THE POTENTIAL OF WHITE LUPIN SEEDS (*LUPINUS ALBUS* L) FROM LOW-ALKALOID VARIETIES, AS A PROTEIN ALTERNATIVE SOURCE, IN THE DIETS OF LAYING HENS

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Abstract

This study assesses the effects of using lupine seeds from low-alkaloid varieties, as alternative protein source for laying hens feeding related the bioproduction performances and egg quality. A total number of 160 Tetra-SL LL commercial layers of 30 weeks of age were distributed in a randomized complete block design, of 4 treatments and 5 replicates of 8 layers per replica (n = 40 layers/treatment). Treatments consisted of 4 input levels (0, 15, 20 and 25% -% of the combined fodder composition) of white lupine seeds (cv. Amiga) for laying hens feeding. The results achieved suggest that white lupine modern varieties seeds can be included into laying hens' diet in a ratio up to 20% of the combined fodder composition, as an efficient substitute for soybean meal proteins, without affecting the egg intensity and the quality of the eggs, the feed intake, feeding efficiency, it can decrease feed costs related to egg yield up to 4.2%. The input of lupine in laying hens feeding in a ratio up to 25% significantly reduces laying intensity, egg weight, egg shell mineral thickness and weight, and increases feed conversion ratio.

Key words: laying hen, lupine seed, performance, egg quality, yolk cholesterol

INTRODUCTION

Nowadays, soy products and by-products are the main protein source for poultry feeding. For all that, in our country, which is not showing optimal pedoclimatic conditions for high soy production, finding out alternative vegetal protein sources is still a concern of the research in this fields. In the past, the use of the white lupine as protein source for poultry feeding was limited due to its high alkaloids content, that negatively affect poultry performance [4], [10]. Nevertheless, in the last decades, researchers from plant improvement field came to develop lupine varieties showing alkaloids values up to zero (0.08-0.12 g /kg; [3]), showing a negligible impact on poultry performances [7]. [13]. Despite the low alkaloid content of lupine seeds, there are other anti-nutritional compounds, which can limit its use for laying hens feeding. The lupine seeds contain a high concentration of phytic phosphorus, form that is not available for poultry [23], but also of NSP (non-starch polysaccharides: 350-410 g NSP/kg lupine grains, of which about 70 g are water soluble NSP - [4]), which prevents the restriction enzymes activity, thereby reducing the feed nutrients digestibility.

The results of the studies related the lupine seeds use for laying hens feeding and its effect on the bioproductive performances and egg quality are inconclusive. According to the data achieved by Rutkowski et al. [21], the lupine seeds can be included into the laying hens feed up to a 20% ratio (200 g lupine/kg combined fodder), without interference with the bioproductive performances and eggs quality. Kubis et al. [9] stated an increment of the daily feed intake next to a linear decrement of egg weight and a parallel gradually increase of the white lupine for laying hens feeding (from 180 g to 240 g and respectively to 300

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g lupine/kg combined fodder). On the other hand, Prinsloo et al. [18], Krawczyk et al. [8] did not confirmed the interference of lupine seeds input up to a 30% ratio for laying hens feed, related to egg number yield, feed intake, feeding efficiency and egg weight. Although these results were not proved by the studies performed by Perez-Maldonado et al. [17] and Hammershoj and Steenfeldt [5], which showed that a ratio of 25 and 30% of white lupine for the laying hens feed interferes with egg weight and egg-mass production.

The aim of the performed study was to ascertain the influence of partial replacement of soybean meal with white lupine seeds free of alkaloids (cv. Amiga) for laying hens feed, related to the bioproductive performances and egg quality yield.

MATERIAL AND METHODS

A total number of 160 layers of the hybrid Tetra-SL LL of 30 weeks age, were weighed and randomly assigned to four treatments, each treatment consisting of five replicates of 8 layers per replica, resulting in a total of 40 layers per treatment. Treatments consisted of 4 input levels (0, 15, 20 and 25%) of white lupine seeds (cv. Amiga), as alternative vegetal protein source for laying hens feeding.

