INFLUENCE OF RAW PEA (*PISUM SATIVUM*) OR BLUE LUPIN SEEDS (*LUPINUS ANGUSTIFOLIUS*) ON THE LEVEL OF SELECTED BIOACTIVE SUBSTANCES IN PORK MEAT*

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Abstract

The study objective was to evaluate the impact of different contributions of pea (*Pisum sativum*) cultivar Hubal and blue lupin (*Lupinus angustifolius*) cultivar Regent on the level of selected bioactive substances in pork meat. One hundred three cross-breed piglets: ♀ (Landrace × Yorkshire) × ♂ Duroc were used. Two experiments were performed, in which pea seeds (experiment I: E1 – 5.0% pea seeds; E2 – 10.0% pea seeds; E3 – 15.0% pea seeds; E4 – 17.5% pea seeds) and blue lupin seeds (experiment II: D1 – 5.0% blue lupin seeds; D2 – 10.0% blue lupin seeds; D3 – 15.0% blue lupin seeds; D4 – 17.5% blue lupin seeds) were used instead of genetically modified soybean meal (SBM-GM). In each of the experiments 50 animals were divided into 5 groups (control – C, and four experimentals), placed in group pens, each for 10 individuals (sex ratio barrows : gilts – 1:1). The animals were weighed and tagged before the experiments. The initial body weight of the pigs at experiment I and II commencement was: 26.7 and 33.5 kg, and at the end of the experiments: 122.0 and 124.0, respectively. In the first experiment (progressive pea “seeds” contribution) the concentration of carnosine was shown to be higher in E4 than E3 and C by 47.3% and 94.2%, respectively. In comparison with group C, the Q10 coenzyme content in groups E1, E2, E3 and E4 was lower by 40.9%, 56.8%, 40.9% and 65.9% respectively. In the experiment II (progressive lupin “seeds” contribution) increased content of all of the investigated bioactive substances was recorded in groups D1-D3 vs C. Significant differences between groups C, D2, D4 for taurine ($P \leq 0.05$; $P \leq 0.01$) and creatine ($P \leq 0.05$) have been recorded. The content of bioactive substances in the *longissimus lumborum* muscle was significantly influenced by legumes, which increased the level of bioactive components of protein fraction. Therefore, it can be concluded that pea (*Pisum sativum*) cultivar Hubal and blue lupin (*Lupinus angustifolius*) cultivar Regent are an alternative to SBM-GM, increasing the nutritional value of pork meat.

Key words: fattening pigs, nutrition, pea seeds, lupin seeds, meat, peptides

Pork meat is the second most-consumed meat type in the world. Predictions of global pigmeat consumption for 2025 indicate that consumption will be 12,5 kg/citizen/year (Szűcs and Vida, 2017). Despite the growing consumer interest in this type of meat, information on its nutritional value, and in particular the content of bioactive substances is scarce (Kołodziej-Skalska et al., 2016).

Bioactive peptides are fragments of amino acid sequences of proteins, remaining inactive in their precursors, yet when freed from their parent proteins by proteolytic enzymes, they may act as modulators for a series of processes occurring in the organism (Darewicz et al., 2008). A biopeptide should exhibit biological effect or effects, which can be confirmed in a specific manner, in addition they should be beneficial for the health (Möller et al., 2008). The level of bioactive substances in the meat is determined by a variety of factors, with the most important including: animal species and breed, husbandry system, nutrition, muscle and cutting type, as well as its

processing technology (Arihara et al., 2001; Pravst et al., 2010; Cardenia et al., 2015; Łukasiewicz et al., 2018). Biologically active compounds found in foods influence human and animal organisms. They may have antioxidative and anticancer effects, they accelerate metabolism, improve digestion and reduce blood pressure and blood glucose levels (Lafarga and Hayes, 2014). Thus, modification of the content of these substances in meat should be of interest to consumers and producers.

