Polish Journal of Veterinary Sciences Vol. 17, No. 1 (2014), 47–52

DOI 10.2478/pjvs-2014-0006

Original article

Biochemical and haematological profile of pheasant hens during the laying period

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Abstract

The present paper provides new experimental data on the biochemical and haematological profile of blood in pheasant hens, and points out the changes in both biochemical and haematological parameters that occur during the laying period. Significant effects of egg laying on both the biochemical and the haematological blood parameters of pheasant hens were found. Biochemical analyses revealed a significant increase in the metabolites cholesterol, uric acid, lactate, the enzyme aspartate aminotransferase (AST) and the minerals calcium and phosphorous, as well as a significant decrease in total protein, albumin and glucose in the course of the laying period. Haematological analyses revealed a significant increase in the count of leukocytes, lymphocytes, eosinophils, basophils and monocytes due to egg laying. In addition, the erythrocyte count and haemoglobin content significantly decreased in the middle of the laying period and then rebounded at the end of the laying period. The haematocrit content gradually decreased till the end of the laying period. All together, the results of this study underline the impact of the reproduction status of pheasant hens on basic blood parameters. The biochemical and haematological values presented in this study may be of help in assessing disease conditions in laying pheasant hens.

Key words: biochemical profile, haematological profile, pheasant, laying period

Introduction

The common pheasant (*Phasianus colchicus*) is a gallicianeous bird, which can be found throughout the world. Although high densities of wild pheasants exist in some areas, there is an increasing number of captive-reared pheasants (Hauptmanova et al. 2006, Voslarova et al. 2006). In breeding facilities (pheasantries) the birds are hatched, hand-reared and subsequently kept in large flocks (Hauptmanova et al. 2006, Voslarova et al. 2006). The common pheasant is of importance as feathered game, and is frequently hunted in Europe. The number of birds available to hunters, therefore, is frequently increased by the release of captive-reared pheasants (Hauptmanova et al. 2006, Voslarova et al. 2006, Chloupek et al. 2009).

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Furthermore, commercial pheasant rearing has developed as an alternative poultry sector for meat production (Kececi and Col 2010, Hrabcakova et al. 2012, Nazifi et al. 2012).

With respect to animal welfare and production quality it is necessary to objectively assess the health of captive-reared pheasants. Blood biochemical and haematological parameters provide highly valuable information on the physiological state of individuals and are, thus, routinely used to determine the health and the general condition of caged birds (Schmidt et al. 2007, Kececi and Cöl 2010). Blood biochemistry in combination with haematology is a cornerstone of the medical diagnosis of organ dysfunction and disease in avian species (Kececi and Cöl 2010). The need for clinical chemistry is underlined by the fact that clinical signs of illness in birds are frequently subtle (Schmidt et al. 2007, Nazifi et al. 2012). Changes, which may be easily overlooked during physical examination, may be discovered by biochemical and haematological examination (Voslarova et al. 2006).

In spite of a large number of papers dealing with blood biochemical and haematological parameters in gallicianeous poultry there is a lack of knowledge of these parameters in feathered game such as the common pheasant. In particular, there is no information concerning the dynamic changes of the biochemical and haematological profile of blood during the laying period of pheasants. It is important to note that, beside pathological drivers, blood biochemical and haematological parameters in birds are influenced by a variety of factors such as age, sex, diet, climatic conditions and the method of rearing (Kececi and Cöl 2010, Suchy et al. 2010, Nazifi et al. 2012). Data from laying hens further underline the impact of physiological conditions, e. g. reproduction, on blood biochemistry and haematology (Strakova et al. 2001, Suchy et al. 2001, Suchy et al. 2004, Pavlik et al. 2007). Thus, to reliably interpret the results obtained by biochemical and haematological examinations of the blood of laying pheasant hens there is a need for knowledge of the dynamic changes in these parameters during egg laying.

The present paper provides new experimental data on the biochemical and haematological profile of blood in pheasant hens and points out the changes in both biochemical and haematological parameters that occur during the laying period.