The layers from the control lot (C) were fed using a standard combined fodder, harboring soybean meal as main protein source. The composition of the combined fodders used in the experimental lots, soybean meals were partially substituted by white lupine free of alkaloids, obtained in the specific pedoclimatic conditions of Transylvania. The chemical composition of the lupine seeds was determined according to AOAC [1], as shown in Table 1.

The metabolizable energy content (ME) of lupine seeds was estimated based on the following equation [15]:

$$MEn = 26.7\ (DM) + 77\ (EE) - 51.22\ (CF),$$

where DM, EE and CF are dry matter, ether extract and crude fiber percentage from lupine seeds.

Table 1 Nutrient composition and metabolizable energy content of lupine seeds (% DM)

Nutrients	DM	Crude Protein	Ether Extract	Crude Fiber	Ash	NFE	ME (kcal/kg)
%	89.72	37.67	11.81	12.59	3.98	33.95	2660.0

DM, dry matter; NFE, nitrogen free-extracts; ME, metabolizable energy

Lupine seeds were included into the combined fodder composition used for the experimental lots in a ratio of 15% (E_{15}), 20% (E_{20}) and respectively 25% (E_{25}), along with an adequate decrease of soybean meal ratio (table 2).

The experiment was performed over a period of 10 weeks, under production conditions at SC Rosbro Avicom SRL. Poultry were housed separately for each replicate of each treatment within one enclosure of 1.5 m^2 area (approx. 1875 cm^2/layer), providing the required conditions of feed intake and egg yield registration. Laying hens breeding was carried out on permanent straw litter floor system, being regularly refreshed.

While performing the researches of productive effect, the same maintaining conditions were assured both for the layers of the control lot and also of the experimental lots, according to the instructions of the management manual of Tetra-SL LL hybrid [24].

During the entire period of the experiment, the temperature from the poultry house was maintained relatively constant (20-22ºC) and the relative humidity varied between 60-70%. Light intensity was set to a 25 lux, assuring 16 hours of light and 8 hours of dark per day. Ventilation rate was calculated for a required air of 2.5 m^3/kg body live weight / hour.

Table 2 The ingredients and chemical composition of experimental diets fed to laying hens

	Dietary treatment[1]			
	C	E_{15}	E_{20}	E_{25}
a) **Ingredients (%)**				
Corn	47.34	41.91	40.09	37.41
Wheat	10.00	10.00	10.00	10.00
Sunflower meal	5.00	5.00	5.00	5.00
Soybean meal (46%)	*22.50*	*12.50*	*9.10*	*5.90*
Lupine seeds	-	*15.00*	*20.00*	*25.00*
Sunflower oil	3.90	4.40	4.60	4.90
DL-Methionine	0.16	0.19	0.20	0.21
Limestone	8.90	8.80	8.80	8.80
Monocalcium phosphate	1.50	1.50	1.50	1.51
Salt	0.20	0.20	0.20	0.20
Vitamin-mineral premix*	0.50	0.50	0.50	0.50
TOTAL	100.00	100.00	100.00	100.00
b) **Calculated Composition (%)**				
ME (kcal/kg)	2800	2801	2800	2805
Crude protein (%)	17.00	17.00	17.02	17.06
Ether extract (%)	6.48	7.81	8.32	8.89
Linoleic acid (%)	2.18	2.69	3.36	4.15
Crude fiber (%)	3.48	4.69	5.10	5.50
Lysine (%)	0.85	0.85	0.85	0.85
Methionine (%)	0.42	0.42	0.42	0.42
Calcium (%)	3.76	3.75	3.75	3.76
Available phosphorus (%)	0.40	0.40	0.40	0.40
Ratio EM/CP	164.70	164.76	164.51	164.42

[1]C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg);

*Providing per kg of diet: Mn 80 mg; Zn 50 mg; Fe 60 mg; Cu 7 mg; Co 0.4 mg; I 2 mg; Se 0.10 mg; choline chloride 230 mg; vitamin A 11000 IU; vitamin D3 2500 IU; vitamin E 50 mg; vitamin K3 3.3 mg; vitamin B1 3 mg; vitamin B2 5 mg; niacin 25 mg; calcium pantothenate 11 mg; vitamin B6 6 mg; vitamin B12 0.08 mg; folic acid 1.7 mg; ME, metabolizable energy.

