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LUPINE IS APPLICABLE IN DIETS FOR LAYER CHICKENS OF PARENTAL FLOCK

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Abstract

Climatic conditions in most Russian regions are unfavorable for the cultivation of soy beans which are considered the best protein source in diets for all types of poultry. Soya yield in Russian soya-producing regions (Far East, Krasnodar Krai, and some other southern territories) cannot provide the growing poultry production with this important protein source. The alternatives for soybean meal and other soya products are therefore in need; domestically selected low-alkaloid cultivars of white and narrow-leaf lupine are increasingly gaining importance as vegetable protein sources. Feed-grade lupine usually contains up to 42 % of crude protein. The disadvantages of lupine are high contents of fiber (12.5-16.0 %) and lignin (0.9 %), and the presence of alkaloids. Alkaloid content in sweet lupine cultivars is 0.008-0.12 %, in bitter cultivars 1-3 %. Chemical and amino acid composition of white lupine modern cultivars was earlier determined, and their efficiency in poultry diets was studied in vivo. It was found that the grain of low-alkaloid cultivars Gamma, Dega, Dikaf 14 can be included into the diets for poultry at 15-20 % dosage. Supplementation of lupine-containing diets with proper enzyme preparations can improve the digestibility of dietary nutrients and poultry performance. Dehulling of lupine grain decreases fiber content and increases protein content in concentrated lupine-based protein feeds; the latter in dehulled lupine is close to that in soybean products. This original research for the first time proves the possibility of soybean and sunflower meals substitution for white low-alkaloid lupine cultivar Dega the in diets for parental flock of laying hens. The trial was performed on 5 groups of parental White Leghorn layers (cross SP 789) from 184 to 365 days of age fed balanced diets containing 0; 5; 7; 10; and 15 % of dehulled Dega lupine grain (39.61 % crude protein, 5.60 % crude fiber). It was found that 5-10 % lupine does not impair livability and productivity parameters in layers. Lupine was found to influence the intensity of lay, egg fertility, hatch of chicks, micromorphology of liver in hens. The substitution of soy for lupine (7, 10, and 15 %) improved egg production and egg weight output per hen by 1.51 and 7.10 %; 3.31 and 1.64 %; 6.56 and 3.64 %, respectively, in compare to control; feed expenses per 1 kg egg weight in these groups was 0.9; 4.07; and 1.81 % lower. The percentages of infertile eggs and early embryonic deaths in using 10 and 15 % lupine were lower compared to control, evidencing the absence of negative impact of lupine Dega on early embryonic development. The highest doses of lupine did not increase the incidence of late embryonic deaths in eggs from layers aged 47 weeks: the percentages of late embryonic deaths and weak chicks from layers fed 15 % lupine were 4 and 5 % vs. 9 and 8 % in control. The histological investigation of liver revealed no significant differences between lupine-fed and control layers; nucleoplasmic ratio in the hepatocytes was similar in all treatments. In all treatments liver had no abnormalities, the connective tissues were poorly developed and located in the peripheral segments of the liver (where it forms a capsule) and near the portal triad. The hepatic plate structure is well developed, and the tortuous plates are radially oriented. The clusters of blood cells are seen in the lumen of the central veins and sinuous capillaries. Hepatocytes are not clearly bordered, polygonal in shape; nuclei centered or sometimes slightly peripherally shifted, round or oviform, has 1-4 nucleoli. Cytoplasm is unevenly stained, granular; lymphoid cells are found in the stroma and parenchyma. The results of histological investigation of liver, productivity parameters in layers, and the efficiency of incubation of eggs obtained evidence that 5-15 % of Dega lupine dehulled grain as a protein source in diets of laying hens from parental flock does not impair

productivity and rendered no cytotoxic effects on the liver of hens. This cultivar of white lupine can be recommended as a dietary protein source both for commercial and parental layer flocks.

