Folia biologica (Kraków), vol. **53** (2005), Supplement

Products and Biopreparations from Alkaloid-rich Lupin in Animal Nutrition and Ecological Agriculture

Danuta CIESIOŁKA, Piotr GULEWICZ, Cristina MARTINEZ-VILLALUENGA, Radosław PILARSKI, Marek BEDNARCZYK and Krzysztof GULEWICZ

Accepted November 4, 2005

CIESIOŁKA D., GULEWICZ P., MARTINEZ-VILLAIUENGA C., PILARSKI R., BEDNARCZYK M., GULEWICZ K. 2005. Products and biopreparations from alkaloid-rich lupin in animal nutrition and ecological agriculture. Folia biol. (Kraków) **53** (Suppl.): 59-66.

This paper presents a new approach to utilization of alkaloid-rich lupin as a high protein and ecological plant. After processing of bitter lupin seeds, many valuable products are obtained, i.e. protein concentrate, high dietary fiber product, raffinose family oligosaccharides (RFOs), and lupin extract. The described debittering process is fully ecological without waste and all obtained products may be utilized in different domains. Therefore, (i) the lupin protein concentrate may be a soybean substitute for feeding animals. (ii) high dietary fiber product improves rheological properties of dough and quality of bakery products, (iii) RFOs are prebiotics, (iv) lupin extract may be used for ecological plant cultivation, as a plant protection preparation and as a medium for the production of yeast, gibberellic acid (GA_3), etc.

Key words: Lupin, proteins, dietary fiber, RFOs, biopreparations, yield factors, prebiotics.

Danuta CIESIOŁKA, Radosław PILARSKI, Krzysztof GULEWICZ, Phytochemistry Laboratory, Institute of Bioorganic Chemistry PAS, Noskowskiego12/14, 61-704 Poznań, Poland.
E-mail: krysgul@ibch.poznan.pl
Piotr GULEWICZ, Cristina MARTINEZ-VILLALUENGA, Instituto de Fermentaciones Industriales (CSIC) Juan de la Cierva 3, E-28006 Madrid, Spain.
Marek BEDNARCZYK, Department of Animal Biotechnology, University of Technology and Agriculture, Mazowiecka 28, 85-084 Bydgoszcz, Poland.

One of the main questions of the contemporary world is deficiency of protein concentrates necessary for the production of fodder as well as chemicalization of agriculture that brings about ecological problems. The deficiency of protein, among other things, is caused by intensification of animal production as well as increase of production of low protein content grains and energetic carbohydrate fodders. Presently, in some countries, environmental pollution (water, air, soil) caused by gigantic development of the industry, production and wide-scale application of chemical preparations in different branches of economy is undoubtedly a menace to human existence. The response of man to the destruction and degradation of his natural environment is the creation of widespread ecological movements, green parties, ecological programs and actions in favor of the environment. One of the programs of the ecological movement is the development of agriculture based on natural products and biological preparations utilized in nutrition and plant cultivation and protection.

1. Lupin as a protein-rich and ecological plant

Lupin, in particular its alkaloid-rich cultivars, may play a very important role in this program. The features distinguishing lupin from other plants in this respect is as follows: (i) it is important from the economic point of view, (ii) it has modest soil and climatic demands, (iii) it does not need nitrogen fertilization, (iv) it provides live nitrogen and significant organic mass in soil after harvest, (v) it improves air-water conditions of soil, (vi) it provides access to macro- and microelements eluted to a sublayer of soil.

80 years ago, when studies of von Sengbusch resulted in the first sweet (alkaloid-low) lupin cultivars, the problem of total utilization of this plant seemed to be solved, and a balanced protein deficiency was merely a question of time. From our time perspective, these expectations have not been confirmed in practice. The lupin alkaloid problem remains the subject of numerous papers and a topic

of wide discussions. On the other hand, numerous investigations have shown that the removal of alkaloids from lupin caused a decrease in resistance and yield in these plants (BACKER & NEHRING 1965; WINK 1984; KAHNT & HIJAZI 1987; KRZYMAŃSKA *et al.* 1988; VON BAER *et al.* 1990; GULEWICZ *et al.* 1994). The enormous vitality of bitter cultivars confirmed the expansion of the Russel lupin in New Zealand. This lupin, introduced in 1952 as an ornamental plant, rapidly spread over the whole southern region of the island and presently constitutes a potential fodder base (SAVAGE *et al.* 1990). In spite of considerable progress in the breeding of sweet cultivars, bitter lupin still plays a substantial role in the protein balance of some countries.

2. Debittering process of alkaloid-rich lupin seeds

Seeds of various species of lupins have been used as food for over 3000 years around the Mediterranean (GLADSTONES 1970) and for as much as 6000 years in the Andean highlands (UAUY *et al.* 1995). People used to soak the seeds in running water to remove most of the bitter and toxic alkaloids and then cook or roast the seeds to make them edible (HILL 1977). Unfortunately, this mode of debittering led to removal of the valuable biological compounds that were significant from an economic point of view. This important problem was resolved with a method described by GULEWICZ 1988, 1991a, 1991b; GULEWICZ *et al.* 2000. A general scheme of this process is presented in Fig. 1. The main products of this process are protein concentrate (PC), high dietary fiber product (HDFP), lupin extract (LE), and raffinose family oligosaccharides (RFOs).

