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Selected blood biochemical indicators of Cherry Valley ducks undergoing fattening in relation to their diet and sex

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ABSTRACT: The aim of the present study was to assess dietary and sex-related effects on selected blood indicators of 40-day-old mallard ducks undergoing fattening. The ducks were divided into three dietary groups (control, L50 and L100). The control (C) group was fed a diet containing soybean meal as the main protein component of the feed. In both experimental groups L50 and L100 soybean meal was replaced with meal of whole seeds of white lupine, Zulika variety, in proportions of 50 and 100%, respectively. With respect to the assessed dietary effect, the serum of the L50 group showed a significantly higher albumin concentration as compared to the C group ($P < 0.05$) accompanied by a significantly lower proportion of γ-globulins in the L50 group as compared to the L100 group ($P < 0.01$). As for the blood plasma, the C group showed a significantly higher concentration of total cholesterol as compared to the L100 group ($P < 0.05$) and a highly significant increase in the triacylglyceride (TAG) level as compared to the L50 and L100 groups ($P < 0.01$). Concerning plasma mineral indicators, ducks of the C group showed a significantly higher calcium concentration ($P < 0.05$) in comparison to the L100 group and a lower phosphorus concentration ($P < 0.01$) in comparison to the L50 as well as L100 group. Further, the C group showed a significantly lower level of potassium (K) as compared to the L50 group ($P < 0.05$) as well as the L100 group ($P < 0.01$). The sex of the ducks significantly ($P < 0.05$) affected the proportion of α-globulins in serum and the magnesium level in the plasma and affected the plasma levels of TAG and K and the activity of aspartate aminotransferase ($P < 0.01$).

Keywords: hybrid mallard duck; dietary white lupine seeds; blood plasma indicators; serum protein fractions

Dietary use of lupine seeds is currently studied for its role in human as well as animal nutrition. Due to its high amount of protein, ranging from 29 to 41% (Strakova et al. 2006; Saastamoinen et al. 2013), which is comparable to the level of protein in soy beans, sweet lupine varieties with low alkaloid levels are currently included as a vegetable protein source in human nutrition as well as in feeds for animals (Zraly et al. 2008; Volek et al. 2014; Geigerova et al. 2017; Prusinski 2017). White lupine varieties are easier to grow under the soil and climatic conditions of the Czech Republic than yellow lupine varieties. In addition, utilisation of white lupine seeds in poultry diets as a replacement for currently expensive soy products also carries financial benefits (Strakova and Suchy 2016).

Recently, the dietary effect of replacement of soybean meal with white lupine meal on fattening performance and carcass value has been studied in broiler chickens and also in hybrid mallard ducks (Suchy et al. 2010; Strakova et al. 2015). The results of the study conducted by Strakova et al. (2015) showed that the complete replacement of soybean meal with meal of white lupine seeds of the Amiga

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variety neither worsens the growth performance nor the carcass composition of the fattened hybrid mallard ducks up to 42 days of age.

Further, other recent studies shown that dietary use of lupine seeds brings certain health benefits in different animal species. Viveros et al. (2007) found that dietary inclusion of white lupine seeds reduces the serum cholesterol level in broiler chickens. The same cholesterol lowering effect was described by Martins et al. (2005) in pigs that were fed blue lupine seeds. Besides this effect of lupine seeds on animal lipid metabolism, other health benefits were also found, for example related to cardiovascular disease prevention. Pilvi et al. (2006) reported a positive effect of lupine proteins on blood pressure and vessel endothelium function in rats with hypertension. Except for the protein content itself, especially the presence of arginine, which is often deficient in poultry feeds (Suchy et al. 2006), seeds of certain varieties of white lupine are appreciated also as a considerable source of n-3 PUFA (Zapletal et al. 2015) and also as a major source of oligosaccharides of the raffinose series, which exert positive prebiotic effects on poultry health (Geigerova et al. 2017).

For optimum inclusion of white lupine seeds in the diet for fattening mallard ducks its effect on animal health must be assessed, and blood indicators may be informative with respect to their specific effects on a given organism. In this respect, blood biochemistry indicator values can play an important role in understanding physiological changes in animals (Yang et al. 2017). The preventive role of blood biochemistry examination in birds consists of monitoring the status of an organism and of studying the physiological functions of the individual organs; both plasma and serum may be used. Tothova et al. (2016) state that heparinised plasma is used more often for blood biochemistry examination in birds, while interpretation of results must take into consideration not only this pre-analytical effect of the examination but also other factors which may affect the biochemical blood profile of birds. These factors include mainly the species, age, sex and laying cycle of birds (Olayemi et al. 2006; Filipovic et al. 2007; Campbell 2012; Eze et al. 2014). The basic biochemical profile of the blood may be complemented with analysis of serum proteins by electrophoresis (SPE). Clinical biochemistry uses electrophoresis as a standard method for separation of individual serum protein fractions (Azim et al. 2004). At present, SPE is used in veterinary medicine as a diagnostic support in determination of various diseases of companion and farm animals (Tappin et al. 2011; Kudelkova et al. 2016), while its potential can also be harnessed in avian medicine (Briscoe et al. 2010; Delk et al. 2015).

