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The effect of feeding raw and germinated *Lupinus luteus* and *Lupinus angustifolius* seeds on the growth performance of young pigs

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ABSTRACT. Two 33-day experiments were conducted. In each experiment, fifty male pigs about 9 kg body weight were divided into five groups and fed *ad libitum* a mixture containing soya bean meal in the control group (SBM), whereas in the experimental diets, 50% (experiment I) or 75% (experiment II) of the soya protein was replaced by the protein of raw or germinated seeds of yellow (RYL and GYL, respectively) or blue (RBL and GBL) lupin. The species of lupin had a significant effect ($p < 0.05$) on feed conversion ratio (FCR) in experiment I, whereas diet and germination significantly affected feed intake in experiment II. In experiment I feed utilization per kg body weight gain was worse ($p < 0.05$) in animals fed on the GYL diet than on GBL diet. In experiment II, intake of the GBL diet was significantly lower ($p < 0.05$) than of the SBM and RYL diets. Consumption of diet GBL was also significantly lower than of diet RBL. The alkaline phosphatase (AP) concentration was significantly lower ($p < 0.05$) in the blood of animals fed the diets with germinated lupin than those containing raw lupin seeds. The concentration of AP in the blood of animals fed the RYL diet was significantly higher ($p < 0.05$) compared with the other diets. The replacement of 50% or 75% of soya bean meal protein by raw or germinated yellow and blue lupin seeds did not significantly affect performance, but the higher level of germinated blue lupin seeds negatively affected feed intake by the pigs.

Introduction

Lupin seeds are a rich source of protein, but their nutritional value in the diet for monogastric animals depends on the content of anti-nutritional factors (ANF). Oligosaccharides of the raffinose family are recognized as the main compounds causing flatulence, non-starch polysaccharides reduce energy utilization by animals, alkaloids usually reduce palatability and feed intake because of their bitter taste and are characterized as compounds with toxic effects (Pastuszewska et al., 2001; Kim et al., 2007). Germination can modify the type and quantity of nutrients in seeds, which can influence the digestibility of protein and amino acids in animals (Sanchez et al., 2005; Sangronis and Machado, 2007). Previous research has revealed an increase in the content of protein and decrease the concentrations of anti-nutritional factors in the sprouts of yellow and blue lupin compared with raw seeds, however, germination negatively influenced standardized ileal digestibility of lysine and methionine in pigs, but improved the digestibility of crude protein (Chilomer et al., 2010, 2012). On the other hand, the palatability of sprouts may be a major problem in their practical use in feeding. Rubio et al. (2002) and Urbano et al. (2005) found similar consumption of germinated and raw legume seeds in rats, but the palatability of lupin sprouts for pigs is not recognized well. The amount of raw lupin seeds must be limited in diets for pigs (Kim et al., 2007). There is no information in the literature about the possibility of using germinated lupin seeds in animal diets as a replacement for soya bean meal. It is hypothesized that germination of lupin seeds could improve their palatability and utilization, which may positively affect the performance of growing pigs. The demonstrated lower digestibility of lysine and methionine may be compensated by a higher proportion of protein and amino acids in dry matter of the products (Chilomer et al., 2012). The observed decrease in the content of alkaloids and oligosaccharides can improve palatability and feed intake and improve the welfare of animals in which the consumption of oligosaccharides is the cause of diarrhoea, bloating and gas production. The aim of the study was to assess: 1) the possibility of using different levels of raw and germinated seeds of two lupin species as a replacement for soya bean meal in diets for growing pigs, and 2) the effect of lupin seed germination on production results and some blood parameters.

Material and methods

Germination

Seed samples (about 200 kg) of blue lupin (*Lupinus angustifolius)* cv. Graf and yellow lupin (*Lupinus luteus)* cv. Lord were bought from the Plant Breeding Station (IHAR, Poland). Seeds of yellow lupin (YL) and blue lupin (BL) were soaked for 30 min with 2.5 g · l^{-1} sodium hypochlorite to reduce microbial degradation during germination. The seeds were then washed with distilled water to a neutral pH. After that, the seeds were germinated in the dark at 24°C for 4 days in distilled water in open containers with a free air supply and watered twice daily. Germinated seeds of yellow and blue lupin were dried in a vacuum dryer at 50°C to about 900 g · kg^{-1} dry matter. Germinated and raw seeds were ground in a laboratory mill (Retch). For the performance experiments the germinated seeds were prepared in portions of about 10 kg.