Nowadays, post-extraction soybean meal is the main source of protein in feed mixes for pigs. According to FEFAC (2015), 90% of the post-extraction soybean meal available on the market and genetically modified plant derivatives are used for feed and consumption purposes. Research shows that genetic modifications do not affect animal production results and the quality and nutritional value of the obtained meat (Padgett et al., 1996; Aurlich et al., 2003; Flachowsky et al., 2005). What is more, no transfer of transgenic DNA to animal tissues could

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be verified (Świątkiewicz et al., 2011). The research results have not convinced some social groups in numerous countries, however, who require the elimination of products created with GM organisms from animal and human nutrition. Thus, alternative sources of proteins for humans as well as livestock are looked for, providing consumption products (meat, milk, eggs) to reduce the use of GM products. Livestock feeding requirements are met by legumes (pea, faba bean, sweet lupins), which can be successfully used in feed mixes for pigs (Sońta and Rekiel, 2017). Leguminosae is a large family with over 18,000 species of climbers, herbs, shrubs and trees. However, the most popular are: peas, broad beans, lentils, soybeans, lupins, lotus, sprouts, mung beans, green beans, etc. (Yorgancilar and Bilgicli, 2014). Legumes are an excellent source of good-quality protein in the range of 20 to 45%, including peas have 17–20% protein, and lupins have 38–45% protein (Kouris-Blazos and Belski, 2016). Protein from legumes is an excellent source of bioactive peptides. Bioactive peptides from enzymes of legume proteins exhibit a spectrum of biological activity, from nutraceuticals to therapeutic potential. Bioactive plant peptides (similar to those found in muscle tissue) fulfill a range of functions, including the protection against pathogens, infection, regulate growth and development. In order to increase the consumption of bioactive peptides, an important strategy is to increase its concentration in vegetable food via optimum yield management. It should be noted that studies concerning the correlations between the introduced agronomic practice and natural levels of peptides are scarce among the numerous scientific reports on the identification and characteristics of the available bioactive plant peptides (Farrokhi et al., 2008;

Belovic et al., 2011; Ortiz-Martinez et al., 2014). What is more, there are no data on the impact of available bioactive peptides from plant protein hydrolysis on human organism in the plant-animal-human chain.

The antioxidant capacity of legumes allows them to inhibit or slow down oxidative processes (Maphosa and Jideani, 2016). The exhaustion of antioxidant systems is one of the reasons for the occurrence of oxidative stress, which results in avalanche production of reactive oxygen species or free radicals. Puppel et al. (2015) point out the direct relationship between the excess of free radicals and the lowering of animal resistance and their susceptibility to diseases. Bearing in mind the above, increasing the level of antioxidants is beneficial from the point of view of animal welfare and meat antioxidant potential.

The study objective was to evaluate the impact of different contributions of the selected legumes: pea (*Pisum sativum*) cultivar Hubal and blue lupin (*Lupinus angustifolius*) cultivar Regent used in pig nutrition on the level of bioactive compounds such as: carnosine, anserine, Q10 coenzyme, taurine and creatine and creatinine in the *longissimus lumborum* muscle.

Material and methods

In line with Polish law and the EU Directive (no. 2010/63/EU) (2010), the experiment did not require approval of the local Ethics Committee, because it was performed at a private farm (in production conditions).

Two experiments on growing pigs were performed, in the nutrition of which pea and blue lupin seeds were used instead of SBM-GM.

Table 1. Contribution (%) of raw materials in feed mixtures administered at stage I and II of fattening in experiment I

Raw feed materials	Groups				
	C	E1	E2	E3	E4
1	2	3	4	5	6
Fattening stage I					
Barley	35.0	30.0	25.0	15.0	5.0
Triticale	24.0	19.2	20.3	22.1	28.3
Wheat	20.0	25.0	25.0	30.0	30.0
Oats	5.0	5.0	5.0	5.0	5.0
Soybean meal GM	13.0	9.7	8.3	6.4	2.0
Rapeseed meal	–	2.5	2.5	2.5	7.8
Pea seeds	–	5.0	10.0	15.0	17.5
Soybean oil	–	0.6	0.9	1.0	1.4
Premix*	3.0	3.0	3.0	3.0	3.0
Analyzed nutritional value (%)					
Dry matter	87.6	87.4	86.9	87.3	87.5
Crude protein	16.4	16.5	16.3	16.5	16.3
Ether extract	2.6	2.7	2.7	2.7	2.8
Crude fiber	3.9	3.9	4.0	4.1	4.1
Crude ash	3.9	4.0	3.9	4.1	3.9

Table 1 – contd.

