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EFFECT OF DIFFERENT PROTEIN SOURCES ON THE MEAT QUALITY OF ZŁOTNICKA SPOTTED PIGS WITH PARTICULAR EMPHASIS OF SLAUGHTER BODY WEIGHT*

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Abstract

The aim of the study was to determine the impact of protein feeding from Fabaceae plants and the final slaughter weight on the quality of meat of native Zlotnicka Spotted pigs. The experiment was carried out on 96 fattening pigs (48 barrows and 48 gilts) of the Złotnicka Spotted breed. Animals were divided into 4 feeding groups: group A – control cereal-soybean mixture, and 3 experimental mixtures containing different types of domestic protein sources (groups B – lupin, C – faba beans and D – peas). All diets were isoproteic and isoenergetic within the fattening phase, i.e. grower and finisher. In addition, pigs were fattened to 3 different slaughter weights: 120, 130 and 140 kg. The type of protein had no effect on fattening and slaughter traits. These traits (feed intake [kg], gaily gain during fattening [g/day], mean backfat thickness [mm]) were influenced by the body weight of the pigs at slaughter (P<0.01). A statistically significant interaction effect was found between feeding group and body weight on the fat, the fatty acid profile of meat (*longissimus lumborum*) and on oxidative stability (thiobarbituric acid reactive substances – TBARS) of meat. Increasing the slaughter weight to 140 kg affects increasing the fat content (P<0.05) of meat (*longissimus lumborum*) primarily in groups of pigs fed on cereal-soy mix (A) and faba beans (C). In addition, fat up to 140 kg and feeding with faba beans (C) and peas (D) has a beneficial effect by reducing the proportion of PUFA n-6/n-3 in meat (P<0.05). Finally, the benefit of feeding pigs with faba beans (C) up to a slaughter weight of 140 kg is the reduction of TBARS of meat (P<0,05). In conclusion, increasing the slaughter weight up to 140 kg and the use in the feed mixture of faba beans improves the nutritional value and oxidation durability of pork.

Key words: pigs, Złotnicka Spotted breed, nutrition, Fabaceae plants, meat quality

In feeding fast-growing, high-lean pigs, the compound feeds mixtures enabling fast growth rates, a short fattening period and the best possible feed conversion efficiency are used. Among the main sources of protein, the post-extracted soybean meal from genetically modified (GM) soybeans, is one of the most popular ones. When feeding slow-growing pigs of native breeds, which do not have such high protein requirements, it is more appropriate to use domestic protein sources. The use of extracted rapeseed meal, or rapeseed cake, a valuable product left over from oil production, in pig feeding has been known and used for years. Fabaceae seeds are far less popular, primarily due to their lower availability on the feed market and lower protein content (Śmiglak-Krajewska, 2022). Fabaceae protein is characterized by a high lysine content, although the amount of sulphur amino acids (methionine and cystine) is not high. However, its digestibility may be lower than in extracted soybean meal, due to the higher amount of fibre (Hanczakowska and Księżak, 2012). The presence of anti-nutritional substances in the seeds of these plants is also a problem (Kasprowicz-Potocka and Frankiewicz, 2013). However, it is a domestic source of protein, which, when properly balanced in the ration, can be a valuable feed in the feeding of pigs, especially those fattened extensively. Based on previous studies (Hanczakowska et al., 2017 a, b; Hanczakowska and Świątkiewicz, 2014), we know that the addition of Fabaceae plants to feed can affect the quality of pig meat. Among the particularly favourable observations, we should mention the improvement of meat water holding capacity, i.e. the reduction of meat juice loss. This feature is very important from the point of view of meat processing technology, having a direct effect also on sensory and culinary qualities, or the quality of the finished product. To date, there have been no nutritional studies involving Fabaceae plants on Złotnicka Spotted pigs. Only a small amount of research has been conducted on pigs of the Puławska breed receiving faba

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bean seeds in the feed mixture (Milczarek and Osek, 2016 a, b; 2017). Also in these studies, an improvement in the water holding capacity of meat was observed, in addition to an improvement in flavour, juiciness and tenderness, and in some cases an increase in the content of polyunsaturated fatty acids (PUFA) or n-3 linolenic acid.

Another factor affecting the quality of pig meat is the length of fattening and body weight at slaughter. This is indicated by a number of studies conducted on many highly productive breeds of pigs (Correa et al., 2006; Strzelecki et al., 2007, Milczarek et al., 2019, Lebret and Čandek-Potokar, 2022). In the case of Spanish native breed pigs, Pugliese et al. (2004) and Pugliese and Sirtori (2012) showed that meat quality is significantly influenced by fattening length. Meat from pigs fattened in free-range conditions up to about 88 kg body weight (487 days of age), compared to meat obtained from animals kept indoors up to about 100 kg body weight (448 days of age), was characterized by greater shear force (raw meat) and darker colour. Janicki and Buzała (2013) demonstrated that the toughness and tenderness of the meat depended on the collagen content, which in turn depended on the breed of the animals, nutrition, muscle activity, slaughter age and even the date of castration. The darker colour of meat is associated with an increase in myoglobin concentration in the muscle, which occurs as animals age (Mayoral et al., 1999).

In addition to the above factors, pig genotype has a significant impact on the technological quality of meat. Mutations with the *RYR1*^{nn} gene can lead to pale, soft, exudative meat, and therefore impair water-holding capacity (Lebret and Čandek-Potokar 2022).

