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The effectiveness of grass pea (*Lathyrus sativus* L.) seeds in pig feed

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Abstract: The aim of this study was to assess the usefulness of raw grass pea seeds in pig feed, and the usefulness of supplementing mixtures that contain raw grass pea seeds with a probiotic and an enzymatic preparation. The study included 96 fattening pigs (Polish Landrace × Polish Large White) × Pietrain that were divided into 4 feeding groups. Two experimental nutritional agents were used. The first was the source of protein in the diet: soybean meal and raw grass pea seeds (amounting to 50% of feed protein). The other experimental agent was mixtures containing raw grass pea seeds supplemented with 1.0% of a probiotic (Toyocerin®) or an enzymatic preparation (Porzyme 9300). Use of raw grass pea seeds accounting for 50% of feed protein did not lower production or slaughter parameters. Mixtures containing raw grass pea seeds supplemented with the enzymatic preparation reduced feed intake, and lowered total protein and metabolic energy consumption per kg of weight. Nonetheless, in consideration of the rearing results, supplementing mixtures of raw grass pea seeds with probiotic seems to be pointless.

Key words: Pig, grass pea seeds, Toyocerin®, Porzyme 9300, growth performance, carcass characteristics

Introduction

The primary source of protein in pig feed in Poland is soybean meal (1). This is a very expensive feed, as the majority of meal available on the market is imported. Little soy is grown in Poland because of an unfavorable climate (2). Consequently, cheaper sources of protein must be considered. Grass pea is an undemanding plant, which is resistant to harsh soil and climate conditions (3), yet provides a large quantity of protein per hectare (>0.5 t ha^{-1}) (4).

A factor that limits the use of grass pea seeds in animal feed is the presence of numerous anti-nutritional agents, especially β-ODAP neurotoxin, which leads to lathyrism (5). Castell et al. (6) reported a significant reduction in food intake and growth performance when grower pigs were fed diets containing 0.55 g of β-ODAP kg^{-1}. High demand for plant protein encouraged geneticists to cultivate some varieties of grass pea with reduced β-ODAP content. Other research resulted in improving the nutritional value of grass pea seeds using thermal and plastic processing: extrusion and expanding (7). Research results indicate the usefulness of grass pea seeds in feeding different animal species, as well as humans. This is related to the favorable chemical composition of the seeds (8), as well their taste.

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According to some researchers (6,8-13), grass pea seeds can partially replace soybean meal and rapeseed meal in animal feed. At the same time it was reported that the proportion of raw seeds should not exceed 15% in a grower mixture or 20% in a finisher mixture for pigs (9). Higher values will lead to a significant reduction in production output. Some authors (14-16) observed that probiotics and xylanase administered orally to fattening pigs are effective growth stimulants.

The aim of the present study was to assess the usefulness of raw grass pea seeds in pig feed, and to determine the usefulness of supplementing mixtures that contain raw grass pea seeds with probiotic and enzymatic preparations.

Materials and methods

The study included 96 (Polish Landrace × Polish Large White) × Pietrain piglets between 25 kg and ca. 100 kg of body weight (BW) that were divided into 4 feeding groups. They were housed in cages (2 per cage), given water ad libitum, and slaughtered at approximately 100 kg of BW.

Two experimental agents were used. The first was the source of protein in the diet: soybean meal and raw grass pea seeds (Table 1). The animals in group 1 were fed standard grower and finisher mixtures. The animals in the experimental groups (2, 3, and 4) were fed mixtures that included a proportion of raw grass pea seeds amounting to 50% of feed protein throughout the fattening period. The other experimental factor was addition of 0.1% Toyocerin® probiotic (group 3) or Porzyme 9300 enzymatic preparation (group 4) to the experimental mixtures. Toyocerine® (Asahi Vet. S.A., Spain) contains 1×10^9 *Bacillus cereus* var. *toyoi* spores per g. The product was officially approved for use in pig feed and can be found in Annex II of Directive 70/524/EEC. Porzyme 9300 (Danisco, Marlborough, UK) is a preparation containing xylanase (4000 U g^{-1}) of minimum activity obtained from *Trichoderma longibrachiatium* bacteria (endo-1,4-beta-xylanase). The composition of the mixtures and the proportion of nutrients present in them are shown in Table 2.

During the study the following production results were analyzed: daily weight gain (in the first and the second fattening period), feed intake, and consumption of total protein and metabolic energy for 1 kg of weight gain.