The dietary effect of white lupine seeds on the blood biochemistry profile of hybrid mallard ducks, including an assessment of the serum protein fractions, has not yet been reported in the literature. Since previous studies have proven the dietary effects of lupine seeds on some biochemical indicators in chickens (Viveros et al. 2007) and other animals (Martins et al. 2005; Bettzieche et al. 2008; Simek et al. 2018), it can be expected that this effect exists also in fattening ducks. For that reason, the purpose of the present study was to assess the dietary effect of partial (50%) and complete replacement of soybean meal with meal of whole seeds of white lupine, Zulika variety, on selected blood plasma biochemistry indicators and serum protein fractions of Cherry Valley ducks undergoing fattening at the age of 40 days. Another objective of the study was to assess the effect of the sex of hybrid mallard ducks on selected blood biochemistry indicators at this age of the birds.

MATERIAL AND METHODS

Animals and experimental design. A total of 180 (90 males and 90 females), 1-day-old hybrid mallard ducks (Cherry Valley) were used in the experiment. Ducklings were randomly placed in three dietary groups (control and two experimental groups with 60 (30 males and 30 females) birds per group. Ducklings from each group were housed in four floor pens (15 birds/pen) covered with wood shavings. Ducks were housed in the accredited experimental stable of the Department of Animal Nutrition and the Department of Animal Husbandry and Animal Hygiene of the University of Veterinary and Pharmaceutical Sciences (UVPS) Brno under controlled housing conditions that fully complied with standards for the fattening of Cherry Valley ducks. Birds were observed twice daily for any signs of illness and behavioural changes. The experimental procedures were approved by the Animal Welfare Committee of the UVPS Brno, project No. 57/2015/2220/FVHE.

A four-phase feeding program (starter, grower I, grower II and finisher) was used. Starter (VKCHS)

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was fed until day nine of the age, grower I (VKCH1) was fed between day ten and day 18 of the age, grower II (VKCH2) was fed from day 19 to day 34 of age and finisher (VKCH3) was fed from day 35 onwards. Feed mixtures were fed to ducks in the form of crumble-pellets and whole pellets until the ninth day of age and from the tenth to the 40th day of age, respectively. The experimental period lasted for 40 days; feed and water were supplied *ad libitum*.

The ducks of the control (C) group were fed diets containing soybean meal as the main protein source in the feed. The experimental groups were fed diets in which soybean meal was replaced either partially (group L50; 50% replacement) or completely (group L100; 100% replacement) with meal of whole seeds of white lupine (*Lupinus albus*), Zulika variety, as an alternative main source of dietary protein. The feed mixtures were composed of the following components: wheat, maize, extracted soybean meal and/or meal of whole seeds of white lupine, soy oil, animal fat, methionine, lysine, threonine, monocalcium phosphate, NaCl and enzymes. The chemical composition of the diets is outlined in Table 1.

Blood sampling and biochemical analyses. At the end of the experiment (day 40), 20 ducks (10 males and 10 females) per group were randomly selected (five birds/pen) for blood biochemistry examination. All ducks were weighed and subsequently their blood was sampled by puncture from the *vena basilica*. Blood samples for biochemistry examination were placed in sample tubes with heparin and blood samples for serum protein fraction examination were placed in sample tubes with coagulation accelerator. After that, the blood samples were transported to the laboratory for examination.

Plasma biochemistry was performed on heparin-stabilised blood centrifuged for 15 minutes at 3000 rpm. Thereafter, blood plasma samples were analysed using a DPC Konelab 20i analyzer® (Thermo Fisher Scientific, Finland). The following biochemical indicators were determined: total protein (TP), glucose, total cholesterol, triacylglycerides (TAG), calcium (Ca), inorganic phosphate (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl) and activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Serum samples (10 µl) were used for manual specification of the individual protein fractions with the Hydragel Protein K20 diagnostic kit (Sebia, France) according to the manufacturer's instructions on 1%