The acreage of Fabaceae plants grown for seed in Poland is not sufficient to cover the demand from the commercial feed market, or to fully replace GM soybean meal (Dzwonkowski, 2016). Using it to cover the needs of a small population of native breeds of pigs may make sense and be optimal for covering the demand for plant-based protein. Stimulating the cultivation of Fabaceae plants through initiatives such as those proposed in the research presented here carries a number of nationwide benefits. It should be emphasized that currently one of the most important problems is the diversification of the protein feed market and the creation of the country's feed independence, especially in the situation of a growing deficit of these expensive feeds. At the same time, there is a growing opposition to the use of feeds with GM seeds in their composition in animal nutrition, despite the fact that scientific research does not confirm the undesirable effects of their use, in terms of both animal health and the safety of the produced food of animal origin (Świątkiewicz et al., 2014; De Vos et al., 2018). In addition, sustaining the biodiversity of crops used for forage production has beneficial effects on soil improvement through nitrogen enrichment and desirable changes in its physical, chemical and biological properties. An important aspect of the interest in native breeds of pigs and domestic protein sources is also to meet the expectations of consumers, who are increasingly looking for unconventional products, of good quality, with health-promoting properties, of known origin, preferably local. Three native breeds of pigs are bred in Poland, including the Złotnicka Spotted breed. This breed was generated after the Second World War based on primitive pigs originating from the area of Vilnius and Novogrudok. They were crosses of primitive Long-eared and Short-eared pigs with predominance of the former and possibly, a share of English Large White breed. This breed was improved only based on the evaluation, selection and pairing for mating. No crossing with other breeds was introduced to improve this breed. Currently it represents the slow growing lard type pig, which until today has retained traits of its primitive ancestors (Szulc et al., 2024).

The aim of the study was to determine the impact of protein feeding from Fabaceae plants and the final slaughter weight on the quality of meat of native Złotnicka Spotted pigs. The quality of meat was confirmed by dietary and technological parameters.

Material and methods

Ethical approval

Ethical Review and Approval was waived for this study, as according to Polish law, Ethical Approval is not required for services within the scope of the Act of 18 December 2003 on animal treatment facilities, as well as agricultural activities, including the rearing or breeding of animals, carried out in accordance with the provisions on the protection of animals, and activities that, in compliance with the practices of veterinary medicine, do not cause pain, suffering, distress, or permanent damage to the body of animals, to an extent equal to or more intense than a needle prick (Act of the Protection of Animals Used for Scientific and Educational Purposes, Legislative Decree 266/2015). The experiment was conducted according to the guidelines of the Declaration of Helsinki and in compliance with the European Union law (Directive 2010/63/EU, received in Poland by Legislative Decree 266/2015) of the European Parliament and of the Council on the protection of animals used for scientific purposes.

Animals, experimental layout, nutrition

The growth experiment was carried out on 96 fattening pigs (48 barrows and 48 gilts) of the Złotnicka Spotted breed. Animals were divided into 4 feeding groups of 24 heads each: group A – control cereal-soybean mixture and 3 experimental mixtures containing different types of domestic protein sources (groups B – lupin, C – faba bean and D – pea) (Table 1).

Table 1. Nutritional composition of the tested plant protein materials

Item (g/kg)	Yellow lupin	Faba bean	Pea
Dry matter	892.3	889.9	880.5
Crude protein	390.0	268.5	210.0
Crude fibre	138.1	73.0	59.0
Ether extract	46.6	13.2	14.0
Crude ash	44.8	36.0	30.0
Lys	19.5	17.7	15.1
Met	2.7	1.9	2.0
Cys	9.0	3.5	3.2
Tre	12.5	9.9	7.9
Trp	3.12	2.4	1.9

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Table 2. Complete feed mixtures used in the experiment

Item (%)	Group A (control)	Group B (lupin)	Group C (faba bean)	Group D (pea)
1	2	3	4	5
GROWER – first fattening stage				
Wheat	20	20	20	20
Barley	34.05	39.49	37.51	33.52
Triticale	20	20	20	20
Wheat bran	8	–	–	–
Rapeseed oil	1	–	–	–
Extracted soybean meal	14	–	–	–
Fish meal	–	2	2	2
Rapeseed cake	–	8	8	8
Yellow lupin	–	8	–	–
Faba bean	–	–	10	–
Pea	–	–	–	14
Phosphate 1-Ca	0.45	0.25	0.25	0.25
Ground limestone	1.5	1.3	1.3	1.3
Salt	0.26	0.23	0.23	0.23
Premix 0.5% PT-1*	0.5	0.5	0.5	0.5
L-lysine	0.22	0.23	0.21	0.20
DL-methionine	0.02	–	–	–
In 1 kg of mixture (g/kg)**				
Crude protein	157	160	156	157
Metabolizable energy (MJ/kg)***	12.7	12.8	12.9	12.9
Lys	9.20	9.23	9.16	9.20
Met + Cys	5.53	5.69	5.50	5.54
Tre	5.44	5.59	5.51	5.48
Trp	1.88	1.71	1.68	1.66
Calcium	7.14	7.12	7.13	7.11
Phosphorus (digestible)	2.55	2.60	2.57	2.54
FINISHER – second fattening stage				
Wheat	13	15	13	12
Barley	39.6	400.56	39.61	35.62
Triticale	13	15	13	12
Hybrid rye	10	10	10	10
Wheat bran	8	–	–	–

Table 2 – contd.

1	2	3	4	5
Rapeseed oil	1	–	–	–
Extracted soybean meal	13	–	–	–
Rapeseed cake	–	7	8	8
Yellow lupin	–	10	–	–
Faba bean	–	–	14	–
Pea	–	–	–	20
Phosphate 1-Ca	0.25	0.22	0.22	0.22
Ground limestone	1.3	1.3	1.3	1.3
Salt	0.21	0.22	0.22	0.22
Premix 0.5% PT-2*	0.5	0.5	0.5	0.5
L-lysine	0.14	0.20	0.15	0.14
In 1 kg of mixture (g/kg)**				
Crude protein	152	151	150	150
Metabolizable energy (MJ/kg)***	12.7	12.7	12.8	13.0
Lys	8.2	8.2	8.2	8.4
Met + Cys	5.2	5.5	5.0	5.1
Tre	5.3	5.1	5.2	5.2
Trp	1.8	1.6	1.6	1.6
Calcium	6.2	6.3	6.2	6.2
Phosphorus (digestible)	1.9	2.0	2.0	1.9

*1 kg of premix contained: grower: betaine – 14,000 mg, vit. A – 1,300,000 IU, vit. D – 400,000 IU, vit. E – 14,000 IU, vit. K – 400 mg, vit. B_1 – 300 mg, vit. B_2 – 800 mg, vit. B_6 – 700 mg, vit. B_{12} – 6,000 µg, niacin – 5,000 mg, folic acid – 100 mg, choline chloride – 30,000 mg, biotin – 30,000 µg, Fe – 20,000 mg, Mn – 8,000 mg, Zn – 17,000 mg, Cu – 4,000 mg, I – 200 mg, Se – 60 mg, Ca – 287.75 g, Mg – 30 g.