Following slaughter, dissection of the right half of each carcass was performed, in accordance with the methods recommended by the Polish Pig Testing Stations (SKURTCh) and described by Różycki (17). Next, the following slaughter analysis parameters were measured: middle length, weight of the ham, kidney, liver, and fat, as well as backfat thickness and loin eye area. Additionally, samples were taken (each weighing ca. 150 g) from the loin, ham, liver, kidney, lungs, and liver.

Table 1. Experimental design.

	Feeding groups			
	I	II	III	IV
Grower	Standard mixture	Raw grass pea seeds (50% of protein)	Raw grass pea seeds (50% of protein) + probiotic Toyocerin	Raw grass pea seeds (50% of protein) + Porzyme 9300
Finisher	Standard mixture	Raw grass pea seeds (50% of protein)	Raw grass pea seeds (50% of protein) + probiotic Toyocerin	Raw grass pea seeds (50% of protein) + Porzyme 9300
Number of piglets in the experiments	24	24	24	24
Number of pigs for dissection	5 G + 5 B	5 G + 5 B	5 G + 5 B	5 G + 5 B

G - gilts, B - barrows

Table 2. Ingredients and chemical composition of the control and experimental diets.

	Feeding groups							
	I		II		III*		IV**	
	grower	finisher	grower	finisher	grower	finisher	grower	finisher
Wheat	30.0	30.0	41.3	21.0	41.3	21.0	41.3	21.0
Barley	50.55	55.7	30.55	59.0	30.55	59.0	30.55	59.0
Grass pea – raw seeds	-	-	15.0	10.0	15.0	10.0	15.0	10.0
Soybean meal	13.0	10.0	10.0	7.2	10.0	7.2	10.0	7.2
Meat-and-bone meal	5.0	1.0	-	-	-	-	-	-
Blood meal	-	1.0	-	-	-	-	-	-
Vitamin-mineral premix[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-lysine	0.05	-	0.20	0.15	0.20	0.15	0.20	0.15
DL-methionine	-	-	0.05	0.05	0.05	0.05	0.05	0.05
Fodder salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	0.10	0.70	0.70	0.80	0.70	0.80	0.70	0.80
Dicalcium phosphate	-	0.30	0.90	0.50	0.90	0.50	0.90	0.50
1 kg feed mixtures contains:								
Crude protein, g	176.3	153.0	170.6	151.2	170.6	151.2	170.6	151.2
Crude ash, g	34.74	28.93	27.49	27.33	27.49	27.33	27.49	27.33
Crude fiber, g	41.51	44.53	40.34	44.39	40.34	44.39	40.34	44.39
Crude fat, g [b]	24.18	21.11	17.58	18.61	17.58	18.61	17.58	18.61
Lysine, g	9.28	7.51	9.20	7.63	9.20	7.63	9.20	7.63
Methionine+cysteine, g	5.75	5.25	5.55	5.08	5.55	5.08	5.55	5.08
Calcium, g	7.76	7.37	7.67	7.20	7.67	7.20	7.67	7.20
Total phosphorus, g	6.45	5.10	6.05	5.03	6.05	5.03	6.05	5.03
Trypsin inhibitor (TI), mg^{-1}	0.032	0.02	4.20	2.11	4.20	2.11	4.20	2.11
β-ODAP, g	0.00	0.00	0.21	0.10	0.21	0.10	0.21	0.10

[a] Composition of premix/kg: vitamin A 400,000 IU, vitamin D_3 66,000 IU, vitamin E (as DL-α-tocopherol acetate) 6000 mg, vitamin K_3 (as menadion sodium bisulfite) 100 mg, vitamin B_1 60 mg, vitamin B_2 150 mg, vitamin B_6 100 mg, vitamin B_{12} 1 mg, nicotinic acid 800 mg, pantothenic acid (as Ca-D-pantothenate) 350 mg, folic acid 15 mg, choline 10,000 mg, betaine 3500 mg, Fe 350 mg, Zn 3650 mg, Mn 3000 mg, Cu 3500 mg, J 75 mg, Co 15 mg, Se 13 mg

[b] The content of crude fat in grass pea seeds is three times lower in comparison to wheat, barley, and soybean meal; raw seeds of grass pea contain of 0.73% of crude fat (personal studies, unpublished)

* Toyocerine (Asahi Vet. S.A./Spain) was added at 0.1% of diets in place of barley

** Porzyme 9300 (Danisco, Marlborough, UK) was added at 0.1% of diets in place of barley

The basic chemical element contents in the mixtures and tissue samples were determined with the use of standard AOAC methods (18). Lysine and methionine + cysteine were analyzed in acid hydrolysates in a color reaction with ninhydrin reagent, using a Beckman-System Gold 126 AA automatic analyzer. The quantity of Ca was measured with the AAS flame technique, using a Unicam 939 apparatus (AA Spectrometer Unicam), and P was measured with the calorimetric method, according to AOAC (18). The activity of trypsin inhibitors was determined with the method described by Kakade et al. (19), whereas the β-ODAP concentration was measured with the HPLC method, as described by Kuo et al. (20). All chemical analyses were performed in triplicate.

