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Biofortification of Meat with Microelements by Biological Dietary Feed Supplements

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Abstract

In the present paper, zootechnical studies on fatteners fed with the new feed supplement formulation based on the soybean meal were undertaken.

Soybean meal was enriched with the following microelements via biosorption process: Cu(II), Mn(II), Fe(II) and Zn(II). The concentration of which in the enriched biomass of soya was as follows: 11.7 ± 1.5; 11.9 ± 1 .5; 5.92 ± 0.77 and 9.06 ± 1.18, respectively. Porkers were divided into three groups: control group feed with fodder supplemented with microelement ions in inorganic form, and two experimental groups: E100% where animals were fed with the fodder supplemented with microelements in biological form of enriched soybean meal, that covered 100% demand for microelements, and E125% group where animals were fed with the fodder supplemented with microelements in biological form of enriched soybean meal, that covered 125% demand for microelements.

Obtained results demonstrated that the use of biological microelement supplements increased the average daily gain of pigs, while reducing the consumption of feed. Also increased carcass weight and carcass yield compared to the control group was observed. Meat from the experimental groups was classified as class E, and studies of pH and EC confirmed that the meat was of good quality. Also, increased juiciness of the meat and red color component of meat were denoted in the experimental in comparison to the control group. Administration of pigs with biologically-bound microelements, resulted in an increased level of iron in the meat by about 10% at the recommended dosage, whereas at elevated dosages, the meat was richer in all microelements by 4.3 to 20.3%.

Keywords: Microelements supplementation; Cu(II), Mn(II), Fe(II), Zn(II); Biosorption; Soybean meal; Porkers

Introduction

In modern animal breeding, increased attention is paid to the quality of feed, including the bioavailability of the individual components, including micronutrients that are essential for the proper development and growth of the organism, as well as in maintaining an adequate level of animal welfare. Providing the required doses of micronutrients in the feed is not a fully comprehensive approach, because account should be taken of their availability. Currently, widely used inorganic salts are characterized by low availability to the animal, which results in high doses and the majority is excreted in manure and consequently lost [1-3]. An alternative to inorganic salts are organic forms of microelements, which are much better absorbed, but the cost of their use is unprofitable for many farmers [4,5]. For this reason, it is necessary to search for new formulations that will combine characteristics desirable by breeders: high bioavailability, a low cost, non-toxicity and ease of use.

Almost half of the contents of soybean meal is well absorbed protein (43-48%) [6], of which 90% are globulin and 10% albumin [7]. The protein in the soybean meal has a balanced amino acid profile [8] and compared to the other feed raw materials of plant origin, it contains high levels of lysine, which makes a particularly useful soybean meal for pigs [6,9].

Biosorption is known as selective and effective method of removing pollutants from waste water [10-12]. In this study the mentioned process was applied as technique of binding metal ions to the biomass of soybean meal which are nutritionally significant for animals. The process of binding microelements to the biomass is based on the ability of biological materials to bind metal ions by either metabolically mediated or purely physico-chemical pathways of uptake [13]. The utilization of biosorption process as a method of enrichment was proposed in the previous papers. The biomass of microalgae, as well as macroalage was enriched with microelements and then tested on the laying hens [14] and swine [15,16].

The aim of the current study was to develop the concept of waste-free technology of producing new formulations with microelements by biosorption. The biomass used was a typical component of compound feed. The new concept provides both a lower cost of manufacture of preparations as compared to previous studies conducted on algae. In this work, for the first time, research was conducted on the preparation obtained in the quarter-technical scale in developed and built specifically for this purpose installation. The utilitarian values of the product were tested in zootechnical study on porkers.

In this paper the zootechnical trials were performed to evaluate the utilitarian properties of new formulation for livestock. The biomass of soybean meal was enriched with microelements: Cu(II), Mn(II), Fe(II) and Zn(II) via biosorption process in quarter-technical pilot plant scale and then sent to the feeding experiments.

