

Evaluation of the Variation of the Contents of Anti-Nutrients and Nutrients in the Seeds of Legumes

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Abstract: *It is assumed that the content of anti-nutrients in legumes seeds and find out the dependency among their content and the amount of important nutrients. The influence of the agri-environmental conditions on concentration of anti-nutrients was evaluated on the basis of the analysis of the material collected from the experiments carried out in the years 2010-2011, located in different regions of Poland. The analyses were performed in the Main Chemical Laboratory of IUNG-PIB Pulawy and in Laboratory of Research Centre for Cultivar Testing (COBORU) in Słupia Wielka, near Poznań. The obtained results indicate that the region of cultivation did not have a significant impact on the concentration of these substances in the seeds of faba bean. Seeds of fodder pea of Muza and Marych cultivars contain significantly less tannins than Roch and Wiato cultivars. In the case of faba bean, however, fewer of these compounds were found at white-flowering cultivars. The average content of total alkaloids in lupine was definitely greater in blue lupine than in yellow lupine. The location of cultivation of yellow lupine did not have an influence on the level of total alkaloids and gramine. Blue lupine collected much less alkaloids in the location of Central Poland and significantly more in the North and West of Poland.*

Keywords: *anti-nutrient substances, legumes, location of cultivation, nutrients, seeds*

I. Introduction

Species belonging to the legumes are important group of major economic importance in the global scale [1]. They are grown for seeds used for the purpose of industry (oil production), consumption and fodder, and also as a raw material for the production of nutritive fodder, green forage, green manure and for reclamation of fallow ground. They are characterized, as one of the few groups of crops, by a positive balance of organic matter in the soil. They are also an excellent forecrop for cereal, industrial and root crops. Climatic conditions in Poland allow to the cultivation of these plants in the whole country [2]. They can be cultivated both in holdings with organic farming and sustainable production system. Because of the economic importance and natural values, they play an important role in crop production. In the last several years, cropping area under cultivation has undergone large changes. The largest area of 385 thousand hectares was taken by legumes in 1989. Their share in the cropping pattern amounted to 3.6%. It was connected to the marketing plan based on the country's self-sufficiency in raw materials for the production of nutritive fodder, in which the legume seeds, were the main source of protein. International situation, which creates problems with obtaining high protein soy pellets is also significant. The interest of farmers in cultivating legumes has been varied over time and depended largely on the demand for seeds and the profitability of their cultivation.

The main factor, which determines the size of cultivation area of this group of plants is the availability and soy pellets price. The introduction of free market economy in the late 1980s and 1990s resulted in many changes in agriculture that affected the evolution of the economic factors, agrarian structure and the associated structures under cultivation. The cultivation area of legumes decreased rapidly in this period. However, in the last 2-3 years, there has been an increase of cultivation area (in the year 2011 over 150 000 hectares and the share in the cropping pattern by more than 1,2%) (data not published).

An important feature of seeds legume is the content of the anti-nutritional substances, which negatively affect the use of nutrients and compounds negatively affected the health and growth of animals called: growth depressing factors [3, 4]. Anti-nutritive and anti-nutritional components have or may have a harmful effect on the nutritive, technological and sensory value of seeds or the products obtained from them. The researches of experiments on these compounds are meant to clarify their role and deepen knowledge about their action, which does not change the fact that their presence limits the full use of high-value protein in the legumes seeds. They cause a decrease in the consumption of feed, as well as a reduction in the use of the nutrients, and often cause a damage to the cells of intestinal epithelium and excessive growth of internal organs [5, 6, 7]. Negative impact on digestion and utilization of nutrients - proteins, carbohydrates and mineral compounds was shown by inhibitors of enzymes: trypsin, chymotrypsin, amylase, phenolic compounds, and especially condensed tannins, most lectin and oligosaccharides [8].

