

IS NARROW-LEAVED LUPINE (*LUPINUS ANGUSTIFOLIUS*) A SUITABLE CRUDE PROTEIN SOURCE FOR RABBIT DIETS? A COMPARISON WITH SOYBEAN MEAL

PETER ŠUFliARSKÝ^{1,2}, ZDENĚK VOLEK^{1,2}, TOMÁŠ TAUBNER², VLADIMÍR PLACHÝ¹, LUKÁŠ ZITA³

¹ Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Kamýcká, 129, 16500, Prague, Czech Republic

² Department of Nutritional Physiology and Animal Product Quality, Institute of Animal Science, Přátelství 815, 104 00, Prague, Czech Republic

³ Department of Animal Science, Czech University of Life Sciences Prague, Kamýcká, 129, 16500, Prague, Czech Republic

Corresponding email address: sufliarsky@af.czu.cz

ABSTRACT

The present study aimed to evaluate the efficacy of narrow-leaved lupine seeds (NLL) as a protein source in rabbit diets in terms of growth performance, digestive enzyme activity, nutrient digestion, and nitrogen excretion and retention. Different varieties of NLL (Boregine, Jowicz, and Rumba) were used as the replacement of soybean meal (SBM) in the present study. The control diet contained 60 g/kg SBM, and the experimental diets, containing 110 g/kg NLL, differed in the varieties used (Boregine, NLLB diet; Jowicz, NLLJ diet; and Rumba, NLLR diet). For the growth performance trial, 160 rabbits (Hyplus PS

19 x PS 40; of both sexes; weaned at 32 days of age) were randomly allocated into 4 groups (40 rabbits per group) and fed one of the diets for a period of 42 days. In addition, 40 rabbits weaned at d 32 (Hyplus PS 19 x PS 40; 10 rabbits per diet) were used for the determination of the coefficients of total tract apparent digestibility (CTTAD) of the diets and to determine nitrogen balance and nitrogen retention. The proteolytic activity tended to be lower in rabbits fed the SBM diet than in those of the other rabbits. The CTTAD of the diets were not affected by the dietary treatments. The losses of nitrogen in faeces were not affected by the dietary treatment. There were higher losses of nitrogen in urine ($P = 0.006$) and also a higher total excretion of nitrogen (by 0.36 g/day; $P = 0.013$) in rabbits fed the NLLJ diet than in rabbits fed the other diets. Consequently, there was a lower nitrogen retention coefficient in these rabbits ($P = 0.008$). There was a higher average daily feed intake in rabbits fed the NLLJ diet than in other rabbits ($P = 0.035$). This corresponded with the worse FCR in rabbits fed the NLLJ diet than in rabbits fed the other diets ($P < .0001$). The present study revealed that the varieties of narrow-leaved lupine seeds, Boregine and Rumba, represent a suitable dietary CP and can fully replace traditionally used SBM for rabbit diets. Negative results in performance, nitrogen excretion, and nitrogen balance in rabbits fed the diet containing variety Jowicz indicate the importance of choosing a suitable variety of NLL for rabbit diets.

Keywords: rabbit; crude protein; narrow-leaved lupine; feed efficiency; nitrogen retention

INTRODUCTION

The effort toward sustainable agricultural practices has intensified in recent years, with a particular focus on finding alternative crude protein (CP) sources for animal feeds. Within the European Union (EU), the drive towards sustainability is paramount, given the need to reduce dependency on imported feed ingredients, mitigate environmental impacts, and ensure food security (Parisi et al., 2020). Lupines, among legumes, represent a promising alternative with their unique nutrient composition. They are crops well-adapted to the temperate climates of Europe and present a promising alternative to traditional protein sources such as soybean meal (SBM) (Musco et al., 2017; Soñta and Rekiel, 2020). Studies up to date have already shown that white lupine seeds (WLS) are a suitable substitution (partial or full) for SBM as a crude protein source in rabbit nutrition (Volek and Marounek, 2009; Volek et al., 2018). Similarly, narrow-leaved lupine seeds (NLL) could become a suitable CP source and extend the range of domestic protein components. The NLL are rich in nutrients (Lemus-Conejo et al., 2023). Currently, a limited number of studies are available regarding the dietary inclusion of NLL in rabbits. In fact, only Volek et al. (2020) evaluated the dietary inclusion of NLL in comparison with the well-studied dietary inclusion of WLS in rabbits. Concerning SBM, there is no information in the literature regarding the substitution of NLL for SBM in rabbit diets.

