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Effect of increasing levels of raw and extruded narrow-leafed lupin seeds in broiler diet on performance parameters, nutrient digestibility and AME_N value of diet

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ABSTRACT. In the first study conducted to investigate the nutritional value of raw and extruded narrow-leafed lupin (*Lupinus angustifolius*) seeds cv. Boruta, 60 male Ross 308 chickens of age 16–20 days were used (Experiment 1). In 35-day performance trial (Experiment 2), 960 1-day-old chickens were randomly allotted to 11 treatments and fed diets containing 0, 50, 100, 200, 250 or 300 g · kg^{-1} diet of raw or extruded narrow-leafed lupin seeds. In the first experiment, extrusion of narrow-leafed lupin seeds led to the decrease of neutral detergent fibre (NDF) concentration. Seed processing increased fat digestibility and nitrogen retention in chickens but had no effect on ileal digestibility of protein and amino acids. There was a tendency to increase the apparent metabolizable energy corrected to zero N balance (AME_N) after lupin extrusion. In the second experiment, the inclusion of increasing levels of raw or extruded seeds into diets quadratically decreased body weight gains (BWGs) of birds, except for broilers fed extruded lupin on days 15–35. The extrusion increased the BWGs of birds in the whole trial period (days 0–35). Increasing levels of raw and extruded narrow-leafed lupin seeds quadratically increased feed conversion ratio during the experiment. So, the extrusion of narrow-leafed lupin seeds had a limited positive effect on the chemical composition of seeds and growth performance of broiler chickens.

Introduction

The growing interest in cultivation and the introduction of legume seeds into animal and poultry diets has been observed in recent years. It was shown that biologically active legume proteins, rich in lysine, have antioxidant properties (Nalle et al., 2011; Smulikowska et al., 2013; Kaczmarek et al. 2014; Hejdysz et al. 2015, 2016a,b; Rutkowski et al., 2015, 2017). Antioxidants (such as selenium and vitamin E) and microelements (Zn, Cu, Mn) supporting the immune system are among other beneficial components of legume seeds (Wasilewko and Buraczewska, 1999; Lampart-Szczapa and Łoza, 2007).

Antinutritional factors present in seeds such as protease inhibitors, alkaloids, α-galactosides and tannins limit inclusion levels of legume seeds in diets, especially for young, growing animals (Smulikowska et al., 1999; Laudadio and Tufarelli, 2011b).

However, optimum inclusion levels of some of these substances are associated with health-promoting activities (Jamroz and Kubizna, 2008).

Different preparation methods of legume seeds, such as dehulling that decreases the content of dietary fibre and phenolic compounds or thermal and baro-thermal processing that reduces the activity of thermal-sensitive proteases and inhibitors, can improve the nutritional value of seeds (Leontowicz et al., 2001; Lampart-Szczapa et al., 2007; Laudadio and Tufarelli, 2011a).

In the previous studies in which yellow lupin seeds were used (raw or extruded) in broiler diets in a share of 5–30%, it was found that the extrusion improved fat digestibility of seeds and nitrogen retention in chickens, and enhanced the apparent metabolizable energy corrected to zero N balance (AME_N) value of seeds (Rutkowski et al., 2015). However, the inclusion of 25% or 30% of either raw or extruded yellow lupin seeds into diets significantly decreased the performance indices of broiler chickens. Using 10% or 20% of extruded seeds positively affected growth and feed conversion ratio of birds in comparison to those fed diets with raw seeds (Rutkowski et al., 2015).

Published data on the usefulness of narrow-leafed lupin (*Lupinus angustifolius*) seeds, as a replacement for soyabean meal, in broiler diets are limited and contradictory (Farrell et al., 1999; Rubio et al., 2003), with some studies showing lowered performance and others reporting performance similar to control diets at inclusion levels of over 200 $g \cdot kg^{-1}$. The positive effect of extrusion on broiler chicken performance was confirmed for yellow lupin seeds. There is no comparative information on the influence of extrusion on the nutritive value of narrow-leafed lupin and broiler chickens performance. Therefore, the objective of this study was to evaluate the effect of increasing levels of raw and extruded narrow-leafed lupin seeds on nutrient utilization, AME_N value and growth performance of broiler chickens.

Material and methods

Lupin seeds processing

Raw or extruded narrow-leafed lupin seeds (*Lupinus angustifolius*) cv. Boruta harvested in 2014 were used. Seeds were obtained from the Plant Breeding Stations in Wiatrowo (Poland). A portion of lupin seeds was extruded using a KMZ 2 extruder (Russia) (500 $kg \cdot h^{-1}$). The extrusion process was carried out under the following conditions: moisture 22%, time 10 s, temperature 135 ± 10 °C and pressure 30 $kg \cdot cm^{-2}$. The extruded seeds were allowed to cool to room temperature, reground to pass through a 3.18 mm sieve and stored at 4 °C.

