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Feeding broiler chickens with practical diets containing lupin seeds (*L. angustifolius* or *L. luteus*): effects of incorporation level and mannanase supplementation on growth performance, digesta viscosity, microbial fermentation and gut morphology

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ABSTRACT. Total 208 day-old female broilers were fed on isonitrogenous and isoenergetic diets containing seeds of sweet narrow-leafed (*L. angustifolius* cv. Bojar – NL) or sweet yellow (*L. luteus* cv. Parys – YL) lupin at the level of 0 (control, C), 10% (days 1–14), and 15% or 25% (days 15–35 of life). The diets were fed unsupplemented or supplemented with mannanase. At the end of second week of life, the birds fed both lupin diets were smaller than control birds, due to lower feed intake ($P < 0.05$). The final body weight ($P < 0.05$) and body weight gain ($P < 0.01$) in birds fed the NL were higher than in those fed the YL diets due to higher ($P < 0.01$) feed intake, but their feed conversion ratio was worse ($P < 0.01$). The higher dietary lupin level did not affect feed intake, but depressed feed utilization. The relative liver weight was greater in birds fed YL than NL. The viscosity of ileal digesta in birds fed the NL diets averaged 3.12 mPas.s and was significantly greater than in control and YL-fed birds. Villi height and crypt depth were lower ($P < 0.05$) at the increased level of dietary lupin. The dietary treatments did not affect the total short-chain fatty acid concentration in ileal and caecal digesta. Mannanase supplementation did not significantly affect any of the measured parameters, but increased the butyrate concentration in caecal digesta ($P < 0.05$). Inclusion of sweet lupin at a 15% level can be accepted in older broiler diets provided with adequate amino acid and fat supplementation.

Introduction

Only two species of sweet lupin are being cultivated in Poland in recent years – narrow-leafed lupin (*L. angustifolius*), which is sometimes referred to as blue lupin, and yellow lupin (*L. luteus*), as these species are more anthracnose-resistant than white lupin (*L. albus*). The supply of lupin seeds in Poland recently increased, but there are no recommendations as to the safe level of these seeds in poultry diets. The main drawback of lupin in the past was its high alkaloids content. In yellow lupin harvested in the years 1991–1994 and analysed by Wasilewko and Buraczewska (1999), the alkaloids content was from 230 to 1300 mg · kg^{-1} (lupinine, gramine and sparteine); in narrow-leafed lupin it ranged from 300 to 440 mg · kg^{-1} (lupanine and 13-hydroxylupanine). Seeds of new Polish cultivars of sweet lupins with

quinolizidine alkaloids contents lower than 200 mg · kg^{-1} (COBORU, 2011) could be a good alternative to soyabean meal, all the more so as chickens are far less sensitive to lupin alkaloids than pigs and rats (Buraczewska et al., 1993).

The proteins of narrow-leafed and yellow lupin are digested to the same extent as those from soyabean meal (Alloui et al., 1994; Steenfeldt et al., 2003; Mieczkowska et al., 2005; Olkowski et al., 2010), but the main problem is non-starch polysaccharides (NSP), as no endogenous NSP-degrading enzymes are present in the broiler gastrointestinal tract. The high viscosity of digesta induced by water-soluble NSP is the main constraint for the use of some feeds in broiler diets. Petterson (2000) reported that narrow-leafed lupin cultivated on a large scale in Australia was used in commercial broiler diets at a maximum 10% level as higher levels could induce the health and environmental problems associated with digesta viscosity and high litter moisture. Kocher et al. (2000) found that a diet containing 350 g · kg^{-1} of Australian narrow-leafed lupin cv. Gungurru induced high viscosity in the duodenal and jejunal digesta of broilers, reaching even 11.6 mPas·s in ileal digesta (382% higher than in the control sorghum-based diet). Viscosity was not affected by addition of two commercial enzyme preparations, but was three times as large after supplementation of the diet with a feed enzyme preparation containing β-glucanase, hemicellulase and pectinase activity.

It was reported that the apparent metabolizable energy, as well as the nutritive value, of European cultivars of narrow-leafed lupin for broilers were lower than yellow lupin (Alloui et al., 1994; Roth-Maier and Paulicks, 2003). Information concerning the digesta viscosity induced by European cultivars of both species of lupin in broilers are scarce, however. Mieczkowska et al. (2005) reported that the viscosity of ileal digesta in birds fed a semi-synthetic diet based on yellow lupin cv. Amulet (550 g · kg^{-1}) was 2.92 mPas·s, and decreased by 37% after dehulling the seeds and by 15% after α-galactase supplementation. Steenfeldt et al. (2003) reported that the ileal viscosity in chickens fed a diet with 200 g · kg^{-1} narrow-leafed lupin cv. Emir was 5.48 mPas·s and was 253% higher than in the control, maize-based diet. It decreased by 15% to 44% after supplementation of different feed enzymes, but still was much higher than the control. The effects of NSP of modern Polish cultivars of lupin on digesta viscosity, development of small intestinal walls, and activity of small intestinal microbiota in chickens are not well recognized.

