



MICROBIAL PHYTASE IMPROVES PERFORMANCE AND BONE TRAITS IN BROILERS FED DIETS BASED ON SOYBEAN MEAL AND WHITE LUPIN (*LUPINUS ALBUS*) MEAL*

Marta Kubiś¹, Sebastian Kaczmarek^{1*}, Marcin Hejdysz¹, Robert Mikuła¹, Zuzanna Wiśniewska¹, Ewa Pruszyńska-Oszmałek², Paweł Kołodziejewski², Maciej Sassek², Andrzej Rutkowski¹

¹Department of Animal Nutrition, Poznan University of Life Sciences,
Wołyńska 33, 60-637 Poznań, Poland

²Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences,
Wołyńska 35, 60-637 Poznań, Poland

*Corresponding author: sebastian.kaczmarek@up.poznan.pl

Abstract

For several years in Europe, there has been a growing interest in the use of native sources of protein (e.g. lupin seeds) in poultry nutrition. The current study aimed to investigate the effectiveness of a microbial phytase in broiler diets with the addition of different levels of white lupin seeds. A total of 480 one-day-old male broiler chickens (Ross 308) were randomly divided into six dietary treatments (10 replications/8 birds per group). The basal diet contained SBM as the main protein source and experimental treatments were prepared with white lupin meal (WLM) at 3 levels (0, 10, and 20%) and with or without phytase inclusion. The experiment was divided into two feeding periods: from 1 to 14 days (starter) and from 15 to 35 days (grower). Diets with phytase addition were deficient in Ca and non-phytate P. All diets were fed in mash form and offered *ad libitum*. On day 35 excreta were collected and on day 36, ten chickens from each group were euthanized and blood, tibia, and digesta samples were collected for further analysis. The 20% addition of WLM negatively increased the content of phytic-P. The results showed that feed conversion ratio (FCR) and body weight gain (BWG) were not affected by phytase inclusion but by the WLM level alone. In addition, birds fed the diet with 20% WLM were characterized by having the lowest BWG and the highest FCR of all groups. There were no significant differences in feed intake (FI) among chickens fed control and experimental treatments. Regardless of the white lupin level addition, phytase addition improved ($P < 0.001$) nitrogen-corrected apparent metabolizable energy (AME_N). In conclusion, the addition of phytase positively influenced the performance and availability of minerals (Ca and P) regardless of the level of WLM used. However, with regard to the use of WLM in poultry nutrition, it can be assumed that 10% addition is safe and does not affect performance.

Key words: broiler, microbial phytase, white lupin meal

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In recent years, there has been a significant increase in the interest of using lupin seeds in monogastric animal diets (Nalle *et al.*, 2012; Kaczmarek *et al.*, 2015). In Poland, the following three varieties of lupins are commonly grown: white, yellow, and narrow-leaved. In the past decades, their use in poultry diets was almost impossible because of their high content of alkaloids and non-starch polysaccharides (NSPs) (Steenfeldt *et al.*, 2003). Thanks to plant breeders efforts, it is now possible to develop varieties that are nearly completely free of alkaloids, which enabled the inclusion of lupins in feed for poultry and other monogastric animals. However, in lupin cultivars, phytates are still found in large amounts, which can negatively affect feed utilization by birds (Kaczmarek *et al.*, 2016; Kubiś *et al.*, 2018).

One of the most expensive mineral feed components in poultry diets is phosphorus (P) (Li *et al.*, 2018). Most of the P content in plants occurs in the indigestible form of phytic-P. The ability to use phytic-P by monogastric animals is very low due to the minimal amount of endogenous phytase in their digestive tract (Maenz and Classen, 1998), and hence, for this reason, the use of exogenous sources of P (calcium phosphate) is necessary to avoid P deficiency in poultry metabolism.

Phytic acid interacts with proteins, amino acids (AAs), and proteolytic enzymes, causing a reduction in AA digestibility. Phytate forms complexes with minerals such as Mg, Ca, Cu, and Zn (which reduces their availability and digestibility). The phytate-calcium complex negatively affects the digestibility of fats; in the gastrointestinal tract (GIT), it combines with fatty acids to form insoluble soaps in the intestinal lumen. Phytic acid can also bind with starch, which leads to a reduction in the digestibility of carbohydrates (Woyengo and Nyachoti, 2013).

