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Dietary effects of the inclusion of white lupine seeds and different types of binders on the blood indicators of young Dwarf Lop rabbits

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ABSTRACT: The aim of the present study was to evaluate the effects of the inclusion of whole seeds of white lupine and different binder types in complete pelleted diets on selected haematological and plasma biochemical indicators of growing dwarf rabbits. The individual litters of Dwarf Lop rabbit kits were randomised after birth into control (n = 12) and experimental (E) groups. The young rabbits of the E group (n = 24) received an experimental pre-weaning diet containing a 25% share of white lupine seeds. At the time of weaning, the live weights of eight-week-old rabbits in the control group and E group were 727 g and 743 g, respectively. The weaned rabbits of the E group were further equally divided into two post-weaning experimental dietary groups (M = 12; G = 12); these diets contained a 20% share of white lupine seeds each and their composition only differed in the type of the binder (molasses or glycerol component). The rabbits of the control group received a commercial pet rabbit diet with no white lupine seeds during the entire experimental period. At the age of eight weeks, the diet showed a significant effect on the level of albumin ($P < 0.05$), A/G ratio ($P < 0.05$) and ALP activity ($P < 0.01$) in plasma of dwarf rabbit kits. Significant effects of diet were found in 15-week old rabbits in relation to the plasma level of albumin ($P < 0.05$), Ca ($P < 0.05$), A/G ratio ($P < 0.05$) and ALT activity ($P < 0.01$). The dietary inclusion of white lupine seeds resulted in beneficial health effects on certain blood indicators of rabbits, and their use in the proportion of 25% in the pre-weaning diet and 20% in the post-weaning diet can be recommended as a suitable feed additive for dwarf rabbits. The use of crude glycerol as binder in the experimental diet had no adverse effect on the blood indicators of the growing rabbits and thus it can be recommended for the manufacture of feed pellets intended for dwarf rabbits.

Keywords: pet rabbit; diet; haematology; plasma biochemistry; white lupine seed

The most popular category in pet rabbits rearing includes the dwarf rabbit breeds, namely the Netherland Dwarf and the Dwarf Lop (Gonzalez-Redondo and Contreraz-Chacon 2012). Considering the strictly non-production purpose of pet rabbits, the most important husbandry considerations are good health status and long life (Prebble 2014). Flaws in the nutrition and feeding of pet rabbits often contribute to the development of dental, gastrointestinal and ethological disorders (Prebble and Meredith 2014). One of the big health problems experienced by pet rabbits is obesity, mainly related to husbandry and dietary factors (Meredith 2012); the metabolic processes involved in the development of rabbit obesity are similar to those in humans (Zhang et al. 2008).

It has recently been found that the dietary inclusion of lupine seeds resulted in health benefits in selected biochemical indicators of lipid metabolism in experimentally reared pigs (Martins et al.

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2005), rats (Sirtori et al. 2004), hamsters (Fontanari et al. 2012) and rabbits (Marchesi et al. 2008). This strategy was then subsequently applied in human studies of metabolic syndrome (Bahr et al. 2015; Harisa and Alanazi 2015).

The use of a suitable binder in the production of pelleted feed is mainly important for the intrinsic pelleting process and subsequently for the quality of the produced pellet; molasses is traditionally the most commonly used binder. Glycerol, a waste by-product of biodiesel production, has recently been successfully used for the nutrition of some farmed animals; dietary use of glycerol improved both the production performance of these animals and the technological and hygienic quality of the pellets (Schroder and Sudekum 1999; Donkin et al. 2009; Kroupa et al. 2011).

As concerns rabbit nutrition, the use of alternative feed components for pellet production has recently been intensely studied exclusively in meat-type rabbit strains. The inclusion of white lupine seeds (*Lupinus albus*) in rabbit diets has proved to have positive effects on meat production, production and quality of milk and health status (Volek and Marounek 2009; Volek et al. 2014). The use of crude glycerol in diets for meat rabbit breeds showed no negative effects on their growth and reproduction (Inigo et al. 2011).

The effect of the dietary inclusion of white lupine seeds on a wider profile of blood indicators in rabbits has thus far been studied only to a limited extent (Marchesi 2008; Al-harbi et al. 2014) and there are no known data for pet rabbits reared under common husbandry conditions. The effect of the dietary inclusion of crude glycerol on the blood indicators of rabbits has not been studied at all so far. Based on the above-mentioned studies, it can be assumed that some physiological indicators of dwarf rabbits might be affected favourably by the dietary inclusion of white lupine seeds. Therefore, the aim of the present study was to evaluate the effects of the inclusion of meal from whole white lupine seeds cv. Dieta and different binder types in complete diets on selected haematological and plasma biochemical indicators in young dwarf rabbits.

