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Nutritional value of selected mixtures of spring cereals with legumes

Wartość pokarmowa wybranych mieszanek zbóż jarych z roślinami bobowatymi grubonasiennymi

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The material for the study comprised three two-component mixtures of spring cereals: barley or triticale with narrow-leaved lupin, and barley with pea, as well as their components derived from pure sowing. The nutritional value of components and mixtures was determined on the basis of the chemical composition and the biological value of protein, determined directly in the model system in rats. Spring cereals and legumes, as well as mixtures composed with their participation, differed significantly in nutrient and antinutrient content. The results showed the significant effect of component selection on the nutritional value of cereal-legume mixtures. The share of narrow-leaved lupin in the mixture contributed to improving the quality and concomitantly increasing the content of protein, and also of dietary fibre. This applies in particular to mixtures with barley. Cereals, due to their high content of starch, increase the energy value of the mixture. Based on chemical indicators and study *in vivo*, a greater protein nutritional value was shown for cereal-legume mixtures than their components from pure sowing. Studies have revealed that a mixture of spring barley, variety Radek, with peas, variety Model, was characterized with the best chemical composition and the highest nutritional value of protein, determined *in vitro* and *in vivo*.

Keywords: spring barley, spring triticale, peas, narrow-leafed lupine, cereal-legume mixtures, nutrients, antinutrients, protein utilization, rats

Materiał badawczy obejmował trzy mieszanki dwuskładnikowe form jarych jęczmienia i pszenżyta z łubinem wąskolistnym i jęczmienia z grochem oraz ich komponenty pochodzące z siewu czystego. Wartość pokarmową zbadano na podstawie składu chemicznego ziarna/nasion i wartości odżywczej białka określonej *in vitro* oraz bezpośrednio w układzie modelowym na szczurach. Zboża jare i rośliny bobowate grubonasienne, jak również mieszanki skomponowane z ich udziałem, różniły się istotnie pod względem zawartości składników odżywczych i antyżywieniowych. Wyniki pokazały znaczący wpływ doboru składników na wartość pokarmową mieszanek zbóż jarych z roślinami bobowatymi. Udział łubinu wąskolistnego w mieszance przyczynił się do wzrostu zawartości białka i poprawy jego jakości, ale jednocześnie zwiększył ilość włókna pokarmowego. Odnosi się to w szczególności do mieszanki z jęczmieniem. Zboża, ze względu na dużą zawartość skrobi, podwyższały wartość energetyczną mieszanki. Na podstawie wskaźników chemicznych i badań bilansowych na zwierzętach stwierdzono większą wartość odżywczą białka mieszanek zbóż z roślinami bobowatymi niż ich komponentów z siewu czystego. Mieszanka jęczmienia jarego, odmiany Radek, z grochem, odmiany Model, charakteryzowała się najlepszym składem chemicznym i najwyższą wartością odżywczą białka, określoną metodami *in vitro* i *in vivo*.

Słowa kluczowe: jęczmień jary, pszenżyto jare, groch, łubin wąskolistny, mieszanki zbożowo-bobowate, składniki odżywcze, składniki antyżywieniowe, wykorzystanie białka, szczury

Introduction

Growing cereal-legume mixtures has a long tradition in Poland, although the scale of production has changed over the years. After a dramatic decrease in the harvest area during the times of political transformation, farmers are again showing increased interest in sowing mixed crops, mainly due to its importance for sustainable and organic agriculture (Kotecki, 2015). Mixed sowing increases the biodiversity of agricultural ecosystems. Because of differences between crop species, plants in the mixture use habitat resources in a complementary way and this results in their compensatory growth and development. Differences in the morphology of mixture components, their potential for the uptake of water and nutrients, and the ability to fix nitrogen from the air by symbiotic bacteria living inside nodules on the roots of Fabaceae plants are reasons why mixtures of spring cereals with legumes usually yield better and more stable than monocultures, especially in slightly poorer habitats (Staniak et al., 2014; Księżak et al., 2016). In cereal-legume mixtures, cereals utilize from the soil nitrogen produced in

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the soil by the symbiotic bacteria of legumes, which decreases demand for mineral fertilizers compared to monocultures. In turn, legumes use cereals as support plants; this applies in particular to mixtures with peas, which have a tendency to lodge (Staniak et al., 2014). In addition, cereal-legume mixtures are less susceptible to weed infestation and more resistant to diseases and pests, and mitigate the negative effects caused by the subsequent sowing of cereals on the same field.