3. Utilization of lupin protein concentrates in animal feeding

The economic significance of the lupin plant as a source of high-protein fodder arises from its modest soil requirement and tolerance of acidic conditions. An interesting feature of lupin is its high protein content and very low content of antinutritional factors, such as phytate, tannins, lectins and protease inhibitors. Moreover, the alkaloids in modern varieties of sweet lupin have been reduced to a very low level (HANCOCK 2003). The content of crude protein in the seeds of different species of lupin (HANCOCK 2003): *L. angustifolius* (28-38%); *L. albus* (34-45%), and *L. luteus* (36-48%) is similar or higher in comparison with soybean (35-40%). Lupin seeds contain two classes of proteins which, according to Osborne's classification, belong to the albumin and globulin fractions. The net protein utilization of lupin was 77% that of egg protein (EGANA *et al.* 1992) and the protein isolate from lupin seeds showed good foaming activity, comparable to egg white (YOSHIE-STARK *et al.* 2004).

Lupin proteins appear to have interesting properties. An *in vitro* study demonstrated the hypocholesterolemic activity associated with stimulation of LDL receptor by a well-defined protein component of the lupin seeds (SIRTORI *et al.* 2004). The addition of lupin to the nutritional dose is ecological since it reduces methanogenesis, thus indirectly contributing to the reduction of greenhouse gas emission (ZAWADZKI *et al.* 2004).

Lupin seeds supplemented with methionine are an essential source of protein in the diet of nonruminant animals, chicken (ERICSON 1985; RUBIO *et al.* 2003), and pigs (LIEBHOLZ 1984). The content of lupin in appropriately composed fodder of ruminants is unlimited. The addition of yellow lupin seeds to the ruminant fodder has a positive influence on the fermentation process in their rumens, shown by a significant increase of the level of total protein, ammonia and lactic acid, especially after the introduction of higher doses of lupin (ZAWADZKI *et al.* 2004). Lupin protein may become a nutritional alternative which will substitute animal-origin fodder (SUDARYONO *et al.* 1999).

The protein concentrates obtained during the debittering process were also the object of studies for many feeding experiments (KARASIŃSKI *et al.* 1988; BEDNARCZYK *et al.* 1989; POTKAŃSKI *et al.* 1992a, 1992b). These experiments conducted on different animals, i.e. ducks, piglets, pigs showed that lupin protein allows for the production level comparable to the control groups where soybean was used as a protein source.

4. Effect of high dietary fiber product on rheological properties of dough and quality of bakery products

As showed in the studies of CIERNIEWSKA *et al.* 1996, the seed cover of *Lupinus albus* after the debittering process contains 89.04% of nonsoluble dietary fiber (NSDF). The main component of NSDF is cellulose (79%). Other, i.e. hemicellulose and lignin, remain at levels of 14 and 7%, respectively. The assessment of suitability of high dietary fiber product and its utilization as a valuable source of dietary fiber was carried out in experimental baking, where 10, 15 and 20% additions of HDFP to the wheat dough were used. It was found that the contribution of HDFP in mixture with wheat flour affects an increase of water absorbability in comparison to the control dough. Also, the advantageous effects of HDFP on rheological properties of dough such as its development, time