Researchers agree that exogenous phytase improves the utilization of mineral components, causing, for example, an increase in the content of crude ash, Ca, and P in the bones of birds (Alabi *et al.*, 2019).

It is also known that phytase can improve the digestibility of the protein, by reducing endogenous AA loss and by an improvement in the retention of dietary AAs (Cowieson *et al.*, 2004). Research conducted over the years has shown that the efficiency of phytase in improving the use of dietary protein is not the same every time and these differences can be due to several reasons. It seems that the most important factor is the location of phytic compounds in the plant. Some plants store phytic acid and protein in the form of globoids located in vacuoles for protein. Depending on the plant species, globoids may contain 40% of the protein and approximately 40% of phytic acid. Their presence may contribute to the reduction of phytase efficiency for the availability of protein, and therefore, it may be assumed that the globoids are partially or completely resistant to the direct action of phytase (Jiang *et al.*, 2001). It is known that corn contains about 90% of its phytate within the germ portion of the kernel. In oilseeds such as soy, phytate is bound to protein bodies and evenly distributed throughout the seed. Phytate in peanuts, cotton seeds, and sunflower seeds is concentrated in substructures called crystalloids or globoids, which are located within the protein body membrane (Biehl and Baker, 1997). In the literature, there are no data on the location of phytates in legume seeds, so it is difficult to predict the phytase effect. The aim of the present study was to determine the effectiveness of phytase in diets containing different inclusion levels of white lupin seeds on broiler performance and mineral bioavailability.

Material and methods

Lupin seeds and phytase

Seeds of white lupin (*Lupinus albus* L., Butan cv.) were obtained from the Plant Breeding Stations located in Przebędowo and Wiatrowo, Poland. The chemical composition of seeds is presented in Table 1.

Table 1. Chemical composition of soybean meal (SBM)* and white lupin meal (WLM) used in the experiment

	SBM (g/kg)	WLM (g/kg)
Dry matter	909	889
Crude protein (N × 6.25)	438	341
Crude fat	30.10	98.70
ADF ¹	6.42	21.80
NDF ²	9.71	23.80
Ca	3.00	3.50
P	6.10	7.25
Phytic-P	3.70	6.30
RFO ³	56.22	99.60
Raffinose	11.40	10.40
Stachyose	44.50	62.80
Verbascose	0.32	26.40
Total alkaloids	(mg/kg)	150
Angustifolia	n.d. ⁴	14.90
Isolupanine	n.d.	4.02
Lupanine	n.d.	106
13–0 H Lupanine	n.d.	24.90

Abbreviations: *hulled soybean seeds were used, ¹ADF, acid detergent fiber; ²NDF, neutral detergent fiber; ³RFO, raffinose family oligosaccharides; ⁴n.d., not detected.

The supplemental phytase used was RONOZYME HiPhos (DSM Nutritional Products, Kaiseraugst, Switzerland) containing 5000 U of phytase activity/g, where 1 U of phytase activity is defined as the quantity of enzyme required to produce 1 mmol of inorganic P/min for 5.1 mmol/L sodium phytate at 5.5 pH and 37°C. The phytase was added on top.

Bird management and sample collection

All animal procedures were conducted in accordance with the guidelines of the Polish Council of Animal Care. The protocol for this study was approved by the Local Animal Care Committee of the Poznan University of Life Sciences.

The experiment was conducted with 480 one-day-old male broiler chickens (Ross 308). Birds were obtained from a commercial hatchery (DanHatch Poland SA, Wolsztyn, Poland). Before being assigned to treatments, 10% of all birds were weighed to estimate their initial body weight (40 ± 2 g). Chicks were randomly distributed into 6 dietary treatments each consisting of 10 replicates of 8 birds. The study consisted of a completely randomized experimental design with three different levels of WLM (0%, 10%, and 20%) and with or without phytase. Birds were reared on wood-shaving litter in 1.2×0.8 m floor pens. For the first 7 days, birds were exposed to light for 24 hours, then the light program was changed to 18 hours of lights and 6 hours of darkness. The temperature was manually controlled, in the first week it was 32°C and then it was gradually reduced until reaching 23°C at the end of the 3rd week of the experiment. The house was ventilated using stirring fans and manually operated curtains. Water was available throughout the experiment *ad libitum*. Each pen was equipped with three nipple drinkers.