Mixtures of legumes with cereals can be used for various purposes (Staniak et al, 2014). Those cultivated for grain are important for farms focused on animal production, and can be used as feed providing significant amounts of energy and protein. Cereal grain is the main source of energy in animal feed due to the high starch content, while legume seeds are a protein-rich component, although peas also contain a considerable amount of starch (Jamroz, 2013). These two components of mixtures differ significantly in the content of basic nutrients (Pastuszewska, 2013; Boros et al., 2015). Moreover, there are significant differences in the content of these components between varieties of cereals and legumes. Legume seeds contain a relatively high (lupin) or very high (pea) level of lysine in the protein, and supplement well the deficiency of this amino acid in cereal protein (Rakowska et al., 1978). Cereals, on the other hand, contain a much higher level of legume protein limiting amino acids of (Pastuszewska, 2013). Thereby, through the complementarity of amino acids, the protein from a mixture of cereals and legumes has a better balanced amino acid composition, and consequently a much higher nutritional value than the individual components of the mixture (Pastuszewska, 2013). The factor limiting the use of nutrients from the cereal-legume mixtures is the presence of antinutrients, which have a negative effect on the growth performance of animals receiving this type of diet. Antinutrients in cereals include dietary fibre, especially its water-soluble fraction, consisting of arabinoxylans and β-glucan (Bach Knudsen, 2014). Antinutrients in legume seeds include besides dietary fibre, also phenolic compounds, especially condensed tannins, alkaloids, phytates and inhibitors of proteolytic enzymes (Brenes et al., 2004). Similar to the level of nutrients, the content of antinutrients in cereal grain and legume seeds varies significantly, not only between species, but also within species (Pastuszewska, 2013; Boros et al., 2015).

The yield volume and quality of harvested crops can be modified by a suitable combination of cereals and legumes in the mixture. For this purpose, the suitability of new varieties of cereals and legumes has to be verified.

The aim of the study was to assess the nutritional value of selected mixtures of barley and triticale with narrow-leaved lupin or peas. These mixtures were derived from experiments carried out under the Multiannual Programme of the IHAR-PIB, which focused on the selection of cereal and legume varieties for mixed sowing in various agroclimatic conditions in Poland. Results were compared with data for components of these mixtures grown in monocultures.

Material and Methods

Research material

Three two-component mixtures were analysed: barley with narrow-leaved lupin (cv. Radek/Rumba), barley with peas (cv. Radek/Model) triticale with narrow-leaved lupin (cv. Sopot/Rumba), as well as the components of these mixtures grown in monocultures. Cultivars were selected for use in mixtures based on their origin (domestic varieties first), high yielding and different resistance to diseases (COBORU, 2017). The research material was harvested in 2017 during COBORU experiments, established in three Stations, for Variety Assessment with different soil and climatic conditions (Dukla, Jelenia Góra, Kościelna Wieś).

Chemical analyses

Chemical analyses were carried out for averaged samples, obtained by pooling the grain/seeds of each combination or mixture components in equal weight proportions, from each of the three grain/seed harvest locations. The weight of the averaged analytical sample was 750g (250g×3 locations). Samples obtained in this way were analysed for the content of crude protein using the nitrogen-to-protein conversion factor of 6.25 (AOAC, 955.04), crude ash (AOAC, 923.03), total lipids (Marchello et al., 1971) and digestible starch (AACC, 76–13) which, when added were the sum of nutrients (SNC).

The amino acid composition of protein, except tryptophan, was analysed using ion-exchange chromatography with the AAA 400 amino acid analyser (INGOS, Czech Republic). Samples were hydrolyzed with 6M HCl at 110°C for 23 h after pre-oxidation of the sulfuric amino acids with performic acid (Mason et al., 1980). Results were expressed in g per 100 g of amino acids. The nutritional value of the protein was estimated based on the calculated chemical score (CS) and the essential amino acids index (EAAI), against the amino acid composition of the FAO protein reference standard (1965) (Rakowska et al., 1978).

Total dietary fibre (TDF) was determined by the enzymatic-chemical method (AACC, 32–25), as the sum of non-starch polysaccharides (NSP), raffinose family oligosaccharides (RFOs), resistant starch (RS), uronic acids (UA), and lignin.

Non-starch polysaccharides were analysed by gas chromatography (Clarus 500, Perkin Elmer). Polysaccharides were determined as the sum of sugars: rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose. In the first stage of the procedure, starch was removed by enzymatic hydrolysis, then samples were centrifuged to obtain insoluble nonstarch polysaccharides (I-NSP) and soluble nonstarch polysaccharides (S-NSP), which were hydrolyzed with 1M sulfuric acid (100°C, 2h). The obtained sugar monomers were converted into volatile alditol acetate derivatives and separated in a capillary quartz column (Rtx – 225, 0.53 mm × 30 m). The sugar separation temperature was 225°C, and the injector and detector temperature was 275°C. Helium was used as the carrier gas.

The content of oligosaccharides was determined by gas chromatography as the sum of raffinose, stachyose and verbascose (Autosystem XL, Perkin Elmer, USA) according to a protocol proposed by Lahuta (2018). Samples (40 – 45mg) were extracted in 50% aqueous ethanol solution (30 minutes, 90°C) containing the in-house reference standard (xylitol, 100μg). The extracted sugars were centrifuged (20 minutes, 4°C, 21000g) and 400 μl of the homogenate was deionized with the mixture (300 μl) of Dowex ions – ion-exchange resin for 45 minutes. After centrifugation, 200 μl of the clear extract was dried (gas chromatography vials containing inserts) in a vacuum centrifuge. Residues of dry samples were converted using a mixture of TMSI and pyridine (1: 1 v/v) (80°C, 40 minutes) on a dry block heater. The obtained trimethylsilicate sugar derivatives were separated in a ZEBRON ZB-1 capillary column ZEBRON ZB-1 (0.25 mm × 15 m). The temperature was 325°C for the injector and 350°C for the detector. The capillary column was heated to temperatures of 150°C to 350°C with varying temperature increments. Helium was used as the carrier gas.