Keywords: white lupine, alkaloids, laying hens, productivity, egg fertility, hatchability, liver histomorphology

Climatic conditions in most Russian regions are unfavorable for the cultivation of sufficient quantity of soybeans (i.e., a worthful crop) required for poultry production. Sunflower meal is a common protein feed in Russia. As for pulse crops, pea, field beans, vetch and lupine bear mentioning [1-4]. Due to lack of protein poultry feed, lupine is of greater interest because of high yield and potential cultivation in areas where soybean growth is impossible, or its yield is low. Some Russian low-alkaloid lupine cultivars are developed to substitute soya products in feed production [5, 6]. In terms of global production, lupine occupies about 1% of cultivation areas. Nowadays, Australia, New Zealand, Poland and Belarus are leaders of lupine cultivation [7-9]. Romanian cultivars of low-alkaloid white lupine were developed recently. Climatic conditions are favorable to cultivate narrow-leaf and yellow lupine in Poland, Belarus and Germany. Its impact on egg production and quality [10-12] associated with improved dietary formulation [13], antinutrient effects [14], as well as application of low-alkaloid cultivars [15], whole and dehulled seeds [16] are still studied. Feed value and potential use of blue lupine is an active area of research in Great Britain [17, 18].

Feed-grade lupine usually contains up to 42% of protein. This is a good source to replenish concentrated feed with crude protein that, in turn, is of great importance to manage deficiency of qualified animal feeds and soybean meal. The disadvantages of lupine are high contents of fiber (12.5-16.0%) and lignin (0.9%), and the presence of alkaloids. Namely, sweet and bitter lupine cultivars contain 0.008-0.120% and 1-3%, respectively. Lupine alkaloids are lupinine, lupanine, sparteine and hydroxylupanine. Lupanine is the most toxic alkaloid quantitatively predominant in most of bitter lupine cultivars. Containing an atom of nitrogen and a primary alcohol group, it can be oxidized to lupininic acid. Lupinine is a main toxic factor of lupine intoxication. At the same time, other alkaloids are nothing but accessory factors of toxicosis. TDL0 of lupinine is 25-28 mg/kg, LD is 29-31 mg/kg [9]. As for Russian white low-alkaloid lupine cultivar Dega, mean total content of alkaloids in whole grain is 0.062% where lupanine (37.1%) and dehydroxylupanine (14.3%) are predominant components. The value increases to 0.080% [3] in dehulled grain.

1. Alkaloid content in Dega lupine cultivar grain (on absolutely dry basis, %) (M±SEM) **[3]**

Parameter	Whole grain	Dehulled grain
Alkaloid content	0.062±0.006	0.080±0,008
Alkaloid composition, %		
sparteine	5.9±0.6	8.5±0.8
ammodendrine	8.5±0.9	5.4±0.5
angustifoline	9.5±1.0	8.1±0.8
isolupaine	9.3±0.9	8.8±0.9
aphillidine	4.7±0.5	5.7±0.6
dehydoxylupanine	14.3±1.4	15.0±1.5
lupanine	37.1±3.7	40.1±4.0
13-oxylupanine	8.5±0.8	6.5±0.6
ether 13-oxylupanine	2.2±0.2	1.4±0.1

Previously we [1, 2, 19, 20] and other researchers [21] studied efficiency of Gamma and Dega cultivars consumed by commercial broilers and layers. According to the studies, up to 20% of lupine can be included into their diet [1, 19].

The remarkable thing is that high content of fiber specific for narrow-leaf and white lupine results in restricted application of high doses in poultry diets. To solve the issue, multi-enzyme preparations [1, 4, 22] or lupine bean dehulling are used. The latter enables both decrease in fiber content in concentrated feed and increased protein level that is equivalent in the dehulled lupine grain and soya products [16, 19].

Despite broader industrial use of lupine to feed commercial broilers and layer flocks, there are no similar national studies in breeding poultry in fact.

It is the first time we demonstrated that white low-alkaloid lupine cultivar Dega can be an efficient substitution for soya and sunflower products to produce concentrated feed for laying hens of parental flock. It was found that 5-15% of Dega lupine dehulled grain did not have a negative effect on layer liver and hepatic micromorphology. Also, this resulted in high livability and productivity parameters, as well as 1-day young crop equivalent to ones associated with soya products.