Table 1. Nutrient composition of the diets

Nutrient (g/kg as fed)	Feed											
	starter VKCHS (day 1–9)			grower 1 VKCH1 (day 10–18)			grower 2 VKCH2 (day 19–34)			finisher 3 VKCH3 (day 35–40)		
	C	L50	L100	C	L50	L100	C	L50	L100	C	L50	L100
Dry matter	879.5	886.6	892.3	883.5	893.1	893.1	883.6	880.9	876.5	879.7	889.3	880.0
Crude protein	209.5	219.8	237.2	188.8	191.6	205.2	159.1	161.0	154.1	157.2	151.5	159.2
Crude fibre	22.9	42.8	56.5	26.8	33.9	56.7	27.7	34.6	40.4	31.7	31.2	33.2
ADF	33.3	46.8	70.2	38.4	43.9	70.8	35.4	43.8	53.6	37.9	48.8	52.7
NDF	102.0	107.4	131.1	95.4	123.6	135.4	107.8	125.6	118.1	116.6	110.4	121.3
ADL	6.1	6.9	7.6	10.1	2.7	5.4	4.9	4.4	4.2	7.4	5.9	6.8
Crude fat	34.7	38.0	51.5	33.5	39.7	49.6	33.2	37.9	45.9	38.1	41.2	43.5
Crude starch	382.5	372.0	317.5	427.5	424.2	370.7	454.2	419.6	410.6	452.2	447.2	439.1
NFE	555.5	533.1	498.6	580.3	579.9	532.2	617.4	602.4	595.1	607.2	627.3	603.6
Organic matter	822.6	833.7	843.7	829.4	845.1	843.7	837.5	835.9	835.5	834.3	851.2	839.5
Crude ash	56.9	52.9	48.6	54.1	48.0	49.4	46.1	45.0	41.0	45.4	38.2	40.5
Inorganic phosphate	6.59	6.49	6.78	6.39	5.79	6.25	5.41	4.83	4.32	4.40	4.09	4.52
NaCl	3.86	4.29	2.95	4.32	4.06	4.12	4.53	4.62	4.44	4.21	4.03	4.68
ME (MJ/kg)	14.2	14.1	14.1	14.5	14.7	14.3	14.7	14.4	14.4	14.7	14.9	14.8

ADF = acid detergent fibre; ADL = acid detergent lignin; C = control diet; L50 = experimental diet; L100 = experimental diet; ME = metabolisable energy; NDF = neutral detergent fibre; NFE = nitrogen-free extractives

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buffered agarose gel sat pH 8.6. Serum samples were applied in the horizontal arrangement, with seven samples on a plate. Electrophoresis resulted in separation of serum proteins into the following individual protein fractions: albumin, α-globulins, β-globulins and γ-globulins. The electrophoresis image was evaluated using automatic densitometry at 570 nm wavelength with densitometer for electrophoresis gels by Sebia and the percentages of individual fractions were presented in the graphic format. After that, the albumin/globulin (A/G) ratio was calculated.

Statistical analysis. Statistical analyses were performed using the STATISTICA CZ version 10 software (StatSoft Inc. 2011). The arithmetic mean and 95% confidence interval were determined for live weight and monitored blood indicators. The Shapiro-Wilk test was used to test the normal distribution of the data within the respective evaluated groups; normality was found in all assessed variables.

Pearson's correlation coefficient was calculated to assess the level of correlation between the live weight (LW) and blood indicators.

A two-way ANOVA was used for determination of the differences between the evaluated groups in the LW and in the evaluated blood indicators which were not significantly correlated with the LW. The following mathematical model was used:

$$Y_{ijk} = \mu + D_i + S_j + (D \times S)_{ij} + e_{ijk}$$

where: Y_{ijk} = k^{th} observation at the i^{th} level of factor D and the j^{th} level of factor S, μ = overall mean, D_i = fixed effect of diet (i = C, L50 and L100), S_j = fixed effect of sex (j = male and female), $(D \times S)_{ij}$ = interaction between the diet and sex, e_{ijk} = random residual error

A significant correlation was found between the LW and the TAG and Mg. ANCOVA was used for determination of differences between the evaluated groups in the TAG and Mg levels, while the LW was used as the covariate. When the ANOVA and ANCOVA showed significant differences among the groups, Tukey's test was used.

RESULTS

Mean LW and values of selected biochemistry indicators in hybrid mallard ducks in the respective dietary groups are shown in Table 2. The diets used for the purpose of this study significantly affected the LW and the levels of TP, albumin, total cholesterol, TAG, Ca, P and K, the proportion of γ-globulins and the values of the A/G ratio.

In the case of LW, the ducks in the L50 and C groups showed significantly higher values as compared to ducks of the L100 group ($P < 0.01$), while the mean LW did not differ between the C and L50 groups ($P > 0.05$). The same trend was found for the plasma TP concentration: significantly higher concentrations were found in the L50 and C groups in comparison to the L100 group ($P < 0.01$). As for the studied serum protein fractions, significant differences were found in the proportion of albumin and γ-globulins. A significantly higher proportion of albumin was found in the L50 group as compared to the C group ($P < 0.05$), whereas the proportion in the L100 group did not differ from the L50 and C groups ($P > 0.05$). A significantly lower proportion of serum γ-globulins was found in the L50 group as compared to the L100 group ($P < 0.01$); its proportion in the C group did not differ from the two experimental groups ($P > 0.05$). Significant changes in the proportions of individual serum protein fractions among respective dietary groups of ducks were also exemplified by the significantly different serum A/G ratios ($P < 0.01$), with its value showing an absolutely identical trend as the intrinsic albumin proportion.

As for the total plasma cholesterol, ducks of the C group showed a higher concentration when compared to ducks of the L100 group ($P < 0.05$), without any significant difference between the L50 group and the C and L100 groups ($P > 0.05$). Similarly, ducks of the C group showed a significantly higher concentration of plasma TAG as compared to both the L100 group and the L50 group ($P < 0.01$); the plasma TAG concentration did not differ between the L50 and L100 groups ($P > 0.05$).