The content of resistant starch was determined colorimetrically according to AACC, 32–40. After removing the digestible starch, the precipitated resistant starch (RS) was dissolved in a 2M KOH solution. Next, the solution was neutralized and the starch was hydrolyzed to glucose in reaction with amyloglucosidase. Glucose was determined using the GOPOD reagent containing glucose oxidase and glucose peroxidase.

The content of uronic acids was determined colorimetrically according to Scott (1979). We used 3,5-dimethylphenol, which is a highly selective reagent with respect to uronic acid derivatives in the presence of concentrated sulfuric acid.

Lignin was determined gravimetrically using a method proposed by Theander and Westerlund (1986), as a dry (105°C, 16h) residue of a sample previously digested with 72% sulfuric acid and incinerated (550°C, 5 h). The content of lignin was calculated based on the loss of dry weight after the incineration of a sample.

Total phenolic compounds (TPC) were determined by extraction with 80% methanol and then 70% acetone, and the Folin-Ciocalteau method (Singleton and Rossi, 1965). Results were expressed as gallic acid equivalent (GAE) in mg per g of dry sample weight.

Condensed tannins were also determined colorimetrically using the vanillin method (Price et al., 1978), and the results were expressed as the catechin equivalent in mg per g of dry sample weight. The content of trypsin inhibitor (TUI) was analysed by colorimetry using the BAPNA substrate, according to the standard procedure proposed by Kakade et al. (1974). Absorbance in the colorimetric assays was measured with a UV-1601 spectrophotometer (Rayleigh, UK).

Animal study

The experimental procedure was approved by the 2nd Local Ethical Committee for Experiments on Animals in Warsaw (Decision no. WAW2/52/2016).

The nutritional value of protein was assessed by the balance method using 40 Wistar outbreed rats, with a mean baseline body weight of 78.30 ± 4 g, allocated to 8 experimental groups. The balance experiment was performed according to the Eggum procedure (1973), in which the tested grain/seeds were the only source of protein in the animal diet (9.4% of protein in air dry weight). Throughout the experimental period the animals had constant access to water, and each day they received 10g of feed that fully covered the demand of growing rats for energy, minerals and vitamins. In the 9-day experiment the first four days were an adaptation period during which the rats adapted to solitary confinement, cage topography (Vivari metabolic cages, Italy) and diet. The remaining days were the balance period, and all faeces and urine from rats were collected.

Nitrogen and dry matter in the faeces and nitrogen in the urine were determined, and then the true protein digestibility (TPD), biological value (BV) of protein, and the net protein utilization (NPU) were calculated. Dry matter digestibility (DMD) was also determined. The amount of metabolic nitrogen in faeces and endogenous nitrogen in urine were calculated based on a formula proposed by Lehman et al. (1968).

Statistical analysis

All chemical analyses were performed in three replicates and the results were expressed as percentage of dry weight. Data were processed using one-way analysis of variance according to the fixed effects model and the Tukey-Kramer multiple comparison procedure. Statistical analysis was performed using Statistica software, version 13.3 (TIBCO Software Inc., 2017).

Results and Discussion

Mixtures with the highest content of legumes were selected for the study, i.e. they contained cereals and about 23% of narrow-leaved lupin, or 34% of peas. The material prepared for mixed sowing contained 40% of cereals, and 60% of legumes (COBORU, 2017). The smaller share of legume seeds in the mixture at the time of harvest in comparison to their share in seed material results from the difference in yield potential between cereals and legumes, and could also be caused by the drop out of legume plants in competition with cereals in the stand (Staniak et al., 2014).

Spring cereals and legumes as well as their mixtures differed significantly in the content of nutrients and antinutrients (Tables 1–5). These differences are mainly due to the species diversity of the spring cereals (Boros et al. 2015) and legumes (Grela et al., 2017). There were small differences between barley grain var. Radek and triticale var. Sopot with regard to nutrients, but significant differences in the content of protein (9.7% vs. 10.1%), lipids (2.8% vs. 2.5%) and ash (2.5% vs.1.9%) (Tab. 1). Greater significant differences between the grain of the examined barley and triticale varieties were found for the content of starch (56.5% vs. 63.9%) and thus for the sum of nutrients (71.6% vs. 78.3%). In contrast to cereal grains, the seeds of narrow-leaved lupin and pea were much more diverse in nutrient content, in particular protein, starch and lipids. Seeds from Rumba narrow-leaved lupin contained 50% more protein (31.3%) than seeds from Model pea (20.8%). These values were within the ranges reported for lupin and peas by other authors (Grela et al., 2017) and depended significantly on the year of harvest in the case of lupin, as well as the variety and sowing date (Kotlarz et al., 2011). Legumes are very diverse in terms of starch content. The content of starch is very low (0.6% - 0.8%) in lupin seeds (Maharjan et al., 2019), but very high in peas

Tabela 1
Table 1

Składniki odżywcze w ziarnie mieszanek zbożowo-bobowatych oraz ich komponentów z siewu czystego (% s.m.)

Nutrient content in cereal mixtures with legumes and their pure components (% d.m.)

