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OCCURRENCE AND BIOLOGICAL ACTIVITY OF LECTINS IN SEEDS OF LEGUMINOUS PLANTS AND CEREALS

WYSTĘPOWANIE I AKTYWNOŚĆ BIOLOGICZNA LEKTYN W NASIONACH ROŚLIN STRĄCZKOWYCH I ZIARNACH ZBÓŻ

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Streszczenie. Materiał do badań stanowiły nasiona roślin strączkowych i ziarna zbóż. Występowanie lektyn określono na podstawie aktywności czerwonych krwinek ludzkich, szczurów laboratoryjnych i baranich traktowanych wyciągami z wymienionych nasion i ziarna oraz owocnika pieczarki (*Agaricus campestris*) jako kontroli metody. Aktywność biologiczną lektyn określono na podstawie największego stopnia rozcieńczenia wyciągu wywołującego jeszcze aglutynację erytrocytów. Badania wykazały występowanie lektyn w nasionach lędźwianiu, grochu i soczewicy oraz ich silniejszy wpływ na aglutynację erytrocytów szczura niż człowieka. Ze zbóż jedynie w życie ozimym Warko stwierdzono występowanie lektyn i słabą aglutynację erytrocytów ludzkich grupy A1 przy braku aglutynacji erytrocytów szczura i barana.

Key words: agglutination, human, lectin, ram, rat.
Słowa kluczowe: aglutynacja, baran, człowiek, lektyny, szczur.

INTRODUCTION

The usefulness of feed in animal nutrition is determined on the basis of its chemical composition, digestibility and assimilation of nutrients. One of the major components of feed is protein, a nutrient with multiple functions in the body. Its usefulness in animal fodder depends not only on its amino acid composition, but also on the impact of anti-nutritional compounds, such as lectins (phytohemagglutinin). The first mention of lectins appeared in late 19th and early 20th century, but the real interest in them started in 1960. The best known, in terms of chemical structure and properties, are lectins of plant origin, but they also may occur in the animal world (Sharon 2007). According to Sharon (2007) and Sharon et al. (1974) lectins have many biological properties in common, yet they represent a diverse group of proteins with respect to size, composition and structure.

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Lectins of plant origin are proteins which bind to oligosaccharides and surface glycoproteids of many cells causing agglutination of erythrocytes and adversely affecting the gastrointestinal tract of animals, particularly monogastric ones. Lectins are resistant to proteolytic enzymes and may react with components of both intestinal content and with epithelial cells, which can lead to growth inhibition and loss of weight, depletion of lipid of carcass and an overgrowth of small intestine and diarrhea, general intoxication, deterioration of digestion and absorption, and high mortality. Lectins may also decrease immunity, lead to endocrine dysfunction and impair the secretion of digestive juices by the intestinal wall (Vasconcelos and Oliveira 2004, Zang et al. 2006, Sharon 2007). A report by Cavallé de Moya et al. (2003) showed that bean lectins have an impact on the weight of certain internal organs, skeletal muscle, and insulin and LDL-cholesterol levels. According to Le Guen et al. (1995) lectins may reduce the apparent ileal digestibility of dry matter and nitrogen in piglets, as shown by research on pea and pea protein isolate, with or without the addition of concentrated antinutritional compounds (lectins and TIA – trypsin inhibitor activity). But not always did those authors obtain digestibility coefficients that were reflected in weight gains.

Lectin toxicity in food stems from the “blood type diet” theory. The basic premise is that ABO blood type is the most important factor in determining a healthy diet. The cornerstone of this theory is that lectins in foods react differently with each ABO blood type. On the other hand, many dieticians, physicians and nutritional scientists claim the theory lacks scientific evidence (Hamid and Masood 2009).

The negative effect of lectins can be overcome by heat treatment (Roy et al. 2010), for example when preparing meals for people. However, subjecting seeds to even high temperatures does not always entirely eliminate the adverse effects of lectins, for example in bean seeds (Peumans and Van Damme 1996). In animal feeds, raw seeds are usually only ground and heated.