Collection trays were installed in floor pens on day 35 for excreta collection. Five excreta samples per treatment (randomly chosen), free from contamination (feed and feathers), were collected per day. One excreta sample represented one randomly chosen pen (eight birds and 5 pooled collections, made over 10 h at 2 h intervals). On day 36, 10 chickens from each group (and each pen/replication) were sacrificed by cervical dislocation, according to the recommendations for euthanasia of experimental animals (Close et al., 1997). From these birds, a blood sample was collected, and then the blood serum was obtained by centrifugation for 10 min at 3500×g, and then frozen at -80°C until further analysis. Next from this same birds the ileum and left tibiae were removed. Ileum digesta were flushed from the terminal ileum (15 cm, adjacent to the ileocecal junction) and pooled (two birds/sample) to obtain sufficient sample for chemical analysis ($n = 10$). Tibiae were kept frozen until further analysis.

Experimental diets

Experimental diet compositions are presented in Table 2. The experiment was divided into two feeding periods: from day 1 to 14 (starter period) and from day 15 to 35 (grower period). All diets were fed in a mash form and provided *ad libitum* from day 1 to day 35. All diets were supplemented with TiO_2 (3 g/kg) to determine digestibility levels. Grower diets with phytase addition were deficient in Ca, available P. Other nutrients met or exceeded Aviagen recommendations for ROSS 308 broiler chickens (AVIAGEN, 2014). Starter diets met or exceeded Aviagen recommendations for ROSS 308 broiler chickens. All diets were formulated to obtain similar digestible AAs level across diets. To maximize the homogeneity of the added phytase, an appropriate amount of phytase was mixed with a small quantity of the feed as a premix, which was then added to the feed to obtain the final concentration. This procedure was followed by the addition of the screened and premixed portion to a stainless-steel horizontal feed mixer (Zuptor 100) for proper mixing of the completed diet. Mixing time was 4 min, mixing band 27.4 rev/min. Before mixing, all ingredients were ground using a Skiold Disc mill (SK2500) with disc distance set at 1.8 mm. In total, six independent starter and grower diets were prepared.

Table 2. Composition and calculated and determined analysis results of the experimental diets

Protein source Enzyme	WLM 0%		WLM 10%		WLM 20%		WLM 0% +		WLM 10% +		WLM 20% +	
	-		-		-		-		-		-	
	1-14d	15-35d	1-14d	15-35d	1-14d	15-35d	1-14d	15-35d	1-14d	15-35d	1-14d	15-35d
1	2	3	4	5	6	7	8	9	10	11	12	13
Feed ingredients (g/kg)												
Maize ^a	532.00	572.70	521.80	546.30	520.20	508.80	531.90	605.10	521.70	577.10	520.10	541.50
WLM ¹	-	-	100.00	100.00	200.00	200.00	-	-	100.00	100.00	200.00	200.00
SBM ²	363.20	323.70	270.00	244.40	170.00	175.30	363.20	323.70	270.00	244.40	170.00	175.30
Soybean oil	59.54	62.60	61.20	66.75	61.50	72.51	59.54	41.00	61.20	46.00	61.50	50.00
Monocalcium phosphate	14.54	13.00	15.20	13.88	16.20	14.65	14.54	4.00	15.20	5.30	16.20	6.00
Vitamin and mineral premix ^b	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	4.63	4.10	4.20	3.52	3.60	2.91	4.63	2.50	4.20	2.00	3.60	1.40
DL-Methionine	3.32	3.13	4.00	3.32	4.00	3.43	3.32	3.00	4.00	3.20	4.00	3.30
Lysine HCl	2.71	2.62	4.00	3.24	5.00	3.56	2.71	2.50	4.00	3.10	5.00	3.40
Titanium dioxide	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Sodium bicarbonate	4.63	2.09	3.60	2.23	3.60	2.24	4.63	2.09	3.60	3.20	3.60	2.80
Sodium chloride	1.50	2.08	1.50	1.93	1.20	1.88	1.50	2.08	1.50	1.30	1.20	1.50
Threonine	1.49	8.90	1.49	0.99	1.70	0.95	1.49	0.89	1.49	0.99	1.70	0.95
Tryptophan	-	-	-	0.32	-	0.68	-	-	-	0.32	-	0.69
HiPhos ^c	-	-	-	-	-	-	0.04	0.04	0.04	0.04	0.04	0.04
Calculated												
AME _N (MJ/kg)	13.00	13.40	13.00	13.40	13.00	13.40	13.00	13.30	13.00	13.32	12.99	13.27
Crude protein	217	203	210	200	203	200	217	206	210	203	203	203
Calcium	8.80	8.20	8.70	8.20	8.70	8.20	8.80	8.50	8.70	8.50	8.70	8.50