Składnik / mieszanka Component / mixture	Białko surowe Crude protein	Skrobia Starch	Lipidy Lipids	Popiół surowy Crude ash	SNC*
Jęczmień jary / Spring barley *Radek*	9,7 ^{g}	56,6^{b}	2,8^{de}	2,5^{d}	71,6^{b}
Pszenżyto jare / Spring triticale *Sopot*	10,1 ^{f}	63,9^{a}	2,5^{e}	1,9^{e}	78,3^{a}
Łubin wąskolistny / Narrow-leaved lupine *Rumba*	31,3 ^{a}	0,7^{g}	7,8^{a}	4,4^{a}	44,2^{d}
Groch / Peas *Model*	20,8^{b}	44,8^{e}	3,2^{c}	3,3^{b}	72,2^{b}
Mieszanka jęczmienia z łubinem / Mixture of barley and lupine *(Radek/Rumba)*	16,0^{d}	40,4^{f}	3,9^{b}	2,8^{c}	63,1^{c}
Mieszanka pszenżyta z łubinem / Mixture of triticale and lupines *(Sopot/Rumba)*	18,2^{c}	47,8^{d}	3,6^{b}	2,6^{d}	72,2^{b}
Mieszanka jęczmienia z grochem / Mixture of barley and peas *(Radek/Model)*	14,9^{e}	51,1^{c}	3,1^{cd}	2,9^{c}	71,9^{b}
Statystyka F / Statistics F	65863	1918,35	630,86	304,44	594,5
Wartość p / p - value	0,000	0,000	0,000	0,000	0,000

**SNC – suma składników odżywczych / SNC – sum of nutrients*
*** Wartości w kolumnach opatrzone różnymi literami różnią się istotnie przy p ≤ 0,05 / Values in the columns with different letters differ significantly at p ≤ 0.05*

(38.6% - 45.3%) (Hejdysz et al., 2015). Similar differences in the content of starch were found in our study. The content of starch was 0.7% in the seeds of Rumba narrow-leaved lupin, and 44.8% in Model peas. On the other hand, narrow-leaved lupin seeds were characterized by a high content of lipids, which was almost 2.5-fold higher than in peas (7.8% vs. 3.2%). Kaczmarek et al. (2014) and Hejdysz et al. (2015) reported even greater differences between narrow-leaved lupin and peas in the content of the energy-rich component measured as an ether extract (5.8% vs. 1.3%). Legume seeds also differed in the content of crude ash, which was 33% higher in narrow-leaved lupin than in peas. Differences in the content of nutrients between narrow-leaved lupin and peas (mainly starch) had a significant impact on the total content of nutrients. For this reason, narrow-leaved lupin seeds had the lowest total content of nutrients (44.2%), while in peas their amount was comparable (72.2%) to that in barley grain. The presence of legume seeds in mixtures with cereals resulted in a very significant increase in the protein content. This effect was particularly clear in mixtures with narrow-leaved lupin. Compared to component-based calculations and the share of components in mixtures, the content of protein was more than 1.3 percentage units higher in the mixture of lupin with barley, and 3.2 percentage units higher in the mixture of lupin with triticale. Barley and triticale plants grown in mixed crops had more nitrogen available in the soil, which was synthesized by symbiotic bacteria of narrow-leaved lupin. Some studies revealed an increased total protein content in the cereal grain as a result of increased nitrogen fertilization (Biel, Jaroszewska, 2016).

The nutritional value of protein depends primarily on the content of amino acids, in particular exogeneous amino acids, and their proportions (Rakowska et al., 1978). Grain of individual cereals species and legume seeds differed significantly in the amino acid composition of the protein (Tab. 2). With regard to the sum of exogenous amino acids, their content was in the range from 34.4 g/100 g of amino acids in the protein from triticale var. Sopot to 44.4 g/100 g of amino acids in the protein from peas var. Model. The content of these amino acids in cereal protein was significantly lower than in the protein from legume seeds (by 15% on average). Considering individual amino acids, differences were observed primarily in the content of lysine, the highest concentration of which was found in pea protein (7.7 g/100 g amino acids), and in narrow-leaved lupin (4.7 g/100 g amino acids). Cereal protein contained significantly less of this amino acid compared to protein from legumes, although the content of lysine in barley was significantly higher (4.0 g/100 g of amino acids) than in triticale (3.3 g/100 g of amino acids). Smaller differences were found in the content of sulfur amino acids, which was significantly higher in barley protein (3.8 g/100 g amino acids) and triticale (3.7 g/100 g amino acids%) than in pea protein (2.6 g/100 g amino acids), and especially in narrow-leaved lupin (2.2 g/100 g amino acids). The amount of threonine was significantly highest in pea protein (3.6 g/100 g amino acids), lower in protein from lupin and barley (3.0 g/100 g amino acids), and lowest in triticale protein (2.6 g/100 g amino acids). Differences in the amino acid composition between cereals and legumes influenced the differences in the quantity of amino acids contained in the protein from mixtures. The content of lysine and threonine was highest in the protein from the mixture of barley and peas (5.3 g/100 g of amino acids, 3.2 g/100 g of amino acids, respectively). With regard to the content of sulfur amino acids, no significant differences were observed between the mixtures, and their protein contained on average 3.6 g/100 g of these amino acids.