Experimental design. The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Poznań (#18/2007).

Each of the two experiments was performed on fifty crossbred pigs with initial body weight (BW) of 9.9 kg and 8.9 kg in experiments I and II, respectively. The experiments were performed in different years. The animals were divided according to body weight into five groups of 10 animals each (5 male, 5 female) and placed in individual pens in one room. Environmental conditions were in agreement with welfare requirements for pigs. The animals were fed *ad libitum* with free access to fresh water. The feed was given in dry mash form. The experiments lasted 33 days to achieve a body weight of about 30 kg.

Five complete starter diets for each experiment were formulated according to GfE (2006) recommendations using the results of our own lupin analysis and ileal digestibility coefficients of crude protein and amino acids (Chilomer et al., 2012). Other component data were taken from GfE (2006) and Degussa (2001). The chemical composition and nutritional value of the raw and germinated lupin seeds and diets are presented in Tables 1, 2 and 3.

Table 1. Chemical composition of raw and germinated lupin seeds

Nutrients	Yellow lupin seeds		Blue lupin seeds	
	raw	germinated	raw	germinated
Crude protein, g · kg^{-1} DM	437	484	340	399
Crude ash g · kg^{-1} DM	52	52	40	44
Crude fibre, g · kg^{-1} DM	162	173	164	171
Ether extract, g · kg^{-1} DM	42	33	49	31
NFE, g · kg^{-1} DM	307	258	407	353
Lysine, g · kg^{-1} protein	45.5	38.4	44.0	34.8
Methionine, g · kg^{-1} protein	5.0	3.9	5.0	4.1
Threonine, g · kg^{-1} protein	32.2	29.9	34.6	32.0
Cystine, g · kg^{-1} protein	19.4	14.1	10.4	8.3

Table 2. Oligosaccharides and alkaloid concentrations in raw and germinated lupin seeds, g · kg^{-1} DM

Parameters	Yellow lupin seeds		Blue lupin seeds	
	raw	germinated	raw	germinated
Total RFOs	130.91	21.69	80.59	7.18
Raffinose	10.91	5.09	9.95	2.11
Stachyose	74.15	15.04	55.78	4.98
Verbascose	45.85	1.55	14.85	0.09
Total alkaloids	0.10	0.07	0.15	0.10
Ammodendrine	3.66	2.59	0.34	0.32
Angustifoline	0.00	0.00	0.66	1.76
Lupanine	2.87	0.08	68.79	52.10
3OH-lupanine	0.00	0.00	0.36	5.20
13OH-lupanine	6.34	4.92	24.06	18.21
13alphatigloxylupanine	0.00	0.00	0.00	17.57
Lupinine	26.83	29.00	0.00	0.00
Sparteine	57.77	53.90	0.00	0.00
17-oxosparteine	2.53	1.40	0.00	0.00
Gramine	0.00	8.11	0.00	0.00

