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# *Effect of raw and extruded flat pea (Lathyrus sativus) seeds in the diet on activity of enzymes and contents of glycogen in the liver of rats*

Wpływ surowych i ekstrudowanych nasion lędźwianu siewnego (*Lathyrus sativus* L.) w diecie szczurów na aktywność enzymów i zawartość glikogenu w wątrobie

Certain legume seeds are rich in protein and could be utilized as important feed ingredients for animals diets. However, because of the presence of toxic and antinutritional factors the benefits from such seeds have not been fully exploited. One of the leguminous plants with high yield potential, drought resistance and the ability to fix atmospheric nitrogen is *Lathyrus sativus* L. The seeds of *Lathyrus sativus* are good source of protein, however deficient in tryptophan and methionine (7).

*Lathyrus* seeds are extensively grown in Podlasie region of East Poland and could become a significant component of general purpose diets for animals of any age (4). A factor which has retarded the utilization of flat seeds as a feed crop is the problem of toxicity and of antinutritional substances (11, 12, 14, 16). It has been known that neurological and osteological symptoms can develop in both animals and humans when *Lathyrus* seeds are consumed as a major proportion of the diet (1). The causative agents for these symptoms termed as osteolathyrism and neurolathyrism are β-aminopropionitrile (BAPN) and β-N-oxalyl-amino-L-alanine (BOAA). For the purpose of elimination of these toxic substances the flat pea seeds are boiled, water extracted, autoclaved or extruded (9, 13).

There has been a great deal of work on lathyrism covering different animal species, but no attempt has been made as yet to present the effect of *Lathyrus* seeds consumption on enzymatic status of liver. The purpose of this work was to evaluate feeding value of raw and extruded flat pea seeds supplemented to the diet on activity of some enzymes and content of glycogen in the liver of rats.

## MATERIAL AND METHODS

The studies were conducted on 30 Wistar rats divided at random into 5 groups and kept in cages, 6 specimens in each. The rats were fed for 4 weeks with the mixtures prepared basing on typical fodder (LSM) for laboratory rats. The rats of goup I were fed control diet (LSM) without supplementation

Tab. 1. Composition of experimental diets

| Constituents (%) | Feeding groups | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Wheat bran | 10 | 10 | 10 | 10 | - |
| Corn | 15 | 15 | 15 | 15 | - |
| Wheat | 30 | 30 | 30 | 30 | - |
| Barley | 28.5 | 25.5 | 21.5 | 21.5 | - |
| Flat pea raw | - | 10 | 20 | - | - |
| Flat pea extruded | - | - | - | 20 | 99 |
| Fish meal | 3 | 1.5 | - | - | - |
| Skim milk powder | 7 | 3 | - | - | - |
| Yeast fodder | 3 | 1.5 | - | - | - |
| Chalk fodder | 1.3 | 1.3 | 1.3 | 1.3 | - |
| Calcium diphosphate | 1.2 | 1.2 | 1.2 | 1.2 | - |
| Mineral-vitamin premix LSM | 1 | 1 | 1 | 1 | 1 |

of flat pea seeds (Tab. 1). The rats of group II were fed a diet with 10% of ground flat pea seeds, and of group III with 20% of ground flat pea seeds (Tab. 1). The rats of group IV received a fodder with 20% of extruded flat pea seeds and of group V only alone extruded flat pea seeds with 1% of Polfamix LSM (Tab. 1). During the experiment rats were ensured free access to fodder and water. After having finished the feeding stage of the research the rats were decapitated and then liver was immediately perfused with 10 $cm^3$ of physiological saline through the portal vein. Liver after excision was weighed and homogenized in the glass Potter homogenizer in the buffer of the same pH and ionic strength as the buffer used for enzymatic activity determinations. The homogenates were centrifuged at 13000 xg for 20 min in the cooled centrifuge. In the supernatans the activity of the following enzymes was estimated: alkaline phosphatase AP (E.C. 3.1.3.1) (6), isocitrate dehydrogenase ICDH (E.C. 1.1.1.42) (6), sorbitol dehydrogenase SDH (E.C. 1.1.1.14) (8), glutamate dehydrogenase GLDH (E.C. 1.4.1.2) (15), lactate dehydrogenase LDH (E.C. 1.1.1.27) (10), γ-glutamyltransferase γ-GT (E.C. 2.3.2.2.) (14), alanine aminotransferase AlAT (E.C. 2.6.1.2) (14) and aspartate aminotransferase AspAT (E.C. 2.6.1.1) (14). Activity of enzymes was expressed in international units (U or mU) and related to protein concentrations in mg. Protein was estimated by the Folin method (3) and the content of glycogen with anthrone method (2). The results were statisticaly analysed using Students t-test.