Materials and Methods

Birds and their treatment

The experiment was performed on approximately 1-year-old (average body weight 0.85 kg) common pheasant hens (*Phasianus colchicus*). In the rearing facility, the birds were housed in external aviaries prior to the experiment. The number of birds in the pheasantry was approximately 900 (parent flock).

From the beginning of the laying period, pheasant hens were housed in a two-tiered cage battery. There was one breeding group in each cage, consisting of one cockerel and five hens. Each laying cage was equipped with a wire floor, five automatic nipple drinkers and a feeder located at the front wall of the cage, with manual administration to feedings. The dimensions of the cage were as follows: 200 cm length, 85 cm depth, 58 cm back height and 70 cm front height. The floor was sloped towards the front wall to enable collection of eggs. The pheasants were fed ad libitum with BZN pelleted feeding mixture (ADW Agro a.s., Krahulov, Czech Republic). The composition of the BZN feeding mixture is shown in Table 1. The cages were kept only under natural light (daylight), with no artificial lighting. The average daily temperature ranged from 10.4°C (April) to 16.3°C (June). Before being placed in the cage, the pheasants were fitted with commercially sold clip-on spectacles (Clip-On Specs, Agrigame, Worcestershire, Great Britain). All pheasants were ringed, i. e. an individually numbered plastic tag was attached to the leg of the bird.

Table 1. Composition of the BZN feeding mixture.

Components	
Dry matter (g/kg)	881.92
ME (MJ/kg)	11.87
Crude protein (g/kg)	154.21
Crude fat (g/kg)	33.19
Crude fibre (g/kg)	27.38
Crude ash (g/kg)	95.95
Lysine (g/kg)	6.95
Methionine (g/kg)	3.80
Threonine (g(kg)	5.39
Methionine – Cysteine (g/kg)	6.64
Ca (g/kg)	25.69
P (g/kg)	7.12
Na (g/kg)	1.56
Cu (mg/kg)	17.14
Se (mg/kg)	0.39
Zn (mg/kg)	143.41
Vit. A (I.U./kg)	15720
Vit. D3 (I.U./kg)	3000
Vit. E (I.U./kg)	58.20

At the beginning of the laying period 15 pheasant hens from 15 randomly selected cages (each hen from a different cage) were randomly selected and sampled for biochemical and haematological analysis of blood. Subsequently, the same hens were sampled after 6 and 12 weeks of laying period. The health of the birds was checked throughout the experiment.

The experimental design was approved by the University of Veterinary and Pharmaceutical Sciences Brno Committee on Animal Care in Research, protocol number 15/2011.

Blood sampling

Blood samples (3 mL) for biochemical and haematological examinations were taken from the *vena basilica* of the left wing, and collected using syringe-needle assemblies that had been flushed with heparin. Heparin was also added in the bottom of the tubes (15 µL of heparin to 3 mL of blood), used for collection of blood. The samples were collected within 1 min of capture to ensure that the levels of the monitored parameters were not affected by any stress induced by pre-sampling handling (Chloupek et al., 2009). Blood sampling was always performed at the same time of day (9:00 a.m.), and its duration did not exceed one hour. The heparinised blood was immediately centrifuged at 837 × g at 4°C for 10 min, and plasma samples were stored at -80°C in 1.5 ml test tubes until the analyses were performed. Samples for haematological examinations were collected into EDTA-treated 1.5 ml test tubes and immediately examined.

Biochemical examinations

Selected plasma biochemical indices (total protein, albumin, cholesterol, glucose, uric acid, lactate, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), calcium, phosphorous) were measured by photometric detection using a KONELAB 20i biochemical analyzer (ThermoFisher Scientific, Pardubice, Czech Republic) and commercial test kits (Biovendor – Laboratorni medicina a.s., Brno, Czech Republic).

