THE EFFECT OF CHEMICAL COMPOSITION OF PEA CONTAINING DIETS ON FEED PREFERENCES BY RATS

Zenon Zduńczyk, Irena Godycka-Kłos, Łucja Brzuzan, Monika Wróblewska, Przemysław Zduńczyk, Jerzy Juśkiewicz
Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, 10 Tuwima Str., PL-10-747 Olsztyn, Poland

Abstract. The present study was designed to determine the extent in which selected pea seeds constituents, including antinutritional factors, affect the free choice and diet intake by rats. In the experiment in which rats were fed free choice of pea containing diet (400 g/kg) the intake of diet depended on the cultivar inclusion. Diet intake was significantly affected by two variables: protein amount from pea seeds (negative relation) and sucrose content in diet (positive correlation). The other variables which significantly increased sensitivity of multiple regression equation were the content of phenolics, phytates or dietary fibre in diet. Activity of trypsin inhibitors or gross energy of pea seeds had no effect on diet intake.

Key words: Peas, rats, diets, digestible.

RACIONO SU ŽIRNIAIS CHEMINĖS SUDĖTIES ĮTAKA ŽIURKIŲ MITYBOS YPATUMAMS

Zenon Zduńczyk, Irena Godycka-Kłos, Łucja Brzuzan, Monika Wróblewska, Przemysław Zduńczyk, Jerzy Juśkiewicz
Gyvulių reprodukcijos ir Maisto tyrimų institutas, Lenkijos mokslų akademija, Mitybos skyrius Tuwima gt., 10-747 Olštynas, Lenkija

Santrauka. Šio tyrimo tikslas buvo nustatyti, kaip rinktinių žirnių sėklų komponentai ir antimitybiniai faktoriai veikia pašaro ėdamumą ir pasisavinimą žiurkių organizme. Eksperimento metu žiurkės buvo šeriamos pasirinktu racionu su žirniais (400 g/kg), raciono pasisavinimas priklausė nuo kultivavimo. Jis buvo ypač veikiamas dviejų kintamųjų: proteino kiekio žirnių sėklose (neigiama koreliacija) ir cukrozės kiekio racione (teigiama koreliacija). Kiti kintamieji, turėję patikimos įtakos regresijos lygčiai, buvo fenolio darinių, fitatų ir ląstelienos kiekis racione. Tripsino inhibitorių ir bendra žirnių sėklų energija pašaro pasisavinimui įtakos nedarė.

Raktažodžiai: žirniai, žiurkės, mityba, pasisavinimas.

Introduction. Peas are considered to be valuable dietary protein source for both animals and humans. On the other hand, the nutritive value of raw pea proteins is relatively low because of its poor digestibility, deficiencies in sulphur amino acid content and of antinutritional factors that might disturb metabolic processes in consuming animals and reduce their ability to effectively utilise nutrients (Le Guen et al., 1995). Results of many studies indicate that trypsin inhibitors seem to be a major factor that causes negative effects (Huisman, 1993). Trypsin inhibitors taken in greater amounts inactivate the (chymo)trypsin in the intestinal tract and disturb pancreatic enzyme secretion (Gatel and Grosjean, 1990). Pea lectins, which bind to the gut wall and may cause gut wall damage (Grant and Driessche, 1993), have a similar negative effect on the utilization of diet components. Less effect is ascribed to other pea seed components including phytates, phenolics or oligosaccharides. Their content can be changed by selection of appropriate cultivars or preparation of pea seeds (Gatel, 1994). For this reason it is needed to determine the limiting values at which particular compounds do not have a negative effect on the nutritional value and consumptive usability of new pea cultivars. The aim of this study was to determine the extent in which selected pea seed constituents, including ANFs, affect the free choice and diet intake by rats.

Material and Methods. The seeds of 9 best cropping pea *(Pisum sativum)* obtained from Polish plant breeding and acclimatization stations were studied. Chemical composition of seeds was determined by standard methods (AOAC, 1990). For ANFs determination the following methods were used: trypsin inhibitors activity according to Kakade et al (1974), phytates according to Oberleas and Harland (1986), total phenolics according to Naczk and Shahidi (1989). Oligosaccharides content (raffinose, stachyose and verbascose) as well as sucrose were determined by the HPLC method following preparation of batches according to Muzquiz et al (1992), whereas total fiber and its fractions according to Asp et al.(1983).