1	2	3	4	5	6
Calculated nutritional value (%)					
Metabolizable energy (MJ/kg)	13.22	13.21	13.21	13.21	13.21
Lysine	1.05	1.06	1.09	1.10	1.10
Methionine + Cysteine	0.64	0.64	0.64	0.63	0.67
Threonine	0.70	0.71	0.71	0.71	0.72
Tryptophan	0.20	0.19	0.19	0.19	0.19
Calcium	0.81	0.82	0.82	0.82	0.86
Phosphorus	0.54	0.55	0.55	0.54	0.58
Sodium	0.17	0.19	0.21	0.23	0.24
Fattening stage II					
Barley	35.0	25.0	15.0	10.0	8.2
Triticale	32.0	26.5	30.0	30.0	30.0
Wheat	10.0	21.6	24.7	30.0	30.0
Oats	10.0	10.0	10.0	6.9	5.0
Soybean meal GM	10.5	6.6	4.8	3.0	-
Rapeseed meal	–	2.5	2.5	2.2	6.0
Pea seeds	–	5.0	10.0	15.0	17.5
Soybean oil	–	0.3	0.5	0.4	0.8
Premix*	2.5	2.5	2.5	2.5	2.5
Analyzed nutritional value (%)					
Dry matter	86.8	86.4	87.0	86.7	86.1
Crude protein	15.3	15.4	15.5	15.2	15.1
Ether extract	2.5	2.6	2.6	2.6	2.7
Crude fiber	4.1	4.2	3.9	4.2	3.9
Crude ash	4.5	4.7	4.5	4.7	4.6
Calculated nutritional value (%)					
Metabolizable energy (MJ/kg)	13.17	13.16	13.17	13.17	13.16
Lysine	0.94	0.94	0.95	0.96	0.97
Methionine + Cysteine	0.60	0.61	0.61	0.60	0.61
Threonine	0.64	0.64	0.64	0.64	0.65
Tryptophan	0.18	0.18	0.18	0.17	0.17
Calcium	0.68	0.70	0.69	0.69	0.71
Phosphorus	0.51	0.51	0.51	0.50	0.52
Sodium	0.15	0.17	0.19	0.21	0.21

*Premix composition: lysine – 12.10%; methionine – 2.65%; threonine – 5.05%; tryptophan – 0.25%; calcium – 20.50%; phosphorus – 1.80%; sodium – 5.00%; iron – 4,000 mg; manganese – 2 400 mg; zinc – 2 600 mg; copper – 800 mg; iodine – 55.0 mg; selenium – 13.50 mg; vitamin A – 260,000 IU; vitamin D₃ – 69,000 IU; vitamin E – 4 700 mg; vitamin K₃ – 68 mg; vitamin B₁ – 68 mg; vitamin B₂ – 170 mg; vitamin B₆ – 105 mg; vitamin B₁₂ – 830 mcg; vitamin C – 1,000 mg; folic acid – 27.00 mg; pantothenic acid – 410 mg; niacinamide B₃ – 690 mcg; biotin – 3,450 mg; choline chloride – 10,000 mg; Aroma, antioxidant: 1b (E320-BHA, E321-BHT, E324 – Ethoxyquin) 550 mg/kg; Enzymes: 4a E-1 640 6 – phytase (EC 3.1.3.2.6 n-5000 FTU/g) 17 500 FTU/kg, (E1600 endo 1,4-beta-xylanase, EC 3.2.1.8 – 22,000 VU/g; 425,000 VU/kg, endo 1.3 beta-glucanase EC 3.2.1.6 – 30,000 VU/g, 57 000 VU/kg); raw material composition: calcium carbonate, monocalcium phosphate, (monophosphate) sodium chloride 1.8.1.9, herbal mix 10 g/kg.

Animals and housing

For two experiments, one hundred three cross-breed piglets: ♀ (Landrace × Yorkshire) × ♂ Duroc were used. The animals were weighed and tagged prior to the experiments. The initial body weight of the pigs at experiment I and II commencement was: 26.7 and 33.5 kg, and at the end of the experiments: 122.0 and 124.0, respectively. Fifty animals were divided into 5 groups for each experi-

ment (control – C, and experimental – E1, E2, E3, E4), and were placed in group pens, each for 10 individuals (sex ratio: barrow : gilts – 1:1). The husbandry conditions complied with the Regulation of the Ministry of Agriculture and Rural Development of 15 February (2010). Experiment I was conducted between 23 December 2016 and 22 March 2017 and experiment II from 10 November 2017 to 23 January 2018.

