Beneficial effects of substituting soybean meal for white lupin (*Lupinus albus*, cv. Zulika) meal on the biochemical blood parameters of laying hens

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**ABSTRACT**

The experiment aimed to determine the effect of substituting 50% of soy protein for lupin protein in feed mixtures intended for the nutrition of high-yield Isa Brown laying hens, on selected biochemical blood plasma indicators. The trial was designed as a long-lasting feeding experiment (51 weeks) and involved a total of 180 hens divided in three experimental groups (control group C and experimental groups WLS and WDLS). At the end of the laying cycle, blood samples were taken from the hens by puncture of the vena basilica and subsequently, the required blood plasma indicators were analysed in the laboratory. The results demonstrated that the long-term feeding of white lupin (variety Zulika) did not affect the molar concentration of plasma inorganic phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), uric acid (UAC) and the activity of alanine aminotransferase (AST). By contrast, the substitution of soy protein for lupin protein in the diets of the experimental groups of laying hens led to the statistically significant (*p* ≤ 0.05) decrease of total plasma protein (TP), total cholesterol (Chol), calcium (Ca) and triacylglycerides (TAG) and to an increase (*p* ≤ 0.05) of alkaline phosphatase activity (ALP).

**HIGHLIGHTS**

- Replacement of soya protein with white lupin protein reduced cholesterol and triacylglycerides in blood plasma
- Feedstuff including white lupin protein did not affect negatively the health status of the layers

**Introduction**

Finding protein feeds capable to completely or partially replace soya products (soybean meal is one of the priorities of European agriculture) became very important after the ban on feeding meat and bone meal feeds to livestock animals. The high price of imported soya commodities, but also the issue of genetically modified crops (which include most varieties of soybean, de Vos and Swanenburg 2018), are heightening pressure on the production of other protein feeds (Al-Harthi et al. 2018; Al-Sagan et al. 2020) and the challenging was thus increase after COVID-19 negative impacts of feed chain and agriculture sector (Hafez and Attia 2020). From this point of view, legumes, in which cultivated species of lupins are included (genus *Lupinus*), may be considered as potential protein crops (Al-Sagan et al. 2020). White lupin varieties contain in fact comparable amounts of crude protein as soybeans as documented by Martinez-Villaluenga et al. (2006). Straková et al. (2006) reported that the protein content of lupin seeds can even be increased by dehulling, when crude protein content may be increased about 20/30%, depending on the variety. The advantage of cultivated lupine varieties is that they contain a small amount of anti-nutritive substances in comparison with other types of legumes (Kurlovich et al. 2002; Sujak et al. 2006). Lupin seed oil is also an important source of polyunsaturated fatty acids (PUFA) (Zapletal et al. 2015), mainly the n/3 and n/6 group. For these reasons, the seeds of cultivated white lupin varieties are a subject of great interest as an alternative source of dietary protein in feed mixtures for animals, e.g. for fattening pigs (Zralý et al. 2008; Kasprzycz-Potocka et al. 2016) or for rabbits (Volek et al. 2018) and broiler chickens (Al-Sagan et al. 2020).
Lupin seeds find wide range of utilisation in feed mixtures for poultry nutrition (Jeroch et al. 2016), primarily in the fattening of broiler chickens (Suchy et al. 2010; Olkowski 2018), or rearing of laying hens (Rutkowski et al. 2017). Drazbo et al. (2014) reached the positive improvement of egg yolk colour and fatty acids spectrum in high-yield laying hens fed with lupin meal. Moreover, results on the improvement of lipid metabolism, with lower blood cholesterol and decrease in mortality are documented in publications dealing with feeding animals with lupin seed meal (Martins et al. 2005; Viveros et al. 2007).

Results from animal feeding experiments are in agreement with the conclusions from experimental studies of human nutrition. They described the positive effect of white lupin seeds on the physiological condition of the human body (diabetes, hypertension, obesity, cardiovascular diseases, high cholesterol level, concentration of blood lipids, blood glucose concentration and colorectal cancer (Martirosyan and Singh 2015)). Lupin proteins in the human diet significantly decreased the serum cholesterol, including LDL (Nowicka et al. 2006; Fontanari et al. 2012), glucose and triacylglyceride levels (Kinder and Knecht 2011).

The aim of this study was to verify the effect of the partial substitution of soybean meal for white lupin meal on health status, mineral equilibrium and biochemical parameters of laying hens.

