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

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KEYWORDS: \textit{Lupinus angustifolius}, \textit{Lupinus luteus}, ileal viscosity, gut morphology, broilers

ABSTRACT. Total 208 day-old female broilers were fed on isonitrogenous and isoenergetic diets containing seeds of sweet narrow-leafed (\textit{L. angustifolius} cv. Bojar – NL) or sweet yellow (\textit{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 (\textit{L. angustifolius}), which is sometimes referred to as blue lupin, and yellow lupin (\textit{L. luteus}), as these species are more anthracnose-resistant than white lupin (\textit{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 $\cdot$ kg$^{-1}$ (lupinine, gramine and spar-teine); in narrow-leafed lupin it ranged from 300 to 440 mg $\cdot$ kg$^{-1}$ (lupanine and 13-hydroxylupanine). Seeds of new Polish cultivars of sweet lupins with……
quinolizidine alkaloids contents lower than 200 mg \cdot 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 \cdot 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 \cdot 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 \cdot 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>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>lupin, g · kg^{-1} diet</td>
<td>n</td>
<td>lupin, g · kg^{-1} diet</td>
<td>n</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>0 (24) 18</td>
<td>0 16</td>
<td>0 16</td>
</tr>
<tr>
<td>CE</td>
<td>Control + enzyme</td>
<td>0 (24) 18</td>
<td>0 16</td>
<td>0 16</td>
</tr>
<tr>
<td>N</td>
<td>Narrow-leafed lupin</td>
<td>100 (40) 34</td>
<td>150 16</td>
<td>150 16</td>
</tr>
<tr>
<td>NE</td>
<td>Narrow-leafed lupin + enzyme</td>
<td>100 (40) 34</td>
<td>150 16</td>
<td>150 16</td>
</tr>
<tr>
<td>Y</td>
<td>Yellow lupin</td>
<td>100 (40) 34</td>
<td>150 16</td>
<td>150 16</td>
</tr>
<tr>
<td>YE</td>
<td>Yellow lupin + enzyme</td>
<td>100 (40) 34</td>
<td>150 16</td>
<td>150 16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component</th>
<th>1–14 day</th>
<th>15–28 day</th>
<th>28–36 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N₁</td>
<td>Y₁</td>
</tr>
<tr>
<td>narrow-leaved lupin</td>
<td>100</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>yellow lupin</td>
<td>100</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>soya meal</td>
<td>344.7</td>
<td>316.9</td>
<td>234.0</td>
</tr>
<tr>
<td>wheat</td>
<td>400.4</td>
<td>423.7</td>
<td>330.5</td>
</tr>
<tr>
<td>rapeseed oil</td>
<td>20</td>
<td>25</td>
<td>55</td>
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<tr>
<td>maize</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>NaCl</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>limestone</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>mono-Ca-phosphate</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>L-Lys (78%)</td>
<td>1.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>DL-Met (98%)</td>
<td>1.6</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>DL-Thr (98%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>min-wit.premix</td>
<td>5˟²</td>
<td>5˟²</td>
<td>5˟²</td>
</tr>
<tr>
<td>Crude protein</td>
<td>215.0</td>
<td>215.0</td>
<td>215.0</td>
</tr>
<tr>
<td>Met, MJ · kg⁻¹</td>
<td>12.1</td>
<td>12.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Lys¹</td>
<td>11.9</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>Met + Cys</td>
<td>8.4</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Thr</td>
<td>7.8</td>
<td>7.9</td>
<td>7.7</td>
</tr>
</tbody>
</table>

1 C – control, N₁, N₂, N₃ – narrow-leaved lupin, Y₁, Y₂, Y₃ – yellow lupin in the amount of 10%, 15% and 25% diet, respectively; each diet was prepared un-supplemented or supplemented with 400 mg (40000 U) of β-D-mannanase (EC 3.2.1.78) added at the expense of wheat; ²³ – provided per kg diet: ÷ IU: vit. A – 12500, vit. D₃ – 5000; mg: vit. E – 50, vit. B₃ – 3, vit. B₆ – 7.5, biotin – 0.2, vit. B₉ – 5, vit. B₁₂ – 0.025, vit. K₁ – 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. D₃ – 4500; mg: vit. E – 70, vit. B₂ – 2, vit. B₆ – 6, biotin – 0.183, vit. B₉ – 4, vit. B₁₂ – 0.017, vit. K₁ – 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 Narasin (Monteban) – 70 mg; ⁴ IU: vit. A – 9000, vit. D₃ – 2500; mg: vit. E – 80, vit. B₂ – 2.25, vit. B₆ – 4.5, biotin – 0.1, vit. B₁₂ – 3, vit. K₁ – 0.015, vit. K₂ – 2.5, nicotinic acid – 30, folic acid – 0.75, pantothenic acid – 3.75, 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.

Feeding broiler chickens with practical diets containing lupin seeds

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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 × 10³ 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 ileo-caecal 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 jejunal tissue, 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 pH340, 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 μm). Helium was used as the carrier gas. The oven was initially kept at 100°C for 2 min, then heated to 150°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 ≤ 0.05 and P ≤ 0.01.