Haematological examinations

The total erythrocyte and leukocyte counts were determined by means of the flask method of dilution and counting corpuscles using a Burker chamber. Haemoglobin levels were determined photometrically using a SPECOL-11 photometer (Analytic Jena, Jena, Germany) and Drabkins solution (Dr. Kulich Pharma s.r.o., Hradec Kralove, Czech Republic) at the 540 nm wavelength, and haematocrit values were determined by means of the centrifuge-based micro-haematocrit technique. Counts of individual leukocyte types (in 200 cells) were computed by classical histological methods using a light microscope with an immersion lens after staining blood smears following Pappenheim with May-Grunwald and Giemsa-Romanowski solutions (Dr. Kulich Pharma s.r.o., Hradec Kralove, Czech Republic). The absolute counts of leukocytes were obtained by multiplication of relative counts and total leukocyte counts.

Statistical analysis

The data were analysed using the UNISTAT 5.1 statistical package (Unistad Ltd., Harrow, Great Britain). For all variables tested normality and homogeneity of variances were checked by means of the Shapiro-Wilk test and the Bartlett-Box test. In the case of not normally distributed data (eosinophils, basophils, HLR, cholesterol, LDH) logarithmic transformations were used for analysis of variance, though actual mean values are presented in the tables. Because the same bursas were measured each day of sampling over the length of the experiment the data were subjected to a repeated measures ANOVA using the general linear model procedure with a factor "Day of sampling" with 3 levels (day 1, day 42, day 84) within the birds in a randomized block design. There were n = 15 pheasants (blocks) in the experiment. When the effect was statistically significant the Tukey-HSD test was performed as a post hoc test for pairwise comparisons of means. A P-value < 0.05 was considered as significant.

Results

Biochemical blood indices analysed include total protein, albumin, cholesterol, glucose, uric acid, lactate, lactate dehydrogenase (LDH), aspartate aminotransferase (AST) as well as the minerals calcium and phosphorous (Table 2). With the exception of LDH all of the mentioned parameters are significantly altered in the course of the egg laying period (Table 2). On the one hand there was a significant increase in cholesterol, uric acid and lactate, the enzyme AST, and calcium and phosphorous (Table 2). A significant decrease in total protein, albumin and glucose was also to observe (Table 2).

Haematological blood indices analysed include haematocrit, haemoglobin and counts of erythrocytes, leukocytes, heterophils, lymphocytes, eosinophils, basophils and monocytes (Table 3). The heterophil to lymphocyte ratio (HLR) was also calculated (Table 3). Significant modifications due to egg laying were observed for all of the mentioned parameters with the exception of the heterophil count and the

Table 2. Selected biochemical indices in pheasant hens (n = 15 / group) fitted with spectacles during the laying period. Data are means ± SEM.

	Group		
Biochemical indices	Day 1	Day 42	Day 84
Total protein (g/l)	49.6 ± 1.34[a]	45.0 ± 1.78[a]	29.2 ± 3.85[b]
Albumin (g/l)	25.7 ± 0.70[a]	21.8 ± 0.71[b]	22.3 ± 0.58[b]
Cholesterol (mmol/l)	5.61 ± 0.57[a]	7.36 ± 0.76[a]	8.93 ± 0.65[b]
Glucose (mmol/l)	23.9 ± 0.85[a]	19.8 ± 0.31[b]	19.7 ± 0.26[b]
Uric acid (μmol/l)	194.4 ± 19.4[a]	256.6 ± 18.9[b]	268.2 ± 14.5[b]
Lactate (mmol/l)	9.82 ± 0.41[a]	10.4 ± 0.45[a,b]	11.9 ± 0.54[b]
LDH (μkat/l)	4.47 ± 0.70[a]	3.91 ± 0.28[a]	3.95 ± 0.39[a]
AST (μkat/l)	3.04 ± 0.44[a]	6.61 ± 0.37[b]	5.82 ± 0.33[b]
Calcium (mmol/l)	3.63 ± 0.22[a]	5.56 ± 0.27[b]	5.90 ± 0.16[b]
Phosphorus (mmol/l)	1.62 ± 0.11[a]	2.10 ± 0.07[b]	2.35 ± 0.09[b]

Means denoted by different letters ([a, b]) are significantly different ($P < 0.05$). LDH = lactate dehydrogenase, AST = aspartate aminotransferase.

