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Changes in haematological and biochemical blood parameters of laying hens of the ROSS 308 parent flock during the hatching period

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ABSTRACT. The aim of this study was to assess the productive health of the meat hybrid ROSS 308 parent flock during the laying season at regular two-month intervals by means of selected haematological (haematocrit, haemoglobin) and biochemical (total protein, glucose, cholesterol, alanine aminotransferase, aspartate aminotransferase, calcium, phosphorus, magnesium, sodium, potassium) parameters. To assess the metabolic profile in a randomly selected set of laying hens (n = 30), blood was collected by vena basilica puncture and stabilized with heparin. The productive capacity of the parental flock was assessed on the basis of hatching egg laying capacity of laying hens (n = 5000). The experiment began after the adaptation period at 23 weeks and ended at 62 weeks of age of laying hens. Laying intensity during the study period ranged from 84.50% (30 weeks of age) to 50.10% (62 weeks of age), with weekly hatching egg production ranging from 5.60 (30 weeks of age) to 3.40 eggs (62 weeks of age). As hatching intensity decreased, the mean values of haematocrit, haemoglobin and aspartate aminotransferase gradually increased. On the other hand, the mean values of total protein, cholesterol, alanine aminotransferase, calcium, phosphorus, magnesium and potassium tended to follow hatching intensity. For plasma glucose and sodium levels, fluctuations in values were observed independent of hatching intensity. Information on the dynamic changes in the metabolic profile of meat hybrid laying hens, occurring during the hatching season, can serve as an important indicator for assessing the health status and welfare of hens.

Introduction

One of the most significant factors affecting the economic prosperity of livestock production is the health of farm animals. In veterinary practice, biochemical blood tests can easily be conducted to examine the health status of an animal (Zhong et al., 2020). These biological values are very important diagnostic indicators of the health and production status, i.e. animal's productive capacity expressed in yield (Musco et al., 2020), feed mixture conversion (Ertl et al., 2015), daily gain and live weight (Cerchiaro et al., 2005), mortality (Mondal et al., 2012), etc. The reciprocal relationship between the metabolic profile, nutrition, age and yield of each farm animal should be considered significant.

Animal health, as well as the quantitative and especially qualitative aspects of animal performance, can be affected up to 70% by nutrition (Zhaleh et al., 2019). Feed quality is essential to ensure adequate growth and development of animals and obtain high-quality products intended for human consumption. Feed is one of the main costs in animal breeding, and understanding the impact of a nutrition strategy on the quality and marketability of animals and their products are essential for the profitability of livestock farming (Szollosi et al., 2021).

Poultry meat is produced conventionally for two main reasons, firstly, it is fast-growing, and secondly, high-yielding modern broiler strains, reach market weight as early as 35 days of age (Schmidt et al., 2009). Both these reasons are constant and justifiable. Globally, production of basic poultry products, i.e. meat and eggs has been dynamically growing during the last two decades. In 2019, world hen eggs production reached 83 million tonnes, an increase of 63% between the two decades, and similarly, chicken meat showed the largest growth in absolute and relative terms since 2000 (101% or 59 million tonnes), and was the most produced type of meat in 2019 (FAO, 2021). These values reflect human consumption based on consumer preference for these high-quality products and a relatively low price compared to other animal protein products. Variable but moderate energy content, highly digestible proteins of good nutritional quality, unsaturated lipids, vitamins and minerals make poultry meat a valuable food (Marangoni et al., 2015).

The nutrition of food animals is currently directed towards maintaining the good health of animals, while taking advantage of their high production capacity. This capacity is primarily determined by the genetic potential and nutrition, which ensure the production of nutritionally valuable, healthy and harmless ingredients and foods of animal origin (Fogarty et al., 2006). Currently, increasing attention is focused on the production of safe foodstuffs, as well as the composition of diets and their impact on human health (Fraeye et al., 2012). Consumers' awareness of the quality and safety of food products is growing rapidly (Kralik et al., 2020).

There were relatively few studies that concerned haematological and biochemical poultry blood indices aimed at obtaining information on dynamic changes in metabolic markers, e.g. during the laying or fattening period under defined housing conditions of hen breeds. Objective assessment of animal health is based on monitoring the haematological and biochemical profile of the organism (Zhong et al., 2020). It should be mentioned that veterinary surgeons also struggle with a lack of data on specific haematological and biochemical blood indices in clinically healthy poultry species. The results of haematological and biochemical screenings in scientific literature are usually presented only as supplemental information (Abdel-Wareth, 2016).