Thus, this study aims to evaluate the efficacy of NLL as a protein source in rabbit diets in terms of growth performance, digestion, digestive enzyme activity, and nitrogen retention. Different varieties of NLL were used for the replacement of SBM in the present study.

MATERIAL AND METHODS

Diets

Different CP sources were used for the formulation of the diets (Table 1). The control diet contained 60 g/kg SBM, and the experimental diets, containing 110 g/kg NLL, differed in the varieties used (Boregine, NLLB diet; Jowicz, NLLJ diet; and Rumba, NLLR diet) (Table 2).

The diets met, except for sulphur amino acids (AA) and threonine, the recommendations of De Blas and Mateos (2020) for the nutrient requirements of fattening rabbits (Table 3). The diets were offered *ad libitum* to rabbits as pellets with a diameter of 3 mm and a length of 5 to 10 mm.

Animals and experimental design: performance trial

For the growth performance trial, 160 rabbits (Hyplus PS 19 x PS 40; of both sexes) weaned at day 32 were randomly allocated to one of the four groups (40 rabbits per group), and each group was fed one of the diets for a period of 42 days. Rabbits were housed in wire-net cages (80 x 60 x 45 cm), with 4 animals per cage. Feed intake was recorded daily, and live weight was recorded weekly, both per cage. Average daily weight gain (ADWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated after the end of the experiment.

Table 1. The chemical composition (% on as fed basis unless otherwise stated) of SBM and selected varieties of NLL used in this study (Boregine, Jowicz, and Rumba)

	SBM	Boregine	Jowicz	Rumba
Dry matter	93.6	87.3	87.3	88.9
Crude protein	42.7	29.4	30.0	27.7
Ether Extract	2.4	5.64	5.14	5.94
Crude fibre	7.61	14.6	12.2	13.7
Neutral detergent fibre	15.1	28.9	27.0	23.4
Acid detergent fibre	10.5	28.4	18.4	17.7
Insoluble dietary fibre	17.32	39.5	40.4	41.5
Soluble dietary fibre	4.10	1.2	0.9	1.8
Ash	7.09	3.28	2.84	3.86
Calcium	2.95	2.34	2.77	3.4
Phosphorus	5.54	4.63	3.42	4.62
Lysine	2.60	1.27	1.36	1.39
Methionine + cysteine	1.28	0.585	0.565	0.619
Threonine	1.70	0.953	0.956	0.945
Total EAA	18.70	10.9	12.3	11.5
α -tocopherol (mg/kg)	2.98	5.61	3.67	4.26
δ -tocopherol (mg/kg)	8.76	1.25	1.59	1.10
γ -tocopherol (mg/kg)	7.29	59.8	54.7	56.2
β -carotene (mg/kg)	0.005	5.79	4.01	5.0
Lutein (mg/kg)	0.242	13.2	9.93	12.6
Zeaxanthin (mg/kg)	0.048	7.20	5.78	8.80

Table 2. Ingredients (% on an as-fed basis) of the experimental rabbit diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

Component	Experimental diets			
	SBM	NLLB	NLLJ	NLLR
Alfalfa meal	28.5	28.5	28.5	28.5
Soybean meal	6	0	0	0
Boregine	0	11	0	0
Jowicz	0	0	11	0
Rumba	0	0	0	11
Wheat bran	33	31	31	31
Sugar beet pulp	9	9	9	9
Oat	15	12	12	12
Barley	6	6	6	6
Vitamin-mineral premix	1	1	1	1
Monocalcium phosphate	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5