In order to reduce the quantities of anti-nutrients in legume seeds, various thermal and hydrothermal processes are used, such as dry heating, steam heating or cooking, autoclaving or mechanical heating (e.g. removing a seed coat). There have been attempts to use some of the industrial methods which combine thermal and mechanical actions such as microionization, extrusion, flaking and pelleting [9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. In addition, [19] indicate that in many cases, the use one method only, may be not effective enough, and it is then necessary to combine two or more methods. At the same time, heating feed for too long and in too high temperature causes the decrease of availability of amino acids and the possibility of binding tannins in complexes with protein. Moreover, carrying out these experiments requires additional financial input, which raises the cost of feed production. Technological processes aimed at improving the nutritive value of legume seeds of have not yet come out of the phase of the experiments. The standards of technological conditions for this treatment have not been developed. Inappropriate conditions cause the decrease in the availability of amino acids, as well as in the nutritional value of the proteins. Mechanical removal of the seed coat of faba bean and lupine causes an increase of the energy value of these seeds and may be economically justifiable only in poultry feeding. There is only little information about the impact of the agri-environmental factors on accumulation of anti-nutrients in the legumes seeds. It was shown with that the environment can affect the activity of trypsin inhibitor [20]. The relationship between content of these compounds in seeds legume and quantity of the important nutrients has also been little recognized.

The aim of the research was to determine the content of anti-nutrients in seeds legume and find out the dependency among their content and the amount of nutrients.

II. Materials And Methods

The influence of the agri-environmental conditions on concentration of anti-nutrients was evaluated on the basis of the analysis of the material collected from the experiments carried out in the years 2010-2011, located in different location of the country.

Samples of all species seeds were marked for the contents of major nutrients (crude fibre - by weight method, crude fat - by Soxhlet's weight method, N - by flow spectrophotometry, crude ash - by weight method at 580° C, sugars and starches).

Field and laboratory experiments were performed on 9 different faba bean cultivars, 13 fodder pea, 8 yellow lupine, 14 blue lupine, derived from harvests.

The determination of alkaloid contamination in yellow lupine (gramine) and blue lupine (total alkaloids) has been made by the Spanish method of capillary gas-liquid chromatography (GLC). Alkaloid extracts were separated by gas chromatography on fused-silica capillary columns (15 m long, 0,25 mm diameter; SE 30 or DB 1; J&W Scientific; obtained from ICT, Frankfurt, FRG). Conditions for GLC: injector: 250° C; detector: 320° C; oven: 150-300° C, 15° C min⁻¹, at 300° C isothermal for 10 min; carrier gas: helium (1 bar); make-up gas: 20 ml min⁻¹ helium; split injection: 1:25. Sparteine was used as an external standard [21].

The seeds of faba bean and fodder pea were marked for the content of tannins by method of [22]. Samples were determined by the vanillin method with using sulphuric acid.

The analyses were performed in the Main Chemical Laboratory and in the Department of Biochemistry and Crop Quality of Institute of Soil Science and Plant Cultivation–State Research Institute in Pulawy and in Laboratory of Research Centre for Cultivar Testing (COBORU) in Słupia Wielka, near Poznań.

III. Results And Discussion

Tannins are important compounds in the seeds of cultivars of faba bean and fodder pea. They cause the reduction of the digestibility of the proteins and carbohydrates, reduce the availability of methionine and iron and deteriorate the feed taste. Seeds of fodder pea of Muza and Marych cultivars contain significantly fewer of these compounds. More of them were found at Roch and Wiato cultivars (Table 1). The seeds of white-flowering cultivars of faba bean including Leo, Kasztelan, Albus, Amulet, and Olga, were characterized by a smaller amount of these compounds, and more of them were found at Boskovic, Sonet, Granit, and Optimal (Table 1). According to [3], the seeds of Ballet cultivar of pea contained significantly more of tannins than Renata and Solara cultivars. [23] recorded large variation in the total oligosaccharides in seeds of cultivars of pea. According to [20], tannins are most often present in the seeds of cultivars of colorful flowers which may be used in the production of feed, although they are often characterized by a lower digestibility of proteins compared to other cultivars of pea. [24] shows that low content of tannins is related to white colour of flowers, which is controlled by at least two recessive genes. [25] state that an alternative method to the chemical method used to reduce of content of tannins is breeding cultivars with a low concentration of tannins, which is controlled by the same gene as the white colour of the flowers. However, according to [20], the seeds of cultivars without tannins have a wide application both in human and animal feeding.