MATERIAL AND METHODS

The experimental procedures were approved by the Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences (UVPS) Brno (No. 66/2016/2230/FVHE).

Animals. The study was performed on a total of 36 rabbits of the Dwarf Lop breed. The rabbits came from a common pet stock with a focus on exhibition activity. The rabbits were housed in an outdoor hutch (pen size 65 cm wide × 60 cm high × 45 cm deep), roofed for protection against unfavourable weather conditions. All rabbits were housed and treated under identical conditions. The rabbit does were vaccinated against rabbit haemorrhagic disease (Castorex, Pharmagal, Slovak Republic) and myxomatosis (MXT, Dyntec, Czech Republic). The young rabbit kits were not vaccinated in order to avoid the potential adverse effects of the vaccines on the kits' metabolism. After weaning, the young rabbits were housed in groups of two animals per hutch. The health status of the rabbits was monitored once daily.

Experimental design and diets. Within the litters, the young rabbit kits were randomised after birth into two dietary groups (control group, n = 12 and experimental group, n = 24). In both dietary groups, the whole litter was housed together with its doe up to the age of eight weeks. Weaning was performed at the age of eight weeks. The young rabbits of the experimental group with lactating does received the experimental pre-weaning diet up to the seventh week of age. Subsequently, a one-week gradual changeover from the pre-weaning to post-weaning diets was performed. The weaned rabbits of the experimental group were equally divided into two post-weaning experimental dietary groups (12 kits per group); the rabbits received these diets until the 15th week of age. Rabbits of the control (C) group received the control diet during the entire experiment (until the 15th week of age). The sex ratio of the raised rabbits was approximately equal in all groups.

A total of four types of pelleted rabbit diets were used in the study. The pelleted diets were analysed in the laboratory of the Department of Animal Nutrition. As for chemical composition of the diets, we determined the content of crude protein, starch, crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), ether extract, gross energy, ash and selected minerals. Crude protein was determined by the Kjeldahl method using a Buchi analyser (Centec Automatika, Czech Republic). Starch was determined using the P3002RS auto-

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matic digital polarimeter (Kruss, Germany). Crude fibre, ADF, NDF and ADL were determined using the ANKOM 220 fibre analyser (O.K. Servis BioPro, Czech Republic). Ether extract was determined using the Soxhlet method. Gross energy was determined using the AC500 Kalorimeter (LECO, s.r.o., Czech Republic). Digestible energy was calculated according to Villamide et al. (2009). Ash was determined by weighing the sample after incineration at 550 °C under prescribed conditions. The calcium, sodium, copper, zinc and manganese contents were determined using the 240 AA atomic absorption spectrometer (Agilent Technologies, USA). The total phosphorus content was determined spectrophotometrically. An acidic solution of ash was treated with molybdovanadate reagent and the absorbance of the solution was measured at 430 nm (Helios Alpha UV-Vis spectrophotometer, Thermo Fisher Scientific Inc.). The ingredients and chemical composition of the respective diets are presented in Table 1. The control diet used was the Berkel-Futter 6008 Light commercial diet for dwarf rabbits (Berkel-Futter, Coesfeld, Germany). Young rabbits of the experimental groups were fed in phases according to their age (pre-weaning diet and post-weaning diets), while all the experimental diets included a significant proportion of the meal from whole white lupine seeds (*Lupinus albus* cv. Dieta). The composition of the two post-weaning experimental diets only differed in the binder type used. Sugar beet molasses (M group) and glycerol from rapeseed oil (G group) were used as binders in the experimental post-weaning diets. Rabbits of all dietary groups received a daily amount of 20–30 g of pelleted feed per kg of live weight (LW) offered regularly once a day. Feed intake did not differ between dietary groups. Meadow hay was offered to the rabbits three times weekly. Unlimited access to drinking water was provided.

Blood sampling. Blood sampling was performed at the age of eight weeks (prior to the diet changeover in the experimental groups) and again at the end of the experiment at the age of 15 weeks. One day before blood sampling, all the rabbits were given their last feed ration consisting of the pelleted diets and meadow hay at 8 p.m. The meadow hay was available during the night. In the morning before the blood sampling, the rabbits were not fed pelleted diets due to its possible undesired effects on the blood indicators. Before the intrinsic blood sampling, the rabbits were weighed and their health