## RESULTS AND DISCUSSION

The results of the activity of liver enzymes and glycogen content in the liver are presented in Tables 2 and 3. A statisticaly significant increase in the activity of γ-glutamyltransferase in the liver of rats of all experimental groups occurred (Tab. 2). The activity of γ-GT in the liver of control rats was 18.65 mU/mg of protein and in the rats of the second group 52.67 mU/mg of protein, while in the third 96.38 mU/mg and in the fourth and in the fifth 110.57 mU/mg and 134.52 mU/mg respectively. Comparing the activity of γ-GT in liver homogenates of control and experimental rats from the second group (10% of raw seeds of flat pea) the increase amounted

Tab. 2. Activity of analysed enzymes in rats liver homogenates (n = 6)

| Enzymes (U/mg protein) | | Feeding groups | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| Alkaline phosphatase (AP) | U/mg protein | 3.78±0.41 | 4.07±0.42 | 3.90±0.40 | 4.66±0.44 | 4.38±0.39 |
| Isocitrate dehydrogenase (ICDH) | U/mg protein | $0.43\pm0.07^{a}$ | $0.36\pm0.07^{ab}$ | $0.27\pm0,05^{b}$ | $0.41\pm0.08^{a}$ | $0.33\pm0.06^{ab}$ |
| Sorbitol dehydrogenase (SDH) | U/mg protein | 5.73±0,92 | 6.31±0.89 | 4.64±0.80 | 6.81±0.91 | 4.35±0.86 |
| Glutamate dehydrogenase (GLDH) | mU/mg protein | 57.73±10.28 | 75.68±12.02 | 52.24±10.40 | 69.28±13.94 | 64.74±10.55 |
| Lactate dehydrogenase (LDH) | mU/mg protein | 91.0±33.0 | 107.1±32.4 | 65.1±29.3 | 116.2±38.6 | 78.8±29.7 |
| Gamma-glutamylotransferase (γ-GT) | mU/mg protein | $18.65\pm4.84^{A}$ | $52.67\pm6.91^{B}$ | $96.38\pm10.26^{C}$ | $110.57\pm10.47^{CD}$ | $134.52\pm10.55^{D}$ |
| Alanine aminotransferase (AlAT) | mU/mg protein | 309.5±29.7 | 277.6±28.3 | 299.4±29.2 | 310.2±32.4 | 312.1±33.9 |
| Aspartate aminotransferase (Asp AT) | mU/mg protein | 130.3±15.6 | 129.6±14.3 | 169.6±24.0 | 155.1±17.5 | 135.7±16.8 |

A, B, C, D - statistically significant difference at $p \leq 0.01$
a, b - statistically significant difference at $p \leq 0.05$

Tab. 3. Protein and glycogen content in the liver homogenates of rats (n = 6)

| Item (mg/g wet tissue) | Feeding groups | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Protein | 112.60±20.75 | 99.10±19.60 | 153.15±24.45 | 94.60±19.70 | 135.15±21.35 |
| Glycogen | 2.40±0.25 | 2.30±0.29 | 2.50±0.27 | 2.80±0.31 | 3.80±0.83 |

to 2.8 times. This value increased 5.2 when 20% of raw flat pea was administered and to 5.9 and 7.2 respectively, when 20% and 100% of extruded seed were administered. All these values when compared with mean control value were significant as well as differences between group II and III and between group IV and V (Tab. 2).

The activity of isocitrate dehydrogenase (ICDH) was lower in the rats of group II by 16.3% and in group III by 37.2%. These lower values of activity were statistically significant at $p \leq 0,05$ respectively. The rats of group IV fed on diet with 20% of extruded flat pea did not show differences in the activity of ICDH with values of control rats.The rats fed on 100% of extruded flat pea showed again a significantly lower activity of ICDH by 23.2% in comparison to control rats (Tab. 2).

The activity of the sorbitol dehydrogenase (SDH) in liver homogenates of the control rats amounted to 5.75 U/mg of protein and was lower by 19% in the rats of group II fed on 20% of ground raw flat pea seeds (Tab. 2). Rats fed on 100% of extruded flat pea seeds showed as well lower values by 20% but both were statistically not significant (Tab. 2). The rats from group II and IV showed a tendency for higher values in the activity of this enzyme but without statistical significance (Tab. 2).

The activity of glutamate dehydrogenase (GLDH) in the control group was 57.73 mU/mg of protein and in all the experimental groups 65.48 mU/mg of protein. The differences of about 20% proved to be statistically irrelevent.

