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EVOLUTION OF THE BLOOD BIOCHEMICAL PROFILE OF THE HY-LINE VAR. BROWN HENS IN NATURAL MOULTING CONDITIONS

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Abstract

The purpose of this paper was to determine the evolution of the blood biochemical profile in 63-week-old Hy-Line var. Brown hens, in natural moulting conditions. A control group that showed no signs of moulting and a spontaneously moulted hen group were monitored for eight weeks. The analysed blood biochemical parameters were: glucose, total protein, uric acid, cholesterol, calcium and phosphate concentrations, and Ca/P ratio. All analysed parameters recorded different levels in the moulting period, compared to those in the period before this physiological process. The hens in the moulting group showed significant ($P<0.01$) variations in serum glucose compared to those in the control group where these variations did not differ significantly. Glucose raised by 19%, uric acid decreased by 2.9% and total serum proteins also decreased by 23.89% compared to the control group. Cholesterol of the moulting group reached its highest values raising with 100% and Ca/P ratio had a decreasing with 5.1% compared to the control group. Thus, it can be stated that the determination of the biochemical profile is useful in the evaluation of the physiological status of moulting hens.

Key words*: Hy-Line hen, biochemical profile, moulting.*

INTRODUCTION

Poultry meat is considered the healthiest because it has a lower fat content compared to other types of meat (Marangoni et al., 2015). Therefore, the worldwide increased demand for chicken was satisfied only by the impressive progress made by primary breeding companies in genetic selection (Buzala et al., 2016) to improve the growth rate, skeletal health, food conversion ratio (Nangsuay et al., 2017) and disease resistance (Parmentier et al., 2012), along with management improvements.

Another important sector of the poultry industry is egg production. Egg is one of the most affordable sources of animal protein, and it is therefore not surprising that the number of laying flocks is growing rapidy in developed countries such as India and China (Bain et al., 2016). The economic importance of raising poultry is given by a whole series of qualities that they have, especially their ability to produce various and valuable animal products, as well as their genetic ability to ensure large productions with low consumption, energy and feed. In some countries, at the end of the first production cycle, chickens are either sold as reform chickens or subjected to forced moulting. The choice to eliminated these reformed hens or to force their feeding by artificially inducing moulting depends on several economic factors. These factors include the availability and cost of replacement hens (North & Bell et al., 1990). Forced induction of moulting after 12 months of intensive production is frequently practiced by the commercial egg industry to increase the poultry productivity, thus inducing a second production cycle. Moulting of avian species can generally be defined as periodic loss and subsequent replacement of feathers. For most bird species, moulting involves resting. For the egg producer, there would be an unprofitable period of low egg production, which means the end of the active life of a flock. Although there are many data in the literature on this metabolic process, there are still questions about the triggers, metabolic processes that occur during moulting, hormonal mechanisms and how they influence the increase in the percentage of laying, including questions concerning the metabolic peculiarities of poultry, preceding or

initiating the moulting process. At the same time, no data are known on the particularities of carbohydrate, protein, lipid metabolism, as well as on the phenomena of degradation of reserves in the body, during moulting. Based on this data, the purpose of this paper was to determine the evolution of the blood biochemical profile in 63-week-old Hy-Line var. Brown hens, in natural moulting conditions.

MATERIALS AND METHODS

The experimental study was carried out in a commercial laying hen farm, located in Brăila county, specialized in raising laying hens, with a capacity of 361,600 cap., which uses as biological material the hybrid Hy-Line var. Brown, housed on the ground. The Hy-Line var. Brown is a highly specialized egg production tetra linear hybrid. This hen line has average body weight at 70 weeks, 2.25 kg, the color of the mineral peel is uniform, dark brown, the technological holding is from 18 to 80 weeks and the egg cycle is 62 weeks (Hy Line International, Galline ovaliole commerciali Hy-line Brown-Management Guide, 2021). There were made two hens' groups with 50 cap. each, a control group that did not show signs of moulting and an experimental group with spontaneously moulted hens that were collected and grouped in the same hall with the same microclimate conditions with the control group. The hens were identified at the beginning of the moulting period. They were collected, grouped, weighed and isolated, all in the same hall. The hens were monitored during the pre-moulting period as well as during the natural moulting period. The monitoring of the two groups was carried out and was considered completed when the hens from the experimental group completed the moulting and feathering process, and the laying percentage reached parameters similar to those of the hens in the control group. Blood samples were taken after the first collection of eggs and before the application of technological treatments. In order to perform the blood biochemical determinations, the venous blood samples were collected on Li-Heparin and centrifuged at 1500 rpm, thus retaining the plasma. Plasma samples were stored at -20°C until processing. The blood samples were collected weekly, both from the control and experimental groups during the moulting period, for eight weeks from the moment of appearance the moulting signs. Catalyst Dx Chemistry Analyzer (IDEXX) and Stat Spin VT Centrifuge (IDEXX) was used for blood biochemical determinations. The laboratory method used is based on the principle of dry biochemistry. The reagents used are integrated by dry biochemistry technology and specific for the tested biochemical parameters equivalent to slide test. Each slide test was made up of overlapping layers. Centrally, a circle was placed on the test slide, indicating where the test sample is pipetted, automatically placed by the analyser (https://www.idexx.com/files/catalyst-dx-operators-guide-en.pdf). The analysed blood biochemical parameters were: glucose, total protein, uric acid, cholesterol, calcium and phosphate concentrations, and Ca/P ratio was calculated.