The hypothesis of the present work was the expected change in the metabolic profile (selected haematological and biochemical parameters) of laying hens depending on hatching egg production during laying, as this period is very demanding and critical in the parent flock.

Material and methods

The experiment complied with the ethical recommendations and permission of the Ministry of Education (No. 26691/2016-4, authorisation No. 45-2016).

Experimental birds, feeding and management

A total of 5000 clinically healthy laying hens (*Gallus gallus f. domestica*) from the parent flock of the ROSS 308 meat hybrid was divided into five groups: five replications, 1000 hens per replication (n = 5000). Laying hens were housed in a hall with laying nests. The ratio of laying hens to cocks was 10:1. During the experiment (breeding of the parent flock), the technological directions for breeding ROSS 308 hens were maintained, including the above sex ratio of birds and controlled light (14 h of light). Ventilation and temperature regime were regulated by the management to maintain animal welfare and productive health, i.e. hatching of viable chickens at the highest possible level. The natality of laying hens during the laying period amounted to 96%. The lying period lasted 39 weeks, and started after the adaptation period at 23 weeks of age of hens and ended at 62 weeks of age.

The parent flock was fed a complete feed mixture dedicated for meat hybrids according to their needs, containing (per one kg of dry matter): g: crude protein 183.50, methionine 4.00, threonine 6.90, arginine 11.90, calcium 39.60, phosphorus 7.30, magnesium 1.10; mg: vitamin E 50.00, biotin 0.25; MJ: ME_N 12.00; IU: vitamin A 13000, vitamin D3 3000.

Blood biochemical indices

Live weight of laying hens and the consumption of feed mixture per laying hen were monitored at two-month intervals. The production capacity of the parent flock was assessed by laying intensity and the number of hatching eggs. Eggs weighing more than 50 g were considered hatching eggs. Blood for the assessment of selected haematological and biochemical indices was collected for the first time at 23 weeks of age (before the laying period) and thereafter at two-month intervals during the laying period. Blood (5 ml) was sampled by vena basilica puncture into a heparinised tube from randomly selected 30 laying hens (3 replications, 10 hens per replication). The ethics committee recommended and approved the number of 30 hens for blood sampling. Subsequently, blood was centrifuged for 15 min at 3000 *g* to separate blood plasma for analysis using a DPC Konelab 20i Thermo Scientific biochemical analyser (Diamond Diagnostics Inc., Holliston, MA, USA) based on photometric determination. The analyses were carried out at the clinical laboratory for small animals of the Veterinary University in Brno. The following haematological: haematocrit (Hk) and haemoglobin (Hb), and biochemical parameters were determined: total protein concentration, glucose, cholesterol, Ca, P, Mg, Na, K, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Statistical analysis

All analyses were performed using Unistat version 5.6 for MS Excel. Data met the assumption of homogeneity of variance. Differences between mean values were evaluated using multiple comparisons (Tukey HSD test), with the significance level set at $P \leq 0.05$. The arithmetic mean and standard deviation were also determined.

Results

Weight gain

The mean body weight at the beginning of the experiment (23 weeks of age) was 2990.30 ± 90.34 g and 4 089.80 ± 24.19 g at the end of the experiment (62 weeks of age), i.e. an increase of 1099.50 g (36.77%) was recorded. The highest laying intensity and the highest weekly production of hatching eggs was observed at 30 weeks of age – 84.50 ± 2.31% and 5.60 ± 0.26, respectively, while the lowest at the end of the experiment, i.e. 50.10 ± 1.68% (a decrease of 34.40%) and 3.40 ± 0.13 (a decrease of 2.20 hatching eggs), respectively. The weight of hatching eggs ranged from 56.70 ± 1.38 g to 70.20 ± 0.31 g and was directly proportional to the increasing age of hens throughout the experiment. In contrast, feed mixture consumption decreased (from 166.00 ± 0.00 g to 156.50 ± 0.58 g per hen per day) with increasing age of hens. The values of production parameters and their changes during the laying cycle of the ROSS 308 parent flock meat hybrids are presented in Table 1.