Reports on lectins in literature concern, for example, their content in feed and food, hemagglutination activity, as well as oral, intradermal and parenteral toxicity. Researchers have also examined the impact of lectins on the growth of animals, their productivity, and the impact on the health of animals and humans (Peumans and Van Damme 1996, Gelencsér et al. 2000, Grant et al. 2000, Vascocelos and Oliveira 2004, Czerwiński et al. 2006, Hamid and Masood 2009). Full information about the dangers of lectins would require long-term and complementary studies, and due to the significant costs it is not always feasible. Hemagglutination activity (HA) is fairly easy to measure (Vascocelos and Oliveira 2004), although rather inaccurate when it comes to predicting the degree of oral toxicity. HA provides information about the presence of lectins in plants and the degree of erythrocyte agglutination. Differences in the agglutination of red blood cells may indicate the different sensitivities of animals and humans to lectins. Determination of HA may be carried out using rabbit erythrocytes, or those from guinea pigs, rats, sheep, cattle or man (Bhatia 1974, Grant et al. 1983, Ruiz-Lopez et al. 2000, Van Nevel et al. 2000, Czerwiński et al. 2006). The use of red blood cells from laboratory rats to determine HA is especially important when conducting model studies on the quality of food and feed proteins.

Lubowicki et al. (2000) observed a negative nitrogen retention when laboratory rats were given experimental mixtures where the only sources of protein were grass pea or lentil seeds. It is possible that those results were associated with the adverse effects of lectins which deteriorate nitrogen digestibility and reduce the availability of amino acids and thus cause negative nitrogen balance (Li et al. 2003, Vasconcelos and Oliveira 2004). Lubowicki et al. (2000) not observe a negative nitrogen retention after administration of lupin. Falcón et al. (2000) ascertained that some lupin proteins may have lectin-like properties, for example leading to the agglutination of red blood cells.

The character of lectin activity in the body and the use of different methods to determine the content or hemagglutinating activity make it difficult to compare results presented in the literature (Champ 2002). As reported by Roy et al. (2010), the variety, cultivation area and method of harvest may also affect the concentration of lectins, although there have not been too many comparative reports between varieties. It should also be remembered that negative results do not necessarily mean the absence of lectins (Peumans and Van Damme 1996). The aforementioned problems and the scarcity of literature on the HA of various leguminous plants and cereals in the erythrocytes of man and animals have been the main reason for this research.

The specific aim was to determine the presence lectins in raw seeds of several plants used for animal feed and human nutrition, which erythrocytes they affect (human, ram, rat), and the extent of their agglutinating effect on the tested erythrocytes, which should enable the comparison of potential sensitivity of man and animals to lectins.

MATERIALS AND METHODS

The basis to determine the presence of lectins from seeds and grains of this study, and from fruiting mushrooms *(Agaricus campestris)* was agglutination erythrocytes from at least one group of human blood or one animal species. Hemagglutination activity (HA) was examined using the anti-globulin test by Coombs et al. (1945a, 1945b).

Plant material and preparation of extracts

The study used extracts from the seeds of 6 varieties of pea (*Pisum sativum*)*,* 2 varieties of grass pea (*Lathyrus sativus),* 4 varieties of sweet lupin (*Lupinus albus* L., *Lupinus angustifolius* L., *Lupinus luteus* L.), 1 variety of lentil (*Lens culinaris*) and 2 varieties of barley (*Hordeum* L.), 1 variety of oat (*Avena* L.), 1 variety of wheat (*Triticum* L.), 2 varieties of triticale (*Triticale*) and 1 variety of rye (*Secale* L.). Extract from the fruiting body of the mushroom (*Agaricus campestris*) was used as the positive control (Table 1). The same seeds of lupin, grass pea and lentil were evaluated in previous studies in terms of biological quality of protein by performing experiments with laboratory rats (Lubowicki et al. 2000). Seeds and grains were obtained from the Seeding Centre in Szczecin, mushrooms were purchased from the producer and qualified by a certified mushroom expert. The seeds and grains were cleaned and rendered free of dust, then stored in tightly closed glass jars at room temperature until used.