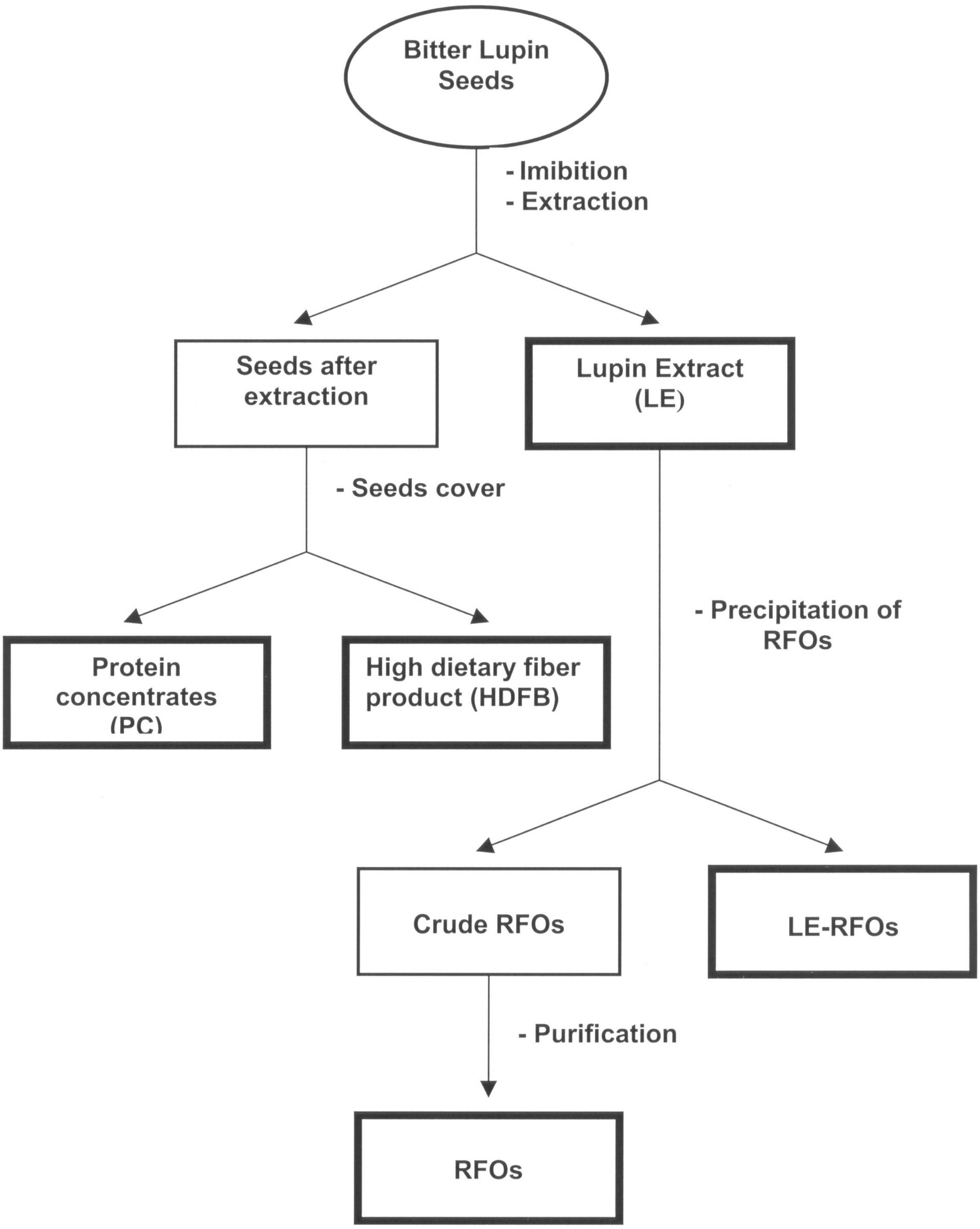

Fig. 1. General scheme of bitter lupin seeds processing.

stability, and index tolerance to kneading were observed. The best organoleptic effect was obtained when a 10% addition of HDFP was used. Furthermore, a delicate structure of crumb was observed. The contribution of HDFP to bread production essentially affected its nutritive value change. On the other hand, an increased dietary fiber rich in non- and soluble substances is very important from dietary point of view.

5. Lupin extract is an important product and raw material in different branches

The lupin extract, that is a by-product of the debittering process, is a rich source of many valuable natural products such as RFOs, alkaloids, free amino acids, peptides, non-volatile organic acids, derivatives of benzoic, phenylacetic and cynamic acids, derivatives of coumarin, aliphatic acids, isoflavonoids and mineral compounds (GULEWICZ 1988; MARKIEWICZ *et al*. 1988; STOBIECKI *et al*. 1993). Studies on the toxicity of different lupin extracts, in case of which the extracts were applied to the stomach of mice and rats, showed that they are non-toxic. Moreover, the studied extracts do not have any cytotoxic effect, as shown in the tests on the viability of mouse thymocytes (STOBIECKI *et al*. 1993; SZCZAWIŃSKA *et al*. 1994). Such lupin extract rich in non-toxic natural products resulted in wide application in different branches.

5.1 Effect of lupin extract on the plants

One of the directions of research on the utilization of lupin extract was the determination of its effect on the development and yield of various plants. These studies have been performed in field experiments for three years on such plants as wheat, triticale, barley, sugar beet root, fodder beet root, winter rape, potato, cucumber, and tomato. The plants were sprayed with doses of 5 -10 kg of extract dry weight per hectare (CWOJDZIŃSKI *et al*. 1989a; POLCYN 1995). In all cases, the extract beneficially influenced yield, although it varied for the tested plants. The highest increase of yield was observed for wheat, triticale, sugar beet root, winter rape, and potato (ca. 24%), while the lowest for fodder beet root (ca. 10%). These results are in accordance with the results reported by KAHNT and HIJAZI 1987,1991, which used Lupinex – water aqueous lupin extract. CWOJDZIŃSKI (*et al*. 1989b) and MICHALSKI (1991) also showed that leaf spraying leads to an essential increase of protein yield and has a beneficial effect on the nutritive value of protein (NPU, Dt, BV). STOBIECKI and GULEWICZ (1991) isolated from lupin an extract fraction called A_4 containing glycosides, nucleosides of purine and pyrimidine bases that strongly stimulated the development of plants (PERETIATKOWICZ *et al*. 1994). These results were confirmed by PRZYBYLAK *et al*. 2005 showing that the removal of raffinose family oligosaccharides from extract of *Lupinus exaltatus* does not have any effect on the development and yield of paprika. The studies of CWOJDZIŃSKI *et al*. (1996) on the effect of lupin extract on the accumulation of nitrate by the root of carrot (*Daucus carota*) clearly showed that the extracts affect the processes connected with collection and accumulation of nitrates by plants, resulting in a decrease of the level of these compounds. These results were confirmed by POLCYN (1995), who studied the effect of lupin extract on accumulation of nitrate in cucumber (*Cucumis sativus* L.). Up to now, studies of the effect of lupin extract on plants have shown that the compounds presented in the extract beneficially interact with photosynthesis, resulting in a more intensive green color of the plants and prolongation of vegetation time of the plants treated with the extracts (CWOJDZIŃSKI *et al*. 1989a; GULEWICZ *et al*. 1994). It is well known that photosynthesis has an essential effect on speed reduction of nitrate that is connected with activity of nitrate reductase. Owing to this, intensive photosynthesis creates unbeneficial conditions for the accumulation of nitrate by plants. Obviously, such interpretation of the mechanism of lupin extract interaction may be so far considered hypothetical requiring sound evidence. Studies in this field should include a series of factors that directly affect photosynthesis, i.e. humidity, light, type of soil and content of nutrients.