Considering the pure components of mixtures, barley grain was characterized by the best nutritional value of protein, as indicated by the score for the limiting amino acid (CS = 58) and the essential amino acid index (EAAI = 77). However, the highest EAAI was found for peas (82), which indicates the high quality of this protein, higher than that of the other components of mixtures. For this reason, the mixture of spring barley and peas had the best balanced amino acid composition of protein and the highest chemical scores defining its nutritional value. The amino acid limiting the utilization of protein was lysine in cereals and their mixtures with narrow-leaved lupin, and the sum of methionine and cystine in legumes and the mixture of cereals with peas. Lysine, as the most deficient amino acid in the protein from barley and triticale, was also indicated by other authors (Boros et al., 2010; Kowieska et al., 2011), and sulfuric amino acids in the protein from narrow-leaved lupin and peas (Kotlarz et al., 2011; Pastuszewska, 2013; Koivunen et al., 2016; Grela et al., 2017). The high concentration of lysine in protein from barley and peas increased its content in the protein from the mixture to 76% of the protein reference standard, hence the limiting amino acids in this mixture were methionine and cystine (CS = 62). Summing up, the nutritional value of protein assessed based on chemical scores was higher for the mixtures of barley and triticale with narrow-leaved lupin, and barley with peas than for the pure components of mixtures.

Cereal grain and legume seeds differed significantly in the content and composition of dietary fibre (TDF), which ranged from 13.0% in triticale grain to 55.2% in lupin seeds (Tab. 3). Barley grain had about 35% more TDF than triticale grain, and peas more than 50% less than lupin seeds. The content of TDF in the mixtures of narrow-leaved lupin with triticale, and barley with peas was similar (21.1% and 20.3%), while the content of fibre was significantly higher (26.0%) in the mixture of barley with lupin. Similar differences in the content of dietary fibre in the grain from spring barley and triticale as well as narrow-leaved lupin and peas have been reported by other researchers, regardless of the genetic origin and place of crop harvest (de Almeida Costa et al., 2006; Boros et al., 2015; Fraś et al., 2016). In the present study, the content of non-starch polysaccharides, the main component of dietary fibre, was highest in narrow-leaved lupin seeds (42.7%), and accounted for more than 77% of TDF. The rest of TDF in lupin seeds were oligosaccharides (7.0%) and uronic acids (3.8%). Compared to lupin seeds, peas contained much less dietary fibre (27.0%), which was composed of approximately 44% NSP, and 25% oligosaccharides. Lignin (11%) as well as uronic acids and resistant starch (approx. 10% each) also had a significant share in the dietary fibre from legume seeds. In contrast to legume seeds, cereal grain contained significantly less TDF, which included NSP and lignin. NSP and lignin accounted for 89% of TDF in triticale, and 93% in barley, and the mean share of NSP in both cereals was 68%. Differences in TDF between the components of mixtures, as well as in the components of TDF, had a significant impact on the content of TDF in legume-cereal mixtures. This concerned in particular NSP, uronic acids and oligosaccharides, whose levels were determined by the proportion of peas or lupin in the mixture. The barley-pea mixture showed significantly more oligosaccharides (2.5%) and resistant starch (1.1%) than the two mixtures of barley with narrow-leaved lupin (mean 30% vs 68%). Moreover, this mixture had less NSP, which accounted for 58.1% of TDF in the mixture, including nearly 24% in the soluble form. Considering mixtures, the content of NSP (15.4%) was significantly lower in the mixture of

Tabela 2
Table 2

Zawartość ograniczających aminokwasów egzogennych (g/100g aminokwasów) i wskaźniki jakości białka ziarna mieszanek zbożowo-bobowatych oraz ich komponentów z siewu

Content of limiting essential amino acids and qualitative protein indices in cereal mixtures with legumes and their pure components (g/100g amino acids)

Zboże Cereal	Lys*	Thr*	Met + Cys*	Suma EAA Sum of EAA*	CS*	EAAI*
Jęczmień jary/Spring barley *Radek*	4,03 ^{d}	3,02 ^{b}	3,80 ^{a}	38,0 ^{c}	58 *lys*	77
Pszenżyto jare/Spring triticale *Sopot*	3,27 ^{e}	2,57 ^{c}	3,73 ^{a}	34,4 ^{d}	47 *lys*	69
Łubin wąskolistny/Narrow-leaved lupine *Rumba*	4,65 ^{c}	3,01 ^{c}	2,23 ^{c}	40,8 ^{b}	39 *met+cys*	71
Groch/Peas *Model*	7,66 ^{a}	3,61 ^{a}	2,56 ^{b}	44,4 ^{a}	45 *met+cys*	82
Mieszanka jęczmienia z łubinem/Mixture of barley and lupine *(Radek/Rumba)*	4,05 ^{d}	3,07 ^{b}	3,56 ^{a}	37,9 ^{c}	58 *lys*	75
Mieszanka pszenżyta z łubinem/Mixture of triticale and lupines *(Sopot/Rumba)*	3,44 ^{e}	2,58 ^{c}	3,71 ^{a}	34,9 ^{d}	49 *lys*	69
Mieszanka jęczmienia z grochem/Mixture of barley and peas *(Radek/Model)*	5,29 ^{b}	3,23 ^{ab}	3,52 ^{a}	40,0 ^{b}	62 *met+cys*	78
Statystyka F/Statistics F	523,03	28,69	93,22	186,5	-	-
Wartość p/p – value	4,03 ^{d}	3,02 ^{b}	3,80 ^{a}	38,0 ^{c}	-	-