Table 1. Ingredients and chemical composition of the diets

Item	Pre-weaning diets (up to week 7)		Post-weaning diets (weeks 8 to 15)		
	control	experimental	control	experimental	
				M	G
Ingredients in 1 kg of diet					
Alfalfa meal (g/kg)	417.0	340.0	417.0	320.0	320.0
Barley (g/kg)	85.0	100.0	85.0	20.0	20.0
Wheat bran (g/kg)	226.0	100.0	226.0	247.0	247.0
Oat (g/kg)	0	100.0	0	130.0	130.0
Oat bran (g/kg)	60.0	0	60.0	0	0
White lupin cv. Dieta seeds (g/kg)	0	250.0	0	200.0	200.0
Malt sprouts (g/kg)	151.0	50.0	151.0	0	0
Sugar beet pulp (g/kg)	29.0	0	29.0	20.0	20.0
Molasses (g/kg)	19.0	30.0	19.0	30.0	0
Glycerol (g/kg)	0	0	0	0	30.0
Mineral premix (g/kg)	0	10.0	0	5.0	5.0
Monocalcium phosphate (g/kg)	1.0	7.0	1.0	15.0	15.0
Calcium carbonate (g/kg)	8.5	10.0	8.5	10.0	10.0
Sodium chloride (g/kg)	3.5	3.0	3.5	3.0	3.0
Chemical composition in 1 kg of dry matter					
Dry matter (g/kg)	1000.0	1000.0	1000.0	1000.0	1000.0
Crude protein (g/kg)	160.5	201.5	160.5	185.9	185.3
Crude fibre (g/kg)	173.2	161.4	173.2	160.6	163.0
ADF (g/kg)	233.6	252.5	233.6	227.5	225.7
NDF (g/kg)	420.0	347.6	420.0	384.5	375.7
ADL (g/kg)	53.0	57.2	53.0	54.9	56.4
Crude fat (g/kg)	26.8	47.4	26.8	47.6	47.4
Crude starch (g/kg)	151.9	180.0	151.9	159.3	155.2
Ash (g/kg)	86.2	87.6	86.2	82.9	89.5
Calcium (g/kg)	11.20	14.41	11.20	11.23	11.11
Inorganic phosphorus (g/kg)	5.70	5.90	5.70	8.90	8.80
Sodium (g/kg)	2.00	1.73	2.00	2.06	2.55
Potassium (g/kg)	18.28	13.53	18.28	16.20	14.75
GE (MJ/kg)	18.3	18.6	18.3	18.5	18.6
DE (MJ/kg)	10.8	11.78	10.8	11.52	11.5

ADF = acid detergent fibre, ADL = acid detergent lignin, DE = digestible energy, G = experimental group with glycerol, GE = gross energy, M = experimental group with molasses, NDF = neutral detergent fibre

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condition was examined. The rabbits included in the present study showed no clinical signs of disease. The blood samples (2 ml) were taken between 9–10 a.m. from the *vena saphaena lateralis* using a 23-gauge sterile needle. The blood was transferred to sample tubes with heparin and transported to the laboratory.

Haematological and biochemical examination. Counts of red blood cells (RBC) and white blood cells (WBC) were determined manually using a haemocytometer, while Hayem's solution or Turck's solution was used as diluting fluid. The haematocrit value (HCT) was determined using the micro-haematocrit method in a micro-centrifuge at 1677 *g* for 10 min. The haemoglobin (HB) concentration was determined using the cyanohaemoglobin method with Drabkin's reagent. Then, erythrocytic indicators such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

Half of the blood sample volume (approx. 1 ml) was centrifuged for 15 min at 805 *g* at a temperature of 22 °C. Biochemical indicators were determined in the blood plasma after centrifugation of heparin-stabilised blood. Samples were analysed using a DPC KONELAB 20i Analyser® (Thermo Fisher Scientific, Finland). The following biochemical indicators were determined: total protein (detection limit (DL) 0.50 g/l; coefficient of variance (CV) 0.91%), albumin (DL 2.00 g/l; CV 1.12%), glucose (DL 0.06 mmol/l; CV 0.67%), creatinine (DL 18.00 µmol/l; CV 1.30%), urea (DL 0.30 mmol/l; CV 2.33%), cholesterol (DL 0.08 mmol/l; CV 0.61%), triacylglycerols (DL 0.02 mmol/l; CV 0.69%), calcium (DL 0.02 mmol/l; CV 1.50%), inorganic phosphate (DL 0.065 mmol/l; CV 1.12%), sodium (DL 20.00 mmol/l), potassium (DL 0.20 mmol/l) and chloride (DL 25.00 mmol/l) as well as the activities of aspartate aminotransferase (DL 0.03 µcat/l; CV 1.38%), alanine aminotransferase (DL 0.7 µcat/l; CV 1.60%), and alkaline phosphatase (DL 0.03 µcat/l; CV 0.67%). Subsequently the albumin : globulin (A/G) ratio was calculated.