The aim of this study was to compare the effects of two lupin species (narrow-leafed and yellow) incorporated into practical broiler diets at one (starter type) or two levels (grower/finisher type), and of mannanase supplementation, on the growth performance and gut parameters of broilers.

Material and methods

Seeds of sweet (low in alkaloids) narrow-leafed lupin (*L. angustifolius* cv. Bojar) and yellow lupin (*L. luteus* cv. Parys) from the 2011 harvest were purchased from ROLNAS, Bydgoszcz (Poland). Seeds were ground in a disc-grinder to pass a 3 mm sieve, analysed according to AOAC (1990), and used in experimental diets as a soyabean meal substitute.

Animals and diets

The experimental procedures were approved by the 3rd Local Animal Care and Ethics Committee in Warsaw. A total of 208 one-day-old Ross 308 broiler females (initial weight 43 ± 2 g) was obtained from a local commercial hatchery and kept in electrically heated battery brooders, 8 birds per cage (replication). The birds were randomly allocated to 6 dietary treatments (Table 1): groups C and CE contained 24 birds, groups N, NE, Y and YE had 40 birds each, and fed the experimental starter di-

Table 1. Experimental scheme, n – number of birds per group

Group	Dietary treatments	Days 1–14 of life		Days 15–28 of life		Days 29–35 of life	
		lupin, g · kg^{-1} diet	n [1]	lupin, g · kg^{-1} diet	n	lupin, g · kg^{-1} diet	n
C	Control	0	(24) 18	0	16	0	16
CE	Control + enzyme	0	(24) 18	0	16	0	16
N	Narrow-leafed lupin	100	(40) 34	150	16	150	16
				250	16	250	16
NE	Narrow-leafed lupin + enzyme	100	(40) 34	150	16	150	16
				250	16	250	16
Y	Yellow lupin	100	(40) 34	150	16	150	16
				250	16	250	16
YE	Yellow lupin + enzyme	100	(40) 34	150	16	150	16
				250	16	250	16

[1] the number of chickens in a group in the 1st week of life is given in parentheses

Table 2. Composition of diets, g · kg^{-1} air dry matter[1]

Indices	Period of feeding												
	1–14 day			15–28 day					28–36 day				
	C	N_{10}	Y_{10}	C	N_{15}	N_{25}	Y_{15}	Y_{25}	C	N_{15}	N_{25}	Y_{15}	Y_{25}
Component													
narrow-leafed lupin	—	100	—	—	150	250	—	—	—	150	250	—	—
yellow lupin	—	—	100	—	—	—	150	250	—	—	—	150	250
soyabean meal	344.7	290.3	276.8	316.9	234.0	175.1	213.3	140.7	289.6	204.1	144.6	183.2	110.8
wheat	400.4	334.4	350.3	423.7	330.5	284.3	356.3	323.7	445.4	362.3	316.7	388.6	355.5
rapeseed oil	20	40	38	25	50	55	45	50	30	48	53	43	48
maize	200	200	200	200	200	200	200	200	200	200	200	200	200
NaCl	3	3	3	3	3	3	3	3	3	3	3	3	3
limestone	11	11	11	11	11	10.7	11.5	11.5	12.9	12.5	12.2	12.9	13
mono-Ca-phosphate	13	13	13	12	12.5	12.7	12	12	11.4	11.8	12	11.3	11.4
L-Lys (78%)	1.3	1.5	1.4	1.6	1.8	1.9	1.8	2.0	1.4	1.8	1.9	1.6	1.8
DL-Met (98%)	1.6	1.8	1.5	1.8	2.2	2.3	1.8	1.6	1.3	1.5	1.6	1.1	1
DL-Thr (98%)	—	—	—	—	—	—	0.3	0.5	—	—	—	0.3	0.5
min.-wit.premix [2,3,4]	5[2]	5[2]	5[2]	5[3]	5[3]	5[3]	5[3]	5[3]	5[4]	5[4]	5[4]	5[4]	5[4]
Calculated nutrients composition													
crude protein	215.0	215.0	215.0	205.0	205.0	205.0	205.0	205.0	195.0	195.0	195.0	195.0	195.0
EM[6], MJ · kg^{-1}	12.1	12.1	12.1	12.2	12.2	12.2	12.2	12.2	12.4	12.4	12.4	12.4	12.4
Lys[6]	11.9	11.9	11.9	11.4	11.4	11.4	11.4	11.4	10.7	10.7	10.7	10.7	10.7
Met + Cys[6]	8.4	8.4	8.4	8.5	8.5	8.5	8.5	8.5	7.6	7.6	7.6	7.6	7.6
Thr[6]	7.8	7.9	7.7	7.5	7.5	7.5	7.5	7.5	7.1	7.1	7.1	7.1	7.1