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Materials and Methods

Biosorption experiments

Soybean meal was enriched separately with a given microelement by the biosorption process. The solutions of Cu(II), Fe(II), Mn(II) and Zn(II) were prepared by dissolving appropriate amounts of $CuSO_4$•5H2O, $FeCl_2$•$4H_2O$, $MnSO_4$•$7H_2O$ and $ZnSO_4$•$7H_2O$ (POCh S.A. Gliwice, Poland) in deionized water. The initial concentration of the ions in the solutions was 300 mg/l. The pH of the solutions was adjusted to 5.0 with 0.1 MHCl/NaOH solution (POCh S.A. Gliwice, Poland) and measured with pH meter equipped with an electrode (InLab413) with the compensation of temperature (Mettler-Toledo Seven Multi; Greifensee, Switzerland). Biosorption experiments were performed in a column reactor with a capacity of 4 l. The process was carried out at ambient temperature. The solution was pumped through the column from the top. The flow rate was 0.072 l/h. The saturation of the bed was determined by controlling the concentration of the microelement ions in the solution coming out from the column. The complete saturation of the bed was reached after 7 h. After the biosorption, the preparations were air-dried at 25°C for 48 h. Samples of biomass were taken before and after the biosorption process to determine the content of the microelements in the enriched soybean meal.

Feeding experiments

Feed: The enriched biomass of soybean meal via biosorption process was investigated as the source of microelements – Cu(II), Fe(II), Mn(II) and Zn(II). The content of Cu, Fe, Mn and Zn in the biomass after biosorption process was presented in Table 1. Basing on the content of micronutrients in enriched biomass, the mass of soybean meal enriched with the elements that should be added to the feed to meet the needs of animals was calculated, taking into account the Polish and European legislation in this area and dietary recommendations [17,18]. Table 1 shows the dose of micronutrients, which are given to animals to cover 100% of their demands for these elements.

Three experimental groups were distinguished: control group (C) and two experimental groups (E100% and E125%). The dose of microelements in the experimental groups was provided at 100% (E100% group) and 125% (E125% group). All feed mixtures fed to pigs have been produced in the Feed Factory in Pawłowice (Poland). Mixtures of all the groups have been produced from the same components, only different premixes were applied. Premixes were produced by Cargill Poland Ltd. (Botulinum, Poland). In the premix, which was added to the feed for the control group, trace elements were provided in the form of inorganic salts and covered requirements of animals were in 100%. The doses of micronutrients have been selected in accordance with the current European Union standards of feeding pigs [17]. Experimental groups received the same premix, but at the production stage, the inorganic forms of zinc, copper, iron and manganese were withdrawn and replaced by enriched soybean meal (Soya-Zn, Soya-Cu, Soya-Fe and Soya-Mn, where 'Soya' is enriched soybean meal in the given microelement) (Table 2). The content of nutrients and feed additives is presented in Table 3. The source of vitamins and microelements was a premix produced by National Research Institute - Experimental Department in Pawłowice. The experimental group was fed with the same feed but microelements were supplemented by soybean meal enriched with microelements by biosorption.

Animals, housing: The duration of the experiment was 13 weeks (91 days), was conducted in Pawłowice Experimental Station of the National Research Institute of Animal Production while the carcass slaughter parameters were evaluated in the Control Station of Pig Slaughter Usefulness. Dewormed (Dectomax® or Ivomec®) porkers (females sex, line 990) (39 heads, 29.8 ± 2.3 kg) were randomly divided into 3 groups: 13 heads in the control group and 13 in the each experimental group. The two different feed compositions according to the different nutritional requirements for growth of the animals, were used. Porkers during the first period of fattening (40-65 kg, 7 weeks (49 days)) were fed with standard Grower feed mixture, and porkers in the second period of fattening (65-105 kg, 6 weeks (42 days)) were fed with the standard Finisher feed mixture. Nutritional value of fodder in specific periods of feeding was presented in Table 3. In the second week of experiment a vaccine to immunize against Porcine circovirus type 2 (PCV2) was provided.

M*	**Recommended supplementation of microelements (mg/kg feed)**	**Content of microelements in enriched soybean meal (mg/g)**	**Mass of enriched soybean meal added to feed-100% (g/kg feed for grower or finisher)**	**Mass of enriched soybean meal added to feed-125% (g/kg feed for grower or finisher)**
Cu(II)	20	11.7 ± 1.5	1.7	2.13
Fe(II)	50	11.9 ± 1.5	4.2	5.25
Mn(II)	20	5.92 ± 0.77	3.4	4.25
Zn(II)	50	9.06 ± 1.18	5.5	6.88

M*	Recommended supplementation of microelements (mg/kg feed)	Content of microelements in enriched soybean meal (mg/g)	Mass of enriched soybean meal added to feed-100% (g/kg feed for grower or finisher)	Mass of enriched soybean meal added to feed-125% (g/kg feed for grower or finisher)
Cu(II)	20	11.7 ± 1.5	1.7	2.13
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Zn(II)	50	9.06 ± 1.18	5.5	6.88

M* - microelement

Table 1: Doses of microelements in zootechnical experiments on fatteners (±SD, N=3).

