Influence of dehulling *Lupinus albus* seeds used in the laying quail (*Coturnix coturnix*) diets on the intestinal viscosity, faecal humidity and blood biochemical parameters

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Abstract. White lupine seeds from low-alkaloid varieties are a valuable alternative source of protein for soybean meal in poultry feed. The effects of using dehulled white lupine seeds in the diet of laying quails on intestinal digesta viscosity, fecal moisture and blood biochemical parameters were analyzed in this research. A total of 200 laying Japanese quails (*Coturnix coturnix japonica*) 10-18 weeks of age were randomly assigned to five groups fed with five types of diets: a control diet (C - based on soybean meal) and four experimental diets incorporating whole lupine seeds in amounts of 200 and 250 g kg^{-1} (WLS_{20} and WLS_{25}, respectively), and dehulled lupine seeds in the amount of 200 and 250 g kg^{-1} (DLS_{20} and DLS_{25}, respectively). The results reveal that seed dehulling did not positively influence ($p>0.05$) the viscosity of intestinal digesta and faecal humidity of quails. Inclusion of lupine seeds (whole or dehulled) in the amount of 250 g kg^{-1} in the quails diet led to an increased ($p<0.05$) value associated with oxidative stress and glutathione peroxidase, compared to the control group. Quails from the groups WLS_{20}, WLS_{25} and DLS_{25} had a higher concentrations ($p<0.05$) of blood proteins than those from control. The components of urea and creatinine increase with the lupine seeds inclusion in diet. The blood indices values, associated with the lipid profile (total lipids, cholesterol and triglycerides - mg dL^{-1}) reveal a decreasing tendency with the increasing proportion of lupine seeds inclusion in feed.
Key Words: blood indices, health status, nutrition, poultry, white lupine.

Introduction. *L. albus* seeds from free alkaloid varieties represent a potential valuable alternative source of protein for soybean meal in farm animal feed (Lucas et al 2015), due to the high content of crude protein (37-42% of dry matter - DM) and crude fat (10-11% of DM) in lupine seeds (Sedláková et al 2016; Musco et al 2017). The proteins of lupine are characterized by a high biological value, being comparable with other vegetal protein sources, but are deficient in sulfur amino acids (Sujak et al 2006; Mierliţă et al 2018). The fats of lupine seeds have a rich profile in unsaturated fatty acids (60-70% of FAME) from the omega 3 and omega 6 series, being rich in oleic and linoleic acid (Suchý et al 2008; Boschin et al 2008). White lupine seeds used in the diet of monogastric animals, especially poultry, contributed to the quality increase of the obtained products (Laudadio & Tufarelli 2011; Park et al 2016).

Researches on laying hens (Kubiś et al 2018) and broiler chickens (Olkowski et al 2005; Mierliță & Popovici 2013; Kaczmarek et al 2016), ducks (Geigerová et al 2017) and turkeys (Krawczyk et al 2015) has clearly shown the possibilities of using lupine seeds as an alternative source of protein for soybean meal. From these studies, it results that live productive performances comparable to using only soybean meal as the main source of protein in feed can be obtained by using lupine seeds up to 20% in the combined feed structure. The increase of the lupine proportion at 25, 30 and even 35% in the feed structure contributed to the occurrence of some problems of the digestion processes, characterized by: the increase of the intestinal digesta viscosity, which determined the intestinal passage time, modified the useful microbiota and gave a viscous consistency, which caused increased manure moisture. All these were associated with a lower degree of feed utilization, causing the decrease of productive performances (Kocher et al 2000; Brenes et al 2002; Steenfeldt et al 2003; Olkowski et al 2005; Konieczka & Smulikowska 2017). Research has shown that this is due to the presence of non-starch polysaccharides (NSP) in lupine seeds, especially soluble fractions, which are found in quantities of up to 80-100 g kg^{-1} (Nalle et al 2011; Hejdysz et al 2018). Birds do not have appropriate endogenous enzymes for the utilization of these compounds; therefore, they exert an antinutritional role (Knudsen 2014).

