475±0.119	0.228±0.057	0.09±0.02	0.0828±0.0207
	Al	118±18	124±19	106±16	138±21	164±25	74.0±11.1
	V	7.54±1.88	6.88±1.72	7.31±1.83	7.73±1.93	8.27±2.07	6.49±1.62

LLD lower limit of detection

analyse the carcass colour, the tip of the surface of the measuring head placed against the surface of the meat sample was investigated.

Calculations and statistical analyses

Shapiro–Wilk test was used to ensure that the data had a normal distribution. Levene's test and Brown–Forsythe test were used to assess the equality of variances in different samples. Significance of differences between the groups were examined with Mann–Whitney *U* test (where the distribution was not normal), Welch (for data that have normal distribution and unequal variance) and *t* test (for data that have normal distribution and equal variance). Three levels of statistical significance were taken into account—at 0.1, 0.05 and 0.001. Statistical significance at $P<0.1$ was regarded as a "trend", while 0.05 and 0.001 showed statistical significance of differences. The arithmetic mean values, standard deviations and *t* tests were carried out with the use of computer software *Statistica* ver. 9.0.

Results

Leeson and Caston (2008) proved that trace minerals are oversupplied in feed formulations. Assuming the enhanced bioavailability of trace minerals in the form of enriched biomass of *S. maxima*, dietary levels of Cu at first stage of experiments (*starter*—where the level of microelement was the highest) were minimized in the experimental group to about 50 % in comparison to the control group (Table 4).

There were no statistically significant differences between the average daily weight gain (ADWG) and average periodic weight gain (APWG), weight gain, average daily (ADFI) and periodic (APFI) of feed intake, as well as feed conversion ratio (FCR) (Table 5). This means that the new dietary supplement with microelements based on the algal biomass did not change its organoleptic properties and yielded similar production effects.

The results of biochemical analyses of blood serum of pigs are presented in Table 6. Table 7 reports the

Table 5 Average production yields in different periods of feeding experiments: starter, grower and finisher

		Control group		Experimental group		Increase (↑)/decrease (↓)%	*P* values
		Mean	SD	Mean	SD		
Starter	BW (kg)						
	Start[a]	20.8	2.16	21.0	2.33	↑ 0.760	0.864
	End[a]	45.3	2.33	45.6	2.65	↑ 0.644	0.778
	APWG (kg)[a]	24.5	1.91	24.6	1.57	↑ 0.545	0.854
	ADWG (g)[a]	942	73.3	947	60.5	↑ 0.531	0.857
	APFI (kg)[c]	49.0	1.08	48.1	2.19	↓ 1.77	0.356
	ADFI (kg)[c]	1.88	0.040	1.84	0.087	↓ 2.21	0.225
	FCR[a]	2.01	0.162	1.96	0.153	↓ 2.45	0.452
Grower	BW (kg)						
	Start[a]	45.3	2.3	45.6	2.7	↑ 0.644	0.778
	End[a]	76.0	2.2	75.5	4.2	↓ 0.614	0.739
	APWG (kg)[a]	30.7	1.8	30.4	2.8	↓ 1.086	0.734
	ADWG (g)[a]	987	60	982	90	↓ 0.557	0.862
	APFI (kg)[c]	81.4	3.8	80.5	4.4	↓ 1.065	0.611
	ADFI (kg)[c]	2.62	0.12	2.59	0.14	↓ 1.340	0.615
	FCR[a]	2.65	0.07	2.66	0.26	↑ 0.567	0.848
Finisher	BW (kg)						
	Start[a]	76.0	2.2	75.5	4.2	↓ 0.614	0.740
	End[a]	107	3	105	5	↓ 1.84	0.256
	APWG (kg)[a]	31.1	2.0	29.6	3.5	↓ 4.85	0.210
	ADWG (g)[a]	1,036	68	986	115	↓ 4.85	0.210
	APFI (kg)[c]	94.9	4.2	94.8	3.7	↓ 0.158	0.273
	ADFI (kg)[c]	3.17	0.16	3.16	0.12	↓ 0.315	0.273
	FCR[a]	3.07	0.21	3.25	0.46	↑ 6.03	0.227
Whole period	BW (kg)						
	Start[a]	20.8	2.2	21.0	2.3	↑ 0.760	0.864
	End[a]	107	3	105	5	↓ 1.84	0.256
	APWG (kg)[a]	86.3	3.9	84.1	5.4	↓ 2.47	0.279
	ADWG (g)[a]	991	45	967	62	↓ 2.47	0.279
	APFI (kg)[c]	225	8	223	6	↓ 0.902	0.273
	ADFI (kg)[c]	2.59	0.09	2.57	0.07	↓ 0.902	0.482
	FCR[a]	2.62	0.10	2.66	0.17	↑ 1.85	0.412