Table 3 Chemical composition and nutritive value (% on an as-fed basis unless otherwise stated) of the experimental rabbit diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets			
	SBM	NLLB	NLLJ	NLLR
Dry matter	87.8	87.9	87.6	87.8
Crude protein	14.7	15.1	15.0	14.8
Neutral detergent fibre	36.3	35.1	35.6	36.8
Acid detergent fibre	18.2	18.3	18.9	18.1
Acid detergent lignin	4.3	4.3	4.4	4.4
Insoluble dietary fibre	35.3	34.3	35	36.4
Soluble dietary fibre	4.9	4.9	3.2	4.1
Ether extract	2.7	2.8	2.8	2.8
Starch	16.4	15.1	15.3	15.4
Lysine	0.72	0.68	0.68	0.67
Methionine + cysteine	0.51	0.48	0.47	0.48
Threonine	0.60	0.58	0.59	0.59
Total essential amino acids	5.4	5.3	5.3	5.0
Ash	7.3	7.3	6.9	7.0
Particle size distribution (%)				
>1.25 mm	13	15	16	16
>0.630 mm	7	7	7	7
>0.315 mm	8	8	8	8
<0.315 mm	72	70	69	69

Health status, expressed as the sanitary risk index (SRI), was calculated according to the definitions of the European Group on Rabbit Nutrition

(Fernández-Carmona et al., 2005) as a sum of ailing and deceased animals, where the animal was recorded as either ailing or deceased only once. The deceases were recorded daily, and ailments were recorded weekly and monitored by individual observations of clinical signs of digestive disorders (diarrhoea, mucus in faeces, abnormal excretion of caecotrophs faeces) or weight loss.

Digestibility trial, nitrogen balance, and nitrogen retention

The 40 rabbits weaned at d 32 (Hyplus PS 19 x PS 40) were used for the determination of the coefficient of total tract apparent digestibility (CTTAD) of the organic matter (OM), CP (N x 6,25), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), and gross energy (GE) of the diets in accordance with the European Group on Rabbit Nutrition (Perez et al., 1995) and to determine nitrogen balance and nitrogen retention in accordance with the study of Volek et al. (2020). Rabbits were randomly allocated into four groups (10 rabbits per group) and individually housed in wire-net metabolic cages (50 x 40 x 42.5 cm). Each group was fed ad libitum on one of the four diets, and after an adaptation period that lasted 28 days, the 4-day collection of the whole production of faeces and urine followed afterwards. At the end of the digestibility trial, all rabbits (64 days old) were slaughtered without previous fasting. After laparotomy, the contents of the whole small intestine were immediately frozen (-80 °C) until analysis. The small intestinal contents were used for the analysis of the hydrolytic activities of pancreatic enzymes and the concentration of sialic acid.

Analytical methods

Protein sources, feeds, urine, and faeces were analysed by AOAC (1984, 2000, 2005) methods. Total amylase, protease, and lipase

activities were analysed according to Taubner et al. (2023) with soluble starch, azocasein, and tributyrin as substrates. The analysis of the sialic acid was performed according to Salcedo et al. (2011). For the quantification of the neutral detergent fibre, the methodology of Mertens (2002) was used. Lignin levels were determined by the solubilization of the cellulose in the sulfuric acid (Robertson and Van Soest, 1981). Gross energy was determined by combustion in an adiabatic calorimeter (C5000 control, IKA-Werke, Staufen, Germany). The quantification of β -carotene, α -tocopherol, δ -tocopherol, and γ -tocopherol in the SBM, lupine seeds, and diets was determined according to the European standards (EN 12822, EN 12823-2) for high-performance liquid chromatography, equipped with a diode-array detector (VP series) (Shimadzu, Kyoto, Japan). The same equipment was used for the quantification of lutein and zeaxanthin in the crude protein sources and diets, following the methodology described by Froescheis et al. (2000).

Statistical analysis

Growth performance, CTTAD of diets, pancreatic enzyme activity, nitrogen balance, and nitrogen retention were examined by one-way ANOVA using the GLM procedure in Statistical Analysis System 9.4 (2012), with type of diet as the fixed effect. For the growth performance assessment, the cage represented the experimental unit. Cages with mortality of 50% and more (i.e., two or more rabbits) were not included in the statistical analysis of growth performance. No corrections concerning the cage feed consumption in cases of morbidity were made. The individual rabbit represented the experimental unit for the CTTAD, nitrogen balance, and nitrogen retention. The data in

tables are presented as least squares means. Health status was assessed using the Fisher's exact test. The individual rabbit was used as the experimental unit. Differences among least squares means with $P \leq 0.05$ were accepted as statistically significant, while $P \leq 0.10$ were considered a trend of significance.