Statistical analysis. Statistical analyses were performed using STATISTICA CZ software version 10 (StatSoft Inc. 2011). Arithmetic means and 95% confidence intervals were determined for the monitored blood indicators. A Shapiro-Wilk test was used to test the normal distribution of the data, and normality was found in all of the blood indicators within the individual dietary groups and rabbit ages. One-way ANOVA was used to determine differences in the monitored indicators at the age of eight weeks and also to determine differences in the monitored indicators at the age of 15 weeks, if these indicators were not significantly correlated with the LW of the rabbits. The differences for these indicators were tested according to the following statistical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where: Y_{ij} = variance associated with parameter a; μ = overall mean; a_i = dietary effect; e_{ij} = error term

When ANOVA showed significant differences among the dietary groups, the HSD test and Tukey's test were used in eight-week-old and 15-week-old rabbits, respectively.

In 15-week-old rabbits, a significant correlation between the LW and WBC, triacylglycerols (TAG) and alanine aminotransferase (ALT) was found. Regarding the significant correlation between the LW and the above-stated blood indicators, ANCOVA was used to determine the differences among dietary groups; LW was used as a covariate. The differences for these indicators were tested according to the following statistical model:

$$Y_{ij} = \mu + a_i + B\,(c_i - m_{ij}) + e_{ij}$$

where: $Y_{ij} = j^{th}$ observation under the i^{th} categorical group; μ = overall mean; a_i = dietary effect of the i^{th} level; B = regression coefficient for i^{th} covariate live weight; $c_i = j^{th}$ observation of the covariate live weight under the i^{th} group; m_{ij} = mean of i^{th} covariate live weight; e_{ij} = error term

Differences among dietary groups were analysed for significance using Tukey's test.

RESULTS

The results of the LW and blood examination of the young eight-week old rabbit kits are presented in Table 2. Diet significantly affected the albumin concentration ($P < 0.05$), A/G ratio ($P < 0.05$) and the activity of alkaline phosphatase ($P < 0.01$). Regarding albumin, we found a decrease in the experimental group (–2.87 g/l) in comparison to the C group. In the case of the A/G ratio we also found a significant decrease in the experimental group (–0.24) when compared to the C group. The activ-

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Table 2. Live weight and blood indicators of young rabbit kits of the Dwarf Lop breed at the age of eight weeks

Indicator	Control group (n = 12)		Experimental group (n = 24)		Significance
	$\bar{x}$	CI	$\bar{x}$	CI	
Live weight (g)	727		743		ns
Haematological examination					
Red blood cells ($\times 10^{12}$/l)	5.99	5.09–6.88	5.96	5.71–6.22	ns
Haematocrit value (l/l)	0.38	0.36–0.40	0.39	0.38–0.41	ns
Haemoglobin (g/l)	126.07	115.39–136.76	129.21	123.87–134.55	ns
MCV (fl)	66.45	58.60–74.30	66.33	64.52–68.14	ns
MCHC (g/l)	331.49	294.19–368.79	328.61	316.75–340.46	ns
White blood cells ($\times 10^{9}$/l)	4.44	3.44–5.44	4.34	3.65–5.02	ns
Biochemical examination					
Total protein (g/l)	50.72	43.74–54.85	51.28	49.58–52.98	ns
Albumin (g/l)	27.23^{b}	25.11–29.35	24.36^{a}	23.22–25.50	*
A/G	1.18^{b}	1.03–1.34	0.94^{a}	0.84–1.04	*
Glucose (mmol/l)	7.01	6.49–7.54	7.44	7.15–7.72	ns
Total cholesterol (mmol/l)	1.14	0.79–1.48	1.18	0.95–1.41	ns
Triacylglycerols (mmol/l)	1.31	0.89–1.72	1.44	1.23–1.66	ns
Creatinin (mmol/l)	61.65	48.52–74.79	63.05	59.72–66.38	ns
Urea (mmol/l)	5.36	4.66–6.07	4.60	3.86–5.34	ns
ALP (µcat/l)	3.51^{B}	2.80–4.23	2.18^{A}	1.87–2.49	**
ALT (µcat/l)	0.59	0.38–0.80	0.52	0.44–0.61	ns
AST (µcat/l)	0.68	0.45–0.91	0.71	0.52–0.90	ns
Calcium (mmol/l)	2.97	2.80–3.14	2.95	2.88–3.01	ns
Inorganic phosphate (mmol/l)	1.60	1.37–1.82	1.60	1.42–1.78	ns
Sodium (mmol/l)	144.82	142.85–146.78	142.02	139.88–144.16	ns
Potassium (mmol/l)	4.60	4.34–4.86	4.58	4.45–4.70	ns
Chloride (mmol/l)	110.38	109.06–111.71	111.28	110.13–112.42	ns

A/G = albumin/globulin ratio, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CI = confidence interval, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, ns = non-significant, $\bar{x}$ = arithmetic mean

[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 ($P < 0.01$**)

ity of alkaline phosphatase (ALP) was significantly lower in the experimental group (–1.33 µcat/l) when compared to the C group.