Table 1. Mean values of selected production indices of the ROSS 308 meat hybrid parent flock (n = 5000)

Parameter	BW, g	Laying intensity, %	PHE, eggs/week	EW, g	CFM, g/hen/day
Month of the clutch (age of hens)					
0 (23rd week)	2990.30 ± 90.34	–	–	–	–
2 (30th week)	3411.80 ± 60.19	84.50 ± 2.31	5.60 ± 0.26	56.70 ± 1.38	166.00 ± 0.00
4 (38th week)	3633.00 ± 25.10	78.60 ± 1.43	5.40 ± 0.10	62.30 ± 0.56	165.50 ± 0.58
6 (46th week)	3784.50 ± 25.05	69.40 ± 1.55	4.80 ± 0.13	65.30 ± 0.47	162.50 ± 0.58
8 (54th week)	3937.80 ± 25.46	59.80 ± 1.55	4.10 ± 0.13	68.00 ± 0.43	159.50 ± 0.58
10 (62th week)	4089.80 ± 24.19	50.10 ± 1.68	3.40 ± 0.13	70.20 ± 0.31	156.50 ± 0.58

BW – body weight of hens, PHE – production of hatching eggs, EW – egg weight, CFM – consumption of feed mixture; data are presented as mean value ± SD (standard deviation)

Haemoglobin and haematocrit

Significant changes in haematological indices were observed during the laying period. The mean haematocrit and haemoglobin values were 0.29 ± 0.03 1/l and 102.97 ± 22.63 g/l, respectively, during the whole laying period. Table 2 shows that the mean haematocrit values oscillated in a very narrow range from 0.28 ± 0.03 to 0.30 ± 0.04 1/l, and the mean haemoglobin values from 93.51 ± 18.65 g to 115.55 ± 28.32 g/l ($P \leq 0.05$). The data demonstrated that the decrease in mean haematocrit and haemoglo-

Table 2. Mean values of monitored haematological indices of the ROSS 308 meat hybrid parent flock (n = 30)

Parameter, unit	Hk, l/l	Hb, g/l
Month of the clutch (age of hens)		
0 (23rd week)	0.30 ± 0.04	115.55 ± 28.32^{A}
2 (30th week)	0.29 ± 0.03	104.53 ± 17.26
4 (38th week)	0.28 ± 0.03	93.51 ± 18.65^{B}
6 (46th week)	0.29 ± 0.02	95.11 ± 21.20^{B}
8 (54th week)	0.30 ± 0.02	112.12 ± 22.54
10 (62nd week)	0.29 ± 0.03	96.98 ± 17.56^{B}

Hk – haematocrit, Hb – haemoglobin; data are presented as mean value ± SD (standard deviation); ^{AB} – means within a column with different superscripts are significantly different at $P < 0.05$

bin content was dependent on clutch intensity. While laying intensity showed a constant decreasing trend (10.18% on average), the haematocrit and haemoglobin values increased from week 46 to week 54, from 0.29 ± 0.02 to 0.30 ± 0.02 1/l (haematocrit), and from 95.11 ± 21.20 g to 112.12 ± 22.54 g/l (haemoglobin; $P \leq 0.05$), and subsequently decreased again to 0.29 ± 0.03 and 96.98 ± 17.56 g/l, respectively.

Organic substances

Total plasma protein had a mean value of 54.33 ± 6.57 g/l during the experiment, with minimum and maximum values of 46.53 ± 4.40 g/l and 60.00 ± 6.75 g/l in week 46 of the experiment, showing a significant ($P \leq 0.05$) decreasing tendency towards the end of the clutch. The mean plasma cholesterol level in the experimental period was 4.53 ± 1.36 mmol/l, and minimum and maximum values were 2.95 ± 0.55 mmol/l and 5.32 ± 1.37 mmol/l. The values followed the intensity during the laying period. The maximum value at 38 weeks of age was significantly higher than at the beginning of the clutch, while the values recorded later were significantly lower.

Plasma glucose, which averaged 12.41 ± 1.60 mmol/l, varied considerably irrespective of laying intensity and age of the hens, with a significant ($P \leq 0.05$) minimum of 11.31 ± 2.08 mmol/l (at 38 weeks of age), and a maximum of 13.61 ± 0.83 mmol/l (at 30 weeks of age) compared to the baseline value.

A similar relationship to Hk and Hb was observed for AST concentration, where laying intensity showed a decreasing trend, and AST concentration tended to vary (Table 3). The mean AST value was 3.64 ± 1.11 µkat/l throughout the monitored period and ranged with significant differences from 3.07 ± 0.54 (38 weeks of age) to 3.97 ± 2.11 µkat/l (62 weeks of age). This trend did not occur for any other monitored biochemical parameter. ALT concentration differed significantly ($P \leq 0.05$) between the beginning of the experiment at 23 weeks of age (0.03 ± 0.01 µkat/l) and 38 weeks (0.28 ± 0.33 µkat/l; maximum value), while the mean value during the experiment was 0.08 ± 1.16 µkat/l.

