Haematological indicators in hybrid mallard ducks (*Anas platyrhynchos*) with regard to the use of meal from whole white lupin seeds in their diet

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Abstract

The objective of our study was to assess the effect of replacing soybean meal with the meal from whole white lupin seeds (*Lupinus albus*) of the Zulika variety in diets on selected haematological indicators in 40-day-old fattened hybrid mallard ducks. A total of 180 Cherry Valley ducks were divided into three groups (E1, E2, and control). The control group was fed a diet containing soybean meal. Soybean meal replaced with 50% and 100% meal of white lupin seeds were used in group E1 and group E2, respectively. At the end of the fattening, 12 ducks (6 males and 6 females) were randomly selected from each group for a haematological examination. From the result of this study, it is clear that the effect of the diet was found only on the slightly varying number of white blood cells and on the proportion of monocytes. Ducks of group E2 showed a slight increase in the total number of leukocytes which was accompanied by a decrease in the percentage share of monocytes ($P < 0.05$). Based on the results, it can be claimed that the replacement of soybean meal with meal from the Zulika variety of whole white lupin seeds in the diet did not have a negative effect on the determined blood indicators. Therefore, whole white lupin seeds were successfully used as the important protein component of the diet for fattening hybrid mallard ducks.

Cherry Valley duck, soybean meal, Lupinus albus, fattening, whole blood

The most common vegetable protein source in poultry feed is soybean meal. Recently, however, the use of home-grown legume seeds is becoming increasingly important as an alternative source to using soybean products, especially in climate areas that are less favourable for the production of soybean (Nalle et al. 2012; Kaczmarek et al. 2016). In this regard, home-grown white lupin seeds (*Lupinus albus*) may effectively be used as an important vegetable protein source in feed used for the fattening of various food animals (Straková et al. 2006; Suchý et al. 2010; Hernández and Roman 2016).

As for duck meat production in the Czech Republic nowadays, mainly the mallard duck hybrids are used for this purpose because of their short-term fattening, performance, carcass quality, and adaptability to indoor housing conditions.

The haematological examination of birds is used as part of the evaluation of their health condition, the same as in mammals. It is especially used for the diagnosis and monitoring of the course of the disease, evaluating the effectiveness of therapy or prognosis of the disease. Another advantage of performing a haematological examination can also be seen in defining physiological reference values of specific indicators in a number of bird species. However, within a specific bird species, there are many particular physiological factors which could influence the haematological profile of healthy birds. These are mainly the nutrition, breed, age, sex, moulting, laying season, and geographical location (Král and Suchý 2000; Okeudo et al. 2003; Olayemi and Arowolo 2009; Knotková et al. 2013). A lack of the amount of relevant physiological values for the specific production

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type or sexual maturity are among the causes which could possibly make it difficult to interpret the results of the haematological examination in birds (Král and Suchý 2000). In addition, for a specific bird species, it is typical that there may be large variability in their leukogram. Whereas, especially the reference values of the white blood count are often stated within a much wider range than in domestic mammals (Knotková et al. 2013).

Previous studies revealed that different legume seeds used as dietary protein sources sometimes may affect haematological profile of poultry and other food animals (Obun 2013; Iqbal et al. 2016), whereas variations in haematological indicators are associated with a wide variety of nutritional and anti-nutritional factors of particular feed components (Osuigwe et al. 2007). Therefore, the objective of our study was to assess the effect of replacing soybean meal with meal from the whole white lupin seeds of Zulika variety in the diets on selected haematological indicators of fattened hybrid mallard ducks.

Materials and Methods

The experimental procedures were approved by the Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences (UVPS) Brno, project no. 57/2015/2220/FVHE.

Animals and nutrition

The experiment was performed in the accredited experimental stable of the Department of Animal Nutrition and the Department of Animal Husbandry and Animal Hygiene of UVPS Brno, under controlled housing conditions.