Group and dose	Feed	Premix	Feed supplement
C100%	grower, finisher	Standard premix with microelements in inorganic form	-
E100%	grower, finisher	Premix with withdrawn inorganic forms of Cu, Mn, Zn and Fe	Soybean meal enriched separately with: Cu(II), Mn(II), Zn(II) oand Fe(II)
E125%	grower, finisher	Premix with withdrawn inorganic forms of Cu, Mn, Zn and Fe	Soybean meal enriched separately with: Cu(II), Mn(II), Zn(II) and Fe(II)

Table 2: Supplementation program in the zootechnical trial on finishers.

Component	Grower	Finisher
	Content (g/kg)	Content (g/kg)
Middlings triticale	300.0	300.0
Middlings barley	160.0	200.0
Middlings corn	200.0	200.0
Middlings soya 46%	140.0	90.0
Middlings rape	80.0	80.0
Bran wheat	65.1	87.5
Plant oil	20.0	9.0
Fodder chalk	10.0	10.0
Premix[1]	10	10
Monocalcium phosphate	6.0	4.5
NaCl	3.5	0.3
Pell-Tech	3.0	0.3
L-Lysine HCl 99%	2.0	2.3
Xylanase (Xynalase 4000G)	0.30	0.3
Phytase (Phyzyme XT)	0.10	0.1
Chemical composition	Content (g)	Content (g)
Crude protein	170.0	154.0
Crude fiber	43.0	45.0
Lysine	9.5	8.6
Methionine + Cystine	6.0	5.6
Threonine	6.2	5.6
Tryptophan	2.1	1.9
Ca	6.6	5.8
P total	6.2	6.0
Na	1.7	1.5
Metabolisable energy[2] (MJ)	129	126

*The composition of the feed was determined by the manufacturer. The content of ingredients per kilogram of feed: vitamin A (retinyl acetate). 700000 IU; Vitamin D3 (cholecalciferol). 50000 IUl; Vitamin E (DL alpha tocopherol acetate). 7000IU;

[1]Microelements supplemented (mg/kg feed): Fe as $FeSO_4$*H_2O 50; Mn as MnO_2 20; Cu as $CuSO_4$*$5H_2O$ 20; Zn as $ZnSO_4$*H_2O 50;

[2]The value declared by the manufacturer of feed.

Table 3: Composition of compound feed for grower and finisher pigs during fattening.

The study was performed in individual rearing pens (100x285 cm, floor covered with small layer of straw), with controlled microclimate (16-180°C). Feed and water were available semi ad libitum. Piggery was equipped with ventilation and central heating.

Sampling: The feeding experiment was conducted for 91 days and was divided into two series, first fattening phase (49 days) and second fattening phase (42 days), respectively. After each series, each animal was weighed. Every day morning the amount of not consumed fodder was recorded. At the end of experiments, 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 02/04/2004 by the persons entitled to professional slaughter and acceptable methods of slaughter and killing of animals (Polish Journal of Laws 2004.70.643). Approved procedure involved use of electronarcosis and exsanguination of pigs. Muscle (longissimus dorsi muscle) and liver samples were homogenized. All samples with the exception of fodder, were kept in the freezer for multielemental analysis.

Analytical methods

Determination of post-slaughter carcass parameters: All the analyses of parameters of meat, except multielemental composition, were performed at Pawłowice Experimental Station of the National Research Institute of Animal Production.

Specified value of pH45 and pH24 of meat, 45 minutes and 24 hours after slaughter was determined using a pH meter (Matthäus), while the measurement of conductivity in the muscle tissue was performed using a conductivity meter (Matthäus). Meat color was determined using Minolta camera in the L and b, and water absorption in accordance with the methodology Grau-Hamm, which is expressed in the ratio of water content to loose water content related to the total. Also, intramuscular fat content was determined by Soxlet SoxtecTM system based on technology of TecatorTM [19].

Determination of elemental composition: Multielemental composition of loin and ham was examined, sampled individually from each pig. The appropriate mass of biological sample (feed – 0.5 g, saya – 0.5 g) materials was digested with 5 mL of concentrated – 65% HNO3 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, 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 ILAC-MRA and Polish Centre for Accreditation according to PN-EN ISO/IEC 17025.