The seeds of the pea cultivars compared were used in the cycle of tests of free diet choice on Wistar rats. Pea seeds were size-reduced in beater mill before including them in the diets. Sieve meshes had a diameter 0.5 mm. In all diets pea meal made 400 g kg'[1] and was supplied with standard set of other components (Table 1). Protein level in diets was completed to 100 g kg"[1] with small addition of casein and identical addition of synthetic amino acids (DL-methionine 1.5, tryptophane 0.5, lysine 1.5 g/kg). The experiment was carried out on Wistar rats from Ifz:JAZ outbreed herd. The rats were housed individually in metal net cages at a room temperature 22-23°C, relative humidity of 60-70 % and 12 h lighting.

Table 1. **Composition of diets with inclusion of pea seeds of various cultivars**

Pea cultivar	Diet ingredients, g/kg				
	Pea seeds	Casein	Soy oil	Synthetic additions*	Maize starch
Ametyst	400	19	75	54.5	451.5
Diament	400	16	75	54.5	454.5
Ergo	400	12	75	54.5	458.5
Hermes	400	25	75	54.5	445.5
Karat	400	15	75	54.5	455.5
Koral	400	20	75	54.5	450.5
Rodan	400	23	75	54.5	447.5
Rubin	400	12	75	54.5	458.5
Szafir	400	15	75	54.5	455.5

*Mineral mixture (NRC, 1976) containing in 100 g: 73.5 g $CaHPO_4$; 8.10 g K_2HPO_4; 6.80 g K_2SO_4; 3.06 g NaCl; 2.10 g $CaCO_3$; 2.14 g $NaHPO_4$; 2.50 g MgO; 558 mg ferric citrate; 81 mg $ZnC0_3$; 421 mg $MnC0_3$; 33.3 mg $CuCO_3$; 0.7 mg KJ and 705 mg citric acid (30 g/kg of diet);

Vitamin mixture (AOAC 1975) containing in l g: 2 000 IU vitamin A; 200 IU vitamin D_3; 10 IU vitamin E; 0.5 mg vitamin K; 200 mg choline; 10 mg p-aminobenzoic acid; 10 mg inositol; 4 mg niacin; 4 mg calcium pantothenate; 0.8 mg riboflavin; 0.5 mg thiamin; 0.5 mg pyridoxine; 0.2 mg folic acid; 0.04 mg biotin; 0.003 mg cobalamine; sucrose (supplement to l g) (20 g/kg of diet); j,

DL-methionine – 2.5 g/kg, DL-tryptophan - 0.5 g/kg, L-lysine - 1.5 g/kg of diet.

Table 2. **Chemical composition and seeds gross energy content in various pea cultivar seeds**

Cultivar	Dry matter g/kg	Gross energy MJ/kg	Crude protein g/kg	Dietary fibrę g/kg	Sucrose g/kg	Oligosacchar ides g/kg	TIA* TIU/mg	Phytates mg/g	Phenołics mg/g
Ametyst	896.2	179.7	232.5	193.9	18.1	33.3	3.82	7.60	0.77
Diament	879.8	181.4	244.8	188.1	12.7	33.8	6.33	9.90	0.78
Ergo	894.0	184.1	250.5	187.5	18.8	40.8	7.32	7.42	0.77
Hermes	879.1	182.0	223.1	175.2	13.8	39.7	4.66	7.28	0.88
Karat	893.8	183.1	243.9	169.2	11.7	37.4	5.69	7.56	1.00
Koral	884.5	183.2	233.6	193.0	20.6	36.3	5.54	8.84	0.90
Rodan	911.8	179.2	220.9	209.9	15.2	43.8	4.58	6.32	0.99
Rubin	877.1	176.2	255.6	161.5	17.8	33.9	2.91	7.24	0.97
Szafir	877.3	186.1	247.6	163.6	18.1	32.2	4.70	8.20	1.04
Mean	888.2	181.7	239.2	182.4	16.3	36.8	5.06	7.82	0.90
SEM	67.6	2.9	12.2	16.1	3.1	3.9	1.32	1.04	0.11

*TIA - trypsin inhibitor activity expressed in trypsin inhibitor units (TIU) per gram dry matter

The experiment was made on 12 rats (body weight 98.5 ± 2.5 g) fed individually for 40 days. Within in 4 periods of 10 days, rats were given 3 of the 9 compared diets to choose. Each time a subgroup of 4 rats was given a 3-diet set changed after 10 days of experiment. This way during 4 cycles rats could choose each diet being also given two other diets with inclusion of pea seeds of the cultivars compared. Diet intake was checked daily and the order of containers with food was changed according to Dayton and Morrill (1974). Special cylindrical food pots with a diameter of 55 mm were used do reduce the spillage of a diet. The diet was covered with a ring. Its opening had a diameter 20 mm. Rats had an access to the diet only through this opening. It fell together loss of diet in a food pot. Thus, spillage from each of the different food pots was smali and when omitted it caused a smali error of the experiment. The fuli food pot was filled up every day with weighted amount of a diet, whereas twice a week it was filled wholly with fresh diet.