The results of the LW and blood examination of the 15-week old rabbits are presented in Table 3. While at the age of eight weeks the rabbit LW was not affected by the consumed diet ($P > 0.05$), in the 15-week old rabbits mean LW differed significantly among the evaluated dietary groups. The rabbits of the M group showed significantly higher LW in comparison to the rabbits of the C group ($P < 0.01$). In addition, significantly higher LW of rabbits in the experimental M group in comparison to the experimental G group was found ($P < 0.05$). A significant effect of the diet was also found at this age in the level of albumin, A/G ratio, Ca and in ALT activity. Rabbits of both experimental groups M and G showed significantly reduced albumin levels ($P < 0.05$) when compared to the C group (–4.29 and –3.56 g/l, respectively); no significant difference in the values of this indicator was found between the experimental groups M and G. The rabbits of the experimental group M showed a decrease in the A/G ratio (–0.34) in comparison to the C group ($P < 0.05$), while in comparison with the two experimental groups M and G the

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Table 3. Live weight and blood indicators of weaned rabbit kits of the Dwarf Lop breed at the age of 15 weeks

Indicator	Dietary group						Significance
	control (n = 12)		molasses (n = 12)		glycerol (n = 12)		
	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	
Live weight (g)	$1075^{A,a}$		$1322^{B,b}$		$1168^{A,B,a}$		**
Haematological examination							
Red blood cells ($\times 10^{12}$/l)	5.89	5.41–6.36	5.34	4.66–6.02	5.62	5.20–6.03	ns
Haematocrit value (l/l)	0.39	0.36–0.41	0.37	0.36–0.39	0.37	0.35–0.39	ns
Haemoglobin (g/l)	126.88	120.62–137.14	131.87	125.60–138.14	130.60	121.00–140.21	ns
MCV (fl)	66.21	64.01–68.40	72.72	61.71–83.72	66.35	60.21–72.50	ns
MCHC (g/l)	334.28	307.75–360.81	353.61	343.03–364.18	355.32	334.22–376.41	ns
White blood cells ($\times 10^{9}$/l)	6.77	5.44–8.09	4.98	3.82–6.13	4.58	3.66–5.49	ns
Biochemical examination							
Total protein (g/l)	56.82	53.67–59.98	55.33	52.26–58.39	54.76	52.00–57.52	ns
Albumin (g/l)	31.29^{b}	28.38–34.19	27.00^{a}	24.87–29.14	27.73^{a}	26.717–29.29	*
A/G	1.33^{b}	1.01–1.65	0.99^{a}	0.84–1.13	$1.05^{a,b}$	0.94–1.16	*
Glucose (mmol/l)	6.77	6.08–7.45	6.64	6.09–7.20	6.93	6.53–7.32	ns
Total cholesterol (mmol/l)	0.99	0.71–1.26	0.97	0.78–1.17	0.86	0.72–1.01	ns
Triacylglycerols (mmol/l)	1.12	0.84–1.40	0.78	0.54–1.02	0.74	0.59–0.90	ns
Creatinin (µmol/l)	85.87	74.26–97.49	85.43	80.54–90.33	84.35	77.53–91.18	ns
Urea (mmol/l)	5.48	4.52–6.43	6.16	5.58–6.74	6.03	5.39–6.67	ns
ALP (µcat/l)	1.96	1.50–2.41	2.20	1.47–2.94	2.47	1.88–3.05	ns
ALT (µcat/l)	1.17^{B}	0.74–1.60	0.46^{A}	0.40–0.52	0.41^{A}	0.35–0.47	**
AST (µcat/l)	0.75	0.48–1.02	0.83	0.54–1.13	0.68	0.47–0.89	ns
Calcium (mmol/l)	3.09^{b}	2.98–3.19	$2.91^{a,b}$	2.77–3.04	2.84^{a}	2.68–3.00	*
Inorganic phosphate (mmol/l)	1.77	1.41–2.12	1.89	1.56–2.22	1.87	1.46–2.28	ns
Sodium (mmol/l)	145.13	141.82–148.43	143.70	142.15–145.25	143.96	142.57–145.35	ns
Potassium (mmol/l)	4.72	4.45–4.99	4.55	4.38–4.71	4.47	4.32–4.61	ns
Chloride (mmol/l)	108.20	105.82–110.70	111.81	110.22–113.40	110.34	107.05–113.64	ns