Petterson (2000) notes that some of the insoluble NSPs are also found in the lupine hulls; therefore, some of these NSPs can be removed if the lupine seeds are dehulled. In addition, by dehulling lupine seeds, most of the crude fiber, which is another antinutritional compound for poultry, is removed with the hulls (Písaříková et al 2008). Arslan & Seker (2002) show that the use of dehulled white lupine seeds in the amount of 150 g kg^{-1} in the feed of Japanese quails aged 1 to 42 days leads to improved growth performance compared to the use of whole seeds. To our knowledge, there is a lack of studies to demonstrate the effects of dehulling lupine seeds used in quail diets on the specific parameters of digestive processes (viscosity of intestinal digestion and fecal moisture) and blood biochemical parameters. Recently, Straková et al (2021) reported the beneficial effects of soybean meal replacement by 50% with dehulled white lupine seeds on the blood biochemical parameters of laying hens.

Therefore, the aim of this paper was to analyze the influence of dehulling white lupine seeds used in the laying quail diets, on the intestinal viscosity, faecal humidity and blood biochemical parameters.

Material and Method. This study was conducted in a breeding farm of laying quails situated in the nord of Transylvania, Romania, between May and July 2020.

Animals and experimental design. 200 laying Japanese quails (*Coturnix coturnix japonica*) 8 weeks of age were randomly assigned to one control group and four experimental groups. Before starting the experiment, a pre-experimental period of 2 weeks was considered, when quails were weighed and relocated on replicas and groups. The experimental period lasted for 8 weeks, from the age of 10 to 18 weeks. Thus, at the beginning of the experiment, equality and uniformity between experimental variants was ensured. Each experimental group consisted in five replicates with 8 quails per replicate (N=40). The quails were kept in standard battery cages, with a surface of 337.5 cm^2 per quail, according to the regulations for poultry maintenance (Directive 98/58/EC). Each battery cage was individually equipped with a feed trough, nipple drinkers, trays for manure collecting, and white and yellow lights. Therefore, the maintenance conditions were identical for each replicate and experimental group.

The medial parameters ensured in the shelter were a 22±0.4°C temperature, 70±0.8% humidity (monitored with a digital thermo-hygrometer) and 0.2 m s^{-1} air draft. The daily lighting regime was 18 hours of light and 6 hours of darkness.

Diets formulation. Five types of diets were randomly assigned to each experimental group (Table 1). The composition and nutritional characteristics of the control and experimental diets are presented in Table 1 (Struți et al 2021). The same diets were used in a different experiment, and it was concluded they provide the nutritional requirements of quails (Struți et al 2021).

All the tested diets were formulated to ensure the standard nutritional requirements of laying quails according to NRC (1994), being iso-energetic and iso-protein (2900 ME kcal kg^{-1} and 20%). The combined fodder associated with the control group (C) had soybean meal as the main protein source (control diet). The experimental groups received different amounts of whole lupine seeds in the diets: WLS_{20} - experimental group with whole lupine seeds (200 g kg^{-1}) and WLS_{25} - experimental group with whole lupine seeds (250 g kg^{-1}); and dehulled white lupine seeds: DLS_{20} - experimental group with dehulled lupine seeds (200 g kg^{-1}) and DLS_{25} - experimental group with dehulled lupine seeds (250 g kg^{-1}). The quails had ad-libitum access to the water source and feed.