The activity of lactate dehydrogenase (LDH) amounted in the rats of control group 91.0 mU/mg of protein and 98.1 mU/mg of protein for rats of all experimental groups without being statistically significant (Tab. 2).

There was no statistically significant differences in the activities of the alkaline phosphatase (AP) and of transaminases between the control and experimental groups. The mean alkaline phosphatase activity of the control group was 3.78 U/mg of protein and of experimental groups 4.25 U/mg of protein (Tab. 2). The mean alanine aminotransferase (AlAT) activity in the rats of control group was 309.5 mU/mg of protein and for rats of experimental groups 299.8 mU/mg of protein. The

mean values of aspartate aminotransferase activity of the same control and experimental groups were 130.3 mU/mg and 150.0 mU/mg respectively (Tab. 2). The glycogen content in the liver of rats from group V which were fed exclusively on extruded flat pea seeds amounted to 3.8 mg/g of tisue and in the rats of the whole rest groups 2.5 mg/g of tissue (Tab. 3).

The excessive consumption of flat pea seeds has long been associated in animals and humans with the disease lathyrism. Recent studies have shown the presence of two factors in flat pea seeds which possess neuropharmacological and pathological action. One of these factors is the β-N-oxalyl-amino-L-alanine (BOAA) and the other β-aminopropionitrile (BAPN) (11, 14). The mechanism of biochemical action of β-aminopropionitrile (BAPN) occurred to be connected with specific inhibition of lysyl oxidase, the enzyme responsible for forming of cross-connections between fibres of collagen and elastine (14).

The role of β-N-oxalyl-amino-L-alanine (BOAA) is up to now unknown, but its causative involvement in acidosis was described (11).

The results of our observations have showed that the substances in the flat pea seeds impact the enzymes of the liver when administered to the diet and fed on rats for four weeks. Experimental diets supplemented with raw and extruded flat pea seeds significantly affected the activity of γ-glutamyltransferase, the enzyme of endoplasmatic reticulum, which is known to be a very sensitive factor of functional state of the liver. This enzyme is connected in some way with the detoxified system of cytochrome P-450 (5). Some of the substances or even known lathyrogens affect stimulation of this system, which was clearly presented in our work.

At the same time the groups of rats fed on the diets supplemented with raw flat pea seeds showed the lower activity of mitochondria isocitrate dehydrogenase, which belongs to mitochondrial type of enzymes. The highest supplementation of the diet with raw flat pea seeds (20%) affected significantly sorbitol dehydrogenase which belongs to cytoplasmatic enzymes. Extrusion of flat pea seeds affected evidently the influence of these antinutritional factors and substances on the analysed enzymes of the liver. Activity of the isocitrate dehydrogenase was in the range of control values when flat pea seeds were extruded before being suplemented to the diet of experimental rats.

## CONCLUSIONS

1. The results of these experiments do not present harmful changes in enzymatic profile of liver and suggest the possibility of supplementation into diet of flat pea seeds in rats.

2. Higher supplementation of the diet with flat pea seeds then 10% should be connected with extrusion because of affecting the enzymes of the liver in rats.

3. The most prominent increase of γ-glutamyltransferase connected with an increased supplementation of flat pea seeds to the diets is intriguing and up to now not clear and it needs a further study.

