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Evaluation of selected mutants of grasspea (*Lathyrus sativus* L.) var. Krab as an ingredient in broiler chicken diet

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ABSTRACT

The aim of the study was to evaluate the nutritional value of *Lathyrus sativus* var. Krab in broiler chickens as well as that of 3 mutants (K-12, K-63 and K-64) developed from var. Krab by mutagenic treatment and selection towards improved agronomic traits. The proximal composition of mutants was similar to that of var. Krab with the exception of a higher protein content in K-12, and lower β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP) and tannin contents in the mutants. Trypsin inhibitor activity ranged from 57 to 68 mg/g crude protein (CP) in mutants, and equaled 72 mg/g CP in Krab variety seeds.

Four test diets composed using the basal diet and evaluated grasspea seeds at a proportion of 6:4 were used in the balance experiment; the control diet without grasspea, and 4 isonitrogenous diets containing 10% grasspea were used in the growth experiment. Apparent total tract protein digestibility and metabolizable energy in mutant K-12 equaled 72% and 9.8 MJ ME/kg DM, respectively, and were similar as in var. Krab but higher than in mutants K-63 and K-64 ($P<0.05$). In chickens fed from day 8 to 40 of age diets containing 10% grasspea, performance, tibia ash content, and tibia ultimate strength did not differ from the control group. However, birds fed the diet with var. Krab seeds had enlarged pancreases ($P<0.05$) and livers in comparison with controls; the weight of these organs was lower ($P<0.05$) in groups fed mutant seeds. Bone and joint deformations indicative of neurolathyrism were found in four birds from the group fed var. Krab, while they were not seen in birds fed the diets with seeds from mutants.

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The results indicate that mutagenic treatment of *Lathyrus sativus* can have a positive effect on the nutritional quality of seeds.

KEY WORDS: *Lathyrus sativus*, mutants, metabolizable energy, trypsin inhibitors, performance, broiler chickens

INTRODUCTION

Grasspea (*Lathyrus sativus* L.), also known under other common names (flat pea, everlasting pea, chickling pea, chickling vetch, khesari), is a very important source of protein grown as a pulse crop in some warm temperate and subtropical areas (Smartt et al., 1994; Chowdhury et al., 2005). It was already in use in Neolithic times and presently is considered a model crop for sustainable agriculture, as it may be grown on non-irrigated marginal land and is more resistant than other pulses to drought and floods, as well as to insect attack (Vaz Patto et al., 2006). The main constraint of grasspea is the presence of neurotoxins, the most abundant of which is β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP), responsible for the irreversible, crippling disease neurolathyrism in humans after prolonged consumption (Briggs et al., 1983; Hanbury et al., 2000).

In a comprehensive review Hanbury et al. (2000) found that the proximal composition of grasspea is similar to field pea and faba beans, with a protein content ranging from 26 to 36%, low fat and high starch contents. Its amino acid profile is similar to other grain legumes, being low in methionine and cystine but rich in lysine (the tryptophan content was not given). Most of the antinutritional factors (ANFs), i.e. trypsin and chymotrypsin inhibitors, amylase inhibitor, tannins, phytates, lectins and oligosaccharides are in quantities similar to those found in other common grain legumes and are unlikely to cause any serious concerns when grasspea is used in animal feeds. The effect of grasspea ANFs on animal performance is still not well understood and is sometimes confounded by ODAP effects.

It seems that the effect of grasspea on animal performance depends mainly on the species and the type of digestive system of the animal. Hanbury et al. (2000) found that horses are susceptible to lathyrism, while sheep and other ruminants can tolerate high levels of grasspea with no reduction in performance, as ODAP and probably other ANFs are broken down in the rumen. Castell et al. (1994) reported that in pigs, an increased inclusion rate of grasspea reduced voluntary feed intake and performance and increased liver and kidney weights. The inclusion level of grasspea had a much greater effect than its ODAP concentration, as no evidence of neurolathyrism could be seen.