Table 3. Composition and nutritional value of diets

Indices	Units	SBM	Experiment I				Experiment II			
			RYL	GYL	RBL	GBL	RYL	GYL	RBL	GBL
Component										
wheat	g · kg^{-1}	498.0	453.0	453.0	453.0	453.0	450.0	450.0	450.0	450.0
barley	g · kg^{-1}	158.5	169.5	195.0	109.0	143.7	161.0	185.5	57.0	110.5
soya bean meal	g · kg^{-1}	280.0	148.0	142.0	163.0	151.0	82.5	75.0	106.5	92.0
raw yellow/blue lupin seeds	g · kg^{-1}	–	160.0	–	205.0	–	240.0	–	310.0	–
germinated yellow/blue lupin seeds	g · kg^{-1}	–	–	145.0	–	175.0	–	220.0	–	265.0
soya oil	g · kg^{-1}	25.0	30.0	25.0	30.0	37.0	26.0	26.0	34.0	40.0
calcium phosphate	g · kg^{-1}	10.0	10.0	10.0	10.0	10.0	10.0	12.0	12.0	12.0
limestone	g · kg^{-1}	14.0	14.0	14.0	14.0	14.0	15.0	15.0	14.0	13.0
NaCl	g · kg^{-1}	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Premix *	g · kg^{-1}	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-lysine (78%)	g · kg^{-1}	4.0	5.0	5.0	5.0	5.0	5.0	5.5	5.0	6.0
D, L-methionine (99%)	g · kg^{-1}	1.0	1.0	1.5	1.5	1.8	1.0	1.5	2.0	2.0
threonine (98%)	g · kg^{-1}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Nutritional value										
ME	MJ · kg^{-1}	13.4	13.4	13.4	13.3	13.3	13.3	13.3	13.3	13.3
crude protein	g · kg^{-1}	204.6	204.5	204.7	204.5	202.5	205.1	205.7	205.0	204.9
digestible lysine	g · kg^{-1}	11.5	11.7	11.1	11.7	11.0	11.4	10.9	11.4	11.1
digestible met + cys	g · kg^{-1}	6.1	6.2	6.2	6.1	6.2	6.2	6.1	6.3	6.1
digestible threonine	g · kg^{-1}	7.5	6.9	6.7	7.0	6.8	6.7	6.4	6.8	6.6
crude protein	g · kg^{-1}	224.3	224.7	220.7	215.4	216.4	213.5	218.2	224.6	213.5
lysine	g · kg^{-1}	12.4	12.4	12.2	12.4	12.4	12.2	12.7	12.8	12.6
methionine + cystine	g · kg^{-1}	7.6	7.0	7.4	7.2	7.0	7.4	7.6	7.8	7.3
threonine	g · kg^{-1}	8.2	8.2	7.8	8.0	7.6	7.6	7.6	7.6	7.8
total alkaloids	mg · kg^{-1}	–	17	10	31	18	24	15	47	27

* mineral and vitamin premix content, per 1 kg; mg: choline chloride 80000, Fe 20000, Cu 32000, Co 80, Mn 8000, Zn 28000, J 160, Se 40, antioxidants (butylated hydroxyanisole, butylated hydroxytoluene) 2 400 000; IU: vit. A 300,000; mg: vit. D_3 14,000, vit. E 300, vit. K_3 300, vit. B_1 800, vit. B_2 600, vit. B_6 5,000, pantothenic acid 4,000; nicotinic acid 400, folic acid; mcg: vit. B_{12} 2,000, biotin 20,000; g: Ca 180;
** calculated value from composition of lupin products

In both experiments, soya bean meal was the main source of protein in the control group (SBM). In the experimental groups, 50% (experiment I) and 75% (experiment II) of the soya bean meal protein was replaced by the protein of raw (RYL and RBL) or germinated (GYL and GBL) seeds of yellow or blue lupin species. The diets were isoenergetic and isonitrogenous. Diets were supplemented with crystalline lysine, methionine and threonine, according to the requirements for ileal digestible amino acids recommended by GfE (2006). Diets containing germinated seeds were supplemented with greater amounts of methionine and lysine than those with raw seeds due to the lower ileal digestibility of these amino acids and the composition of diets. Individual daily feed intake (FI) and body weight at the beginning and end of the trial were recorded; the health status of the animals was monitored. Average daily gains (ADG) and feed conversion ratio (FCR) were estimated. Samples of blood from 6 animals of each group were collected at the end of experiment II.

Chemical analysis

Diets and components were analysed in duplicate for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), ash (CA) and AA using AOAC (2005) methods. For chemical analysis, the samples were grounded to pass through a 0.5-mm sieve. N-free extractives (NFE) were calculated:

$$NFE = DM - (CP + CA + CF + EE)$$

The raffinose family of oligosaccharides was assayed according to Muzquiz et al. (1992) and alkaloids according to Muzquiz et al. (1994) in raw and germinated lupin seeds. Samples of blood from the jugular vein from 6 animals of each group were collected on the last day of experiment II into test tubes with a coagulant. Samples were centrifuged and the separated serum was frozen at –20°C and stored until analysis. The blood serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GP) were determined using kits from Pointe Scientific, Poland.

Statistical analysis

A 2 × 2 factorial analysis with a control treatment was performed (ANOVA) with germination and species and their interactions as factors. The significance of differences among means was calculated using the Duncan test at $p < 0.05$. The statistical analysis was performed using SAS 9.1 Software.