Inorganic substances

Plasma Ca values were at their minimum at the beginning of the experiment (2.66 ± 0.17 mmol/l), and increased ($P \leq 0.05$) up to 38 weeks of age (6.71 ± 1.16 mmol/l) and then slowly decreased ($P \leq 0.05$) to 5.71 ± 1.28 mmol/l (Table 4). The same trend was observed for P and Mg, where the minimum values measured at the beginning of the experiment were 1.92 ± 0.37 and 0.93 ± 0.07 mmol/l, respectively, and each subsequent measured value was significantly higher, reaching the maximum at 46 weeks of age (3.58 ± 0.59 and 1.51 ± 0.09 mmol/l, respectively). Na levels were fluctuating during the observation period, with a minimum of 142.52 ± 4.80 mmol/l and a maximum of

Table 3. Mean values of monitored organic substances in the metabolic profile of the ROSS 308 meat hybrid parent flock (n = 30)

Parameter, unit	TP, g/l	Glu, mmol/l	Chol, mmol/l	ALT, µkat/l	AST, µkat/l
Month of the clutch (age of hens)					
0 (23rd week)	46.53 ± 4.40^{A}	12.61 ± 2.26^{A}	2.95 ± 0.55^{A}	0.03 ± 0.01^{A}	3.75 ± 0.53
2 (30th week)	54.17 ± 4.62^{B}	13.61 ± 0.83	4.39 ± 0.85^{B}	0.04 ± 0.01	3.52 ± 1.11
4 (38th week)	53.98 ± 5.10^{B}	11.31 ± 2.08^{B}	5.32 ± 1.37^{B}	0.28 ± 0.33^{B}	3.07 ± 0.54^{A}
6 (46th week)	60.00 ± 6.75^{B}	12.71 ± 0.66	5.08 ± 1.33^{B}	0.04 ± 0.02	3.78 ± 0.72
8 (54th week)	55.31 ± 5.33^{B}	11.58 ± 0.87	4.90 ± 1.07^{B}	0.03 ± 0.00	3.74 ± 0.55
10 (62nd week)	55.97 ± 5.04^{B}	12.65 ± 0.89	4.52 ± 1.39^{B}	0.03 ± 0.00	3.97 ± 2.11^{B}

TP – total protein, Glu – glucose, Chol – cholesterol, ALT – alanine aminotransferase, AST – aspartate aminotransferase; data are presented as mean value ± SD (standard deviation); ^{AB} – means within a column with different superscripts are significantly different at $P < 0.05$

Table 4. Mean values of monitored inorganic substances in the metabolic profile of the ROSS 308 meat hybrid parent flock (n = 30)

Parameter, unit	Ca, mmol/l	P, mmol/l	Mg, mmol/l	Na, mmol/l	K, mmol/l
Month of the clutch (age of hens)					
0 (23rd week)	2.66 ± 0.17^{A}	1.92 ± 0.37^{A}	0.93 ± 0.07^{A}	142.52 ± 4.80^{A}	3.93 ± 0.36
2 (30th week)	6.12 ± 0.98^{B}	2.73 ± 0.48^{B}	1.44 ± 0.13^{B}	147.28 ± 3.42^{B}	3.93 ± 0.35
4 (38th week)	6.71 ± 1.16^{B}	3.40 ± 0.56^{B}	1.45 ± 0.14^{B}	144.74 ± 1.84	3.84 ± 0.46
6 (46th week)	6.70 ± 1.27^{B}	3.58 ± 0.59^{B}	1.51 ± 0.09^{B}	147.19 ± 4.63^{B}	3.96 ± 0.40
8 (54th week)	5.54 ± 0.82^{B}	3.29 ± 0.40^{B}	1.46 ± 0.15^{B}	146.71 ± 2.01^{B}	3.87 ± 0.35
10 (62nd week)	5.71 ± 1.28^{B}	2.99 ± 0.59^{B}	1.46 ± 0.17^{B}	145.50 ± 3.07^{B}	3.75 ± 0.30

data are presented as mean value ± SD (standard deviation); ^{AB} – means within a column with different superscripts are significantly different at $P < 0.05$

147.28 ± 3.42 mmol/l. Higher values, compared to the beginning of the experiment, were recorded in each sampling week, but they were significantly different ($P \leq 0.05$) only in weeks 30, 46 and 54. The mean K concentration over the entire monitored period was 3.88 ± 0.38 mmol/l and fluctuated from 3.75 ± 0.30 to 3.96 ± 0.40 mmol/l, reaching a maximum at 46 weeks of age.