RESULTS AND DISCUSSIONS

As shown in Table 1, the control group have been constant values for total serum proteins during the eight weeks of monitoring.

Table 1. The evolution of total serum proteins in the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks							
	Day 1	63	64	65	66	67	68	69	70
C	54.9 ±7.9	50.0 ±6.8	56.2 ±6.4	48.98 ±5.7	59.2 ±6.0	55.9 ±4.9	48.9 ±4.4	50.2 ±4.2	53.01 ±4.2
M	31.1± 6.1	28.1± 6.02	32.98± 6.01	30.1± 4.1	38.01± 5.89	47.05± 3.2	44.1± 4.2	44.9± 3.1	45.03± 3.2

C= control group; M=moulting group; Normal protein reference values: 35-40g/L

The values obtained in the case of the moulting group showed significant differences compared to the control group ($P > 0.5$), as follows: in the first week of monitoring, the value recorded was 28.1 ± 6.02, which continues to increase to 45.03 ± 3.2 in the last week, a fact explained in the literature by changing metabolism as a result of stress. The same results regarding the differences between the control and the experimental group were found in other studies, such as Arora et al. (2011) which reported on the Japanese quail a decrease in total plasma protein 3.50 g/dL vs. 5.56 g/dL during moulting compared to the pre-moulting period, returning to a value of 5.89 g/dL after moulting

(Arora et al., 2011). Puvadolpirod et al., 2000 reported an increase in total plasma protein levels in young hens after administration of glucocorticoid hormone preparations for stress induction (Puvadolpirod et al., 2000).

The increase in total serum protein has been reported by Puvadolpirod et al. (2000) following the administration of glucocorticoid hormone preparations for stress induction (Puvadolpirod et al., 2000).

The uric acid recorded in the present study, in the case of the control group, showed constant values throughout the monitoring period, respectively 4.3 ± 1.8 mg/dL in the first week and 4.8 ± 1.05 mg/dL in the eighth week. The group subjected to natural moulting, compared to the control, showed lower values, especially in the first week, 1.3 ± 0.15 (mg/dL), a period characterized by the phenomenon of profuse moulting, caused by stress, and metabolic changes (Table 2).

Table 2. The evolution of serum uric acid during in the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks								
	Day 1	63	64	65	66	67	68	69	70
C	4,2 ±1,6	4,3 ±1.8	4,8 ±1,1	4,6 ±1,2	4,7 ±1,3	5,04 ±1.5	4,4 ±1,5	4,6 ±0,96	4,8± 1,05
M	1,3 ±0,2	1,3 ±0,15	1,9 ±0,21	2,8 ±0,33	3,9 ±0,6	4,2 ±0,87	4,4 ±0,96	4,5 ±0,76	4,3 ±0,5

C= control group; M=moulting group; Normal acid uric reference values:1-7/l

Starting with the second week of monitoring, the concentration of uric acid increased steadily, which towards the end of the monitoring reached values close to those of the control group, namely 4.3 ± 0.5 mg/dL, which showed the end of the moulting period, regulating the metabolism to normal and physiological parameters as well as establishing the normal percentage of eggs.