Information on the metabolizable energy value and effects of feeding grasspea in poultry are scarce. Latif et al. (1976) reported that the AME value of *L. sativus* for poultry was 11.3 MJ/kg DM, while Chowdhury et al. (2005) estimated it at 12 MJ/kg. Low et al. (1990) did not find any symptoms of lathyrism in chickens fed a diet with 82% of *L. sativus*, while Rotter et al. (1991) reported that performance of broiler chickens decreased with increased *L. sativus* levels in the diet.

The variation existent in the *Lathyrus* genus along with modern breeding techniques are providing considerable potential for rapid improvement of *Lathyrus* varieties, while their drought and salt tolerance and resistance to biotic stress are valuable traits that can be used for the improvement of related legume species (Vaz Patto et al., 2006). At the Institute of Plant Genetics, Polish Academy of Sciences, mutation breeding was applied to create additional genetic variability that may be used in the development of cultivars with improved specific agronomic traits, such as better lodging resistance due to change in plant habit and higher seed yield (Rybiński, 2003). It may be hypothesized that mutagenesis also can have a positive or adverse effect on chemical composition and ANFs content of seeds and on utilization of seeds for animal consumption.

The aim of the study was to evaluate the nutritional value for broiler chickens of selected *Lathyrus sativus* mutants developed by mutagenic treatment of Krab variety seeds.

MATERIAL AND METHODS

Materials

The seeds of grasspea (*Lathyrus sativus* L.) var. Krab and 3 mutants developed at the Institute of Plant Genetics, Polish Academy of Sciences, Poznań were grown under the same environmental conditions and were harvested in 2004. The initial material for mutagenic treatments constituted seeds of the Polish variety, Krab. As a result of seed irradiation with helium-neon laser light at a dose of 30 min and mutation induction through the use of two chemomutagenes, sodium azide (NaN_3) and N-nitroso-N-methyl urea (MNU), a selection of mutants towards the improvement of agronomic traits such as better lodging resistance due to change of plant habit from prostrate to semi-erect, more seeds per pod, higher seed yield was performed in generation M_2 (Rybiński, 2003). In generation M_3, the progeny of selected forms was sown together with the initial variety in order to verify the changes observed in generation M_2. All mutants, as well as variety Krab, had white flowers and cream-coloured seeds. Three mutants (K-63, K-64 and K-12) with an average seed yield of 3.09, 3.12 and 2.93 t./ha, respectively, as compared to yield of Krab variety 2.66 t./ha were selected. After multiplication, the seeds of these mutants as well of their initial variety, Krab, were used in experimental diets.

Diets

In Experiment 1 (digestibility trial) a basal diet (B) containing no grasspea served as the reference (Table 1). Four test diets (T) were composed of diet B mixed with grasspea seeds at a proportion of 6:4 on a DM basis. The reference and test diets were mixed with 3 g Cr_2O_3 per kg DM as a marker. In Experiment 2 (growth trial), the control diet did not contain grasspea, while experimental diets contained 100 g/kg grasspea var. Krab or 3 selected mutants (Table 1). Seeds were ground and analysed prior to inclusion into experimental diets. All diets were cold pelleted on CL-2 CPM Laboratory Pellet Mill.

Table 1. Chemical composition of grasspea var. Krab and selected mutants

Component	Krab var.	Grasspea mutants		
		K-63	K-64	K-12
Dry matter, g/kg	879	873	874	876
Crude protein, g/kg DM	242	268	269	296
Crude ash, g/kg DM	30.7	33.2	30.3	31.5
Crude fat, g/kg DM	7.0	6.1	6.9	6.4
Crude fibre, g/kg DM	65.6	67.3	68.0	59.7
N-free extractives, g/kg DM	655	625	626	606
Acid-detergent fibre, g/kg DM	87.6	95.7	92.1	86.7
Neutral-detergent fibre, g/kg DM	332	348	344	332
Starch, g/kg DM	412	419	440	411
Soluble sugars, g/kg DM	55.1	62.4	49.9	54.1
P total, g/kg DM	4.09	4.38	3.87	4.64
P phytic, g/kg DM	2.32	2.70	2.38	2.76
Gross energy, MJ/kg DM	19.2	19.2	19.4	19.5
TIA[2], mg/g DM	17.4	15.4	16.1	20.0
TIA,[2a] , mg/g crude protein	71.9	57.5	59.8	67.6
B-ODAP[1], mg/kg DM	152	127	131	100
Tannins, g/kg DM	3.8	3.3	2.8	2.9