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13
Available phosphorus	4.50	4.20	4.50	4.20	4.50	4.20	4.50	0.42	4.50	0.42	4.50	0.42
Digestible lysine	12.00	11.00	12.00	11.00	12.00	11.00	12.00	11.00	12.00	11.00	12.00	11.00
Digestible sulfur amino acids	8.90	8.40	8.90	8.40	8.90	8.40	8.90	8.40	8.90	8.40	8.90	8.40
Digestible tryptophan	1.90	1.80	1.90	1.80	1.90	1.80	1.90	1.90	1.90	1.80	1.90	1.80
Digestible threonine	7.90	7.30	7.90	7.30	7.90	7.30	7.90	7.40	7.90	7.40	7.90	7.40
Determined												
Gross energy (MJ/kg)		18.80		18.80		18.80		18.70		18.62		18.62
Crude protein		211		210		202		209		208		210
Calcium		7.80		7.60		8.00		6.30		6.40		6.40
Phytic-P		2.52		2.74		3.17		2.40		2.64		3.02

Abbreviations: ¹WLM, white lupin meal; ²SBM, soybean meal; +, phytase added; –, no phytase added. ^aSee Table 1 for detailed raw material composition. ^bVitamin-mineral premix provided per kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; Na, 1.6 g; vitamin A, 8250 IU; vitamin D₃, 1000 IU; vitamin E, 11 IU; vitamin B₁₂, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg. ^c2000 IU/kg, see Material and methods section for product details.

Chemical analyses

Representative samples of WLM, SBM, and diets were ground to pass through a 0.5 mm sieve for chemical analyses. WLM and SBM were analyzed for dry mass, crude protein (CP), crude fat (CF), acid detergent fiber (ADF, expressed inclusive of residual ash), and neutral detergent fiber (NDF, with heat-stable amylase and expressed inclusive of residual ash) by using methods 934.01, 976.05, 920.39, 942.05, and 973.18, respectively, according to AOAC (2005). Diets, digesta, and excreta samples were analyzed in duplicate. Prior to analysis, digesta and excreta samples were homogenized using Stomacher homogenizer (Interscience, Saint-Nom-la-Bretèche, France), freeze-dried using Christ 1825 Medizinische Apparatebau 326 (Martin Christ GmbH, Osterode, Germany), and ground (1-mm screen). Titanium dioxide (TiO_2) levels in both grower diets and excreta were determined according to the method of Short et al. (1996). Gross energy (GE) of diets and excreta was determined using an adiabatic bomb calorimeter (KL 12Mn, PRECYZJA-BIT PPHU, Bydgoszcz, Poland) standardized with benzoic acid. Alkaloid content in white lupin seeds was determined at the Plant Variety Testing Laboratory in Słupia Wielka. Alkaloids were extracted from lupin flour with trichloroacetic acid followed by methylene chloride using a gas chromatograph (GC17A, Shimadzu, Kyoto, Japan) equipped with a capillary column (Phenomenex, Torrance, CA, USA), as described previously by Muzquiz et al. (1996). Raffinose family oligosaccharides in WL seeds and SBM were extracted and analyzed by high-resolution gas chromatography, as described previously by Zalewski et al. (2001). Phytic-P in raw materials and diets was determined using the method described by Reichwald and Hatzack (2008). After thawing of the left tibia (one bird per replication) and removal of the muscle tissue, the percentage of tibia ash and Ca content was determined on a fat-free dry-weight basis, in accordance with AOAC (2005).

The concentrations of the following blood serum hormones were investigated using commercial RIA kits: total thyroxine (Thyroxine [T4] kit, RIA—Cis International), free thyroxine (Free Thyroxine [fT4] kit, RIA—Cis International), total triiodothyronine (Triiodothyronine [T3] kit, RIA—Cis International), and free triiodothyronine (Free Triiodothyronine [fT3] kit, RIA—Cis International).