[a] *t* test

[b] Cochran–Cox test

[c] Mann–Whitney *U* test

parameters determining the value of the slaughter carcass in the experiment. Statistical differences were found in the case of Chol t and for Chol LDL, it was 9.43 % and 17.05 % lower in the experimental group, respectively. Additionally, the decrease of ALAT, TG and creatine kinase (CK) by 11.3, 14.6 and 21.8 %, respectively, as well as increase of globulin concentration by 13.5 %, was also observed, but these differences were not statistically significant.

Balance and digestibility trials

In balance and digestibility trials, amount of consumed feed, the mass of excreted faeces and urine samples were determined. Also, nitrogen balance was performed. The results are presented in Table 8. An increased amount of faecal excretion by 11 % ($P<0.1$) in the experimental group compared to the control group and increased dry weight by 6 % ($P<0.1$) were denoted.

Table 6 Biochemical indicators in blood serum of pigs, mean±SD

Parameter	Unit	Control group	Experimental group	Increase (↑)/decrease (↓) %	*P* value
AST[a]	U L^{-1}	63.0±10.6	60.9±13.8	↓ 3.38	ns
ALAT[a]	U L^{-1}	37.3±14.7	33.1±4.0	↓ 11.3	ns
Chol t[a]	mmol L^{-1}	2.65±0.24	2.40±0.29	↓ 9.43	**
Chol HDL[b]	mmol L^{-1}	1.06±0.12	1.02±0.34	↓3.22	ns
Chol LDL[a]	mmol L^{-1}	1.28±0.16	1.05±0.14	↓ 17.05	**
LDH[b]	U L^{-1}	903±154	935±156	↑ 3.57	ns
GTP[a]	U L^{-1}	34.6±8.9	34.8±6.0	↑ 0.433	ns
CK[b]	U L^{-1}	1,482±1,250	1,159±944	↓ 21.8	ns
Urea[a]	mmol L^{-1}	5.71±0.75	5.45±1.17	↓ 4.60	ns
Creatinine[a]	μmol L^{-1}	120±13	126±14	↑ 5.20	ns
LA[b]	mmol L^{-1}	4.49±2.00	4.62±2.06	↑ 2.92	ns
Glucose[b]	mmol L^{-1}	5.13±0.86	5.43±0.48	↑ 5.81	ns
TG[a]	mmol L^{-1}	0.261±0.062	0.223±0.065	↓ 14.6	ns
TP[a]	g L^{-1}	65.6±6.5	67.3±4.9	↑ 2.65	ns
Albumin[a]	g L^{-1}	41.8±3.1	40.4±3.0	↓ 3.51	ns
Globulin[b]	gL^{-1}	23.8±6.1	27.0±5.2	↑ 13.5	ns
Na[b]	mmol L^{-1}	142±1	144±4	↑ 1.62	ns
K[a]	mmol L^{-1}	5.31±0.54	5.24±0.41	↓ 1.21	ns
Cl[b]	mmol L^{-1}	102±3	102±2	↓ 0.186	ns
Ca[b]	mmol L^{-1}	2.59±0.13	2.61±0.12	↑ 0.967	ns
Mg[a]	mmol L^{-1}	0.872±0.066	0.879±0.091	↑ 0.803	ns
P[a]	mmol L^{-1}	3.03±0.14	2.98±0.44	↓ 1.55	ns
Fe[b]	mmol L^{-1}	25.8±8.5	22.6±3.2	↓ 12.5	ns
Zn[b]	mmol L^{-1}	10.3±1.3	11.5±5.9	↑ 12.0	ns
Cu[a]	mmol L^{-1}	38.5±4.7	41.8±5.6	↑ 8.59	ns

ns no statistical differences

**$P<0.05$

[a] *t* test

[b] Mann–Whitney *U* test

Additionally, digestibility coefficients (%) were compared (Table 9). There were no statistically significant differences between the digestibility coefficients of dry organic matter, total protein, total fat and crude fibre between the control and the experimental group. Dry matter digestibility of the control group differed statistically when compared with the experimental group. The difference was at 1.6 %. Statistical differences related to the coefficient of digestibility of crude ash and digestible nitrogen-free extract that in the experimental group were lower by 24 % ($P<0.001$) and 1 % ($P<0.1$).