Nutrient digestibility and growth performance trials

Experiment 1. The experiment was carried out on 60 16-day-old Ross 308 male chickens, reared from birth in cages and fed basal diets (Table 1). At 16 day of age, birds were randomly allotted to individual cages and assigned to three dietary treatments, 20 replicates in each. Broilers from control group were fed a basal diet, whereas broilers in experimental groups – two experimental diets containing 80% of the basal diet and 20% of raw or extruded lupin. For the determination of total or ileal digestibility, 3 $g \cdot kg^{-1}$ of TiO_2 was added to diets as an indigestible marker. All diets were offered *ad libitum* in a mash form. During the 5 days of accommodation, birds were also fed experimental diets. On days 19 and 20, the excreta of chickens were individually collected twice a day and immediately frozen for further analyses (n = 20 per treatment). On day 21, all chickens from each group were sacrificed by cervical dislocation and their ileums were removed. Digesta was flushed from the terminal ileum (15 cm, near the ileocecal junction) and pooled (two birds per sample) to provide adequate quantities for chemical analyses.

Table 1. Composition of basal diet, $g \cdot kg^{-1}$ (Experiment 1)

Components	$g \cdot kg^{-1}$
Maize	600.0
Soyabean meal (CP 44%)	292.6
Soyabean oil	41.6
Fish meal	29.4
Monocalcium phosphate	10.3
Chalk (<2 mm)	5.1
Premix[1], broiler	10.0
NaCl	2.9
$NaHCO_3$	0.1
DL-methionine	2.7
L-lysine	1.7
L-threonine	0.6
TiO_2	3.0
Analysed	
metabolizable energy, $MJ \cdot kg^{-1}$	12.85
crude protein	218.0
ether extract	71.9

[1] provided per kg diet: IU: vit. A 11250, cholecalciferol 2500; mg: vit. E 80, menadione 2.50, vit. B_{12} 0.02, folic acid 1.17, choline 379, D-pantothenic acid 12.5, riboflavin 7.0, niacin 41.67, thiamine 2.17, D-biotin 0.18, pyridoxine 4.0, ethoxyquin 0.09, Mn 73, Zn 55, Fe 45, Cu 20, I 0.62, Se 0.3, salinomycin 60

Experiment 2. In total, 960 1-day-old male Ross 308 chickens (initial individual weight: 42 ± 3 g) were randomly assigned to 11 dietary treatments (10 cages per replicate, 8 birds per treatment). In the experiment, in a control group 20 received replications were included. Birds were obtained from a commercial hatchery (DanHach Poland, Wolsztyn, Poland). Chickens were kept in cages (0.5 m^2, 8 birds in each) on straw litter. The environmental conditions were typical for broiler rearing; the lighting program in the first 7 days was 24 h · d^{-1}, and then, 18 h light:6 h darkness. The temperature was maintained at 32 °C during the first week and was gradually decreased to ~23 °C by the end of the third week.

Diets and drinking water were provided *ad libitum*. The chickens were fed starter (0–14 days of age) and grower diets (15–35 days) in a mash form. Diets containing 21–22% of crude protein (CP) and 12.6–13 MJ · kg^{-1} of metabolizable energy were used. Diets contained narrow-leafed lupin seeds (cv. Boruta) (50, 100, 200, 250 and 300 g · kg^{-1} diet) instead of soyabean meal (Tables 2 and 3). In treatments R50, R100, R200, R250 and R300, diets contained raw seeds, whereas in treatments E50, E100, E200, E250 and E300, analogous quantities of extruded lupin seeds were used. The control diet (C) did not contain lupin seeds.

Body weight and feed intake (FI) were monitored after 14 days and at the end of the experiment, with the cage serving as the experimental unit. Before weighing, the birds were fasted for 4 h. Mean weight gain, FI and feed conversion ratio (FCR) were used to determine the growth performance following any necessary corrections for mortality. Mortality in the experiments was very low and averaged 1.1%.