The results of the free diet choice can be summarized as follows: - average intake of each diet set given to 4 rats for 10 days (Table 3) and average intake of each diet by 12 rats given diets in 4 sets (Table 4);

- relative diet intake expressing the per cent difference (positive or negative) in the intake of one diet compared to average intake of two other simultaneously given diets (Tables 3 and 4).

The results were statistically handled determining simple and multiple correlations between the number of diet components from pea seeds and average diet intake.

Results and Discussion. The seeds of the pea cultivars compared slightly differed in gross energy value and a bit more in protein content (Table 2). Average total protein content (239.2±12.2 g/kg) was lower from the value accepted as mean (250 g/kg dry matter) in European pea cultivars (Gatel, 1994). The cultivars compared were characterized by a similar amino acid composition of protein, with average EAAI 69.3 ± 0.5, which was discussed in detail in a separate study (Zdunczyk et al., 1997). Much more pronounced differences, discussed in that study, were found in dietary fiber content and its fractions, sucrose and oligosaccharides as well as antinutritional substances (trypsin inhibitors, phytates, phenolics). Compared to literature data the activity of trypsin inhibitors and phytates content were higher (Gdala et al., 1992), whereas phenolics and oligosaccharides content was lower (Saini, 1989).

Table 3. **Results of preference test of diets with inclusion of seeds of various pea cultivars**

Diet set	Pea cv.	Diet intake		Diet set	Pea cv.	Diet intake	
		total* g/rat	relative** %			total*	relative**
I-1	Ametyst	36.6±13.4	-32.6	III-1	Ergo	39.8±20.0	-31.1
	Diament	60.2±19.7	+42.3		Hermes	59.6±27.3	+24.9
	Rubin	48.1±14.5	-0.7		Rubin	55.7±21.3	+12.1
I-2	Hermes	65.5±13.9	+26.8	III-2	Ametyst	75.6±17.7	+70.6
	Rodan	49.3±14.8	-17.8		Karat	40.5±12.1	-34.6
	Szafir	54.2±17.5	-5.6		Rodan	48.2±9.1	-17.1
I-3	Ergo	52.1±17.2	+15.0	III-3	Diament	20.9±11.8	-66.9
	Karat	37.4±15.5	-29.0		Koral	78.3±12.0	+113.0
	Koral	53.2±8.7	+19.0		Szafir	52.6±10.4	+6.0
II-1	Ametyst	43.5±22.7	-23.7	IV-1	Koral	53.4±20.7	+2.9
	Hermes	57.2±10.0	+14.2		Rodan	66.0±27.0	+44.7
	Koral	56.7±29.1	+12.7		Rubin	37.9±10.2	-26.6
II-2	Karat	52.2±12.5	-8.3	IV-2	Ametyst	61.8±21.0	+20.5
	Rubin	52.1±14.6	-8.5		Ergo	41.3±6.5	-32.9
	Szafir	61.7±17.7	+18.3		Szafir	61.3±35.0	+18.9
II-3	Diament	28.0±17.7	-59.1	IV-3	Diament	42.7±21.8	-12.6
	Ergo	63.6±9.1	+25.7		Hermes	52.7±20.6	+20.1
	Rodan	73.2±17.3	+59.9		Karat	45.0±15.0	-5.7

*during 10 days
**Intake of a given diet (%) in relation to average intake of two diets fed simultaneously

Table 4. **Intake of diets containing seeds of various pea cultivars** (mean values for 4 cycles of free diet choice test)

Pea cv.	Diet intake, g/rat/10 days	Relative diet intake, %
Koral	60.40±20.38	+31.5
Rodan	59.16±19.99	+ 13.0
Hermes	58.75±17.73	+21.6
Szafir	57.43±20.21	+ 9.4
Ametyst	54.35±23.26	+ 5.2
Ergo	49.18±16.20	- 8.5
Rubin	48.43±15.61	- 9.8
Karat	43.78+16.35	-28.5
Diament	37.95±22.50	-32.5

Diet intake depended both on the pea variety used in a given diet and the diet set given to choose (Table 3). Relatively high variability in the intake of particular diets could partially result from the method of diet giving. Accepted after Dayton and Morrill (1974) the principle of a daily change in the order of diet containers made rats seek for the diet preferred most likely by means of multiple trials. It can be also supposed that the differences in chemical composition of diets with inclusion of various pea cultivars were not very distinct in relation to the assumed mechanism of metabolic regulation of food intake (York, 1990). According to Naim et al (1985) rats choose a semi-synthetic diet in autoselection condition more due to sugar and fat content than sensorial factors.