In the Southern part of the Poland, the seeds of pea cultivars accumulated significantly less of these compounds, and substantially more in the North-East and the Midwest. Correlation analysis showed that crude

fibre, starch, tannins, and the soil pH of adversely affect the protein content in seeds of fodder pea, but it is positively correlated with quantity of sugars and soil complex (Table 5). The increase the crude fibre content in the seeds is significantly affected by the soil complex, but it is reduced by the concentration of sugars. Accumulation of tannins is induced by the content of sugars and soil pH. The amount of sugars and starch are adversely affected by the soil pH and the total precipitation during the growing season. The location of cultivation did not have a significant influence on the concentration of these substances in faba bean seeds (Table 4). Tannins and sugars found in seeds of faba bean cause a temporary limit in the content of protein, crude fibre and crude ash and increase in the amount of crude fat. Protein content in seeds was increased by the content of crude fibre but reduced by crude fat. The content of the evaluated nutrients in the faba bean seeds was insignificantly impacted by the total precipitation during the growing period and soil pH.

Alkaloids occurring in yellow and blue lupine can act as stimulants, anesthetics, and even poisons. They affect central nervous system causing its paralysis, and furthermore, they may cause severe stomach pains and vomiting. If consumed in small quantities, they decrease the absorption of feed and growth of animals. Seeds of cultivars of yellow lupine contain a similar amount of alkaloids. More of them were found at cultivars of blue lupine such as Mirela and Karo (Table 2). Other cultivars contained a significantly smaller amount of them. A greater quantity of alkaloids in the seeds of Mirela and Karo cultivars was recorded in previously researches by [26]. In addition, the average total alkaloids in lupine were definitely higher in the seeds of blue lupine than at yellow lupine. The indole alkaloid gramine is toxic to animals and may play a defensive role in plants, especially if it occurs in significant quantities. All evaluated cultivars of yellow lupine contain little of gramine, but its slightly higher amount was found at Parys cultivar (Table 3). [27] consider that breeding cultivars of lupine with low content of alkaloids (called: sweet) is one of the greatest achievements of breeders. New lupine cultivars usually contain less than 200 mg kg⁻¹ of alkaloids [27]. The location, where lupine was grown did not have an effect on the level of total alkaloids and gramine in the seeds. The data obtained from the correlation analysis suggests that the contents of crude fibre and crude fat adversely affect the protein content of lupine, and that the soil complex is positively correlated with protein content. Increase the crude fibre content in seeds is significantly affected by the content of crude fat and forecrop after, which lupine is cultivated. The amount of crude fat was reduced by the total of precipitation during the growing season. Accumulation of alkaloids was induced by soil quality and the sugars - by forecrop. According to [28], there is a correlation among the level of alkaloids in lupine and the level of precipitation in the period of flowering of plants. According to this author, the drought in this period significantly increases the level of alkaloids in lupine. A similar tendency of changes in the content of alkaloids in white lupine (Hetman cultivar) and blue lupine (Saturn cultivar) was observed by [29]. Much less of these compounds were collected from the seeds of blue lupine in the region of Central of Poland and significantly more in the North and West of Poland. The protein content in blue lupine, similarly as in yellow lupine, was negatively correlated with crude fat content, crude fibre content and the soil pH. An increase in the crude fibre content in seeds was positively affected by quantity of sugars, and adversely affected by agro-environmental factors. Crude fat content was positively correlated with soil quality and negatively correlated with the content of sugars, forecrop and total of precipitation. The quantity of alkaloids and starch was not correlated with any of the evaluated nutrients and habitat conditions.