Table 2. Ingredient composition and nutrient content of starter diets containing different levels of narrow-leafed lupin meal in raw (R) or extruded form (E), g · kg^{-1} (Experiment 2)

Component	Starter										
	Control	R50	R100	R200	R250	R300	E50	E100	E200	E250	E300
Maize	468.0	448.4	422.6	368.9	359.1	326.7	448.4	422.6	368.9	359.1	326.7
Raw narrow-leafed lupin	-	50.0	100.0	200.0	250.0	300.0	50.0	100.0	200.0	250.0	300.0
Extruded narrow-leafed lupin	-	-	-	-	-	-	-	-	-	-	-
Soya oil	56.0	60.0	66.0	81.0	82.0	86.0	60.0	66.0	81.0	82.0	86.0
Soyabean meal (CP 44%)	306.5	272.0	242.0	180.0	138.0	115.8	272.0	242.0	180.0	138.0	115.8
Rapeseed meal (CP 34.9%)	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
DDGS (CP 36%)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Limestone (<2 mm)	4.4	4.4	4.3	4.4	4.6	4.5	4.4	4.3	4.4	4.6	4.5
Monocalcium phosphate	13.8	13.5	13.2	12.8	13.0	14.0	13.5	13.2	12.8	13.0	14.0
Premix[1], broiler	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$NaHCO_3$	3.6	4.0	2.9	3.6	3.6	3.6	4.0	2.9	3.6	3.6	3.6
NaCl	1.0	0.6	1.1	1.0	1.0	1.0	0.6	1.1	1.0	1.0	1.0
L-lysine HCl (98%)	3.7	3.9	3.9	4.4	4.4	4.0	3.9	3.9	4.4	4.4	4.0
DL-methionine	1.5	1.7	1.7	2.0	2.1	2.2	1.7	1.7	2.0	2.1	2.2
L-threonine	0.9	0.9	1.1	0.9	1.0	0.9	0.9	1.1	0.9	1.0	0.9
L-valine	0.5	0.5	1.0	0.7	0.9	1.0	0.5	1.0	0.7	0.9	1.0
L-tryptophan	0.1	0.1	0.2	0.3	0.3	0.3	0.1	0.2	0.3	0.3	0.3
Calculated											
metabolizable energy, MJ · kg^{-1}	12.14	12.11	12.11	12.12	12.14	12.11	12.11	12.11	12.12	12.14	12.11
lysine (digest.)	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8
methionine (digest.)	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
threonine (digest.)	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
calcium	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6
sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
chlorine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Analysed											
crude protein, g · kg^{-1}	218.0	219.0	220.0	218.0	221.0	220.0	220.0	221.0	219.0	220.0	221.0

DDGS – dried distillers with solubles; [1] provided per kg diet: IU: vit. A 11250, cholecalciferol 2500; mg: vit. E 80, menadione 2.50, cyanocobalamine 0.02, folic acid 1.17, choline 379, D-pantothenic acid 12.5, riboflavin 7.0, niacin 41.67, thiamine 2.17, D-biotin 0.18, pyridoxine 4.0, ethoxyquin 0.09, Mn 73, Zn 55, Fe 45, Cu 20, I 0.62, Se 0.3, salinomycin 60

Table 3. Ingredient composition and nutrient content of grower diets containing different levels of narrow-leafed lupin meal in raw (R) or extruded form (E), g · kg^{-1} (Experiment 2)

Component	Grower										
	Control	R50	R100	R200	R250	R300	E50	E100	E200	E250	E300
Maize	500.0	473.3	451.9	400.0	385.1	357.1	473.3	451.9	400.0	385.1	357.1
Raw narrow-leafed lupin	-	-	-	-	-	-	-	-	-	-	-
Extruded narrow-leafed lupin	-	50.0	100.0	200.0	250.0	300.0	50.0	100.0	200.0	250.0	300.0
soya oil	79.0	85.0	89.0	102.0	106.0	107.0	85.0	89.0	102.0	106.0	107.0
soyabean meal (CP 44%)	253.2	224.1	192.0	130.0	90.0	65.8	224.1	192.0	130.0	90.0	65.8
rapeseed meal (CP 34.9%)	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
DDGS (CP 36%)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
limestone (<2 mm)	3.3	3.1	3.1	3.1	3.2	3.3	3.1	3.1	3.1	3.2	3.3
monocalcium phosphate	14.9	14.6	14.4	14.0	14.5	15.0	14.6	14.4	14.0	14.5	15.0
Premix[1], broiler	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$NaHCO_3$	3.6	4.0	2.9	3.6	3.6	3.6	4.0	2.9	3.6	3.6	3.6
NaCl	1.0	0.7	1.1	1.0	1.0	1.0	0.7	1.1	1.0	1.0	1.0
L-lysine HCl (98%)	3.0	3.1	3.2	3.5	3.5	3.9	3.1	3.2	3.5	3.5	3.9
DL-methionine	1.2	1.3	1.4	1.6	1.8	1.9	1.3	1.4	1.6	1.8	1.9
L-threonine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
L-valine	0.1	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
L-tryptophan	0.1	0.1	0.2	0.3	0.3	0.3	0.1	0.2	0.3	0.3	0.3
Calculated											
metabolizable energy, MJ · kg^{-1}	12.76	12.69	12.75	12.71	12.78	12.74	12.69	12.75	12.71	12.78	12.74
lysine (digest.)	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
methionine (digest.)	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
threonine (digest.)	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
calcium	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7
sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
chlorine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Analysed											
crude protein, g · kg^{-1}	210.0	209.0	211.0	213.0	212.0	209.0	212.0	213.0	209.0	212.0	209.0