Finisher: betaine – 8,000 mg, vit. A – 1,300,000 IU, vit. D – 300,000 IU, vit. E – 14,000 IU, vit. K – 300 mg, vit. B_1 – 200 mg, vit. B_2 – 800 mg, vit. B_6 – 400 mg, vit. B_{12} – 6,000 µg, niacin – 4,000 mg, folic acid – 50 mg, choline chloride – 6912.44 mg, biotin – 20,000 µg, Fe – 20,000 mg, Mn – 8,000 mg, Zn – 17,000 mg, Cu – 4,000 mg, I – 100 mg, Se – 60 mg, Ca – 305.61 g, Mg – 30 g.

**Crude protein and amino acids content are results of the chemical analysis, Ca and P content are calculated values.

***Metabolizable energy was calculated using equation from Hoffmann and Schiemann (1980).

Pigs were fattened to 3 different slaughter weights: 120, 130 and 140 kg. The weight group consisted of 8 heads in each of the experimental groups. During fattening, animals between 30 and 80 kg body weight were fed a grower diet, and from 80 kg body weight to the established final slaughter weight (120/130/140 kg) a finisher diet. All diets were isoproteic and isoenergetic, meeting the requirements according to the Polish standards of pig feeding (Grela and Skomiał, 2020) (Table 2). After the animals were slaughtered, the parameters of selected carcass quality traits were determined, the meat content of the carcass was measured with an optical probe device (Sydel CGM), and meat samples (*longissimus lumborum*) were taken from the section behind the last rib vertebra for chemical analysis and evaluation of meat quality parameters

Analyses and measurements carried out

Basic chemical analyses of tested domestic protein sources (seeds of yellow lupin, faba bean and pea) and feed mixtures were performed according to standard methods (AOAC, 2005). Amino acids were analysed with an AAA 400 automatic analyser (INGOS Ltd., Prague, Czech Republic).

Basic chemical analyses (dry matter, crude protein, crude fat) of meat samples were performed according to standard methods (AOAC, 2007).

The fatty acid profile of the feed mixture and meat was estimated using gas chromatography with extraction of lipids from tissues according to the methods of Folch et al. (1957). The fatty acid profile was determined on an Rtx2330, 105 m × 0.32 mm × 0.2 µm column (Shimadzu GC-2010 Plus). All the analyses were performed in duplicate and mean values are given. The fatty acids results were presented as grams per 100 grams of the total determined fatty acids (in relative percentage).

Thiobarbituric acid reactive substances (TBARS) were analysed in the meat samples after 2 months of storage at –18°C using a modified method proposed by Pikul et al. (1989). Briefly, 10 g of comminuted sample was homogenized with 50 mL of 4% perchloric acid with butylated hydroxytoluene (BTH) additive. After filtering, 5 mL of the filtrate was mixed with 5 mL of 2-thiobarbituric acid (0.02 M). The solution was heated in a sealed tube in a boiling water bath for 1 h, then cooled under running cold water for 10 min. The measurement was carried out at 532 nm against a calibration curve including a blank sample.

Table 3. Fatty acid profile of experimental finisher diets (g per 100 g of all fatty acids determined)

Fatty acid	Group A	Group B	Group C	Group D
Hexadecane (C16:0)	21.56	15.26	16.44	16.09
Octadecane (C18:0)	2.09	2.12	1.95	2.28
Cis-9-octadecene (C18:1 *n*-9)	18.23	33.96	35.45	36.89
Cis, cis-9,12-octadecadiene (C18:2 *n*-6)	47.8	37.32	35.96	34.25
∑ SFA	24.6	19.03	19.34	19.27
∑ UFA	75.4	80.97	80.66	80.73
∑ MUFA	18.6	34.56	36.01	37.46
∑ PUFA	56.8	46.41	44.65	43.27
∑ PUFA n-6	47.85	37.37	36.02	34.29
∑ PUFA n-3	8.95	9.04	8.63	8.98
∑ DFA	77.49	83.09	82.62	83.02
∑ PUFA n-6/n-3	5.35	4.13	4.17	3.82

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, PUFA n-6 acids: C18:2, C20:4, gamma18:3; PUFA n-3 acids: C18:3, EPA, DHA; Desirable fatty acids (DFA): (UFA)+C18:0.

The acidity of the meat was controlled with a pH-meter Matthäus pH Star twice: 45 min after slaughter (pH_{45}) and after 24 h of cooling at +4°C (pH_{24}).

The colour of the meat was assessed with the L* a* b* system using a Minolta C-300 colorimeter (Japan). Lightness (L*), redness (a*), and yellowness (b*) were measured in fresh samples (24 h after slaughter).

Deoxyribonucleic acid (DNA) was isolated from hair follicles collected from the animals and for determination of individual polymorphism in the *RYR1* gene. Genomic DNA was isolated using the Sherlock kit (A & A Biotechnology). The quality and concentration of the obtained DNA were assessed on a Nanodrop apparatus. Polymorphic variants of the c.1843C>T mutation under study (rs118192172) were determined by PCR-RFLP using the *HinP*I restriction enzyme (Fujii et al., 1991).