**Lys – lizyna/lysine; Thr – treonina/threonine; Met – metionina/methionine; Cys – cysteine/cysteine; EAA – aminokwasy egzogenne/essential amino acids; CS – wskaźnik aminokwasu ograniczającego/chemical score; EAAI – indeks aminokwasów ograniczających/the essential amino acid index*

*** Wartości w kolumnach opatrzone różnymi literami różnią się istotnie przy p ≤ 0,05/Values in the columns with different letters differ significantly at p ≤ 0.05*

lupin with triticale, but 25% higher in the mixture with barley. The high content of fibre in narrow-leaved lupin seeds is considered to be the main factor limiting its wider use in feeding monogastric animals (Kaczmarek et al. 2014).

Phenolic compounds are also antinutrients, and tannins from this group have a particularly negative impact on the growth performance of animals. These compounds, found mainly in the seed husk, form indigestible complexes with proteins in the gastrointestinal tract, leading to reduced protein retention (Brenes et al., 2004). According to Brenes et al. (2004), protein digestibility is negatively correlated with the tannin content in feed. Tannins also give the feed an acrid and bitter taste, which reduces feed intake. In the analysed material the total content of phenolic compounds was in the range from 1.2 mg/g to 3.4 mg/g, and the content of tannins from 0.0 mg to 0.3 mg/g (Tab. 4). The highest content of phenolic compounds was found in peas (3.4 mg/g) and barley grain (2.6 mg/g). Moreover, the content of condensed tannins in these crops was the highest (0.19 mg/g in peas and 0.30 mg/g in barley). Similar levels of tannins for these two crop species were reported by Xu et al. (2007) and Hejdysz et al., (2015), and they were in the range of 0.0–1.71 mg/g for peas and 0.41–0.99 mg/g for barley. Considering the content of TPC, values measured for lupin seeds, barley and triticale grains were consistent with the results presented in other studies (Zhu et al., 2015; Fraś et al., 2016; Król et al., 2018). The amount of phenolic compounds determined in peas was much higher than the values reported by other authors (Xu et al., 2007; Piecyk et al., 2012), which probably results from differences in crop varieties used in the research, as well as different habitats of crop production. Wang et al. (1998) reported that over 30% of the content of phenolic compounds in peas is determined by the crop variety, 18% depends on the environment, and the remaining 52% depends on interactions between the environment and genotype. Differences in the content of phenolic compounds in cereal grains and legume seeds grown in monocultures had a significant effect on the variation in their content in legume-cereal mixtures. The content of TPC in

Tabela 3
Table 3

Zawartość składników włókna pokarmowego (% s.m.) w ziarnie mieszanek zbożowo-bobowatych oraz ich komponentów z siewu czystego

Content of dietary fibre constituents (% d.m.) in cereal mixtures with legumes and their pure components

Zboże Cereal	I-NSP*	S-NSP*	NSP*	Lignina Lignin	UA*	RS*	RFOs*	TDF*
Jęczmień jary/Spring barley Radek	7,7 *ef*	4,2 *a*	11,9 *d*	4,4 *a*	0,3 *e*	0,4 *c*	0,6 *d*	17,5 *d*
Pszenżyto jare/Spring triticale *Sopot*	6,5 *f*	2,5 *c*	9,0 *e*	2,6 *d*	0,3 *e*	0,3 *c*	0,7 *d*	13,0 *e*
Łubin wąskolistny/Narrow-leaved lupine *Rumba*	39,7 *a*	3,0 *b*	42,7 *a*	1,7 *e*	3,8 *a*	0,0 *e*	7,0 *a*	55,2 *a*
Groch/Peas *Model*	10,6 *cd*	1,4 *d*	12,0 *d*	3,0 *c*	2,6 *b*	2,6 *a*	6,8 *a*	27,0 *b*
Mieszanka jęczmienia z łubinem/Mixture of barley and lupine *(Radek/Rumba)*	15,2 *b*	4,1 *a*	19,3 *b*	3,1 *c*	1,4 *c*	0,4 *c*	1,8 *c*	26,0 *b*
Mieszanka pszenżyta z łubinem/Mixture of triticale and lupines *(Sopot/Rumba)*	12,4 *c*	3,0 *b*	15,4 *c*	2,6 *d*	1,2 *d*	0,3 *d*	1,7 *c*	21,1 *c*
Mieszanka jęczmienia z grochem/Mixture of barley and peas *(Radek/Model)*	8,9 *de*	2,8 *bc*	11,8 *d*	3,8 *b*	1,1 *d*	1,1 *b*	2,5 *b*	20,3 *c*
Statystyka F/Statistics F	671,064	131,672	687,303	549,77	1350,62	4316,90	2632,14	469,89
Wartość p/p – value	0,000	0,000	0,000	0,000	0,000	0,00	0,00	0,000

