
PhD THESIS

Improving the use of lupine seeds (*Lupinus albus* L.) in the poultry feeding by dehulling and addition of enzymes

(SUMMARY OF Ph.D. THESIS)

PhD student **Dănuț Ioan Struți**

Scientific coordinator **Prof. univ. dr. habil. Daniel Mierliță**



SUMMARY OF THE THESIS

The doctoral thesis entitled „Improving the use of lupine seeds (*Lupinus albus* L) in the poultry feeding by dehulling and addition of enzymes" is structured in two main parts: the bibliographic study part and the personal contributions part. The doctoral thesis includes a number of 9 chapters, to which the references are added. The full work includes a total number of 162 pages, 58 tables, 50 figures and 182 reference sources.

Part I of the thesis - **The current state of knowledge** - includes two chapters, in which a literature review of the knowledge in the field of the present doctoral thesis research is made: Chap. 1 „*Chemical composition and nutritional quality of lupine seeds*" and Chap. 2 „*Possibilities to improve the efficiency of lupine seeds utilization in the feeding of monogastric animals*". Part 1 contains 22 pages, which represent 13,58 % of the total thesis volume, and a number of 7 tables and 4 figures.

Part II of the thesis - **Personal contributions** - includes a number of five chapters: Chap. 3 „*Aim, objectives and research organization*"; Chap. 4 „*Materials and methods of the research*"; Chap. 5 „*The influence of dehulling on the nutritional quality of white lupine seeds*"; Chap. 6 „*The influence of dehulling white lupine seeds used in the feeding of laying quails, on the bioproductive performances and quality of eggs*" and Chap. 7 „*The influence of enzyme addition in lupine-based feed compounds, on the bioproductive performances and eggs quality at quails*". Part II contains a number of 106 pages, which represent 65,43 % of the total thesis volume, and a number of 51 tables and 47 figures.

Chapter 8 presents the general conclusions and recommendations, and Chapter 9 presents the elements of originality and innovative aspects of the doctoral thesis.

Intensive poultry breeding is dependent by conventional vegetal protein sources, represented mainly by soybean meal, due to the high nutritional qualities suitable for their nutritional requirements. In the context of the restriction of animal meal use in feeding farm animal species, the limited ecological possibilities to cultivate soybeans in different pedo-climatic areas, as well as the tendency to limit the use of genetically modified organisms in animal feed, it is necessary to assess new unconventional vegetal protein sources with a high biological value, which can be obtained locally and at lower costs. Therefore, according to the literature, *L. albus* seeds from low-alkaloid varieties are a suitable alternative to soybean meal, both nutritionally and economically, but also ethically, because they do not come from genetically modified crops. However, the main impediment of lupine seeds is the high level of crude cellulose (15-20% of DM) and non-starch polysaccharides (25-30% of DM), compounds which cannot be degraded in the small intestine of poultry due to the lack of specific enzymes, thus reducing the use of feeds. Some research report that thorough dehulling of seeds can remove 60-70% of the crude cellulose content as well as some of the non-starch polysaccharides (PÍSAŘÍKOVÁ et al., 2008; SAEZ et al., 2015), contributing to the increase of the nutritional value of lupine seeds. However, the largest amount of non-starch polysaccharides and oligosaccharides is concentrated in

the kernels, so it is necessary to apply complementary methods that lead to a significant improvement of the feed use (OLKOWSKI, 2011). The lack of specific endogenous enzymes, in the case of using lupine seeds in poultry feed, leads to the premises of a exogenous specific enzymes intake in the compound feeds, in order to increase the use of lupine seeds (MERA-ZÚÑIGA et al., 2019). Studies from the literature are limited and only partially show the effects of seeds dehulling (LAUDADIO and TUFARELLI, 2011b) and addition of enzymes in the lupine-based compound feeds (LEE et al., 2016) on productive performances in laying hens, but those related for laying quails are missing.