Results

The health status of the experimental animals during the period of the trial was good. The average performance results are presented in Table 4, blood parameters in Table 5. In experiment I, there were no effects of diet or germination on BW, FI, or ADG. Lupin species had a significant effect on FCR ($p < 0.05$). The animals fed on a diet containing germinated yellow lupin seeds utilized more feed ($p < 0.05$) per kg of body gain compared with animals offered the diet with germinated blue lupin seeds. In experiment II, effects of diet and germination ($p < 0.05$) were found only on FI. No species effects were observed. Feed intake of diet GBL was significantly lower ($p < 0.05$) than of diets SBM and RYL. The feed intake of diets containing germinated blue lupin seeds was significantly lower than of that with raw blue lupin seeds. There was no effect ($p > 0.05$) of diet in which 75% of soya bean meal protein was replaced by yellow or blue lupin products on the concentrations of GP, AST and ALT in the blood serum of pigs. The AP concentration was significantly lower ($p < 0.05$) in the blood of animals fed diets with germinated seeds than in those given raw lupin seeds. The blood AP concentration animals fed the RYL diet was significantly higher ($p < 0.05$) compared with the other diets.

No interaction of germination × species effect was found ($p > 0.05$) in any group.

Discussion

In the presented experiments, the same parts of lupin components (raw and germinated seeds) as in experiments published previously by Chilomer et al. (2010, 2012) were used. There was no problem with the condition or health of the animals during the experiments. It is commonly known that germination usually reduces feed palatability. The concentration of palatable sugars, especially sucrose, is strongly reduced during germination (Chilomer et al., 2010). Moreover, the alkaloids in lupin seeds are recognized as the main factor responsible for reduction of feed intake (Kim et al., 2007). Previous experiments of Chilomer et al. (2012) showed that

Table 4. Performance results of experiments I and II

Experiment	Parameter	Diets					SEM	*P* value			
		SBM	RYL	GYL	RBL	GBL		dietary effect	germination effect	species effect	interaction G × V
I	Initial BW, kg	9.9	9.8	10.1	10.0	9.8	0.1	–	–	–	–
	Final BW, kg	33.4	32.3	31.7	33.6	32.2	0.5	NS	NS	NS	NS
	FI, $g \cdot d^{-1}$	1181	1233	1163	1218	1136	15	NS	NS	NS	NS
	ADG, $g \cdot d^{-1}$	711	680	657	714	678	10	NS	NS	NS	NS
	FCR, $kg \cdot kg^{-1}$	1.67	1.84	1.79^{C}	1.71	1.68^{c}	0.02	NS	NS	0.048	NS
II	Initial BW, kg	8.9	9.0	8.9	8.9	8.9	0.1	–	–	–	–
	Final BW, kg	29.0	26.6	27.6	26.3	25.6	0.6	NS	NS	NS	NS
	FI, kg	1179^{A}	1136^{A}	1115	1027^{B}	$948^{a,b}$	24	0.045	0.015	NS	NS
	ADG, g	609	568	552	526	522	11	NS	NS	NS	NS
	FCR, $kg \cdot kg^{-1}$	1.95	2.05	2.04	1.99	1.91	0.04	NS	NS	NS	NS

BW – body weight, FI – feed intake, ADG – average daily gain, FCR – feed conversion ratio. Values in rows with letters of different size (aA – for dietary effect, bB – for germination effect, cC – for species effect) differ significantly at $p < 0.05$; NS – not significant

Table 5. Blood parameters in experiment II

Parameter	Diets					SEM	*P* value			
	SBM	RYL	GYL	RBL	GBL		dietary effect	germination effect	species effect	interaction G x V
AP, $IU \cdot l^{-1}$	232.3^{a}	$338.6^{A,B,C}$	$244.5^{a,b}$	$236.2^{a,c}$	283.3^{a}	7.2	0.001	0.006	0.006	NS
GP, $IU \cdot l^{-1}$	58.0	65.2	68.4	59.6	60.7	3.3	NS	NS	NS	NS
ALT, $IU \cdot l^{-1}$	58.7	67.2	65.1	58.5	72.9	4.1	NS	NS	NS	NS
AST, $IU \cdot l^{-1}$	75.1	72.6	62.2	75.6	81.7	4.7	NS	NS	NS	NS