The fatty acid profile was determined in the finisher diet used before slaughter (Table 3). The experimental feed mixtures were characterized by a higher content of unsaturated fatty acids, mainly monounsaturated fatty acids (MUFA) as a consequence of the difference in the content of C18:1 acid (34–37 vs. 18.23 g/100 g), and a lower content of polyunsaturated fatty acids (PUFA) n-6 shaped by differences in the content of C18:2 acid (34–37 vs. 47.8 g/100 g). The level of PUFA n-3 acids was similar in all groups, at around 9 g/100 g. The PUFA n-6/n-3 ratio was lower in the experimental groups (3.8–4.2 vs. 5.35), while the amount of acids included in the desirable fatty acids (DFA) group was higher in the experimental groups (83 vs. 77). The lower amount of saturated fatty acids (SFA) in the experimental mixtures was due to differences in the content of C16:0.

Statistical analysis

The effects of experimental factors (feeding and slaughter weight) on the analysed traits of basal composition, oxidative stability and fatty acid profile in the loin, as well as fattening and slaughter performance of 96 Złotnicka Spotted fatteners were evaluated using analysis of variance with interaction according to the model:

$$Y_{ijkl} = \mu + a_{i + bj + ck} + d_l + f_{(ab)} + e_{ijkl}$$

Where: y_{ijkl} – $_{ijkl}$ observation, μ – mean value, a_i – fixed effect of ith experimental group (A, B, C, D), b_j – fixed effect of jth slaughter weight (120, 130, 140), c_k – fixed effect of kth sex (1,2), d_l –fixed effect of *RYR1* genotype (*1, 2, 3*), $f_{(ab)}$ – interaction between factors (experimental group, slaughter weight), e_{ijkl} – random error. Statistically significant differences between group means were determined at P<0.05 and P≤0.01 using the NIR test. The software used for the analyses was STATISTICA version 12 (StatSoft, Inc., 2014).

Results

Table 4 shows the mean values, standard deviations (SD) for the traits analysed, as well as the effect of feeding group, slaughter weight, sex and *RYR1* genotype, and the interaction between the factors (body weight * feeding group).

The effect of feeding group, slaughter weight, sex and *RYR1* genotype on fattening and slaughter performance

The fattening and slaughter performance of the Złotnicka Spotted pigs was most influenced by the slaughter weight and sex of the animal. There was no significant effect of dietary factor and the *RYR1* genotype on these traits (Table 4). Animals fattened to a body weight of 140 kg achieved statistically significantly the highest daily gain compared to other individuals (Supplementary Table 4 a). Increasing the slaughter weight of animals from 120 to 130 kg and then to 140 kg was associated with a statistically significant increase in feed intake (120<130, 140;P ≤0.01) and a decrease in dressing percentage (120>130, 140; P≤0.01). It was also shown that animals at a slaughter weight of 140 kg were characterized by statistically significantly higher average backfat thickness and lower loin eye height, compared to animals slaughtered at 130 kg (P≤0.01 and P<0.05, respectively).

Table 4. Mean values (± SD) of analysed traits of fattening and slaughter performance of pigs and the quality of meat (*longissimus lumborum* muscle) and the effects of experimental factors and gender and *RYR1* genotype

Trait	n	Mean	SD	P-value				
				Feeding group (a)	Body weight (b)	Sex (c)	*RYR1* (d)	$f_{(ab)}$
Fattening and slaughter performance of pigs								
Age at slaughter (days)	96	290.7	38.69	ns	ns	**	ns	ns
Feed intake (FI) (kg)	96	433.4	78.23	ns	**	ns	ns	ns
Fattening (days)	96	173.5	35.32	ns	ns	**	ns	ns
Daily gain during fattening (g/day)	96	0.60	0.12	ns	*	**	ns	ns
Feed conversion ratio (FCR) (kg/kg)	96	4.34	0.76	ns	ns	ns	ns	ns
Slaughter body weight (kg)	96	130.1	8.14	ns	ns	**	ns	ns
Dressing percentage (%)	96	77.48	0.76	ns	**	ns	ns	ns
Mean backfat thickness (mm)	96	3.69	0.72	ns	**	**	ns	ns
Loin eye width (cm)	96	9.71	0.63	ns	ns	ns	ns	ns
Loin eye height (cm)	96	5.85	0.67	ns	*	ns	ns	ns
Loin eye area (cm^2)	96	41.74	5.73	ns	ns	ns	ns	ns
Carcass meat content (%)	96	44.44	5.12	ns	*	**	ns	ns
Quality of meat								
Colour L*	96	50.17	4.98	ns	ns	ns	*	ns
Colour a*	96	19.44	1.66	ns	ns	ns	ns	ns
Colour b*	96	2.88	1.57	ns	ns	ns	*	ns
pH_{45}	96	6.45	0.12	ns	ns	ns	ns	ns
pH_{24}	96	5.44	0.13	ns	ns	ns	ns	ns
Crude protein (%)	96	24.07	0.93	ns	ns	ns	ns	ns
Crude fat (%)	96	2.45	0.77	ns	*	**	*	*
Dry matter (%)	96	26.99	1.03	ns	**	**	ns	ns
TBARS (mg/kg)	96	0.34	0.09	ns	**	*	*	*
Profile of selected fatty acids (g/100 g)								
C16:0	96	25.43	1.46	ns	ns	**	ns	ns
C18:0	96	13.19	0.91	ns	**	ns	ns	ns
C18:1 n-9	96	44.91	2.94	*	**	ns	ns	ns
C18:2 n-6	96	6.26	1.71	**	**	**	**	*
∑SFA	96	40.61	2.06	ns	**	**	ns	*
∑UFA	96	59.39	2.06	ns	**	**	ns	*
∑MUFA	96	49.79	3.06	ns	**	*	ns	ns
∑PUFA	96	9.61	3.01	**	**	**	*	*
∑PUFA n-6	96	8.94	2.87	**	**	**	**	*
∑PUFA n-3	96	0.68	0.16	ns	**	**	ns	ns
∑DFA	96	72.59	1.52	ns	ns	**	ns	ns
∑PUFA n-6/n-3	96	13.11	2.02	**	*	**	*	*

Ns – not significant; *P<0.05; **P≤0.01.