A/G = albumin/globulin ratio, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CI = confidence interval, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, ns = non-significant, $\bar{x}$ = arithmetic mean

[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 ($P < 0.01$**)

A/G ratios were not significantly different. Both experimental groups M and G showed a highly significant decrease in ALT activity ($P < 0.01$) in comparison to the rabbits of the C group (–0.71 and –0.76 µcat/l, respectively), while values did not differ significantly between the experimental groups ($P > 0.05$). In the case of Ca concentration, group G showed a significantly lower plasma level ($P < 0.05$) in comparison to the rabbits of the C group (–0.25 mmol/l); the Ca concentration in the G group was not significantly different from its concentration in the M group.

DISCUSSION

The values of RBC, HCT, HB, MCV and MCHC found in young dwarf rabbits at the ages of eight and 15 weeks are in accordance with the physiological reference ranges for healthy rabbits (Vennen and Mitchell 2009; Wesche 2014). Concerning WBC in the present study, especially in the case of the eight-week-old rabbit kits, slightly lower values were generally found in comparison to the above-mentioned reference ranges. This trend may be associated with a dwarf breed effect, since in

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our previous study on six-month-old Dwarf Lop females we found WBC concentration to range from 4.0 to 5.9 × 10^9/l (Simek et al. 2017a). In addition, Wesche (2014) stated that young rabbits tend to have lower values of WBC, which was also confirmed by the study performed by Jeklova et al. (2009) on SPF New Zealand White rabbits. In this respect, the values of WBC found in the present study may be considered physiological. The dietary inclusion of white lupine seeds was found to result in a non-significant decrease (P = 0.051) in WBC in young rabbits in both experimental groups at the age of 15 weeks in comparison to rabbits of the control. Al-harbi et al. (2014) found a completely opposite dietary effect of white lupine seeds on WBC: rabbits fed a white lupine mono diet showed elevated WBC. Considering the strictly toxicological character of their study, these results cannot be compared to our results from balanced rabbit diets, where the white lupine seeds represented only one of the dietary components used. In addition, the dietary inclusion of another leguminous species, jack beans (*Canavalia ensiformis*), also showed an effect on rabbit WBC. While inclusion of jack beans in doses of 100 g/kg and 200 g/kg in the diet resulted in a decrease of WBC, a dose of 300 g/kg in the diet elevated the WBC level (Bamikole et al. 2000). It seems that the inclusion of a certain proportion of some leguminous plant seeds in diets for growing rabbits may reduce the intrinsic value of WBC.

The determined levels of total protein (TP) and albumin in all monitored groups were within physiological ranges (Vennen and Mitchell 2009; Wesche 2014). The plasma TP level was not significantly affected by diet in eight-week-old as well as 15-week-old rabbits. On the other hand, diet was found to significantly affect the albumin level in the present study. At the time of weaning, the eight-week old rabbits in the experimental group showed a decrease in their albumin level in comparison to the C group ($P < 0.05$), while a significant lowering of the albumin plasma level was also found at the age of 15 weeks in both experimental groups M and G. These changes in the proportions of different of protein fractions were also reflected in a change in the A/G ratio; values found within all studied groups remained within the physiological reference range stated by Wesche (2014). Generally, there is a lack of nutritional studies dealing with the dietary effect of white lupine seeds on rabbit protein metabolism. The determination of plasma proteins in rabbits may be used for the evaluation of total protein reserve in the organism, including an evaluation of their nutrition status and hepatic function, while general interpretations of changes in the protein profile (values of TP and albumin) in rabbits are similar to other mammals. Except for immunoglobulins, most plasma proteins are synthesised in hepatocytes, with albumin representing the largest proportion (Eckersall 2008). Regarding the components and nutritional composition of the diets fed to the experimental rabbit groups in the present study, one can assume that the decreased albumin levels were likely caused by the inclusion of the white lupine seeds in the diet. This finding is also in accordance with the results of our previous preliminary study where increasing proportions of white lupine seeds in diets for growing dwarf rabbits resulted in decreased levels of plasma albumin, without significant changes in the level of plasma TP (Simek et al. 2017b). The particular proportion of plasma or serum globulins can be determined by electrophoresis (Vavricka et al. 2009), while classification into individual globulin fractions also depends on the analytical technique used (Tothova et al. 2016). Electrophoresis for the measurement of blood serum proteins has recently been used in veterinary studies evaluating the effects of nutrition on the health status of rabbits and some other farmed animals (Rupic et al. 1999; Viveros et al. 2007; Kudelkova et al. 2016).