The results obtained were statistically analyzed (SEM, SD, and effects of diet) by ANOVA and Duncan's multiple range test, using Statistica v.6.0 software (21). P values <0.05 were considered significant. An asterisk indicates a statistically significant difference between the control and experimental groups.

Results

Using a mixture containing raw grass pea seeds amounting to 50% of feed protein during both fattening periods did not lower daily weight gain or the following slaughter parameters: slaughter output, middle length of the carcass, weight of the ham, and the loin eye area (Table 3). The animals fed a mixture containing grass pea seeds enriched with Toyocerin® (group 3) had a significant ($P < 0.05$) increase in the thickness of the fatback, as compared with the remaining experimental groups. It should be noted that hypertrophy of the liver or the kidneys was not observed in any of the experimental groups.

Grass pea seeds amounting to 50% of feed protein in group 2 did not significantly affect daily weight gain during either of the 2 fattening periods and did not contribute to differences in feed intake between the 2 fattening periods (Table 3). Supplementation with Toyocerin® in group 3 did not significantly influence daily weight gain; however, it led to a significant ($P < 0.05$) increase in feed consumption per kg of weight gain in the second fattening period. Supplementation with Porzyme 9300 enzymatic preparation in group 4 resulted in a significant ($P < 0.05$) reduction in feed consumption per kg of weight gain in both fattening periods, while at the same time its effect on daily weight gain was not significant.

Supplementation of pig feed mixtures with raw grass pea seeds did not contribute to higher consumption of total protein or metabolic energy per kg of weight gain during either fattening period in group 2, nor did supplementation with the probiotic in group 3 significantly affect these parameters. Yet, in the group fed mixtures containing raw grass pea seeds a statistically significant ($P < 0.05$) reduction in protein and energy consumption was observed, in comparison with the control group.

Table 4 shows the dry matter, total protein, crude ash, and crude fat content in the ham, loin, liver, kidneys, lungs, and spleen in the fattening pigs. A statistically significant ($P < 0.05$) effect of raw grass pea seeds amounting to 50% of feed protein (group 2) on the content of the basic chemical elements in the examined tissues was not observed. Yet, supplementing the diet with a probiotic (group 3) or an enzymatic preparation (group 4) significantly reduced the content of all the examined chemical elements in the kidney, as compared to groups 1 and 2.

Discussion

Two experimental agents were used in the present study. The first was the source of protein in the diet: soybean meal and raw grass pea seeds. The other experimental factor was supplementation with 0.1% Toyocerin® probiotic or Porzyme 9300 enzymatic preparation. Porzyme 9300 is an enzymatic preparation used in pig feed (14). It contains xylanase of minimum activity (4000 U g^{-1}). On the other hand, Toyocerin® is a probiotic preparation administered to animals in order to stabilize their intestinal microflora (22,23). Some researchers (15,16) observed that probiotics administered orally to fattening pigs were effective growth stimulants, replacing in-feed antibiotics that are banned in the European Union.

Raw grass pea seeds contain a number of anti-nutrients, such as tannins, protease inhibitors, phytates, alkaloids, and lathyrogens (5). Such substances must be eliminated from the organism, which involves mainly the liver and the kidneys. Studies (6,9) have revealed that hypertrophy of these organs occurred in fattening pigs fed large amounts of raw grass pea seeds (30% in grower mixture and 20% in finisher mixture). The cause for the increased weight of the liver and kidneys could be ascribed to an intensified process of eliminating anti-nutrients from the organism.

Table 3. Results of performance and pig carcass characteristics (mean ± sd).