At the same time, high plasma uric acid levels indicate the use of dietary protein for energy needs or conversion to other compounds (lipids) but may indicate a decrease in the body's protein during starvation. The results from this study are comparatively with those of Dunkley et al., 2007, who recorded that the plasma concentration of uric acid was minimal (2.7 mg/dL) after five days of starvation, again reaching values comparable to those of the control (at 5 mg/dL) after 12 days of starvation (Dunkley et al., 2007).

Regarding glucose, there were differences between glucose levels before and during the moulting period, with a higher level during the latter. In the case of the control group, a value of 197 ± 46 mg/dL was recorded in the first week of monitoring, and will increase steadily to 206 ± 25 mg/dL. The values of the group undergoing natural moulting were higher than those of the control group, respectively 153 ± 32 mg/dL in the first week, following that starting with the fourth week (212 ± 40 mg/dL) to increase and reach in the last week values of 214 ± 24 mg/ dL (Table 3).

Table 3. The evolution of blood glucose in the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks								
	Day 1	63	64	65	66	67	68	69	70
C	189 ±41	197 ±46	203 ±44	210 ±42	187 ±32	186 ±30	196 ±39	201 ±25	206 ±25
M	153 ±32	162 ±36	166 ±50	188 ±44	212 ±40	235 ±40	220 ±25	210 ±20	214 ±24

C= control group; M=moulting group; Normal glucose reference values C-145-198 mg/dL, M-130-270 mg/dL

Arora et al. (2011) reported that hens in the normal physiological period of egg production had higher blood glucose levels compared to hens in the moulting period, an increase of 31.93% and respectively 120.09% compared to post-moulting birds (Arora et al., 2011). McCormick et al. (1984) noted that liver glycogen has reached the minimum allowable levels due to zinc interfering with insulin secretion (McCormick et al., 1984). Hanafy et al., 2001 showed that there are no changes in blood biochemistry except for increased alkaline phosphate levels (Hanafy et al., 2001). The ovaries showed a reduction in the number of follicles, cessation of ovulation and hyperplasia of the germinal epithelium.

Regarding the calcium values on the first day of monitoring, in the case of the control group, was recorded 22.4 ± 6.1 mg/dL, a value that was constant during the first four weeks. This, in the following weeks and until the end of the monitoring, registered a decrease, but insignificant, compared to the first four weeks, reaching in the eighth week a value of 19.9 ± 3.1 mg/dL (Table 4).

The values of serum calcium, recorded in the case of the group subjected to natural moulting, were lower than those of the control group, respectively 9.1 ± 2.3 mg/dL on the first day of

monitoring, values that remained constant during the first four weeks, and which continued to increase gradually and steadily over the next four weeks, reaching 16.67 ± 3.2 mg/dL in week eight. Regarding the phosphorus monitoring, it showed variable values during the eight weeks of monitoring, in the case of the group subjected to natural moulting, being recorded in the first week values of 2.4 ± 0.7 mg/dL, so that in week five it reaches at values of 3.7 ± 1.2 mg/dL. The values obtained in our monitoring were similar with the literature results, which attributed the decrease in serum calcium to ovarian involution and uterine regression, due to the phenomenon of profuse moulting. Gildersleeve et al., 1983 reported low concentrations of this mineral, along with low concentrations of inorganic phosphorus, transaminases (GPT) and albumin (Gildersleeve et al., 1983). Brake and Thaxton (1979) showed that both total plasma calcium (Ca) and inorganic plasma phosphate (P) showed decreases in moulted hens compared to those that did not go through this process (Brake and Thaxton, 1979). They attributed this decrease in total plasma protein and total calcium to the loss of estrogen-dependent plasma complex phosphor-lipoprotein. One of the proteins in this complex is fosvitin 52, which binds a large proportion of total plasma calcium. Loss of fosvitine decreases total calcium concentrations to 5-6 mg/100 ml (Brake and Thaxton, 1979). Sexual rest led to the cessation of yolk synthesis, decreased total plasma proteins and total calcium levels. In addition, a decrease in estrogen levels is thought to cause a decrease in plasma inorganic phosphate.