Extracts were prepared as follows: the dried seeds and grains were milled in a Foss Tecator Knifetec 1095 mill, then an isotonic solution (0.9% NaCl) was added in proportions of 1g ground meal into 9 ml of isotonic solution, then incubated for 1 hour at room temperature, shaken for 3 minutes and frozen at –20ºC. Before the test, the preparations were thawed, incubated for 1 hour at 37ºC, shaken for 3 minutes and centrifuged. Pure isotonic solution (0,9% NaCL) was a negative control *per se*.

Hemagglutination assay

The biological activity of lectins was established on the basis of the maximum degree of dilution of the extract that was still causing further agglutination of erythrocytes. Human blood were obtained from the Regional Blood Donor Center in Szczecin, ram blood were obtained from the Regional Veterinary Station in Szczecin and the rat blood were taken from left heart ventricle *post mortem* animals used in a previously conducted experiment (Lubowicki et al. 2000). Rams came from a commercial farm, typical for NW Poland, where animals are kept at pasture, and were under the supervision of a veterinarian who monitored the health status of the entire herd. The experiment on two month old laboratory Wistar rats was approve by the Local Ethical Committee for Experiments on Animals from the West Pomeranian University of Technology in Szczecin. Blood (10 ml each sample) was obtained from 3 groups of healthy persons (5 donors per each blood group) and 1 groups of rams (5 individuals) and 1 group of laboratory rats (80 individuals). An agglutination test was performed individually for each individual duplicate analyses. A single result in Table 1 corresponds to the maximum dilution at which agglutination in the group occurred most frequently. Results were reproducible to a high degree within the group.

A 5% suspension was prepared from standard human erythrocytes washed 3 times from groups A_1, B and 0, and also from rat and ram erythrocytes. Extracts (five drops each) from subsequent dilutions at an exponential rate (q = 1/2) were placed onto glass sheets. To the series of dilutions, standard erythrocyte suspensions were added; namely – to the 1st series of A_1 erythrocytes, to 2nd group B, 3rd group 0, 4th group of rat erythrocytes, and the 5th group of ram erythrocytes. The results were read after 30 minutes incubation in a humid chamber at room temperature.

RESULTS

Negative control didn`t resulted in agglutination of erythrocytes. Extracts from the fruiting body of the mushroom used as a positive control resulted in agglutination of rat erythrocytes even at a dilution of 1/128, while to a much lesser extent it agglutinated ram erythrocytes (1/32). All groups of human blood cells were agglutinated at a maximum extract dilution of 1/16.

The research showed the presence of lectins in the seeds of the leguminous plants: pea, lentil and sweet pea, and weak presence of lectins in the seeds of the winter rye, Warko variety. Pea lectins agglutinated red cells in varying degrees depending on their variety and the selected erythrocytes. Extracts from the seeds of white lupine, yellow lupin, blue lupin and tested varieties of oat, wheat, triticale and barley did not show the presence of lectins.

Rat erythrocytes used to demonstrate the presence of lectins were usually more agglutinated than human erythrocytes of group A_1, B and O. None of the tested lectin extracts of leguminous seeds and cereal grains used for mixtures did agglutinate ram erythrocytes. Lectin from the seeds of winter rye "Warko" were weak (1/2) and showed some effect only on human erythrocytes of Group A_1.

DISCUSSION

Own research (Table 1) shows the presence of lectins in the seeds of leguminous plants, and an absence in the grains of cereals, except rye. Extracts from the pea had agglutinated erythrocytes across a wide range of dilutions from 1/2 (humans) to 1/32 (rats). Extract from grass pea seeds had a higher hemagglutinating activity than the lentil (1/16–1/32 against 1/8–1/16). Peumans and Van Damme (1996) reviewed the results obtained by different authors comparing the action of lectins of seeds of selected crops. The most toxic seeds were the beans (concentration of lectins were 1–10 g · kg^{-1}). Lectins present in lentil (0.1–1 g · kg^{-1}) and pea (0.2–2 g · kg^{-1}) were found to be slightly toxic, and their detrimental effect was completely eliminated by heat treatment.