	Feeding groups				SEM	Effects of diet
	I	II	III	IV		
Fattening days	101	101	101	101	0.03	NS
Body weight, kg						
Initial	25.2 ± 1.6	25.2 ± 1.4	25.1 ± 1.4	25.1 ± 1.3	0.03	NS
Final	100.1 ± 2.1	100.0 ± 2.3	100.2 ± 1.8	101.0 ± 2.5	0.25	NS
Dressing percentage, %	83.0 ± 5.1	81.4 ± 4.2	82.7 ± 4.7	82.8 ± 4.9	1.14	NS
Carcass length, cm	83.4 ± 4.7	83.4 ± 4.3	83.3 ± 4.1	82.5 ± 4.1	0.47	NS
Proper ham weight, kg	6.5 ± 0.4	6.5 ± 0.7	6.8 ± 0.4	6.6 ± 0.4	0.10	NS
Loin eye area, cm^2	48.3 ± 2.4	47.1 ± 1.8	48.4 ± 2.0	48.7 ± 2.1	1.32	NS
Backfat thickness, cm**	1.49^{a}	1.51^{a}	1.94^{b}	1.55^{a}	0.18	*
Leaf fat, kg	0.67^{b} ± 0.05	0.61^{a} ± 0.04	0.66^{b} ± 0.05	0.66^{b} ± 0.03	0.13	*
Kidney weight, g	150.0 ± 12.1	155.8 ± 13.4	156.7 ± 11.1	146.7 ± 18.5	6.83	NS
Liver weight, g	1658 ± 122.1	1673 ± 102.8	1683 ± 99.1	1667 ± 87.9	0.04	NS
Daily live weight gain, g						
Fattening period I	607 ± 28.1	606 ± 29.9	615 ± 28.4	620 ± 28.5	16.38	NS
Fattening period II	873 ± 31.2	870 ± 27.4	889 ± 26.8	880 ± 32.1	24.32	NS
Fattening	740	738	752	750	15.25	NS
Feed intake per kg live weight gain						
Fattening period I	3.27^{b} ± 0.2	3.31^{b} ± 0.1	3.32^{b} ± 0.08	3.02^{a} ± 0.1	0.06	*
Fattening period II	3.53^{b} ± 0.2	3.57^{b} ± 0.2	3.67^{c} ± 0.1	3.44^{a} ± 0.13	0.10	*
Fattening	3.40^{b}	3.44^{b}	3.50^{c}	3.23^{a}	0.05	*
Protein intake, g per kg live weight gain						
Fattening period I	566.4^{b} ± 24.1	564.7^{b} ± 12.7	566.4^{b} ± 14.1	515.2^{a} ± 23.9	10.83	*
Fattening period II	536.0^{ab} ±15.0	539.8^{ab} ±25.1	554.9^{b} ± 24.3	520.1^{a} ± 12.4	10.66	*
Fattening	551.2^{b}	552.3^{b}	560.7^{b}	517.7^{a}	7.68	*
Energy intake, MJ per kg live weight gain						
Fattening period I	41.92^{b} ± 2.3	42.27^{b} ± 3.4	42.40^{b} ± 4.2	38.56^{a} ± 3.4	0.82	*
Fattening period II	48.50^{b} ± 2.9	44.62^{ab} ± 2.1	45.88^{ab} ± 2.0	43.00^{a} ± 3.2	0.88	*
Fattening	45.21^{b}	43.45^{ab}	44.14^{b}	40.78^{a}	0.62	*

* $P < 0.05$

NS- non-significant

a, b, c- means in the same row with different superscripts differ significantly ($P \leq 0.01$)

** mean from 5 measurements

Table 4. Dry matter, crude protein, crude ash, and crude fat percentages of meat and organs samples (mean ± sd).