Results

Growth performance

Yellow lupin cv. Parys contained more nutrients – crude protein and fat – than narrow-leaved lupin (Table 3). Experimental diets were formulated to contain the same level of apparent metabolizable energy (AMEₓ), assuming that the level of AMEₓ of narrow-leaved 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-leaved lupin
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).

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

<table>
<thead>
<tr>
<th>Dietary treatments</th>
<th>Jejunal mucosa</th>
<th>ileal digesta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>villus height</td>
<td>villus width</td>
</tr>
<tr>
<td>Control</td>
<td>1065</td>
<td>159 150</td>
</tr>
<tr>
<td>Control with enzyme</td>
<td>1050</td>
<td>138 148</td>
</tr>
<tr>
<td>Lupin species (L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>1035</td>
<td>166 151b</td>
</tr>
<tr>
<td>L. luteus</td>
<td>1011</td>
<td>163 145a</td>
</tr>
<tr>
<td>Lupin level, g · kg⁻¹ diet (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>1059a</td>
<td>165 151b</td>
</tr>
<tr>
<td>250</td>
<td>986b</td>
<td>163 145b</td>
</tr>
<tr>
<td>Enzyme supplement (E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without enzyme</td>
<td>1014</td>
<td>165 148</td>
</tr>
<tr>
<td>with enzyme</td>
<td>1031</td>
<td>163 148</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>44.5</td>
<td>9.2 3.8</td>
</tr>
</tbody>
</table>

Interactions (P value)  
L × D
L × E
d x E
L × D × E

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.

plmentation 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

<table>
<thead>
<tr>
<th>Dietary treatments</th>
<th>Total SCFA</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Iso-butyrate</th>
<th>Valerate</th>
<th>Iso-valerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>141.2</td>
<td>102.9</td>
<td>6.6</td>
<td>28.7</td>
<td>1.03a</td>
<td>1.18</td>
<td>0.79a</td>
</tr>
<tr>
<td>Control with enzyme</td>
<td>157.9</td>
<td>114.7</td>
<td>8.9</td>
<td>28.9</td>
<td>1.59a</td>
<td>1.90</td>
<td>1.77b</td>
</tr>
<tr>
<td>Lupin species (L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>156.0</td>
<td>114.8</td>
<td>8.9b</td>
<td>28.8</td>
<td>1.10</td>
<td>1.50</td>
<td>0.80</td>
</tr>
<tr>
<td>L. luteus</td>
<td>160.1</td>
<td>120.6</td>
<td>6.9a</td>
<td>29.8</td>
<td>0.90</td>
<td>1.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Lupin level, g · kg⁻¹ diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>156.4</td>
<td>117.8</td>
<td>7.1a</td>
<td>28.2</td>
<td>1.00</td>
<td>1.40</td>
<td>0.80</td>
</tr>
<tr>
<td>250</td>
<td>159.7</td>
<td>117.6</td>
<td>8.7b</td>
<td>30.4</td>
<td>0.90</td>
<td>1.50</td>
<td>0.70</td>
</tr>
<tr>
<td>Enzyme supplement (E)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without enzyme</td>
<td>153.0</td>
<td>115.3</td>
<td>8.2</td>
<td>26.5b</td>
<td>0.99</td>
<td>1.50</td>
<td>0.70</td>
</tr>
<tr>
<td>with enzyme</td>
<td>163.1</td>
<td>120.2</td>
<td>7.7</td>
<td>32.1a</td>
<td>0.97</td>
<td>1.50</td>
<td>0.80</td>
</tr>
</tbody>
</table>
| 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.

mements 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 supplementations 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.
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% yel-
low 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; Steen-
feldt et al., 2003; Olkowski et al., 2005; Olkowski,
2011). Sometimes results were even more adverse.
Olkowski et al. (2001) reported that apart from de-
creased feed intake and growth rate, some chickens
fed a diet containing 40% narrow-leafed lupin in the
first week of life showed signs of muscle paralysis
and skeletal deformity. Despite digesta viscosity not
having been measured in the last study, it can be rec-
ognized 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, diges-
ta 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 (NSPs) and oligosaccharides
characteristics found in the current study had detrimental
consequences on the feed conversion ratio, and they
were deeper in broilers fed with narrow-leafed lu-
pin compared with yellow lupin. The increase of
crypt depth may be connected with the greater vis-
cosity generated by narrow-leafed lupin. Formerly,
Iji et al. (2001) reported that crypt depth in the jeju-
dum 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 lu-
men. 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. Gener-
ally, shortening of the villi decreases the surface area
for nutrient absorption (Montagne et al., 2003). The
changes in intestinal morphology and digesta char-
acteristics 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 consti-
tute about 320 and 120 (L. luteus) to 400 and 76
(L. angustifolius) g · kg⁻¹ of seed DM, respectively

In the current study, there were interesting in-
teractions among lupin level and species. Increas-
ing the yellow lupin level in the diet resulted in
lower feed intake, BWG and final body weight,
while increasing the narrow-leafed lupin level re-
sulted 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 ad-
versely 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 pro-
tein 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 nei-
ther 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 il-
eum measured in the last study was very low and
amounted to about 24 mMol per bird. In the cur-
rent 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 lu-
pin compared with yellow lupin. The increase of
crypt depth may be connected with the greater vis-
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(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; Olkowska 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., 2010; Olkowski, 2011).

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.

Acknowledgements

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