[1] C – control, N_{10}, N_{15}, N_{25} – narrow-leafed lupin, Y_{10}, Y_{15}, Y_{25} – yellow lupin in the amount of 10%, 15% and 25% diet, respectively; each diet was prepared unsupplemented or supplemented with 400 mg (64000 U) · kg^{-1} of β-D-mannanase (EC 3.2.1.78) added at the expense of wheat; [2,3,4] provided per kg diet: [2] IU: vit. A. 12500, vit. D_3 5000; mg: vit. E – 50, vit. B_1 – 3, vit. B_2 – 7.5, biotin – 0.2, vit. B_6 – 5, vit. B_{12} – 0.025, vit. K_3 – 3, nicotinic acid – 50, folic acid – 1.75, Ca-pantothenate – 12.5, choline – 400, Mn – 90, Zn – 90, Se – 0.3, Co – 0.3, Cu – 15, Fe – 60, J – 1; Ca – 0.95 g, endo-1,4-β-xylanase (EC 3.2.1.8) – 1500 U, 6-phytase (3.1.1.26) – 1500 FTU mg; coccidiostat Maxiban 100 mg; [3] IU: vit. A – 10000, vit. D_3 – 4500; mg: vit. E – 70, vit. B_1 – 2, vit. B_2 – 6, biotin – 0.183, vit. B_6 – 4, vit. B_{12} – 0.017, vit. K_3 – 2.8, nicotinic acid – 50, folic acid – 1.58, Ca-pantothenate – 12, choline – 333, Mn – 107, Zn – 93, Se – 0.3, Cu – 15, Fe – 50, J – 1.1, Co – 0.3; Ca – 1.01 g; endo-1,4-β xylanase (EC 3.2.1.8) – 67 EPU, 3-phytase (EC 3.1.3.8) – 333 FTU; 6-phytase (EC 3.1.1.26) – 1000 FTU; endo-1,3 (4) β-glucanase (EC 3.2.1.6) – 67 U, coccidiostat Narasine (Monteban) – 70 mg; [4] IU: vit. A – 9000, vit. D_3 – 2500; mg: vit. E – 80, vit. B_1 – 2.25, vit. B_2 – 4.5, biotin – 0.1, vit B_6 – 3, vit. B_{12} – 0.015, vit. K_3 – 2, nicotinic acid – 30, folic acid – 0.75, pantothenic acid – 9.375, choline – 300, Mn – 70, Zn – 60, Se – 0,15, Cu – 6, Fe – 40, J – 0.6, Co – 0.25; Ca 0.45 g; endo-1,4-β-xylanase (EC 3.2.1.8) 1500 EPU; 6-phytase (EC 3.1.1.26) 1500 FTU

ets (Table 2). Room temperature was maintained at 30°C for the first 3 days and thereafter gradually reduced according to normal management practices. A light cycle of 18 h light and 6 h darkness was maintained throughout the study, birds had free access to feed and water. On the 8th day of life the broilers were deprived of feed for 4 h and weighed. Eighteen birds from the control group and 34 birds from the experimental groups with body weights close to the group average were placed in individual cages, each bird considered a replication. On the 15th day of life, the birds were weighed, the N, NE, Y and YE groups were divided into 2 groups of similar average body weights and fed either the diets with 150 g or with 250 g lupin per kg, respectively (Table 2).

Diets were formulated to provide nutrients according to the Polish recommendations for broilers (Smulikowska and Rutkowski, 2005) and to contain the same amount of metabolizable energy and crude protein within each set (Table 2). The control diet was based on wheat, maize and soyabean meal (449 g CP · kg^{-1}). Ground lupin (*L. angustifolius* and *L. luteus*) seeds were used to formulate lupin-containing diets. The levels of lupin were 100 g · kg^{-1} in starter-type diets, and 150 or 250 g · kg^{-1} in grower/finisher-type diets. Lupins replaced part of the soyabean meal and wheat; rapeseed oil, lysine and methionine were added to create isonitrogenic and isoenergetic diets. All diets contained the following enzymes added with commercial premixes: starters: endo-1,4-β-xylanase (EC 3.2.1.8) 1500 U, 6-phytase (3.1.1.26)1500 FTU; growers: endo-1,4-β xylanase (EC 3.2.1.8) 67 EPU, 3-phytase (EC 3.1.3.8) 333 FTU, 6-phytase (EC 3.1.1.26) 1000 FTU, endo 1,3 (4) β-glucanase (EC 3.2.1.6) 67 U; finishers: endo-1,4-β-xylanase (EC 3.2.1.8) 1500 EPU and 6-phytase (EC 3.1.1.26) 1500 FTU (activities per kg are given according to the manufacturers' declarations, Adisseo and Huevpharma). Each diet was prepared unsupplemented or supplemented