Watering was carried out by using of automatic nipple type drinkers (production SKA). Fodder supply and eggs harvesting were carried out daily, manually.

Along the experiment, the following parameters were recorded: layers initial and final weight (for each replicate); daily egg production, separately for each replica; weekly combined fodder intake and feeding efficiency (for each reply); egg weight with an accuracy of 0.1 g, daily measured for 5 eggs of each replica; egg yolk, egg white and egg shell weight, weekly determined for 5 eggs of each replica; egg shell thickness (mm) at the equator of the egg, measured every two weeks for 5 eggs of each replica, using a micrometer screw with an accuracy of 0.01 mm.

Eggs were collected for a period of 8 consecutive days from all of the pens under each of the 4 treatments (40 eggs/treatment) during week 9 and 10 for egg yolk cholesterol analysis. The egg yolks were separated and stored at -20^{0}C until analyzed.

Egg yolks were analyzed for total cholesterol content by using the Washburn and Nix method [25]. Cholesterol was separated from fat after saponification with KOH and extraction with ethyl ether. The sample was subjected to chromatographic analysis under the following conditions: the length of a glass column, 1m, internal diameter, 4 mm; film thickness, 0.25 µm; temperature of detector, 300^{0}C; temperature of injector, 290^{0}C; temperature of column, 260^{0}C; carrier gas, argon, flow rate, 50 cm^3/min [8]. Egg yolk cholesterol content was calculated and expressed as milligrams per gram of yolk.

The results were compared by analysis of variance (ANOVA) with diet as main effect (SAS 9.1, 2000; SAS Institute Inc., Cary, NC, USA) [22]. ANOVA for productive performances, egg quality and total cholesterol content of the yolk, included the main effect of diet and week and the interaction between these two main effects. When the diet effect was significantly, the means were separated using the Tukey test. The significance level to detect statistical differences was set at p <0.05 for all outcomes.

RESULTS AND DISCUSSIONS

The average layer`s body weight (Table 3) ranged between 1.59 to 1.61 kg/head at the beginning and 1.85-1.91 kg/after 10 weeks of the trial, with no significant differences between the treatments. Similar results were obtained previously by Lee et al. [11], using blue lupine for laying hens feed, as whole grains or shelled beans (15% of the combined fodder composition). However, Rubio et al. [19] reported that layer`s body weight, fed with lupine beans in a ratio up to 40% of the combined fodder, was lower than in the control lot, but lupine beans were up to 40% of the combined fodder, compared to our study in which lupine was not more than 25% of the layer`s feed.

Table 3 data indicates lower laying intensity values for the lots fed with 15% and 20% lupine, but closer to those showed by the control lot (lower with 1.59 respectively 2.04 percentage points), meanwhile an average egg yield much lower was achieved for the layer lot fed with 25% lupine, both compared to C (5.16 percentage points), but also to E_{15} and E_{20} (lower with 3.57 and respectively 3.12 percentage points). Rutkowski et al. [21] reported similar results, by registering a substantial drop in egg yield, when yellow lupine in a 25% ratio was used for laying hens feeding, compared to the control lot or to the lots in which yellow lupine used in a ratio of 10 % and respectively 20% of the compound fodder composition.

Table 3 Effect of experimental diets on productive performance and egg parameters of laying hens

Item	Dietary treatment[1]				SEM[2]	p-values[*]
	C	E_{15}	E_{20}	E_{25}		
Layer's weight (kg):						
- initial	1.59	1.60	1.61	1.60	0.011	0.109
- final	1.88	1.91	1.85	1.81	0.010	0.853
Feed intake (g/d/bird)	109.64	112.11	112.90	112.10	0.924	0.327
Egg production (%)	96.39^a	94.80^b	94.35^b	91.23^c	1.621	0.032
Egg weight (g)	59.95^a	60.51^b	59.57^a	57.76^c	0.752	0.014
Egg mass (g/d/bird)	57.78^a	57.36^a	56.80^a	52.69^b	0.874	0.029
Feed efficiency: - g/egg	115.74^b	120.35^a	119.70^a	122.99^a	0.143	0.044
- g/g	1.830^b	1.885^{ab}	1.894^{ab}	1.939^a	0.024	0.038
Cost of feed (lei/kg)	1.028	0.974	0.958	0.942	-	-
Feed costs (% as against C)	-	98.50	95.79	97.36	-	-