[1] β-oxalyl-diaminopropionic acid; [2]trypsin inhibitor activity expressed as mg pure trypsin inhibited per gram dry matter of grains or [2a] per gram crude protein of grains

Chickens and management

One-day-old female Cobb 500 broiler chickens were purchased from a commercial hatchery. They were housed in electrically heated battery cages and provided with continuous light and free access to feed and water. The chickens were fed on a starter-type control diet containing no grasspea (Table 1) until the beginning of experiments. Two experiments were performed, both were conducted in compliance with the European Union regulations concerning the protection of experimental animals. The Local Ethics Committee approved the study protocol.

Experiment 1

In the digestibility trial, forty 3-week-old chickens with a mean initial body weight of 918 g were used. Birds were placed in individual balance cages. They were randomly allocated to 5 groups, 8 birds per group, and given 90 g/bird/day of the respective balance diets (Table 2) in three meals. After two days of preliminary feeding the birds were fasted for 14 h, then given the same diets for 3 days and again fasted for 14 h. Feed intake was recorded daily, while during the last 86 h of the experiment, excreta were quantitatively collected, frozen and kept at -18°C until further analysis.

Table 2. Amino acid composition of grasspea var. Krab and selected mutants, g/16 g N

Amino acid	Krab var.	Grasspea mutants		
		K-63	K-64	K-12
Lysine	6.02	5.89	6.00	5.82
Methionine	0.85	0.83	0.80	0.80
Cystine	1.72	1.56	1.51	1.59
Threonine	3.42	3.39	3.28	3.34
Tryptophan	0.89	0.89	0.89	0.88
Valine	4.16	4.16	3.94	4.07
Isoleucine	3.73	3.63	3.36	3.69
Leucine	5.93	5.93	5.75	5.92
Phenylalanine	3.78	3.64	3.72	3.79
Tyrosine	2.86	2.80	2.65	3.03
Histidine	2.39	2.38	2.01	2.35
Arginine	6.95	7.06	7.16	7.35
Aspartic acid	9.92	9.80	9.96	9.90
Glutamic acid	15.65	15.89	15.84	15.66
Serine	4.15	4.18	4.01	4.19
Proline	4.85	4.83	4.08	4.61
Glycine	3.69	3.65	3.59	3.56
Alanine	3.85	3.86	3.72	3.72

Experiment 2

Sixty 7-day-old chickens with a mean initial body weight of 173 g were randomly allocated into 5 groups, 12 birds per group and placed in individual cages. Experimental diets (Table 1) were provided *ad libitum:* starter diets between days 7 and 35 of age, finisher, diets between days 35 and 40 of age. Body weight and feed intake were measured in weekly intervals after 4 h feed deprivation. After conclusion of the growth trial, the chickens received the same diets *ad libitum*, on the next day they were weighed and sacrificed by cervical dislocation, the abdominal cavity was opened and the liver, pancreas, kidneys and abdominal fat were excised and weighed. The contents of the ileum and caeca were collected and pooled by segment for every 2 birds. After emptying, the caeca were weighed. Digesta from caeca were mixed with deionized water (1:1 w/w). Ileal and caecal digesta were centrifuged at 10.000 *g* for 10 min using a Beckman centrifuge (model J2-21 with J-20 rotor) at 4°C and the viscosity of the supernatant (0.5 ml aliquot) was immediately measured with the use of a Brookfield Digital cone/plate viscometer (model LVDV II+, Brookfield Engineering Laboratories, Stoughton, MA, USA). Readings were expressed in centipoise (1cP=1MPa·s). The right tibias were collected, cleaned of all exterior tissue and frozen at -18°C until analysis.

Measurements of tibia strength and tibia ash content

Tibias were weighed and analysed for strength by the shear force measurement with a Texture Analyzer TA-XT2i (Stable Micro Systems). After the shear test, the tibias were dried, crushed and defatted in refluxing ethyl ether in a Soxlet apparatus for 48 h. The defatted tibias were oven dried and ashed in ceramic crucibles for 24 h at 600°C. Ash content was expressed as a percentage of the fat-free, moisture-free tibia weight.