Raw seeds of Polish pea varieties contained lectins at a concentration range between 0.27 and 0.75 g · kg^{-1} (Gelencsér et al. 2000). The hemagglutinating activity of this pea had been examined using rabbit erythrocytes by Valdebouze et al. (1980), and was 10% of the HA of soya seeds, while the HA of sweet lupine seeds was practically undetectable, similar to our results (Table 1).

Bhatia (1974) observed that the extracts from grass pea and lentil seeds reacted with the guinea pig, and did not agglutinate with human erythrocytes (group A) nor erythrocytes of the cow, chicken, sheep, rabbit, frog or rat. This is not confirmed by our results where extracts from grass pea and lentil resulted in the agglutination of human red blood cells (A_1, B and O) and rat red blood cells.

Peumans and Van Damme (1996) showed the presence of lectins in rye, barley and wheat, while in cereal grains there was only a little lectin (below 0.01 g per 1 kg) with high thermal stability – harmful in raw grains. In processed seeds, lectins may still be harmful (barley, rye) and are definitely harmful in wheat. Literature data show the presence of lectins in *Triticale.*

Hariharan and Rajagopal Rao (1978) obtained hemagglutinating activity of triticale lectins in rat and rabbit erythrocytes but not in human erythrocytes. Liener (1986) showed the biological activity of lectins in wheat, barley and oat. In our study, a low HA was only observed in rye (1/2, A_1 human erythrocytes), but was not observed for other cereals.

Agglutination of human cells by an extract of *Agaricus campestris* occurred at a maximum dilution of 1/16. Pea lectins agglutinated human erythrocytes in various dilutions, from ½ to 1/32, depending on variety. Piątek (1998) observed the agglutination of human erythrocytes from group A_1 after a 1/16 dilution of *Agaricus campestris* extract, and groups B and O at 1/32, and the 1/8 and 1/16 dilution of pea extract, respectively. Czerwiński et al. (2006) obtained the same hemagglutination titre for pea (the lowest concentration of lectin which showed agglutination), equalling 10 µg · ml^{-1} erythrocytes of groups A, B, O. It was about 4–8 times greater for soya seeds.

Table 1. Hemagglutination tests of extracts from the examined seeds, grain and Agaricus mushroom, using human, rat and ram erythrocytes*

Tabela 1. Test hemaglutynacyjny wyciągów z badanych nasion, ziarna i pieczarki z wykorzystaniem erytrocytów człowieka, szczura i barana*

Item Wyszczególnienie	Extract from the *Agaricus fruiting* body and seeds Wyciąg z owocni i nasion	Agglutination of erythrocytes Aglutynacja z erytrocytami				
		human (group) człowiek (grupy)			rat szczur	ram baran
		A_1	B	0		
Fruiting body – control – Owocnia – kontrola						
1	Mushroom Pieczarka *Agaricus campestris*	1/16	1/16	1/16	1/128	1/32
Seeds of leguminous plants – Nasiona roślin motylkowatych						
1	Field pea coloured seeds of cv. Dawo Groch pastewny kolorowo kwitnący Dawo	1/4	1/8	1/2	1/32	0
2	Edible pea white seeds of cv. Piast Groch jadalny biało kwitnący Piast	1/16	1/8	1/4	1/32	0
3	Field pea white seeds of cv. Kier Groch pastewny biało kwitnący Kier	1/16	1/8	1/4	1/32	0
4	Edible pea white seeds of cv. Agra Groch ogólnoużytkowy Agra	1/16	1/8	1/16	1/16	0
5	Edible pea white seeds of cv. Tambo Groch ogólnoużytkowy Tambo	1/16	1/16	1/16	1/32	0
6	Field pea coloured seeds of cv. Idol Groch pastewny Idol	1/16	1/16	1/16	1/16	0
7	Grass pea cv. Derek Lędźwian siewny Derek	1/16	1/16	1/16	1/32	0
8	Grass pea cv. Krab Lędźwian siewny Krab	1/16	1/16	1/32	1/32	0
9	White lupine cv. Bardo Łubin biały Bardo	0	0	0	0	0
10	White lupine cv. Butan Łubin biały Butan	0	0	0	0	0
13	Yellow lupine cv. Legat Łubin żółty Legat	0	0	0	0	0
14	Narrow-leaved lupine cv. Polonez Łubin wąskolistny Polonez	0	0	0	0	0
15	Lentil cv. Anita Soczewica jadalna Anita	1/16	1/8	1/8	1/16	0
Cereal grain – Ziarna zbóż						
1	Barley spring cv. Rambo Jęczmień jary Rambo	0	0	0	0	0
2	Barley winter cv. Kroton Jęczmień ozimy Kroton	0	0	0	0	0
3	Naked oat cv. Akt Owies nagi Akt	0	0	0	0	0
4	Wheat winter cv. Sakwa Pszenica ozima Sakwa	0	0	0	0	0
5	Wheat spring cv. Migo Pszenżyto jare Migo	0	0	0	0	0
6	Triticale winter cv. Bogo Pszenżyto ozime Bogo	0	0	0	0	0
7	Rye winter cv. Warko Żyto ozime Warko	1/2	0	0	0	0