## REFERENCES


1. Briggs C., Parreno N., Campbell C.: Phytochemical assessment of *Lathyrus* species for the neurotoxic agent, β-N-oxalyl-L-α-β-diaminopropionic acid. Plant. Med. **47**, 188, 1983.
2. Carrol N., Longley R., Roe J.: The determination of glycogen in liver and muscle by use of antrone reagent. J.Biol. Chem. **220**, 283, 1956.
3. Chandra P., Appel W.: Metody badawcze stosowane w biologii molekularnej. PWN, Warszawa 1978, pp. 76-77.
4. Grela E.: Skład chemiczny i użyteczność pokarmowa nasion lędźwianu. Przegląd Hodowlany. **62**, 9, 1994.
5. Hanke J., Lutz W.: Biochemia, toksykologia i diagnostyka laboratoryjna wątroby. IMP im. J. Nofera, Łódź 1986, pp. 195-197.
6. Homolka J.: Biochemia kliniczna. PZWL, Warszawa 1971, pp. 512-514, pp. 520-522.
7. Kay D.: Crop and Product Digest. Food Legumes. Tropical Product Institute, London 1976, No 3, p.115.
8. Kokot F.: Metody badań laboratoryjnych stosowane w klinice. PZWL, Warszawa 1969, pp. 342-343.
9. Mohan V., Nagarajan V., Gopalan C.: Simple practical procedures for the removal of toxic factors in *Lathyrus sativus* (Khesari dhal). Ind. Jour. Med. Res. **54**, 410, 1966.
10. Ostrowski W.: Wybrane metody z chemii klinicznej. PZWL, Warszawa 1974, pp. 246-253.
11. Rao S., Sarma P.:Neurotoxic action of β-N-oxalyl-L-α, β-diaminopropionic acid. Biochem. Pharmac. **16**, 218, 1967.
12. Rotter R., Marquardt R., Campbell C.: The nutritional value of low lathyrogenic lathyrus *(Lathyrus sativus)* for growing chicks. Can. J.Anim.Sci. **70**, 739, 1990.
13. Rotter R, Marquardt R., Low R., Briggs C.: Infuence of autoclaving on the effects of *Lathyrus sativus* fed to chicks. Can. J. Anim. Sci. **70**, 739, 1990.
14. Roy D., Bhat R.: Variation in neurotoxin, tripsin inhibitors and susceptibility to insect attack in varietes of *Lathyrus sativus* seeds. Envir. Physiol. Biochem. **5**, 172, 1975.
15. Szczeklik E.: Enzymologia kliniczna. PZWL, Warszawa 1974, pp. 224-225, pp. 239-240, pp.255-256.
16. Tekle-Haimanot R., Abegaz B., Wuhib E., Kassina A., Kidane Y, Kebede N., Alemu T., Spencer P.: Pattern of *Lathyrus sativus* (grass pea) consumption and beta-N-oxalyl-α-β-diaminoproprionic acid ( β-ODAP) content of food samples in the lathyrism endemic region of Northwest Ethiopia. Nutrition Research **13**, 1113, 1993.


## STRESZCZENIE

Badania przeprowadzono na 30 szczurach rasy Wistar podzielonych losowo na pięć grup po 6 sztuk. Grupa I – kontrolna otrzymywała paszę standardową, grupa II paszę z udziałem 10% surowej śruty z nasion lędźwianu siewnego, III grupa paszę z udziałem 20% surowej śruty z nasion lędźwianu, zaś IV paszę z udziałem 20% ekstrudowanej śruty z nasion lędźwianu, a w V zastosowano jedynie ekstrudowaną śrutę lędźwianową z 1% dodatkiem Polfamiksu LSM. Po 4 tygodniach żywienia wszystkie szczury dekapitowano, a w homogenatach wątrobowych oznaczono: zawartość białka, aktywność fosfatazy zasadowej (AP), dehydrogenazy izocytrynianowej (ICDH), dehydrogenazy sorbitolowej (SDH), dehydrogenazy glutaminianowej (GLDH), dehydrogenazy mleczanowej (LDH),

gamma-glutamylotrans-peptydazy (γ-GT) oraz aminotransferazy alaninowej (AlAT) i asparaginianowej (AspAT). Określono także zawartość glikogenu metodą antronową.

Stwierdzono istotny ($p \leq 0,01$) wzrost aktywności gamma-glutamylotransferazy, dodatnio skorelowany z ilością dodanej do paszy śruty z nasion lędźwianu zarówno surowej, jak i i ekstrudowanej. Aktywność γ-GT wynosiła średnio: 18,65 mU/mg białka w grupie I (kontrolna), 52,67 m U/mg białka w grupie II, 96,38 mU/mg białka w III, 110,57 mU/mg białka w IV oraz 134,52 mU/mg białka w grupie V.

Aktywność dehydrogenazy izocytrynianowej szczurów grupy kontrolnej wynosiła średnio 0,43 U/mg białka i obniżała się w grupach żywionych śrutą lędźwianową. Aktywność dehydrogenazy sorbitolowej w wątrobach szczurów wynosiła średnio 5,73 U/mg białka dla grupy kontrolnej i ulegała obniżeniu o 19% w grupie III i o 24% w grupie V, zaś w grupie II oraz IV była zwiększona około 10% w stosunku do grupy kontrolnej. Wszystkie stwierdzone różnice nie zostały potwierdzone statystycznie. Nie stwierdzono również istotnych różnic w aktywności pozostałych enzymów wątrobowych.

W grupie V otrzymującej w paszy wyłącznie ekstrudowaną śrutę lędźwianową stwierdzono podwyższony poziom glikogenu (3,8 mg/g tkanki) w porównaniu do pozostałych grup szczurów (średnia 2,5 mg/g tkanki).

Brak statystycznie istotnych zmian w aktywnościach analizowanych enzymów, uznawanych jako indykatorowe dla homeostazy biochemicznej wątroby, wskazuje na możliwość stosowania nasion lędźwianu siewnego, zwłaszcza poddanych ekstruzji, jako składnika pasz w żywieniu zwierząt.