Table 1

Composition and nutritional characteristics of the laying quail (*Coturnix coturnix japonica*) diets

Specification	*C*	*Experimental diets*			
		WLS₂₀	*WLS₂₅*	*DLS₂₀*	*DLS₂₅*
Composition of feed (%)					
Maize (8% Cp)	46.03	41.85	41.1	49.03	49.75
Triticale (11.4% Cp)	10	10	10	10	10
Soybean meal (46% Cp)	33	16.5	12.3	12	6.8
Lupine whole seeds	-	20	25	-	-
Lupine dehulled seeds	-	-	-	20	25
Sunflower oil	3.2	3.85	3.8	1.1	0.5
DL-Methionine	0.02	0.05	0.05	0.05	0.05
L-lysine HCl	-	-	-	0.07	0.15
Limestone	5.25	5.25	5.25	5.25	5.25
Vitamin–Mineral premix	2.5	2.5	2.5	2.5	2.5
TOTAL	100	100	100	100	100
Nutritional characteristics (calculated values)					
Metabolizable energy (kcal/kg)	2901	2906	2904	2903	2903
Crude protein (%)	20.02	20.04	20.03	20.02	20.04
Ether extract (%)	5.83	7.9	8.23	5.55	5.42
Crude fiber (%)	2.88	4.55	4.98	2.66	2.61
Lysine (%)	1.02	1.03	1.01	1	1
Methionine (%)	0.45	0.45	0.45	0.45	0.45
Methionine+cysteine (%)	0.8	0.81	0.81	0.83	0.83
Calcium (%)	2.5	2.5	2.5	2.5	2.5
Available phosphorus (%)	0.43	0.44	0.44	0.43	0.43

Note: C – control diet; WLS_{20} – experimental diet with 200 g kg^{-1} whole lupine seeds; WLS_{25} - experimental diet with 250 g kg^{-1} whole lupine seeds; DLS_{20} - experimental diet with 200 g kg^{-1} dehulled lupine seeds; DLS_{25} - experimental diet with 250 g kg^{-1} dehulled lupine seeds. Diets were formulated according to NRC (1994). Vitamin–Mineral premix: vitamin A 480000 IU kg^{-1}, vitamin B1 60 mg kg^{-1}, vitamin B2 200 mg kg^{-1}, vitamin B4 16.800 mg kg^{-1}, vitamin B5 441 mg kg^{-1}, vitamin B6 200 mg kg^{-1}, vitamin B9 60 mg kg^{-1}, vitamin B12 0.6 mg kg^{-1}, vitamin D3 95000 IU kg^{-1}, vitamin E 1200 IU kg^{-1}, vitamin H 4 mg kg^{-1}, vitamin K3 100 mg kg^{-1}, vitamin PP 2400 mg kg^{-1}, copper 600 mg kg^{-1}, iron 2160 mg kg^{-1}, iodine 48 mg kg^{-1}, manganese 3720 mg kg^{-1}, selenium 6 mg kg^{-1}, zinc 2844 mg kg^{-1}, calcium 145 g kg^{-1}, phosphorus 123 g kg^{-1}, chlor 7.1%, sodium 5.5%; DL-methionine 54.72 g kg^{-1}, BHT, propil galat (E310), etoxiquin.

Analysis of faecal humidity. The faecal humidity (%) was determined weekly (in the same day), the fresh faeces resulting after one hour being collected from each replicate and group (n=5 per group), followed by the determination of the dry matter in the oven (by dehydration: 100 - DM%).

Analysis of intestinal viscosity. At the end of the experiment, from one quail of each repetition (5 birds per group), the digestive content of the terminal part of the small intestine was emptied and the samples were prepared according to the method of Konieczka & Smulikowska (2017). The determination of the intestinal viscosity was performed with the Brookfield viscometer (model LVDVE, Brookfield Engineering Laboratories, MA 02346) equipped with coaxial cylinders and a mobile rotor, the values being expressed in centipoise (cP).