*The lack of agglutination is denoted by 0 (zero); fractions (e.g. 1/16) denote the greatest dilution at which agglutination was still occurring.

* W tabeli oznaczano brak aglutynacji „0" (zerem), a ułamkiem np. 1/16 podano największe rozcieńczenie, przy którym jeszcze wystąpiła aglutynacja.

Based on the obtained results (Table 1), we may generally suppose that the A_1 blood group in man is more sensitive to lectins of pea, grass pea, lentil and rye, than an individual with group B or O.

In this study, extracts from the seeds of plants that agglutinated human erythrocytes also agglutinated rat erythrocytes, and in many cases their impact on rat erythrocytes was much stronger. These results confirm the findings of Grant et al. (1983) for the raw seeds of various species of edible leguminous plants.

We found no haemagglutination activity (HA) in ram erythrocytes, which perhaps indicates a lack their of sensitivity to lectins material of this study. However, Paduano et al. (1995) found some, although low, haemagglutination activity of Uniharvest var. narrowleaf lupin (14 units mg^{-1} dry weight). They also observed a complete loss of HA after 24 h presence of lupin meal in the rumen of sheep.

Rabbit erythrocytes are a frequently used indicator of haemagglutination activity. Van Nevel et al. (2000) used rabbit erythrocytes subjected to the activity of protease and determined weaker hemagglutination activity in lupines (20 and 41) than in soybean grist (1024 and 2048 units mg^{-1}).

Other authors (Ruiz-Lopéz et al. 2000) demonstrated the positive response of sheep erythrocyte (defibrinated and treated with trypsin solution) to *Lupinus mexicanus* lectins, and also of rabbit erythrocytes to *Lupinus reflektus* lectins, in both cases at a maximum dilution of the lupin extract. In the same study, *Lupinus excaltatus* showed no haemagglutination activity. These examples indicate the possible presence of lectins in lupins, which was not confirmed by our research. Research by Kłos et al. (2008) on selected fractions of narrow--leaved lupin globulins confirm the lack of HA for human erythrocytes of the groups A, B, 0.

Vasconcelos and Oliveira (2004) suggest it is possible to predict the oral toxicity of lectins on the basis of their ability to selectively bind various types of erythrocytes. In this way, their haemagglutination activity could be the basis of *in vitro* studies on the potential toxicity of lectins. They infer on the basis of Grant et al. (1983, 1995) that lectins that agglutinate a wide range of red blood cells should normally have a similar oral route toxicity as rats. Lectins that agglutinate only rabbit erythrocytes need not agglutinate the erythrocytes of rats, whether treated with enzymes or not. However, the lack of a significant number of reports that confirm these relationships means that it is not a certain solution, especially considering the fact that haemagglutination activity alone does not indicate the degree of toxicity of lectins, which can only be determined in experiments on animals.