Analysing the effect of sex (Supplementary Table 4 b) on fattening and slaughter traits, it was found that gilts, compared to barrows, were characterized by statistically significantly lower daily weight gains during fattening (P≤0.01) and therefore a longer fattening period and later slaughter age (P≤0.01). There were no statistically significant differences in feed conversion ratio (kg/kg) between the analysed sexes. However, it was shown that gilts were characterized by lower average backfat thickness (P≤0.01) and higher meat content in the carcass (P≤0.01) than barrows.

The effect of feeding group, slaughter weight, sex and RYR1 genotype on meat quality

For meat quality expressed in terms of its colour and pH level, there was no statistically significant effect of dietary, slaughter weight and sex factors on changes in the values of these traits. Examining the effect of the *RYR1* genotype (Supplementary Table 4 c) only showed that individuals carrying the T allele of this gene were characterized by higher lightness (L* colour) and yellowness (b*) in meat taken from loin compared to those with the *CC* genotype (*CC*<*CT*; P<0.05).

Analysing the basic chemical composition and oxidative stability (TBARS) of *longissimus lumborum* meat, it was found that there was no significant effect of dietary factor on the mean values of these traits (Table 4 and Supplementary Table 4 d). The slaughter weight (Supplementary Table 4 a) and gender of the animals (Supplementary Table 4 b) had the greatest influence on the analysed traits. At the same time, a statistically significant effect of the interaction between body weight and nutrition group on the fat and TBARS was found. This is discussed in detail in the next subsection. It was found that fattening to a slaughter weight of 140 kg had an effect on increasing fat and dry matter content and decreasing TBARS values in meat in all feeding groups. In the case of sex of animals, it was shown that the meat of gilts, compared to barrows, was characterized by statistically significantly lower fat and dry matter content (P≤0.01) and higher TBARS value (P<0.05). Since the *RYR1 TT* genotype occurred in only 4 individuals, the results of statistical analyses for this genotype were not included in the discussion. The *RYR1* genotype (Supplementary Table 4 c) had an effect on the TBARS value. Loin meat from individuals carrying mutations in this gene (*CT*), compared to meat from mutation-free individuals (*CC*), had a higher TBARS value (*CT*>*CC*, P<0.05).

The effect of feeding group, slaughter weight, sex and RYR1 genotype on the fatty acid profile

The changes in the fatty acid profile were mainly influenced by the weight of fattening pigs (Supplementary Table 4 a) and their sex (Supplementary Table 4 b). Nutritional factor (Supplementary Table 4 d) and RYR1 genotype (Supplementary Table 4 c) affected only PUFA fatty acids, including PUFA n-6. At the same time, a statistically significant effect of the interaction between body weight and nutrition group on the fatty acid profile was found. This is discussed in detail in the next subsection. The sex of the fattened animals had a statistically significant effect primarily on reducing the content of SFA acids, including C16:0 acid (gilts<barrows; P<0.01), increasing the content of PUFA acids, including PUFA n-6 and PUFA n-3 (gilts<barrows; P<0.01), and DFA acids and PUFA n-6/n-3 ratio (gilts<barrows; P<0.01). Analysing the effect of *RYR1* genotype on the fatty acid profile showed that individuals with the T allele of this gene in their genotype were characterized by a higher content of PUFA acids, including PUFA n-6 and a higher value of PUFA n-6/n-3 ratio (*CT*>*CC*, P<0.05).

Table 5. Effect of feeding group (A, B, C, D) and slaughter weight (120/130/140 kg) on the basic chemical composition and oxidative stability (TBARS) of *longissimus lumborum* loin meat (mean values, SD)

Slaughter weight (kg)		A		B		C		D	
		mean	SD	mean	SD	mean	SD	mean	SD
120	n	8		8		8		8	
	Protein (%)	24.34	0.59	23.77	0.50	24.00	0.46	24.11	1.18
	Fat (%)	1.95 by	0.42	2.32	0.91	2.23 y	0.64	2.73 a	1.62
	Dry matter (%)	26.88	0.64	26.49 y	1.00	26.84 b	0.74	27.82 a	1.11
	TBARS (mg/kg)	0.37	0.05	0.38	0.06	0.34 x	0.10	0.40	0.03
130	n	8		8		8		8	
	Protein (%)	23.90	0.75	23.79	1.10	24.02	0.66	24.37	1.47
	Fat (%)	1.93 y	0.37	2.41	0.89	2.37	0.50	2.60	0.60
	Dry matter (%)	26.12 Y	0.93	26.61 y	1.06	26.83	0.91	27.03	0.95
	TBARS (mg/kg)	0.34	0.14	0.33	0.06	0.36 x	0.10	0.36	0.07
140	n	8		8		8		8	
	Protein (%)	24.35	1.00	24.51 a	0.75	23.47 b	1.10	24.25	1.09
	Fat (%)	2.67 x	0.61	2.66	0.68	2.98 x	0.49	2.54	0.64
	Dry matter (%)	27.58 X	1.20	27.62 x	0.88	26.88	1.07	27.28	0.98
	TBARS (mg/kg)	0.34 a	0.17	0.33	0.07	0.24 yb	0.09	0.30	0.03

Means in rows marked with different letters a, b are statistically significantly different at P<0.05; Means in columns within each trait and different body weight marked with: lowercase letters x, y are statistically significantly different at P<0.05; uppercase letters X, Y are statistically significantly different at P≤0.01.