DDGS – dried distillers with solubles; [1] provided per kg diet: IU: vit. A 11250, cholecalciferol 2500; mg: vit. E 80, menadione 2.50, cyanocobalamine 0.02, folic acid 1.17, choline 379, D-pantothenic acid 12.5, riboflavin 7.0, niacin 41.67, thiamin 2.17, D-biotin 0.18, pyridoxine 4.0, ethoxyquin 0.09, Mn 73, Zn 55, Fe 45, Cu 20, I 0.62, Se 0.3, salinomycin 60

Chemical analyses

Representative samples of both raw and extruded lupin seeds and experimental diets were ground to pass through a 0.5 mm sieve. The AOAC International (2007) methods 934.01, 976.05, 920.39, 942.05 and 973.18 were used for dry matter (DM), CP, ether extract (EE), acid detergent fibre (ADF) and neutral detergent fibre (NDF) analyses, respectively. All samples were analysed in duplicate.

The amino acid (AA) content was determined using an AAA-400 Automatic Amino Acid Analyzer (Ingos, Prague, Czech Republic) with ninhydrin for post-column derivatization. The samples were hydrolysed with 6 N HCl for 24 h at 110 °C (procedure 994.12; AOAC International, 2007). Gross energy (GE) was determined using an adiabatic bomb calorimeter (KL 12Mn; Precyzja-Bit PPHU, Bydgoszcz, Poland) standardized with benzoic acid. Titanium dioxide was determined according to Short et al. (1996), with samples prepared according to the procedure of Myers et al. (2004).

Raffinose family oligosaccharides were analysed by gas-liquid chromatography as described by Zalewski et al. (2001). Phytate phosphorus was determined using the procedure of Haug and Lantzsch (1983). The water extract viscosity (WEV) of lupin seeds was measured *in vitro*. Prior to the determination of WEV, lupin samples were ground in a mill using a 0.5 mm sieve. One gram of sample was mixed with 5 ml of distilled water for 1 h at 40 °C. The samples were centrifuged at 10 000 *g* for 10 min at 4 °C, the supernatant was withdrawn, and viscosity was determined in a Brookfield Digital DV-II+ cone/plate viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) maintained at 40 °C at a shear rate of 12 s^{-1}. The WEV units are mPas · s = cP = 1 × 100 dyne · s · cm^{-2}.

The energy values of diets were calculated based on the European Tables of Energy Values of Feeds for Poultry (World's Poultry Science Association, 1989) and Polish Poultry Feeding Standards (Smulikowska and Rutkowski, 2005).

Calculations of nutrient digestibility

Using CP as an example, the following equation was used for the calculation of digestibility (apparent ileal digestibility and apparent total tract digestibility), N retention and dry matter (DM) retention as well as AME_N of various dietary components of the basal and experimental diets:

$$DC = \{1 - [(TiO_{2[g \cdot kg^{-1} diet]} / TiO_{2[g \cdot kg^{-1} DIG\ or\ EX]}) \times (CP_{[g \cdot kg^{-1} DI\ or\ EX]} / CP_{[g \cdot kg^{-1} diet]})]\} \times 100\%$$

$$AME_{N[kcal \cdot kg^{-1}]} = [GE_{[kcal \cdot kg^{-1} of\ EX]} \times (TiO_{2[g \cdot kg^{-1} diet]} / TiO_{2[g \cdot kg^{-1} EX]})] - 34.4 \times [(N_{[g \cdot kg^{-1} EX]} - (TiO_{2[g \cdot kg^{-1} diet]} / TiO_{2[g \cdot kg^{-1} EX]}]$$

where: DC – digestibility coefficient, DIG – digesta, EX – excreta, GE – gross energy, N – nitrogen and 34.4 – energy equivalent of uric acid nitrogen (Hill and Anderson, 1958).