5.2 Lupin extract in plant protection

In a search of a new non-chemical mode of plant protection, attention is paid to biological methods such as utilization of natural resistance of plants or secondary metabolites, e.g. lupin alkaloids that are a "chemical weapon" of plants (BACKER & NEHRING 1965; WINK 1984). This assumption has been confirmed by numerous phenomena, i.e. higher resistance of bitter lupins to infection, recurring increase of alkaloid content in stress conditions, e.g. mechanical damage of plants. The studies of KRZYMAŃSKA (1967) on the role of alkaloids in the resistance of some lupin varieties against pea aphid (*Acyrthosiphon pisum Harris*) clearly confirmed their inhibiting influence on the development of this pest population. The abovementioned studies initiated experiments on the effect of lupin extract on the feeding and development of potato-beetle population. The selection of insects was not accidental since it greatly damages potato cultivation causing considerable economic waste. In the climatic conditions of Poland, a number of larvae in L_4 phase are generally a decisive factor in potato-beetle harmfulness. These larvae need about 80% of the nourishment required by L_1, L_2 and L_3 larvae together. The studies of KRZYMAŃSKA *et al*. (1988) showed that lupin extract causes important inhibition of the development of potato-beetle larvae L_2 phase and that the level of action depends on alkaloid content. In experiments on the feeding preference of adult insects, in the first stage, the beetles chose the non-sprayed leaves. After 48h, all of the nonsprayed leaves were eaten, while the leaves sprayed with lupin extract were not attacked by insects. WYROSTKIEWICZ *et al*. (1996) observed reduced feeding behaviour of the colorado beetle and the large white butterfly under the influence of alkaloid lupin fractions A_1 and A_5 obtained by the method described by STOBIECKI *et al*. (1993). The extract from *Lupinus angustifolius* and fractions A_1 and A_5 also inhibited the feeding of large white butterfly larvae. The extracts reduced the populations of butterfly parasitoid (*Apanteles glomeratus* L.). The effect of lupin extract and its alkaloid fractions A_1 and A_5, on feeding and development of *Pieris brassicae* L. and *Pieris rapae* L. was a subject of the FOLKMAN'S *et al*. (2002) studies. The results showed that in the case of large cabbage white fraction A_1 was the most efficient preparation that resulted in a low level of feeding, high value of absolute deterrence factor, low weight of pupae, and low rate of transformation of larvae into pupae. Lupin extract (non processed) stimulated feeding of large cabbage white larvae, which resulted in negative value of the deterrence factor (–9.8090), high weight of pupae, and rate of transformation. The weight increase of small cabbage white larvae was the lowest in the case of the A_1 fraction, however, it was not correlated with the amount of the eaten feed (ADF=1.247). Feeding with cabbage sprayed with A_1 fraction and extract as well as 13-hydroxylupanine perchlorate caused damage to pupae of small cabbage white. There is no significant influence of the used alkaloid perchlorates on feeding and development of the tested species. Owing to this, an active substance, but only in a form acceptable by pest, is able to perform strong biological activity analogously to the dependence of drug efficacy upon its formula. Finding in lupin extract a system that enables the activation of the genes of proteinase inhibitors and other proteins suggest that this polypeptide, besides alkaloids, may play a major role in the defence of various plants against insects as well (RADŁOWSKI *et al*. 1997).

The studies of SAS-PIOTROWSKA *et al*. (1996) on the growth and development of phytopathogenic fungi that affected potato leaf diseases (*Alternaria solani*, *Cladosporium herbarum*, *Collectotrichum coccodes*, *Verticillium alboatrum*) and also the studies on the fungi that affected potato tuber diseases (*Fusarium coerelueum*, *Fusarium sulphureum*, *Phoma exigua* var. *foveata, Phoma exigua* var. *exigua*, *Rhizoctonia solani*) on the media supplemented with extracts from *Lupinus albus*, *Lupinus luteus*, *Lupinus angustifolius* and its alkaloid fractions A_1 and A_5 confirmed fungistatic effect of the used preparations. The activity of the preparations on the leaf pathogens was stronger that on potato tuber pathogens. The strongest effect of the alkaloid fractions was noted for the colonization of potato leaf fungi. An interaction between pathogen species and type preparation was observed. These studies show that the lupin extracts may be potential ecological preparations in plant protection.

TYSKI *et al*. (1988) observed full inhibition of bacterial (*B. thuringensis*, *B. subtilis*, *S. aureus*) growth at concentrations of lupin extracts of 5 to 10 mg/ml for the assay. This is equivalent to the concentration of the major alkaloids in the extract – e.g. lupanine, about 2.5 mM. At similar concentrations, nearly complete inhibition of the translation process was observed (KORCZ *et al*. 1987). The coincidence of the observed effects, namely a bacteriostatic and an inhibitory effect at the same molar concentration of alkaloids, appears interesting. This is a concentration of about 1 order of magnitude below the physiological concentration of alkaloids in the seeds (WINK *et al*. 1984).

5.3 Pharmacological properties of lupin extract

The effect of extract from *Lupinus angustifolius* L. on arterial blood pressure, bioelectrical activity of heart, motor activity as well as its hypnotic activity constituted a subject of several papers (SZCZAWIŃSKA *et al*. 1994; ALI 1996; BOBKIEWICZ-KOZŁOWSKA *et al*. 2005). It was shown that the lupin extract, despite considerable content of alkaloids (about 11% dry weight), shows a significant decrease of arterial blood pressure of rats in the doses of 1/40 and 1/20 of LD_{50}. The extract demonstrates a barely perceptible influence on bioelectrical activity of the heart (the pulse per minute decreased in a period of 30-60 min of observation time). The lupin extract decreases the motor activity of rats, however, hypnotic activity was not observed. In search of the compounds responsible for the effects on the cardiovascular system and central nervous system, only 13-hydroxylupanine succeeded in attenuating the heart rate and only sparteine was affected by inhibiting locomotor activity. On the basis of the obtained results, it can be concluded that not only alkaloids, but other compounds of lupin extract might be involved in the pharmacological lupin seeds extracts. The studies of BŁASZCZYK *et al*. (1994) on the influence of lupin extracts on immunotropic properties showed that the extracts from bitter and sweet lupin seeds possess immunosuppressive activity in rosette forming cells (E-RFC). It has been proven that the procedure used in utilization of lupin protein is very advantageous because it simultaneously allows the removal of substances which are responsible for decreasing the resistance of the animal organism.