Table 2. Contribution (%) of raw materials in feed mixtures administered at stage I and II of fattening in experiment II

Raw feed materials	Groups				
	C	D1	D2	D3	D4
Fattening stage I					
Barley	36.6	12.0	11.0	5.4	2.3
Triticale	15.0	30.0	30.0	33.0	35.0
Wheat	25.0	33.7	35.0	35.0	33.0
Oats	5.0	3.0	–	–	–
Soybean meal GM	15.0	10.5	8.0	5.5	2.0
Rapeseed meal	–	2.5	2.5	2.5	6.0
Blue lupin seeds	–	5.0	10.0	15.0	17.5
Soybean oil	0.4	0.3	0.5	0.6	1.2
Premix*	3.0	3.0	3.0	3.0	3.0
Analyzed nutritional value (%)					
Dry matter	87.7	87.4	87.3	87.1	87.4
Crude protein	16.3	16.4	16.3	16.2	16.4
Ether extract	2.6	2.6	2.7	2.7	2.8
Crude fiber	3.9	3.9	4.2	4.2	4.1
Crude ash	3.9	4.1	4.1	4.1	3.9
Calculated nutritional value (%)					
Metabolizable energy (MJ/kg)	13.23	13.19	13.20	13.21	13.20
Lysine	1.09	1.06	1.04	1.03	1.05
Methionine + Cysteine	0.66	0.65	0.64	0.63	0.64
Threonine	0.72	0.71	0.70	0.70	0.71
Tryptophan	0.21	0.20	0.19	0.19	0.18
Calcium	0.87	0.89	0.89	0.89	0.91
Phosphorus	0.53	0.52	0.51	0.50	0.51
Sodium	0.17	0.18	0.18	0.18	0.18
Fattening stage II					
Barley	36.7	12.0	11.4	8.8	5.8
Triticale	20.0	30.0	30.0	33.0	35.0
Wheat	20.0	32.3	35.0	35.0	33.0
Oats	8.0	7.5	3.0	–	–
Soybean meal GM	12.8	8.2	5.6	3.1	–
Rapeseed meal	–	2.5	2.5	2.5	5.6
Blue lupin seeds	–	5.0	10.0	15.0	17.5
Soybean oil	–	–	–	0.1	0.6
Premix*	2.5	2.5	2.5	2.5	2.5
Analyzed nutritional value (%)					
Dry matter	87.6	87.9	87.4	87.5	88.0
Crude protein	15.4	15.6	15.3	15.2	15.3
Ether extract	2.2	2.1	2.3	2.2	2.3
Crude fiber	3.9	3.8	4.2	4.0	4.1
Crude ash	4.0	3.9	4.0	4.1	4.3
Calculated nutritional value (%)					
Metabolizable energy (MJ/kg)	13.20	13.19	13.19	13.19	13.20
Lysine	0.98	0.95	0.96	0.96	0.96
Methionine + Cysteine	0.63	0.63	0.61	0.60	0.60
Threonine	0.66	0.65	0.65	0.64	0.65
Tryptophan	0.19	0.19	0.18	0.17	0.17
Calcium	0.74	0.75	0.76	0.76	0.78
Phosphorus	0.50	0.49	0.48	0.47	0.48
Sodium	0.14	0.15	0.15	0.15	0.16

*Premix composition – see: Table 1.

Nutrition

Complete mixes in bulk, administered *ad libitum*, with constant access to water were used in the 2-stage fattening (experiment I: 88 days; experiment II: 73 days) (Table 1 and 2). The pigs were fed with balanced feed, as per the recommendations (Grela and Skomiał, 2015).

Amino acid content determinations were performed with the gas chromatography method at the Central Agro-ecological Laboratory of the Lublin University of Life Sciences, according to the internal procedures of the laboratory (CLA/PLC/20, CLA/PLC/34) (Table 3).

Table 3. Amino acid content in pea and blue lupin seeds (mg/g d.w.)