Chemical analysis

Prior to analysis the excreta were dried for 12 h at 60°C, kept open for 48 h and ground to pass a 1 mm sieve. The chemical composition of grasspea seeds, diets and excreta was determined according to AOAC (1990). Crude fat was determined by ether extraction, phytate P was measured according to Tangkongchitr et al. (1981), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined with a Fibertec System M (Tecator) according to Van Soest and Wine (1967) and Van Soest (1973). ODAP content was determined according to Briggs et al. (1983). Trypsin inhibitor activity was analysed according to Kakade et al. (1974) and expressed as mg pure trypsin inhibited per gram dry matter of product. Tannin content was measured according to the method of Tyczkowska (1977). Amino acid analyses were performed with an automatic Beckman 6300 HP Amino Acid Analyzer after acid hydrolysis, methionine and cystine after oxidation with performic acid, and tryptophan after hydrolysis with BaOH. Faecal N in excreta was determined according to Ekman et al. (1949), chromic oxide in diets and excreta was estimated spectrophotometrically following wet ashing according to Hinsberg et al. (1953). Gross energy was measured on a Parr adiabatic oxygen bomb calorimeter KL-10.

Calculations and statistics

In Experiment 1 the total tract apparent digestibility (CTTAD) of dietary protein, nitrogen and organic matter retention and apparent metabolizable energy (AME_N) value of diets were calculated relative to the ratio of Cr_2O_3 to the content of the nutrient in question or gross energy in feed and excreta. AME_N was corrected to zero nitrogen balance using 34.96 kJ/g N retained. Respective values for grasspea (GP) seeds were calculated by assuming additivity of the values of the basal (B) and the test (T) diets, from the formula: AME_N GP = AME_N B - (1-X) AME_N T/X, where X = the level of inclusion of GP in the T diet. In experiment 2 the body weight gain (BWG) and feed conversion ratio (FCR) were calculated for a period from 8 to 40 days of age, the weight of organs was calculated relative to live body weight before slaughter.

The results were subjected to one-way analysis of variance (ANOVA) generated by Statgraphics ® ver. 5.1 software (SAS, 1994-2001).

RESULTS

The weight of 1000 seeds of mutants K-63 and K-64 was about 200 g, var. Krab 160 g, while of mutant K-12 145 g. The proximal composition of mutant seeds was similar as in var. Krab, with the exception of a higher protein content in K-12. The ODAP and tannin contents as well as trypsin inhibitor activity (expressed per g crude protein) were lower in the mutant than in Krab seeds (Table 3). The protein composition of mutants K-63 and K-12 was similar, while K-64 contained less isoleucine, leucine, tyrosine, histidine and proline than var. Krab seeds. Krab and mutant seeds were equally low in sulphur-containing amino acids, especially methionine (Table 2).

In the digestibility trial, chickens ate the basal diet and test diets containing 40% grasspea equally well. Nitrogen retention was between 10 and 14% in all evaluated grasspea seeds. Organic matter retention was significantly ($P<0.05$) higher in K-12 than in var. Krab, while apparent total tract protein digestibility was higher ($P<0.05$), AME_N value and energy metabolizability were significantly higher ($P<0.01$) in K-12 than in both remaining mutants (Table 4).

Performance was similar in all experimental groups in the growth experiment (Table 5). However, one bird in group K-63 died with symptoms of ascites and eight birds were rejected. Four rejections seem not to be connected with experimental treatments (one bird in the control and one in the K-63 group were rejected due to unresorbed yolk sac, one bird in the control group had a distended crop, one bird in the Krab group had a distended gizzard), while the reason for rejections of the four remaining birds from the Krab group in the fifth week of life were bone and joint deformations,