with 0.4 g (64000 U) per 1 kg mannanase in the form of a coated granulate (Hemicell®HT) containing β-D-mannanase (EC 3.2.1.78) 160×10^3 U · g^{-1} according to the producer's declaration (ChemGen). The preparation was added to wheat and mixed with the diets prior to cold pelleting on a CL-2 CPM laboratory pellet mill.

Birds were fed starter diets from days 1 to 14, growers until day 28, finishers until slaughter on day 36 of life; feed intake and body weight were measured between days 8–35 of life, body weight gain (BWG) and feed conversion ratio (FCR) were calculated and analysed separately for the periods days 8–14 and 15–35 of life.

Sampling procedures, chemical and histological analysis

On the next day after completion of the growth assay, live body weight (LBW) was measured without previous fasting and the birds were killed by cervical dislocation. The abdominal cavity was opened and the liver (with gall bladder), gizzard and abdominal fat were excised and weighed. The luminal content of the ileum (1 cm distal of Meckel's diverticulum to the ileocaecal junction) and caecum were pooled by segment for every two chickens and homogenized. Ileal digesta was sampled for pH, dry matter (DM), viscosity and short-chain fatty acid (SCFA) analysis, caecal digesta only for SCFA analysis. From seven randomly selected birds per group, 3 cm segments of jejunum, starting 5 cm anterior to Meckel's diverticulum, were taken for histological examination.

The pH was determined immediately in the ileal contents mixed with deionized water (1:1 w/w), using a digital pH-meter (WTW pH/340, Germany) and pH standard WTW-82362 Weilheim (model STP4) at room temperature. The DM analysis was done according to procedure 930.15 (AOAC, 1990). Samples of ileal digesta were cooled on ice, centrifuged (10 000 *g,* 10 min, 4°C) using a Beckman centrifuge (model J2-21 with J-20 rotor) 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) at 40°C. Readings were expressed in mPa·s.

The ileal and caecal digesta samples were converted to their respective sodium salts by adjusting the pH to 8.2 using 1 M NaOH, and stored at –20°C. The SCFA analysis was done according to the procedure described by Barszcz et al. (2011) using isocaproic acid as an internal standard on an HP 5890 Series II gas chromatograph (Hewlett Packard, Waldbronn, Germany) with a flame-ionization detector (FID) and Supelco Nukol fused silica capillary column (30 m × 0.25 mm internal diameter, film 0.25 mm). Helium was used as the carrier gas. The oven was initially kept at 100°C for 2 min, then heated at 10°C per 10 min to 140°C and held for 20 min. The injector temperature was maintained at 220°C, while detector was kept at 250°C. Concentrations of individual SCFA were estimated in relation to the internal standard using a mixture of SCFA standard solutions.

Segments of jejunal tissue were placed in Bouin's solution, dehydrated and embedded in paraffin wax. Serial histological 5 μm sections were cut on a microtome, mounted on slides, stained with haematoxylin, counter-stained with eosin and examined under a light microscope Zeiss Axio Star Plus (Carl Zeiss, Göttingen, Germany) and image analysis programme Axio Visio LE Rel. 4,5 (Carl Zeiss, 2002–2005).

Calculations and statistical analysis

The weight of organs was calculated relative to LBW before slaughter. Body weight gain (BWG), feed intake and feed conversion ratio were calculated separately for the periods days 8–14 and 15–35 of age due to the different factorial arrangement. It was 2 × 2 in the first period, taking into consideration the main effects of lupin species and mannanase supplement, and 2 × 2 × 2 in the second period taking into consideration the main effects of lupin species, lupin level, and mannanase supplement. Additionally, in both periods the results were subjected to one-way analysis of variance to compare the data obtained from lupin-fed chickens with control groups. The main effects of dietary treatments and their interactions were determined by one-way, two-way, or three-way ANOVA and differences between treatments were analysed *post hoc* by the least significant differences test using STATGRAPHICS® Centurion XVI ver. 16.1.03 software (Statistical Graphic Corp., 1982–2010). The effects were considered to be significant at $P \leq 0.05$ and $P \leq 0.01$.