Our purpose was to evaluate potential application of the white lupine cultivar Dega as a substitution for soya and sunflower products to feed layers of a parental flock.

Techniques. The trial was performed on 5 groups of parental layers (cross SP 789; each group included 30 hens) aged 184-365 days in the vivarium of Zagorskoe Genetic Selection Centre of the Federal Scientific Center All-Russian Research & Technology Institute of Poultry RAS (Moscow Province) during the 6-month productive life. Poultry were held on dedicated cages (Pyatigorsksel'mash ZAO, Russia). Feed was given manually. Dega white lupine toasted dehulled grain was included into poultry diet. Feeding and management conditions (concentrated feed nutritional value, seating standards, light, temperature and moisture conditions, feeding and drinking spaces) complied with appropriate guidelines (All-Russian Research & Technology Institute of Poultry, 2015) within all the test. Layers of group I (i.e., controls) consumed balanced crumbled complete concentrated lupine-free feed (primary diet, PD). To replace soya products, groups II, III, IV and V were fed with concentrated feed containing 5%, 7%, 10% and 15% of lupine.

Layers aged 34 and 47 weeks were artificially inseminated to obtain hatching eggs. The insemination was carried out as per a standard method using a new patented medium to dilute cock semen (patent RU no. 2637774 C2, insemination dose – 0.1 ml, dilution – 1:3). 100 eggs collected from each group were placed in an experimental incubator (Danki, Belgium). Using a dedicated probe (accuracy < 0.1 °C), 37.7 °C and 37.2 °C was maintained during preincubation (days 1-18) and hatcher period (days 19-21). Relative humidity values were 52-53% and 52-75%, respectively.

We considered main zootechnical parameters such as initial and final weight (individual weighing), livestock livability and egg production. Feed consumption and expenses (per a hen, 10 eggs and 1 kg of eggs) were calculated. Incubation parameters (fertility and hatch), vitamin and carotenoid hepatic levels, hepatic chemical composition, as well as calcium, phosphorus and manganese shinbone levels were assessed as per effective GOST standard methods [23].

At the end of the test hepatic samples were collected from the lateral side of the right hepatic lobe of laying hens (n = 15, 3 hens were selected in each group) within 1 hour after the slaughter. These samples were fixed in 10% aqueous solution of neutral formalin. The material was washed with current water, dehydrated in ethanol of increasing concentration and condensed with paraffin. Paraffin 5-8 μm sections were produced by a RMD-3000 semi-automatic rotary microtome (Kreonika OOO, Russia). Deparaffinized sections were stained with hematoxylin and eosin. General morphological picture was evaluated with a Micromed-3 (LOMO, Russia) light microscope. Micrometry was performed with a DSM 300 ocular camera (Hangzhou Scopetek Opto-Electric Co., Ltd, China) and ScopePhoto 3.1 software (China) (https://scopephoto.software.informer.com/3.1/). Major and minor diameters of hepatocytes and their nuclei, volume of cells, cytoplasm and their nuclei, as well as nucleoplasmic ratio were evaluated. Volume

of hepatocytes and their nuclei was assessed using C. Tasca's formula [24]: $\pi/(6 \times L \times B^2)$, where L is a major cell (nuclear) diameter (μm) and B is a minor cell (nuclear) diameter (μm).

The findings were processed according to Lakin [25] and Plokhinskii [26]. STATISTICA 10 (StatSoft, Inc., USA) software was applied. Means (*M*) and errors of means (±SEM) were assessed. To calculate significance of differences, *t*-test was used. Differences were considered as statistically significant at $p \leq 0.05$.

Results. See primary parameters of Dega chemical composition compared with other white lupine cultivars in the Table 2. Certainly, protein, fat and carotenoid levels can vary depending on climatic conditions. In general, the table demonstrates nutritional value of modern national white lupine cultivars completely.