Amino acids	Pea seeds cv. Hubal	Blue lupin seeds cv. Regent
Lysine	15.30	12.30
Methionine	1.83	1.66
Leucine	15.20	16.70
Isoleucine	8.50	9.38
Histidine	5.34	6.69
Phenylalanine	10.20	9.38
Threonine	8.30	8.79
Tryptophan	1.62	7.56
Valine	9.57	9.24
Arginine	18.70	29.00
Tyrosine	6.05	8.07
Cysteine	3.66	4.40
Alanine	9.23	8.86
Glycine	9.01	10.20
Aspartic acid	25.10	24.80
Glutamine	41.10	59.90
Serine	10.80	12.10
Proline	9.74	10.90

Analysis of bioactive substances

After the completion of the fattening, all animals were subject to slaughtering according to the procedures of the plant. After 24-hour cooling at the temperature of +4°C, samples of the *longissimus lumborum* muscle (MLL) (approx. 0.5 kg) were collected for the study from the right half carcasses. Approximately 200 g of muscle sample was ground in a machine with revolving blades and mixed. The material was secured and frozen.

The levels of bioactive peptides – carnosine, anserine (Łukasiewicz et al., 2015), Q10, creatine, creatinine, taurine (Purchas et al., 2004) in the muscles were determined using the reverse-phase high-performance liquid chromatography RP-HPLC Agilent 1100 (Agilent Technologies, Waldbronn, Germany) and a Jupiter C18 300A column (Phenomenex, Torrance, CA, USA). The mobile phase A comprised of acetonitrile mixture with water (30:70) and 0.1% TFA acid (both reagents by Merck), phase B – acetonitrile mixture with water (70:30) and

0.1% TFA. The flow through-column was 1.4 ml/min., at UV detection of 214 nm. The qualitative analysis was performed using the calibration against the models: carnosine Lot # CBB7948; anserine Lot # BCBF45160, Q10 Lot # SLBT6669, taurine Lot # BCBJ5953V, creatine Lot # CBAD0780, creatinine Lot # CBBG4255 (Sigma-Aldrich, St Louis, MO, USA).

Statistical analysis of results

The results were statistically elaborated using the IBM SPSS Statistics 21 package, independently for the experiment I and II. The Shapiro-Wilk test was used to check the normal distribution. Differences between the groups were tested with the Kruskal-Wallis test. The tables present mean result and standard deviation. The differences were determined statistically significant at $P \leq 0.05$ or $P \leq 0.01$.

Results

Production results of this experiment already published in the journal *Animals* (Table 4) indicate that there is no contraindication to the use of pea and blue lupin seeds at 5.0% to 17.5% as alternative feed materials to SBM-GM in fattening pigs (Sońta et al., 2020). These results are beneficial from both environmental and consumer perspectives, hence the continued research towards bioactive peptides.

Experiment I

The lowest carnosine content was determined in group E2, and the highest in group E4 (Table 5). Highly statistically significant differences were confirmed between groups E3 vs C, E1 and E2, and E4 vs C, E1 and E2. Carnosine content in the meat of the experimental fattening pigs E3 and E4 in comparison with control C was higher by 47.3% and 94.2%. The content of anserine was lowest in group E2, and in groups E3 and E4 approximately 2-fold higher vs. E2 ($P \leq 0.01$). The differences between E2 vs E3 and E4 were 100% and 88%, respectively.

The Q10 coenzyme content in all experimental groups was significantly lower than in control ($P \leq 0.01$), approx. 2-fold, and up to 3-fold (group E4 vs C). In comparison with group C, the Q10 coenzyme content in groups E1, E2, E3 and E4 was lower by 40.9%, 56.8%, 40.9% and 65.9% respectively and it was not a directional correlation. The lowest taurine content in the tested *longissimus lumborum* muscle samples was recorded in the control group and in the experimental E2 group. The highest level of taurine was determined in the meat of pigs from group E3. Highly statistically significant differences (at $P \leq 0.01$) were determined between groups E3 vs C, E1 and E2, as well as E4 vs C and E2, and at $P \leq 0.05$ between E1 and E4. Creatine level was at its highest in group E2, and lowest in group E3. Differences at $P \leq 0.01$ were demonstrated between groups C vs E3 and E4; E1 vs E3; E2

vs E3 and E4. The highest creatinine content was recorded for the *longissimus lumborum* muscle samples of the pigs from group C, and the lowest from E4 ($P \leq 0.01$).