Calculations and statistical analyses

Obtained results underwent statistical analysis. The arithmetic mean values (( ), standard deviations (SD) and significance of differences between the groups were examined with the use of computer software Statistica ver. 9.0. Data were tested for normality (Shapiro– Wilk's test). In the case of normal distribution, the significance of differences between the groups was examined with a t-test. If the distribution was not normal, U-Mann–Whitney test was then applied. All the data are reported as means followed by standard deviation (SD). Differences were considered statistically significant at a probability $P < 0.05$

Post-slaughter carcass parameters and the meat content of elements were analyzed by analysis of variance (ANOVA) using Tukey's honest significance test.

Results and Discussion

In the present work the new concept of the production of feed additives with microelements by biosorption was proposed. The term «feed additive» is used in practice in relation to the registered formulations. In this paper we used the term «feed additive» in respect of the preparation, which de facto is a candidate for a feed additive. As the biomass which is the carrier of microelements, we proposed the material (soybean meal), which is a commonly used ingredient of compound feed. Thus, contrary to previous studies, the problem of costs related with obtaining additional components of feed (microalgae and macroalgae) was reduced by using a typical feeding material. This significantly reduced the costs of the preparation.

The first reports about the possibility of the use of soybean meal in animal nutrition originate from the early twentieth century. Osborne and Mendel [20] studied the possibility of using soybeans in animal nutrition, performing experiments involving rats. With the introduction of the ration of unprocessed soybeans, the animals showed a slight increase. The animals were given the processed soybean: milled grains were mixed with distilled water to a pulp consistency («trick mush»), then the mixture was heated on a steam bath and allowed to dry at 80-90°C. It was observed that the resulting product stimulated growth of the animal. Chemical composition of soybean meal is presented in Table 4.

Poland is one of the leaders of pork production in the European Union and the consumption of pork in the country per capita is higher than the average consumption of meat in the EU [21]. The main purpose of feeding studies conducted with finishers was to evaluate the effect of enriched soybean meal on characteristics of fattening and slaughter, as well as on meat quality and its mineral composition. Zootechnical trials on fatteners have been conducted with the use of the formulations prepared by biosorption in a quarter-pilot plant scale. Before supplementation of the feed, the enriched soybean meal was analyzed by ICP-OES in order to determine multielemental analysis for the content of e.g. toxic and nutritional elements in the produced supplements. The analysis showed that the level of toxic elements was lower than the levels permitted by the Polish legislation [22].

Ingredients	Content
Dry matter (g/kg)	880*
Metabolisableenergy (MJ/kg)	17.6**
Crude protein (g/kg)	471*
Crude fat (g/kg)	56*
Crude fiber (g/kg)	33*
Crudeash (g/kg)	64*
Ca (g/kg)	4.4**
P (g/kg)	7.5**
Saccharose (g/kg)	63**
Starch (g/kg)	51-69***
Cellulose (g/kg)	71***

*Wang et al. [31]

**Baker et al. [32]

***Jezierny et al. [33]

Table 4: Chemical composition of soybean meal.

Effect of enriched soybean meal on fattening and slaughter characteristics

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. Pigs performance and animal health were very good in all groups throughout the experiment. None of pigs was excluded from the experiment.

The value of slaughter is called the quantity and quality of raw meat and fat, which can be obtained from the animal. In order to assess the impact of enriched soybean meal on fattening and slaughter characteristics, the measurement of body weight of finishers on the day of start of the experiment and after 7 and 13 weeks of fattening was conducted. Table 5 shows the evaluation of carcass slaughter value. There were no statistically significant differences between groups in body weight of porkers on the first day of fattening. After the first phase of fattening (7 weeks), the animals in both experimental groups E100% and E125% had a higher mean body weight as compared to the control group by about 8.0 and 4.7% and the difference between the control group and the group E100% was significant (P=0.0957). After the first stage of fattening, higher average daily gain in the two experimental groups was also observed, 15.2 and 8.2%, respectively for the groups E100% and E125%, and the difference between the control group and the E100% group was statistically significant (P =0.0437). In turn, the average daily feed intake in the first phase of fattening was significantly lower in the group E125% than in the control group (P=0.000611) and in experimental E100% (P=0.00139) and the calorific value of feed at