The plasma calcium concentration was significantly higher in the C group as compared to the L100 group ($P < 0.05$). On the other hand, significantly lower plasma P concentrations were found in ducks of group C as compared to the two experimental groups ($P < 0.01$), while there was no difference in Ca concentration between the L50 and L100 groups ($P > 0.05$). The plasma concentration of K in ducks of the C group was significantly lower than in the L100 group ($P < 0.01$) as well as the L50 group ($P < 0.05$), while its concentration did not differ between the L50 and L100 groups ($P > 0.05$).

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Table 2. Biochemical indicators, live weights and protein fractions of fattened mallard ducks in relation to the diet

Item	Diet						Significance
	control		L50		L100		
	mean	CI	mean	CI	mean	CI	
Number	20		20		20		
Live weight (kg)	3.44[B]	3.34–3.54	3.38[B]	3.27–3.48	3.14[A]	3.09–3.20	**
Total protein (g/l)	32.6[B]	31.63–33.61	32.3[B]	31.29–33.28	29.1[A]	27.40–30.82	**
Albumin (%)	49.1[a]	48.41–49.91	50.5[b]	49.96–51.06	49.7	49.10–50.37	*
α-globulin (%)	27.3	26.77–27.85	27.1	26.62–27.58	27.0	26.24–27.83	ns
β-globulin (%)	19.1	18.16–20.10	18.0	17.53–18.46	18.3	17.54–19.02	ns
γ-globulin (%)	4.86	4.53–5.18	4.39[A]	4.14–4.64	4.99[B]	4.70–5.27	**
A/G	0.96[A]	0.93–0.99	1.02[B]	1.00–1.05	0.99	0.96–1.02	**
Glucose (mmol/l)	12.8	12.47–13.20	13.3	12.75–13.76	13.0	12.73–13.30	ns
Cholesterol (mmol/l)	4.50[b]	4.19–4.82	4.29	4.10–4.53	4.00[a]	3.70–4.30	*
TAG (mmol/l)	1.28[B]	1.00–1.56	0.95[A]	0.79–1.10	0.74[A]	0.66–0.81	**
ALP (μkat/l)	14.4	13.27–15.52	14.3	13.10–15.58	13.1	11.78–14.51	ns
ALT (μkat/l)	0.70	0.65–0.75	0.78	0.68–0.88	0.70	0.63–0.80	ns
AST (μkat/l)	0.92	0.75–1.10	0.80	0.65–0.95	0.77	0.64–0.90	ns
Calcium (mmol/l)	2.95[b]	2.91–3.00	2.87	2.82–2.93	2.86[a]	2.81–2.90	*
Phosphate (mmol/l)	1.98[A]	1.83–2.13	2.55[B]	2.49–2.62	2.52[B]	2.42–2.62	**
Magnesium (mmol/l)	0.86	0.83–0.90	0.85	0.82–0.87	0.82	0.80–0.83	ns
Sodium (mmol/l)	157.3	155.75–158.92	157.2	155.88–158.45	155.8	154.35–157.31	ns
Potassium (mmol/l)	2.89[A,a]	2.64–3.14	3.26[A,B,b]	3.01–3.51	3.47[B]	3.17–3.76	**
Chlorine (mmol/l)	105.2	103.70–106.63	105.0	103.91–106.01	105.6	104.48–106.67	ns

A/G = albumin/globulins ratio; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = 95% confidence interval; L50 = experimental diet; L100 = experimental diet; ns = non significant; TAG = triacylglycerides

*[a,b]Means within a row with different superscript letters differ significantly ($P < 0.05$)

**[A,B]Means within a row with different superscript letters differ significantly ($P < 0.01$)

Mean LW and values of selected biochemistry indicators of hybrid mallard ducks in relation to their sex are shown in Table 3. At the age of 40 days, duck sex significantly affected the serum proportion of α-globulins and plasma concentrations of TAG, AST, Mg and K. As for the serum α-globulins, their proportion was significantly higher in the females as compared to the males ($P < 0.05$). Females showed significantly higher plasma concentrations of TAG ($P < 0.01$) and Mg ($P < 0.05$) and highly significantly lower plasma activity of AST and plasma K levels in comparison to males ($P < 0.01$).

The effect of interaction between duck sex and diet (Table 3) on the LW and concentrations of TP, TAG, P and K, and activities of ALP and ALT was proven to be highly significant ($P < 0.01$) in the plasma and the effect on the plasma glucose concentration was also found to be significant ($P < 0.05$).

As follows from Table 4, a significant correlation between the LW and the observed blood biochemistry indicators was confirmed only for the values of TAG and Mg, while their plasma concentrations were positively correlated with the LW value itself in the examined ducks ($P < 0.05$).