2. Chemical and amino acid compositions of Russian lupine cultivar grain (on absolutely dry basis, %)

Content, %	Start	Manovitskii	Gamma	Delta	Dega	Deter	Desnyanskii
Moisture	9.68	9.44	8.66	8.45	8.61	8.65	8.78
Protein	34.18	34.93	37.75	33.12	33.81	35.12	32.18
Fiber	10.68	10.92	10.76	10.21	10.86	9.95	10.19
Fat	9.37	9.28	9.95	11.25	9.79	9.90	11.75
Ash	3.37	3.09	3.32	3.12	3.31	3.11	3.05
Calcium	0.308	0.321	0.350	0.335	0.354	0.360	0.380
Phosphorus	0.300	0.359	0.300	0.310	0.310	0.300	0.300
Carotenoids	27.33	27.25	28.77	17.71	28.47	23.17	23.42
Lysine	1.37	1.41	1.44	1.43	1.41	1.57	1.54
Histidine	0.79	0.80	0.94	0.85	0.81	0.92	0.94
Arginine	2.84	2.91	3.34	2.99	2.97	3.17	2.73
Aspartic acid	3.22	3.16	3.10	2.96	2.89	3.35	3.01
Threonine	1.27	1.24	1.34	1.27	1.18	1.23	1.18
Serine	1.66	1.75	1.81	1.72	1.65	1.70	1.55
Glutamic acid	6.42	6.95	7.63	7.28	7.17	6.78	6.22
Proline	0.93	1.13	1.04	1.69	1.46	1.30	1.40
Glycine	1.17	1.22	1.28	1.19	1.18	1.18	1.12
Alanine	0.96	1.03	1.19	1.62	1.02	0.94	0.96
Cystine	0.47	0.39	0.44	0.48	0.45	0.45	0.51
Valine	1.03	1.06	1.19	1.09	1.08	1.08	1.03
Methionine	0.42	0.32	0.39	0.40	0.37	0.39	0.36
Isoleucine	1.05	1.13	1.32	1.17	1.18	1.21	1.02
Leucine	2.01	2.14	2.44	2.16	2.22	2.35	2.18
Tyrosine	1.31	1.40	1.60	1.36	1.44	1.55	1.25
Phenylalanine	1.07	1.11	1.24	1.28	1.09	1.20	1.01

N o t e. Presented findings resulted from the test performed in the Testing Center (Federal Scientific Center All-Russian Research & Technology Institute of Poultry RAS).

See results of chemical and toxicological tests, as well as amino acid composition of Dega dehulled grain in the Table 3.

It was found that 5-15% of Dega lupine grain fed within the 6-month productive life did not decrease productivity and livability of layers. Thus, the livestock livability was 96.67-100 %. Mortality of controls, as well as poultry in groups III and V consumed 7% and 15% of lupine, respectively, was 3.33%. It was not associated with a food factor (see Table 4). Poultry consumed lupine-containing concentrated feed willingly. Despite higher productivity of layers in groups III, IV and V, as well as increased nutrient removal with eggs, weight of these 52-week hens was by 7.25%, 3.52% and 8.69% higher than in controls (differences between test and control poultry are significant if $p \leq 0.5$). Along with this, layers in groups III, IV and V consumed by 0.90%, 4.07% and 1.81% less of feed per 1 kg of eggs. In general, lupine substitution for conventional protein sources in concentrated feed (groups III, IV and V) increased egg production and, in turn, egg output and weight per a layer by 1.51%, 7.10% and 3.31%, respectively, as well as by 1.64%, 6.56% and 3.64%, respectively.