Haemagglutination assay results (Table 1) showed particularly strong agglutination of rat erythrocytes (from 1/16 to 1/32) by extracts from seeds, which is even more pronounced given the action of the extract of mushrooms used as the control (1/128). It may signify high sensitivity of these animals to lectins. These results confirm the findings of Grant et al. (1983), who recorded the hemagglutination of rat erythrocytes (previously treated with protease) at a greater sample dilution than in the case of rabbit erythrocytes, and to a lesser extent than bovine and human erythrocytes. This higher sensitivity of rat erythrocytes to lectins should be taken into account in laboratory tests concerning the quality of protein in lectin-containing feed and food consisting of raw seeds.

According to Grant et al. (1983) protein in seeds of different species of beans with high toxicity associated with high hemagglutination ability has a negative net protein utilization

coefficient (NPU). In contrast, the protein of lentils, peas and some species of beans, classified as non-toxic, obtained positive values (from 43 to 62). Lubowicki et al. (2000) obtained positive coefficients for lupins (from 46 to 66), and for the grass pea Krab variety (44), and negative values for varieties for Derek grass pea and Anita lentils, with the largest weight loss was observed after feeding animals with lentils. This is inconsistent with the results of our hemagglutination assay (Table 1) since the lesser hemagglutination ability of lentils (1/16) compared to grass peas (1/32) produced an inverse relationship in relation to the aforementioned values. This can be explained by the greater share of lentil seeds in the experimental mixtures, i.e. 38.5 compared to 33.7 and 31.7% dry matter, respectively (Lubowicki et al. 2000), the greater toxicity of lentil lectins (Vasconcelos and Oliveira 2004) and the activity of other antinutritional compounds. Extracts from two varieties of grass pea (Table 1) agglutinated rat erythrocytes at the same dilution 1/32, suggesting an identical response in rats in research on the biological assessment of protein (Lubowicki et al. 2000). However, in this study grass pea varieties induced divergent reactions in rats which could have resulted from differences in the concentrations of lectins and also other antinutritional compounds, e.g. lathyrogens: ODAP (ß-N-oxalyl-L-diaminopropionic acid) and BAPN (beta--amino-propionitrile). Deshpande and Campbell (1992) showed that these substances can reduce the productivity of monogastric animals, mainly associated with growth of animals and the use of seed feeds.

CONCLUSION

In conclusion, human, rat and sheep erythrocytes did not reveal the presence of lectins in the seeds of lupins and cereal grains except rye. The observed differences suggest that the pea variety may influence the HA of lectins.

Red blood cells of laboratory rats were most agglutinated under the influence of lectins of the mushroom *Agaricus campestris* used as the control, then much less by grass peas, peas and lentils. Rat erythrocytes agglutinated the most, and as such they are the best indicator of biological activity of lectins among the examined red blood cells. Due to the high sensitivity of rat erythrocytes to the presence of lectins, it is recommended that research on the protein quality of the seeds of grass peas, peas and lentils conducted on rats should take into account their harmful effect on protein utilization in foods and feedstuffs. Generally, higher HA of red blood cells from A1 group may indicate a higher sensitivity of people with this blood group to lectins of the examined leguminous plants and rye, than individuals with blood groups B and O.

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Abstract. The material in this research consisted of leguminous plants and cereals. The occurrence of lectins was determined on the basis of the activity of red blood cells in humans, laboratory rats and rams treated with extracts of the seeds and grain of the aforementioned plants, using the extract of the fruiting body of the mushroom *Agaricus campestris* as control. The biological activity of lectins was determined on the basis of the maximum degree of dilution of the extract that was still causing further agglutination of erythrocytes. The results showed the presence of lectins in the seeds of grass peas, peas and lentils and their stronger agglutinating effect on red blood cells in rats than humans. With regard to cereals, lectins were detected only in winter rye with weak agglutination of group A_1 erythrocytes in humans, and no detectable effects in erythrocytes of rats and rams.