Table 10 shows the daily balance and nitrogen retention. There were no statistically significant differences between the amount of N excreted in faeces and urine, in relation to the retention of N, both in relation to the consumed N, and relative N digested were at a similar level.

Discussion

In order to assess the health status of animals fed with diet containing supplement of enriched *S. maxima*, basic research of serum biochemical parameters was undertaken. This allowed the clinical assessment of individual organs. Biochemical parameters concern diagnostic profile of selected organs. Commonly used to study organ profiles include renal profile, liver profile, bone, cardiac lipid and thyroid. Comparison of the veterinary standards gave information on the general health of animals fed with a premix of inorganic salts, as a source of micronutrients and animal fed with the feed, prepared on the basis of enriched *S. maxima* biomass.

To assess the condition of the liver, the following parameters were taken into account: AST, ALT, GGT, albumin, lactate dehydrogenase (LDH) and cholesterol. AST and GGT are enzymes produced in the liver. The increase in AST is an indicator of the liver disease. Its lower value in serum indicates a better condition and less stress on the liver. Introduction of microalgal biomass as a source of micronutrients to animal diet caused AST decrease by about 3.38 %, while in the case of GGT, an increase, but less than 0.5 %. This is consistent with a reduction in total cholesterol levels by 10 % ($P<0.05$), which was observed in the experimental group. LDL is the principal carrier of cholesterol

Table 7 Evaluation of carcass slaughter value, mean ± SD

Specification	Unit	Group		Increase (↑)/ decrease (↓) %	*P* value
		Control	Experimental		
Content in muscle					
Water[a]	%	72.4±1.1	72.4±0.6	↑ 0.0511	ns
Fat[a]	%	3.24±0.89	3.28±0.69	↑ 1.11	ns
Protein[a]	%	23.3±0.7	23.1±1.0	↓ 0.804	ns
Evaluation of carcass slaughter value					
Carcass weight[a]	kg	90.7±2.8	90.5±5.8	↓ 0.198	ns
Carcass yield[a]	%	54.7±2.3	55.1±3.3	↑ 0.658	ns
Area of loin eye[c]	cm^2	38.9±4.8	39.0±6.5	↑ 0.316	ns
Marbled[a]	pkt	1.75±0.26	1.90±0.52	↑ 8.57	ns
Weight of liver[a]	g	1,724±227	1,647±183	↓ 4.46	ns
Backfat thickness					
Sacralis I[a]	mm	20.2±3.1	20.6±3.4	↑ 1.98	ns
Sacralis II[a]	mm	13.8±2.6	12.8±2.9	↓ 7.25	ns
Sacralis III[a]	mm	16.2±3.58	15.0±2.9	↓ 7.41	ns
Dorsal[a]	mm	20.2±5.1	22.2±3.3	↑ 9.90	ns
Shoulder[a]	mm	38.7±6.7	41.4±5.7	↑ 6.98	ns
Physical and chemical parameters of carcass					
pH[a]		6.28±0.24	6.39±0.23	↑ 1.74	ns
pH'[a]		5.51±0.09	5.52±0.16	↑ 0.254	ns
Conductivity[a]	$mS\,cm^{-2}$	3.97±1.15	3.17±0.54	↓ 20.2	*
Lightness—*L**[a]		50.8±1.7	50.1±2.1	↓ 1.54	ns
Redness—*a**[a]		4.49±0.72	5.08±0.76	↑ 13.0	*
Yellowness—*b**[a]		0.325±0.898	0.0584±1.2527	↓ 82.0	ns
Leakage natural[a]	%	5.46±2.13	3.60±1.34	↓ 34.0	**
Water adsorption[a]	%	32.9±0.9	31.2±2.4	↓ 5.04	ns