AME_N of all experimental diets was calculated using the previous equations and was corrected to zero nitrogen balance using 34.4 kJ · g^{-1} N retained (Hill and Anderson, 1958).

The following equation was used to calculate the digestibility coefficients of various dietary components and the AME_N level of lupin seeds:

$$DC = (DC_{[CP\ pea\ diet]} \times C_{[g \cdot kg^{-1} CP\ lupin\ diet]} - D_{[CP\ basal\ diet]} \times C_{[g \cdot kg^{-1} CP\ basal\ diet]} \times 0.20) / (C_{[g \cdot kg^{-1} CP\ lupin\ diet]} - C_{[g \cdot kg^{-1} CP\ basal\ diet]} \times 0.20) \times 100\%$$

$$AME_{N[kcal \cdot kg^{-1}]} = (AME_{N[kcal \cdot kg^{-1} lupin\ diet]} - (AME_{N[kcal \cdot kg^{-1} basal\ diet]} \times 0.8) / 0.2)$$

where: DC – digestibility coefficient of CP in narrow-leafed lupin seeds; $DC_{[CP\ lupin\ diet]}$ – digestibility coefficient of CP in narrow-leafed lupin diet; $C_{[g \cdot kg^{-1} CP\ lupin\ diet]}$ – concentration of CP in narrow-leafed lupin diet; 0.20 – amount of investigated narrow-leafed lupin in narrow-leafed lupin diet; $D_{[CP\ basal\ diet]}$ – digestibility coefficient of CP in basal diet; $C_{[g \cdot kg^{-1} CP\ basal\ diet]}$ – concentration of CP in basal diet; $AME_{N[kcal \cdot kg^{-1}]}$ – metabolizable energy of narrow-leafed lupin seeds; $AME_{N[kcal \cdot kg^{-1} lupin\ diet]}$ – metabolizable energy of narrow-leafed lupin diet; $AME_{N[kcal \cdot kg^{-1} basal\ diet]}$ – metabolizable energy of basal diet; and 0.80 – amount of basal diet in narrow-leafed lupin diet.

Statistical analysis

Experiment 1. All data were earlier explored to discard any possible outliers. Statistical analyses were performed using procedures of SAS® v. 9.1 package (SAS Institute Inc., Cary, NC, USA) (distribution analyses; outliers were defined as observations that were in the distance bigger than 3 times standard deviation). The obtained results were subjected to Student t-test analysis, at the level of $P < 0.05$.

Experiment 2. Statistical calculations were conducted using the SAS® v. 9.1 package (SAS Institute Inc., Cary, NC, USA). Both the mean values and the standard error of the mean were calculated for all traits. Differences among treatments and experimental factors were determined by using the two-way linear model of analysis of variance:

$$Y_{ijk} = \alpha_j + \beta_k + (\alpha\beta)_{jk} + e_{ijk}$$

where: Y_{ijk} – value of analysed trait; α_j – constant effect of i^{th} lupin seed; β_k – constant effect of j^{th} extrusion; $(\alpha\beta)_{jk}$ – interaction between α and β; and e_{ijk} – effect of experimental error.

Orthogonal contrasts were used to establish the difference between treatments. The used contrasts were control treatment vs other five dietary treatments (50, 100, 200, 250 and 300 g of lupin seeds per kg of feed) as well as the contrast for linear (L) and quadratic (Q) effects. All data are presented as means with pooled standard error of the mean.

Results

Experiment 1. The extrusion of narrow-leafed lupin seeds led to the decrease in NDF content by 49 g · kg^{-1} DM (Table 4). The GE, crude fat, crude ash, CP and AA contents were similar in both raw and extruded seeds. The contents of alkaloids and oligosaccharides were not affected by seed processing (Table 4).

The extrusion of lupin did not significantly affect DM retention, but there was a tendency to improve DM retention ($P = 0.088$). It increased the apparent total tract digestibility of crude fat ($P < 0.05$) from 73.1 to 86.5% as well as nitrogen retention from 45.7 to 55.5% in comparison to raw seeds (Table 5). Seed extrusion resulted in a tendency to enhance the apparent AME_N value of narrow-leafed lupin by about 0.5 MJ · kg^{-1} ($P = 0.075$).

The dietary inclusion of extruded lupin seeds did not significantly affect the ileal digestibility of CP (Table 5) but extruded lupin seeds were characterized by lower digestibility coefficients ($P < 0.01$) for methionine and proline.