**Materials and methods**

**Experimental bird, feeding and management**

A total of 180 Isa Brown laying hens was randomly divided into three groups: the control group (C) and two experimental groups (WLS and WDLS). Each group was comprised of 60 hens (6 replications, 10 hens per replication). Hens were housed in colony layer cages (ten hens per cage) located in a certified stable for layers farming starting from the age of 17 weeks; the initial body weight was 1456 g (C), 1441 g (WLS) and 1443 g (WDLS) on average. The laying cycle lasted 12 months, and started at the 20th week of age of the hens. The experiment was performed in accordance with technological directions on breeding Isa Brown laying hens, with a controlled light, ventilation and temperature regime involving 16 hours of light. For each hen was available a life space of 750 cm², 12 cm of trough feeder, two accessible nipple drinkers, hen-roost and space for grubbing and dust bathing. The experiment was in conformity with the ethical recommendations and with the permission of the Ministry of Education (number 26691/2016-4, authorisation number 45-2016).

Hens were fed with three complete feed mixtures during the 12 months lasting laying period: N1 start (the 1st month of laying), N1 (from the 2nd to the 5th month of laying), N2 (from the 6th to the 12th month of laying) ad libitum. Feedstuff were formulated to respond the demands of developing animal body and balanced mainly for proteins and calcium and contained soybean meal, corn, wheat, soybean oil, whole lupin seeds and dehulled lupin seeds and premix of vitamins and minerals. The feed mixtures used for group C contained only soybean meal as a protein source; in the diets of the experimental groups, 50% of the soybean meal was substituted on protein content basis for an alternative protein source – whole white lupin seed meal (group WLS) and dehulled white lupin seed meal (group WDLS), both of Zulika variety. Commercially produced complete feed mixtures were administered in a loose state from the trough feeders. The nutritional composition of the feed mixtures is in Table 1. The feedstuff formula was not disclosed by the producer.

**Blood biochemical indices**

The health status of the animals was monitored daily by checking their general organic functions and feed intake. The biochemical examination of the blood plasma was performed in the 12th month of the laying period (end of the experiment) on 20 laying hens (10 hens per replication) from each group (C, WLS, and WDLS). The number of 20 hens for blood sampling was recommended and approved by the ethics committee. Blood (5 mL) was sampled by puncture of the vena basilica into heparinised tubes. Then the blood was centrifuged for 15 min at 3000 xg to separate blood plasma. The analyses took place at the clinical laboratory for small animals, University of Veterinary and Pharmaceutical Sciences Brno. Total protein concentration (TP), glucose (Glu), total cholesterol (Chol), triacylglycerides (TAG), uric acid (UAC), calcium (Ca) inorganic phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), alkaline phosphatase activity (ALP) and alanine aminotransferase (AST) were determined in the blood plasma by the DPC Konelab 20i – ThermoScientific biochemical analyser based on the photometric determination principle.
Table 1. Nutrient composition of the feed mixtures (as fed basis).

| Parameter                  | C      | WLS 50% | WDLS 50% | WLS 50% | WDLS 50% |
|---------------------------|--------|---------|----------|---------|----------|
| Crude protein             | 161.00 | 149.00  | 129.00   | 139.00  | 137.00   |
| Crude fat                 | 30.50  | 44.90   | 39.70    | 19.10   | 28.60    |
| Crude ash                 | 126.00 | 110.00  | 115.00   | 105.00  | 98.30    |
| Crude fibre               | 25.80  | 34.70   | 24.10    | 29.20   | 31.40    |
| Sugar                     | 29.60  | 31.10   | 23.20    | 33.00   | 42.40    |
| Starch                    | 438.00 | 432.00  | 475.00   | 486.00  | 470.00   |
| Fat                       | 40.20  | 34.00   | 35.70    | 33.40   | 29.70    |
| Protein                   | 9.15   | 7.17    | 7.63     | 6.53    | 8.41     |
| Linoleic acid             | 12.60  | 16.21   | 12.76    | 8.53    | 9.74     |
| Zn                        | 8.31   | 6.46    | 4.75     | 6.64    | 5.63     |
| Methionine                | 63.33  | 52.5    | 4.00     | 4.64    | 4.76     |
| Lysine                    | 3.70   | 3.62    | 3.49     | 2.46    | 3.06     |
| Threonine                 | 10.50  | 9.15    | 7.17     | 7.63    | 8.41     |
| Arginine                  | 1.14   | 2.20    | 1.85     | 0.66    | 1.44     |
| Uric acid                 | 8.65   | 8.85    | 8.45     | 7.70    | 6.50     |
| Mg                        | 8.31   | 6.46    | 4.75     | 6.64    | 5.63     |
| Cu                        | 87.40  | 82.20   | 102.00   | 80.70   | 89.70    |
| Linoleic acid             | 12.60  | 16.21   | 12.76    | 8.53    | 9.74     |
| α-Linolenic acid          | 1.14   | 2.20    | 1.85     | 0.66    | 1.44     |
| Ca                        | 147.30 | 150.00  | 147.70   | 244.00  | 190.60   |
| Mn                        | 130.30 | 133.80  | 126.00   | 90.40   | 112.00   |
| Mg                        | 8.65   | 8.85    | 8.45     | 7.70    | 6.50     |
| Zn                        | 87.40  | 82.20   | 102.00   | 80.70   | 89.70    |
| Ca                        | 147.30 | 150.00  | 147.70   | 244.00  | 190.60   |
| Mn                        | 130.30 | 133.80  | 126.00   | 90.40   | 112.00   |