Calculations and statistical analyses

Apparent ileal digestibility (AID) of protein and nitrogen-corrected AME_N value of the diets were calculated relative to the ratio of TiO_2 to the content of the nutrient to be determined in the feed or digesta (excreta).

Using CP, the following equation was used to calculate the digestibility (D), AID, or apparent total tract digestibility of diets:

$$\text{Digestibility} = \left\{ 1 - \left[\left(\frac{\text{TiO}_2 \text{ diet}}{\text{TiO}_2 \text{ digesta or excreta}} \right) \times \left(\frac{\text{CP}_{\text{digesta or excreta}}}{\text{CP}_{\text{diet}}} \right) \right] \right\}$$

where the level of TiO_2 and AME_N in diets and digesta are expressed in grams per kilogram.

$$AME_N \text{ MJ/kg} = GE_{\text{excreta}} - GE_{\text{excreta}} x \left(\frac{TiO_{2\text{diet}}}{TiO_{2\text{excreta}}} \right) - 0.0344 x \left\{ N_{\text{diet}} - \left[N_{\text{excreta}} x \left(\frac{TiO_{2\text{diet}}}{TiO_{2\text{excreta}}} \right) \right] \right\}$$

where GE represents the gross energy [MJ/kg], N represents nitrogen, and TiO_2 is the dietary marker. AME_N was corrected to zero nitrogen balance using 34.4 MJ/kg N, as described by Hill and Anderson (1958).

Two-way analysis of variance was performed using the R environment (R Development Core Team, 2014) with the “agricolae” package (de Mendiburu, 2014), according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the measured dependent variable, μ is the overall mean, α_i is the effect of protein source, β_j is the effect of phytase, $\alpha\beta$ is the interaction between protein source and phytase, and ε_{ij} is the random error. Means were compared pairwise using Duncan's multiple range test. If there were no significant interactions ($P > 0.05$), the main effects were discussed. All data are presented as means and pooled standard error of the mean (s.e.m.).

Results

The overall mortality of approximately 4% (0–35 days of the experiment) was not related to dietary treatment at any phase measured (data not shown). The level of CP in all diets was very similar, but they differed in the content of phytic-P (Table 2). The inclusion of 20% of WLM in the ratio increased the content of phytic-P from 2.52 g/kg to 3.17 g/kg. On the basis of determined phytic-P values (Table 1), lupin seeds contained over 47% phytic-P in the diet. The diets had a comparable energy density (13.35 MJ/kg) because of varying levels of soybean oil (41–72 g/kg).

From day 1 to day 35, there were no interactions between any of the main treatment parameters (Table 3). Birds from all groups were characterized by similar FI during the whole experiment. The feed conversion ratio and BWG were not affected by phytase inclusion but only by WLM level. Birds fed with 20% inclusion of WLM had the lowest BWG and highest FCR of all the groups.

The results of determination of the effect of WLM level in diet and enzyme addition on the AID of protein, P, and AME_N of diet from days 1 to 35 are presented in Table 4. The use of phytase improved AME_N ($P < 0.001$) regardless of the lupin level inclusion. The interactions between the trial factors ($P < 0.001$) for AME_N were confirmed. There was no effect of AID of protein in all groups. Apparent ileal digestibility of P was affected by enzyme addition ($P < 0.001$). Phytase has a highly statistically significant effect on increasing the phosphorus digestibility. Tibia bone ash content was similar across all levels of WLM ($P > 0.05$), whereas Ca content of the tibia was different ($P < 0.05$). Phytase inclusion influenced tibia ash and Ca tibia content ($P < 0.001$). Birds fed with diets supplemented with 20% white lupin had the highest tibia Ca content ($P < 0.05$). Thus, there was no interaction between protein source and phytase addition for all tibia traits.