Blood parameters. At the end of the experiment corresponding to 18 weeks of age, a quail was sampled randomly from each replicate of the group after an 5 hour no feed interval (n=5 per group). Blood samples were collected in the lithium-heparin tubes. The samples were transferred to the hematological analysis laboratory, being centrifuged at

3000x rpm for 30 min to separate the blood plasma, then stored at -20°C, until the following parameters were analyzed: hematocrit - Hct (%); hemoglobin - Hb (g dl^{-1}); erythrocytes - Ery (mL mm^{-3}); superoxide dismutase - SOD (U gHb^{-1}); glutathione peroxidase - GPx (U gHb^{-1}); total protein (g dL^{-1}); albumin (g dL^{-1}); γ–Globulin (g dL^{-1}); Urea (mg dL^{-1}); creatinine (mg dL^{-1}); total lipids (mg dL^{-1}); cholesterol (mg dL^{-1}); trigiliceride - TGs (mg dL^{-1}); alanine aminotransferase - ALAT (U L^{-1}); aspartate aminotransferase - ASAT (U L^{-1}). The samples were analyzed to highlight the quail health status and some parameters of nutrients metabolism, as a result of the applied dietary treatments.

Statistical analysis. The statistical analyses of the results and the graphical display of the data were performed using Microsoft Excel 2013. To determine the significance of the dehulling effect on the faecal humidity, intestinal viscosity and blood parameters, ANOVA single-way was used. The Tukey Honest significant difference (HSD) test was applied as a post-hoc test to detect the differences between two treatments. The confidence level used was set at 0.05 for all analyses. Data were expressed as mean ± standard deviation (SD).

Results and Discussion

Faecal humidity and intestinal viscosity. The utilisation of whole and dehulled lupine seeds in the laying quails diet led to an increase in fecal moisture and viscosity of the intestinal digestive content ($p<0.05$) compared to the control group, without lupine in feed (Table 2). The dehulling of lupine seeds had a negative influence on the fecal moisture, which it increased, even if the differences were not statistically suported. The inclusion of a high amount of whole and dehulled lupine seeds in the structure of quail feed (250 g kg^{-1}) led to an increased ($p<0.05$) fecal moisture (Table 2).

Table 2

Influence of dehulling lupine seeds used in the quail (*Coturnix coturnix japonica*) diets, on the humidity of faeces evolution (mean±SD)

Week	*C*	*Experimental treatments*				*Anova single-way*	
		WLS_{20}	*WLS_{25}*	*DLS_{20}*	*DLS_{25}*	*F-value*	*P-value*
W-1	$77.42±2.61^{a}$	$75.52±1.32^{a}$	$76.38±1.77^{a}$	$76.57±1.94^{a}$	$78.81±2.34^{a}$	1.845	0.160
W-2	$73.20±1.27^{a}$	$73.79±2.59^{a}$	$73.00±1.60^{a}$	$74.46±1.70^{a}$	$76.38±2.74^{a}$	1.738	0.181
W-3	$73.25±1.55^{a}$	$74.79±1.26^{ab}$	$76.77±1.99^{b}$	$75.89±2.68^{ab}$	$76.93±1.26^{b}$	3.492	0.026
W-4	$71.83±0.83^{a}$	$76.86±5.31^{a}$	$75.64±2.78^{a}$	$73.17±2.05^{a}$	$75.24±3.25^{a}$	1.984	0.136
W-5	$76.45±2.21^{a}$	$75.05±1.29^{a}$	$77.11±1.76^{a}$	$76.69±4.13^{a}$	$76.16±2.77^{a}$	0.439	0.779
W-6	$74.35±0.49^{a}$	$74.75±0.97^{a}$	$79.43±2.65^{b}$	$78.01±2.33^{ab}$	$77.48±2.66^{ab}$	5.720	0.003
W-7	$77.70±1.64^{a}$	$78.23±3.81^{a}$	$78.59±1.11^{a}$	$77.34±1.95^{a}$	$80.98±1.81^{a}$	2.013	0.131
W-8	$73.03±1.24^{a}$	$75.13±3.05^{ab}$	$76.84±2.85^{ab}$	$77.54±3.27^{ab}$	$78.56±2.69^{b}$	3.257	0.033
Mean	$74.65±2.57^{a}$	$75.51±2.89^{ab}$	$76.85±2.51^{bc}$	$76.21±2.84^{abc}$	$77.57±2.85^{c}$	9.037	0.000