Table 6. Effect of feeding group and slaughter weight on the profile of selected fatty acids (g/100 g) in loin (*longissimus lumborum*)

	A		B		C		D	
	mean	SD	mean	SD	mean	SD	mean	SD
120 kg								
C16:0	25.13	1.79	26.10	2.18	25.61	1.02	26.42 x	2.02
C18:0	13.31	0.83	13.78 X	0.74	13.58	0.86	14.11 Xx	0.92
C18:1 n-9	42.50	2.69	42.84 Yy	2.62	43.48 Y	2.12	43.41 y	3.22
C18:2 n-6	7.88 xa	2.25	6.67	1.66	6.99 X	1.61	6.05 b	2.18
∑SFA	40.26 b	2.43	41.80 x	2.56	41.10	1.70	42.43 Xa	2.80
∑UFA	59.74 a	2.43	58.20 y	2.56	58.90	1.70	57.57 Yb	2.80
∑MUFA	47.28 y	3.07	47.89 Y	2.59	48.13 Y	2.40	48.13 y	3.24
∑PUFA	12.47 xa	4.27	10.32	2.97	10.77 X	2.82	9.44 b	3.83
∑PUFA n-6	11.71 xa	4.03	9.64	2.81	10.05 X	2.70	8.69 b	3.60
∑PUFA n-3	0.76	0.25	0.68	0.16	0.73 x	0.13	0.75	0.24
∑DFA	73.06	1.87	71.98	2.31	72.48	1.14	71.68	2.03
∑PUFA n-6/n-3	15.41 Aax	1.30	14.12 A	1.67	13.67 xAb	1.43	11.28 B	1.94
130 kg								
C16:0	25.15	0.97	24.89	2.23	25.30	0.97	25.48	1.04
C18:0	12.80	0.89	13.18	1.10	13.19	1.21	13.18 y	0.78
C18:1 n-9	43.48	3.28	45.90 x	2.60	44.98	3.80	45.71	1.61
C18:2 n-6	7.43 a	1.75	6.10	1.33	6.25	1.64	5.56b	1.25
∑SFA	39.90	1.66	40.08	2.95	40.59	2.05	40.75	1.67
∑UFA	60.10	1.66	59.92	2.95	59.41	2.05	59.25	1.67
∑MUFA	48.49	3.73	50.53	2.65	49.78	3.83	50.62	1.78
∑PUFA	11.61 xa	3.43	9.39	2.17	9.63	2.89	8.63 b	2.33
∑PUFA n-6	10.88 xa	3.27	8.72	2.08	8.94	2.76	8.09 b	2.42
∑PUFA n-3	0.72	0.18	0.66	0.10	0.69	0.13	0.67	0.15
∑DFA	72.90	1.15	73.10	2.27	72.59	0.85	72.42	1.16
∑PUFA n-6/n-3	14.95 Aa	1.84	13.06 b	1.54	12.72 B	1.77	11.99 B	1.69
140 kg								
C16:0	25.54	0.85	25.11	1.01	25.51	1.37	25.02 y	1.25
C18:0	12.82	0.77	12.57 Y	0.62	13.16	0.67	12.77 Y	0.76
C18:1 n-9	45.11	1.88	46.69 X	2.69	47.46 X	2.56	46.80 x	1.62
C18:2 n-6	6.00y	1.76	5.76	1.52	4.88 Y	0.44	5.70	1.46
∑SFA	40.52	1.08	39.63 y	1.43	40.64	1.69	39.83 Y	1.38
∑UFA	59.48	1.08	60.37 x	1.43	59.36	1.69	60.17 X	1.38
∑MUFA	50.47 x	2.34	51.77 X	2.92	52.10 X	2.32	51.65 x	1.74
∑PUFA	9.02 y	2.90	8.61	2.48	7.25 Y	0.84	8.53	2.24
∑PUFA n-6	8.41 y	2.76	7.97	2.32	6.68 Y	0.82	7.87	2.10
∑PUFA n-3	0.60	0.14	0.64	0.18	0.57 y	0.04	0.66	0.14
∑DFA	72.30	0.97	72.95	1.10	72.52	1.34	72.94	1.42
∑PUFA n-6/n-3	13.73 Aay	1.46	12.93	2.91	11.60 yB	1.15	11.83 b	0.89

Means in rows marked with: different lowercase letters a, b are statistically significantly different at $P<0.05$; different uppercase letters A, B are statistically significantly different at $P\leq0.01$. Means in columns for a given trait at different body weights marked with different: lowercase letters x, y are statistically significantly different at $P<0.05$; uppercase letters X, Y are statistically significantly different at $P\leq0.01$.

The interaction effect between feeding group and body weight on the basic chemical composition, oxidative stability (TBARS) and fatty acid profile of meat

Based on statistical analysis, there was a statistically significant interaction effect between feeding group and body weight on the basic chemical composition and oxidative stability (TBARS) of meat (*longissimus lumborum*), which is shown in Table 5.

Analysing in detail the effect of body weight and feeding group on the chemical composition of loin meat, it was shown that, compared to fatteners in the control group, fatteners fed with mixture D up to a body weight of 120 kg had a higher fat content ($P<0.05$). It was also found that an increase in this parameter occurred as the body weight of the fatteners increased to 140 kg in feeding groups A and C ($P<0.05$). In the case of TBARS, a statistically significant reduction in its value was found only in the group of fatteners fed experimental mixture C up to a body weight of 140 kg. The obtained TBARS value in this group of animals was lower in relation to both individuals with a slaughter weight of 120 kg and 130 kg ($P<0.05$).

