
PhD THESIS

Studies on the efficiency of using lupine seeds (*Lupinus albus* L.) as alternative protein source in poultry feeding

(SUMMARY OF Ph.D. THESIS)

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SUMMARY

The PhD thesis entitled "Studies on the efficiency of using lupine seeds (*Lupinus albus* L.) as alternative protein source in poultry feeding" is structured in two parts: the bibliographical one and the own research one, respectively in 7 chapters to which general conclusions, recommendations, innovative and originality aspects, next to the studied bibliographic references were added. The work consists in a number of 151 pages, 58 tables, 32 figures and 119 bibliographic titles.

The 1st part–**Literature review** structure is made up of two chapters, the literature review of researches referenced in the field aimed in the present PhD thesis, namely: CHPT. I. *Nutritional characterization of lupine seeds from low-alkaloid varieties* and CHPT. II. *Lupine seeds use in animal feeding and human nutrition*. Part I contains 20 pages, which represents 13,24% of the total volume of the PhD thesis.

The IInd part of the thesis – **Personal contribution**, comprises five chapters: CHPT. III. *Aim, objectives and research planning*; CHPT. IV. *Materials and research methods*; CHPT. V. *Studies regarding the cultivation pretability, chemical composition and nutritional value of white lupine seeds obtained in the specific agroclimatic conditions of Transylvania*; CHPT. VI. *Influence of white lupine seeds used in laying hens feeding on bioproductive performances and egg quality*; CHPT. VII. *Influence of white lupine seeds used in broilers feeding on bioproductive performances and meat quality*.

In the context of ban of animal flavours in poultry feeding, ban of cultivation of genetically modified plants (GMOs) and restriction tendency of using genetically modified soy products and by-products, which are mainly provided by high-priced imports, white lupine from improved varieties is still a promising alternative in insuring the on-farm vegetable protein production for poultry feeding in our country. Thereby, **the main purpose of the research carried out was to assess the white lupine (*Amiga* variety) cultivation pretability in the specific agroclimatic conditions of our country and to study the possibilities of using it in various poultry species feeding (laying hens and broilers).**

To achieve the proposed goal, the research aimed **four objectives**. **The first one was to assign white lupine from the varieties with low alkaloids content (*Lupinus albus*, *Amiga* variety) cultivation pretability, productivity and economic efficiency in the specific agroclimatic conditions of the hilly area of Transylvania.** On this line, white lupine *Amiga* variety culture (seeds were imported from Netherlands) was attained on an area of 0.87 ha, under production conditions, in Sălaj County, in 2018. The specific technology stand in line when attaining and maintaining the culture, being pursued the following: agronomic characteristics of the culture, seed yield, as well as production costs for obtaining one kg of seeds, or one kg of crude protein and respectively gross profit achieved by cultivating this species, which is not a traditional culture in Romania.

The second objective was to assess the chemical composition and nutritional evaluation of white lupine seeds free of alkaloids.

Seeds yielded in the own experimental field were subjected to laboratory analyses in order to assess crude chemical composition and to evaluate protein and fat

quality by means of chemical methods, determining amino acid and fatty acid content respectively.

The third objective was to ascertain the effect of partial replacement of soybean meals with white lupine seeds free of alkaloids in laying hens feeding on bioproductive and economic performances, and egg quality. Accordingly, live body weight, egg production, feed intake, feed conversion ratio, poultry health status and feed costs related egg production were pursued during the experiment. The physico-chemical egg quality indicators analyzed in fresh eggs were: average weight of egg and egg components, mineral eggshell thickness and chemical crude composition of egg white and yolk.

To highlight some effects of white lupine use, on some biochemical parameters of eggs quality, fatty acid profile of yolk fat (including cholesterol content), sanogenous lipid indices of yolk fat and non-enzymatic lipophilic antioxidants content of yolk were assessed.

In order to evaluate oxidative stability of yolk fat during the storage period, eggs were stored in refrigerator at 2-4°C, for a period of 20 and 40 days respectively, considered similar to the storage time of eggs in shell by the consumer. At the end of each storage period, yolk was analysed for α -tocopherol and malondialdehyde (MDA) content as final peroxidation product of fats and especially of polyunsaturated fatty acids.