Table 3. Ingredients and nutrient composition of diets, g/kg

Item	Exp. 1	Experiment 2 - dietary treatments									
	Basal diet	control		Krab var.		K-63 mutant		K-64 mutant		K-12 mutant	
		1-35 d	36-40 d	8-35 d	36-40 d	8-35 d	36-40 d	8-35 d	36-40 d	8-35 d	36-40 d
Ingredients											
grass pea	-	-	-	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
wheat	369.6	358.1	402.7	287.8	332.0	294.8	339.1	294.8	339.1	302.7	347.0
soyabean meal	370.0	361.0	305.8	331.3	276.5	324.3	269.4	324.3	269.4	316.4	261.5
maize	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
limestone	9.0	9.0	10.5	8.5	10.5	8.5	10.5	8.5	10.5	8.5	10.5
monocalcium phosphate	12.0	12.0	12.0	12.5	12.0	12.5	12.0	12.5	12.0	12.5	12.0
rape seed oil	30.0	50.0	60.0	50.0	60.0	50.0	60.0	50.0	60.0	50.0	60.0
L-lysine, 78%	0.4	0.7	-	0.7	-	0.7	-	0.7	-	0.7	-
DL-methionine, 98%	1.0	1.2	1.0	1.2	1.0	1.2	1.0	1.2	1.0	1.2	1.0
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Premix[1,2]	5.0[1]	5.0[1]	5.0[2]	5.0[1]	5.0[2]	5.0[1]	5.0[2]	5.0[1]	5.0[2]	5.0[1]	5.0[2]
Calculated											
crude protein	223.7	218.4	200.0	218.4	200.0	218.4	200.0	218.4	200.0	218.4	200.0
Lys	12.34	12.04	10.19	12.28	10.41	12.18	10.37	12.22	10.40	12.16	10.31
Met + Cys	8.16	8.18	7.51	8.03	7.37	7.99	7.32	7.98	7.30	8.00	7.32
Thr	8.24	8.03	7.28	8.03	7.25	8.08	7.23	7.97	7.20	7.95	7.20
Trp	2.68	2.61	2.36	2.54	2.30	2.56	2.29	2.56	2.29	2.52	2.27

[1] supplied per kg diet, IU: vit. A 13500 , vit. D_3 3500; mg: vit. E 40, vit. B_1 3, vit. B_2 8, biotin 0.1, vit. B_6 5, vit. B_{12} 0.03, vit. K 4, niacine 60, folic acid 1.75, Ca pantothenate 15, choline 175, Mg 100, Mn 80, Zn 70, Co 0.3, Se 0.2, Cu 15, Fe 70,I 1, salinomycin 60, Barox Dry 10, flavomycin 7, Ronozyme P (xylanase) 300, Rovabio AP (phytase) 50; g: Ca 1.885

[2] supplied per kg diet, IU: vit. A 11000, vit. D_3 3000; mg: vit. E 35, vit. B_1 1.5, vit. B_2 6, biotin 0.05, vit. B_6 3, vit. B_{12} 0.02, vit. K 2, niacine 40, folic acid 1.25, Ca pantothenate 10, choline 400, Mg 100, Mn 70, Zn 50, Co 0.3, Se 0.2, Cu 10, Fe 60, I 1, Barox Dry 10; g: Ca 1.29

Table 4. Nutrients digestibility and metabolizable energy value of grasspea var. Krab and selected mutants in 3 week-old chickens (Experiment 1)

Item	Krab var.	Grasspea mutants			SEM
		K-63	K-64	K-12	
Crude protein digestibility, % [1]	71.6^{bc}	67.8^{a}	68.6^{ab}	71.8^{c}	1.06
Nitrogen retention, %[1]	13.7	9.97	10.7	13.4	2.36
Organic matter retention, %[1]	47.4^{a}	49.4^{ab}	49.8^{ab}	54.8^{b}	2.11
$AME_{N,}$ MJ/kg DM	9.14^{bAB}	7.63^{aA}	7.25^{aA}	9.79^{bB}	0.31
AME_N/GE, %	47.6^{bAB}	39.7^{aAB}	37.4^{aA}	50.2^{bB}	1.62

^{ab,AB} means in a column with different superscripts are significantly different: ^{ab} P<0.05, ^{AB} P<0.01

[1] total tract apparent digestibility or retention; AME_{N} - apparent metabolizable energy corrected to zero nitrogen balance; AME_N/GE - gross energy metabolizability

Table 5. Performance of broilers, 8-40 day of age (Experiment 2)