Since the fatty acid profile was found to be significantly influenced by the interaction between body weight and feeding group, this relationship was analysed in detail (Table 6). It was shown that increasing the slaughter weight of fatteners from 120 to 140 kg contributed to a statistically significant increase in the content of MUFA acids (A and D – $P<0.05$; B and C – $P\leq0.01$) in the loin meat of all animals. It was also found that in feeding groups B, C and D, where domestic protein sources were used, there was an increase in C18:1 acid content (D – $P<0.05$; B and C – $P\leq0.01$) as slaughter weight increased to 140 kg. In addition, in animals in feeding groups B and D, an increase in slaughter weight was associated with a decrease in the content of SFA acids (B – $P<0.05$; D – $P\leq0.01$), including C18:0, and an increase in the total content of UFA acids (B – $P<0.05$; D – $P\leq0.01$). However, with an increase in slaughter weight of fatteners in feeding groups A and C, there was a decrease in the content of PUFA acids, including PUFA n-6, and in group C there was an additional decrease in PUFA n-3. As a result, loins in feeding group C from fatteners with a slaughter weight of 140 kg were characterized by the lowest PUFA n-6/n-3 acid ratio, which was statistically significantly lower compared to fatteners with a slaughter weight of 140 kg from control group A ($P\leq0.01$). The effect of feeding on the change in the value of the PUFA n-6/n-3 ratio in loin meat was also found in feeding group D. Individuals fed with the addition of peas were characterized by a lower value of PUFA n-6/n-3 ratio compared to the other feeding groups (A, B, C) fattened to a body weight of 120 kg ($P<0.01$). At higher slaughter weights (130 and 140 kg), the value of this ratio in group D was still statistically significantly lower, but only compared to control group A ($P<0.05$).

Discussion

The Złotnicka Spotted pigs included in the study are one of the three native pig breeds included in the country's genetic resources conservation programme. The fattening and slaughter performance of native breed pigs significantly differs from the analogous performance characterizing high-producing breeds. In the performance tests of pigs in Poland, body weight gains of Złotnicka Spotted gilts are 220–240 g lower than those of high-producing breeds. Similarly, the meatiness of Złotnicka Spotted pigs is more than 10% lower than that of high-producing breeds (Żak, 2022). The obtained body weight gains and carcass meatiness determined post-slaughter, as in the case of performance testing, differ significantly from analogous traits found after slaughter in high-producing breeds. Differences in weight gain exceed 200 g, and in carcass meat percentage 11 percentage points. This is due to genetic determinants characteristic of the native breed. Furthermore, it is also a consequence of the implementation of the programme for the conservation of pig genetic resources, according to which it is not permitted to improve performance traits such as daily weight gains, backfat thickness, or carcass meat percentage. The level of fattening and slaughter traits of the pigs included in the study should be considered typical for the Złotnicka Spotted breed (Szulc et al., 2024).

Effect of Fabaceae plants and slaughter weight of pigs on fattening and slaughter performance and meat quality

As shown in the presented study, in the feeding of Złotnicka Spotted pigs, the replacement of GM extracted soybean meal with seeds of Fabaceae plants (lupin, faba bean or pea) did not impair their fattening and slaughter performance. Similarly, in studies by White et al. (2015) and Grabež et al. (2020), where the pigs were fed Fabaceae seeds (faba bean, pea) as alternative protein sources to soybean-containing compound feeds, there was no negative effect on changes in growth rate, feed intake, dressing percentage and carcass fat content. Also a study by Stødkilde et al. (2023) showed no negative effect of feed mixtures containing dried green protein (from perennial ryegrass, lucerne and red clover), faba bean and rapeseed cake on pig fattening performance. In the case of faba bean, Gatta et al. (2013) found that the pyrimidine antinutritional compounds present in this plant do not accumulate and do not affect pig growth. In the literature, it is often found that fattening pigs with legumes will not differ from fattening with extracted soybean meal, provided that the feeds are balanced in terms of nutritional value, including digestible amino acid content (White et al., 2015).

Fattening and slaughter traits in the studies presented here were significantly affected by extending pig fattening to a body weight of 140 kg. An increase in feed intake during fattening, an increase in average backfat thickness, or a decrease in dressing percentage and meatiness in heavy fatteners is an obvious effect (Fischer et al., 2006 a; Lebret and Čandek-Potokar, 2022). It should be noted that in the presented studies, pigs fattened to a body weight of 140 kg were characterized by the fastest daily gain, which probably affected feed conversion, which was not statistically significantly different from the control group fatteners.

The results of a number of experiments showed that there was no negative but positive effect of the use of Fabaceae seeds in the finisher diet on improving meat quality. Bocian et al. (2016) showed that replacing extracted soybean meal with a mixture of Fabaceae seeds (peas, lupins) had no negative effect on meat quality of fattening pigs. Milczarek and Osek (2017), on the other hand, showed a favourable effect of replacing soybean seeds in the diet of pigs (by 10% and 20%) with low-tannin faba bean seeds on the free water content (WHC) of meat, which increased by more than 2%, and on the reduction of juice loss from meat (Milczarek and Osek, 2016 a). In turn, Sońta et al. (2017) found that partial replacement of extracted soybean meal, at 7.5% with yellow lupin seed, resulted in a significant increase (by 1%) in meat juice loss. Our studies also show a positive impact of feeding protein of Fabaceae plants on the quality of meat. Feeding pigs with feed with faba beans up to a body weight of 140 kg was associated with an increase in the fat content of the carcass and a favourable decrease in the oxidative stability (TBARS) of the loin meat of Złotnicka Spotted pigs. Different results were obtained by Fischer et al. (2006 b) with Pietrain × Landrace crossbred pigs. It was shown that the increase in body weight from 110 kg to 160 kg did not increase intramuscular fat (IMF) content, but mainly decreased collagen content. High slaughter weight of fattening pigs (135 to 160 kg) was also associated with a slight increase in electrical conductivity and juice loss from the *longissimus dorsi* muscle and a decrease in cooking loss. Later slaughter age was also associated with darker meat with significantly higher red pigment content and lower water content. From a sensory point of view, loins from fatteners with a slaughter body weight of 135 kg were best evaluated (Fischer et al., 2006 b). In addition, a study by Czyżak-Runowska et al. (2015) showed statistical differences in WHC loss depending on slaughter weight, in the direction of both its increase and decrease depending on the genotype of hybrid pigs of the F_1 generation. The same authors also found higher values of meat colour lightness (L*) for pigs slaughtered at higher body weights. The aforementioned authors found no statistical differences between fattening pigs slaughtered at low and high body weights for such meat quality traits as pH_{45} and pH_{24} of the *longissimus dorsi* muscle, which was similar to the results observed in the presented study.