Note: SD – standard deviation; different superscript letters in the same row show significant differences ($p<0.05$); C – control group, without lupine; WLS_{20} – experimental diet with 200 g kg^{-1} whole lupine seeds; WLS_{25} - experimental diet with 250 g kg^{-1} whole lupine seeds; DLS_{20} - experimental diet with 200 g kg^{-1} dehulled lupine seeds; DLS_{25} - experimental diet with 250 g kg^{-1} dehulled lupine seeds; W-1 to W-8 – experimental weeks.

The utilisation of whole and dehulled lupine seed in the laying quail diets led to an increase in viscosity of the intestinal digestive content ($p<0.05$) compared to the control group, without lupine in the feed (Figure 1). The dehulling of lupine seeds had a negative influence on digestive processes, because it lead to an increase tendency of the intestinal digesta viscosity, especially when the dehulled lupine was used in the amount of 250 g kg^{-1} in the diet, even if the differences were not statistically suported ($p>0.05$).

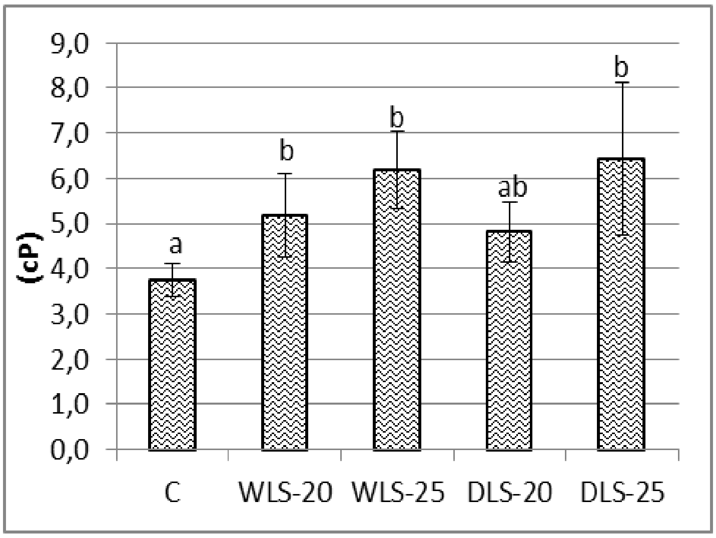

Figure 1. Influence of dehulling lupine seeds used in quail feed on the intestinal viscosity (centipoise-cP); C – control group; WLS-20 - experimental group with 200 g kg^{-1} whole lupine seeds in feed; WLS-25 - experimental group with 250 g kg^{-1} whole lupine seeds in feed; DLS-20 - experimental group with 200 g kg^{-1} dehulled lupine seeds in feed; DLS-25 - experimental group with 250 g kg^{-1} dehulled lupine seeds in feed; a-b - different superscript letters above bars show significant differences ($p<0.05$); bars represent the standard deviation.

Biochemical blood parameters. The results regarding blood biochemical parameters, which are indicators of the metabolic processes status of feed nutrients are presented in Table 3.

The introduction of whole or dehulled lupine seeds in the quail diet did not affect ($p>0.05$) the hematological parameters such as: hematocrit (%), hemoglobin (g dL^{-1}), eritrocytes (mL mm^{-3}), albumin (g dL^{-1}) and γ-globulins (g dL^{-1}). The inclusion of lupin seeds (whole or dehulled) in the amount of 250 g kg^{-1} in the quail diets led to an increased ($p<0.05$) value associated with oxidative stress, SOD and GPx compared to the control group, without lupine.