Experiment II

Table 6 presents the results of the mean content of the tested bioactive substances in the *longissimus lumborum* muscle. The highest values for all of the studied bioac-

tive substances were determined in group D2, fed with 10% addition of blue lupin. The lowest carnosine content was determined for group C. The content of anserine, Q10 coenzyme, taurine, creatine and creatinine was lowest in the *longissimus lumborum* muscles obtained from E4 pigs. Significant differences were recorded between groups C, D2, D4 for taurine ($P \leq 0.05$; $P \leq 0.01$) and creatine ($P \leq 0.05$).

Table 4. Daily body weight gains and meatiness of fatteners (Experiment I and II) ($\bar{x}$, SD) Soñta et al. (2020)

Item	Groups ¹					P-value
	C	E1/D1	E2/D2	E3/D3	E4/D4	
Experiment I						
Initial body weight (kg)	26.4±1.1	27.1±0.8	26.9±0.9	26.5±0.8	26.5±1.0	0.336
Final body weight (kg)	123.4±9.6	123.0±8.7	124.8±7.5	122.1±7.3	116.5±9.7	0.255
Average daily body weight gain (g)	1104±119	1090±97	1113±87	1086±80	1022±108	0.294
Meatiness (%)	60.0±0.8	60.6±1.9	60.1±2.3	60.4±2.0	59.7±2.4	0.854
Experiment II						
Initial body weight (kg)	33.5±1.4	33.1±1.5	33.9±1.5	33.7±1.8	33.4±1.5	0.810
Final body weight (kg)	125.5±8.7	123.5±5.8	125.1±4.9	126.6±7.7	121.1±7.4	0.452
Average daily body weight gain (g)	1260±118	1238±82	1249±70	1272±107	1201±92	0.529
Meatiness (%)	58.9±1.9	58.9±2.9	58.9±2.0	59.2±2.5	58.1±2.5	0.878

¹C – soybean meal; E1 – 5.0% pea seeds; E2 – 10.0% pea seeds; E3 – 15.0% pea seeds; E4 – 17.5% pea seeds; D1 – 5.0% blue lupin seeds; D2 – 10.0% blue lupin seeds; D3 – 15.0% blue lupin seeds; D4 – 17.5% blue lupin seeds.

Table 5. Content of bioactive substances in the *longissimus lumborum* muscle (mg/g) ($\bar{x}$, SD)

Item	Groups ¹					P-value
	C	E1	E2	E3	E4	
Carnosine	2.43 Aa±0.93	2.44±1.09	2.12 EF±0.49	3.58 ACE±1.41	4.72 BDF±1.65	0.005
Anserine	0.23±0.09	0.22±0.14	0.17 AB±0.06	0.34 A±0.29	0.32 B±0.11	0.035
Q10 coenzyme	0.044 ABCD±0.011	0.026 A±0.005	0.019 B±0.001	0.026 C±0.007	0.015 D±0.001	0.001
Taurine	0.17 Aa±0.02	0.22±0.15	0.17 DE±0.02	0.37 ACD±0.17	0.30 Bea±0.12	0.002
Creatine	2.46 AB±0.97	2.05±1.12	2.49 DE±0.93	1.13 ACD±0.44	1.78 BE±0.37	0.005
Creatinine	1.48 ABCD±0.38	0.74 A±0.51	0.57 B±0.32	0.68 C±0.31	0.47 D±0.14	0.002

¹C – soybean meal; E1 – 5.0% pea seeds; E2 – 10.0% pea seeds; E3 – 15.0% pea seeds; E4 – 17.5% pea seeds.

A, A – means in the lines marked with the same upper case letters differ significantly at $P \leq 0.01$.

a, a – means in the lines marked with the same lower case letters differ significantly at $P \leq 0.05$.