Effect of Fabaceae plants and slaughter weight on health-promoting qualities of meat (*longissimus lumborum*) – fatty acid profile

In the presented own research, it was shown that, regardless of nutrition, increasing the slaughter weight of fattening pigs from 120 to 140 kg primarily affects the favourable increase in the content of MUFA acids in the meat of all experimental animals. In addition, in the control group (A) and experimental group C (faba bean), it was found that as the slaughter weight of the fatteners increased, the content of PUFA acids, including PUFA n-6, decreased, resulting in a favourably lower PUFA n-6/n-3 ratio in both groups in fatteners with a slaughter weight of 140 kg. This is consistent with the results of Fischer et al. (2006 b), where it was shown that an increase in the body weight of fatteners from 110 to 160 kg contributed primarily to an increase in MUFA acids (including oleic C18:1 n-9) and a decrease in PUFA acids (including C18:2, C18:3 and C20:4).

The health-promoting quality of meat and fat is determined primarily by nutrition, but also by the housing system for pigs (free-range, indoor) during fattening (Sarmiento-García and Vieira-Aller, 2023). Native breeds are kept on individual family farms, often with access to free range. They are usually fed with feed produced on their own farm, including, among other things, grains and Fabaceae seeds, root crops, green fodder and silage (Hanczakowska and Świątkiewicz, 2015; Szyndler-Nędza et al., 2021). Such nutrition translates into an increase in meat's vitamin A and E content and a modification of the fatty acid profile of intramuscular fat and backfat, in a direction more favourable to human nutritional needs, including higher levels of unsaturated fatty acids, especially MUFA (including C18:1 (n-9)) and lower levels of saturated fatty acids (SFA) (Andrés et al., 2001). The use of green fodder and haylage in pig diets has also been shown to favourably affect the fatty acid profile of meat, including a significant reduction in the thrombogenic index (TI) (Szyndler-Nędza et al., 2021). The level of this index indicates susceptibility to clot formation in blood vessels (Ghaeni et al., 2013).

In the study presented here, the use of lupin seeds in experimental group B, at a rate of 10% in the feed mixture, had an effect on reducing the PUFA n-6/n-3 ratio only in fatteners with a slaughter weight of 130 kg. In general, the lack of a clear effect of the applied feed mixture on the fatty acid profile of meat was probably due to the low proportion of lupin in the ration. Seoni et al. (2021) demonstrated that partial replacement of extracted soybean meal with lupin seeds in different amounts (5, 10, 15%), contributed to a linear increase ($P<0.01$) in the level of C18:3 n-3 and long-chain homologues in backfat and intramuscular fat (IMF), resulting in a decrease in the PUFA n-6/n-3 ratio in fat and meat of pigs. The most favourable feed mixtures in our study, turned out to be mixture C containing faba bean seeds and mixture D containing pea seeds. They changed the fatty acid profile in the meat of the pigs studied. Feeding these mixtures to fattening pigs up to 140 kg body weight had a favourable effect on reducing not only TBARS (mixture C), but also the ratio of PUFA n-6/n-3 (mixtures C and D) in the meat. In contrast, Gatta et al. (2013) found that in order to partially replace extracted soybean meal in feed, it seems more interesting to feed pigs with faba bean seeds than peas. Feeding faba beans was positively associated with higher polyphenol content and higher phytoestrogen concentrations in the plasma and muscle of pigs. Also in studies by Milczarek and Osek (2016 b, 2017), in the meat of fattening pigs fed a feed mixture with 10% or 20% low-tannin faba bean seeds, there was a favourable increase in the content of the most valuable exogenous fatty acids, i.e. C18:2 n-6 linoleic acid and C18:3 n-3 linolenic acid, and a decrease in the PUFA n-6/n-3 ratio. Similarly, a study by Grabež et al. (2020) found that a good source of protein in feed, instead of extracted soybean meal, is rapeseed meal along with faba bean seeds. In this study, the use of rapeseed meal with faba bean in the diet of fattening pigs improved the colour of the meat, increased the content of amino acids and sweet-tasting metabolites in the meat, and thus had a positive effect on its quality. In the case where fattening pigs were fed a feed mixture containing seeds of various Fabaceae plants, it was shown that the replacement of extracted soybean meal with peas and lupins, had a beneficial effect primarily on the ratio of essential amino acids in the meat of fattening pigs (Cebulska et al., 2021). In fattening pigs fattened to a slaughter weight of 160 kg, the replacement of extracted soybean meal with a mixture of Fabaceae plants (faba bean, lupin) and potatoes had a beneficial effect on increasing the amount of PUFA n-3 fatty acids, but only in raw maturing ham (Mordenti et al., 2012).

Summing up the results, it was concluded that, on the fattening and slaughter performance of pigs, there was no effect of protein source, but body weight at slaughter (120, 130, 140 kg). Animals with a slaughter body weight of 140 kg were characterized by the statistically significantly highest feed intake, daily gain during fattening and greater backfat thickness. No effect of body weight on feed conversion ratio (kg/kg) was found. Source of protein and slaughter body weight have a combined effect primarily on the quality of meat. It was found that animals fattened with experimental mixture C (with faba beans) and mixture D (with peas) to a slaughter weight of 140 kg were characterized by the meat with the most favourable (lowest) values in terms of PUFA n-6/n-3 fatty acid ratios. In addition, pigs fattened with mixture with faba beans (C) to a slaughter weight of 140 kg were favourably characterized by the lowest value of meat oxidative stability (TBARS) among all dietary groups. In conclusion, increasing the slaughter weight up to 140 kg and the use in the feed mixture of faba beans improves the nutritional value and oxidation durability of pork

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