Therefore, **the aim of the research was to improve the use of white lupine seeds (*Lupinus albus*, cv. Amiga) in the feeding of laying quails (*Coturnix coturnix japonica*) by dehulling of seeds and addition of enzymes in the compound feeds.**

In order to achieve the proposed aim, the research has three main objectives. **The first objective was to determine the influence of dehulling on the raw chemical composition and nutritional qualities of lupine seeds from low-alkaloid varieties.** For dehulling the lupine seeds, a dehuller machine of original concept was used, which achieved an optimal mechanical separation of the seeds morphological components. Next, the proportion of the lupine seeds morphological components was assessed, followed by the determination of the raw chemical composition from whole and dehulled seeds, and hulls. The evaluation of the proteins and fats quality of the whole and dehulled lupine seeds was performed on the basis of the amino acids profile, determined by liquid column chromatography (HPLC), and respectively of the fatty acids content, determined by gas chromatography (GC).

The second objective was to evaluate the effect of dehulling lupine seeds used in the quail feeding on the bioproductive performances and egg quality. Therefore, the research regarding the productive performances was carried out in production conditions for a period of 8 weeks, being organized five experimental groups, composed of five replicas of 8 quails/replica, resulting in a number of 40 quails/group. In order to evaluate the effects of dehulling as a method for improving the use of lupine seeds for poultry, five types of compound feeds were prepared and distributed randomly to the five groups. The combined feed (diet) associated to the control group (LC) had soybean meal as a main source of protein, while for the experimental groups, soybean meal was partially replaced by white lupine seeds inclusion in different proportions in feed: 20% whole seeds (LI₂₀) and 25% whole seeds (LI₂₅); 20% dehulled seeds (LD₂₀) and 25% dehulled seeds (LD₂₅).

During the conducted research, the following aspects were taken into account when evaluating the bioproductive performances: the evolution of the quails body weight mass, laying eggs intensity, feed intake and the use of feed, health and feed costs associated with egg production. Also, certain physico-chemical parameters of the fresh eggs quality were determined, such as: egg weight and its components weight, albumen index, yolk index, albumen Haugh index, mineral shell thickness and the raw chemical composition of the albumen and yolk. In order to evaluate the nutritional quality of the eggs, the fatty acids content of the yolk fats was determined by gas chromatography and the sanogenic lipidic indices were calculated. Also, the cholesterol and carotenoids content of the yolk was determined by the gas-chromatography and liquid chromatography analyses, respectively. In order to establish the effect on the digestion processes, the faecals humidity was determined,

and at the end of the experiment, the viscosity of the intestinal content, the weight and the length of the gastrointestinal tract segments were evaluated. Also, at the end of the experiment, the main blood biochemical indicators were determined by laboratory analyses.

The research regarding the bioproductive effect was carried out in production conditions at a private breeding farm and the laboratory analyses were performed in private research units as well as in specialized laboratories within USAMV Cluj-Napoca.

The third objective was to evaluate the effect of the enzymes addition in white lupine-based compound feeds on the productive performances and egg quality at quails. Consequently, the experiments were carried out in production conditions for a period of 8 weeks, by organizing six experimental groups, consisting of five replicates/group, with 7 quails/replica, thus resulting a number of 35 birds/group. For each experimental group, one of the six types of compound feed was randomly assigned, as follows: LC- (combined feed without lupine and without enzymes); LC+ (combined feed without lupine, with added enzymes); LI₂₀- (combined feed with 20% lupine, no enzymes); LI₂₀+ (combined feed with 20% lupine, with added enzyme); LI₂₅- (combined feed with 25% lupine, no enzymes); LI₂₅+ (combined feed with 25% lupine, with added enzyme). In the control groups (with or without enzymes), the main source of protein in feed was soybean meal, and in the experimental groups (LI, with or without enzymes) the soybean meal was partially replaced with whole lupine seeds, which were introduced in the feeds for quails in the proportion of 20% and 25%, respectively. Enzymes were added in feed as a commercial preparation of Hostazyme X (0,2 g/kg NC) containing: endo 1,4 β-xylanases, endo 1,3 (4) β-glucanases, proteases, α-amylases, galactosidases .

In order to achieve the proposed objectives, the same bioproductive performances and quality indicators of eggs, similar to those mentioned in the previous objective, were analyzed during the research.