Values in rows with letters of different size (aA – for dietary effect, bB – for germination effect, cC – for species effect) differ significantly at $p < 0.05$; NS – not significant after 4-day germination, the total alkaloid content in germinated seeds was reduced by 30% and 50% in the seeds of yellow and blue lupin, respectively, but this did not improve feed intake. The differences in FI were associated rather with lupin species. The tolerated level of lupins in diets for pigs is dependent both on the total amount of dietary alkaloids and their origin (Kim et al., 2007). Therefore, differences in alkaloid composition between lupin species may explain, at least in part, the variation in feed intake response. Yellow and blue lupin seeds differ significantly in the content of particular alkaloids both before and after germination (Chilomer et al., 2012). Alkaloids during germination are degraded and used as a nitrogen source in the biosynthesis of new, more bioactive compounds, such as other alkaloid types and esters (Sanchez et al., 2005). In the previous part of this study performed by Chilomer et al. (2010, 2012), higher levels of angustifoline and 3OH-lupanine and the appearance of 13α-tigloxylupanine in germinated blue lupin seeds were noted, whereas in germinated yellow lupin seeds, higher concentrations of lupinine and appearance of gramine were observed. Before, several palatability problems had been reported in rats, chickens and rainbow trout fed with diets containing gramine, sparteine and lupanine (Pastuszewska et al., 2001; Serrano et al., 2011). The main toxic effects of alkaloids result in disturbances of the central nervous system, digestive processes, and the immune system. These effects are supported by the current observations. In experiment II, pigs receiving diet RYL showed a significantly higher level of serum AP (338.6 IU $\cdot$ l^{-1}; in 5 animals the value exceeded 300 IU $\cdot$ l^{-1}), while the reference level for pigs is 92-294 IU $\cdot$ l^{-1}. Generally, a high level of AP in the blood indicates biliary obstruction and may be a symptom of liver cancer. The liver is the main organ involved in the detoxification and degradation of toxic substances, thus, it might be crucial for the prevention of alkaloid poisoning that could reflect high serum AP concentrations (Serrano et al., 2011).

Germination increases the protein level compared with raw seeds, but essential amino acid concentrations in protein are lower in germinated than in raw seeds (Donangelo et al., 1995; Rubio et al., 2002; Rodriguez et al., 2008; Chilomer et al., 2010). Moreover, Chilomer et al. (2012) found that germination strongly reduced standardized ileal digestibility coefficients (SID) of methionine (17% for yellow lupin and 9% for blue lupin) and lysine (5%). In the present work, germination had no impact on ADG and FCR, due to the similar nutritional values for all of the mixtures (isoenergetic and isonitrogenous) supplemented with crystalline amino acids. The lack of positive effects of germination on feed utilization may be a result of the presence of not only newly formed alkaloids, but also other components in the germinated lupin seeds. Non-starch polysaccharides are not degraded during germination and can be responsible for the reduction of nutrient digestibility and energy utilization (Kim et al., 2007). In germinated seeds, an increase of total fibre, cellulose and polysaccharides rich in glucose and mannose has also been observed (Donangelo et al., 1995). Furthermore, the appearance of glycosylated peptides, which are resistant to proteolytic enzymes, could be responsible for the lower digestibility and utilization of germinated lupin seed amino acids compared with raw seeds observed in pigs. On the other hand, in the current study an effect of species on FCR was found. Raw or germinated yellow and blue lupin seeds differed strongly in the concentration of nutrients (Chilomer et al., 2012). Yellow lupin products contain more crude protein and NFE but less alkaloids than blue lupin. In comparison with the present results, Urbano et al. (2005) found that the 4-day germination of pea seeds had a positive impact on feed intake, weight gain, and protein effective ratio in rats. Rubio et al. (2002) using faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*) in diets for rats, found that germination improved dry matter and nitrogen digestibility, but did not affect weight gain or feed efficiency.

The replacement of 50% soya bean meal protein by raw or germinated yellow and blue lupin seeds did not significantly affect pig performance. Substitution of 75% of protein of soya bean meal by germinated blue lupin seeds negatively affected only feed intake. Many authors found that lupin seeds can be used as a substitute for soya bean meal in pig diets without negative effects on gains (Kim et al., 2007). There is no information, however, about using germinated lupin seeds in diets for pigs. In the present paper, the use of germinated lupin seeds in diets for growing pigs did not significantly lower pig performance in comparison with soya bean meal, but in comparison with raw lupin seeds it did not positively impact performance, either. Germination engaged time and money for drying germinated seeds, but in light of the obtained results there is no economic justification for this.

Conclusions

The germination of yellow and blue lupin seeds, carried out over four days at 24ºC, did not positively impact the performance of growing pigs when compared with feeding raw seeds. We do not recommend using germinated lupin seeds as a replacement for soya bean meal in growing pig nutrition.

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