Parameter		J*	Feeding group		
			C	E100%	E125%
Body weight	Initial	kg	29.8 ± 1.8	29.7 ± 2.8	30.1 ± 2.4
	After 7 weeks	kg	*64.0 ± 6.4*^{A}	*69.1 ± 5.4*^{A}	67.0 ± 6.3
	After 13 weeks	kg	102.6 ± 6.0	107.5 ± 5.9	107.3 ± 9.4
Average daily weight gain (I phase)		g	*697 ± 114*^{a}	*803 ± 82*^{a}	754 ± 123
Daily weight gain (II phase)		g	919 ± 56	916 ± 72	960 ± 110
Daily weight gain (all period)		g	800 ± 61	855 ± 58	849 ± 102
Daily feed intake (I phase)		kg	*2.08 ± 0.04*^{a}	*2.07 ± 0.05*^{b}	*1.98 ± 0.08*$^{a.b}$
Daily feed intake (II phase)		kg	*2.81 ± 0.05*$^{a.b}$	*2.96 ± 0.12*^{a}	*2.96 ± 0.18*^{b}
Feed conversion ratio	I phase	kg	*3.06 ± 0.53*^{a}	*2.45 ± 0.12*^{a}	2.69 ± 0.18
	II phase	kg	3.07 ± 0.21	3.25 ± 0.24	3.11 ± 0.29
	all period	kg	*3.11 ± 0.33*^{A}	*2.91 ± 0.16*^{A}	2.74 ± 0.66
Carcass weight		kg	*77.9 ± 5.2*$^{a.A}$	*83.8 ± 5.4*^{a}	*83.3 ± 7.4*^{A}
Carcass yield		%	*75.9 ± 1.9*$^{a.A}$	*77.9 ± 2.4*^{a}	*77.6 ± 1.2*^{A}
The meat content in the carcase		%	57.8 ± 2.1	57.7 ± 2.4	58.0 ± 1.8
Back fat thickness	Dorsal	cm	3.0 ± 0.3	2.9 ± 0.5	3.1 ± 0.6
	Shoulder	cm	1.5 ± 0.3	1.7 ± 0.4	1.5 ± 0.4
	Sacralis I	cm	2.1 ± 0.3	2.1 ± 0.5	2.1 ± 0.6
	Sacralis II	cm	1.3 ± 0.4	1.3 ± 0.3	1.2 ± 0.5
	Sacralis III	cm	2.2 ± 0.5	2.0 ± 0.8	2.1 ± 0.7
Weight of liver		kg	1.99 ± .022	1.99 ± 0.22	2.05 ± 0.22

$^{a.b.c}$Statistical significant differences p≤0.05

$^{A.B.C}$Significant differences p≤0.1

J* - unit.

Table 5: Evaluation of carcass slaughter value, mean ± SD (N=13).

this stage was smaller in both experimental groups than in the control group of which in the group E100% was 19.9% and the difference was statistically significant (P=0.0179).

After the second phase of fattening (after 13 weeks of the experiment), animals in the experimental group had higher body weight as compared to the control group by 4.8 and 4.6%, respectively for the groups of E100% and E125%, but the differences were not statistically significant. The average daily gain in the second phase of fattening in E125% group was comparable to the control group, but in the E100% group was higher than in the control group by 4.5%. Average daily feed intake in the second phase of fattening was higher in both experimental groups by 5.3% as compared to the control group and the differences were statistically significant (P=0.0153 for the group E100% and P=0.0147 for the group E125%). Feed consumption in this phase in the group E100% was higher than in the control group by 5.9%, while in the group E125% was comparable with the control group. Nevertheless, taking into account the duration of the entire fattening, feed consumption per 1 kg of body weight was the highest in the control group, in the experimental groups was lower in the group E125% by 6.4% , and significantly lower by 11.9% in the group E100% (P=0.0880). The results indicate that animals from both experimental groups make better use of feed provided during fattening, which is important for farmers from an economic point of view.