Statistical analysis of the obtained results was done using the statistical program GraphPad Prism version 8.3.0 (538) and the ANOVA test. The Tukey Honest Significant Difference (HSD) test was applied to determine the statistical significance of the differences between the two data sets. The significance threshold used to express the differences was set at 5% ($p < 0,05$) in all analyses; respectively at 1% ($p < 0,01$) and 0,1% ($p < 0,001$) when the differences were inherently significant and very significant, respectively. Data are presented as an average of n determinations. Determination coefficients (R^2) were established and show the degree of the experimental data concentration around the regression line.

Based on the results obtained in our research carried out to improve the use of lupine seeds (*L. albus*) in the feeding of laying quails (*Coturnix coturnix japonica*) by seeds dehulling and adding enzymes in the combined feeds, the following conclusions were formulated:

(1) Conclusions regarding the influence of dehulling on the chemical composition and nutritional qualities of white lupine seeds.

- The dehuller machine of original concept allowed an optimal separation of the morphological components (kernel and hulls) of lupine seeds (*L. albus*, cv. Amiga). After dehulling, the kernel represents 82,3% and the hulls 17,7% of the whole seed weight.

- Dehulling of white lupine seeds from low-alkaloids varieties led to the improvement of raw chemical composition, a significant increase of crude protein content being recorded, similarly with the decrease in crude fibre content, which can lead to an increase in the nutritional value of dehulled seeds.
- By dehulling lupine seeds, the protein content in certain amino acids increased, especially in amino acids considered to be limiting for animal production, namely lysine (5,73 vs. 4,99 g/16g N) and methionine (0,55 vs. 0,48 g/16g N). The nutritional value of proteins from dehulled seeds, assessed on the basis of the standards used, is superior to those from whole seeds, both being considered an optimal source of proteins for broilers and less so for fattening pigs.
- The proportion and ratio of fatty acids in the structure of white lupine seeds fats do not change ($p>0,05$) due to the seeds dehulling. There is a high proportion of monounsaturated fatty acids (55,16–55,72% of FAME) and polyunsaturated fatty acids (27,23 - 27,86% of FAME) in the fats structure; therefore, lupine seeds can be considered a good source of unsaturated fatty acids (approx. 83%). The values recorded by the determined sanogenic lipidic indices indicate a high nutritional quality of the fats, given the high PUFA content. Seeds dehulling does not change ($p>0,05$) the nutritional qualities of fats.

(2) Conclusions regarding the influence of dehulling white lupine seeds used in the laying quails feeding on the bioproductive performances and eggs quality.

- Dehulling of lupine seeds and their use in the laying quails feed in the amount of 200 g/kg (LD₂₀) led to obtaining comparable bioproductive performances (quails body weight mass, laying rate, feed intake, the use of feed) with those obtained by quails from the control group (LC), in which only soybean meal was used as the main source of protein.
- The similar bioproductive performances of quails from the groups in which whole lupine seeds were used in the amount of 200 g/kg (LI₂₀) and dehulled seeds in the amount of 250 g/kg (LD₂₅), demonstrates that dehulling has made possible to increase the amount of lupine in the laying quails feed from 200 g to 250 g/kg in feed, which contributed to the reduction by 41,2% of the amount of soybean meal used. Compared to the control group (LC - without lupine), the seeds dehulling allowed the reduction of the proportion of soybean meal in quail feed by 63,6% (in the case of the LD₂₀ group) and by 79,4% (in the case of LD₂₅), without affecting the laying rate, the eggs weight and the use of feed.
- Dehulling of white lupine seeds used in the amount of 200 g/kg in laying quails feed (LD₂₀) led to an increase of the eggs weight ($p<0,05$) compared to the weight of the eggs from the groups in which whole lupine seeds were used in the amount of 200 g/kg (LI₂₀) and was comparable to the eggs weight obtained from the group without lupine (LC).
- The partial replacement of soybean meal with whole and dehulled lupine seeds resulted in a reduction of the cost price per 1 kg of combined feed up to 24,27%, which contributed to a substantial reduction in the feed costs for egg production (lei/egg, respectively lei/kg egg mass). Thus, compared to the control group (LC), feed costs related to the egg production decreased by up to 21,7%, the lowest feed costs being recorded in the case of groups where dehulled lupin

seeds were used (LD₂₀, LD₂₅).