Table 3. Performance of birds fed diets with soybean meal (SBM) and white lupin meal (WLM), supplemented without (-) or with (+) phytase

% of WLM	Phytase addition	1-14 days			15-35 days			1-35 days		
		BWG ¹ g/bird	FI ² g/bird	FCR ³ g feed/g gain	BWG g/bird	FI g/bird	FCR g feed/g gain	BWG g/bird	FI g/bird	FCR g feed/g gain
0%	-	396	483	1.22	1805	2589	1.41	2200	3051	1.38
10%	-	397	488	1.23	1780	2561	1.45	2202	3065	1.41
20%	-	362	478	1.36	1685	2579	1.53	2047	3069	1.50
0%	+	382	470	1.23	1844	2594	1.41	2226	3083	1.40
10%	+	391	506	1.30	1766	2645	1.46	2157	3124	1.44
20%	+	332	453	1.35	1600	2591	1.58	1992	3079	1.55
Pooled s.e.m. ⁵		5.00	6.00	0.01	14.00	13.00	0.01	17.00	15.00	0.01
P-value		<0.001	0.166	<0.001	<0.001	0.609	<0.001	<0.001	0.829	<0.001
0%	-	385	483	1.27	1757	2577	1.47	2148	3062	1.43
+		368	477	1.29	1757	2610	1.49	2125	3096	1.46
0%		389 a	477	1.22 b	1824 a	2591	1.41 c	2214 a	3066	1.39 b
10%		394 a	497	1.26 b	1773 a	2603	1.46 b	2178 a	3095	1.42 b
20%		347 b	465	1.35 a	1673 b	2585	1.56 a	2020 b	3074	1.53 a
WLM level		<0.001	n.s. ⁴	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.	<0.001
Phytase		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction terms										
WLM level × phytase	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Abbreviations: ¹BWG, body weight gain; ²FI, feed intake; ³FCR, feed conversion ratio; ⁴n.s., not significant ($P>0.05$); ⁵Pooled s.e.m., pooled standard error of the mean, +, phytase added; -, no phytase added.

a, b, c - $P<0.05$.

Table 4. Influence of WLM level (0%, 10% or 20%) supplemented without (–) or with (+) phytase on apparent metabolizable energy (AME_N) of diet, apparent ileal protein digestibility, apparent ileal retention (nitrogen, phosphorus, and calcium), and tibia bone characteristic (ash, calcium, and phosphorus)

% of WLM	Phytase addition	AME _N (MJ/kg)	Apparent ileal digestibility (%)		Bone characteristic (g/100 g)	
			crude protein	phosphorus	ash	calcium
0%	–	13.10 bc	78.50	45.90	55.00	40.70
10%	–	12.90 c	76.50	39.60	55.50	39.20
20%	–	12.00 d	76.80	32.30	55.80	45.10
0%	+	14.10 a	77.00	50.80	56.50	53.40
10%	+	13.50 b	75.70	48.10	58.30	60.90
20%	+	12.40 d	77.80	46.50	58.20	64.30
Pooled s.e.m. ²		27.44	0.50	8.70	0.32	1.66
P-value		<0.001	0.70	<0.001	<0.001	<0.001
Main effects						
	–	12.70	77.30	39.30 b	55.40 b	41.90 b
	+	13.30	76.80	48.50 a	57.70 a	59.40 a
0%		13.60	77.70	48.40	55.70	47.00 b
10%		13.20	76.10	43.90	56.90	50.70 ab
20%		13.20	77.30	39.40	57.00	54.70 a
WLM level		n.s. ¹	n.s.	n.s.	n.s.	<0.05
Phytase		<0.001 ¹	n.s.	<0.001	<0.001	<0.001
Interaction terms						
WLM level × phytase	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.

Abbreviations: ¹n.s., not significant (P>0.05); ²Pooled s.e.m., pooled standard error of the mean, +, phytase added; –, no phytase added.
a, b, c – P<0.05.

Table 5. Influence of WLM level (0%, 10% or 20%) supplemented without (–) or with (+) phytase on free triiodothyronine (fT3), triiodothyronine (T3), free thyroxine (fT4), and thyroxine (T4)

% of WLM	Phytase addition	fT3 (nmol/l)	T3 (nmol/l)	fT4 (nmol/l)	T4 (nmol/l)
0%	–	3.20	1.18	10.10	7.07
10%	–	3.15	1.15	10.50	6.81
20%	–	3.79	1.23	10.40	11.60
0%	+	3.11	1.35	10.00	6.63
10%	+	3.03	1.29	10.10	7.84
20%	+	3.39	1.49	10.00	10.70
Pooled s.e.m. ²		0.076	0.043	0.292	0.429
P-value		0.09	0.0173	0.751	0.063
Main effects					
0%	–	3.38	1.19	10.30	8.47
10%	+	3.36	1.37	10.10	8.39
20%		3.16b	1.26	10.10	6.85 b
WLM level		3.09b	1.22	10.30	7.33 b
Phytase		3.86a	1.36	10.20	11.11 a
		<0.01	n.s.	n.s.	<0.01
		n.s. ¹	n.s.	n.s.	n.s.
Interaction terms					
WLM level × phytase		n.s.	n.s.	n.s.	n.s.