ns no statistical differences
*$P<0.1$; **$P<0.05$
[a] *t* test

around the body and was reduced by 17.05 % ($P<0.05$) in the pigs fed with the treatment diets. HDL cholesterol is a major transporting lipoprotein cholesterol in the blood. Its effect, reducing blood cholesterol levels, is to remove the excess from the cells and transport to the liver where it is metabolised. HDL cholesterol makes up to 40 % of total cholesterol, and its concentration in the serum of pigs from the experimental group did not differ statistically from the serum of the control group. Dehydrogenase activity (LDH) levels should be in the range 575–3,294 U L^{-1}. Increased activity may indicate liver disease. There were no statistically significant differences between the experimental and the control group. ALT is an organ non-specific enzyme that is involved in the metabolism of proteins. Increased activity of serum ALT levels above 43 U L^{-1} indicates liver and pancreatic cancers. In the experiment, no statistically significant differences between groups were found. ALT activity was at 33 U L^{-1}.

In the assessment of cardiac profile, the following parameters were taken into account: AST, ALT, CK, LDH, Na and K. The activity of CK levels in healthy pigs should be on the level 50–3,531 U L^{-1}. Exceeding this value may indicate a muscle injury. For the experimental group, CK was smaller by 21.8 %, when compared with the control group, but the difference was not statistically significant. AST and ALT ratios have been discussed as part of the liver profile. Differences between the remaining discussed indicators were less than 5 % and were not statistically significant. It can be concluded that the use of the preparation from *S. maxima* had no effect on the cardiac profile of the animals.

The level of urea, sodium, potassium and creatinine is used to assess renal profile. Creatinine concentration in plasma is the result of the production and excretion and is directly dependent on muscle mass and the efficiency of excretory function. Its concentration in serum should be in the range 88.4–238.7 μmol L^{-1}. Higher concentration may indicate poisoning by organic and inorganic compounds. The concentration of urea in healthy pigs should be in the range 3.32–6.64 mmol L^{-1}. Urea is the final product of protein metabolism in the

Table 8 Daily quantities of faeces and urine excreted by fatteners during the experiment (4 days), mean ± SD

	Specification	Unit	Group			*P* value
			Control	Experimental	Increase (↑)/decrease (↓) %	
Faecal	Excreted[a]	g	765±75	845±53	↑ 10.6	*
	Dry matter[a]	%	32.7±3.0	31.4±3.1	↓ 4.16	ns
	Dry matter[a]	g	249±11	264±12	↑ 5.96	*
Urea	Excreted[a]	g	5,095±1,035	4,732±1,203	↓ 7.12	ns
	N[a]	%	0.427±0.116	0.478±0.118	↑ 11.9	ns
	N excreted[a]	g	20.8±1.9	21.5±1.2	↑ 3.55	ns

ns no statistical differences
*$P<0.1$
[a] *t* test

body. It is excreted by the kidneys and reflects the level of kidney function. In the experiment, there was no statistically significant differences between the experimental and the control groups. K and Na concentration in the serum of healthy pigs ranged, respectively, 4.4–5.6 mmol L^{-1} and 139.1–156.5 mmol L^{-1}. Treatment with algae had no effect on their level in the serum. Introduction to the diet of animals in the form of trace elements bound to microalgae biomass did not affect kidney function.

Conversion of lactate (gluconeogenesis: lactic acid (LA) → glucose) is important in maintaining acid–base equilibrium. No differences between the concentrations of LA in the blood serum of the experimental and the control groups indicate that the microelement preparation based on *Spirulina* does not interfere with carbohydrate metabolism. There was also a higher concentration of glucose in serum, which could be an indication of better energy-efficient metabolism. Glucose affects proteinous and energetic processes.

Differences in defining the activity and concentration of various indicators were at the level of individual variability. Statistically significant differences at $P<0.05$ were noted for cholesterol; the level was lower in the experimental group. Blé-Castillo et al. (2002) also found a reduction in the level of liver fat after using *S. maxima* in the high fat diet.