RESULTS AND DISCUSSION

The chemical composition of protein sources and the formulation of the experimental diets used in the present study

The proximate composition of the NLL varieties is within the range reported by the other authors (Bartkiene et al., 2016; Ferchichi et al., 2021). All tested NLL varieties contained lower levels of CP, soluble dietary fibre, and ash and higher levels of EE, NDF, ADF, and insoluble dietary fibre than the SBM. These findings are in accordance with Lagos and Stein (2017).

The diets used in this study were formulated to have similar limiting AA levels. Because of the possible influence of dietary inclusion of synthetic AA on the digestibility of AA/CP in rabbits (Taboada et al., 1994; de Blas et al., 1998), synthetic AA were not added to the diet used. For this reason, the levels of limiting AA in the experimental diets used in this study were slightly below the dietary recommendations for growing-fattening rabbits (de Blas and Mateos, 2020).

Hydrolytic activity of pancreatic enzymes and concentration of sialic acid

The hydrolytic activity of pancreatic enzymes (Table 4) did not differ significantly among dietary treatments, except for the proteolytic

activity, which tended to be lower in rabbits fed the SBM diet than in those of the other rabbits (on average by 193 mg azocasein/g dry matter digesta/h; $P = 0.10$).

Table 4. Pancreatic enzyme activity and concentration of sialic acid in small intestine of rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

Experimental diets					
	SBM	NLLB	NLLJ	NLLR	P-value
Rabbits (n)	10	10	10	10	
Live weight ¹ (g)	2356	2286	2334	2265	0.714
Amylolytic activity ²	9.02	6.00	6.11	6.08	0.177
Proteolytic activity ³	684.7	922.6	883.1	828.3	0.104
Lipolytic activity ⁴	8.5	8.6	10.3	10.6	0.174
Sialic acid ⁵	2.37	2.43	1.95	3.27	0.291

¹at d 64 of age; ²at mg sugar/g dry matter digesta/h; ³mg azocasein/g dry matter digesta/h; ⁴mmolbutyrate/g dry matter digesta/h; ⁵μg sialic acid/g dry matter sample.

This finding is more likely related to anti-nutritional factors (ANFs), such as trypsin inhibitors (TI). In this context, Torres et al. (2005) detected no trypsin inhibitors in NLL, whereas the major ANFs in SBM is TI, which may influence the proteolytic activity and subsequently the apparent ileal digestibility of AA and CP, as detected in pigs (Woyengo et al., 2017; Chen et al., 2020). Although the concentration of TI in our study was not determined, a study by Hoffmann et al. (2019) suggest that TI concentration below the threshold (4 mg/g) can negatively affect digestibility of nutrients and growth performance in chickens. In

this context, further experiments should be carried out to evaluate the threshold of TI for rabbits fed diets containing CP sources with TI.

Coefficients of total tract apparent digestibility of the experimental diets, nitrogen balance, and nitrogen retention

Data describing the CTTAD of the diets, nitrogen balance and nitrogen retention are presented in table 5. The CTTAD of OM, CP, EE, NDF, and ADF were not significantly affected by the dietary treatments. The CTTAD of starch was slightly lower in rabbits fed the NLLJ than in those of other rabbits (on average by 0.008; $P = 0.048$), a finding which is related with a higher ADF/starch ratio in this diet (Gidenne et al., 2000). No significant differences were observed between treatments concerning nitrogen intake. The losses on nitrogen in faeces were not affected by the dietary treatment. There were higher losses of nitrogen in urine (on average by 0.36 g/day; $P = 0.006$) and also a higher total excretion of nitrogen (by 0.36 g/day) in rabbits fed the NLLJ diet than in rabbits fed the other diets. Consequently, there was a lower nitrogen retention coefficient in these rabbits. These findings may be related to the different AA/CP balances of the diets used in the present study (Volek et al., 2021).