The average weight of the carcass after the experiment was higher in both experimental groups as compared to the control group by about 4.8% in E100% and the difference was statistically significant (P=0.0478), and by 4.6% in E125%, in which the difference was significant (P=0.0706). The average weight gain over the entire experiment was higher in both experimental groups by 6.9 and 6.1%, but the differences were not statistically significant. Yield from slaughter of animals in E100% group was higher as compared to the control group of fatteners by 2.6% and the difference was statistically significant (P=0.0300), while the dressing percentage of fatteners in E125% group was significantly higher by 2.2% (P=0.0637). According to the Decree of the Minister of Agriculture and Rural Development from 12 June 2003 concerning the quality of pig carcasses weighing from 60 to 120 kg of slaughter, differentiation between six classes (S, E , U, R, O, P) depending on the conformation was established. Both, the carcasses of the control group and experimental groups were in a range of 55-60%, according to the classification can be qualified to the E-Class, since the average meat content of the carcass in both experimental groups was slightly higher compared to the control group. The results indicate that the carcasses obtained met current standards and could be used by the meat industry. Similar results were obtained in the work of Saeid et al., [16], who examined the impact of micronutrient-enriched biomass of microalgae Spirulina sp. on the characteristics of fattening and slaughter fattening. Carcasses from the experimental group were classified as Class E, while the carcasses of the control group to a lower Class U.

There were also slight differences between the groups in backfat thickness, but the differences were not statistically significant. The greatest difference was observed between the control group and a group E125% in backfat thickness at the back, where the thickness of the fat in the group E125% was higher by 13.3% than in the control group. Liver weight in the experimental group of animals E100% by weight was equal to the livers of animals in the control group, while the mass of liver of animals from E125% was slightly higher than the control group (by 3.0%), but the difference was not statistically significant. This may indicate, that trace elements given in the form of enriched soybean meal did not burden the liver to a higher extent than the microelements provided in the form of inorganic salts.

The results indicate that administration of microelements in the form of enriched soybean meal had a positive impact on the characteristics of fattening and slaughter of animals in the experiment. In 2012, in the European Union the relative price of feed for livestock production accounted for 55.3%. Reduced feed intake was recorded during fattening, while larger daily increase signifies lower costs of livestock production, which is an important parameter for producers and farmers from the economic point of view.

Table 6 shows the parameters defining the quality of meat obtained from animals in the experiment. An important characteristic of pork is its pH. In the process of maturing, the meat is acidified (pH decreases). It is believed that the most favorable pH for consumption, and processing is in the range of pH 5.6-5.8 [23]. The higher the pH value, even greater than 6.0, the meat has better water absorption, intense color, improved taste and improved microbial stability. Due to the pH of the meat and its distinguished characteristics, it is categorized to the following classes [23,24]: RFN (Reddish, Firm, Non-exudative), PFN (Pale, Firm, Non-exudative), RFE (Reddish-Pink, Firm, Exudative), RSE (Reddish, Soft, Exudative), PSE (Pale, Soft, Exudative), DFD (Dark, Firm, Dry), AM (Acid Meat).

The most desirable is the meat of the class of RFN, so-called of normal quality. There is no single criterion by which to include meat to the above classes. Literature reference parameter ranges, according to which the meat is classified, differ to some extent. Literature reports that pH1 meat, measured after 45 minutes after slaughter, should be above 5.6 (some indicate that greater than 6.0). In the experiment pH1, measured after 45 minutes, had a value of approximately 6.0 in all the groups, which proves its good quality, the meat in the control group and in the group E100% was characterized by a slightly higher pH value than the meat from the group E125%. In turn, the value of the measured pH2 after 24 hours was less than 5.5 which indicates the class of PSE [24], but the value of pH2 was higher in the experimental group as compared to the control group, which indicates better quality of meat derived from animals fed with diet containing enriched soybean meal.

Conductivity, measured after 45 minutes (EC1) and after 24 hours (EC2) after slaughter is also an indicator of meat quality. According to German research stations, receiving the meat EC1 values below 4.3 mS/cm proves the high class of meat [23]. For all three groups of the