The objective of pig rearing is the production of meat. The value of the product determines the value of animal slaughter—the quantity and quality of the meat, to a lesser extent fatty material. The results of Lisiak et al. (2005) indicated that in 2005, carcass yield achieved in Poland was 52.6 %, with an average carcass weight of 85.7 kg. Carcass yield in the experimental group was 55.1 % and was approximately 1 % higher as compared with the control group. According to the classification of SEUROP (Commission Regulation (EC) 2008) on pig carcasses weighing from 60 to 120 kg from the slaughterhouse, which distinguishes between six classes (S, E, U, R, O, P) depending on the conformation, the same number of carcass of the experimental group as well as control group can be classified into class E (yield in the range 55–60 %), while the rest of carcass of the control and experimental group was classified as U (yield range 50–55 %).

Increased surface of area of loin eye is desirable (Olszewski 2007). The resulting differences between this parameter for the control and the experimental group was statistically insignificant. Marbling reflects the amount and distribution of intramuscular fat in muscle cross section. Moderate marbling, uniformly distributed, is a desired quality characteristics (Olszewski 2007). Juiciness of meat is closely correlated with water absorption and the amount of intramuscular fat. Meat with high water absorption is more juicy. Also strongly reticular is meat with large amounts of intramuscular fat which is more succulent than meat from young animals with a small amount of fat (Lisiak et al. 2005). Meat from the experimental group was characterized by a higher marbling by about 9 % (ns) compared with the control group and significantly lower

Table 9 Daily balance and nitrogen retention, mean ± SD

Specification	Group		Increase (↑)/decrease (↓) %	*P* value
	Control	Experimental		
Taken in the feed (g)[a]	50.2±0.0	50.2±0.0		–
Excrete in the faeces (g)[a]	6.32±0.74	5.83±0.50	↓ 7.65	ns
Excrete in the urine (g)[a]	20.8±1.6	21.5±1.2	↑ 3.53	ns
Retention (g)[a]	23.1±1.6	22.9±1.2	↓1.08	ns
Retention relative to N collected (%)[a]	46.0±3.2	45.5±2.4	↓ 1.08	ns
Retention relative to N digested (%)[a]	52.6±3.5	51.5±2.6	↓ 2.15	ns

ns no statistical differences
[a] *t* test

Table 10 Digestibility coefficients, %, mean ± SD

Specification	Group		Increase (↑)/ decrease (↓) %	*P* value
	Control	Experimental		
Dry matter[a]	86.2±1.32	84.9±0.726	↓1.58	*
Organic dry matter[a]	88.1±1.19	87.4±0.826	↓0.889	ns
Total protein[a]	87.4±1.81	88.4±1.01	↑1.11	ns
Total fat[a]	77.0±3.42	77.0±5.28	↑0.0649	ns
Crude fibre[a]	22.0±8.19	17.6±4.95	↓20.1	ns
Ash[a]	50.2±4.36	38.3±4.33	↓23.6	***
Digestible nitrogen-free extract[a]	91.9±0.763	91.0±0.910	↓0.997	*

Ns no statistical differences

*$P<0.1$; ***$P<0.001$

[a] *t* test

natural leakage ($P<0.05$). Five measurements were made for back-fat thickness; average back-fat thickness of the carcass of the experimental group was about 3 % (ns) higher.

Liver weight of the experimental group was lighter by 5 % as compared with the control group; the difference was not statistically significant, but smaller liver may indicate a smaller load on the liver, which was confirmed by serum biochemical indices, where the AST, ALT and GGT were lower in the group fed with the algal preparation.

Six classes of meat quality are distinguished based on pH, colour and texture of pork (Brzóska 2001). Normal meat quality—red, firm, normal (RFN)—has a bright red colour that is stable, its texture is firm and water is well-bound. Undesirable defects of meat are described as follows: pale, soft, exudative (PSE); acid, soft, exudative (ASE) and reddish pink, soft, exudative (RSE). This category of meat is characterized by increase of acidity as compared to the typical watery meat (PSE), and its colour is darker than the PSE and acidic meats. DFD meat is characterized by a dark colour, very good stability and low storage stability (Brzóska 2001). Table 11 reports the physicochemical quality criteria that allow to classify meat to the appropriate group. According to the given range of parameters presented in Table 11, four meat carcasses from the control group were classified as RFN while in the experimental group, seven meat carcasses were classified as RFN.