DISCUSSION

In the 40-day-old ducks examined in the present study, the L50 and C groups showed higher LW values in comparison to the L100 group. The values of LW found in the present study are similar to those observed by Baltic et al. (2015) in 49-day-old Cherry Valley ducks and slightly higher than the values recorded by Strakova et al. (2015) in 42-day-old Cherry Valley ducks (3.03–3.14 kg) fat-

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Table 3. Biochemical indicators, live weights and protein fractions of fattened mallard ducks in relation to sex

Item	Sex				Significance	
	male		female			
	mean	CI	mean	CI	sex × diet	sex
Number	30		30			
Live weight (kg)	3.33	3.25–3.41	3.31	3.23–3.40	**	ns
Total protein (g/l)	31.1	29.70–32.44	31.6	30.67–32.53	**	ns
Albumin (%)	49.9	49.38–50.56	49.6	49.11–50.14	ns	ns
α-globulin (%)	26.7	26.24–27.27	27.5	27.12–27.96	ns	*
β-globulin (%)	18.7	18.12–19.53	18.2	17.61–18.60	ns	ns
γ-globulin (%)	4.75	4.47–5.02	4.74	4.52–4.96	ns	ns
A/G	1.00	0.97–1.02	0.99	0.97–1.01	ns	ns
Glucose (mmol/l)	13.1	12.77–13.43	13.0	12.66–13.28	*	ns
Cholesterol (mmol/l)	4.37	4.10–4.64	4.16	3.96–4.36	ns	ns
TAG (mmol/l)	0.89	0.77–1.00	1.09	0.88–1.30	**	**
ALP (μkat/l)	13.8	12.71–14.94	14.1	13.18–14.98	**	ns
ALT (μkat/l)	0.75	0.68–0.82	0.71	0.66–0.76	**	ns
AST (μkat/l)	0.94	0.81–1.10	0.72	0.63–0.80	ns	**
Calcium (mmol/l)	2.90	2.86–2.94	2.89	2.85–2.94	ns	ns
Phosphate (mmol/l)	2.37	2.23–2.50	2.34	2.21–2.47	**	ns
Magnesium (mmol/l)	0.83	0.81–0.84	0.86	0.84–0.88	ns	*
Sodium (mmol/l)	156.6	155.44–157.80	156.9	155.76–158.10	ns	ns
Potassium (mmol/l)	3.56	3.35–3.76	2.85	2.69–3.02	**	**
Chlorine (mmol/l)	105.8	104.90–106.75	104.6	103.69–105.60	ns	ns

A/G = albumin/globulins ratio; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = 95% confidence interval; ns = non-significant; TAG = triacylglycerides
*$P < 0.05$; **$P < 0.01$

Table 4. Pearson's correlation coefficients between the live body weight and blood biochemical indicators and protein fractions of fattened mallard ducks

Indicator	LW	Indicator	LW
Total protein	0.215	ALP	–0.007
Albumin	0.002	ALT	–0.098
α-globulin	–0.161	AST	0.195
β-globulin	0.194	calcium	0.062
γ-globulin	–0.007	phosphorus	–0.218
A/G	–0.017	magnesium	0.273*
Glucose	0.010	sodium	0.107
Cholesterol	–0.008	potassium	–0.040
Triacylglycerides	0.315*	chlorine	–0.073

A/G = albumin/globulin ratio; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LW = live weight
*$P < 0.05$

tened with diets including meal from whole seeds of white lupine, Amiga variety. As for the sex in the present study, no effect on the intrinsic value of LW at 40 days of age was confirmed. In the study conducted by Strakova et al. (2015), male ducks showed a higher mean value of LW as compared to female ducks (3.15 vs 3.02 kg).

Most of the available studies on duck blood biochemistry focus mainly on the evaluation of biochemistry indicators in serum. Generally, there is a lack of studies targeting biochemistry indicators in the blood plasma of domestic mallard ducks.

In birds, like in mammals, the specification of total plasma proteins is used for evaluation of the total protein reserve of the organism including assessment of their nutritional status and hepatic function. However, the plasma TP concentration in birds is only approximately half of the TP level in mammals. Campbell (2012) specifies the physiological limits for TP levels in various bird species

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to range from 25 to 45 g/l, with albumin representing 40–50% of the TP level. In the present study, the TP values observed in the respective groups of fattening mallard ducks were generally in accordance with the above-mentioned physiological range. Similar TP values were reported also by Olayemi et al. (2006) in adult Nigerian ducks (32.3 g/l) and by Zeng et al. (2015) in 35-day-old meat-type ducks (32.6 g/l). Serum and plasma TP values in ducks are affected mainly by the duck genotype, age and nutrition (Chen et al. 2016; Rath et al. 2017; Sinha et al. 2017). As for the duck gene pool, the TP values found in the present study were lower than those observed by Franco et al. (2010) in the Indian Runner breed and by Ismoyowati and Sumarmono (2016) in 12-week-old mallard ducks. In the present study, the plasma values of TP were significantly affected by the duck diet; complete replacement of soybean meal with white lupine seed meal in the L100 group resulted in a reduced TP value as compared to the L50 and C groups. Similarly, Viveros et al. (2007) found a significant decrease in the serum TP values of broiler chickens fed with seeds of white lupine (Multolupa variety) in the dose of 400 g/kg of feed. As for the effect of the bird sex on the plasma protein levels, the available findings differ to some extent. Yaqub et al. (2013) reported varying sex-related effects on plasma proteins in birds, mainly in relation to their breed. No sex-related effect on TP levels was found in the mallard ducks of the present study. Similarly, no significant effect of sex on the TP level was reported by Oladele et al. (2001) who studied local duck breeds and also by Joshi et al. (2015) who studied Khaki Campbell ducks. By contrast, recent studies have found evidence for sex-related effects on the plasma TP level in domesticated chickens and turkeys (Oladele et al. 2000; Gattani et al. 2016).