Urea is the end-product of nitrogen metabolism (Meuten 2012). Even though the crude protein level of the pre-weaning experimental diet in the present study was higher than that in the C group, in the eight-week-old rabbits a slight decrease in the plasma urea concentration was found in the experimental group as compared to the C group ($P > 0.05$). Urea concentration was not significantly different among the rabbit groups at the age of 15 weeks, while a slightly higher urea concentration was found in 15-week-old rabbits in comparison to the 8-week-old rabbits; within all of the presently evaluated rabbit groups, its concentration was within the physiological range (4.6–10.7 mmol/l) for healthy rabbits reported by Vennen and Mitchell (2009). Creatinine is a nitrogenous waste product of the high-energy substance creatine found in muscles, which is commonly transferred into the blood (Harcourt-Brown 2002). In the present study, no significant dietary effect on the creatinine concentration was found. The plasma creatinine concentration is positively

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correlated with the muscle mass (Banfi and Fabbro 2006; Meuten 2012), which is in full agreement with the results of the present study where the growing rabbits at the age of 15 weeks showed considerably higher creatinine levels in comparison to the values found at the age of eight weeks.

The levels of plasma glucose measured in rabbits of the present study were neither affected by the diet at the age of eight weeks nor at the age of 15 weeks, while slightly higher values were found in younger rabbits at the age of eight weeks when compared to the rabbits at 15 weeks of age. Glucose levels in the present study fell within the range (4.2–8.2 mmol/l) established for healthy pet rabbits according to Harcourt-Brown and Harcourt-Brown (2012).

The TAG concentration in eight-week old rabbits of the present study did not differ between the control and the experimental group. Nevertheless, in the 15-week old rabbits an insignificantly lower value was found in both experimental groups (M and G) as compared to the control group ($P > 0.05$). In this respect, our results are in accordance with the findings of Marchesi et al. (2008), who described a TAG-lowering effect of white lupine in laboratory rabbits. They found that a significant decrease in the plasma TAG concentration occurred after feeding of the white lupine diet for 60 days. This finding is to a certain extent in agreement with the results found in the present study for dwarf rabbits, where at the age of eight weeks, i.e. after about 35 days of feeding of the experimental diet, the plasma TAG values still did not change; however, at the age of 15 weeks the TAG decrease was already apparent in both experimental groups when compared to the C group. Therefore, it may be assumed that long-term intake of white lupine in the rabbit diet is necessary for manifestation of marked changes in lipid metabolism indicators. A number of recent animal and human studies have focused on the dietary effect of lupine on lipid metabolism. Marchesi et al. (2008) found that the dietary inclusion of white lupine also showed a significant lowering effect on the total plasma cholesterol in rabbits. A study performed on pigs revealed that the lower plasma level of total cholesterol that was observed was caused by the reduced intestinal absorption of cholesterol (Martins et al. 2005). In another study, the dietary inclusion of white lupine was found to significantly change the fractions of total cholesterol in rat plasma, while the VLDL + LDL cholesterol levels decreased (Sirtori et al. 2004). In the present study, no significant dietary effect of white lupine on the total plasma cholesterol level of dwarf rabbits at the ages of eight and 15 weeks was found. It can be assumed that significant effects of dietary supplementation of lupine seeds on the blood cholesterol level can be achieved by feeding for a longer period or by the use of specific lupine protein isolates.

Alkaline phosphatase is a cellular enzyme bound to the cell membrane (Campbell 2012). The activity of the ALP enzyme in rabbits of the present study showed a significant decrease at the age of eight weeks in the experimental group as compared to the C group, while at the age of 15 weeks no difference in its value among the observed groups was found. The ALP activity values found in the present study were higher than those stated by Vennen and Mitchell (2009); however, Campbell (2012) noted that the effects of age, breed and strain should also be taken into consideration. In the present study, generally higher values of ALP activity were found in eight-week-old rabbits in comparison to 15-week-old rabbits, which is agreement with Wesche (2014), who states that younger growing rabbits show physiologically higher plasma ALP activity.