Previous research on the pharmacological activity of lupin compounds have been restricted to pure alkaloids (NOWACKI & WĘŻYK 1960; MAZUR *et al*. 1966ab). Unfortunately, the method of their isolation, as well as the form in which they were administered to the animal, may give rise to factual doubts. In contrast to the "pure alkaloids", the extract is a pool of native, mutually interacting native compounds of low toxicity. Thus, studies on the pharmacological activity of the extract and its fractions are currently carried out in many laboratories and should supply a comprehensive answer for its possible applications in the pharmacological industry.

5.4 Lupin extract in biotechnology

In the industrial biosynthesis of protein yeast, constituting a rich source of easy bio-availability of protein, vitamins of B group and mineral salt, are utilized most frequently. Up to now, the main raw material in yeast production has been beet molasses, vinase, sulfite liquors, wood hydrolysate, whey and other products of the agricultural industry. The studies of TROJANOWSKA *et al*. (1991) on utilisation of lupin extract showed that this by-product may be a valuable raw material in yeast production. It was shown that lupin extract used as a culture medium for 22 strains of yeast caused the production of biomass on a different level. Simultaneously, 5 yeast strains were found (*Rhodotorula rubra*, *Torulautilis*, *Torula cremoris*, *Torulopsis utilis* and *Ja 36*) for which lupin extract was an excellent cultural medium for the production of biomass. Furthermore, these studies show that the obtained biomass contains ca. 40% of protein in d.w. and that digestibility was on the level of ca. 82%. This protein also showed a rich amino acid composition and all of its technological parameters corresponded to the Polish norms of yeast fodder.

The studies of GULEWICZ *et al*. (1994) showed that lupin extract may be a promising medium for gibberellic acid (GA_3) production by *Fusarium moniliforme* competitive with many other media which have been used for that purpose.

The influence of different concentrations of lupin extract on cultivar P-20 of *Pleurotus ostreatus* mycelium was the subject of GAPIŃSKI'S *et al*. (1996) studies. The experiment was done on agar-agar and wheat straw chaff basis. The addition of mycelium agar of lupin extract had a profitable influence on the growth of *Pleurotus ostreatus* mycelium. The growth of the mycelium depended on the level of the lupin extract used. The addition of lupin extract to sterilized wheat straw had no effect on mycelium *Pleurotus ostreatus* growth. The alkaloids presented in the lupin extract had no inhibitory effect on the development of mycelium.

5.5 Prebiotics from lupin extract

As shown earlier (GULEWICZ 1988; MARKIEWICZ *et al*. 1988), the main components of lupin extract are raffinose family oligosaccharides (RFOs) – ca. 50% in dry weight. Although RFOs perform a very important physiological function from the nutritional point of view, RFOs are considered as antinutritional factors because they are not hydrolyzed by mucosal enzymes in the small intestine of monogastric animals. They are fermented in the large intestine with liberation of gas causing arduous flatulence (CRISTOFARO *et al*. 1974; SAINI & GLADSTONES 1986; PRINCE *et al*. 1988). The opinion about the total function of RFO in nutrition has recently changed due to the elaboration of a simple and rapid method of their isolation and purification from legume extracts, including lupin one (GULEWICZ *et al*. 2000; MARTINEZ-VILLALUENGA *et al*. 2004). It allows for wide studies of these sugars in terms of their prebiotic activity. The investigations on the effect of lupin RFO preparations on a number of bifidobacteria performed on rats and chicken (GULEWICZ *et al*. 2002; LISOWSKI *et al*. 2003), as well as on a chicken embryo model (VILLALUENGA *et al*. 2004; PILARSKI *et al*. 2005), showed that these short chain sugars have prebiotic activity comparable to well known commercial preparations: fructooligosaccharides (FOS) and inulin. Although these studies do not show the beneficial effect of the tested oligosaccharides injected *in ovo* on chicken broiler meat traits, the fact of maintaining high bifidobacteria number during the experiment has multi-way health importance. As it is known, oligosaccharides as prebiotics contribute to health in many ways: (i) they influence proliferation of bifidobacteria and reduce detrimental ones; (ii) they reduce toxic metabolites and detrimental enzymes; (iii) they prevent pathogenic and autogenous diarrhea; they prevent constipation by stimulating intestinal peristalsis and by increasing faecal moisture with osmotic pressure; (iv) they alleviate the detoxifying load of liver; (v) they reduce the serum cholesterol level; (vi) they reduce blood pressure; (vii) they show anticancer activity; (viii) they influence production of vitamins B_1, B_2, B_6, B_{12}, nicotic and folic acids (AMMERMAN *et al.* 1988; BAILEY *et al.* 1991; CHOI *et al.* 1994; WALDROUP *et al.* 1993; TOMOMATSU and papers cited therein, 1994). In the light of our studies, the application of oligosaccharides to the chicken diet can be successfully replaced by injecting these compounds *in ovo* in very low doses. Moreover, taking into consideration health care only, this approach is of high significance and, therefore, cannot be disregarded.

References

AMMEMAN E. G., QUARLES P. V., TWINING J. R. 1988. Broiler response to the addition of dietary fructooligosaccharides. Poultry Sci. **67**: 34-41.

ALI O. A. 1996. The pharmacological activities of seed extracts from selective lupinus species *(L. angustifolius* L., *L. albus* L., *L. luteus* L.). Ph.D. Thesis, Ali O.A ed. Department of Pharmacology, University of Medical Sciences, Poznań.