3. Chemical composition of Dega dehulled grains (on air-dried basis, %)

Parameter	Value
Chemical composition	
Moisture content, %	8.20
Crude protein, %	39.61
Crude fiber, %	5.60
Crude fat, %	9.74
Crude ash, %	3.64
Soluble protein, %	88.59
Acid number, mg KOH/g	4.42
Peroxide number, %	0.013
Non-protein nitrogen, %	0.36
Anino acid composition, %	
Aspartic acid	4.18
Threonine	1.42
Serine	1.86
Glutamic acid	8.60
Proline	1.49
Glycine	1.54
Alanine	1.36
Valine	1.65
Isoleucine	1.82
Methionine	0.24
Leucine	2.90
Tyrosine	1.80
Phenyl alanine	1.59
Lysine	2.08
Histidine	0.90
Arginine	4.03
Cystine	0.51
Leucine	2.90
Vitamins, pigments	
Carotenoids, μg/g	32.56
Vitamin E, μg/g	7.30
Minerals	
Calcium, %	0.243
Phosphorus, %	0.410
Manganese, mg/kg	1324.50
Toxic elements	
Lead, mg/kg	0.13
Cadmium, mg/kg	0.002
Arsenic, mg/kg	0.15
Mycotoxins	
Zearalenone, μg/kg	2.06
Ochratoxin, μg/kg	< 1.05
Fumonisin, μg/kg	Not detected
T-2 toxin, μg/kg	3.84
HT2 toxin, μg/kg	9.78
Deoxynivalenol, μg/kg	17.44
Aflatoxin B_1, μg/kg	Not detected
General toxicity	Non-toxic

Note. Presented findings resulted from the test performed in the Testing Center (Federal Scientific Center All-Russian Research & Technology Institute of Poultry RAS).

It is a well-known fact that lupine contains less essential amino acids than soybeans. Although lysine, methionine and threonine deficiency can be compensated with synthetic amino acids, lack of other amino acids can lead to decreased layer productivity (including reduced egg weight). As tabulated in the Table 4, 5-15% of lupine did not result in substantial differences between mean egg weight in control and test groups.

Mineral metabolism intensity providing a stable poultry skeleton, good quality of eggshell and certified hatching eggs is of great importance for parental flock layers. Maximum elastic strain (i.e., a specific parameter of eggshell quality) demonstrated a specified value (23.00 μm) in 34-week test layers. 47-week test layers showed the decreased value as compared with controls (28.05-24.35 μm vs. 28.77 μm). At the same time, group V consumed 15% of lupine demonstrated better eggshell quality. Nevertheless, it worth mentioning that eggshell thickness in all the layers complied with all the specifications of the cross during the observation.

4. Zootechnical parameters of parental flock layers (cross SP 789) consumed different doses of Dega lupine dehulled grain for 6-month productive life (*M*±SEM, vivarium of Zagorskoe Genetic Selection Centre, All-Russian Research & Technology Institute of Poultry RAS, Moscow Province)

Parameter	Group I (контроль)	Group II (5 % lupine)	Group III (7 % lupine)	Group IV (10 % lupine)	Group V (15 % lupine)
Age, days	182-364 сут (26-52 нед)				
Livestock livability, %	96.67	100	96.67	100	96.67
Weight, g:					
start of the test	1621.67±23.54	1613.67±24.43	1592.73±18.19	1590.87±18.02	1599.87±18.02
end of the test	1719.83±40.79	1777.33±41.13	1844.48±51.23*	1780.33±32.59*	1869.31±47.34*
Consumed feed:					
1 hen/day, g	114.8	114.78	114.81	115.68	115.9
per 10 eggs, g	1.403	1.458	1.413	1.356	1.403
per 1 kg of eggs/layer, kg	2.21	2.29	2.19	2.12	2.17
Total number of eggs/layer, pcs	144.13	142.50	146.3	154.37	148.8
Egg production intensity, %	79.19	78.73	80.83	85.29	82.21
Mean egg weight, g	64.44	63.75	64.52	64.11	64.69
Egg output/layer, kg	9.288	9.08	9.440	9.897	9.626
Elastic strain, μm:					
34 weeks	24.10±1.39	23.78±1.37	22.90±1.34	24.67±2.10	22.40±0.98
47 weeks	28.77±2.06	28.05±1.39	27.86±1.83	27.97±1.61	24.35±1.36
Calcium level in eggshell of 47-week hens, %	37.09	37.29	37.39	37.60	37.34
Egg shell thickness, μm:					
34 weeks	418.50±12.15	431.27±9.61	409.74±6.92	411.67±11.70	390.28±6.10
47 weeks	378.72±8.69	343.33±18.47	400.66±15.29	407.73±11.40	394.31±8.08
364-day hen shinbone contains:					
ash, %	58.00	57.30	58.62	60.37	57.75
Ca, %	20.30	20.32	20.80	21.18	20.29
P, %	9.74	9.50	9.88	10.05	9.61
Mn, mg%	1.72	1.80	1.85	2.33	2.99

N o t e. Individual values and mean group values are presented.
* Differences between initial and final test values are statistically significant at $p \leq 0.05$.