Results

Growth performance

Yellow lupin cv. Parys contained more nutrients – crude protein and fat – than narrow-leafed lupin (Table 3). Experimental diets were formulated to contain the same level of apparent metabolizable energy (AME_N), assuming that the level of AME_N of narrow-leafed lupin was 8.4 MJ · kg^{-1}, of yellow lupin, 9 MJ · kg^{-1}. This made it necessary to add more oil into the diets with narrow-leafed lupin

Table 3. Composition of lupin seeds, g · kg^{-1} DM

Ingredient	Narrow-leafed lupin (*L.angustifolius* cv. Bojar)	Yellow lupin (*L. luteus* cv. Parys)
Dry matter	890.9	871.4
Crude protein (Nx6.25)	354.3	412.4
Crude fat	59.6	54.5
Crude ash	37.0	54.5
Crude fibre	178.9	155.1
N-free extractives	370.7	311.2

than to those with yellow lupin (Table 2). The statistical calculations were done for the data from the second week of feeding starter diets, but at the end of the first and second weeks of life, the birds fed both lupin diets were smaller than the control birds, due to lower feed intake *(P* < 0.05). The birds fed diets with narrow-leafed lupin ate more feed but had worse FCR than birds fed the diets with yellow lupin *(P* < 0.01). Mannanase supplementation did not significantly affect any of the measured parameters (Table 4).

In the following 3 weeks, feed intake and BWG were higher, but FCR worse in birds fed diets with narrow-leafed lupin than with yellow lupin (Table 5). The final BWG was numerically worse in chickens fed narrow-leafed lupin diets by 3% and in chickens fed diets with yellow lupin by 4.5%, respectively. The final body weight *(P* < 0.05) and BWG *(P* < 0.01) in groups fed diets with narrow-leafed lupin were higher than in birds fed yellow lupin diets due to the higher *(P* < 0.01) feed intake, but it resulted in a worse FCR *(P* < 0.01). There were no significant effects of increased dietary lupin level on feed intake and body weight, but the FCR was worse in groups fed higher level of lupin. There were, however, significant interactions between lupin species and their dietary level: the increase in the level of yellow lupin in the diet resulted in lower feed intake, lower BWG and final body weight, while the increase of narrow-leafed lupin resulted in higher feed intake, BWG and final body weight. The birds fed yellow lupin diets had bigger livers than the birds fed narrow-leafed variety. Gizzard weight was larger *(P* < 0.05) in birds fed lupin diets in comparison with the control group, while abdominal fat averaged up to 1% of BW and did not differ among treatments (data not shown). Mannanase supplementation did not significantly affect any measured performance parameters or organ weights (Table 5).

Table 4. Results of control groups and main effects of dietary treatments on performance of chickens between 8 and 15th day of age

Dietary treatments	Initial BW, g	Final BW g	BWG g	Feed intake, g	FCR, kg feed per kg BWG
C - Control	179	497	316	417	1.32
CE - Control with enzyme	180	480	300	406	1.35
Lupin species					
L. angustifolius	169^{B}	464*	294	387^{B*}	1.32^{B}
L. luteus	164^{A*}	453*	289	367^{A*}	1.27^{A*}
Enzyme supplement					
without enzyme	168	462*	294	380*	1.29
with enzyme	165*	454*	289	375*	1.30
Pooled SEM	2.7	6.4	5.1	6.0	0.01

^{a,b,A,B} within columns, for control groups and each main effect, means with different superscripts are significantly different at *P* < 0.05 and *P* < 0.01, respectively; * significantly different from control at *P* < 0.05; all interactions insignificant

Gut parameters

The viscosity of ileal digesta in birds fed narrow-leafed lupin averaged 3.12 mPas·s and was significantly higher than in control birds and birds fed diets with yellow lupin (Table 6). The dietary treat-

Table 5. Results of control groups and main effects of dietary treatments on performance (15–35 day of life) and organs weight in 36-day-old chickens

Dietary treatments	Initial BW, kg	Final BW, kg	BWG, kg	Feed intake, kg	FCR, kg feed per kg BWG	Liver, g · 100 g^{-1} LBW	Gizzard, g · 100 g^{-1} LBW
Control	0.497	2.16	1.66	2.60	1.56	2.26	1.06
Control with enzyme	0.480	2.17	1.69	2.65	1.57	2.40	1.03
Lupin species (L)							
L. angustifolius	0.464	2.11^{b}	1.65^{B}	2.57^{B}	1.56^{B}	2.21^{A}	1.33*
L. luteus	0.453*	2.07^{a}	1.61^{A}	2.42^{A*}	1.50^{A*}	2.34^{B}	1.33*
Dietary lupin level, g · kg^{-1} (D)							
150	0.459*	2.10	1.65	2.49	1.52^{a}	2.27	1.29*
250	0.458*	2.08	1.62*	2.50	1.54^{b}	2.27	1.37*
Enzyme supplement (E)							
without enzyme	0.463*	2.09	1.62	2.48	1.53	2.29	1.35*
with enzyme	0.454*	2.09	1.64	2.51	1.53	2.26	1.31*
Pooled SEM	0.0082	0.032	0.026	0.041	0.02	0.06	0.06
Interactions (*P* value)[1]							
L × D	ns	0.02	0.01	0.05	ns	ns	ns