For the purposes of a more detailed biochemical profile of TP we separated the individual protein fractions by means of serum protein electrophoresis. In the present study, the serum proteins of the fattened hybrid mallard ducks were divided into the following individual protein fractions: albumin, α-, β- and γ-globulins. In healthy birds, like in mammals, the albumin fraction constitutes the largest serum protein fraction of the TP. The mean level of the albumin fraction across the three dietary groups of ducks in the present study was 49.8%. The L50 group showed the highest level (50.5%), while a significantly lower proportion of albumin was found in the C group (49.1%). In addition, the serum electrophoresis revealed that the α-globulin fraction (27.1%) prevailed over the β-globulin fraction (18.5%), while the γ-globulin fraction represented only a small fraction (4.8%) in ducks of the present study. This finding is in accordance with the assertion of Campbell (2012), who stated that a prevalence of the α-globulin fraction is typical of water birds while in psittacine birds the β-globulin fraction prevails. The partial dietary inclusion of meal from whole white lupine seeds in the L50 group of the present study resulted also in a decrease of the γ-globulin fraction as compared to the L100 group. The increased proportion of albumin in the L50 group as compared to the C group was reflected also in the changed albumin/globulins ratio with the C group showing a lower value than the L50 group. According to Campbell (2012) the value of the A/G ratio is affected by the presence or absence of transthyretin (pre-albumin) in the albumin fraction. In the present study, we found generally higher A/G ratio values within all dietary groups of fattened hybrid mallard ducks than those observed in mallard ducks by Olayemi et al. (2006) and Zeng et al. (2015). Besides that, concerning the sex-related effect on the individual serum protein fractions in ducks of the present study, males showed a reduced α-globulin fraction when compared to females.

Basal metabolic regulation of glucose in birds is similar to mammals, with certain quantitative differences (Lumeij 2008). The dietary hypoglycaemic effect of white lupine seeds has been confirmed recently in broiler chickens, with serum glucose level decreasing with an increasing proportion of white lupine seeds in the diet (Viveros et al. 2007). This finding is not in accordance with the results of the present study: the measured glucose concentrations did not differ among the respective dietary groups of hybrid mallard ducks. As for the effect of hybrid mallard duck sex on the plasma glucose concentration, no effect was proven in the present study. Fairbrother et al. (1990) found a significant increase of serum glucose in female as compared to male non-reproductive wild mallard ducks. In addition, glucose concentrations found in mallard ducks in the present study are in general accordance with the values (11.1–13.8 mmol/l) reported by Franco et al. (2010) in blood plasma. Similar values of plasma glucose (8.1–12.9 mmol/l) were also found in domestic mallard ducks by Murray (2011). Our results of plasma glycaemia are in agreement

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with the findings of Koga et al. (2004), who found plasma glucose concentrations to range from 11.9 to 16.5 mmol/l in 7-to-10-month-old male crossbred mallard ducks. On the other hand, Ismoyovati and Sumarmono (2016) found a mean plasma glycaemia of only 6.1 mmol/l.

Cholesterol is an important precursor of steroid hormones, vitamin D and bile acids and is also a component of cell membranes and bile micelles. Animals may receive cholesterol from the diet or synthesize it in the organism (Bruss 2008). A cholesterol-lowering effect of lupine seeds served in the diet has been proved in many animal species. Viveros et al. (2007) found that dietary inclusion of lupine seeds reduces a serum cholesterol level in broiler chickens. This finding is in full accordance with the results of the present study showing total plasma cholesterol concentration decreasing with the increasing proportion of white lupine seeds in feed; this decrease was found to be significant in ducks of the L100 group as compared to ducks of the C group. In addition, Sirtori et al. (2004) found that the decrease in total plasma cholesterol as a consequence of the dietary use of lupine seeds was caused by decreases of the LDL and VLDL cholesterol fractions, whereas this cholesterol-lowering effect of lupine seeds was associated with stimulation of the LDL receptors. Concerning sex of the 40-day-old mallard ducks of the present study, no effect on the plasma cholesterol concentration was found. Besides, the values of total plasma cholesterol in hybrid mallard ducks of the present study are identical with the value observed by Farhat and Chavez (2000) in 7-week-old females of the Pekin ducks (4.5 mmol/l) and are in accordance with the physiological reference values (2.7–6.3 mmol/l), stated by Fudge (2000). Rather the higher plasma cholesterol concentration (6.2 mmol/l) was found by Ismoyovati and Sumarmono (2016).