Of course, eggshell quality depends on consumption of calcium, phosphorus and vitamin D_3 by laying hens. Also, worse eggshell quality and skeleton status can be caused by manganese deficiency or poor digestibility. We found that lupine is a natural source of manganese organic compounds [27]. Manganese level was assessed in white and narrow-leaf lupine cultivars cultivated in various Russian regions with different level of the element in soil. For example, Gamma white lupine whole, uncoated and coated grains contain 491.1 mg/kg, 941.63 and 115.05 mg/kg of Mn, respectively [27]. Dega cultivar also demonstrates increased accumulation of manganese (1324.5 mg/kg on air-dried basis). Our findings correspond with data reported by other researchers. In particular, manganese content in Dega grain is 1428.0±20 mg/kg on absolutely dry basis [3].

As lupine dosage in layer diet increased (see the Table 4), shinbone manganese pooling rose from 1.72 mg% (controls) to 2.99 mg% (group V, 15% of lupine). There were no significant intergroup differences detected between calcium and phosphorus levels in shinbone and eggshell. However, according to the test, lupine included into the diet induced 0.20%, 0.30%, 0.51% and 0.25% higher calcium levels in eggshell of 47-week hens (groups II, III, IV and V, respectively) as compared with controls.

As known, healthy embryonic development during the early incubation influences on quantitative and, most significantly, qualitative results of incubation. As for our test, all the groups demonstrated high egg fertility (94-100%) for all ages (see the Table 5). Amongst other things, it was associated with cock semen dilutant quality used for insemination [28]. Along with this, groups IV and V showed lower number of infertile eggs than controls. Together with low incubation mortality (blood ring), the result indicates absence of negative impact of

10-15% of Dega lupine dehulled grain on early embryonic development. Moreover, we did not mention increased mortality in the groups during the late incubation of eggs obtained from 47-week layers. Therefore, group V consumed 15% of lupine in the diet demonstrated 4% and 5% of late embryonic deaths and weak chicks vs. 9% and 8% in controls, respectively.

5. Biocontrol parameters (%) of egg incubation in parental flock layers (cross SP 789) consumed different doses of dietary Dega lupine dehulled grain (n = 100, vivarium of Zagorskoe Genetic Selection Centre, All-Russian Research & Technology Institute of Poultry RAS, Moscow Province)

Parameter	Group I (контроль)	Group II (5 % lupine)	Group III (7 % lupine)	Group IV (10 % lupine)	Group V (15 % lupine)
Poultry aged 34 weeks					
Infertile eggs	4	6	1	0	0
Blood rings	5	0	2	7	1
Missed	0	2	1	1	1
Late embryonic death	10	15	9	16	4
Weak	2	5	8	7	2
Hatchability	84.38	81.91	87.88	75.0	94.0
Total hatched	81	77	87	75	94
Condition chicks, pcs	79	72	79	68	93
Poultry aged 47 weeks					
Infertile eggs	6	-	1	5	4
Blood rings	2	1	0	1	3
Missed	2	0	0	0	0
Late embryonic death	9	10	11	7	4
Weak	8	11	2	10	5
Hatchability	86.17	89	88.89	91.58	92.71
Total hatched	81	89	88	87	89
Condition chicks, pcs	73	78	86	77	84

6. Hepatic chemical composition (%) in layers (cross SP 789) consumed different doses of dietary Dega lupine dehulled grain (on air-dried basis, vivarium of Zagorskoe Genetic Selection Centre, All-Russian Research & Technology Institute of Poultry RAS, Moscow Province)

Parameter	Group I (контроль)	Group II (5 % lupine)	Group III (7 % lupine)	Group IV (10 % lupine)	Group V (15 % lupine)
Protein	52.34	49.66	46.20	46.93	48.38
Fat	32.31	33.72	39.66	40.32	36.05
Ash	3.88	3.68	3.90	3.88	3.95

Note. According to the method, differences are significant if variations of protein and fat levels are higher than 1% and 2%, respectively. Thus, increase in fat levels is significant in groups III, IV and V.