Means within a row with different superscripts are different (P<0.05);
[1]C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg);
[2]SEM: standard error of the mean; [*]effect of experimental diet.

Contrary to these results, Krawczyk et al. [8] confirmed the negative effect of yellow lupine beans used in a ratio of 30% for laying hens feeding, on the laying intensity.

The study performed by Park et al. [16] showed that blue lupine beans input for laying hens feeding in a ratio of 11, 16.5 and 22% even resulted in an increase of the laying intensity compared to the control lot (94.1% in control lot and 95.2, 96.0 and 96.2% respectively in the experimental lots, which received lupine in feed, as alternative protein source. The discrepancy observed between different studies may be due on one hand to the different species or variety of lupine used in the studies, and on the other hand to the combined fodder formulation strategies. For instance, Nalle et al. [14] found that white lupine varieties show high differences between them related to nutrient content (from 34.1 to 41.2% crude protein) and energy value (from 6.3 to 8.4 MJ/kg DM).

The results of the present study indicate that modern varieties white lupine seeds may be included into laying hens' diet in a ratio up to 20% of the combined fodder composition, as an efficient substitute of soybean meal proteins, without interfering with feed intake and feeding efficiency. The input of white lupine free of alkaloids for laying hens feeding in a ratio up to 25% decreases significantly the laying intensity and rise up the feed conversion ratio value.

The trend of decreasing bioproduction performances (laying intensity, feed intake and feeding efficiency) in the experimental lots compared to the control lot is due to high levels of NAP (non-starch polysaccharides), NDF and ADF from lupine beans, which interfered with nutrients digestion and absorption from feed, thereby reducing the energy and nutritional value of feed [9].

Gradually substitution of soybean meal for laying hens feeding with modern varieties white lupine beans, led to a gradual decrease in price of a kg combined fodder, substantially reducing the feeding costs of one egg or egg mass kg production. The lower feed costs, were recorded in experimental group E_{20}, which means that white lupine beans input in a ratio of 20% for laying hens feeding is most benefit economically. Lupine ratio increment into the combined fodder composition up to 25% (experimental lot E_{25}), even if led to the combined fodder price decrease, due to the significantly decrement of production performances (decrement of laying intensity and egg weight and increment of daily feed intake), the feeding costs increased compared to the case when lupine represented 20% of the combined fodder composition. Even in such conditions, feeding costs were lower in the experimental group E_{25} compared to the control group (C) and even to the experimental group E_{15} (15% lupine in feed).

Lupine input for laying hens feeding in a ratio up to 20% (% of the combined fodder weight), do not interfere with egg weight (case of lots E_{15} and E_{20}). Although daily feed intake has not changed during the experiment, egg weight and daily egg mass production decreased when white lupine ratio into laying hens feeding raised up from 20 to 25% (case of lot E_{25}) (table 3). Similar results were indicated by Kubis et al. [9], which found an increment of the daily feed intake and a linear decrement of laying intensity and eggs weight, along with a gradual increase of white lupine amount for laying hens feed (from 180 g to 240 g and 300 lupine g/kg combined fodder). Instead, Krawczyk et al. [8] proved the interference of yellow lupine beans input in a ratio of 30% for laying hens feeding, on egg weight and egg mass production. However, these results are not confirmed by the studies of Perez-Maldonado et al. [17] and Hammershoj and Steenfeldt [5], which have shown that white lupine hens input for layers feeding in ratio of 25 and 30% negatively affect both egg weight and egg mass production.

The input of lupine for laying hens feed does not affect egg white and yolk ratio, instead the use of lupine in a 25% ratio of the combined fodder composition (the case of lot E_{25}) resulted in a significant decrement in mineral shell weight (Table 4).