Item	Dietary treatment					SEM
	control	Krab var.	grasspea mutants			
			K-63	K-64	K-12	
Feed intake, kg	3.82	3.98	3.88	3.87	3.77	0.07
BWG, kg	2.24	2.30	2.28	2.21	2.18	0.05
FCR, kg feed/kg BWG	1.71	1.73	1.70	1.75	1.74	0.04
Birds rejected, n	2	5	2	0	0	

all differences insignificant

Table 6. Organs weight, relative to the live body weight before slaughter (LBW), viscosity of ileal and caecal digesta and tibia parameters in 41-day old broilers (Experiment 2)

Item	Dietary treatment					SEM
	control	Krab var.	grasspea mutants			
			K-63	K-64	K-12	
Liver, g/100 g LWB	2.31^{bc}	2.50^{c}	2.13^{ab}	1.97^{a}	1.97^{a}	0.11
Pancreas, mg/100 g LWB	159^{a}	212^{d}	197^{cd}	183^{bc}	179^{b}	6.6
Kidneys, mg/100 g LWB	740	796	835	797	749	33
Abdominal fat, g/100 g LWB	1.31	1.29	1.38	1.41	1.17	0.13
Empty caeca, mg/100 g LWB	384^{a}	370^{ab}	356^{ab}	364^{ab}	326^{b}	19
Ileal digesta viscosity, mPas·s	1.99^{ab}	1.64^{a}	2.15^{b}	2.01^{ab}	2.30^{b}	0.13
Caecal digesta viscosity, mPas·s[1]	1.24	1.63	1.81	1.71	1.84	0.35
Tibia ash, % [2]	38.2^{ab}	39.5^{b}	39.6^{b}	39.8^{b}	37.2^{a}	0.78
Tibia ultimate strength, N/100 g	17.1	15.0	15.4	16.8	14.9	0.93

[1]caecal digesta were mixed with distilled water 1:1

[2] on fat-free, moisture-free basis

^{ab} means in a column with different superscripts are significantly different at P<0.05

which made it impossible for the birds to walk. The health of all broilers fed diets with K-64 and K-12 mutants and the remaining birds in the other groups was satisfactory, there were no signs of bone deformations. Tibia weight and tibia ultimate strength did not differ significantly between groups, while tibia ash content was lowest (P<0.05) in group K-12 (Table 6).

The relative mass of the pancreas in all birds fed the diets with grasspea was significantly higher than in control birds (P<0.05), the largest pancreases were in the group fed the diet with var. Krab, followed by mutants K-63, K-64, and K-12. The relative weight of the liver was lower in groups fed diets with mutants K-64 and K-12 than in the control and Krab groups. The viscosity of ileal and caecal digesta in birds fed grasspea diets was not significantly different from the control group, but in groups provided mutants K-63 and K-12, ileal viscosity was higher than in the group fed with var. Krab (Table 6).

DISCUSSION

The proximal composition and ANFs content of grasspea evaluated in the present study was within the range of data found in the literature and reported by Hanbury et al. (2000) in their comprehensive review of the potential of *Lathyrus sativus* grain for use as animal feed, while the ODAP content was rather at the lowest limits reported in the study. Similarly low ODAP contents in grasspea grown in Poland were reported previously by Grela and Winiarska (1998). Amino acid composition was within the range of values reported by Low et al. (1990) and Hanbury et al. (2000), the tryptophan content was 0.89 g/16 g N. Hanbury et al. (2000) concluded that the protein content in grasspea was generally higher than in field pea and faba bean, while amino acid profiles were similar. For monogastric species, these legumes are deficient in methionine and cystine, but are rich in lysine. In poultry diets, grain legumes complement cereals, which have higher levels of methionine and cystine but lower levels of lysine, so on the basis of protein composition grasspea can have similar applications as other legumes.

In the current study, protein digestibility in all groups fed diets containing 40% grasspea, as well as nitrogen retention were lower than in the group fed a reference diet, resulting in an apparent crude protein digestibility of grasspea of 70% and apparent nitrogen retention of 12%, on average. The protein digestibility measured for pea varieties (*Pisum sativum*) grown in Poland, evaluated in the same laboratory a few years ago, was from 75 to 88% (Smulikowska et al., 2001). The very low nitrogen retention was caused by a very low methionine content.