Table 5. Coefficients of total tract apparent digestibility (CTTAD) for the experimental diets, and nitrogen balance a retention in rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				P-value
	SBM	NLLB	NLLJ	NLLR	
Rabbits (n)	10	10	10	10	
CTTAD ²					
Organic matter	0.587	0.600	0.577	0.599	0.653
Crude protein	0.626	0.675	0.672	0.655	0.173
Ether extract	0.915	0.898	0.879	0.898	0.131
Starch	0.952 ^a	0.953 ^a	0.943 ^b	0.948 ^a	0.048
Neutral detergent fibre	0.262	0.266	0.281	0.283	0.737
Acid detergent fibre	0.123	0.144	0.125	0.120	0.816
Nitrogen balance ² (g/day)					
N intake	3.98	3.90	3.98	3.79	0.678
N excretion in faeces	1.49	1.24	1.35	1.31	0.213
N excretion in urine	0.55 ^b	0.72 ^{ab}	0.95 ^a	0.49 ^b	0.006
Total N excretion	2.05 ^{ab}	1.96 ^{ab}	2.30 ^a	1.80 ^b	0.013
N retention					
Retained N ² (g/day)	1.94	1.94	1.69	1.99	0.171
Coefficient of N retention ⁴	0.49 ^{ab}	0.50 ^a	0.42 ^b	0.52 ^a	0.008

¹ Determined in rabbits between 60 and 64 days of age.

² As nitrogen intake – total nitrogen excretion (faeces + urine).

⁴ As retained nitrogen/nitrogen intake.

Growth performance of rabbits

The growth performance of rabbits is shown in Table 6. In general, except for the rabbits fed the NLLJ diet, the CP level in the diets used

provided a satisfactory outcome in terms of growth performance, a finding that is in line with Maertens et al. (1997). There were no significant differences in terms of the final live weight of the rabbits at 74 days of age, or ADWG as well. There was a higher ADFI in rabbits fed the NLLJ diet than in other rabbits (on average by 10 g/day; $P = 0.035$). This corresponded with the worse FCR in rabbits fed the NLLJ diet than in rabbits fed the other diets (on average by 0.34; $P < .0001$). These findings are apparently related to the lower CTTAD of starch, and the lower coefficient of nitrogen retention detected in rabbits fed the NLLJ diet.

Table 6. Growth performance during the fattening period (32 to 74 days of age) in rabbits¹ fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				
	SBM	NLLB	NLLJ	NLLR	P-value
Live weight (g)					
At 32 days of age ¹	758	754	746	747	0.871
At 74 days of age	2556	2555	2464	2502	0.554
AWDG (g)					
32-74 days of age	42.8	42.5	40.9	41.6	0.507
ADFI (g)					
32-74 days of age	140 ^b	135 ^b	146 ^a	134 ^b	0.035
FCR					
32-74 days of age	3.27 ^b	3.17 ^b	3.56 ^a	3.22 ^b	<.0001

¹40 rabbits per group at the beginning of experiment

Table 7. Morbidity, mortality and sanitary risk index (SRI) of rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

Experimental diets					
	SBM	NLLB	NLLJ	NLLR	P-value
Morbidity					
32-74 days of age	5	3	9	5	0.304
Mortality					
32-74 days of age	1	6	1	3	0.124
SRI					
32-74 days of age	6	9	10	8	0.768

CONCLUSION

The varieties of narrow-leaved lupine seeds, Boregine and Rumba, represent a suitable dietary CP and can fully replace traditionally used SBM for rabbit diets. Negative results in performance, nitrogen excretion, and nitrogen balance in rabbits fed the diet containing variety Jowicz indicate the importance of choosing a suitable variety of NLL for rabbit diets.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic) [institutional support MZE-RO0723]; the Ministry of Education, Youth and Sports of the Czech Republic (Prague, Czech Republic) [“S” Grant].