Table 3. Haematological indices in pheasant hens (n = 15/group) fitted with spectacles during the laying period. Data are means ± SEM.

	Group		
Haematological indices	Day 1	Day 42	Day 84
Haematocrit (l/l)	0.39 ± 0.01[a]	0.33 ± 0.01[b]	0.32 ± 0.01[b]
Haemoglobin (g/l)	126.5 ± 3.4[a]	102.4 ± 2.3[b]	129.8 ± 2.1[a]
Erythrocytes (T/l)	3.91 ± 0.19[a]	2.51 ± 0.08[c]	3.32 ± 0.08[b]
Leukocytes (G/l)	14.3 ± 1.26[a]	19.7 ± 1.71[a]	32.9 ± 2.86[b]
Heterophils (G/l)	2.48 ± 0.61[a]	1.61 ± 0.26[a]	3.18 ± 0.59[a]
Lymphocytes (G/l)	11.6 ± 1.06[a]	17.8 ± 1.57[b]	29.2 ± 2.33[c]
Eosinophils (G/l)	0.07 ± 0.01[a]	0.17 ± 0.04[b]	0.16 ± 0.01[b]
Basophils (G/l)	0.07 ± 0.01[a]	0.13 ± 0.02[b]	0.18 ± 0.02[c]
Monocytes (G/l)	0.08 ± 0.01[a]	0.12 ± 0.01[a]	0.16 ± 0.01[b]
HLR	0.27 ± 0.08[a]	0.10 ± 0.03[a]	0.10 ± 0.01[a]

Means denoted by different letters ([a, b, c]) are significantly different ($P < 0.05$). HLR = heterophil to lymphocyte ratio

HLR (Table 3). Haematological parameters which increased in the course of the laying period were the leukocyte count, the lymphocyte count, the eosinophil count, the basophil count and the monocyte count (Table 3). The eosinophil count reached its maximum at day 42, in the middle of the laying period, and then remained constant, whereas the counts of lymphocytes, basophils and monocytes and the total leukocyte count increased until the end of the laying period (day 84) (Table 3). The erythrocyte count significantly decreased at day 42 in the middle of the laying period and then partially rebounded at the end of the laying period (day 84) (Table 3). A similar decline could be seen for the haemoglobin content, but this totally rebounded at day 84. The haematocrit content gradually decreased till the end of the laying period (Table 3).

Discussion

The present paper attempts to address the issue concerning the lack of data on biochemical and haematological blood parameters in pheasant hens during the laying period. It emerged that both the biochemical and the haematological profile of the blood of the hens is modified due to egg laying.

The biochemical and haematological blood profile of gallicianeous birds is influenced by a variety of factors. One of these factors is the reproduction status. Studies performed with laying hens as well as female Bronze turkeys during the laying period consistently report an alteration of blood biochemistry as well as blood haematology due to egg laying (Strakova et al. 2001, Suchy et al. 2001, Suchy et al. 2004, Pavlik et al. 2007, Schmidt et al. 2010). Knowledge of these

changes in blood parameters is of importance for a proper evaluation of biochemical and haematological examinations during the egg laying period. However, in spite of the increasing number of captured-reared pheasants there are no data concerning the impact of egg laying on blood biochemistry and haematology of pheasant hens. The present study was performed to overcome this inadequacy of data.