Discussion

The obtained data suggest that the degree of changes during different intensities of laying and production of hatching eggs varied, and the monitored indicators (haematological and biochemical) were fluctuating according to the intensity of laying. During the observation period, the production of hatching eggs was at the level of 95.60% of the total egg production. This number was higher than the mean value (78.54%) reported by Çabuk et al. (2006), however, this could be due the fact that the latter authors conducted their study under more adverse living conditions for birds.

The blood parameters of layers are considered decisive and proper indicators of poultry health status. They change over the course of a bird's life for many reasons, including illness (Fudge, 2000), various stress sources (Bedanova et al., 2007) and, finally, genotype (Gayathri and Hedge, 2006). The changes observed in the present study have confirmed these facts. Alterations coincided with individual periods of hens' laying season, which put an enormous strain on the body, and had an impact on erythropoiesis, expressed by a reduction in the haematocrit value, i.e. the proportion of erythrocytes in the total blood content.

There is a negative correlation between egg production and plasma cholesterol levels. Egg production culminated at 38 weeks of age, when plasma cholesterol value was only 4.39 ± 0.85 mmol/l. From the age of 39 weeks, plasma cholesterol levels increased, while egg production decreased. The concentration of plasma cholesterol is one of the health status indicators of hens that can be affected by various factors, including selection (Cunningham et al., 1974), feeding (Straková et al., 2021; Ciurescu et al., 2022), oil (Saied et al., 2022) or enzyme (Al-Harthi et al., 2018) addition. Marks and Washburn (1991) found that breeding selection could lower plasma cholesterol levels by one third. Krás et al. (2013) reported that plasma cholesterol concentrations of laying hens depended on the level of metabolized energy in the feed ration, and decreased with the content of metabolized energy. The results of the latter authors contradicted our findings, but in the current study, the birds were fed the same feed mixture throughout the experiment.

In the present study, the values of plasma protein were significantly increased during the entire experimental period, 9.36 g/l on average until week 46 of the clutch compared to the beginning of the laying period. This result was consistent with the findings of Suchý et al. (2004), who concentrated on the metabolic profile of meat-type hybrids during the laying period. These authors recorded an increase in total protein levels (11.05 g/l on average), but the experiment was shorter, and lasted only 25 weeks of the laying period. From week 46 of the clutch, plasma protein values decreased significantly. We speculated that this could be due to a lower metabolic load on the layers.

Puvadolpirod and Thaxton (2000) linked plasma cholesterol and glucose levels in laying hens to stress. These authors found that the mean levels of plasma glucose during the laying period ranged from 12.48 to 14.08 mmol/l, and that blood glucose concentration in the second half of egg laying period increased. Glucose levels in the present study were very unstable and changed with each sampling (8 weeks). Values increased from the beginning of the experiment to fall to a value of 11.31 mmol/l in (week 38 of laying), subsequently increased to 12.71 mmol/l (week 46 of laying), and then decreased again. Glucose levels appeared to be related not only to stress, but also to energy expenditure and nutrient intake.

Trace elements are essential in the nutrition of laying parental flocks. They affect the health of poultry, and their deficiency can cause many diseases, as well as affect the quality and thickness of hatching eggshells, which is vital for the proper embryo development. Primarily, plasma calcium and phosphorus levels represent important diagnostic parameters often used to monitor eggshell quality. Řezáč et al. (2000) found that the mean levels of calcium and phosphorus in the blood plasma of layers were 6.50 and 1.70 mmol/l, respectively. In the present study, mean plasma calcium levels were approximately 14.31% lower, despite the fact that dietary calcium saturation was the same in both studies. In contrast, phosphorus plasma levels were approximately 75.88% higher. The reason was that the diet in this study was richer in phosphorus compared to the study of Řezáč et al. (2000), i.e. 7.30 g/kg and 2.10 g/kg, respectively. In general, trace element should not be overdosed in the diet because, as mentioned by Korish and Attia (2020), they are stored in chicken meat, and meat products, feed, and litter, as well as laying hens' eggs frequently contain their significant quantities.

ALT and AST values did not change markedly during the experiment. The values were still within physiological range, which was positive, as higher AST values are a sign of hepatic disorders.

Conclusions

The results of the monitored haematological parameters showed interesting dynamic changes in the blood plasma of the ROSS 308 parent flock associated with the production of hatching eggs. These changes reflected differences in laying intensity in some biochemical indices. The results can be easily applied in veterinary practice, as well as in avian haematology and biochemistry in order to expand, complete and update data concerning the blood plasma metabolic profile of the ROSS 308 parent flock during the laying season.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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