REFERENCES

- AOAC International. 1985. Official methods of analysis. Official Methods of Analysis of AOAC, 14th ed. Washington, DC, USA.
- AOAC International. 2000. Official methods of analysis. Official Methods of Analysis of AOAC, 17th ed. Washington, DC, USA.
- AOAC International. 2005. Official methods of analysis. Official Methods of Analysis of AOAC, 18th ed. Gaithersburg, MD, USA.
- Bartkiene, E., Bartkevics, V., Starkute, V., Krungleviciute, V., Cizeikiene, D., Zadeike, D., Juodeikiene, G., Maknickiene, Z. 2016. Chemical composition and nutritional value of seeds of *Lupinus luteus* L., *L. angustifolius* L. and new hybrid lines of *L. angustifolius* L. *Zemdirbyste-Agriculture*, 103(1), 107-116. <https://doi.org/10.13080/z-a.2016.103.014>
- Chen, J., Wedekind, K., Escobar, J., Vazquez-Añón, M. 2020. Trypsin Inhibitor and Urease Activity of Soybean Meal Products from Different Countries and Impact of Trypsin Inhibitor on Ileal Amino Acid Digestibility in Pig. *Journal of the American Oil Chemists' Society*, 97(10), 1151-1163. <https://doi.org/10.1002/aocs.12394>
- de Blas, C., Mateos, G. G. 2020. Feed formulation. In: C. de Blas, J. Wiseman (Eds.), *Nutrition of the rabbit* (pp. 222-232). CABI. <https://doi.org/10.1079/9781845936693.0222>
- de Blas, J. C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J., Méndez, J. 1998. Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science and Technology*, 70(1-2), 151-160. [https://doi.org/10.1016/S0377-8401\(97\)00063-1](https://doi.org/10.1016/S0377-8401(97)00063-1)
- Ferchichi, N., Toukabri, W., Vrhovsek, U., Nouairi, I., Angeli, A., Masuero, D., Mhamdi, R., Trabelsi, D. 2021. Proximate composition, lipid and phenolic profiles, and antioxidant activity of different ecotypes of *Lupinus albus*, *Lupinus luteus* and *lupinus angustifolius*. *Journal of Food Measurement and Characterization*, 15(2), 1241-1257. <https://doi.org/10.1007/s11694-020-00722-8>
- Fernández-Carmona J., Blas E., Pascual J.J., Maertens L., Gidenne T., Xiccato G., García. 2005. Recommendations and guidelines for applied nutrition experiments in rabbits. *World Rabbit Science*, 13(4), p. 209-228. <https://doi.org/10.4995/wrs.2005.516>
- Folch, J., Lees, M., Stanley, G.H. S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226(1), 497-509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5)

- Froescheis, O., Moalli, S., Liechti, H., Bausch, J. 2000. Determination of lycopene in tissues and plasma of rats by normal-phase high-performance liquid chromatography with photometric detection. *Journal of Chromatography B: Biomedical Sciences and Applications*, 739, 291-299. [https://doi.org/10.1016/S0378-4347\(99\)00562-9](https://doi.org/10.1016/S0378-4347(99)00562-9)
- Gidenne, T. 2000. Recent advances in rabbit nutrition: Emphasis on fibre requirements. A review. *World Rabbit Science*, 8(1), 23-32. <https://doi.org/10.4995/wrs.2000.414>
- Hoffmann, D., Thurner, S., Ankerst, D., Damme, K., Windisch, W., Brugger, D. 2019. Chickens' growth performance and pancreas development exposed to soy cake varying in trypsin inhibitor activity, heat-degraded lysine concentration, and protein solubility in potassium hydroxide. *Poultry Science*, 98(6), 2489-2499. <https://doi.org/10.3382/ps/pey592>
- Lagos, L. V., Stein, H. H. 2017. Chemical composition and amino acid digestibility of soybean meal produced in the United States, China, Argentina, Brazil, or India. *Journal of Animal Science*, 95(4). <https://doi.org/10.2527/jas2017.1440>
- Lemus-Conejo, A., Rivero-Pino, F., Montserrat-de la Paz, S., Millan-Linares, M. C. 2023. Nutritional composition and biological activity of narrow-leaved lupins (*Lupinus angustifolius* L.) hydrolysates and seeds. *Food Chemistry*, 420. <https://doi.org/10.1016/j.foodchem.2023.136104>
- Maertens, L., Luzi, F., De Groote, G. 1997. Effect of dietary protein and amino acids on the performance, carcass composition and N-excretion of growing rabbits. *Annales de Zootechnie*, 46(3), 255-268. <https://doi.org/10.1051/animres:19970306>
- Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in breakers or crucibles: collaborative study. *Journal of AOAC International* 85, 1217-1240.
- Musco, N., Cutrignelli, M. I., Calabrò, S., Tudisco, R., Infascelli, F., Grazioli, R., Lo Presti, V., Gresta, F., Chiofalo, B. 2017. Comparison of nutritional and antinutritional traits among different species (*Lupinus albus* L., *Lupinus luteus* L., *Lupinus angustifolius* L.) and varieties of lupin seeds. *Journal of Animal Physiology and Animal Nutrition*, 101(6), 1227-1241. <https://doi.org/10.1111/jpn.12643>
- Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angelis, A., Piccolo, G., Pinotti, L., Schiavone, A., Terova, G., Prandini, A., Gasco, L., Roncarati, A., Danieli, P. P. 2020. Protein