Experiment 2. There was observed the significant effect of the level of narrow-leafed lupin seed

Table 4. Chemical composition of narrow-leafed lupin seeds (cv. Boruta), in dry matter basis

Indices	Lupin seeds	
	raw	extruded
Dry matter, g · kg^{-1}	886.2	889.5
Crude ash	37.8	38.1
Crude protein	385.8	389.3
ADF	214.3	219.7
NDF	259.2	210.5
Gross Energy, MJ · kg^{-1}	20.73	20.78
WEV, cP	1.61	1.68
Amino acids, % protein		
Asp	8.91	8.92
Thr	3.15	3.12
Ser	4.11	4.14
Glut	23.77	23.81
Pro	6.52	6.54
Cys	1.11	1.14
Met	0.47	0.49
Gly	4.01	4.03
Ala	3.33	3.37
Val	3.72	3.70
Iso	3.68	3.62
Leu	6.64	6.66
Tyr	3.07	3.08
Phe	3.46	3.50
His	2.91	2.93
Lys	4.49	4.53
Arg	11.65	11.67
Antinutrients		
total alkaloids, mg · kg^{-1}	44.0	43.0
angustifoline, % of total alkaloids	12.5	12.5
izolupaninine, % of total alkaloids	4.6	4.6
lupanine, % of total alkaloids	56.2	56.1
130H lupanine, % of total alkaloids	26.7	26.8
oligosaccharides, g · kg^{-1}	87.7	88.0
rafinose, g · kg^{-1}	12.0	11.8
stachyose, g · kg^{-1}	56.1	56.3
verbascose, g · kg^{-1}	19.6	19.9
total P, g · kg^{-1}	7.23	7.22
phytate P, g · kg^{-1}	4.42	4.21
non-phytate P, g · kg^{-1}	2.81	3.01

NDF – neutral detergent fibre, ADF – acid detergent fibre, WEV – water extract viscosity

Table 5. Coefficients of total tract apparent digestibility of fat, dry matter and nitrogen retention, AME_N as well as apparent ileal nutrient digestibility of narrow-leafed lupin seeds cv. Boruta in chickens (Experiment 1)

Indices	Lupin seeds		SEM	Significance
	raw	extruded		
Dry matter retention, %	59.1	62.4	1.01	0.088
Nitrogen retention, %	45.7	55.5	2.11	0.048
Apparent total tract crude fat digestibility, %	73.1	86.5	1.64	<.0001
AME_N, MJ · kg^{-1}	8.80	9.36	0.07	0.075
Apparent ileal digestibility				
dry matter	61.3	64.4	1.80	0.267
crude protein	81.7	82.8	0.89	0.396
Asp	81.4	80.6	1.01	0.615
Thr	78.9	79.1	1.01	0.906
Ser	80.8	80.0	1.02	0.632
Glut	89.3	88.2	0.69	0.322
Pro	87.8	81.0	0.96	0.006
Cys	80.9	83.0	1.52	0.367
Met.	95.1	93.6	0.32	0.007
Gly	78.7	79.5	1.27	0.704
Ala	84.2	84.5	0.89	0.858
Val	83.2	82.1	0.98	0.481
Iso	84.9	84.0	0.85	0.507
Leu	86.6	83.8	1.36	0.190
Tyr	80.4	82.3	0.82	0.154
Phe	85.4	84.6	0.75	0.474
His	83.3	81.1	1.12	0.206
Lys	88.0	87.7	0.74	0.774
Arg	91.6	91.2	0.56	0.770

SEM – standard error of mean, AME_N – apparent metabolizable energy corrected to zero N balance

in all three examined periods (days 0–14, 15–35 and 0–35). Increasing level of raw narrow-leafed lupin in the diet for broiler chickens decreased quadratically the body weight gain (BWG) during all studied experimental periods (Table 6). In the case of extruded seeds, the decrease of BWG was not confirmed for period from days 15 to 35 of the experiment. There was no effect of extrusion on BWG from days 0 to 14 as well as from days 15 to 35 of the experiment, whereas in the whole experimental period (days 0–35), the positive effect of extrusion was confirmed. Birds fed different lupin levels in extruded form were characterized by higher BWG than birds fed raw seeds. The interaction between extrusion and inclusion levels on BWG was not found in the study.

There was no effect of lupin inclusion as raw form on FI. Birds fed extruded lupin were characterized by quadratical decrease of FI from days 0 to 14 of the experiment ($P < 0.037$), whereas in the remaining periods, there was no statistically significant impact ($P > 0.05$). Extrusion did not change FI in the whole experimental period. Furthermore, no interaction was found between experimental factors.