Abbreviations: ¹n.s., not significant (P>0.05); ²Pooled s.e.m., pooled standard error of the mean; a, b, c – P<0.05; +, phytase added; –, no phytase added.

The results presented in Table 5 show that after the addition of 20% white lupin seeds to the diet, the concentration of fT3 ($P<0.01$) and T4 ($P<0.01$) in plasma increased significantly.

Discussion

The present study shows that by adding 10% WLM to chicken diets, results can be very similar to those when birds are fed SBM. In comparison to these results, a previous study by Kaczmarek *et al.* (2016), carried out using the same variety of white lupin, suggested that after the addition of 10% WLM, a reduction in BWG can be expected. In the present study, when the lupin level was doubled (up to 20%), BWG decreased and FCR increased significantly. This finding is in agreement with the results of previous studies by Steinfeldt *et al.* (2003) who fed birds 20% of narrow-leaved lupins and Kaczmarek *et al.* (2016) who fed 20% of WLM; in both studies, the addition of lupin seeds negatively influenced BWG and FCR. In contrast, Nalle *et al.* (2012) using white lupin and Kaczmarek *et al.* (2015) using narrow-leaved and yellow lupin did not notice the negative impact of 20% lupin addition on birds' performance. These differences between studies may result from different varieties of lupins used in diets or from an unbalanced diet in terms of energy and AAs (Nalle *et al.*, 2012). The high content of RFO in lupin seeds may also affect the reduction of production – their total amount is almost twice as high as in SBM. According to Leske *et al.* (1991), high concentrations of RFO in the GIT may contribute to water retention, which in turn affects the passage rate, leading to adverse effects on the absorption and utilization of nutrients and low AME_N . Bedford (1996) suggested that oligosaccharides of legumes can affect transit of GIT digesta because of its hygroscopic properties by increasing the intestinal osmolarity. Another factor of the discrepancy in the obtained results may be NSP content (different between species and varieties) (Gdala and Buraczewska, 1996). It is well known that water-soluble NSPs affect the viscosity of the digesta, which negatively affects the digestibility of fat and AME_N , thereby contributing to the reduction of production results. In the present study, the content of water-soluble NSPs was not determined, but based on previous studies (Gdala and Buraczewska, 1996; Knudsen, 1997), it can be assumed that their content is about 15% in WLM. The addition of lupin affects AME_N negatively, with 10% of WLM causing statistically significant changes. Previous studies reported similar results and also showed that the lupin seeds increase intestinal viscosity and thus reduce the value of AME_N (Steenfeldt *et al.*, 2003; Kaczmarek *et al.*, 2015). The present study showed that the addition of lupin also changed the viscosity of the intestinal contents. This can be assumed based on a previous study in which high content of water-soluble NSPs was confirmed in the seeds of white lupin (Gdala and Buraczewska, 1996).

The higher concentrations of ADF and NDF in the seeds of white lupin could have an irritating effect on the intestinal mucosa surface and thus increase the production of mucins. For example, previous studies carried out on pigs and rats con-

firmed that the CF of some plants interacts with the mucin layer, causing an increase in its volume (Montagne et al., 2003; Salgado et al., 2002). An increase in the content of mucins on the mucosal surface may adversely affect the absorption of nutrients, and thus the production results. It was also shown that the increase in mucin production led to an increase in endogenous losses, which additionally leads to the deterioration of production results (Montagne et al., 2003).