The findings obtained demonstrate marked effects of egg laying on the biochemical and haematological blood parameters of pheasant hens. The laying period makes high demands on the bird's metabolism. This is reflected by a significant increase in cholesterol, uric acid, lactate, the enzyme AST, and calcium and phosphorous and, simultaneously, a significant decrease in total protein, albumin and glucose. All together, the data display an increase in energy requirements of the pheasant hens due to egg laying. In addition, the alterations in blood biochemical indices indicate an enhancement in the turnover of minerals, proteins and cholesterol, which are accumulated in the eggshell, egg white and egg yolk. The values measured for cholesterol and calcium clearly exceed the so far published values of these parameters in non-laying pheasants (Llyod and Gibson 2006, Masek et al. 2007, Suchy et al. 2007, Speranda et al. 2008, Chloupek et al. 2009, Kececi and Cöl 2010, Suchy et al. 2010, Nazifi et al. 2012).

Studies of laying hens as well as laying Bronze turkeys, in accordance with the data gained in the present study, revealed significant increases in blood content of cholesterol, uric acid, calcium and AST (Suchy et al. 2001, Suchy et al. 2004, Pavlik et al. 2007, Schmidt et al. 2010). These modifications, therefore, seem to be characteristic features of the biochemical blood profile of gallicianeous birds during the laying period. Only ambiguous data are available concerning blood glucose profiles of layers (Suchy et al. 2001, Suchy et al. 2004, Pavlik et al. 2007). This may be due to differences in the carbohydrate content of the feed as well as various time points of blood sampling in the context of feed intake. There are no data concerning the blood parameters lactate and LDH during the laying period either in laying hens or in laying turkeys. The generality of the lactate and the LDH blood profiles of laying pheasant hens seen in the present study, therefore, needs to be checked for other gallicianeous birds.

Laying birds are described as showing a marked increase in plasma total protein concentration just before egg production (Pavlik et al. 2007). According to the data of the present study this can be confirmed for pheasants. The measured total protein content in the plasma of laying pheasant hens was considerably higher than the total protein content described for non-laying pheasant hens of the same age (Schmidt et al. 2007, Kececi and Cöl 2010). The increase in plasma total protein is believed to result from an estrogen-induced proteosynthesis, which is needed to allocate vitellogenin and lipoproteins for yolk production (Schmidt et al. 2007). In contrast to data from laying hens and laying turkeys (Suchy et al. 2001, Suchy et al. 2004, Pavlik et al. 2007, Schmidt et al. 2010) the de-novo synthesis of proteins in the pheasant hens examined was not sufficient to satisfy the high demands due to egg laying, leading to a marked decrease in plasma total protein at the end of the laying period.

Egg laying not only impacts the metabolism of pheasant hens but also affects blood haematological indices. According to the data of the present study both the blood parameters associated with oxygen transport (erythrocytes, haemoglobin, haematocrit) and immune cells from the blood are subject to modification during the laying period. Opposite effects were observed for red blood cells and white blood cells. The number of erythrocytes and the haemoglobin content markedly decreased in the middle of the laying period but rebounded at the end of the laying period. Correspondingly, there was a significant decrease in blood haematocrit values due to egg laying. The data are in accordance with data from laying hens, indicating that the metabolic stress associated with egg laying is accompanied with a decrease in erythropoesis in gallicianeous birds (Strakova et al. 2001). In contrast, there was a significant increase in the count of white blood cells, namely lymphocytes, eosinophils, basophils and monocytes. The effect of egg laying on leukocyte counts has already been described by a study on layers (Strakova et al. 2001). However, according to this study in hens egg laying is accompanied by a decrease in the number of white blood cells (Strakova et al. 2001). Thus, more research is needed to elucidate the interrelations of leukocyte counts and reproduction status with respect to factors such as stress or species of gallicianeous bird.

The results of this study provide first insights into pheasant biochemistry and haematology due to egg laying. The data underline the impact of the reproduction status of pheasant hens on basic blood parameters. It can be stated that the laying period entails significant modifications to both biochemical and haematological blood indices in pheasants. Thus, the reproduction status should be considered when evaluating blood profiles of this feathered game. The biochemical and haematological values presented in this study may be of help in assessing disease conditions in laying pheasant hens.

Acknowledgements

This study was supported by research project number MSM6215712402 Veterinary Aspects of Food Safety and Quality.

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