VON BAER D., LAMPERTI L., ROSS E., PUENTES P., VON BEAR E., VATH D., WEISSMAN U. 1990. Potential of *L. mutabilis* in comparison with *L. albus.* Results of the season 1989/90 in the south of Chile. Proc. 6th Inter. Lupin Conf. Temuco Pucon, Chile. Abs. 3.

BACKER M., NEHRING K. 1965. Handbuch der Futtermittel, P. Parey, Verlag, Hamburg & Berlin: 216-305

BAILEY J. S., BLACKENSHIP L. C., COX N. A. 1991. Effect of fructooligosaccharides on *Salmonella* colonization in the chicken intestine. Poultry Sci. **70**: 2433-2438.

BEDNARCZYK M., KARASIŃSKI D., MICHALSKI Z., MARKIEWICZ M., GULEWICZ K. 1989. The influence of lupin alkaloids on reproductive features of ducks. Lupin Newsletter **13**: 66-74.

BŁASZCZYK B., STOBIECKI M., KOWALCZYK-BRONISZ S. H., GULEWICZ K. 1994. Immunotropic activity of lupin seeds extracts and fractions from *Lupinus angustiofolius* and *Lupinus albus.* Arch. Immun. Therapiae Exp. **42**: 147-153.

BOBKIEWICZ-KOZŁOWSKA T., ALI O. A., GULEWICZ K., WYSOCKA W. 2005. Species-dependent pharmacological properties of lupinus seeds extract. Proc.11th Int. Lupin Conf. Guadalajara, Mexico Abstract 116.

CHOI K. H., NAMKUNG H., PAIK I. K. 1994. Effect of dietary fructooligosaccharides on the suspension of intestinal colonization of *Salmonella typhimurium* in broiler chicken. Korean J. Anim. Sci. **36**: 271-284.

CIERNIEWSKA A., GÓRECKA D., KAWKA A., GULEWICZ K. 1996. Chemical composition of seeds covers of *Lupinus albus* var. Bac and their influence on dough bread properties. (In: Lupin: Investigations and Prospect Utilization. Frencel I., Gulewicz K. eds. Poznań: 300-305. (In Polish).

CRISTOFARO E., MOTTLI F., WHURMANN J. J. 1974. Involvement of raffinose family of oligosaccharides in flatulence. (In: Sugars in Nutrition, H. L. Sepple & K. W. McNutt eds, New York, Academic Press): 313-363.

CWOJDZIŃSKI W., MICHALSKI Z., NOWAK K., GULEWICZ K. 1989a. Studies on the influence of bitter lupin extract on the yield of different cultivated plants. Lupin Newsletter **13**: 46-54.

CWOJDZIŃSKI W., MICHALSKI Z., GULEWICZ K. 1989b. Estimation of nutritive value of protein obtained from plant treated with lupin extract. Lupin Newsletter **13**: 22-28.

CWOJDZIŃSKI W., GULEWICZ K., NOWAK K. 1996. Extract from bitter lupin seeds as a factor limiting accumulation of nitrates by carrot roots. (In: Lupin: Investigations and Prospect Utilization. Frencel I., Gulewicz K. eds. Poznań: 254-265. (In Polish).

EGANA J. I, UAUY R., CASSORLA X., BARRERA G., YANEZ E. 1992. Sweet lupin protein quality in young men. Nutr. **122**: 2341-2347.

ERICSON J. P. 1985. Sweet white lupin (cultivar *L. ultra*) in broiler and turkey diets. Foodstuffs **57**: 21-24.

FOLKMAN W., SZERECHAN J., GULEWICZ K. 2002. Preparation of alkaoid-rich lupin in plant protection: an effect of the preparations on feeding and development of *Pieris brassicae* L. and *Pieris rapae* L. J. Plant Protec. **42**: 143-155.

GAPIŃSKI M., WO NIAK W., GULEWICZ K. 1996. Effect of extracts from the lupin seeds (*Lupinus angustifolius)* on the mycelium growth (*Pleurotus ostreatus*) (In: Lupine: Investigations and Prospect Utilization. Frencel I., Gulewicz K. eds Poznań): 266-271 (In Polish).

GLADSTONES J. S. 1970. Lupins as a crop plant Fild Crop Abstracts **23**: 123-148.

GULEWICZ K. 1988. Studies on utilisation of protein and other compounds from bitter lupin seeds. Habilitation Theses Gulewicz K., Polish Academy of Sciences, Poznań. (In Polish).

GULEWICZ K. 1991a. Polish patent No 152748. Mode of debittering of lupin seeds.

GULEWICZ K. 1991b. Polish patent No153195. Mode of debittering of lupin seeds.

GULEWICZ K., RATAJ-GURANOWSKA M., ŁUKASZEWSKA N., MICHALSKI Z. 1993. Giberellic acid production by *Fusarium monifliforme* on lupin seed extract. Acta Microbiol. Pol. **43**: 73-77.

GULEWICZ K., PERETIATKOWICZ M., BRATEK-WIEWIÓROWSKA D., WIEWIÓROWSKI M. 1994. Bitter lupin seeds and straw as raw materials in ecological agriculture. Proc. 7th Int. Lupin Conf. Evora, Portugal: 312-332.

GULEWICZ P., CIESIOŁKA D., FRIAS J., VIDAL-VALVERDE C., FREJNAGEL S., TROJANOWSKA K., GULEWICZ K. 2000. Simple method of isolation and purification of α-galactosides from legumes. J. Agric. Food Chem. **48**: 3120-3123.

GULEWICZ P., SZYMANIEC S., BUBAK B., FRIAS J., C. VIDAL-VALVERDE J., TROJANOWSKA K., GULEWICZ K. 2002. Biological activity of α-galactosides from *Lupinus angustifolius* L. and *Pisum sativm* L. seeds. J. Agric. Food Chem. **50**: 384-389.