The seeds of cultivar of multiuse pea contain traces of tannins. The accumulation of protein was induced by the quality of soil and total precipitation, but negatively impacted by sugars and soil pH. The accumulation of crude fibre was enhanced by greater total of precipitation, but limited by soil pH. Increase in the content of sugars was caused by the soil pH and forecrop, but limited by soil quality, total precipitation and starch content.

IV. Conclusions

Seeds of fodder pea of Muza and Marych cultivars contain significantly less tannins than Roch and Wiato cultivars. In the case of faba bean, however, fewer of these compounds were found at white-flowering cultivars such as Leo, Kasztelan, Albus, Amulet and Olga. Multipurpose cultivars of peas contain traces of tannin. In the southern part of the country, seeds of pea accumulated much less of these compounds, while significantly more in the North-Eastern of Poland and the Midwest. The region of cultivation did not have a significant impact on the concentration of these substances in the seeds of faba bean.

The average content of total alkaloids in lupine was definitely greater in blue lupine than in yellow lupine. Seeds of yellow lupine cultivars contained a similar amount of alkaloids, while the cultivars of blue lupine such as Mirela and Karo were characterized by their higher amount than other cultivars.

All the evaluated cultivars of yellow lupine contained little of gramine and only slightly more of it was found at Parys cultivar. The region of cultivation of yellow lupine did not have an influence on the level of total alkaloids in the seeds and gramine. Blue lupine collected much less alkaloids in Central of Poland and significantly more in the North and West of Poland.

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Table 1. The content of tannins in seeds of faba bean cultivars in the 2010-2011 years (% d.m.)

Cultivar	2010	Homogeneous groups	Cultivar	2011	Homogeneous groups
Leo	0.062	x	Kasztelan	0.06144444	x
Kasztelan	0.06428571	x	Amulet	0.06544444	x
Albus	0.06742857	x	Albus	0.06644444	x
Amulet	0.06828571	x	Olga	0.067	x
Olga	0.06942857	x	Leo	0.06766667	x
Bobas	0.6161429	x	Bobas	0.5703333	x
Sonet	0.6438571	x	Optimal	0.6743333	x
Granit	0.7068571	x	Granit	0.6756667	x
Optimal	0.744	x			

Table 2. The content of tannins in the seeds of faba bean depending on the location of cultivation in the 2010-2011 years (% d.m.)

Location (place and direction of Poland)	2010	Homogeneous groups	Location (place and direction of Poland)	2011	Homogeneous groups
Radostowo; N	0.2844444	x	Radostowo; N	0.214625	x
Rarwino; NW	0.3125556	x	Zybiszów; SW	0.2555	x
Karżniczka; N	0.3191111	x	Głubczyce; SW	0.256625	x
Zybiszów; SW	0.3455556	x	Kochcice; S	0.2665	x
Przeclaw; SE	0.3482222	x	Rarwino; NW	0.2705	x
Głubczyce; SW	0.3703333	x	Karżniczka; N	0.272375	x
Pawłowice; S	0.386	x	Pawłowice; S	0.291	XX
			Przeclaw; SE	0.29375	XX
			Wróćkowo; NE	0.4085	x

Table 3. The correlation coefficients among the content of nutritional substances, tannins and some agronomic factors in faba bean

Characteristic	protein	crude fibre	crude fat	crude ash	tannins	sugars
crude fibre	0.9607*					
crude fat	-0.2775	-0.0628				
crude ash	0.9787	0.9516	-0.2842			
tannins	-0.9816	-0.9552	0.2697	-0.9758		
sugars	-0.9844	-0.9760	0.3933	-0.9707	0.9809	
soil pH	-0.0476	-0.0374	-0.0938	0.0072	0.0250	0.2383
precipitations for growing season	0.1633	0.1548	-0.1469	0.1690	-0.1250	-0.1436

*- numbers in bold indicate significant differences (for $\alpha = 0,05$)

Table 4. The content of tannins in the seeds of fodder pea depending on the location of cultivation (% d.m.)