LBW – live body weight; ^{a,b,A,B} within columns, for control groups and each main effect, means with different superscripts are significantly different at: *P* < 0.05, *P* < 0.01, respectively; * significantly different from control group at *P* < 0.05; [1]all other interactions insignificant

Table 6. Results of control groups and main effects of dietary treatments on morphometry in jejunal mucosa (n=7) and ileal digesta characteristics (n=8) in 36-day-old chickens

Dietary treatments	Jejunal mucosa			Ileal digesta		
	villus height, µm	villus width, µm	crypt depth, µm	DM, %	pH	viscosity, mPas·s
Control	1065	159	150	18.2	7.82	2.47
Control with enzyme	1050	138	148	18.6	7.72	2.51
Lupin species (L)						
L. angustifolius	1035	166	151^{b}	18.0^{a}	7.70	3.12^{B*}
L. luteus	1011	163	145^{a}	18.4^{b}	7.78	2.60^{A}
Lupin level, g · kg^{-1} diet (D)						
150	1059^{a}	165	151^{a}	18.1	7.78	2.74
250	986^{b}	163	145^{b}	18.4	7.69	2.99
Enzyme supplement (E)						
without enzyme	1014	165	148	18.2	7.70	2.85
with enzyme	1031	163	148	18.2	7.78	2.88
Pooled SEM	44.5	9.2	3.8	0.23	0.138	0.223
Interactions (*P* value) [1]						
L × D	ns	ns	ns	ns	ns	ns
L × E	ns	ns	ns	0.04	ns	ns
D × E	ns	ns	ns	0.03	ns	ns
L × D × E	ns	ns	ns	0.04	ns	ns

^{a,b,A,B} within columns, for control groups and each main effect, means with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$, respectively; * significantly different from control group at $P < 0.05$

ments did not affect the pH of ileal digesta, while the ileal dry matter content was lower ($P < 0.05$) in birds fed narrow-leafed lupin in comparison with yellow lupin. There were interactions between lupin species and level, and mannanase supplementation: at a lower level of dietary lupin, digesta DM was greater, while at the higher level it was smaller; addition of mannanase to yellow lupin diets decreased the DM of ileal digesta, while adding it to narrow-leafed lupin diets, increased it.

The crypt depth of jejunal mucosa was greater in birds fed narrow-leafed lupin than yellow lupin ($P < 0.05$) and villi height and crypt depth were lower ($P < 0.05$) at the higher level of dietary lupin in comparison with the lower one. Mannanase supplementation did not significantly affect any morphometric parameters (Table 6).

The dietary treatments did not influence the total SCFA concentration in ileal digesta, which averaged 32.5 ± 3.3 mmol · g^{-1} in control and lupin-fed birds (data not shown).

In caecal digesta (Table 7), the total SCFA concentration also did not differ between the control and lupin-fed birds, and averaged 156.4 ± 9.1 mmol · g^{-1}. The major component of SCFA was acetate, 74% on average, and the minor components were butyrate and propionate, 18.7% and 5% on average, respectively, followed by small amounts of valerate, isobutyrate and isovalerate. The dietary treatments affected the propionate concentration, which was higher in birds fed the diet with narrow-leafed than yellow lupin ($P < 0.05$), and higher on the diet with 250 g than with 150 g of lupin per kg. The butyrate concentration increased ($P < 0.05$) after mannanase supplementation of the lupin diets.

Discussion

The yellow lupin cv. Parys contained more crude protein and crude ash and less fat than the narrow-leafed lupin cv. Bojar. Seed nutrients were within the range determined previously for older Polish cultivars of both species (Wasilewko and Buraczewska, 1999), since the modification of the lupin genotypes was directed rather towards improvement of agronomical traits and lowering the alkaloid content. According to COBORU (2013), seeds of yellow lupin cv. Parys used in the present study may contain about 150 mg alkaloids per 1 kg, the seeds of narrow-leafed lupin cv. Bojar, about 140 mg.