N1, N1, N2: type of feed mixtures; C: control diet; WLS: whole lupin seeds; WDLS: whole dehulled lupin seeds; WLS: whole lupin seeds; WDLS: whole dehulled lupin seeds. WLS and WDLS = experimental diets with substitution of 50% of soybean meal for lupin meal seed.

Statistical analysis

Differences among health status, mineral equilibrium and biochemical parameters of three dietary groups were evaluated using multiple comparisons (Tukey-HSD test), with the significance level set at $p \leq 0.05$. To assure normal distribution of the data there was used the single-factor analysis of dispersion (ANOVA – Analysis of Variance). All analyses were performed using the Unistat version 5.6 for MS Excell.

Results

Body weight of the hens and vital functions

To determine the individual body weight gain, all birds were weighed at the beginning and at the end of the trial. The mean body weight at the end of the experiment was 2163 g in group C (increase of 707 g; 49%), 2070 g in group WLS (increase of 629 g; 44%) and 2037 g in group WDLS (increase of 594 g; 41%).

Diet did not negatively affect any general organic function of the hen. All hens were healthy and no differences on feed intake were found. During the experimental period, no mortality was detected and at the end of the trial all the animals were returned to the commercial farm for the following laying cycle.

Blood nutrients concentration

From the results shown in Table 2, it is apparent that the long-lasting feeding of the experimental feed mixtures containing lupin meal affected the monitored nutrients in blood plasma of the laying hens. The values of total plasma protein in group WLS (58.35 ± 5.82 g L$^{-1}$) and in group WDLS (55.49 ± 5.26 g L$^{-1}$) were significantly lower ($p \leq 0.05$) in comparison with the C group (62.21 ± 3.64 g L$^{-1}$). Feeding of diets based on lupin meal also significantly reduced ($p \leq 0.05$) the molar concentration of total plasma cholesterol in the experimental groups of hens in both groups, WLS (5.85 ± 2.21 mmol L$^{-1}$) and WDLS (5.63 ± 1.44 mmol L$^{-1}$) in comparison with the control group (7.73 ± 1.90 mmol L$^{-1}$). Diets containing lupin meal decreased ($p \leq 0.05$) the concentration of triacylglycerides in the blood of the experimental groups of hens in group WLS (24.59 ± 11.35 mmol L$^{-1}$) and in group WDLS (20.96 ± 9.90 mmol L$^{-1}$) contrary to (32.09 ± 10.81 mmol L$^{-1}$) in group C. By contrast, values of plasma glucose were significantly ($p \leq 0.05$) higher in the experimental groups of hens, in group WLS...
showed significant reduction (about 1.5% in Na and 2.6% in Cl for sodium and chlorine there was increase of both groups, (on average about 9.6 and 5.7%). By contrast, a modest decrease in the mean values of phosphate (10.01 ± 2.15 mmol L$^{-1}$) in comparison with the control group (12.06 ± 1.75 mmol L$^{-1}$). In any of this above mentioned parameter was not proved statistical difference between groups WLS and WDLS.

**Blood mineral concentration**

The results of micro and macro elements concentration analyses from the blood plasma are reported in Table 3. From these results, it is apparent that the long-term feeding of a lupin meal diet to the laying hens did not significantly affect the plasma concentrations of the monitored indicators (phosphate, magnesium, sodium, potassium and chlorine), although modest decrease in the mean values of phosphate and magnesium was observed in the experimental groups, (on average about 9.6 and 5.7%). By contrast, for sodium and chlorine there was increase of both these macroelement (about 1.5% in Na and 2.6% in Cl). Only the concentration of calcium in blood serum, showed significant reduction ($p \leq .05$) from $7.81 \pm 0.66$ mmol L$^{-1}$ in the group C to $7.02 \pm 1.33$ mmol L$^{-1}$ in the group WDLS.