The inclusion of narrow-leafed lupin seeds in raw and extruded forms into diet for broilers did not affect FCR in young birds (days 0–14), whereas in the remaining two studied periods (days 15–35 and 0–35) the quadratical increase of FCR was

Table 6. Growth performance of birds fed diets containing narrow-leafed lupin seeds in raw or extruded form

Indices	Raw							Extruded							Significance						
															E	LU	E × LU	raw		extruded	
	0	50	100	200	250	300	SEM	0	50	100	200	250	300	SEM				L	Q	L	Q
BWG, g																					
0–14 d	437	429	422	424	417	421	2.43	437	438	424	410	409	402	3.31	0.271	0.042	0.367	0.316	0.022	0.198	<.001
15–35 d	1421	1430	1361	1333	1347	1343	11.4	1421	1485	1358	1388	1355	1371	11.2	0.072	<.001	0.599	0.901	<.001	0.111	0.325
0–35 d	1882	1859	1785	1736	1759	1762	12.6	1882	1900	1833	1822	1782	1771	12.3	0.039	0.004	0.760	0.444	<.001	0.238	0.003
FI, g																					
0–14 d	546	530	532	539	542	542	3.87	546	546	539	516	525	540	4.25	0.623	0.832	0.355	0.532	0.875	0.838	0.037
15–35 d	2456	2430	2444	2340	2409	2432	13.9	2456	2500	2403	2464	2392	2471	15.9	0.225	0.489	0.294	0.724	0.073	0.755	0.439
0–35 d	2991	2959	2951	2883	2950	2968	16.8	2991	3046	2942	2981	2921	3011	17.3	0.240	0.507	0.578	0.895	0.193	0.684	0.303
FCR, $g \cdot g^{-1}$																					
0–14 d	1.27	1.24	1.27	1.26	1.28	1.29	0.01	1.27	1.25	1.28	1.26	1.28	1.35	0.01	0.562	0.186	0.306	0.540	0.105	0.278	0.084
15–35 d	1.71	1.71	1.79	1.83	1.77	1.81	0.01	1.71	1.71	1.74	1.75	1.77	1.81	0.01	0.374	0.402	0.738	0.909	<.001	0.475	0.041
0–35 d	1.60	1.60	1.66	1.69	1.66	1.68	0.01	1.60	1.61	1.63	1.67	1.65	1.70	0.01	0.223	0.438	0.562	0.662	<.001	0.275	0.002

SEM – pooled standard error of mean, E – extrusion effect, LU – narrow-leafed lupin level effect, E × LU – interaction between E and LU, L and Q – linear and quadratic effect of increasing doses of lupin seeds in the diet, respectively, BWG – body weight gain, FI – feed intake, FCR – feed conversion ratio; each value represents mean of 10 replicates (except of control groups in which n = 20)

confirmed regardless lupin seed form. There was no effect of extrusion on FCR; moreover, an interaction between experimental factors was also not confirmed.

Discussion

During the past few years, modifications of lupin genotypes were focused on the improvement of agronomical traits, as well as on lowering the alkaloid content and increasing CP levels. The chemical composition of narrow-leafed lupin cv. Boruta used in this study was similar to older Polish cultivars of narrow-leafed lupin (Gdala and Buraczewska, 1996). Australian cultivars of lupin, as demonstrated by Nalle et al. (2011), are characterised by lower CP and similar EE concentration in comparison to our results. The narrow-leafed lupin cultivar used in this study was characterized by at least 10-fold lower alkaloids content in comparison to the older narrow-leafed cultivars (Gdala and Buraczewska, 1996). The total oligosaccharide content determined in our study was higher than that reported by Gdala and Buraczewska (1996). According to previous research by Lahuta and Górecki (2011), the content of oligosaccharides in lupin seeds increases under drought conditions, so the observed differences could result from different environmental conditions during growth.

Compared to yellow lupin (Wasilewko and Buraczewska, 1999; Rutkowski et al., 2016), narrow-leafed lupin seeds used in this study contained about 2 times more total alkaloids (44 $mg \cdot kg^{-1}$) with the highest concentrations of lupamines and angustifoline. Although the contents of oligosaccharides were similar to those of narrow-leafed lupin and yellow seeds, the amount of phytate P was 7.0 $g \cdot kg^{-1}$ and higher in the latter than 4.42 $g \cdot kg^{-1}$ in the former. In the earlier study of Rutkowski et al. (2016), the use of extrusion of yellow lupin seeds led to relatively minimal changes in nutrient composition, including AAs and antinutritional factors. Only the content of phytate P was reduced. In the present study, processing had no effect on chemical composition of narrow-leafed lupin seeds, except NDF content. Lower concentrations of this ingredient in extruded seeds had also been confirmed in our earlier study (Hejdysz et al., 2016a,b) and by other researchers (Diaz et al. 2006; Sobota et al., 2010). According to Sobota et al. (2010), low concentration of NDF in extruded seeds is caused by the decomposition of insoluble fractions into smaller molecules that are soluble in the NDF solution. These smaller soluble molecules were probably appearing in the nitrogen free extract. Changes in the ratio of soluble to insoluble fibre fractions in extruded seeds have been demonstrated in other studies (Martín-Cabrejas et al., 1999).