Feeding diets that deviate in components from a standard diet raises the question of whether such diets lead to reduced growth performance, thus caus-

Table 7. Results of control groups and main effects of dietary treatments on short-chain fatty acids (SCFA) concentration (µmol/g) in caecal digesta (n=8) in 36-day-old chickens

Dietary treatments	Total SCFA	Acetate	Propionate	Butyrate	Iso-butyrate	Valerate	Iso-valerate
Control	141.2	102.9	6.6	28.7	1.03^{a}	1.18	0.79^{A}
Control with enzyme	157.9	114.7	8.9	28.9	1.59^{b}	1.90	1.77^{B}
Lupin species							
L. angustifolius	156.0	114.8	8.9^{b}	28.8	1.10	1.50	0.80
L. luteus	160.1	120.6	6.9^{a}	29.8	0.90	1.40	0.60
Lupin level, g · kg^{-1} diet							
150	156.4	117.8	7.1^{a}	28.2	1.00	1.40	0.80
250	159.7	117.6	8.7^{b}	30.4	0.90	1.50	0.70
Enzyme supplement							
without enzyme	153.0	115.3	8.2	26.5^{a}	0.99	1.50	0.70
with enzyme	163.1	120.2	7.7	32.1^{b}	0.97	1.50	0.80
Pooled SEM	9.09	6.90	1.22	3.87	0.15	0.21	0.16

^{a,b,A,B} within columns, for control groups and each main effect, means with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$, respectively; all interactions insignificant

ing subsequent economic losses for the producer. In the present study, 10% inclusion of narrow-leafed or yellow lupin into the starter-type diet reduced body weight by 6% and 8% at the 1st week of life and by 7% and 9% at the 2nd week of life, respectively, due to lower feed intake in comparison with the control group. During the following 3 weeks, the BWG in groups fed 25% yellow lupin was lower than in the control group by 5% due to 8% lower feed intake, while the negative effects in groups fed 15% yellow lupin and both levels of narrow-leafed lupin were negligible. In some studies, inclusions of 5%–20% of lupin seed showed little negative impact on growth performance (Roth-Maier and Paulicks, 2003; Orda et al., 2006), while inclusions of 35% or 40% lupin seeds between days 1–21 of life generally decreased performance (Rubio et al., 2003; Steenfeldt et al., 2003; Olkowski et al., 2005; Olkowski, 2011). Sometimes results were even more adverse. Olkowski et al. (2001) reported that apart from decreased feed intake and growth rate, some chickens fed a diet containing 40% narrow-leafed lupin in the first week of life shown signs of muscle paralysis and skeletal deformity. Despite digesta viscosity not having been measured in the last study, it can be recognized as a major cause of these negative effects.

Kocher et al. (2000) reported that in 24-day-old broilers fed a diet with 35% narrow-leafed lupin cv. Gungurru, in comparison with control birds, digesta viscosity was higher by 199% in the duodenum, by 246% in the jejunum, and by 382% in the ileum, while in birds fed the same level of white lupin, the increase in digesta viscosity was insignificant. The high viscosity of digesta is induced by soluble non-starch polysaccharides of specific physico-chemical characteristics. Digesta viscosity greatly depresses fat digestion and absorption, as well as the absorption of fat-soluble vitamins; the most detrimental effects are observed in very young birds (Smulikowska, 1998).

In the present study, the viscosity of ileal digesta in birds fed the diet containing 25% narrow-leafed lupin averaged 3.4 mPas·s and was higher in comparison with control birds (2.5 mPas·s) and birds fed diets with 25% yellow lupin (2.6 mPas·s), but these values were not as high as the 5.5 mPas·s found on the diet containing 20% narrow-leafed lupin cv. Emir determined by Steenfeldt et al. (2003), 11.6 mPas·s on the diet containing 35% narrow-leafed lupin cv. Gungurru measured by Kocher et al. (2000), or 32.7 mPas·s on the diet containing 40% narrow-leafed lupin, vs the 3.5 mPas·s on the diet containing 40% yellow lupin measured by Olkowski et al. (2005).

In the current study, there were interesting interactions among lupin level and species. Increasing the yellow lupin level in the diet resulted in lower feed intake, BWG and final body weight, while increasing the narrow-leafed lupin level resulted in higher feed intake, BWG and final body weight. Recently Roura et al. (2012) reported that chickens have well-developed bitter taste receptors. As yellow lupin used in the present study probably contained more alkaloids than narrow-leafed lupin, the diets with a higher level of yellow lupin can adversely affect the taste and consumption of a diet. The significant enlargement of the liver in birds fed yellow lupin diets in comparison with narrow-leafed ones may confirm this conclusion.