Parameter	Unit	Experimental group		
		C	E100%	E125%
pH_1		6.18 ± 0.42	6.19 ± 0.50	5.97 ± 0.49
pH_2		5.30 ± 0.07	5.37 ± 0.14	5.39 ± 0.11
Conductivity after 45 min (EC_1)	mS/cm	*1.76 ± 0.67*[a]	2.11 ± 0.68	*3.46 ± 2.60*[a]
Conductivity after 24 h (EC_2)	mS/cm	2.35 ± 0.82	3.43 ± 1.55	3.82 ± 2.09
Color of the meat after 45 min				
Lightness - L* a		47.38 ± 4.70	45.61 ± 3.47	47.41 ± 4.91
Redness - a* a		*16.13 ± 0.86*[A]	*16.86 ± 0.81*[A]	16.68 ± 0.70
Yellowness - b* a		2.39 ± 1.07	2.24 ± 1.27	2.38 ± 1.45
Color of the meat after 24 h				
Lightness - L* a		*53.07 ± 3.20*[a]	50.07 ± 4.78	*48.96 ± 4.04*[a]
Redness - a* a		*18.34 ± 1.61*[A]	*19.90 ± 1.82*[A]	19.61 ± 1.97
Yellowness - b* a		8.83 ± 2.46	8.87 ± 2.01	8.56 ± 1.75
Water adsorption	%	*18.80 ± 4.19*[A]	19.68 ± 3.73	*22.37 ± 3.27*[A]
Marbled	%	1.16 ± 0.27	1.01 ± 0.16	1.03 ± 0.20

[a.b.c]Statistical significant differences p≤0.05

[A.B.C]Significant differences p≤0.1

Table 6: The parameters defining the quality of meat obtained from animals in the experiment (±SD. N=13).

conductivity measured 45 minutes after the slaughter, it was less than 4.3 mS/cm and for the control group the lowest value was reached and the difference between the control group and the E125% was statistically significant. After 24 hours, the electrical conductivity in all groups was less than 4 mS/cm, which is also an indicator of meat quality [25]. Literature reports that tainted meat has a higher PSE as compared with normal meat conductivity measured 24 h after slaughter [26,27]. In the experiment, the higher value of EC2 as compared to the EC1 meat was characterized by both, the control group and similarly the experimental groups. The EC2 of meat of the control group was higher than the value of EC1 by 33.5%, in the experimental group E100% by 62.6%, while the experimental group meat from E125% only by 10.4%. Similarly, the research of Chmiel et al., [27], demonstrated that the EC2 for normal grade meat classified as RFN, may be in a wide range: between 2.3 and 11.5 mS/cm, so the value of this ratio cannot directly indicate the class of the tested meat. However, studies show that there is a correlation between higher values of EC2 and meat tendency to have defects of PSE or partially PSE.

Water absorption of meat is a parameter closely related to its juiciness and low water absorption is reported in the literature as one of the most common defects of meat [28]. In both experimental groups, water holding capacity of meat increased by 4.7% in the E100% and 19.0% in the E125% and the difference between these groups and the control group was significant (P=0.0501). Michalak [29] observed the opposite effect: the administration of enriched biomass of algae to fatteners caused deterioration of the water holding capacity of meat. The results obtained indicate that the meat from animals the diet of which was supplemented with enriched soybean meal was juicier as compared with meat derived from animals, to which microelements in the inorganic form were supplemented in feed, particularly when used in high doses as compared with a biological microelements. Enriched soybean meal was also in this case the carrier of ions better acceptable by animals than algae enriched with micronutrients by biosorption.

Another characteristic indicating the juiciness of the meat is intramuscular fat content (called marbling). In the experimental groups, a decrease of marbling was by 12.9% (group E100%) and by 11.2% (group E125%). Michalak [29] observed a similar effect, marbling of the meat in the experimental group fed with microelements bound with seaweeds was lower as compared to the control group.

Important for consumers parameter determining the quality of pork meat color is determined 45 minutes and 24 hours after slaughter. The meat color was defined in the system CIE L*a*b* (L - brightness , and - a reference to red, b - in the direction of saturation of yellow). Brightness meat color (L*) 45 minutes after the slaughter in the control group was similar to the value of this parameter in the group E125%, while in the group E100% the value of the parameter L* was lower. All values were below 50, which corresponds to the high-quality meat presented by Joo [30]. While the work Chmiel et al. [27] reports that the meat of the highest class - RFN may have brightness L* in the range of 45.4 - 55.9. In the experiment, the lowest brightness color 24 hours after slaughter, had the meat of the group E125% (48.96), a slightly higher value had meat from the group E100% (50.07), and the highest had meat from the group E125% (53.07). The parameter a*, in terms of the chromaticity in the range green◊red, was higher in both experimental groups as compared to the control group, both after 45 minutes and after 24 hours after slaughter, and the differences for E100% group were significant (P=0.0590 and P=0.0968, respectively). This suggests that the meat of the experimental groups was more intensively red than meat from the control group, which is an important criterion for the selection of meat by consumers. The parameter b* chromaticity considering terms of blue◊yellow, was comparable in the control group and E125% after 45 minutes of slaughter, while in the group E100% had a value slightly lower. However, after 24 hours of slaughter, this parameter had a value comparable to the control group and E100%, while in group E125% had a value slightly lower. Differences between the groups were not statistically significant.