Alanine aminotransferase and aspartate aminotransferase (AST) are intracellular cytosolic enzymes. Cell damage, especially hepatocyte damage, leads to their leakage from disintegrating cells, which increases the plasma levels of these enzymes (Conkova et al. 2001; Campbell 2012). In the present study, a significant decrease in ALT enzyme levels was found in both experimental groups in comparison to the C group at the age of 15 weeks. The effect of the dietary inclusion of white lupine seeds in the complete pelleted feed on the plasma activity of ALT and AST enzymes has not yet been studied in rabbits. In a toxicological study conducted by Al-harbi et al. (2014), the lupine seed mono diet was found to increase ALT enzyme activity. On the other hand, the partial dietary inclusion of lupine showed a significant lowering effect on ALT values in laboratory animals (Osman et al. 2011; Stanek et al. 2015) as well as in humans (Harisa and Alanasi 2015; Bouchoucha et al. 2016); these latter findings are in accordance with the results of the present study. On the basis of the findings of the above-mentioned studies and the results of the present study, one can assume that the partial supplementation of white lupine seeds in diets may have a favourable effect on the cell life cycle, protecting them against excessive disintegration.

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Nevertheless, for a significant manifestation of this effect a longer dietary administration is needed; in the present study differences in ALT activity between the studied rabbit groups were not yet noticeable at the age of eight weeks. In addition, the decreased plasma activity of liver enzymes has recently been described in laboratory rabbits after the administration of monosodium L-glutamate, while a hepatoprotective effect was only found after nearly two months of its dietary administration (Okoye et al. 2016). Concerning the AST plasma activity in the present study, no difference in its values was found among the dietary groups.

With respect to the mineral profile indicators of the blood plasma that were monitored in the present study, no differences were found between the control and the experimental groups of eight-week old rabbits. However, the 15-week old rabbits fed with experimental diets were found to have lower values of calcium (Ca) in comparison to the C group; the decline in the plasma Ca concentration of the G group in comparison to the C group was found to be significant. In addition, the rabbits in both experimental groups at this age also showed a non-significant increase in the plasma concentration of inorganic phosphate (P) in comparison to the C group. Due to the unique metabolism of Ca and P in rabbits, their plasma levels are affected by the intrinsic levels of Ca and P in the diet, with the ideal dietary Ca/P ratio being 1/1 to 2/1 (Lowe 2010). Although in the present study the dietary Ca/P ratio in the pre-weaning period was higher in the experimental group (2.44/1) in comparison to the C group (1.96/1), the plasma Ca/P ratio in the eight-week-old rabbits was nearly identical in both groups (1.84/1 and 1.86/1, respectively). On the other hand, the different dietary Ca/P ratio in the post-weaning period led to altered levels in the plasma of 15-week old rabbits; the lower dietary Ca/P ratio in the M and G diets (1.26/1) resulted in lower plasma Ca/P ratios in rabbits of the M and G groups (1.54/1 and 1.51/1, respectively) in comparison to the C group (1.75/1). It seems that also the age of the growing rabbits plays an important role in the metabolism of Ca and P. Although both experimental groups of rabbits at the age of 15 weeks in the present study showed lower Ca plasma levels, its values were within the physiological range established for healthy rabbits at the age of 15 as well as eight weeks (Vennen and Mitchell 2009; Wesche 2014). White lupine seeds are known to be a substantial source of phosphate. Strakova et al. (2006) found significant differences in P levels between the seeds of particular varieties of white lupine (4.6–8.6 g/kg), while the Dieta variety harboured higher levels (7.3 g/kg). This fact must be taken into consideration when including lupine seeds in rabbit diets. Inorganic P plasma levels in the rabbits of the present study were slightly higher at the age of 15 weeks in comparison to eight weeks of age; its values in all studied rabbit groups were within the physiological range established for rabbits (Gillett 1994; Wesche 2014). As for the sodium, potassium and chloride plasma levels in the dwarf rabbits of the present study, no significant dietary effect on their values was found; their values did not even differ with respect to the age of growing rabbits and fell within the range established for healthy rabbits according to Gillett (1994) and Wesche (2014).

In conclusion, the feeding of an experimental pre-weaning diet resulted in significant changes in the plasma albumin level, A/G ratio and ALP activity in eight-week old Dwarf Lop rabbits. In response to the feeding of experimental post-weaning diets, 15-week-old rabbits showed significant changes in the plasma albumin level, A/G ratio, ALT activity and Ca level. All measured blood indicators of dwarf rabbits were in the respective physiological ranges for healthy rabbits and no adverse clinical signs were recorded during the study. The inclusion of white lupine seeds in the dose of 250 g/kg for the pre-weaning diet and in the dose of 200 g/kg for the post-weaning diet can be advantageous for dwarf rabbits. However, further research is needed to understand certain specific effects of the white lupine seeds on the rabbit physiology. The use of crude glycerol as binder in the experimental diet did not show any adverse effects on the blood indicators of the growing dwarf rabbits and its use in the dose of 30 g/kg of diet can thus be recommended for the manufacture of feed pellets used for dwarf rabbits.

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