From the table of intake results of all diets (Table 4) it follows that the difference between the diet taken in the greatest amount (Koral - 60.40±20.38 g) and the least amount was about 60% (Diament - 37.95±22.50 g). Statistical analysis showed that the intake of particular diets was determined by their chemical composition and mainly the amount of protein from pea seeds (negative relation) and sucrose content (positive relation). Average diet intake was expressed by the following regression equation:

$$Y = 146.24 + 4.648\ x_1\ 1.424\ x_2 \pm 4.03\ g\ (R^2 = 0.734,\ P \leq 0.05)$$

where x_1 stood for sucrose content brought by pea seeds (g/kg) and x_2 for protein content from pea seeds (g/kg). Positive correlation between the quantity of diets intake and contained in them sucrose was in accordance with the results quoted in the study of Naim et al. (1985).

Table 5. **Amount of selected components derived into the diets from pea seeds and their influence on diets intake.**

Component	Variable	Average	Range	Correlation*
Diet intake, g	(Y)	52.16±7.82	37.95 - 60.40	-
Sucrose, g/kg	(x_1)	5.79±1.08	4.18 - 7.29	0.542
Crude protein, g/kg	(x_2)	84.99+3.91	78.4 - 89.7	-0.626
TIA, TUI/mg	(x_3)	1.80+0.47	1.02 - 2.62	-0.336
Total phenols, g/kg	(x_4)	0.32+0.04	0.27 -0.37	0.316
Phytates, g/kg	(x_5)	2.77±0.35	2.31 - 3.48	-0.431
TDF, g/kg	(x_6)	64.86±6.39	56.70 - 76.50	0.253
Oligosaccharides, g/kg	(x_7)	13.11+1.54	11.33 - 16.01	0.282
Gross energy, kJ/g	(x_8)	6.45±12	6.18 - 6.58	0.230
$Y = 146.24 + 4.648\ x_1 - 1.\ 424\ x_2 \pm 4.03$				0.734^x
$Y = 146.38 + 4.505\ x_1 - 1\ .370\ x_2 - 2.20\ x_3 \pm 4.23$				0.707^x
$Y = 55.40 + 3.\ 747\ x_1 - 8.893\ x_2 \pm 6.65$				0.274
$Y = 156.33 + 4.485\ x_1 - 1.324\ x_2 - 6.379\ x_3 \pm 3.44$				0.806^{xx}
$Y = 185.38 + 5.052\ x_1 - 1.678\ x_2 - 0.308\ x_6 \pm 3.87$				0.775^{xx}
$Y = 118.96 + 4.959\ x_1 - 1.412\ x_2 + 76.437\ x_4 \pm 2.46$				0.900^{xx}
$Y = 155.94 + 4.456\ x_1 - 1.314\ x_2 - 0.550\ x_3 - 6.1\ 13\ x_5 \pm 3.82$				0.760^x
$Y = 144.73 + 5.300\ x_1 - 1.610\ x_2 + 1.41\ x_3 + 74.338\ x_4 - 0.197\ x_6 + 2.77$				0.874^x

*Correlation coefficient with diet intake; limiting value for it 0.666 ($P \leq 0.05$)
[x]Significant at $P \leq 0.05$; [xx]Significant at $P \leq 0.01$

Diet intake was not affected by trypsin inhibitors activity x_3 but was affected by the content of phenolics x_4, phytates x_5, and dietary fiber x_6. This conclusion can be drawn from the equation of multiple correlations with these variables. The diet intake determined (Y) was most accurately expressed by the following regression equation:

$$Y = 118.96 + 4.959\ x_1 - 1.412\ x_2 + 76.437\ x_4 \pm 4.03\ g\ (R^2 = 0.900,\ P \leq 0.01)$$

in which protein and sucrose content (x_1 and x_2) as well as the amount of phenolics (x_4) from pea seeds were included. Positive coefficient value at x_4 indicates that phenolics not only did not decrease but even increased the diet intake of the white-flowered cultivars studied. Determined regression coefficients indicate that along with increase in dietary fiber content there occurred a tendency to increase in diet intake. Consumed diets contained from 56.7 to 76.5 g/kg of pea dietary fiber, the amount which should not inhibit the fodder intake by rats. The same results have been indicated in other authors' contributions (Lopez-Guisa et al., 1988, Schrijver and Conrad, 1992) where the same or bigger fiber fraction in diets was used. It was found that the increase of phytates content resulted in decrease in diet intake. Possible negative effect of phytates on diets acceptance can be due to the changes in physical properties of seeds. It is accepted that interaction between phytic acid, divalent cations (Ca, Mg) and pectins can result from both hard water-impermeable seed coat and hard seed texture (Hincks et al., 1986). No effect was found of oligosaccharides content or gross energy value of pea seeds on diet intake. It was probably due to the slight differences in oligosaccharide content and caloric values of diets.