Effect of soybean meal enriched with microelements on the composition of meat

One of the main objectives of the zootechnical trials on fatteners was to determine whether administration of enriched soybean meal to fatteners has an impact on the content of trace elements in meat. In previous studies, Michalak [29] and Saeid et al. [16] observed an increased content of micronutrients in meat of fattening pigs fed with a diet containing micronutrients in the biological form. It has been hypothesized that the administration of the new formulation to animals can serve as a method for increasing the density of micronutrients in foods of animal origin. The experiment was designed in order to check how the recommended and increased dosage of microelements in a new form affects the accumulation of these elements in meat (loin and ham).

Table 7 shows the content of trace elements in the loin and ham. The copper content of loin in E100% group was comparable to the control group and in the E125% group was higher by 10.4%, but the differences were not statistically significant. Cu(II) content in ham in the E100% group, was slightly lower compared to the control group, whereas in the E125% increased by 12.2%, but the differences were not statistically significant. The iron content in the ham and loin increased in both experimental groups as compared to the control group: in a loin by 9.1% in E100% and by 20.4% in E125%, in ham by 8.0% in E100% and 10.4% in E125%. The manganese content in the loin from E100% group was significantly lower than in the control group 7.5% (P=0.328), while the content of Mn(II) in ham in this group was also lower by 3.9%, but the difference was not statistically significant. However, in the group E125%, content of this element increased by 9.0% and 4.3% in loin and ham, respectively. Differences between groups were not statistically significant. The content of Zn(II) in the loin and ham in E100% group was comparable to the control group, but the content of this element in the meat from E125% group was higher by 7.8% and 11.4% in loin and ham, respectively. The results indicate that administration of micronutrients in the biological form bound with soybean meal to fatteners resulted in an increase in iron content in the meat of about 10% at the recommended dosage, whereas at elevated dosages the meat was richer in all trace elements by 4.3 to 20.3%.

Group	Content of microelement [mg/kg]			
	Cu(II)	Fe(II)	Mn(II)	Zn(II)
Loin				
Control	0.639 ± 0.137	3.95 ± 0.653	0.082 ± 0.008	12.0 ± 1.3
E100%	0.639 ± 0.097	4.31 ± 1.102	*0.076 ± 0.011*[a]	*11.8 ± 1.2*[A]
E125%	0.705 ± 0.201	4.75 ± 1.353	*0.090 ± 0.019*[a]	*13.0 ± 1.5*[A]
Ham				
Control	0.784 ± 0.144	6.20 ± 1.45	0.103 ± 0.014	15.8 ± 3.0
E100%	0.756 ± 0.173	6.69 ± 1.73	0.098 ± 0.022	16.4 ± 3.3
E125%	0.880 ± 0.175	6.84 ± 0.93	0.107 ± 0.022	17.6 ± 2.7

[a,b,c]Statistical significant differences p ≤ 0.05

[A,B,C]Significant differences p ≤ 0.1.

Table 7: The content of trace elements in the loin and ham (mg/kg ± SD; N=13).

Conclusions

The study was conducted with the participation of zootechnical finishers in order to assess the impact of new formulations on the parameters of slaughter and meat quality. Animals from experimental groups made better use of feed collected in both phases of fattening. It has been shown that the use of biological microelement additives increased the average daily gain in the first phase of fattening (15.2%), while a significant reduction in feed consumption (to 19.9%) was observed. In the second phase of fattening, average daily feed intake in the experimental groups was higher than in the control group, but feed consumption per 1 kg of body weight was lower in both groups. Supplementation with enriched soybean resulted in the increase in carcass weight by approximately 5% and significantly increased slaughter yield. Meat from the control group and the experimental groups was classified to the class E (meat quality). In the experimental groups, increased water holding capacity of meat (19.0%) was denoted, providing for its juiciness. pH and conductivity measurements confirmed its good meat quality. There was also darker red color of meat observed. Supplementation of fatteners diet with biological form of microelements in a recommended dosage, increased iron content in the meat of about 10%., while with an increased dosage of the bio-preparation increased the content of microelements from 4.3 to 20.3%.

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