Table 6. Content of bioactive substances in the *longissimus lumborum* muscle (mg/g) $\bar{x}$, SD)

Item	Groups ¹					P-value
	C	D1	D2	D3	D4	
Carnosine	2.47 A±0.46	3.23±0.86	3.60 a±0.98	2.90±0.79	2.50±0.76	0.048
Anserine	0.33±0.09	0.39±0.07	0.41±0.11	0.34±0.09	0.31±0.08	0.149
Q10 coenzyme	0.064±0.014	0.071±0.016	0.080±0.018	0.066±0.015	0.059±0.013	0.087
Taurine	0.20 A±0.03	0.23±0.03	0.26 Aab±0.03	0.21 b±0.03	0.19 A±0.03	0.006
Creatine	2.03±0.24	2.25±0.26	2.47 ab±0.31	2.40±0.96	1.93 b±0.29	0.020
Creatinine	1.06±0.22	1.18±0.25	1.33±0.28	1.09±0.23	0.98±0.21	0.114

¹C – soybean meal; D1 – 5.0% blue lupin seeds; D2 – 10.0% blue lupin seeds; D3 – 15.0% blue lupin seeds; D4 – 17.5% blue lupin seeds.

A, A – means in the lines marked with the same upper case letters differ significantly at $P \leq 0.01$.

a, a – means in the lines marked with the same lower case letters differ significantly at $P \leq 0.05$.

Discussion

Legumes are considered the second most important food source after cereals. Legumes are tasty, easily absorbable and nutritionally valuable (Pettersson et al., 1997; Messina, 1999), providing bioactive compounds, such as protein, nutritional fiber, phenolic compounds, flavonoids, antioxidants, tocopherols, carotenoids, minerals and vitamins, which are responsible for preventing numerous diseases of civilization, including diabetes, cancers, cardiovascular diseases and other chronic disorders (Barbana and Boye, 2010; Amarowicz and Pegg, 2008; Frias et al., 2005). Tables 5 and 6 show changes in the concentration of carnosine for progressive pea and lupin contributions. The lowest content of carnosine was reported as 2.43 mg/g (Table 5, C group), while the highest was 4.72 mg/g (Table 5; E4 group). Carnosine was significantly ($P \leq 0.01$) influenced by legumes. Easter and Baker (1977) reported that carnosine content in pork meat ranges between 2.40 and 4.66 mg/g, which is confirmed by the obtained results. Considering that the carnosine homeostasis regulation has been poorly studied, it proves difficult to explain unambiguously its progressive increase in the meat in experiment I. Our results are consistent with the earlier research (Cornet and Bousset, 1999), showing that the white muscle had higher carnosine content than the dark muscle. According to the model of homology by Vistoli et al. (2006), the carnosinase activity in the serum is stimulated by citrate. Schulz et al. (1989) observed that cAMP inhibits carnosine synthase activity. This study suggests that carnosine concentration is metabolically regulated.

Oxidative intermediate and oxidative slow twitch muscle fibers have been found in the *longissimus dorsi* muscle of wild pigs compared to domesticated pig breeds (Essén-Gustavsson and Lindholm, 1984). Therefore, the lower anserine content in pigs is likely correlated with the more pronounced oxidative metabolism, both in terms of the performed kinetic activity as well as the used hybrid. The lowest content of anserine was reported as 0.17 mg/g (Table 5, E2 group), while the highest was 0.41 mg/g (Table 6; D2 group). Anserine was significantly ($P \leq 0.05$) influenced by legumes. A reverse trend was observed for anserine – meat with the highest carnosine content was characterized by the lowest anserine content. In a summary, in reality the content of the mentioned peptides is not only influenced by the kinetic activity and the fiber type, but also the breed, sex, age or husbandry system (Abe and Okuma, 1995), as well as nutrition.

Taurine shows numerous physiological functions (osmotic pressure regulation, protection of cells against free radicals, influence on the development of the brain and the retina), it is also beneficial for cardiac muscle operation (Pasantés-Morales et al., 1990). Meat is a rich source of this compound, however its concentration depends on the meat and muscle type. Franco et al. (2010) reported that the masseter muscle, followed by *semimembranosus*, and *biceps femoris*, had the highest concentration

of taurine, where taurine accounted for 40, 35 and 31% of the total free fraction, respectively. Taurine content in pork meat depends on the muscle type, and it is highly variable (22.5–162.5 mg/100 g). The lowest content of taurine was reported as 0.17 mg/g (Table 5, C group), while the highest was 0.37 mg/g (Table 6; E3 group). Taurine was significantly ($P \leq 0.01$) influenced by legumes. Taurine is synthesized in mammalian organisms from sulfur amino acids, methionine and cysteine (Pasantés-Morales et al., 1990; Kulasek et al., 2004; Marušić et al., 2013). In the present experiment, the higher taurine content was perhaps influenced by the elevated supply of these amino acids in the mixes, through the increasing contribution of (pea) seeds in the nutrition in experiment I, groups E1-E4 vs C. Post-extraction soybean meal was the sole protein source in the control mix (present study). As stated by Biel (2011), the content of methionine and cysteine in genetically modified post-extraction soybean meal is lower than in legume seeds, which may result in reduced taurine synthesis.