Viveros et al. (2007) found a significant TAG-lowering effect of lupine seeds in broiler chickens, which is in accordance with the results of the present study, where both experimental groups of mallard ducks showed a significant reduction of TAG levels in comparison to the C group. Bettzieche et al. (2008) state that the hypotriacylglycerolaemic effect of lupine seeds is caused by downregulation of fatty acid synthesis genes and upregulation of genes involved in TAG hydrolysis. In the present study, 40-day-old females showed a higher TAG plasma concentration as compared to males. A sex-related effect on blood TAG concentration in mallard ducks has not yet been described in the literature. However, in the case of poultry, similar findings were found recently in adult indigenous Siamese fighting chickens (Sribhen et al. 2006) and also in 5-month-old pheasants (Kececi and Col 2010); in both studies, females showed higher TAG levels as compared to males of these species. The TAG plasma concentrations in mallard ducks reported here are in accordance with those found by Koga et al. (2004). A considerably higher concentration of plasma TAG (3.0–3.2 mmol/l) was found by Farhat and Chavez (2000) and also by Ismoyovati and Sumarmono (2016). Besides that, TAG plasma values in the present study were positively correlated with the LW of hybrid mallard ducks ($r = 0.32$).

Birds show increased ALP activity mainly as a consequence of increased osteoblastic activity, while it may also be observed as a consequence of hepatocyte damage. On the other hand, this elevation possesses only limited explanatory value in relation to liver health (Lumeij 2008). In the present study, no differences in plasma ALP activity among the respective dietary groups of mallard ducks were found, while a slightly decreased ALP activity ($P > 0.05$) was found in ducks fed with a higher proportion of white lupine seeds in the diet. Moreover, we found no significant sex-related effect on plasma ALP activity values; this finding is in agreement with Fairbrother et al. (1990) who also did not prove this effect in mallard ducks. The intrinsic values of ALP activity in mallard ducks stated in available literature references differ considerably. The values of ALP activity observed in the present study are closest to the level found by He et al. (2013) in the serum of 35-day-old Cherry Valley ducks (12.1 μkat/l). A slightly higher value of the ALP activity (15.7 μkat/l) was found by Chen et al. (2014) in the serum of 14-day-old male ducks. A considerably lower reference range of plasma ALP activity (0.90–3.18 μkat/l) as well as serum ALP activity (0.87–3.43 μkat/l) was reported by Franco et al. (2010).

Alanine aminotransferase is a cytoplasmic enzyme whose highest activity is found in hepatocytes (Campbell 2012). In the present study, no dietary effect on plasma ALT activity was found in the different groups of ducks. Furthermore, no sex-related effect on ALT activity was found in hybrid mallard ducks of the present study either, which is in

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accordance with an older study by Fairbrother et al. (1990). Values of ALT activity observed in the present study are similar to those found by Zeng et al. (2015) in male ducks (0.69 µkat/l). Slightly lower ALT activities (0.50–0.53 µkat/l) were found by He et al. (2013) and also by Zhu et al. (2014) in 42-day-old domestic mallard ducks. Aspartate aminotransferase is a cytoplasmic enzyme. Both ALT and AST enzymes are sensitive to liver and muscle diseases. Increased ALT and AST plasma levels are detected when the function of the liver or muscles is impaired, whereas they are detected in the blood plasma earlier as well as later than other liver enzymes. In the present study, an insignificant decrease in the AST activity in both dietary experimental groups as compared to the C group was found. Further, we found higher AST activity in males compared to females, which is in accordance with the findings of Gattani et al. (2016) who proved the same sex-related effect on AST activity in adult turkeys and of Panigrahy et al. (2017) who observed also this trend ($P < 0.05$) in Vanaraja chickens at the end of their fattening. Previous studies on poultry revealed that increased AST activity in blood was induced by different kinds of stress (Huff et al. 2008; Chand et al. 2018; Zapletal et al. 2018), which was connected with possible loss of membrane integrity of some somatic cells as a result of physical exhaustion of the birds. Since ducks in the present study were penned always as a single-sex flock, it is possible that the increased plasma AST activity in males at the end of fattening could be related to their higher physical activity due to the establishment of a hierarchy in male flocks at this age. Besides that, values of AST activity found in the present study are slightly higher than those mentioned by Franco et al. (2010) for duck plasma (0.24–0.58 µkat/l). Lower AST activity (0.25 µkat/l) was also observed by He et al. (2013). In contrast, higher AST activities (1.20–1.92 µkat/l) were found by Chen et al. (2014) and Zeng et al. (2015).