Our results are confirmed by previous studies performed by Rutkowski et al. [20] [21], which concluded that yellow lupine input for laying hens feed in high amounts (25-30%) did not change egg yolk and egg white percentage in the egg structure, in turn led to a significant decrease of the mineral egg shell weight.

Other authors [6], [10] proved that lupine had no effect on the morphological structure of eggs. Opposite to these results, as well as those obtained by us and other authors [20], [21], Drazbo et al. [2] concluded that blue lupine input for laying hens feeding in a ratio of 10 or 20% (% of feed weight) increases the mineral egg shell weight in the egg structure.

Mineral egg shell thickness is not influenced by the white lupine input up to a ratio of 15% (case of lot E_{15}) for the laying hens feed, instead the increment of the white lupine amount of 20% and 25% (E_{20} and E_{25} experimental lot) in the combined fodder composition negatively affected mineral egg shell thickness, which decreased with 1.5% and 4% respectively related control lot.

Table 4 Effect of experimental diets on egg quality of laying hen

Item		Dietary treatment[1] C	E_{15}	E_{20}	E_{25}	SEM[2]	p-values*
Egg weight (g)		60.16	60.43	59.70	58.38	1.265	0.518
Yolk weight (g)		14.68	14.65	14.55	14.34	0.428	0.372
Albumen weight (g)		39.63	40.11	39.54	38.63	0.636	0.183
Shell weight (g)		5.85[a]	5.67[a]	5.61[a]	5.37[b]	0.191	0.038
Shell thickness (mm)		0.399[a]	0.397[a]	0.393[b]	0.383[c]	0.014	0.026
Total cholesterol	- (mg/g yolk)	16.35	15.84	15.40	15.71	0.417	0.087
	- (mg/yolk)	240.02	232.06	224.07	225.28	12.385	0.164

Means within a row with different superscripts are different (P<0.05);
[1]C: control diet with no lupine seed, E_{15}: diet containing lupine seed (150 g/kg), E_{20}: diet containing lupine seed (200 g/kg), E_{25}: diet containing lupine seed (250 g/kg);
[2]SEM: standard error of the mean; *effect of experimental diet.

Our results are asserted by the previously studies of Kubis et al. [9], who found that by incrementing the white lupine amount for laying hens feeding from 240 g up to 180 g, respectively 300 g/kg feed the mineral shell thickness significantly decreased. The authors endorse this relationship between mineral shell thickness and white lupine amount from the feed, with the high phytic phosphorus amount of lupine beans (approx. 86% of the total phosphorus of lupine), which negatively influences the absorption and metabolic use of calcium from feed. However, Krawczyk et al. [8] concluded that the input of yellow lupine in high amounts, up to 300 g/kg feed for laying hens feeding does not affect the mineral shell quality of eggs.

Moreover, Drazbo et al. [2] concluded that blue lupine input in a ratio up to 10 or 20% (% of feed weight) for laying hens feeding, improves eggs quality, and also results in an increment of the mineral shell thickness and its resistance to breakage. The authors state that the oligosaccharides present in the lupine seeds have the ability to stimulate bifidobacteria proliferation processes into the colon and short chain fatty acids production, thereby increasing the absorption rate of minerals, especially calcium an important component of eggshells.

Our study could not prove a correlation between the presence of lupine for layers feeding and egg yolk cholesterol levels, the refore it stands in line with the results previously achieved by Krawczyk et al. [8]. Milinsk et al. [12] consider that a significant reduction of cholesterol level in egg is not possible due to a physiological control mechanism, which brings to an end egg production, when the amount of cholesterol deposed in the yolk is not sufficient for embryo survival.

CONCLUSIONS

The use of lupine seeds free of alkaloids as an alternative and efficient protein source in a ratio up to 20% for laying hens feeding and an adequate decrease of soybean meal ratio seems to have no negative effect on egg quality and yield. Lupine input for laying hens feedingina ratio of 25% significantly reduces laying intensity and increases feed conversion ratio value.

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