**Activity of blood plasma enzymes**

The results indicated (Table 4) that the addition of lupin meal to the diet did not significantly affected the activity of AST. On the contrary, the average activity of ALP rose in the experimental groups and a significant difference ($p \leq .05$) was found between the values of ALP activity in group C ($2.71 \pm 1.20$ µkat L$^{-1}$) and WDLS ($6.48 \pm 6.05$ µkat L$^{-1}$).

**Discussion**

A way to easily screen the health status of the animal is perform biochemical blood test (Zhong et al. 2020). By this method, metabolic disorders related to nutrition can be detected, even in the preclinical stage of a disease. Results of this experimental study show that the tested diets, affected some of the indicators in the metabolic blood profile of the hens during long-term feeding. As mentioned by Jeroch et al. (2016) and Al-Sagan et al. (2020), lupin seeds are a great benefit, especially in poultry nutrition, thanks to their unique amino acid composition, characterised by a high content of arginine, which is for poultry one of the essential amino acids. Using blue lupin (*Lupinus angustifolius*) as the main protein source in the diets for laying hens and its beneficial effect on the weight gain, size and number of eggs (yolk colour included) were observed by Lee et al. (2016). They did not find any significant differences between the groups fed with soya or lupin protein. Similarly, in this study the weight of laying hens increased in all three groups nearly identically (in the range of 41/49%). Conversely, Laudadio and Tufarelli (2011) pointed to the fact that feeding laying hens with a feed mixture containing lupin induced positive changes in the yolk colour of the eggs. Krawczyk et al. (2015) as well as Rutkowski et al. (2017) reported that it is possible to incorporate lupin into the diet for laying hens as a partial replacement for soya meal without any negative effect on the yield of laying hens or the physico-chemical or sensory properties of the eggs. Experiments using diets with lupin protein were also done in fattening chickens (Suchy et al. 2010; Geigerova et al. 2017; Al-Sagan et al. 2020), where the influence of lupin protein on production parameters was observed. These, above mentioned authors who were focussing on the production parameters of poultry, found mostly benefits for substitution of soya meal for lupin meal.

There exist only few studies, that focussing on the health of the animals after the feeding with a diet containing lupin seeds. Straková et al. (2007) observed changes in the yield parameters of laying hens, together with changes in the values of glucose and cholesterol, in an experiment focussed on the substitution of animal protein (fish meal) for plant protein (two experimental feed mixtures – with soybean protein and lupine protein). Both parameters were significantly lower in groups fed with mixtures containing either soya or lupin. The lower values of glucose in

| Table 3. Concentration of inorganic substances in the blood plasma ($n = 20$). |
|---------------------------------|------|------|------|
| Parameter                   | C    | WLS  | WDLS |
| Calcium, mmol L$^{-1}$       | $7.81 \pm 0.66$ | $7.60 \pm 0.94$ | $7.02 \pm 1.33$ |
| Phosphate, mmol L$^{-1}$     | $1.99 \pm 0.23$ | $1.84 \pm 0.33$ | $1.76 \pm 0.51$ |
| Magnesium, mmol L$^{-1}$     | $1.50 \pm 0.17$ | $1.44 \pm 0.14$ | $1.39 \pm 0.24$ |
| Sodium, mmol L$^{-1}$        | $144.82 \pm 4.60$ | $146.44 \pm 4.51$ | $147.50 \pm 6.14$ |
| Potassium, mmol L$^{-1}$     | $3.64 \pm 0.30$ | $3.70 \pm 0.33$ | $3.58 \pm 0.49$ |
| Chlorine, mmol L$^{-1}$      | $112.09 \pm 3.41$ | $114.75 \pm 4.33$ | $115.32 \pm 5.62$ |

| Table 4. Activity of blood plasma enzymes ($n = 20$). |
|---------------------------------|------|------|------|
| Parameter                     | C    | WLS  | WDLS |
| Alkaline phosphatase, µkat L$^{-1}$ | $2.71 \pm 1.20$ | $4.94 \pm 4.62$ | $6.48 \pm 6.05$ |
| Alanine aminotransferase, µkat L$^{-1}$ | $3.43 \pm 0.98$ | $3.59 \pm 1.67$ | $3.27 \pm 0.87$ |

$^a$Means within a row with different superscript letters differ significantly ($p \leq .05$). C: control diet; WLS: whole lupin seeds; WDLS: whole dehulled lupin seeds. WLS and WDLS = experimental diets with substitution of 50% of soybean meal for lupin seed meal.
the blood serum were probably due to the shorter duration of the experiment, which lasted for 22 weeks. In present study, the long-term feeding with the diets containing lupin meal decreased the values of plasma protein in the experimental groups and increased values of plasma glucose concentration levels when compared with the control group.