As known, decrease in egg production and livability of layers, as well as less qualified incubation eggs observed during the late productive life are associated with higher incidence of fatty liver [23, 29]. Increased hepatic fat level detected in test layers consumed concentrated feed with different lupine content (see the Table 6) resulted in an additional histological liver investigation to determine safe dosage of lupine to be included into a diet.

7. Гистоморфометрические показатели печени у кур-несушек кросса СП 789 при разных дозах обрушенного зерна люпина сорта Дега в рационе (M±SEM, виварий СГЦ «Загорское» ФНЦ ВНИТИП РАН, Московская обл.)

Parameter	Group I (контроль)	Group II (5 % lupine)	Group III (7 % lupine)	Group IV (10 % lupine)	Group V (15 % lupine)
Volume, μm^3:					
hepatocyte	511.73±36.04	494.18±27.61	383.00±24.45	420.56±25.21	496.00±30.59
nucleus	45.07±2.41	41.2±2.30	38.73±2.32	38.57±2.02	41.66±2.48
cytoplasm	466.66±35.31	452.98±27.14	344.62±23.99	381.99±24.46	454.32±30.10
Nucleoplasmic ratio	0.10±0.01	0.09±0.01	0.12±0.01	0.11±0.01	0.10±0.01
Trabecules, μ	18.92±0.53	19.72±0.44	18.43±0.40	18.82±0.48	18.79±0.47
Sinusoids, μ	4.16±0.19	4.63±0.20	4.46±0.19	4.42±0.21	4.69±0.22

The morphometric research of hepatic histostructure did not detect any

significant differences between control and test hens. Nucleoplasmic ratio in hepatocytes was equivalent (see the Table 7).

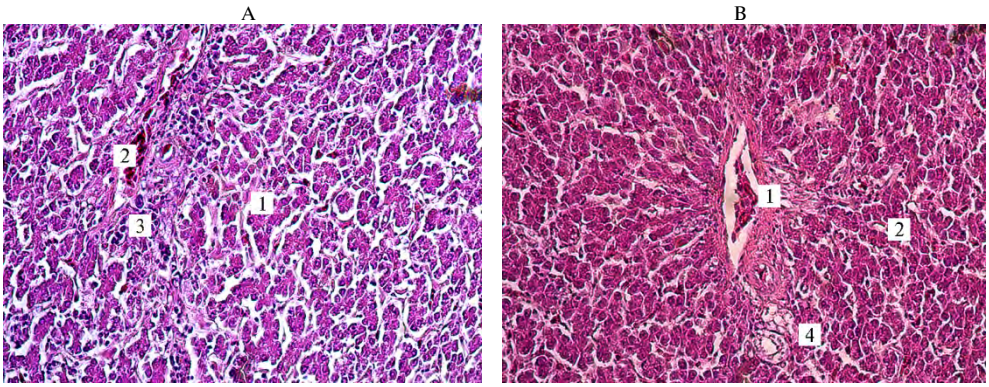

Fig. 1. Hepatic micromorphology in 365-day layers (cross SP 789) consumed dietary Dega lupine dehulled grain: A — lupine-free diet, B — lupine-containing diet (5%); 1 — hepatocytes, 2 — central vein and erythrocytes, 3 — lymphocytes, 4 — sinusoids. Hematoxylin and eosin staining, light microscope (Micromed-3, LOMO, Russia), magnification ×300.