In monogastric animals the digestibility of legume protein depends mainly on the content of trypsin inhibitors and tannins (Huisman and Jansman, 1991; Hanbury et al., 2000). In the current study, trypsin inhibitor activity (TIA) was, on average, 17 mg/g DM (64 mg/g protein) in the evaluated grasspea mutants, while in peas evaluated previously (Smulikowska et al., 2001) it was about 1 mg/g DM (4.5 mg/g protein). Tannin content (3.2 g/kg DM, on average) in grasspeas was higher than in white-flowered peas (0.25 g/kg DM, on average) but was much lower than in coloured-flowered peas (8.7 g/kg DM, on average). It seems that low protein digestibility and low protein retention measured in grasspeas in the current study depended mainly on very high trypsin inhibitor activity, comparable to the level in raw soya beans, which is from 50 to 85 mg/g protein (Huisman and Jansman, 1991).

The metabolizable energy of evaluated grasspea seeds was from 7.2 to 9.8 MJ/kg DM. It is much lower than 12.5 MJ/kg DM, on average, reported for pea varieties (Smulikowska et al., 2001). It seems that the values 11.3 MJ ME/kg of *L. sativus* for poultry estimated by Latif et al. (1976) and Hanbury et al. (2000), as well as 12 MJ TME/kg estimated by Chowdhury et al. (2005) might be too high.

Data concerning the feeding value of grasspea for broilers is scarce. Sharma et al. (2003) included *Lathyrus* grains grown in India at a level of 111 or 222 g per kg diet, from day 7 of age for a period of 4 weeks. Protein digestibility of *Lathyrus*-containing diets was lower than in the control diet and some birds given the diet with higher level of *Lathyrus* showed ruffled feathers, stunted growth and abnormal gait in comparison with the chickens fed the control diet. Low et al. (1990) and Rotter et al. (1991) using *Lathyrus* seeds of low or medium ODAP content grown in Canada at a level 800 g/kg or 200, 400, 600 and 800 g/kg diet reported depressed performance and dry matter digestibility only at the higher levels of inclusion, but none of the classical symptoms of lathyrism or increased mortality.

In the current study, grasspea seeds were included at a level of 100 g/kg in practical diets balanced in terms of amino acid and energy contents. Neither the seeds of var. Krab nor the mutants affected performance parameters, while bone mineralization was worse in chickens fed the diet with mutant K-12 seeds in comparison with var. Krab or mutants K-63 and K-64. Bone and joint deformations indicative of neurolathyrism were observed in four birds from the group fed var. Krab, whereas they were not seen in birds fed diets with seeds from mutants.

In the current study, higher pancreas weights in chickens fed diets with grasspea than in the control group pointed to the negative effect of an increased level of trypsin inhibitors and tannins. The relative weight of the pancreas was highest in the group fed the diet with Krab seeds, lowest in the group fed the diet with mutant K-12 seeds. Ingested trypsin inhibitors and tannins reduce the level of active trypsin in the gut, which is followed by enhanced cholecystokinin-pancreozymin hormone production that stimulates the acinar cells of the pancreas to produce more digestive enzymes. The final effect is hypertrophy and hyperplasia of the pancreas (Huisman and Jansman, 1991). Improvement of the nutritive value of grasspea for monogastric animals can be obtained after thermic treatment, such as extrusion cooking (Grela and Winiarska, 1998). The ileal viscosity measured in chickens fed diets with evaluated grasspea seeds was low, comparable to the control group and similar to that measured in chickens fed diets with pea (Smulikowska et al., 2001). This indicates that the non-starch polysaccharides of grasspea have a rather low viscosity.

In the current study, birds fed the diets with grasspea mutants had lower pancreas and liver weights, but higher ileal digesta viscosity than birds fed diets with var. Krab seeds. This points to changes in the level or physicochemical structure of some grain components due to mutagenesis.

CONCLUSIONS

The results of the current study point to the need to verify the effects of mutation breeding on the nutritional quality of seeds.

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