Table 4. The evolution of serum calcium in the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks								
	Day 1	63	64	65	66	67	68	69	70
C	22,4 ±6,1	22,1 ±4,1	21,5 ±4,2	20,5 ±4,2	21,2 ±3,3	19,6 ±3,1	19,7 ±3,3	19,8 ±2,6	19.9 ±3,1
M	9,1 ±2,3	8,5 ±1,6	10,01 ±2,5	9,5± 2,2	11,98 ±2,1	14,7 ±3,02	15,98 ±3,5	16,05 ±3.6	16,67 ±3,2

C= control group; M=moulting group; Normal serum calcium reference values 520 mg/dL

At the same time, it is plausible that intestinal secretion of estrogen and parathyroid hormone (PTH) is responsible for elevated serum calcium levels in hens that are subjected to moulting, and its decrease is due to eggshell formation (Sykes, 1971). Regarding the Ca/P ratio, in the case of the control group, similar values were recorded during the eight weeks of monitoring, respectively 7.3 in the first week, with a constant but not decrease, up to 6.5 in the last week. The values of the group subjected to natural moulting were comparatively lower, compared to the control group, respectively 3.7 in the first week and 4.4 in the second, values which, however, started to increase with week five, reaching the end of the monitoring at 5.7, values close to the control group, in the same period. The recordings from the eighth week of monitoring the batch subjected to natural moulting tend to increase, to values similar to those of the control batch from the first week, which is attributed to the completion of the moulting process and the training of the wool in the case of Ca/P ratio (Table 5).

Table 5. The evolution of Ca/P ratio din the blood of the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks								
	Day 1	63	64	65	66	67	68	69	70
C	7,4	7,3	7,1	7,2	6,6	6,6	6,5	6,5	6,5
M	3,2	3,7	4,4	5,1	4,98	5,2	5,2	5,6	5,7

C = control group; M = moulting group; Normal Ca/P ratio reference values 3/5 L

There are differences between the cholesterol valuesbetween the control group and the one subjected to natural moulting.

In the case of the control group, the cholesterol values had constant variables during the eight weeks of monitoring but there were no differences between them, respectively 87 ± 20 mg/dL in the first week of monitoring, with constant values until week eight (96 ± 20 g/dL) (Table 6).

However, the same cannot be said about the values of the experimental group, which had significant and visible differences compared to the control group. The values recorded were higher compared to the other batch, respectively 205 ± 48 mg / dL, values that remained high and constant for three weeks, and which began to decrease, reaching 82 ± 16 mg / dL, in the last week monitoring. Gyenis et al. (2006) showed that plasma levels of total cholesterol and LDH cholesterol are constant and similar until the age of 17 weeks of laying hens, when

various developments are observed, LDH-cholesterol decreasing significantly (Gyenis et al., 2006). Kuenzel (2003) reported that during the night moult, a much more pronounced decrease in body weight was recorded (Kuenzel 2003). Halaby 1997 used for the induction of moulting, raw bitter mistletoe seed, which caused the cessation of egg production within 2 weeks of the first administration (Halab, 1997). It has been suggested that the complete withdrawal of food leads to the immediate cessation of egg production because the diet causes changes in the normal physiology with a preference for serum cholesterol, triglycerides and very low-density lipoproteins (VLDL), which are important for egg production (Baranczuk et al., 1995; Peebles et al., 2004). However, there are individual variations between birds and this theory is not generally valid.

Higher serum cholesterol levels have been found in hens near natural moulting. Large variations have been reported from chicken to hen in terms of serum levels (Griminger, 1976) which could make it difficult to determine the level of cholesterol in the blood.

Glucose raised by 19%, uric acid decreased by 2.9% and total serum proteins also decreased by 23.89% compared to the control group. Cholesterol of the moulting group reached its highest values raising with 100% and Ca/P ratio had a decreasing with 5.1% compared to the control group.

Table 6. The evolution of the serum cholesterol (mg/dL) in the natural moulting hens during 8 weeks of monitoring vs. the control

	Age in weeks								
	Day 1	63	64	65	66	67	68	69	70
C	86±22	87±20	97±15	75±15	98±18	102±20	82±16	84±17	96±20
M	106±38	205±48	195±37	182±39	127±25	128±21	97±17	84±16	82±16

C= control group; M = moulting group; Normal cholesterol reference values 3/5

CONCLUSIONS

With the exception of total serum proteins, all biochemical parameters recorded different levels in chickens in the moulting period, compared to those in the period before this physiological process.

Glucose raised by 19%, uric acid decreased by 2.9% and total serum proteins also decreased by 23.89% compared to the control group. Cholesterol of the moulting group reached its highest values raising with 100% and Ca/P ratio had a decreasing with 5.1% compared to the control group.

The determination of the biochemical profile is useful in the evaluation of l physiological status of moulting hens.

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