Table 11 The division of classes of pork due to the pH, colour and consistency (Murray 1995)

Criterion	Groups of meat quality				
	RFN	PSE	RSE	ASE	DFD
pH	>6.3	≤5.5	5.9–6.3	>6.3	>6.3
pH′	5.5–5.7	≤5.5	≤5.5	≤5.5	>6.3
Conductivity	≤8	≤8	≤8	≤8	≤5
Drip loss (%)	2–5	>5	>5	>5	<2
Lightness (L^*)	43–50	>50	43–50	>50	<43

The colour of meat is expressed in CIE $L^*a^*b^*$ (Ganczarski 2012). An important effect of changes in meat quality is the water retention associated with the brightness of colour. The colour of meat is one of the most important characteristic for consumer meat evaluation (Przybylski et al. 2008). Introduction to animal diet of algal preparation had a statistically significant effect on meat colour. The parameter a^* in the experimental group was greater by 13 % ($P<0.1$) and L^* by 1.5 % (ns) lower, which means that the meat had more intensive red colour.

Lower quality of meat usually means an increased deterioration of water absorption and increased meat juice leakage, too light colour and its variable saturation and poorer taste value, including mainly improper slices structure, sometimes toughness (fibrosity). Therefore, water holding capacity of meat is one of the most frequently mentioned disadvantages. Poor water holding capacity of meat affects the cost of meat production and culinary products (Strzyżewski et al. 2008; Murray 1995; Kajak et al. 2007). Use of the algal product had beneficial effect on colour, natural leakage and pH (not a significant change), while lower water holding capacity was observed.

In conclusion, the results reported in the present work showed that the introduction of micronutrients to the diet of animals, bound with the biological matrix in the form of *S. maxima*, had advantageous effect on reared swines: improved profile of the liver, lowering LDL cholesterol by 23 % ($P<0.05$) and total cholesterol by 10.5 % ($P<0.05$). The increase of parameter a^* of 13 % ($P<0.05$) and the reduction of natural leakage by 34 % ($P<0.05$), which improved the technological assessment of carcass grade class, were found. Four meat carcasses from the control group were classified as RFN while in the experimental group are seven.

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References

Abril R, Garrett J, Zeller SG, Sander WJ, Mast RW (2003) Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. Part V: target animal safety/toxicity study in growing swine. Regul Toxicol Pharm 37:73–82

Barclay B (1986) Microalgae Culture Collection 1984–1985. SERI/SP-232-2863

Blé-Castillo JL, Rodríguez-Hernández A, Miranda-Zamora R, Juárez-Oropeza MA, Dıaz-Zagoya JC (2002) *Arthrospira maxima* prevents the acute fatty liver induced by the administration of simvastatin, ethanol and a hypercholesterolemic diet to mice. Life Sciences 70:2665–2673

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315–331

Brzóska F (2001) Jakość mięsa wieprzowego. Trzoda Chlewna 39:112–116

Commission Regulation (EC) (2008) Commission Regulation (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcases and the reporting of prices thereof. Official Journal L 337, 16/12/2008 P. 0003-0030

Crist RH (1981) Nature of bonding between metallic ions and algal cell walls. Environ Sci Technol 5:1212–1220

Fredriksson S, Elwinger K, Pickova J (2006) Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chem 99:530–537

Ganczarski J (2012) CIE LAB http://lumen.iee.put.poznan.pl/do_pobrania/Wsp_chromat/CIE_Lab.pdf. Accessed on 30 April.

Illman AM, Scragg AH, Shales SW (2000) Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme Microb Technol 27:631–635

Kadukowa J, Vircikova E (2005) Comparison of differences between copper bioaccumulation and biosorption. Environ Int 31:227–232

Kajak K, Przybylski W, Jaworska D, Rosiak E (2007) Charakterystyka jakości technologicznej, sensorycznej i trwałości mięsa wieprzowego zróżnicowanej końcowej wartości pH. Żywność Nauka Technologia Jakość 1:26–34

Korniewicz D, Dobrzański Z, Kołacz R, Hoffmann J, Korniewicz A, Antkowiak K (2012) Effect of various feed phosphates on productivity, slaughter performance and meat quality of fattening pigs. Med Weter 68:353–358

Korol W (2002) Mineralne dodatki paszowe-regulacje prawne: Polska-UE. Pasza Przem 4:18

Leeson S, Caston L (2008) Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. Anim Feed Sci Techno 142:339–347