- hunger of the feed sector: the alternatives offered by the plant world. *Italian Journal of Animal Science*, 19(1), 1204-1225. <https://doi.org/10.1080/1828051X.2020.1827993>
- Pérez, J.M., Lebas, F., Gidenne, T., Maertens, L., Xiccato, G., Parigi-Bini, R., Dalle Zotte, A. 1995. European reference method for in vivo determination of diet digestibility in rabbits. *World Rabbit Science*, 3(1), p. 41-43. <https://doi.org/10.4995/wrs.1995.239>
- Robertson, J.B., Van Soest, P.J. 1981. The Detergent System of Analysis. In: James, W.P.T. and Theander, O. (Eds.), *The Analysis of Dietary Fibre in Food*, Chapter 9, Marcel Dekker, New York, 123-158.
- Salcedo, J., Lacomba, R., Alegría, A., Barbera, R., Matencio, E., Lagarda, M. J. 2011. Comparison of spectrophotometric and HPLC methods for determining sialic acid in infant formulas. *Food Chemistry*, 127(4), 1905-1910. <https://doi.org/10.1016/j.foodchem.2011.02.069>
- SAS, 2012. *SAS/STAT User's Guide (Release 9.4)*. SAS Inst. Inc., Cary, NC, USA.
- Soñta, M., Rekiel, A. 2020. Legumes - use for nutritional and feeding purposes. *Journal of Elementology*, 25(3), 835-849. <https://doi.org/10.5601/jelem.2020.25.1.1953>
- Taboada, E., Mendez, J., Mateos, G. G., de Blas, J. C. 1994. The response of highly productive rabbits to dietary lysine content. *Livestock Production Science*, 40(3), 329-337. [https://doi.org/10.1016/0301-6226\(94\)90099-X](https://doi.org/10.1016/0301-6226(94)90099-X)
- Taubner, T., Skřivan, M., Englmaierová, M., Malá, L. 2023. Effects of hemp seed and flaxseed on enzyme activity in the broiler chicken digestive tract. *Animal*, 17(4). <https://doi.org/10.1016/j.animal.2023.100765>
- Torres, A., Frias, J., Vidal-Valverde, C. 2005. Changes in chemical composition of lupin seeds (*Lupinus angustifolius*) after selective α -galactoside extraction. *Journal of the Science of Food and Agriculture*, 85(14), 2468-2474. <https://doi.org/10.1002/jsfa.2278>
- Volek, Z., Adámková, A., Zita, L., Adámek, M., Plachý, V., Mlček, J., Marounek, M. 2021. The effects of the dietary replacement of soybean meal with yellow mealworm larvae (*Tenebrio molitor*) on the growth, nutrient digestibility and nitrogen output of fattening rabbits. *Animal Feed Science and Technology*, 280. <https://doi.org/10.1016/j.anifeedsci.2021.115048>
- Volek, Z., Ebeid, T. A., Uhlířová, L. 2018. The impact of substituting soybean meal and sunflower meal with a mixture of white lupine seeds and rapeseed meal on rabbit doe milk yield and composition,

- and the growth performance and carcass traits of their litters. *Animal Feed Science and Technology*, 236, 187-195. <https://doi.org/10.1016/j.anifeedsci.2017.12.011>
- Volek, Z., Marounek, M. 2009. Whole white lupin (*Lupinus albus* cv. Amiga) seeds as a source of protein for growing-fattening rabbits. *Animal Feed Science and Technology*, 152(3-4), 322-329. <https://doi.org/10.1016/j.anifeedsci.2009.05.003>
- Volek, Z., Uhlířová, L., Zita, L. 2020. Narrow-leaved lupine seeds as a dietary protein source for fattening rabbits: a comparison with white lupine seeds. *Animal*, 144, 881-888. <https://doi.org/10.1017/S1751731119002350>
- Woyengo, T. A., Beltranena, E., Zijlstra, R. T. 2017. Effect of anti-nutritional factors of oilseed co-products on feed intake of pigs and poultry. *Animal Feed Science and Technology*, 233, 76-86. <https://doi.org/10.1016/j.anifeedsci.2016.05.006>