**I-NSP – nierozpuszczalne nieskrobiowe polisacharydy/insoluble nonstarch polysaccharides; S-NSP – rozpuszczalne nieskrobiowe polisacharydy/soluble nonstarch polysaccharides; NSP – nieskrobiowe polisacharydy/nonstarch polysacchrides; UA – kwasy uronowe/uronic acids; RS – skrobia oporna/resistant starch; RFOs – cukry z rodziny rafinozy/raffinose family oligosaccharides; TDF – włókno pokarmowe ogółem/total dietary fibre*
*** Wartości w kolumnach opatrzone różnymi literami różnią się istotnie przy p ≤ 0,05/Values in the columns with different letters differ significantly at p ≤ 0.05*

both mixtures with barley was significantly higher (by 33% on average) than in the mixture of triticale with lupin. On the other hand, the content of tannins in mixtures with barley was similar (mean 0.19 mg/g), while no tannins were found in the mixture of triticale with lupin. In our study we also analysed the level of trypsin units inhibited (TUI), which are Bowman–Birk type inhibitors. The negative effect of TUI consists in inhibiting the activity of pancreatic enzymes, i.e. trypsin and chymotrypsin, by forming inactive complexes of these proteolytic enzymes. Moreover, a prolonged intake of feed with TUI leads to hypertrophy of the pancreas and increases the deficiency of sulfur amino acids (Winiarska-Mieczan, 2007). The analysed material was characterized by significant differences in TUI, both between the components of mixtures and between mixtures, despite the low levels of TUI. Considering the pure components of mixtures, the value of TUI was highest in peas (1.1 mg/g) and barley grain (0.9 mg/g), and lowest in lupin seeds (0.1 mg/g) and triticale (0.5 mg/g). Differences in TUI between mixtures were determined by the components used in mixtures. TUI was highest for the mixture of barley with peas (1.1 mg/g), lower for the mixture of barley with lupin (0.9 mg/g), and lowest for the mixture of triticale with lupin (0.5 mg/g).

Differences in the chemical composition and amino acid profile of barley and triticale grain proteins, as well as narrow-leaved lupin and peas, and consequently in their mixtures, were reflected in the differences between qualitative protein indices obtained in the balance experiment with rats (Tab. 5). The highest protein digestibility was found for triticale grain (TPD = 91.9%), and narrow-leaved lupin seeds (TPD = 90.2%), as well as a mixture of these components (TPD = 91.6%). TPD was significantly lower in animals fed diets based on barley (TPD = 84.8%) or peas (TPD = 84.3%) as well as their mixture (TPD = 85.5%), and the mixture of barley with narrow-leaved lupin (TPD = 86.8%). Of note is the high digestibility of protein from the narrow-leaved lupin, obtained despite the high content of TDF in lupin seeds. This could be attributed to the methodology widely used for the determination of protein digestibility, in which the experimental diets are isoprotein diets (9.4% protein), and therefore differ in the amount of the tested material depending on the protein content (Eggum, 1973). In the case of the lupin-based diet, the share of lupin seeds was about 30%, which in comparison to cereal-based diets, consisting of more than 90% of cereal grains, constituted a small part of it. The content of TDF in the lupin-based diet was therefore comparable to the content of TDF in other

Tabela 4
Table 4

Zawartość związków fenolowych (TPC) oraz inhibitora trypsyny (TUI) w ziarnie mieszanek zbożowo-bobowatych oraz ich komponentów z siewu czystego

Content of phenolic compounds and trypsin inhibitor in cereal mixtures with legumes and their pure components

Zboże Cereal	TPC	Taniny Tannins	TUI
Jęczmień jary/Spring barley Radek	2,6[b]	0,30[a]	0,9[b]
Pszenżyto jare/Spring triticale *Sopot*	1,2[f]	0,03[c]	0,5[d]
Łubin wąskolistny/Narrow-leaved lupine *Rumba*	2,0[d]	0,00[c]	0,1[e]
Groch/Peas *Model*	3,4[a]	0,19[b]	1,1[a]
Mieszanka jęczmienia z łubinem/Mixture of barley and lupine *(Radek/Rumba)*	2,4[c]	0,18[b]	0,7[c]
Mieszanka pszenżyta z łubinem/Mixture of triticale and lupines *(Sopot/Rumba)*	1,6[e]	0,00[c]	0,5[d]
Mieszanka jęczmienia z grochem/Mixture of barley and peas *(Radek/Model)*	2,4[c]	0,20[b]	1,0[a]
Statystyka F/Statistics F	353,69	352,911	620,59
Wartość p/p – value	0,000	0,000	0,000