Kocher et al. (2000) reported that the ileal protein digestibility of narrow-leafed lupin was about 0.85, while jejunal and ileal NSP digestibilities were 0.03 and 0.12, respectively. This proved that neither were lupin NSP digested in the upper part nor were they fermented in the lower part of the small intestine. Indeed, the production of SCFA in the ileum measured in the last study was very low and amounted to about 24 mMol per bird. In the current study, ileal and caecal SCFA concentrations did not increase with dietary lupin inclusion, which indicates that lupin NSPs are resistant to microbial fermentation in young poultry.

In the present study, the crypts in the jejunum were deeper in broilers fed with narrow-leafed lupin compared with yellow lupin. The increase of crypt depth may be connected with the greater viscosity generated by narrow-leafed lupin. Formerly, Iji et al. (2001) reported that crypt depth in the jejunum of chickens fed diets with highly viscous guar gum or gum xanthan increased compared with the control diet. Montagne et al. (2003) indicated that in non-ruminant animals an increased crypt depth points to faster cell turnover and can be associated with increased water secretion into the intestinal lumen. Indeed, in the present study the water content of ileal digesta in chickens fed narrow-leafed lupin was higher. The villi height and crypt depth were lower on the higher level of lupin inclusion. Generally, shortening of the villi decreases the surface area for nutrient absorption (Montagne et al., 2003). The changes in intestinal morphology and digesta characteristics found in the current study had detrimental consequences on the feed conversion ratio, and they indicated that the use of lupin, especially narrow-leafed seeds, in broiler diets should be limited.

The main components of lupin seeds are non-starch polysaccharides (NSPs) and oligosaccharides (α-galactosides of the raffinose series), they constitute about 320 and 120 (*L. luteus*) to 400 and 76 (*L. angustifolius*) $g \cdot kg^{-1}$ of seed DM, respectively

(Gdala and Buraczewska, 1996). Lupin NSPs are highly branched pectic polysaccharides called rhamnogalacturonans in which (1-4)-α-D-galacturonan chains are interrupted by insertion of (1-2)-β-L-rhamnose residues, and long side-chains are constituted by D-galactose, L-arabinose, D-xylose, L-fucose and D-glucuronic acid (Cheetham et al., 1993). In comparison with cereal NSPs, they are much more complicated structures. Neither NSPs nor oligosaccharides are hydrolyzed by digestive enzymes of chickens, which has prompted the use of enzyme preparations having pectinase and α-galactosidase activity to improve the nutritive value of lupin diets and minimize the problems associated with wet litter. The effects of supplementing lupin-containing diets with different enzymes do not seem to be very encouraging, however. In earlier studies in our laboratory we found slight improvement of the AME value of some lupin cultivars due to pectinase and α-galactosidase supplementation, but without an effect on bird performance (Alloui et al., 1994), while supplementation of a diet containing 30% white lupin with pectinase and α-galactosidase increased the viscosity of caecal digesta and depressed organic matter retention and AME value (Mieczkowska et al., 2004). Hughes et al. (2000) found that an increased level of NSPs isolated from narrow-leafed lupin kernel in a sorghum-casein based diet depressed bird performance and AME value and raised ileal viscosity and excreta moisture in a dose-dependent manner. Supplementation of the diets with feed enzymes exhibiting pectinase, α-galactosidase, arabinoxylanase and β-glucanase activity improved dietary AME value and reduced excreta moisture at the lower level of inclusion, but increased the soluble NSP content of digesta and ileal viscosity at the higher level of inclusion. In some trials, a slight improvement of performance in chickens fed diets with 40% yellow lupin was reported due to enzyme supplementation (Rubio et al., 2003; Olkowski et al., 2010; Olkowski, 2011), while at lower levels of lupin inclusion the enzyme effects were small (Steenfeldt et al., 2003) or insignificant (Orda et al., 2006).

In the current study, supplementation of practical diets containing commonly used feed enzymes with an additional feed enzyme having mannanase activity affected neither performance nor most measured parameters, apart from the caecal butyrate concentration, which was higher in birds fed supplemented diets in comparison with unsupplemented ones. That, together with the interactions between lupin species and level, and mannanase effect on the dry matter content of ileal digesta, may indicate that some lupin NSPs can be solubilized due to the hydrolytic action of mannanase increasing the osmotic pressure in ileal digesta and involving microbial fermentation in the caeca.

Conclusions

Narrow-leafed lupin generated higher digesta viscosity in comparison with yellow lupin; this effect can induce some morphological changes in small intestinal walls. It seems that the inclusion of either of the lupin seeds into starter-type diets cannot be recommended as it negatively affects the performance of broilers. Inclusion of sweet lupin at a 15% level can be accepted in older broiler diets provided with adequate amino acid and fat supplementation.

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