Cultivar	Content	Homogeneous groups	Location (place and direction of Poland)	Content	Homogeneous groups
Muza	0.06716882	x	Pawłowice; S	0.3119797	x
Marych	0.07041882	x	Kościelec; Central	0.3537525	XX
Milwa	0.2023688	x	Świebodzin; W	0.3558404	XX
Gwarek	0.3782688	x	Tomaszów Bol.; W	0.3622988	XX
Klif	0.4157188	XX	Głodowo; Central	0.3748434	XX
Hubal	0.4158688	XX	Białogard; NW	0.3812525	XX
Sokolik	0.4332188	XX	Wyczechy; N	0.3815071	XX
Model	0.4441922	XX	Bobrowniki; W	0.3853821	XX
Eureka	0.4641688	x	Ruska Wieś; SE	0.3920042	XX
Pomorska	0.4706688	x	Cicibór Duży; E	0.4219654	XX
Turnia	0.4847637	XX	Marianowo; E	0.482158	x
Roch	0.5440188	XX			
Wiato	0.5763188	x			

Table 5. The correlation coefficients among the content nutritional substances, tannins and some agronomic factors in seeds of fodder pea cultivars

Characteristic	protein	crude fibre	tannins	sugars	starch
crude fibre	-0.1505				
tannins	-0.1620	0.0000			
sugars	0.2728	-0.1902	0.1329		
starch	-0.4571	0.2730	-0.0625	-0.1907	
soil complex	0.1746	0.2266	-0.0598	-0.1148	0.2141
soil pH	-0.2464	0.0159	0.2265	-0.1283	-0.5945
forecrop	0.2010	-0.0858	-0.0342	0.2807	-0.2141
precipitation for growing season	-0.0408	-0.0732	0.1083	-0.0150	-0.4566

*- numbers in bold indicate significant differences (for $\alpha = 0,05$)

Table 6. The content of total alkaloids in seeds of yellow lupine cultivars depending on cultivar and location of cultivation (% d.m.)

Cultivar	Content	Homogeneous groups	Location (place and direction of Poland)	Content	Homogeneous groups
Baryt	0.01296481	X	Marianowo; E	0.01194179	X
Parys	0.01335641	X	Bobrowniki; W	0.01322236	X
Talar	0.01713766	X	Głodowo; Central	0.01382236	X
Perkoz	0.01902516	X	Uhnin; E	0.01510902	X
Taper	0.01929391	X	Świebodzin; W	0.01579893	X
Dukat	0.02106266	X	Ruska Wieś; NE	0.01593569	X
Lord	0.02578766	X	Nowy Lubliniec; SE	0.01998464	X
Mister	0.02915016	X	SESSSSSESESE	0.0247275	X
			Cicibór Duży; E	0.02485607	X
			Nowa Wieś Ujska; W	0.03008464	X
			Tomaszów Bol.; W	0.03146236	X

Table 7. The content of gramine in the seeds of yellow lupine cultivars depending on cultivar and location of cultivation (% d. m.)

Cultivar	Content	Homogeneous groups	Location (place and direction of Poland)	Content	Homogeneous groups
Lord	-0.0003843506	X	Uhnin; E	-0.0002545094	X
Mister	-0.0003843506	X	Sulejów; Central	-0.0001656205	X
Talar	-0.0003843506	X	Bobrowniki; W	-0.0001545094	X
Perkoz	-0.0003843506	X	Nowy Lubliniec; SE	-0.00009863636	X
Dukat	-0.0003843506	X	Świebodzin; W	-0.00008435065	X
Taper	0.0006307062	X	Głodowo; Central	-0.00003228716	X
Parys	0.003036956	X	Ruska Wieś; NE	0.00005660173	X
			Marianowo; E	0.0001870779	X
			Cicibór Duży; E	0.0008727922	X
			Nowa Wieś Ujska; W	0.0009156494	X
			Tomaszów Bol.; W	0.001501364	X