HANCOCK J. 2003. 100% organic livestock feeds – preparing for 2005. Organic Centre Wales, Institute of Rural Studies, University of Wales Aberystwyth, Ceredigion.

HILL G. D. 1977. The composition and nutritive values of lupin seeds. Nutrition Abstracts and Reviews, B. **47**: 511-519.

KAHNT G., HIJAZI L. A. 1987. Effect of bitter lupin extract on growth and yield of different crops. J. Agronomy & Crop Sci. **159**: 320-328.

KAHNT G., HIJAZI L. A. 1991. Use of lupinex to increase crop yield and improve harvest quality with lesser nitrogen fertilization. J. Agr. Crop Sci. **166**: 228-237.

KARASIŃSKI D., BEDNARCZYK M., PERETIATKOWICZ M., GULEWICZ K. 1988. The influence of alkaloids in seeds of *Lupinus angustifolius* on the growth and some meat features of ducks. Bull. Pol. Acad. Sci. **36**: 215-224.

KORCZ A., PULIKOWSKA J., MARKIEWICZ M., TWARDOWSKI T. 1987. Species specific inhibitory effect of lupin alkaloids on translation in plant. J. Plant Physiol. **128**: 433-442.

KRZYMAŃSKA J. 1967. The role of alkaloids in resistance of some lupinus cultivars on *Acyrthosiphon pisum*. Bull. IOR. **36**: 237-247. (In Polish).

KRZYMAŃSKA J., WALIGÓRA D., MICHALSKI Z., GULEWICZ K. 1988. Observation on the influence of spraying potatoes with lupin extract on the feeding and development of potato-beetle population. Bull. Pol. Acad. Sci. **26**: 13-24.

LIEBHOLZ J. 1984. A note on methionine supplementation of pig grower diets containing lupin-seed meal. Anim. Prod. **38**: 515-517.

LISOWSKI M., LEWANDOWSKA M., BEDNARCZYK M., GULEWICZ P., GULEWICZ K. 2003. Prebiotic properties of pea -galactoside preparation applied to chicken *per os.* Bull. Pol. Acad. Sci. **51**: 291-298.

MARKIEWICZ M., KOLANOWSKA A., GULEWICZ K. 1988. The chemical composition of debittered lupin seeds and their extract. Bull. Pol. Acad Sci. **36**: 11-22.

MARTINEZ-VILLALUENGA M. C., FRIAS J., GULEWICZ K., VIDAL-VALVERDE C. 2004. Improved method to obtain pure – galactosides from lupin seeds. J. Agric. Food Chem. **52**: 6920-6922.

MAZUR M., POLAKOWSKI P., SZADOWSKA A. 1966a. The pharmacological studies of lupanine, and 13-hydroxylupanine. Acta Phys. Pol. XVII, 2, 299.

MAZUR M., POLAKOWSKI P., SZADOWSKA A. 1966b. The pharmacological studies of 17-oxolupanine, aminooxide lupanine and 17-hydroxylupanine. Acta Phys. Pol. XVII: 311.

MICHALSKI Z. 1991. Effect of lupin extract on quantity and quality of yield of wheat and triticale. In: Ph.D. Thesis, University of Technology and Agriculture, Bydgoszcz. (In Polish).

NOWACKI E., WĘŻYK S. 1960. Toxicity of alkaloids in lupins for the rabbit (*Oryctolagus curiculus* L.). Rocz. Nauk Rol. Ser. Zootech. **75**: 385-399.

PERETIATKOWICZ M., CIESIOŁKA D., STOBIECKI M., GULEWICZ K. 1994. Biological activity of extract from bitter lupin seeds. Proc. 7th Inter. Lupin Conf. Evora, Portugal, 197-200.

PILARSKI R., BEDNARCZYK M., LISOWSKI M., RUTKOWSKI A., BERNACKI Z., WARDEŃSKA M., GULEWICZ K. 2005. Assessment of effect of α-galactosides injected during embryogenesis on the selected chicken traits. Folia biol. (Kraków) **52**: 13-20.

POLCYN J. 1995. Effect of extract from seeds of *L. angustifolius* cv. Mirela on yield and chemical composition of cucumber (*Cucuimis sativus*) cv. Polan F1 cultivated in cropland. In: Ph.D. Thesis, Polcyn, University of Technology and Agriculture, Bydgoszcz. (In Polish).

POTKAŃSKI A., FRANKIEWICZ A., KRAJNA J., GULEWICZ K. 1992a. Effect of application of partly debitered blue lupin seeds on rearing results of piglets. Lupin Newsletter **16**: 13-19.

POTKAŃSKI A., FRANKIEWICZ a., KRAJNA J., GULEWICZ K. 1992b. Influence of semi-bitter lupin seeds of different levels of debittering on fattening results of pigs. Lupin Newsletter **16**: 25-35.

PRINCE K. R., LEWIS J., WYATT G. M., FENWICK G. T. 1988. Flatulence-causes, relation to diet and remedie*s*. Nahrung **32**: 609-62.

PRZYBYLAK J. K., CIESIOŁKA D., WYSOCKA W., LOPEZ P. M. G., LOPEZ M. A. R., WYSOCKI W., GULEWICZ K. 2005. Alkaloid profiles of Mexican wild lupin and an effect of alkaloid preparation from *Lupinus exaltatus* seeds on growth and yield of paprika (*Capsicum annumm* L.). Ind. Crops & Prod. **21**: 1-7.

RADŁOWSKI M., BARTKOWIAK S., GULEWICZ K., GIEL-PIETRASZUK M., MUCHA P., REKOWSKI P., KUPRYSZEWSKI G., BARCISZEWSKI J. 1997. Systemin – an inducer of proteinase inhibitor synthesis can be responsible for biological activity of a lupin extract against insects. J. Plant Physiol. **150**: 220-223.