In some studies (Diaz et al., 2006), the beneficial effect of extrusion on digestibility coefficients of nutrients and reduction of the activity of thermolabile antinutritive substances was established. Additionally, in the study conducted by Rutkowski et al. (2016) and in the present one, the use of extrusion improved the crude fat digestibility and nitrogen retention in broiler chickens fed diet containing legume seeds. Interestingly, extrusion significantly improved AME_N value of yellow lupin but in a lesser extent modulated the AME_N value of narrow-leafed seeds (only tendency was confirmed). This effect may be the consequence of changes of the physicochemical structure of dietary fibre components and improved fat digestibility

In this study, extrusion did not affect the ileal digestibility of CP and most AAs in broilers fed narrow-leafed lupin. These results are in contrast with those observed by Leontowicz et al. (2001) and in disagreement with the earlier study on yellow lupin by Rutkowski et al. (2016). Such discrepancies were probably due to no effect of extrusion on phytate P content in seeds, which was confirmed in the study on yellow lupin. The negative correlation between the concentration of phytate P and CP and AA digestibility was confirmed in a study on faba bean (Hejdysz et al., 2016a). Some authors have speculated that the mechanism of phytate action on CP and AAs can be explained by the presence of protein–phytate complexes in feedstuffs, the *de novo* formation of binary and ternary protein–phytate complexes in the digestive tract, phytate inhibition of proteolytic enzymes, and exacerbation of endogenous AA losses (Selle et al., 2006). It is difficult to explain why extrusion did not affect the phytic acid content in narrow-leafed lupin seeds, whereas lower phytate P concentration was confirmed in the studies on faba bean, pea and yellow lupin (Hejdysz et al., 2016a,b; Rutkowski et al., 2016).

The positive experience of many authors concerning the effect of lupin extrusion on growth performance of poultry (Diaz et al., 2006; Lampart-Szczapa et al., 2007; Rutkowski et al., 2016) has been partly confirmed in this study. Better BWG of broilers fed diet with extruded lupin obtained in our study was probably caused by higher apparent total tract digestibility of crude fat and AME_N (tendency). Therefore, the negative effect of increasing lupin antinutritional factors in the diet with extruded seeds on bird BWG was lower in comparison to broilers fed diets with increasing level of raw narrow-leafed lupin. Extrusion of narrow-leafed lupin seeds did not positively affect the FCR in chickens fed diets with increased levels of seeds, which could be explained by no effect of extrusion on the content of antinutritional factors in lupin seeds and particle size of extruded narrow-leafed lupin seeds. According to Lv et al. (2015), particle size distribution of feed raw materials could affect nutrient utilization. The particle size of extruded narrow-leafed lupin after reground to pass through a 3.18 mm sieve was very fine. In more recent years, however, it has been thought that a large particle size aided by some structural components is beneficial to gizzard functions and gut development (Svihus et al., 2004; Choct, 2009). Therefore, it can be speculated that the one of factors responsible for the lack of positive impact of extrusion on broiler chicken performance is the particle size of extruded seeds.

Increasing level of narrow-leafed lupin seeds in diet depressed the growth performance in broiler chickens. Similar to the study presented by Steenfeldt et al. (2003), the substitution of soyabean meal and maize with 200 g narrow-leafed lupins per kg of broiler diets depressed BW and FCR. In comparison to our earlier study on yellow lupin (Rutkowski et al., 2016), broiler chickens tolerated 2 times lower concentration of narrow-leafed lupin than yellow lupin in the diet. Kaczmarek et al.(2014) showed a negative correlation between WEV and AME_N of narrow-leafed lupin seeds, as well as AA digestibility. Therefore, it could be speculated that the negative effect of narrow-leafed lupin could be connected to increased ileal digesta viscosity.

Conclusions

The extrusion of narrow-leafed lupin seed had a limited effect on its chemical composition. Extrusion influenced positively body weight gains of broiler chickens, whereas the effect of extrusion on feed intake and feed conversion ratio was not confirmed. An important observation was that the increasing levels of raw and extruded narrow-leafed lupin seeds quadratically worsened the body weight gain and feed conversion ratio during the experiment.

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