Although the concentration of P in the grower II diet in the L50 and L100 groups was slightly lower than in the C group and also lower in the L50 group than in the C group in the finisher diet, at the end of the experiment the plasma P concentration was significantly higher in the L50 and L100 groups as compared to the C group. In addition, the L100 group, when compared to the C group, showed a slight decrease in the plasma Ca concentration, which was manifested by a slight decrease of the Ca/P ratio in the L100 group (1.13/1) as compared to the C group (1.49/1); this ratio in the L50 group was identical with the ratio found in the L100 group. Zhu et al. (2018) found reduced serum concentrations of Ca and P in Cherry Valley ducks as compared to ducks fed diet with the common dietary P level (0.45% vs 0.60%), an effect that was caused by the lower level of dietary P. Although the P concentrations in the grower II diets of the L50 and L100 duck groups of the present study were slightly lower (0.43–0.48%) as compared to the C group (0.54%), a slight decrease in the plasma concentration was observed only in the case of Ca in the experimental groups, while the P concentration increased significantly in these groups. It seems that dietary use of lupine seeds may cause an increase in the plasma P concentration of hybrid mallard ducks. A similar result was obtained recently in a study conducted by Simek et al. (2017) in which dietary inclusion of white lupine seeds resulted in the elevation of the plasma P concentration in dwarf rabbits. Values of the plasma Ca concentration in ducks of the present study are in general agreement with those observed by Martinez-Haro et al. (2011) in Indian Runner ducks and He et al. (2013) in Cherry Valley ducks and also with those observed by Rath et al. (2017) in Pekin ducks. Plasma Ca values reported in these studies ranged from 2.70 to 3.32 mmol/l. In the case of the plasma P concentration in hybrid mallard ducks of the present study, values were higher than those found by Martinez-Haro et al. (2011) in Indian Runner ducks (1 mmol/l) and lower than those found by Orban et al. (1999) in 42-day-old White Pekin ducks (4.4 mmol/l). Out of the other observed mineral indicators of blood plasma in ducks of the present study, only the K concentration was affected by the diet. The ducks of both experimental groups showed higher K concentrations than ducks of the C group. Moreover, K levels as those of Cl in the plasma of hybrid mallard ducks of the present study are in full accordance with the values found by Franco et al. (2010). Sodium plasma concentrations in the present study are slightly higher than the values (137–142 mmol/l) found by Franco et al. (2010). On the other hand, a slightly higher Na concentration (167 mmol/l) was found by Olayemi et al. (2006). As for plasma Mg levels in hybrid mallard ducks of the present study, values were slightly higher than those found by Martinez-Haro et al. (2011) and He et al. (2013), who reported Mg concentrations ranging from 0.63 to 0.72 mmol/l. As for a sex-related effect on the levels

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of mineral indicators in duck blood plasma, 40-day-old female ducks showed higher Mg levels and considerably lower K levels as compared to males. Our finding concerning the higher Mg plasma levels in female ducks cannot be compared with similar studies, since the sex-related effect on Mg blood concentration has not yet been studied in any detail in the literature concerning mallard ducks. Besides that, plasma concentrations of Mg were positively correlated with the LW of hybrid mallard ducks in the present study (r = 0.27). The significant effect of duck sex on the plasma K concentration found in the present study is not in agreement with the results reported by Okeudo et al. (2003), who did not prove this effect in seven-month-old muscovy ducks. In a recent study conducted by Skarkova and Havlicek (2017), the authors found that application of low doses of oestradiol resulted in reduced K concentrations in the plasma of laying hens. Therefore, it is possible that the increased plasma K level in female ducks as compared to males in the present study might be related to the onset of changes in endogenous secretion of the developing reproductive organs of the female hybrid mallard ducks at this age. Moreover, the higher plasma K concentration in males (3.56 mmol/l) found in the present study are within the physiological range defined for adult males of mallard ducks (2.7–5.0 mmol/l) stated by Murray (2011).

In conclusion, the assessment of the dietary effects of duck serum reported here showed a significantly higher proportion of albumin in the L50 group as compared to the C group, and further, a lower proportion of γ-globulin in the L50 group as compared to the L100 group. Concerning the blood plasma, the complete replacement of soybean meal with lupine meal showed significant cholesterol- and TAG-lowering effects as compared to the control group, while a TAG-lowering effect was also demonstrated in the case of partial replacement of soybean meal in the diet compared to the control group. Regarding the plasma mineral indicators, ducks of the C group showed significantly lower P and K concentrations as compared to ducks of the L50 and L100 groups, and on the other hand, the C group showed higher Ca concentrations as compared to the L100 group. Considering the results of the biochemistry examination, it can be stated that dietary replacement of soybean meal with lupine meal of Zulika variety had no negative effect on the assessed indicators of the blood, and thus, the used meal of whole white lupine seeds was a suitable protein component of feed for fattening of Cherry Valley ducks. In addition, as for the sex-related effect in hybrid ducks, females when compared to males showed a significant increase in the α-globulin fraction in serum and also increased plasma concentrations of TAG and Mg, while males when compared to females showed significantly higher AST activity and K concentrations.

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