The remaining bioactive substances determined in the present study also exhibit health-promoting properties. The Q10 coenzyme content in pork meat ranges between 13 and 45 mg/100 g (Pravst et al., 2010), which is not confirmed by the obtained results, because the lowered level has been reported. The lowest content of Q10 was reported as 0.015 mg/g (Table 5, E4 group), while the highest was 0.080 mg/g (Table 6; D2 group). Q10 coenzyme was significantly ($P \leq 0.01$) influenced by legumes. Tyrosine takes part in its synthesis, which has been confirmed in the present study (higher contribution of tyrosine in lupin seeds vs pea seeds likely determined the obtained result), as well as numerous vitamins (Folkers, 1996). However, variations in the coenzyme content have been observed for various species. The highest concentration of this coenzyme is found in the meat of ruminants (cattle and sheep), between 1.1 and 3.1 mg/100 g, with the level of 3.92 mg/100 g in cattle liver. The presence of Q10 coenzyme in the organism is necessary for the normal course of numerous biochemical transformations. It is responsible for the production of high-energy compounds, used by the organism to supply the energy necessary for vital processes. This substance enhances the immune system, exhibits antioxidative action and prevents coronary heart disease; therefore, the increase in coenzyme Q10 obtained in the present experiments is beneficial to consumers.

The action of creatine and creatinine consists of a rapid supply of energy to muscles for their operation, which occurs through ADP to ATP conversion. Creatine synthesis occurs within the liver, kidneys and pancreas, and the organism utilizes arginine, methionine and glycine for this purpose (Mora et al., 2010). Creatine is dissolved to creatinine in the presence of water, low pH and elevated temperature (Pischel and Gastner, 2007), which may have practical significance for thermal processing of meat and production of processed products. The cited authors determined creatine and creatinine level in pork

meat at 414 and 8.21 mg/100 g, respectively, which is not confirmed by the obtained results, because the lowered level of creatine has been reported. The lowest content of creatine was reported as 1.13 mg/g (Table 5; E3 group), while the highest was 2.49 mg/g (Table 5; E2 group). Creatine was significantly ($P \leq 0.01$) influenced by legumes. Having analyzed the results of the present study as well as the selected, available literature devoted to the topic (Pravst et al., 2010), we could agree with the claim of Arihara et al. (2001), who believe that pork meat is a good source of bioactive substances. The cited authors provide the content of certain bioactive substances in pork meat: creatine (247–347 mg/100 g), carnosine (211–419 mg/100 g), taurine (50–72 mg/100 g), Q10 coenzyme (approx. 2 mg/100 g).

Conclusions

The impact of feed minerals used in the nutrition of animals for slaughter on the quality of raw meat is an interesting issue. For this reason, the present study analyzed the content of several bioactive compounds in the pork meat obtained from the studied fattening pigs. An increase in the level of bioactive substances is highly beneficial from the health standpoint. The content of bioactive substances in the *longissimus lumborum* muscle was significantly influenced by legumes. Studies have shown that 10.0% blue lupin seeds had the most beneficial effect. Therefore, it can be concluded that pea (*Pisum sativum*) cultivar Hubal and blue lupin (*Lupinus angustifolius*) cultivar Regent is an alternative to SBM-GM, increasing the nutritional value of pork meat.

Author contributions

Conceptualization, M.S. and A.R.; methodology, M.S., M.Ł. and K.P.; software, M.B. and J.W.; validation, M.B. and J.W.; formal analysis, M.S., J.W. and M.B.; investigation, M.S., M.Ł. and A.R.; resources, M.Ł. AND K.P.; data curation, J.W. and M.B.; writing – original draft preparation, M.S., M.Ł. AND A.R.; writing – review and editing, M.S., M.Ł. and A.R.; visualization, M.S.; supervision, A.R.; project administration, M.Ł.; funding acquisition, M.S. and A.R. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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