	Feeding groups				SEM	Effects of diet
	I	II	III	IV		
Loin, %						
Dry matter	27.35 ± 3.85	27.49 ± 4.01	27.33 ± 4.02	27.44 ± 3.76	0.15	NS
Crude protein	23.30 ± 3.98	23.37 ± 3.55	23.32 ± 4.01	23.36 ± 3.94	0.15	NS
Crude ash	1.44^{b} ± 0.02	1.44^{b} ± 0.02	1.34^{a} ± 0.01	1.30^{a} ± 0.02	0.02	*
Crude fat	2.61 ± 0.03	2.63 ± 0.04	2.61 ± 0.05	2.67 ± 0.07	0.04	NS
Ham, %						
Dry matter	25.56 ± 3.99	25.64 ± 4.11	25.74 ± 3.55	25.78 ± 4.13	0.14	NS
Crude protein	22.23 ± 4.02	22.20 ± 3.78	22.26 ± 3.12	22.36 ± 4.00	0.13	NS
Crude ash	1.42 ± 0.02	1.39 ± 0.01	1.41 ± 0.01	1.42 ± 0.03	0.01	NS
Crude fat	1.97 ± 0.04	2.01 ± 0.04	2.01 ± 0.05	1.95 ± 0.04	0.03	NS
Liver, %						
Dry matter	26.06 ± 4.97	26.09 ± 4.55	25.98 ± 3.98	26.11 ± 4.09	0.18	NS
Crude protein	20.55 ± 3.76	20.58 ± 3.98	20.60 ± 4.11	20.53 ± 3.99	0.14	NS
Crude ash	1.68 ± 0.03	1.65 ± 0.03	1.62 ± 0.04	1.69 ± 0.05	0.02	NS
Crude fat	3.73 ± 0.07	3.77 ± 0.08	3.69 ± 0,08	3.79 ± 0.10	0.07	NS
Kidney, %						
Dry matter	17.31^{b} ± 1.5	17.26^{b} ± 1.1	16.28^{a} ± 1.1	16.29^{a} ± 1.3	0.13	*
Crude protein	13.90^{b} ± 1.0	13.92^{b} ± 1.1	13.55^{a} ± 1.0	13.59^{a} ± 0.09	0.12	*
Crude ash	1.49^{b} ± 0.1	1.52^{b} ± 0.09	1.19^{a} ± 0.05	1.21^{a} ± 0.05	0.01	*
Crude fat	1.76^{b} ± 0.07	1.79^{b} ± 0.05	1.50^{a} ± 0.09	1.43^{a} ± 0.1	0.03	*
Lung, %						
Dry matter	20.10 ± 1.5	20.13 ± 1.5	20.07 ± 1.3	20.15 ± 1.7	0.08	NS
Crude protein	17.41 ± 1.4	17.34 ± 1.2	17.42 ± 1.5	17.46 ± 0.9	0.06	NS
Crude ash	1.16 ± 0.08	1.17 ± 0.10	1.14 ± 0.12	1.12 ± 0.09	0.01	NS
Crude fat	1.46 ± 0.11	1.58 ± 0.12	1.47 ± 0.09	1.48 ± 0.13	0.04	NS
Spleen, %						
Dry matter	21.24 ± 1.9	21.26 ± 1.4	21.23 ± 1.8	21.28 ± 1.5	0.06	NS
Crude protein	18.07 ± 1.1	18.07 ± 1.3	18.00 ± 0.9	18.11 ± 1.4	0.07	NS
Crude ash	1.58 ± 0.01	1.62 ± 0.01	1.58 ± 0.02	1.57 ± 0.01	0.02	NS
Crude fat	1.53^{a} ± 0.02	1.51^{a} ± 0.03	1.50^{a} ± 0.01	1.70^{b} ± 0.01	0.03	*

* $P \leq 0.05$

NS – non-significant

a, b - means in the same row with different superscripts differ significantly ($P \leq 0.01$)

Trombetta et al. (11) reported that using a mixture containing raw grass pea seeds amounting to 50% of pig feed protein during both fattening periods did not result in lower daily weight gain or slaughter parameters. Similar results were observed by Grela et al. (12) in guinea pigs. According to some other researchers, higher proportions of raw grass pea seeds in the diets of fattening pigs (6,9) and chickens (5,10) lead to lower slaughter parameters. It should be also noted that Winiarska-Mieczan (8) reported that raw grass pea seeds amounting to 50% and 100% of protein in the diet of fattening pigs did not result in lower organoleptic properties of ham or loin meat.

A study on fattening pigs (9) reported a positive correlation between the amount of raw grass pea seeds in the diet and subcutaneous adipose tissue thickness. The researcher observed a significant reduction in fat deposition in animals fed grass pea seeds amounting to 100% of feed protein, as compared to animals administered a 50% lower dose of grass pea, which can be explained by the presence of some substances limiting the use of energy elements in the feed. At the same time, Winiarska-Mieczan (24) showed that partial replacement of soybean meal and rapeseed meal with raw grass pea seeds did not significantly affect the fatty acids profile in pig fatback, indicating its organoleptic properties.

In conclusion, grass pea seed is much cheaper than the widely used soybean meal. Using grass pea amounting to 50% of feed protein in pig fattening did not lead to lower production or slaughter parameters. Castell et al. (6) reported a significant reduction in food intake and growth performance when grower pigs were fed diets containing 0.55 g of β-ODAP kg^{-1}. In the present study experimental mixtures contained 0.1-0.21 g of β-ODAP kg^{-1}. Supplementing the mixture containing raw grass pea seeds with Porzyme 9300 enzymatic preparation resulted in lower feed, total protein, and metabolic energy consumption per kg of weight gain. Nortey et al. (14) reported that the release of nutrients with xylanase supplementation might reduce feed and nutrient intake because extra released nutrients might trigger a feedback mechanism to reduce feed intake as the result of a glucostatic or aminostatic response. Nonetheless, in consideration of production output, supplementing mixtures containing raw grass pea seeds with Toyocerin® seems pointless, as it only leads to increasing the cost of fattening pigs.

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