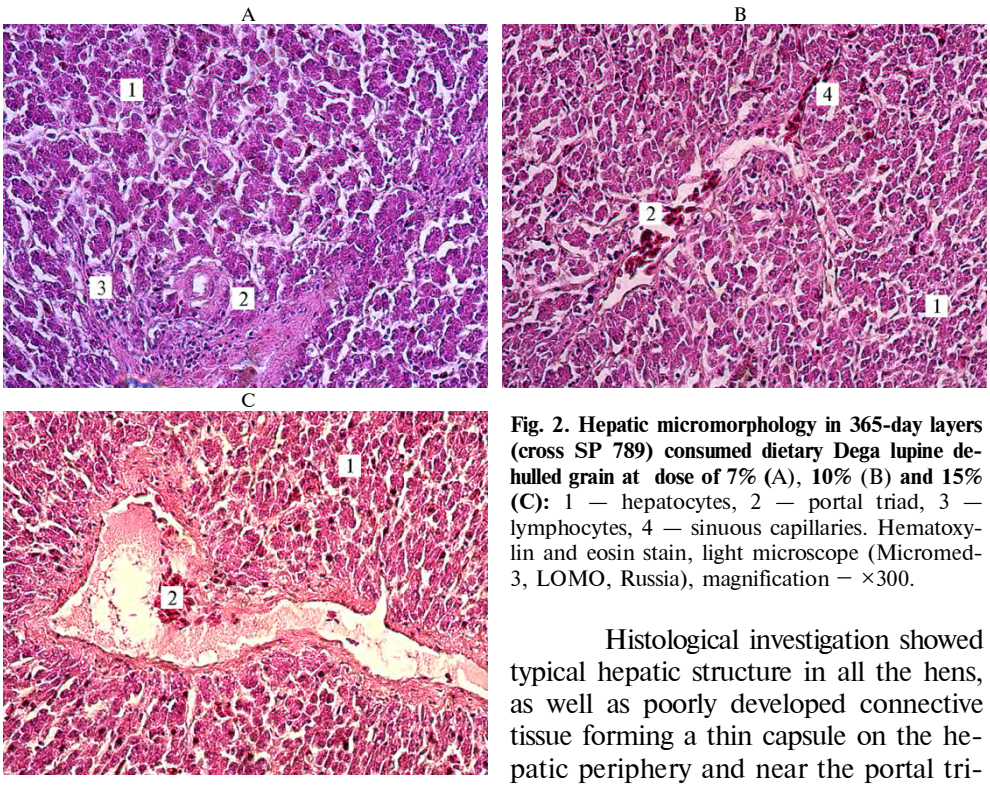

Fig. 2. Hepatic micromorphology in 365-day layers (cross SP 789) consumed dietary Dega lupine dehulled grain at dose of 7% (A), **10%** (B) **and 15% (C):** 1 — hepatocytes, 2 — portal triad, 3 — lymphocytes, 4 — sinuous capillaries. Hematoxylin and eosin stain, light microscope (Micromed-3, LOMO, Russia), magnification – ×300.

Histological investigation showed typical hepatic structure in all the hens, as well as poorly developed connective tissue forming a thin capsule on the hepatic periphery and near the portal triad. The hepatic plate structure is well developed, and the tortuous branchy plates are radially oriented. The clusters of blood cells are seen in the lumen of the central veins and sinuous capillaries. Hepatocytes are not clearly bordered, polygonal in shape; nuclei centered or sometimes slightly peripherally shifted, round or oviform, have 1-4 nucleoli. Cytoplasm is unevenly stained, granular; lymphoid cells are found in the stroma and parenchyma (Fig. 1, 2). Our data on hepatic histological structure comply with findings of other authors [30-34].

Thus, 5-15% of lupine dehulled grain included into the diet of parental flock layers as a protein source did not reduce any zootechnical and incubation pa-

rameters. Poultry demonstrated a long-term egg productivity. Moreover, test livestock livability was similar to controls even after high lupine dose consumption. Histological results confirm absence of evident cytotoxic effect of lupine on hepatic histostructure in layers corresponding with their zootechnical and incubation parameters. This brings us to recommend Dega white lupine to be applied as a feed-grade protein source both in commercial and breeding poultry.

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