- No changes ($p > 0,05$) were observed in the weight of egg albumen and yolk in the whole egg structure, as a result of dehulling lupine seeds and their use in quail feeding, but there was a significant reduction in the weight of the mineral shell and its thickness ($p < 0,05$).
- Dehulling of white lupine seeds did not affect the eggs physical quality indices or the raw chemical composition of the egg albumen and yolk. The use of lupine seeds in quails diets led to obtaining eggs with a higher carotenoids content and a more intense pigmentation of yolk, compared to eggs from the group without lupine ($p < 0,05$).
- The use of dehulled lupin seeds in quail feed has led to an improvement of the fatty acids profile of yolk fats, by decreasing the proportion of saturated FA and increasing those of unsaturated FA, but especially polyunsaturated FA. Thus, the concentration of omega-3 FA increased, considered beneficial for human health, which resulted in higher values of atherogenic and thrombogenic indices. The yolk content in cholesterol was not influenced by the lupine inclusion in quail feed, although there was a trend to decrease the cholesterol level in eggs from groups of quails fed with whole lupine seeds (by 9,40%) or dehulled (by 11,26%).
- Dehulling lupine did not significantly affect fecal moisture and intestinal digesta viscosity due to the high content of non-starch polysaccharides in kernel, which has the capacity to retain large amounts of water and increase the intestinal viscosity. To reduce the negative effect of non-starch polysaccharides on the digestion and the use of feed, further research was needed to add specific enzymes in feed.
- The use of whole lupine seeds in the quails diet has led to an increase in the weight and length of some internal organs with a role in digestive processes, such as: the proventricole, small intestine, caecum, colon, liver; while seeds dehulling led to a decrease of these parameters, being comparable to those recorded in the control group (no lupine in feed).
- The analysis of the determined hematological indices reveal a normal physiological status of the quails, which means that the introduction of lupine seeds (whole or dehulled) in feed did not affect the health of birds. Total lipids, cholesterol and plasma triglycerides reveal a decreasing trend as the proportion of lupine seeds in the diet increases. The plasma concentration of urea and creatinine increased with the increase of lupine seeds proportion in quail feed, which reflects a lower amino acid balance of the proteins from lupine compared to those from soybean meal.

(3) Conclusions regarding the influence of enzyme supplementation of the combined feeds containing white lupine seeds on bioproductive performances and eggs quality at quails.

- The addition of enzymes in combined feeds for laying quails which contain whole white lupine seeds in the amount of 200 g/kg and 250 g/kg improved the bioproductive performances of quails, especially laying rate ($p < 0,05$). Therefore, the addition of enzymes allowed the inclusion of lupine seeds up to 250 g/kg in feed, which reduced the proportion of soybean meal in the feed structure by

62,7% (from 33,0% - the group without lupine, at 12,3% - the group with 25% lupine + enzymes), without affecting the quails body weight mass, laying rate, daily feed intake, the use of feed and the eggs weight.