Table 8. The correlation coefficients among the content of anti-nutritional substances (alkaloids and gramine), nutritional substances and some agronomic factors in yellow lupine seeds

Characteristic	protein	crude fibre	crude fat	alkaloids	gramine	sugars	starch
crude fibre	-0.3102						
crude fat	-0.2341	0.1771					
alkaloids	0.0794	0.0390	0.0276				
gramine	0.1012	-0.0151	0.1470	-0.0857			
sugars	-0.0632	0.0329	-0.1096	0.0821	-0.0002		
starch	-0.0216	0.1851	0.2196	0.4489	-0.8310	0.1732	
soil complex	0.1734	-0.0365	0.1214	0.1982	0.0773	0.0643	-0.0116
soil pH	-0.0048	-0.1115	-0.0660	0.0621	0.0352	-0.1299	-0.0327
forecrop	0.0118	0.2253	-0.2162	0.0335	-0.0395	0.1806	0.0533

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precipitation for growing season	0.0645	0.0089	-0.2250	0.0438	-0.0339	-0.0022	0.0409
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*- numbers in bold indicate significant differences (for $\alpha = 0,05$)

Table 9. The content of total alkaloids in the seeds of blue lupine depending on the cultivar and location of cultivation (% d.m.)

Cultivar	Content	Homogeneous groups	Location (place and direction of Poland)	Content	Homogeneous groups
Dalbor	0.001373278	X	Głodowo; Central	0.05260664	X
Heros	0.005573278	X	Wyczechy; N	0.1526019	XX
Regent	0.006265688	X	Nowy Lubliniec, SE	0.1580213	XX
Neptun	0.01197759	X	Wróćnikowo; N	0.1593477	XX
Graf	0.01252997	X	Rarwino; NW	0.1638559	XX
Zeus	0.01368712	X	Marianowo; S	0.1752227	XX
Kalif	0.01399188	X	Cicibór Duży; S	0.1759497	XX
Baron	0.0179676	X	Bobrowniki; W	0.1789386	XX
Kadryl	0.02164426	X	Kawęczyn; Central	0.1870463	XX
Sonet	0.02206331	X	Ruska Wieś; NS	0.215429	X
Boruta	0.03128712	X	Kościelec; Central	0.2293867	X
Bajor	0.0469395	X			
Mirela	1.062815	X			
Karo	1.084401	X			

Table 10. The correlation coefficients among the content of anti-nutritional substance (alkaloids), nutritional substances and some agronomic factors in blue lupine seeds

Characteristic	protein	crude fibre	crude fat	sugars	starch	alkaloids
crude fibre	-0.2684					
crude fat	-0.2468	0.0026				
sugars	-0.0086	0.1783	-0.1332			
starch	-0.0433	0.0280	0.0394	-0.2848		
alkaloids	0.0959	-0.0297	-0.0774	-0.2143	-0.1884	
soil complex	-0.1057	-0.1607	0.1289	0.1332	-0.0551	-0.0363
soil pH	-0.1621	-0.1455	0.0564	0.0162	0.1160	0.0145
forecrop	0.1254	-0.2861	-0.1650	0.0212	-0.0734	-0.0002
precipitation for growing season	-0.0243	0.0067	-0.3635	0.1781	0.0509	0.0069

*- numbers in bold indicate significant differences (for $\alpha = 0,05$)

Table 11. The correlation coefficients among the content of nutritional substances and some agronomic factors in seeds of multiuse pea cultivars

Characteristic	protein	crude fibre	sugars	starch
crude fibre	0.0922			
sugars	-0.2003	-0.1138		
starch	-0.1804	0.1276	-0.4830	
soil complex	0.1541	-0.0194	-0.1269	0.1601
soil pH	-0.2713	-0.1710	0.2993	0.0941
forecrop	-0.0835	-0.0212	0.3148	-0.1695
precipitation for growing season	0.3875	0.1928	-0.3945	0.1588

*- numbers in bold indicate significant differences (for $\alpha = 0,05$)