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008a) Biofuels from microalgae. Biotechnol Prog 24:815–820

Li Y, Wang B, Wu N, Lan CQ (2008b) Effects of nitrogen sources on cell growth and lipid production of *Neochloris oleoabundans*. Appl Microbiol Biotechnol 81:629–636

Lipstein B, Hurwitz S (1980) The nutritional value of algae for poultry dried *Chlorella* in broiler diets. Br Poult Sci 21:9–21

Lisiak D, Pater A, Borzuta K (2005) Analiza zmian wartości rzeźnej tuczników w pierwszym półroczu 2003, 2004 i 2005 roku. Trzoda Chlewna 11:8–12

Martínez-Fernández E, Acosta-Salmón H, Southgate PC (2006) The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. Aquaculture 257:491–503

Martínez-Fernández E, Southgate PC (2007) Use of tropical microalgae as food for larvae of the black-lip pearl oyster *Pinctada margaritifera*. Aquaculture 263:220–226

Michalak I, Zielińska A, Chojnacka K, Matuła J (2007) Biosorption of Cr(III) by microalgae and macroalgae: equilibrium of the process. Am J Agri Biol Sc 2:284–290

Mosulishvili LM, Kirkesali EI, Belokobylsky AI, Khizanishvili AI, Frontasyeva MV (2002) Experimental substantiation of the possibility of developing selenium—and iodine—containing pharmaceuticals based on blue–green algae *Spirulina platensis*. J Pharm Biomed Anal 30:87

Mrvcic J, Stanzer D, Stehlik-Tomas V, Škevin D, Grba S (2007) Optimization of bioprocess for production of copper-enriched biomass of industrially important microorganism *Saccharomyces cerevisiae*. J Biosci Bioeng 103:331–337

Muller-Feuga A (2000) The role of microalgae in aquaculture: situation and trends. J Appl Phycol 12:527–534

Murray AC (1995) The evaluation of muscle quality. In: Morgan Jones SD (ed) Quality and grading of carcasses of meat animals. CRC, New York, pp 83–107

Navarro N, Yúfera M, García-Gallego M (2001) Use of freeze-dried microalgae for rearing gilthead seabream, *Sparus aurata* L., larvae. II. Biochemical composition. Hydrobiologia 452:69–77

Olszewski A (2007) Technologie przetwórstwa mięsa. WNT, Warszawa

Parpinello GP, Meluzzi A, Sirri F, Tallarico N, Versari A (2006) Sensory evolution of egg products and eggs laid from hens fed diets with different fatty acid composition and supplemented with antioxidants. Food Res 39:47–52

Polish Academy of Sciences (2005) Feeding standards for poultry and swine. Institute of Animals Physiology and Feeding, Warsaw

Ponis ERR, Parisi G (2003) Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 221:491–505

Przybylski W, Jaworska D, Czarniecka-Skubina E, Kajak-Siemaszko K (2008) Ocena możliwości wyodrębniania mięsa kulinarnego o wysokiej jakości z uwzględnieniem mięsności tuczników, pomiaru barwy i pH z zastosowaniem analizy skupień. Żywność Nauka Technologia Jakość 4:43–51

Rak L, Morzyk K (2007) Chemiczne badania mięsa. Wrocław, Wyd. AR

Richmond A (ed) (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science, Oxford

Sardi L, Martelli A, Lambertini L, Parisini P, Mordenti A (2006) Effects of a dietary supplement of DHA-rich marine algae on Italian heavy pig production parameters. Livest Sci 103:95–103

Schlösser UG (1982) Sammlung von Algenkulturen. Pflanzenphysiologisches Institut der Universität Göttingen. Ber Dt Bot Ges 95:181–276

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Strzyżewski T, Bilska A, Krysztofiak K (2008) Zależność pomiędzy wartością pH mięsa a jego barwą. Nauka Przyr Technol 2:1–9

Waldenstedt L, Inborr J, Hansson I, Elwinger K (2003) Effect of astaxanthin-rich algal meal (*Haematococcus pluvalis*) on growth performance. Anim Feed Sci Technol 108:119–132

Zhi-Yong L, Si-Yuang G, Lin L (2003) Bioeffects of selenite on growth of *Spirulina platensis* and its biotransformation. Bioresource Technol 89:171–176