The values of the cholesterol molar concentration in the blood plasma, were significantly reduced. Either Han et al. (2010) observed lower cholesterol levels while feeding lupin meal, but in the egg yolk. The positive effect of lupin protein on production and health was not apparent only in hens and chickens. Similar findings were reported by Jerabek et al. (2018), who confirmed the effect of feeding diet containing lupin on lower levels of cholesterol and triacylglyceride values in the blood plasma of fattened ducks. Feeding diet with lupin to turkeys, positively reflected the development and function of their gastrointestinal tract and also the absorption of nutrients from the feed which is closely linked with better production parameters (Zdunczyk et al. 2016).

For most of the mineral considered (P, Mg, Na, K and Cl) no differences were found between control and experimental groups. Despite this fact, lupin seeds are the source of a variety of macro and micro elements, as reported Straková et al. (2006). Karnpanit et al. (2017) proved that lupin seeds contain a relatively low level of Ca. This can be the explanation for lower concentrations of plasma Ca in the group fed with a diet containing dehulled lupin seed hulls in this trial. In lupin seed hulls is contained a significantly higher amount of minerals comparing with the endosperm. As a consequence, it is appropriate to properly optimise the Ca content in lupin feed mixtures for laying hens. Zhu et al. (2019) reduced the amount of trace elements to 30% and 50% in feed mixtures for male Ross-308 broilers (fed with this diet from the age of one day till the slaughter) and found that this change did not significantly affect the growth, feed conversion rate, or meat quality. Birds receiving diets containing reduced levels of trace elements had diminished excretions of Mn and Zn throughout the entire period (p < .01) and droppings of such broilers, fed with this feed mixtures, were not loading the environment too much with trace elements residues. Also Korish and Attia (2020) assessed the concentrations of Fe, Cu, Zn, Mn, Se, Co, Cr, Pb, Cd, and Ni in chicken meat and meat products, feed, and litter, as well as laying hens’ eggs, feed and litter to monitor the quality of products on the market and their safety for human consumption and their results indicated that there were significant levels of most of the trace elements and heavy metals in the different meat sources.

The lupin meal in the diet did not affect the alanine aminotransferase (AST) activity in the blood plasma of the experimental groups which is positive because higher values of AST are sign of hepatic disorders. Even though, the mean AST values in the plasma of the hens were higher than values in the study of Jerabek et al. (2018). This slight increase cannot be considered as harmful for the health of the layers because the values were still in physiological range and the difference may also have reason in variation of biochemical parameters between hens and ducks. The higher activity of alkaline phosphatase (ALP) was in the blood plasma of the experimental groups. This was probably as the consequence of lower molar concentration of plasma Ca in the blood of the hens.

Similar results were validated by scientists in human nutrition studies. Inclusion of lupin protein in human diet caused the decrease of the total cholesterol levels in blood. This effect was more apparent in people with severe hypercholesterolaemia (Bährl et al. 2015). Fontanari et al. (2012) also highlighted the decrease of lipoproteins density in humans after the consumption of lupin. Kinder and Knecht (2011) reported that the main protein components of lupin seeds are conglutins which apart from their nutritional values, have a potential therapeutic effect on cholesterol and blood glucose levels. Pavanello et al. (2017) found a positive effect of the consumption of lupin protein concentrate on insulin resistance and lowering blood pressure in all of the 25 people who consumed this enriched diet for 12 weeks.

**Conclusions**

The results of this study show that the replacement of soya protein for lupin protein did not affect the health of the laying hens negatively. The molar concentration of mineral equilibrium in the blood plasma of experimental groups did not differ from the molar concentration of the control group with exception of calcium values which were slightly lower. The statistically significant reduction of cholesterol and triacylglycerides in blood plasma is positive and in the prior team research Timova et al. (2020) was affirmed that lupin protein diet can also reduce the cholesterol levels in the egg yolk.

**Ethical approval**

The experiment was in conformity with the ethical manner and with the permission of the Ministry of Education (number 26691/2016-4, authorisation number 45-2016).
Disclosure statement
No potential conflict of interest was reported by the author(s).

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