A total of 180 (90 ♀ and 90 ♂) Cherry Valley hybrid mallard ducks, which were equally divided into three groups [experimental 1 (E1), experimental 2 (E2), and control (C)], were used in the experiment. Ducklings from each group were housed in 4 floor pens (15 birds/pen) covered with wood shavings. Ducks in group C were fed a diet containing soybean extract meal as the main protein component of the diet. Soybean meals replaced with 50% and 100% meal of white lupin seeds (Zulika variety) were used in group E1 and group E2, respectively. A 4-phase feeding programme was used, where the ducks were fed a starter (VKCHS) up to the 9th day of age, grower I (VKCH1) from the 10th to the 18th day of age, grower II (VKCH2) from the 19th to the 34th day of age, and the finisher (VKCH3) from the 35th day to the end of the experiment at the 40th day of age. The complete feed mixtures were composed of the following components: maize, wheat, soybean extract meal or meal from whole white lupin seeds, soy oil, animal fat, threonine, methionine, lysine, monocalcium phosphate, NaCl, and enzymes. In feed, we determined the content of dry matter, crude protein, crude fat, crude starch, crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), crude ash, nitrogen-free extractives (NFE), organic matter (OM) and gross energy. Dry matter was determined by weight upon drying the sample at 105 °C under prescribed conditions. Crude protein was determined by Kjeldahl method using Buchi analyser (Centec Automatika, Czech Republic). Crude fat was determined by Soxhlet method. Crude starch was determined using the Automatic Digital Polarimetr P3002RS (Krüss, Germany). Crude fibre, ADF, NDF and ADL were determined by ANKOM 220 fibre analyzer (O.K. Servis BioPro, Czech Republic). Crude ash was determined by weighing the sample after incineration at 550 °C under prescribed conditions. Gross energy was determined by the Kalorimetr AC500 (LECO, s.r.o., Czech Republic). Then we calculated the NFE and OM content. Chemical compositions of the diets are presented in Table 1, while nutrient compositions of diets corresponded with general nutrition requirements for hybrid mallard ducks in the particular age. Feed was fed to the ducks in the form of crumble-pellets and entire pellets till the 9th day of age and from the 10th to the 40th day of age, respectively. During the course of the entire experiment, feed and water were available *ad libitum*.

Blood sampling and haematological analysis

At the end of the fattening (day 40) 12 ducks (6 ♀ and 6 ♂) per group (3 birds/pen) were randomly selected for a haematological examination. The ducks were weighed and then their blood was taken by puncturing the vena basilica. The blood samples were stabilized by heparin, while haematological indicators were determined in the whole blood immediately after sampling.

The count of red blood cells (RBC) and white blood cells (WBC) was determined manually using a haemocytometer, while Natt and Herrick's solution was used as a diluting fluid. The haematocrit value (HCT) was performed by the microhaematocrit method after centrifuging. The haemoglobin concentration (HGB) was determined using the cyanohaemoglobin method with Drabkin's solution. The following erythrocyte indicators were determined: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

The blood smears were stained using LEUCODIFF 200 (Erba Lachema s.r.o., Czech Republic). Differential leukocyte counts were performed manually categorizing and counting according to the morphologic and staining characteristics, while the first 100 WBC observed on the each stained blood smear were counted and classified. Determination was performed by microscopic examination under a ×100 objective lens with immersion oil.

Table 1. Nutrient composition of the diets.

Nutrient (g/kg of feed as fed)	Feed											
	VKCH S (1 to 9 day)			VKCH 1 (10 to 18 day)			VKCH 2 (19 to 34 day)			VKCH 3 (35 to 40 day)		
	C	E1	E2	C	E1	E2	C	E1	E2	C	E1	E2
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 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
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
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
GE (MJ/kg)	16.5	16.7	17.2	16.4	16.8	17.1	16.4	16.6	16.6	16.5	16.8	16.6
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

C: control diet; E1: experimental 1 diet; E2: experimental 2 diet; ADF: acid detergent fibre; NDF: neutral detergent fibre; ADL: acid detergent lignin; NFE: nitrogen-free extractives, GE: gross energy; ME: metabolizable energy.

The following indicators of leukogram patterns were determined: the percentage share of granulocytes (GRA), lymphocytes (LYM), and monocytes (MON) from the total number of WBC.

Statistical analysis

Statistical analyses were performed using the STATISTICA CZ version 10 software (StatSoft Inc. 2011). The arithmetic mean and its 95% confidence interval were determined in the live weight (LW), RBC, WBC, HCT, HGB, MCV, MCH, and MCHC, while the arithmetic mean and the standard error of the mean (SEM) were determined in the proportion of GRA, LYM and MON. Shapiro-Wilk test was used to test normal distribution of the data within respective dietary groups; normality was found in all assessed indicators. One-way ANOVA was used to determine differences in all haematological indicators. Differences among dietary groups were analysed for significance using Tukey's test.