The results of the AID of protein in the present study confirm that the lupin protein is digested in the same degree as soybean meal protein (Steenfeldt et al., 2003). Moreover, none of the lupin levels used in the study reduced the digestibility of the protein; therefore, these results are comparable to those obtained by Kaczmarek et al. (2016). However, reduction in protein digestibility was noted only when 25% of white lupin seeds was added to the diet. Interestingly, when 20% of yellow lupin was added to the chicken diet, the protein digestibility was higher in the chicken diet than in the SBM-based control group (Kaczmarek et al., 2015). These results may suggest that the NSPs contained in the lupin seeds do not adversely affect the digestibility of protein (Annison et al., 1996).

Furthermore, the addition of exogenous phytase influenced the AME_N , increasing its total value by approximately 143 kcal/kg. It can be assumed that phytase caused the breakdown of complexes composed of phytates and feed components (proteins, AAs, carbohydrates, and starch) (Woyengo and Nyachoti, 2013). Phytic acid affects the deterioration of starch utilization; one of the possible mechanisms of this process is the binding of Ca atoms through inositol hexaphosphate (IP6) in plant cells, which are a cofactor for α -amylase (Cowieson et al., 2004). The addition of an exogenous phytase reduces the negative effect of IP6 by binding different atoms (including Ca), making more starch available or activating the enzymes necessary for its digestion, thereby contributing to the increase of AME_N .

It can be assumed that a 20% WLM supplement for diets resulted in a decrease in their nutritional value, which is indicated by an increase in FCR and a decrease in final BWG. Another result that seems to confirm this change was in the concentration of thyroid hormones in the blood plasma. Changes in food availability appear to be the natural environmental factors that have the greatest influence on thyroid function. When 20% WLM was added to the diet, the thyroxine (T4) content increased ($P < 0.05$), which is also confirmed by earlier studies in which the level of T4 in the blood sample of birds increased during starvation or restricted access to feed (Reyns et al., 2002). The T3 content was similar after adding 20% WLM to the diet; this result is in contrast to that obtained by Reyns et al. (2002) who reported that feed restriction leads to decreased circulating T3. It can be speculated that the increased level of fT3 in the blood may be due to the increased content of thyroxine in the blood because T3 in the body is formed from T4. Additionally, Darras et al. (1995) reported that either feed restriction or starvation causes decreases in circulating T4 concentrations, although the effects on T3 are more variable. However, the effects on metabolism and the relative roles of T4 and T3 in metabolic stimulation have not been fully investigated in birds. Another confirmation of the decrease in the nutritional value of diets with WLM is the decrease in AME_N value. Thus, lowering of the AME_N may result from several factors as follows: high content of ADF and NDF in

WLM and a significant increase in the content of phytic acid in diets with the addition of white lupin. Both these factors blocked large amounts of nutrients present in feed for the digestive tract of birds. The addition of phytase has partially offset the negative impact of WLM on the availability of nutrients, which in turn resulted in an increase of AME_N in the groups with the inclusion of enzymes.

With the increase of WLM content in the diet, Ca digestibility and content in the bones decreased. Thus, it is related to the increasing content of phytic acid that can interact with dietary minerals and other compounds (Cowieson *et al.*, 2006). In lupin seeds, two-third of all P is in phytic form and inclusion of 20% WLM increased the content of phytic acid in the diet by approximately 25%. To reduce feed costs associated with the need to use P in mineral form (inorganic phosphate) and to reduce environmental pollution resulting from the excretion of unabsorbed P to the soil, an exogenous phytase is added to the broiler feed. The addition of phytase significantly improved the content of crude ash and Ca in the tibia bones. It can be assumed that the phytase caused the breakdown of phytic compounds, which contributed to better digestion of P contained in the feed, which in turn contributed to better bone mineralization. These findings are in agreement with previous studies (Chung *et al.*, 2013; Kaczmarek *et al.*, 2015). Exogenous phytase caused dephosphorylation of IP₆, to which minerals (P and Ca) previously bound in the phytate-mineral complexes were released. Released minerals could be absorbed in the digestive tract and then participate in the bone mineralization process. On the basis of obtained results, it can be assumed that the location of phytate in the seeds of white lupin favors the action of exogenous phytase.

In conclusion, regardless of the WLM level in broiler diets, the addition of phytase enhances the availability of mineral compounds. Additionally, performance is not affected by WLM inclusion until it exceeds 10%. To obtain knowledge about phytate storage in legumes, as well as the effect of lupins soluble NSPs on physiological parameters and performance of broilers, and the effect of phytase on protein digestibility, further study is required.

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