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

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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 m³. 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.

Keywords Bioavailability · Biosorption · Copper · Mineral feed additives · *Spirulina maxima* · Cyanobacteria · Swine

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 (Mrvic 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, metabolic 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 m³, 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 CuSO₄·5H₂O 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 C₀ 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
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

| 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) |
| Cryptothecodinium 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) |

### 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     |
| 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      |
| Metabolisable energy         | MJ    | 13.60   | 13.25   | 13.25    |
| Net energy                   | Kcal  | 2,340   | 2,280   | 2,281    |
| 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     |
| Ca                           | %     | 0.73    | 0.68    | 0.60     |
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| Crude fibre                  | %     | 3.0     | 2.8     | 3.5      |
| Crude fat                    | %     | 5.0     | 3.1     | 3.2      |
| Crude ash                    | %     | 5.1     | 4.3     | 3.7      |
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| Digestible P                 | %     | 0.34    | 0.30    | 0.25     |
| Phytase FTU                  |       | 500     | 510     | 425      |

*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 %.

b Vitamins supplemented: vitamin A (retinyl acetate), vitamin D₃ (cholecalciferol), vitamin E (DL alpha tocopherol acetate), vitamin K (bisulphite menadione sodium), vitamin B₁ (thiamine mononitrate), vitamin B₂ (riboflavin), vitamin B₃ (nicotinic acid), vitamin B₅ (D-calcium pantothenate), vitamin B₆ (pyridoxine hydrochloride), vitamin B₁₂ (cyanocobalamin), vitamin B₁ (biotin), vitamin C (ascorbic acid), folic acid (folic acid), choline (choline chloride)
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 4 | The content of the control and the experimental feed for pigs (mg kg$^{-1}$ ± measurement uncertainty) |
|---------|------------------------------------------------------------------------------------------------------|
|         | Starter                                                                                             |
|         | Control                                                                                             |
| Microelements |                                                                                                        |
| Cu      | 13.3±2.0                                                                                           |
| Fe      | 206±31                                                                                             |
| Zn      | 56.9±8.5                                                                                           |
| Co      | 0.576±0.144                                                                                       |
| Mn      | 82.0±123                                                                                           |
| Mo      | 2.96±0.74                                                                                          |
| Cr      | 0.681±0.170                                                                                       |
| Se      | 3.31±0.58                                                                                          |
| B       | 167±25                                                                                             |
| Alkali and alkaline earth metal |                                                                                                       |
| K       | 3,550±532                                                                                          |
| Ca      | 3,111±467                                                                                          |
| Mg      | 788±118                                                                                           |
| Na      | 848±127                                                                                           |
| Ba      | 4.8±1.2                                                                                           |
| Toxic elements |                                                                                                       |
| As      | 3.0±0.6                                                                                           |
| Cd      | LLD                                                                                                |
| Ni      | 0.758±0.152                                                                                       |
| Pb      | 0.5±0.109                                                                                         |
| Other elements |                                                                                                       |
| Be      | LLD                                                                                                |
| Sr      | 8.8±2.2                                                                                           |
| Ti      | 3.76±0.94                                                                                         |
| Tl      | 0.137±0.034                                                                                       |
| Al      | 118±18                                                                                             |
| V       | 7.54±1.88                                                                                         |
| Grower                                                                                     |
| Control                                                                                             |
| Experimental |                                                                                                       |
| Finisher                                                                                           |
| Control                                                                                             |
| Experimental |                                                                                                       |

LLD lower limit of detection
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 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\(^b\)      | 45.3          | 2.33               | 45.6                      | 2.65     | ↑ 0.644  | 0.778   |
| APWG (kg)\(^c\) | 24.5          | 1.91               | 24.6                      | 1.57     | ↑ 0.545  | 0.854   |
| ADWG (g)\(^d\) | 942           | 73.3               | 947                       | 60.5     | ↑ 0.531  | 0.857   |
| APFI (kg)\(^e\) | 49.0          | 1.08               | 48.1                      | 2.19     | ↓ 1.77   | 0.356   |
| ADFI (kg)\(^f\) | 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\(^b\)      | 76.0          | 2.2                | 75.5                      | 4.2      | ↓ 0.614  | 0.739   |
| APWG (kg)\(^c\) | 30.7          | 1.8                | 30.4                      | 2.8      | ↓ 1.086  | 0.734   |
| ADWG (g)\(^d\) | 987           | 60                 | 982                       | 90       | ↓ 0.557  | 0.862   |
| APFI (kg)\(^e\) | 81.4          | 3.8                | 80.5                      | 4.4      | ↓ 1.065  | 0.611   |
| ADFI (kg)\(^f\) | 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\(^b\)      | 107           | 3                  | 105                       | 5        | ↓ 1.84   | 0.256   |
| APWG (kg)\(^c\) | 31.1          | 2.0                | 29.6                      | 3.5      | ↓ 4.85   | 0.210   |
| ADWG (g)\(^d\) | 1,036         | 68                 | 986                       | 115      | ↓ 4.85   | 0.210   |
| APFI (kg)\(^e\) | 94.9          | 4.2                | 94.8                      | 3.7      | ↓ 0.158  | 0.273   |
| ADFI (kg)\(^f\) | 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\(^b\)      | 86.3          | 3.9                | 84.1                      | 5.4      | ↓ 2.47   | 0.279   |
| APWG (kg)\(^c\) | 991           | 45                 | 967                       | 62       | ↓ 2.47   | 0.279   |
| ADWG (g)\(^d\) | 225           | 8                  | 223                       | 6        | ↓ 0.902  | 0.273   |
| ADFI (kg)\(^f\) | 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
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
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 \( \text{U L}^{-1} \). 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 \( \text{U L}^{-1} \) indicates liver and pancreatic cancers. In the experiment, no statistically significant differences between groups were found. ALT activity was at 33 \( \text{U L}^{-1} \).

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 \( \text{U L}^{-1} \). 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.

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 \( \mu \text{mol L}^{-1} \). 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 7 Evaluation of carcass slaughter value, mean ± SD |
|--------------------------------------------------------|
| Specification                 | Unit | Group | Increase (↑)/ decrease (↓) % | \( P \) value |
|--------------------------------|------|-------|-------------------------------|--------------|
| **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\(^d\)                 | pkt  | 1.75±0.26 | 1.90±0.52                      | ↑ 8.57       | ns            |
| Weight of liver\(^d\)         | 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\(^a\)            | mScm\(^{-2}\) | 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

\( *P<0.1; **P<0.05 \)

\( t \) 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 carcases 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 carcases 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 carcases 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)*        | 50.2±0.0      | 50.2±0.0                   | –       |
| Excrete in the faeces (g)*     | 6.32±0.74     | 5.83±0.50                  | ↓ 7.65  | ns      |
| Excrete in the urine (g)*      | 20.8±1.6      | 21.5±1.2                   | ↑ 3.53  | ns      |
| Retention (g)*                | 23.1±1.6      | 22.9±1.2                   | ↓ 1.08  | ns      |
| Retention relative to N collected (%)* | 46.0±3.2  | 45.5±2.4                   | ↓ 1.08  | ns      |
| Retention relative to N digested (%)* | 52.6±3.5 | 51.5±2.6                   | ↓ 2.15  | ns      |

*ns no statistical differences

*a t test

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

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

*ns no statistical differences

*a t test

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

**Table 9** Daily balance and nitrogen retention, mean ± SD

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*ns no statistical differences

*a t test
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.

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 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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