Results

Values of physiological ranges for determined haematological indicators in mallard ducks are stated in Table 2.

The mean LW and selected haematological indicators of the mallard ducks for both sexes in the respective groups are presented in Table 3. The live weight of ducks at the age of 40 days did not differ among the respective groups ($P > 0.05$). The situation was also similar in the case of the determined haematological indicators, except for the WBC ($P < 0.05$). As for the WBC, its highest value was found in mallard ducks in group E2, where this value was significantly higher than in the mallard ducks in group E1 ($P < 0.05$).

Table 2. Reference haematology values for mallard ducks.

Indicator	Range
RBC (× 10^{12}/l)	2.0–3.4
HCT (l/l)	0.298–0.668
HGB (g/l)	114.0–156.0
MCV (fl)	148.0–200.0
MCH (pg)	30.0–78.0
MCHC (g/l)	292.0–316.0
WBC (× 10^{9}/l)	23.4–24.8
Granulocytes (%)	31.0–42.0
Lymphocytes (%)	54.0–66.0
Monocytes (%)	2.0–4.0

Combined data from Doubek et al. (2003); Campbell et al. (2010)
RBC: red blood cells; HCT: haematocrit; HGB: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cells.

Regarding differential leukocyte counts in the ducks of our study, only the percentage share of MON showed significantly different proportions between the respective dietary groups (Table 4), with their lower proportion found in the ducks of group E2 compared to the ducks in groups C and E1 ($P < 0.05$).

Discussion

With regard to the LW of the mallard ducks at the end of the fattening in our experiment, their values (3.3–3.4 kg) are generally in accordance with the results found by Chang et al. (2016) in 35-day-old Cherry Valley male ducks (3.1 kg).

The RBC value is an oxygen supply indicator for the organism and also it serves to determining the values of MCV and MCH (Brockus 2011). Zeng et al. (2015) assessed the effect of using cottonseed meal as a replacement of soybean meal in the diet of meat type ducks. These authors found that various proportions of cottonseed meal replacement in the diet did not have an effect on the values of the RBC, which is in accordance with the findings in our study, where meal from whole white lupin seeds was used to replace soybean meal. Besides, in our study, the values of RBC in the mallard ducks within all monitored groups were slightly higher than those found by He et al. (2013) in 35-day-old Cherry Valley hybrid ducks. On the other hand, the values of RBC that we found are somewhat lower than the values found by Eze et al. (2014) in 21-day-old domestic mallard ducks. Concerning the values of the HCT, the values that we found corresponded to the values found by He et al. (2013). Examination of the HCT is typically performed as a part of the haematological examination, given that the HCT value

Table 3. Haematological indicators and live weights of fattened mallard ducks in relation to the diet.

Item	Diet						Significance
	Control		Experimental 1		Experimental 2		
	Mean	CI	Mean	CI	Mean	CI	
Number	12		12		12		
LW (kg)	3.35	3.24-3.47	3.30	3.20-3.41	3.29	3.22-3.36	ns
RBC (× 10^{12}/l)	2.62	2.350-2.882	2.61	2.311-2.914	2.80	2.269-3.327	ns
HCT (l/l)	0.355	0.3386-0.3714	0.350	0.3324-0.3676	0.340	0.3205-0.3595	ns
HGB (g/l)	118.4	112.68-124.04	115.7	110.23-121.22	113.9	105.71-122.17	ns
MCV (fl)	138.6	124.60-152.58	137.8	120.73-154.88	130.2	108.25-152.22	ns
MCH (pg)	46.2	41.60-50.78	45.5	40.15-50.88	43.3	36.67-49.95	ns
MCHC (g/l)	334.0	320.62-347.29	331.3	319.02-343.50	335.7	315.96-355.39	ns
WBC (× 10^{9}/l)	16.36^{a,b}	11.696-21.020	14.63^{a}	11.986-17.264	21.67^{b}	16.535-26.799	*

CI: 95% confidence interval; LW: live weight; ns: non significant; RBC: red blood cells; HCT: haematocrit; HGB: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cells.
*; ^{a,b}: Means within a row with different superscript letters differ at ($P < 0.05$).

Table 4. Leukogram of fattened mallard ducks in relation to the diet.