The fourth objective was to ascertain the effect of partial replacement of soybean meals with white lupin seeds free of alkaloids in broilers on bioproductive and economic performances and meat production quality. Body weight dynamics, feed intake and feed conversion ratio, livestock losses and broilers health status were assessed during the experiment. Furthermore, next to the bioproductive performances, we have also pursued: economic issues, namely fodder costs/kg weight gain and the 'European Efficiency Factor - EEF'.

To highlight the effect of lupine seeds input in broilers feeding, a various number of physiological status indicators were determined: dry matter content of faeces dynamics (%), viscosity of intestinal content (cP), intestine relative length (cm/kg live weight) and relative weight of digestive tract segments and the annex glands (g/kg live weight).

In order to determine the lupine effect on the health status and metabolic processes, blood samples were collected at the end of the experiment, from which laboratory analyses were carried out to set up the main blood biochemical indices specific to protein, lipid and enzyme profile.

The quality of meat production was estimated based on: slaughter yield, body regions of high economic value ratio in carcass structure, crude chemical composition of meat and fatty acid profile of intramuscular fats and fat deposit.

The researches of productive effect were performed under production conditions at SC Rosbro Avicom SRL (Bihor County).

A total of 160 laying hens Tetra-SL LL commercial hybrid, of 30-weeks of age were randomly assigned to four lots, each lot consisting of five replicas of 8 hens per replica (40 hens per lot). Lots were randomly assigned to one of the four treatments consisting of four input levels (0, 15, 20 and 25%) of white lupine seeds (Amiga variety), as alternative source of vegetable protein in laying hens feed.

In the experiment carried out on broilers Ross 308 commercial hybrid, a number of 200 non-sexed chickens of one day of age were used, being assigned into four lots, each lot consisting of 50 heads. Lots were randomly assigned to one of the four treatments, which consisted of four input levels (0, 8, 16 and 24%) of white lupin seeds (Amiga variety) as an alternative source of vegetable protein in broilers feed.

The primary data resulted from the performed researches were processed by means of well known statistical methods. Variance analysis, Pearson correlations, regression analysis, "Boxplot" diagram (distribution of values) were used as data processing methods. ANOVA (Variance Analysis) technique, ANCOVA (ANalysis of VOCAriances) technique, t-test (Student) and Dunn test were used to assess the significance of the differences between means, as mathematical models of calculation. Statistical processing was performed using the Microsoft Office 2010 software package (Microsoft Inc., USA).

The results of the own researches concerning the white lupine cultivation pretability in the specific agroclimatic conditions of Transylvania and its use as alternative protein source in various poultry species (laying hens and broilers) sustainable feeding, highlighting the influence on the performances and quality of the production yielded, a set of **conclusions** have been drawn, summarised in the following:

- *Conclusions related to the cultivation pretability, chemical composition and nutritional value of white lupine seeds obtained in the specific agroclimatic conditions of Transylvania:*
 - The white lupine Amiga variety behaves well in the agroclimatic conditions of the hilly area of Transylvania, yielding a production of 2942 kg grains/ha, even in the context of less favorable climatic conditions which characterized the year of 2018 (rainfall excess from the flowering and pods development stage). We consider that the assessment of white lupine of improved varieties cultivation pretability, productivity and efficiency in the agroclimatic conditions of the hilly area of Transylvania during one year does not ensure the required certainty to state clear conclusions, further studies being still needed in this regard.
 - The specific agroclimatic conditions of Transylvania and of 2018 did not radically influenced the crude chemical composition and alkaloids level in Amiga variety white lupine seeds, compared to the results reported in other studies previously conducted in various regions of Europe. The white lupine seeds analyzed showed a high protein and fat content, respectively 37.67% protein and 11.8% crude fat (% of DM). The laboratory analyses performed reveal a high content of crude cellulose (12.59%) and a low alkaloid content (0.019%) of the lupine seeds obtained.
 - Lupine seeds protein is a good source of lysine (5.2 - 6.3 g/16 g N), but deficitary in other essential amino acids, mostly in sulfur and tryptophan amino acids. This aspect is confirmed by the high CS_{Lys} value (85.28%), but the sulfur amino acids were limiting amino acids ($CS_{Met + Cys}$: 38.07%), when egg protein, the nutritional requirements of adult man, the nutritional requirements of broilers or the nutritional requirements of fattening pigs were used as standards. Regardless of the standard used, in all the above cases Met + Cys were limiting amino acids. All these conclude that white lupine seeds are rather a good protein source for broilers feeding and stand any

less for the nutritional requirements of essential amino acids for adult human diet, but also for pigs fattening (20-50 kg).