Quails from WLS_{20}, WLS_{25} and DLS_{25} had a higher concentration ($p<0.05$) of blood proteins than birds from the control. Other components of the plasma protein profile (urea and creatinine) increased with lupine seeds inclusion, the increases being higher in the case of dehulled lupine usage (Table 3).

The blood indices values associated with the lipid profile (total lipids, cholesterol and triglycerides - mg dL^{-1}) reveal a decreasing tendency with the increasing proportion of lupine seed inclusion in feed. Moreover, the utilisation of dehulled seeds in the amount of 250 g kg^{-1} in feed determined the lowest level of lipids ($p<0.05$), cholesterol ($p<0.05$) and plasma triglycerides ($p<0.05$) in quails (Table 3).

An increasing tendency of blood indices associated with the enzymatic profile (ALAT – alanin-aminotransferase; ASAT – aspartat-aminotransferase) was found with the lupine increasing percentage of seed inclusion in the diet, the highest values being recorded at the expermintal groups where dehulled lupine seeds were added (Table 3).

Table 3

Influence of dehulling lupine seeds used in quail (*Coturnix coturnix japonica*) diets on the biochemical blood parameters (mean±SD)

Parameters (n=5)	*C*	*Experimental treatments*				*Anova single-way*	
		WLS_{20}	*WLS_{25}*	*DLS_{20}*	*DLS_{25}*	*F-value*	*P-value*
Hct %	$35.4±3.65^a$	$39.6±2.07^a$	$36.36±1.94^a$	$39.00±0.71^a$	$39.6±4.34^a$	2.394	0.085
Hb g dL^{-1}	$10.78± 0.9^a$	$11.5±0.41^a$	$11.72±1.07^a$	$11.92±0.6^a$	$11.9±1.62^a$	1.085	0.391
Ery mL mm^{-3}	$2.95± 0.51^a$	$3.59±0.38^a$	$3.2±0.65^a$	$3.47±0.23^a$	$3.81±0.8^a$	1.858	0.157
SOD U gHb^{-1}	$682.2±75.75^a$	$692.14±55.59^a$	$832.04±41.22^b$	$770.16±64.03^{ab}$	$832.2±41.32^b$	8.091	0.000
GPx U gHb^{-1}	$116.8±6.42^a$	$117.28±8.41^a$	$125.46±14.53^{ab}$	$130.2±12.25^{ab}$	$145.42±17.87^b$	4.341	0.011
Proteins g dL^{-1}	$3.78±0.2^a$	$4.87±0.37^b$	$4.46±0.3^{bc}$	$4.07±0.24^{ac}$	$4.71±0.18^b$	14.353	0.000
Albumin g dL^{-1}	$1.9±0.33^a$	$1.65±0.16^a$	$1.76±0.16^a$	$1.69±0.16^a$	$1.87±0.19^a$	3.295	0.131
γ -Globulin g dL^{-1}	$0.51±0.03^a$	$0.5±0.04^a$	$0.53±0.03^a$	$0.55±0.02^a$	$0.58±0.01^a$	0.205	0.932
Urea mg dL^{-1}	$22.24±2.49^a$	$24.04±4.65^a$	$27.7±12.18^a$	$33.02±10.29^a$	$34.26±10.21^a$	1.828	0.163
Creatinine mg dL^{-1}	$0.32±0.04^a$	$0.6±0.11^b$	$0.61±0.07^b$	$0.57±0.09^b$	$0.63±0.19^b$	6.669	0.001
Total lipids mg dL^{-1}	$1298.6±170.46^a$	$1127.28±177.76^{ab}$	$972.4±125.7^{bc}$	$1123.98±173.57^{ab}$	$817.98±126.26^{cd}$	6.707	0.001
Cholesterol mg dL^{-1}	$241.54±35.58^a$	$237.62±36.83^a$	$187.12±47.34^{ab}$	$202.02±33.87^{ab}$	$174.84±31.53^{bc}$	4.781	0.005
Triglyceride mg dL^{-1}	$988.54±84.16^a$	$807.74±100.7^b$	$637.78±76.93^{bc}$	$750.34±123.11^b$	$539.62±55.78^c$	17.651	0.000
ALAT U L^{-1}	$8.04±2.7^a$	$13±2.41^{ab}$	$17.82±1.68^{abc}$	$21.66±9.04^{bc}$	$28.60±11.35^c$	6.894	0.001
ASAT U L^{-1}	$78.56±1.62^a$	$76.94±10.35^a$	$81.9±1.28^a$	$87.76±8.15^{ab}$	$102.22±13.2^b$	7.441	0.001