- The eggs physical qualities, as well as the raw chemical composition of the albumen and yolk, were not influenced by the addition of enzymes in the combined feeds containing 20 - 25% whole lupine seeds (LI₂₀₊ and LI₂₅₊); the obtained results were similar to those of the control groups (LC- and LC+), which had exclusively soybean meal as the main source of protein. Even to the groups where enzymes were added in the feed, the mineral shell thickness was lower compared to the control batch ($p < 0,05$), with or without the addition of enzymes, which means the enzymes did not influence the digestibility and absorption of calcium from feed.
- The partial substitution of soybean meal from the laying quails feed with lupine seeds, with or without the exogenous enzymes addition, has led to a decrease in the content of fatty acids with atherogenic effect (C16: 0, C18: 0) in the structure of yolk fats, in the favor of omega-3 fatty acids (C18: 3 n-3, C22: 5 n-3, C22: 6 n-3) which positively influenced the ratio of PUFA/SFA, h/H (hypocholesterolemic/Hypercholesterolemic FA) as well as the atherogenic (AI) and thrombogenic (TI) index. An increase in the value of the PUFA/SFA and h/H ratio associated with the decrease in the value of AI and TI, recorded at the groups where lupine seeds were used in feed, indicates a higher nutritional quality of yolk fats, analyzed in terms of the consumer health impact. The improvement of the yolk fats quality is also supported by the reduction of the cholesterol content, in the case of quails in the feed in which lupin seeds were added. The enzymes addition in quails feed did not conclusively influence the FA profile, sanogenic lipidic indices, and cholesterol content of eggs.
- The eggs yolk provided from the quail groups in the feed of which the lupine seeds were used had a significantly higher carotenoids content, which ensured a higher yolk color intensity ($p < 0,05$), but the addition of enzymes did not have a conclusive effect on these parameters.
- The use of enzymatic preparations in lupine-based combined feeds had a positive effect on digestive processes, as it decreased ($p < 0,05$) fecal moisture and viscosity of the intestinal digesta compared to groups without enzymes, due to the efficiency of exogenous enzymes that improved the digestibility and the use of non-starch polysaccharides. All this led to a decrease ($p < 0,05$) in the size and weight of the internal organs involved in digestion, compared to the use of whole lupine seeds without the enzymes in feed.
- The addition of enzymes in quail feeds contributed to a higher utilization of proteins from feed, as evidenced by the decrease in urea and plasma creatinine ($p < 0,05$), probably due to increased energy intake as a results of improving the non-starch polysaccharides digestion. In addition, there was a tendency to decrease the level of total lipids and triglycerides in the blood.

The specialized literature recommends utilization of white lupine seeds in poultry feeds and especially of laying quails, in a proportion of maximum 20% in the combined feed structure. In the studies carried out in this doctoral thesis, we managed to increase the proportion of lupine seeds in the laying quails feed to 25% (% of

weight), through the successfully application of one of the two proposed methods: dehulling the seeds or by addition of specific enzymes. We consider it appropriate and necessary to continue the research in this field by adding specific enzymes in the structure of combined feed containing 25-30% dehulled lupine seeds, which could completely replace soybean meal in the laying quails feed. We also recommend extending the studies to other poultry species and production categories, in particular laying hens and broiler chickens.