- The oleic acid (C18:1 n-9) was the dominant fatty acid of white lupine seeds fat composition assessed (48.8%). The linoleic acid (C18:2 n-6) (20.12%) stand out followed by the linolenic acid (C18:3 n-3) (9.98%) from the polyunsaturated fatty acids. The high content of PUFA indicates that white lupine may be a potential source of fat, which may favourably influence the fatty acid profile of fats in animal agro-food products with a sanogenous effect for consumers. Moreover, the high content of linoleic and linolenic acids make lupine seeds a good source of essential fatty acids for human nutrition and animal feeding.

- The results achieved by assessing the chemical composition and nutritional value of lupine seeds, support the possibility of using it in animal feeding (and even in adult humans), mixed with other protein fodders, along with an appropriate level of essential amino acids (mainly sulfur amino acids) and the maximum allowed level of crude cellulose for monogastric species.

- *Conclusions related to the influence of white lupine seeds used in laying hens feeding on bioproductive performances and egg quality:*

- White lupine seeds of modern varieties may be introduced in laying hens feeding up to 20 % in the combined fodder structure as an effective substitute for soybean meals proteins, with no significant influence on laying intensity, egg weight, feed intake and feed conversion ratio. The input of white lupine free of alkaloids in laying hens feed up to 25% lead to a significant decrease of laying intensity and increases feed conversion index value.

- The input of lupine in laying hens feed led to a decline of the price of one kg of combined fodder, substantially reducing the feeding costs value afferent for the production of an egg or one kg egg mass. The lowest feeding costs were recorded in case of the experimental lot LE₂₀, meaning that a 20% input of white lupine seeds in laying hens feed would be the most economically beneficial.

- Lupine use in laying hens feeding shows no influence on egg white and yolk percent of egg structure, while a 25 % input in the combined fodder structure (case of the lot LE₂₅) led to a significant decrement of egg weight and mineral shell thickness.

- Partial replacement of soybean meals with white lupine seeds in laying hens feed conducted to an improvement of fatty acid profile of yolk fat, respectively to an enhanced quality, analyzed throughout its effect on the consumer health status. Eggs harvested from laying hens of experimental lots showed higher content of polyunsaturated fatty acids in omega-3 (ALA, EPA and DHA) and omega-6 (LA and AA) series, as well as fatty acids with hypocholesterolemic effect (C18:1 + PUFA), regardless of lupine input ratio in feed. However, no correlation between lupine input in hens feed and yolk cholesterol level could be determined.

- White lupine input in laying hens feed in various percent showed no positive influence on omega-6/omega-3 fatty acids ratio, instead led to a decrement of the atherogen index (AI) and the thrombogen index (TI) values, but also to a significant increase of the h/H ratio (hypocholesterolemic/Hypercholesterolemic fatty acids). A higher h/H index value associated with lower AI and IT values indicates a higher nutritional value of yolk fats, which have a lowering effect of plasma cholesterol and diminishment of the possibility of atheroma plaques and/or blood thrombus formation in consumers.

- The results achieve dprove that polyunsaturated fatty acids level and type, α -tocopherol amount and egg storage period have significantly influenced egg oxidative stability. Yolk α -tocopherol content showed significantly higher values in experimental lots (LE₁₅, LE₂₀ and LE₂₅) compared to the control one (LC), both in fresh eggs and after storage, for 20 and 40 days respectively, at 2-4°C, which led to an increase in yolk fatoxidative stability.

• *Conclusions related to the influence of white lupine seeds used in broilers feeding on bioproductive performances and meat quality:*

- The dynamics of body mass accumulations during the experimental period attests the fact that lupine is well harnessed by broilers, especially in a maximum input of 16% in the combined fodder structure.

- By analyzing the main production indices, that characterize the biological-productive effect of a combined fodder (average weight gain, average combined fodder intake and feed conversion index), we conclude that lupine input in broilers feed led to a decrease of growth rate up to 9,68%, feed intake up to 3,73% and therefore an increase of feed conversion index value up to 6.60%. The lowest bioproductive performances were recorded in the broilers of the experimental lot LE₂₄, in which the highest percent of white lupine was used. Total weight gain and feed conversion index did not showed significant changes ($p>0.05$) in broilers of the experimental lots LE₈ (8% lupine in feed) and LE₁₆ (16% lupine in feed), related to the ones of the control lot (LC), allowing us to conclude that lupine input in broilers feed up to 16% has no significant influence on their productive performances.