Note: SD – standard deviation; different superscript letters in the same row show significant differences ($p<0.05$); Hct - hematocrit; Hb-hemoglobin; Ery - erythrocytes; SOD - superoxide dismutase; GPx - glutathione peroxidase; ALAT – alanine-aminotransferase; ASAT – aspartate-aminotransferase; C – control group, without lupine; WLS_{20} – experimental diet with 200 g kg^{-1} whole lupine seeds; WLS_{25} - experimental diet with 250 g kg^{-1} whole lupine seeds; DLS_{20} - experimental diet with 200 g kg^{-1} dehulled lupine seeds; DLS_{25} - experimental diet with 250 g kg^{-1} dehulled lupine seeds.

Faecal humidity and intestinal viscosity. The increase of feces humidity (p=0.000) with the gradual inclusion of whole and dehulled lupine seeds in quail diets (Table 2) is possible due to the high content of soluble NSPs, which have a high capacity for water retention (Mierliță & Popovici 2013; Knudsen 2014). Dehulling of lupine seeds did not significantly influence (p>0.05) the feces moisture and intestinal digesta viscosity (Table 2 and Figure 3). However, there is a tendency of value increase for these two parameters, especially in the case of the DLS_{25} group, due to content of soluble NSPs in lupine seeds, whose concentration increased as a result of dehulling. The effect of increasing feces moisture and intestinal digesta viscosity was also observed at broiler chickens fed with white and blue lupine (Mierliță & Popovici 2013; Konieczka & Smulikowska 2017). High humidity of intestinal digesta is induced by soluble NSP from lupine seeds (Smulikowska et al 2014). Whole seeds of white lupine contain soluble NSP in the amount of 28.8-65.9 g kg^{-1} (Nalle et al 2011; Hejdysz et al 2018). Increasing the viscosity of intestinal digesta depreciates the nutrient absorption, especially affecting the digestion and absorption of fats and fat-soluble vitamins (Smulikowska 1998), aspects that lead to decreased productive performances. These soluble NSPs exert an antinutritional effect on monogastric animals, determining the increases of the intestinal passage time and giving a viscous consistency, which causes increased manure moisture (Písaříková & Zralý 2010; Konieczka & Smulikowska 2017). According to Smulikowska et al (2014), the use of 250 g kg^{-1} whole blue lupine seeds in the feed of broiler chicken aged 15-35 days led to an intestinal viscosity of 3.4 cP, which is lower than the value obtained in the present research (6.18 cP). Steenfeldt et al (2003) report for broiler chickens an intestinal viscosity level close to the value obtained by us (5.5 vs. 5.16 cP) when using similar amounts of whole lupine in feed, 200 g kg^{-1}. Compared to our results, other studies report higher values of intestinal viscosity for broiler chickens, showing an increase as the amount of lupine seeds in the diet increases. In this sense, Kocher et al (2000) report a value of 11.6 cP when they used the whole blue lupine seeds in the amount of 350 g kg^{-1} in diet, and Olkowski et al (2005) report a value of 32.7 CP when they used blue lupine in the amount of 400 g kg^{-1} in feed. Deficiencies can be eliminated (or diminished) if specific enzymatic preparations such as α-galactosidase, β-glucanase, arabinoxylanase, pectinase are added in the lupine-containing poultry combined feeds (Brenes et al 2002, 2005; Olkowski et al 2010).