Item (%)	Diet						Significance
	Control		Experimental 1		Experimental 2		
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	
Number	12		12		12		
GRA	36.33 ± 2.200		35.67 ± 2.805		36.58 ± 3.513		ns
LYM	61.42 ± 2.288		61.75 ± 2.722		62.58 ± 3.456		ns
MON	2.25^{b} ± 0.494		2.58^{b} ± 0.336		0.84^{a} ± 0.297		*

S.E.M.: standard error of the mean; ns: non significant; GRA: granulocytes; LYM: lymphocytes; MON: monocytes
*; ^{a,b}: Means within a row with different superscript letters differ at ($P < 0.05$).

expresses the proportion of erythrocytes from the total blood volume. The HCT value also serves to calculate the basic erythrocyte indices (MCV and MCHC), while these indices are consequently used to partially evaluate the morphology of the erythrocytes and also the saturation level of haemoglobin in the erythrocyte. Particularly the MCV and MCHC can then be useful for determining an anaemia classification (Lassen and Weiser 2004; Tvedten 2010). Generally, it can be said that the values of HGB, MCV, MCH, and MCHC found in our study were lower in comparison with the values found by Li et al. (2012) in 21-day-old Cherry Valley ducks and also by He et al. (2013); in our study, we found slightly lower values of HGB, MCV, and MCH in group E2 ($P > 0.05$). In addition, within all the analysed dietary groups in our study, the HGB values are in accordance with the physiological values presented by Carpenter (2012) in the mallard ducks. The values of MCHC in our study are then in compliance with the values published by Mahmoud (2015) in 3-month-old ducks.

The determination of WBC and differential leukocyte counts are among one of the most important haematological examinations, providing us with information about the health condition of individuals. Values of WBC in respective groups of mallard ducks in our study were slightly lower than those found by Olayemi and Arowolo (2009) in adult Nigerian ducks in the dry season. On the other hand, the values of WBC in our study were slightly higher than those found by Olayemi et al. (2003) in 8–10-week-old Nigerian ducks and also by Olayemi and Arowolo (2009) in adult Nigerian ducks in the wet season.

The values of MON found in the groups C and E1 of our study correspond with the values presented by Campbell et al. (2010). A significantly lower value of MON in group E2 is similar to that found by Olayemi and Arowolo (2009) and also by Mahmoud (2015). Since there is a wide reference interval presented in literature for the values of MON percentage share in Anseriformes, it is necessary to assess adequately the findings with regard to the factors that could influence its proportion. Besides, MON is among the largest cells in bird blood and for this reason they can be identified sometimes mistakenly as LYM, especially as a large type of LYM (Doubek et al. 2003; Campbell et al. 2010; Saha and Geissmann 2011). Moreover, a significantly higher MON value (6.2%) in 21-day-old Cherry Valley ducks was found by Li et al. (2012). These authors used an automatic blood counter analyzer to determine the individual leukocyte types (LYM, GRA, and MON). The considerably higher MON value and LYM value (83.9%) presented in a study by Li et al. (2012) could be related more to the different method of determining differential leukocyte counts. In this regard, it can be stated that the comparison of values of leukogram found by the traditional manual enumeration based on morphological characteristics using a microscope with their values, which were determined by the automatic determination using an analyzer, can be sometimes greatly misleading. Such a comparison can affect negatively

the intrinsic diagnostics process of the examined poultry. Moreover, recently there has been found that precise differentiation of avian leukocytes can be effectively performed by the flow cytometry method which identifies particular leukocyte subpopulations mainly on the basis of their different surface receptors (De Boever et al. 2010; Seliger et al. 2012; Bílková et al. 2017). Besides, our results of the differential leukocyte counts in Cherry Valley hybrid ducks showed that LYM are the most abundant type of leukocytes, which is in accordance with the previous findings of Li et al. (2012) and Eze et al. (2014). The LYM and GRA values found in our study are generally in accordance with those stated by Campbell et al. (2010) for mallard ducks, while their percentage shares did not differ from the respective groups of ducks in our study ($P > 0.05$).

With the exception of WBC, the mean values of the monitored haematological indicators within all 3 dietary groups in our study were found in their common physiological range defined for the mallard ducks. Based on the results of the haematological examination, it can be concluded that the replacement of soybean meal with meal from the Zulika variety of whole white lupin seeds in the diet did not have a negative effect on the determined blood indicators. Therefore, whole white lupin seeds were successfully used as an important protein component of the diet for the fattening of hybrid mallard ducks.

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