Conclusion

The results of the experiments indicate that variety diversification in the chemical composition of the seeds of white-flowered pea cultivars affected food preference of rats. Diet intake was significantly affected by two variables: protein amount from pea seeds (negative relation) and sucrose content in diet (positive correlation). The third variable highly significantly increasing sensitivity of multiple regression equation was the content of phenolics, phytates or dietary fiber in diet. Diet intake was not affected by trypsin inhibitors activity, oligosaccharides content or gross energy value of pea seeds.

References

1. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Edition 1990. Chapter 32.

2. Asp N.G., Johansson C.G., Hallmer H., Siljestrom M., Rapid enzymatic assay on insoluble and soluble dietary fiber. J. Agric. Food Chem., 1983. 31, 476-482.

3. Dayton A.D., Morrill J.Z., A model to estimate the palatability of several calf starter rations. J. Dairy Sci., 1974. 57, 482-484.

4. De Schrijver R., Conrad S., Availability of calcium, magnesium, phoshorus, iron, and zinc in rats fed oat bran containing diets. J. Agric. Food Chem., 1992. 40, 166-1171.

5. Gatel F., Protein ąuality of legume seeds for non-ruminant animals: a literature review. Anim. Feed Sci. Technol., 1994. 45, 317-348.

6. Gatel F., Grosjean F., Composition and nutritive value of peas for pigs: a review of European results. Livest. Prod. Sci., 1990. 26, 155-175.

7. Gdala J., Buraczewska L., Grala W., The chemical composition of different types and varieties of pea and the digestion of their protein in pigs. J. Anim. Feed Sci., 1992. 1, 71-79.

8. Grant G., van Driessche E., Legume lectins: physicochemical and nutritional properties. In: Recent advances of research in antinutritional factors in legume seeds. Proc. 2nd Int. Workshop on ANFs in legume seeds. Wageningen, EAAP Publication, 1993. 70, 219-233.

9. Hincks M. J., Stanley D. W., Multiple mechanism of bean hardening. J. Food. Technol., 1986. 21, 731-740.

10. Huisman J., Effect of tripsin inhibitors on monogastric animals. Grain Legumes, 1993. 1, 20-21.

11. Kakade M.L., Rackis J.J., Mac Ghee J.E., Puski C., Determination of trypsin activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem., 1974. 51, 376-382.

12. Le Guen M.P., Huisman J., Gueguen J., Beelen G., Verstegen M.W.A., Effects of a concentrate of pea antinutritional factors on pea protein digestibility in piglets. Livest. Prod. Sci., 1995. 44, 157-167.

13. Lopez-Guisa J.M., Harned M.C., Dubielzig R., Rao S.C. and Marlett J.A., Processed oat hulls as potential dietary fiber sources in rats. J. Nutr., 1988.118, 953-962

14. Muzquiz M., Rey C., Cuadrado C., Fenwick G.R., 1992. Effect of germination on the oligosaccharide content of lupin species. J. Chromatogr. 607, 349-352.

15. Naim M., Brand J.G., Kare M.R., Carpenter R.G., Energy intake, weight gain and fat deposition in rats fed flavored, nutritionally controlled diets in a multichoice ("Cafeteria") design. J. Nutr., 1985. 115, 1447-1458.

16. Oberleas D., Harland B.F., Analytical methods for phytate. In "Phytic Acid: Chemistry and Applications" (E. Graf, ed.). 1986. pp. 77-100. Pilatus, Minneapolis, Minnesota..

17. Saini H.S., Legume seed oligosaccharides. In: Recent advances of research in antinutritional factors in legume seeds. Huisman J., Van der Poel T.F.B., Liner I.E. (Eds), Pudoc Wageningen, 1989. 329-341.

18. York D.A., Metabolic regulation of food intake. Nutr. Rev., 1990. 48, 64-70.

19. Zduńczyk Z., Godycka I., Amarowicz R., Chemical composition and content of antinutritional factors in Polish cultivars f peas. Plant Food Hum. Nutr., 1997. 50, 37-45.