Biochemical blood parameters. Blood parameters are currently determined in animal nutrition research because they are a good way to find out if the dietary treatments applied influence certain metabolic indices that could lead to the appearance of pathological conditions. The analysis of the selected hematological parameters indicates a normal physiological status of quails, regardless of the applied dietary treatment. This conclusion is supported by all analyzed blood indices, which are within the normal physiological limits specific for the laying quail (Konca et al 2014; da Silva et al 2017).

The results obtained in the current research reaveal that using dehulled white lupine seeds in the quails diet influences the blood metabolic indices of proteins, lipids and carbohydrates (Table 3).

Total plasma lipids, cholesterol and triglycerides highlight a decreasing trend as the proportion of lupine seeds in the diets increases. Through seed dehulling, the plasma concentration of these compounds decreases due to the high quality of fats from lupine seeds, which have an increased level of polyunsaturated fatty acids from the n-3 and n-6 series (Mierliţă 2017). In this regard, Konca et al (2014) concludes that ingredients rich in polyunsaturated fatty acids (such as hemp seed) can be used in laying quail feeds to reduce blood cholesterol levels and to obtain eggs enriched in omega-3 fatty acids. Similar to our results, Straková et al (2021) highlight a significantly decreased level of triglycerides and plasma cholesterol when 50% of soybean meal is replaced by dehulled white lupine seeds in the diet of laying hens. The values obtained for plasma cholesterol are in the value range of 130-279 mg dL^{-1} reported by the literature for laying quails (Konca et al 2014; da Silva et al 2017).

Creatinine is the form of creatine elimination from the organism and is formed by amino acid deamination (Mierliţă 2017). In the present research, higher values of creatinine were registered in the quail groups that received lupine in their feed (whole and dehulled seeds) compared to the group without lupine.

Urea, which is a final product of protein metabolism, has not been influenced by the use of lupine seeds in the quail diet, even if there is a tendency for increase of values as the proportion of lupine inclusion increases. The higher plasma urea levels result from the deamination of amino acids and their use as an energy source, especially in the case of proteins with low biological value from the feed (Mierliţă 2017).

SOD is an enzyme with an antioxidant role that prevents (and reduces) oxidative stress and the accumulation of oxidative by-products of fatty acids in the eggs (Mierliţă 2017). In the present research, SOD has higher values ($p<0.05$) in quails from the groups who received lupine seeds in the dose of 250 g kg^{-1} in feed (WLS_{25} and DLS_{25}) compared to quails from the control group and WLS20.

In the present research, GPx has higher values for the quails that received dehulled lupine seeds in quantities of 250 g kg^{-1} compared with quails fed the diet without lupine, because lupine seeds have a high content of unsaturated and especially polyunsaturated fatty acids. GPx is an enzyme whose level increases when oxidative stress is induced by the increase of unsaturated fatty acids in the blood (Mierliţă 2017).

ALAT and ASAT present increased values only for quails fed dehulled lupine in a proportion of 25% in diet, which suggests the presence of unbalanced amino acids in lupine proteins, and, thus, a high amount of alanine and aspartic acid has been deaminated and used as an energy source in the organism (Mierliţă 2017).

Conclusions. In conclusion, the results reveal that seed dehulling and use in the quail diet did not positively influence ($p>0.05$) the intestinal digesta viscosity and faecal humidity. Inclusion of lupin seeds in the amount of 250 g kg^{-1} (whole or dehulled) in the quail diet led to an increased ($p<0.05$) value of the oxidative stress and glutathione peroxidase. The components of urea and creatinine increase with the lupine seeds inclusion in diet. The blood indices values associated with the lipid profile (total lipids, cholesterol and triglycerides reveal a decreasing tendency with the increasing proportion of lupine seed inclusion in feed.

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