**TPC – związki fenolowe ogółem/total phenolic compounds; TUI – inhibitor trypsyny/trypsin unit inhibitor*
*** Wartości w kolumnach opatrzone różnymi literami różnią się istotnie przy p ≤ 0,05/Values in the columns with different letters differ significantly at p ≤ 0.05*

diets. Protein digestibility measured by the BV index was lowest for narrow-leaved lupin (64.2%). Significantly higher BV was found for protein from peas (73.6%), triticale (81,8%), and the highest for barley (92.6%). The digestibility of barley protein was similar to that of casein protein (91.9%), which was the reference protein in the control group. BV depends on the amount of exogenous amino acids in the protein (Rakowska et al., 1978; Pastuszewska, 2013), hence the low concentration of sulfur amino acids in the lupin protein and lysine in the triticale protein was probably responsible for low BV in our study. The high nutritional value of protein from the mixtures of barley with narrow-leaved lupin or peas calculated based on chemical scores was confirmed in *in vitro* studies. The utilization of protein was highest for the mixture of barley with peas (80.6%), lower for the mixture of barley with narrow-leaved lupin (77% - difference not significant), and lowest (70%) for the mixture of triticale with lupin. Net protein utilization is determined based on protein digestibility and its biological value, and therefore the NPU value was highest for pure cereals (more than 75%), and the mixture of barley and peas (68.9%). The content of dietary fibre and some of its constituents had a negative impact on the NPU value. There was a significant correlation ($p<0.05$) between NPU and TDF ($r = -0.820$), I-NSP ($r = -0.825$), UA ($r = -0.895$) and RFOs ($r = -0.840$). The analysed cereals, legumes, and their mixtures also differed significantly in terms of dry matter digestibility (DMD). Differences in DMD were similar to those in the content of TDF and ranged from 84.2% for the barley diet, to 92.5% for the triticale diet. DMD, which determines the energy value of legume seeds, was most strongly influenced by the content of fibre, a non-digestible component of animal feed (Pastuszewska, 2013).

In conclusion, the study revealed that mixtures of legumes with cereals can be a valuable component

Tabela 5
Table 5

Wskaźniki strawności suchej masy (DMD) i białka (TPD) oraz jego przyswajalności (BV) i wykorzystania netto (NPU) mieszanek zbożowo-bobowatych i ich komponentów

Indicators of digestibility of dry matter (DMD) and protein (TPD) and their bioavailability (BV) and net utilization (NPU) of cereal-legume mixtures and their pure components

Diety/Diets	TPD		BV		NPU		DMD	
Dieta kontrolna/Control diet								
Kazeina Casein	100,0[a]	± 0,7	91,9[a]	± 1,7	92,2[a]	± 2,2	92,5[a]	± 0,2
Diety doświadczalna/Experimental diets								
Jęczmień jary/Spring barley *Radek*	84,8[d]	± 2,5	92,6[a]	± 1,6	78,5[b]	± 1,3	84,2[e]	± 1,2
Pszenżyto jare/Spring triticale *Sopot*	91,9[b]	± 1,2	81,8[b]	± 1,5	75,1[b]	± 1,6	89,9[c]	± 0,5
Łubin wąskolistny/Narrow-leaved lupine *Rumba*	90,2[bc]	± 2,9	64,2[e]	± 3,1	57,8[f]	± 2,0	91,9[ab]	± 1,6
Groch/peas *Model*	84,3[d]	± 2,2	73,6[cd]	± 4,2	62,1[ef]	±4,8	90,4[bc]	± 0,4
Mieszanka jęczmienia z łubinem/ Mixture of barley and lupine *(Radek/Rumba)*	86,8[cd]	± 1,5	77,0[bc]	± 2,3	66,8[cd]	± 1,1	86,7[d]	± 1,0
Mieszanka pszenżyta z łubinem/ Mixture of triticale and lupine *(Sopot/Rumba)*	91,6[b]	± 1,3	70,1[d]	± 1,5	64,[2de]	± 0,7	90,8[abc]	± 1,0
Mieszanka jęczmienia z grochem/ Mixture of barley and peas *(Radek/ Model)*	85,5[d]	± 0,9	80,6[b]	± 1,4	68,9[c]	± 0,9	87,2[d]	± 0,5
Statystyka F/Statistics F	43,2		89,09		126,26		50,2	
Wartość p/p – value	0,000		0,000		0,00		0,000	

TPD – strawność rzeczywista białka/true protein digestibility; BV – wartość biologiczna/biological value; NPU – wykorzystanie białka netto/net protein utilization; DMD – strawność suchej masy/dry matter digestibility Wartości w kolumnach opatrzone różnymi literami różnią się istotnie przy p ≤ 0,05/Values in the columns with different letters differ significantly at p ≤ 0.05* of feed for livestock. The yield and healthiness of mixed crops, as well as their nutritional value, can be modified through the appropriate selection of mixture components. These mixtures usually yield better than their components grown in monocultures (COBORU, 2017), hence the measurable effect of mixed crops is a higher yield of nutrients, mainly protein and starch, per unit of harvest area.

Conclusions

1. The study showed a significant effect of the component selection on the nutritional value of cereal-legume mixtures. The use of narrow-leaved lupin in mixtures increased the quality and the content of protein, and also of dietary fibre. This applies in particular to mixtures with barley.
2. Chemical scores indicated that protein nutritional value was higher for cereal-legume mixtures, particularly those containing peas, than for components grown in monocultures.
3. The high nutritional value of cereal-legume mixtures was confirmed directly in an *in vivo* animal study.
4. The best chemical composition and the highest nutritional value of protein, determined *in vitro* and *in vivo,* were found for the mixture of spring barley var. Radek with peas var. Model.

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