RUBIO L. A., BRENES A., CENTENO C. 2003. Effects of feeding growing broiler chickens with practical diets containing sweet lupin (*Lupinus angustifolius*) seed meal. Br. Poult. Sci. **44**: 391-397.

SAINI H. S., GLADSTONES J. S. 1986. Variability in the total and component galactosyl sucrose oligosaccharides of *Lupinus* species. Aust. J. Agric. Res. **37**: 157-166.

SAS-PIOTROWSKA B., ANISZEWSKI T., GULEWICZ K. 1996. An evidence for fungistatic activity of some lupin preparations from alkaloid-rich lupin seeds on potato pathogenic fungi. Bull. Pol. Acad. Sci. **44**: 41-47.

SAVAGE G. P., HILL G. D., GIBBS H. M. 1990. Alkaloid content of Russel lupinus and their grazing potential. Proc.6th Inter. Lupin Conf. Temuco Pucon, Chile. Abs.18.

SIRTORI C. R., LOVATI M. R., MANZONI C., CASTIGLIONI S., DURANTI M., MAGNI C., MORANDI S., D'AGOETINA A., ARNOLDI A. 2004. Proteins of white lupin seed, a naturally isoflavone-poor legume, reduce cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. J. Nutr. **134**: 18-23.

STOBIECKI M., GULEWICZ K. 1996. The agent stimulating the growth of plants Polish patent No PL 169914 B1.

STOBIECKI M., BŁASZCZYK B., KOWALCZYK-BRONISZ H., GULEWICZ K. 1993. The toxicity of seed extracts and their fractions from *Lupinus angustifolius* L. and *Lupinus albus* L. J. Appl.Toxicol. **13**: 347-352.

SUDARYONO A., TSVETNENKO E., EVANS L. H. 1999. Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. Aqua. Nutr. **5**: 277-285.

SZCZAWIŃSKA K., BOBKIEWICZ T., KOZARYN K., PERETIATKOWICZ M., GULEWICZ K. 1994. Some pharmacological properties an extract from bitter lupin (*L. angustifolius*) seeds. Proc. 7th Inter. Lupin Conf. Evora, Portugal: 201-203.

TOMOMATSU H. 1994. Health effects of oligosaccharides. Food Technol. **48**: 61-65.

TROJANOWSKA K., MARKIEWICZ M., KOLANOWSKA A., CZACZYK K., MUELLER A., GULEWICZ K. 1991. Lupin extract as raw material in biosynthesis of protein feed on the microbiological way. Biotechnologia **3-4**: 131-136. (In Polish).

TYSKI S., MARKIEWICZ M., GULEWICZ K., TWARDOWSKI T. 1988. The effect of lupin alkaloids and ethanol extracts from lupin seeds of *Lupinus angustifolius* on selected bacteria strains. J. Plant Physiol. **133**: 240-242.

UAUY R., GATTAS V., YANEZ E. 1995. Sweet lupins in human nutrition. (In: Word Review of Nutrition and Dietetics Simopoulos A.M. ed.) **77**: 75-88.

VILLALUENGA C. M., WARDEŃSKA M., PILARSKI R., BEDNARCZYK m., GULEWICZ K. 2004. Utilization of the chicken embryo model for assessment of biological activity of different oligosaccharides. Folia biol. (Kraków) **52**: 135-142.

WALDROUP A. L., SKINNER J. T., HIERHOLZER R. E., WALDROUP P. W. 1993. An evaluation of fructooligosaccharides in diets for broiler chicken and effects on Salmonella contamination of carcasses. Poultry Sci. **72**: 643-650.

WĘGOREK W., KRZYMAŃSKA J. 1968. Biochemical causes of resistant some lupin cultivars on *Acyrthosiphon pisum.* Prace Naukowe IOR **10**: 7-30.

WĘGOREK W., KRZYMAŃSKA J. 1971. Further studies on resistant of lupin on *Acyrthosiphon pisum.* Prace Naukowe IOR **13**: 8-23.

WINK M. 1984. Chemical defense of lupin. Mollusc-repelent properties of quinolizidine alkaloids. Z. Naturforsch. **39c**: 553-558.

WINK M., HEINEN H. J., VOGT H., SCHIEBEL H. M. 1984. Cellular localization of quinolizidine alkaloids by laser desorption mass spectrometry (Lamma 1000). Plant Cell Rep. **3**: 230-233.

WYROSTKIEWICZ K., WAWRZYNIAK M., BARCZAK T., ANISZEWSKI T., GULEWICZ K. 1996. An evidence for insecticide activity of some preparations from alkaloid-rich lupin seeds on colorado beetle (*Leptinotarsa decemlineata* Say) larvae of the large white butterfly (*Pieris brassicae* L.) black aphid (A*phis fabae* Scop.) and on their parasitoids (Hymenoptera: Parasitica) population. Bull. Pol. Acad. Sci. **44**: 29-39.

YOSHIE-STARK Y., BEZ J., WADa Y., WASCHE A. 2004. Functional properties, lipoxygenase activity, and health aspects of *Lupinus albus* protein isolates. J. Agric. Food Chem. **52**: 7681-7689.

ZAWADZKI W., KOTECKI A., CZERSKI A., WINCEWICZ E., KOZAK M., JESZKA J., GRACZYK S. 2004. Nutritional value and fermentation course of ground seeds of yellow lupin of markiz and teo types in the rumen fluid in conditions of *in vitro*. Elec. J. Pol. Agric. Univ. **7**: (2).