SELECTIVE REFERENCES

- 1) ARSLAN, C., SEKER, E., 2002, Effects of processed white lupin seed (*Lupinus albus* L.) on growth performance of japanese quail, *Revue de Médecine Vétérinaire*, 153, 643-646.
- 2) BRENES, A., SLOMIN, B., MARQUARDT, R., GUENTER, W., VIVEROS, A., 2003, Effect of enzyme addition on the digestibilities of cell wall polysaccharides and oligosaccharides from whole, dehulled, and ethanol-extracted white lupins in chickens, *Poultry Science*, 82, 1716-1725.
- 3) CHIOFALO B., LO PRESTI, V., CHIOFALO, V., GRESTA, F., 2012, The productive traits, fatty acid profile and nutritional indices of three lupin (*Lupinus spp.*) species cultivated in a Mediterranean environment for the livestock, *Animal Feed Science & Technology*, 171, 230.
- 4) CRISTE, F.L., MIERLIȚĂ, D., SIMEANU, D., BOISTEANU, P., POP, I.M., GEORGESCU, B., NACU, G., 2018, Study of fatty acids profile and oxidative stability of egg yolk from hens fed a diet containing white lupine seeds meal, *Revista de Chimie (Bucharest)*, 69, 2454-2460.
- 5) DRAŽBO, A., MIKULSKI, D., ZDUŃCZYK, Z., SZMATOWICZ, B., RUTKOWSKI, A., JANKOWSKI, J., 2014, Fatty acid composition, physicochemical and sensory properties of eggs from laying hens fed diets containing blue lupine seeds, *Poultry Science*, 78, 245-252.
- 6) JEROCH, H., KOZŁOWSKI, K., SCHÖNE, F., ZDUŃCZYK, Z., 2016, Lupines (*Lupinus spp.*) as a protein feedstuff for poultry. Varieties, composition and nutritional values for poultry, *European Poultry Science*, 80.
- 7) LAUDADIO, V., TUFARELLI, V., 2011b, Influence of substituting dietary soybean meal for dehulled-micronized lupin (*Lupinus albus* cv. Multitalia) on early phase laying hens production and egg quality, *Livestock Science*, 140, 184-188.
- 8) LEE, M.R., PARKINSON, S., FLEMING, H.R., THEOBALD, V.J., LEEMANS, D.K., BURGESS, T., 2016, The potential of blue lupins as a protein source, in the diets of laying hens, *Veterinary and Animal Science*, 1-2, 29-35.
- 9) LUCAS, M.M., STODDARD, F.L., ANNICCHIARICO, P., FRÍAS, J., MARTÍNEZ-VILLALUENGA, C., SUSSMANN, D., DURANTI, M., SEGER, A., PETER, M.Z., PUEYO, J.J., 2015, The future of lupin as a protein crop in Europe, *Frontiers in Plant Science*, 6.
- 10) MERA-ZÚÑIGA F., PRO-MARTÍNEZ, A., ZAMORA, N., SOSA, M., GUERRERO, R., MENDOZA, P., CUCA, G., LÓPEZ, R., CHAN, D., BECERRIL, P., VARGAS, A., BAUTISTA, O.J., 2018, Soybean meal substitution by dehulled lupine (*Lupinus angustifolius*) with enzymes in broiler diets, *Asian-Australasian Journal of Animal Sciences*, 32, 564-573.

- 11) MIECZKOWSKA, A., JANSMAN, A.J.M., KWAKKEL, R.P., SMULIKOWSKA, S., 2005, Effect of dehulling and α -galactosidase supplement on the ileal digestibility of yellow lupin based diets in broiler chickens and adult roosters, *Journal of Animal & Feed Sciences*, 14, 297–304.
- 12) MIERLIȚĂ, D., SIMEANU, D., POP, I.M., CRISTE, F., POP, C., SIMEANU, C., LUP, F., 2018, Chemical composition and nutritional evaluation of the lupine seeds (*Lupinus albus* L.) from low-alkaloid varieties, *Revista de Chimie*, 69, 453-458.
- 13) OLKOWSKI, B.I., JANIUK, I., JAKUBCZAK, A., 2010, Effect of enzyme preparation with activity directed towards degradation of non-starch polysaccharides on yellow lupine seed based diet for young broilers, *Acta Veterinaria Brno*, 79, 395–402.
- 14) OLKOWSKI BOGUSLAW, 2011, Lupin as primary protein source in young broiler chicken diets: Effect of enzymes preparations catalyzing degradation of non-starch polysaccharides or phytates, *World Journal of Microbiology and Biotechnology*, 27, 341–347.
- 15) PARK, J.H., LEE, S., KIM, I.H., 2016, Effects of lupin seed supplementation on egg production performance, and qualitative egg traits in laying hens, *Veterinarni Medicina*, 61, 701–709.
- 16) SUJAK, A., KOTLARZ, A., STROBEL, W., 2006, Compositional and nutritional evaluation of several lupin seeds, *Food Chemistry*, 98, 711–719.
- 17) SMULIKOWSKA, S., KONIECZKA, P., CZERWINSKI, J., MIECZKOWSKA, A., JANKOWIAK J., 2014, Feeding broiler chickens with practical diets containing lupin seeds (*L. angustifolius* or *L. luteus*): effects of incorporation level and mannanase supplementation on growth performance, digesta viscosity, microbial fermentation and gut morphology, *Journal of Animal and Feed Sciences*, 23, 64–72.
- 18) VOLEK, Z., BUREŠ, D., UHLÍŘOVÁ, L., 2018, Effect of dietary dehulled white lupine seed supplementation on the growth, carcass traits and chemical, physical and sensory meat quality parameters of growing-fattening rabbits, *Meat Science*, 141, 50–56.