- Lupine input in broilers feed led to a decline of the price of one kg of combined fodder and the feeding costs, for obtaining one kg weight gain. The lowest feed costs were registered in the case of LE₁₆ experimental lot, meaning that a white lupine seeds input of 16% in broilers feed is the most economically beneficial. This experimental variant would allow to increase economic efficiency, mainly in small farms (family-type farms), which posses the technical and material facilities required for on-farm white lupine cultivation and seed production at low production costs.

- The European Efficiency Factor (EEF) value was calculated for a more complex quantification of white lupine use in broilers feed efficiency, proving once again that white lupine can be introduced in 16% in broilers feeding, without significantly influencing broilers final average weight and feed conversion index, yet enhancing economic efficiency by lowering feeding costs for one kg weight gain, by 3.67%.

- Lupin seeds input in broilers in a ratio up to 16% showed no influence on slaughter yield, carcass quality determined throughout the body regions of high economic value ratio (chest) from its structure, nor meat chemical composition of chest and thigh. Rising up lupine ratio in feed structure to 24% (the case of LE₂₄ experimental lot) showed a negative influence on these indicators.

- Best quality of carcass fat, assessed throughout its influence on human health and defined as an augmented PUFA percent and an decreased SFA one, was recorded in case of LE₁₆ experimental lot of broilers, when a 16% lupine input was used compared to the control lot. Linoleic acid (C18:2, n-6) was best represented of PUFAs, while α -linolenic acid (C18:3, n-3) ratio, considered to be deficitary in human rational diet, increased in LE₁₆ experimental lot, by 31.2% in chest, 13.8% in thigh and 48.5% in intra-abdominal fat. All these allow us to conclude that moderate levels of

white lupine can be used in broilers feed as alternative protein source, providing at the same time an important intake of polyunsaturated fatty acids, mostly α -linolenic acid, concurring to enhance broiler meat sanogenous quality.

- Physiopathological variables monitored in reply to white lupine input in broilers feed included: faeces moisture, digesta viscosity, key organs morphometry involved in digestive processes and blood biochemical indices. The results of this study suggest that faeces moisture and intestinal digesta increases at once with lupine ratio increment in broilers feed. Intestinal content viscosity increased by 61.7% - 144.3% in experimental lots, compared to the control one, which could cause changes related to intestinal transit time, increases nutrient loss by decreasing digestibility and changes in the absorption processes physiology of nutrients.

- Changes in the morphometry of the main digestive organs (increase of relative weight of goiter, gizzard and small intestine and relative length of small intestine and cecum respectively) are an adaptive mechanism for using a high antinutrients content fodder (mainly, non-starch polyglucids and crude cellulose). Thus, broilers in experimental lots developed mechanisms to extract the maximum nutritional benefits from a lower quality feed ratio, by increasing the digestive tract size and weight to enhance the absorption capacity.

- Urea, creatinine and uric acid blood concentrations, considered as relevant markers of protein metabolism and synthesis processes in poultry body, increased directly proportional to the lupine ratio in broilers feed, suggesting that some of the feed proteins, probably due to amino acid imbalance, were deaminated and used as energy source. This conclusion is confirmed by the enhanced value of liver enzymes, GPT (glutamate pyruvate transaminase) and GOT (glutamate oxalo-acetate transaminase), in broilers of the experimental lots, indicating an intensification of feed amino acid catabolism.

- Plasma concentrations of total lipids and cholesterol decreased significantly in broilers from lots having white lupine input, which is an advantage as it stand for the premise of decreasing cholesterol deposition in meat and thus declining the incidence of coronary heart disease in humans following chicken meat consumption.

- The performed researches revealed the pretability of cultivating white lupine from modern genotypes free of alkaloids in the hilly area of Transylvania.

The seeds yielded are of interest for poultry feeding, due to nutrient content, mainly in proteins, showing high essential amino acids and polyunsaturated fatty acid contents, and also the experimental results proved that **lupine seeds can be introduced in a 20% ratio in laying hens feed and 16% in broilers feed** (% in the combined fodder structure), accordingly to the adequate reduction of soybean meals ratio, with no influence on production performances, feed intake, feed conversion ratio, production quality and poultry health status. Lupine seeds also provide an important intake of polyunsaturated fatty acids, mostly α -linolenic acid, concurring to enhance egg sanogenous quality and chicken meat.

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