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The effect of multiple ACTH injections on certain biochemical parameters in Yellow Hungarian laying hens

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ABSTRACT

The aim of this study was to investigate the changes of blood metabolites in response to repeated ACTH injections in Yellow Hungarian laying hens. A total of 36 laying hens aged 56 weeks were divided into two groups. Control group (9 hens) were given 0.2 cm^3 saline and experimental group (27 hens) were given 20 IU/kg adrenocorticotropin (ACTH) for four consecutive days into the thigh muscle. Blood samples were obtained on Day 0, 1, 2, 3 and 4. After the cessation of ACTH or saline, blood samplings were continued on Day 5, 7, 9 and 11. Mean, standard deviation and coefficient of variation (CV%) were calculated from levels detected in each individual, for each parameter investigated. Multiple range tests were used to determine significant differences between control and experimental groups. Glucose (26.7 mmol/l) and cholesterol level (4.1 mmol/l) increased in short time after ACTH treatment but values returned to the range of the control (12.6 mmol/l és 2.6 mmol/l) after the cessation of ACTH treatment as triglyceride (16.3 g/l) and protein content (60.0 g/l) increased and decreased slowlier after the ACTH administration (13.5 g/l and 51.8 g/l). The obtained set of results from the Yellow Hungarian breed can serve as reference values to evaluate harmful stress effects in the future.
(Keywords: stress, Yellow Hungarian laying hens, blood biochemistry values, ACTH)

ÖSSZEFOGLALÁS

Néhány élettani paraméter változása többszöri ACTH kezelés hatására az őshonos sárga magyar tyúkállományban

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Vizsgálataink során a többszöri adenokortikotróp (ACTH) hormon adagolás hatását vizsgáltuk az őshonos sárga magyar tyúkállományban. Összesen 36 tojótyúkot (56 hetes korúak) két csoportba osztottak (kontroll: n=9 és kísérleti: n=27). A kontroll csoport egyedei 0,2 cm^3 fiziológiás NaCl-oldatot, a kísérleti csoport egyedei 0,2 cm^3 ACTH-oldatot kaptak intramuszkulárisan. A tojótyúkoktól a 0., az 1., a 2., a 3. és a 4. napon vért vettek, majd a NaCl- és az ACTH-kezelés megszűnte után az 5., a 7., a 9. és a 11. napon a vérvételek folytatódtak. Az eredmények értékelése során átlag, szórás és CV% értéket, valamint a kontroll és a kísérleti csoport eredményei közötti szignifikáns különbséget értékeltük. Az ACTH kezelés után rövid idővel nagymértékben megnőtt a vérplazma glükóz- (26,7 mmol/l) és koleszterintartalma (4,1 mmol/l), majd a hormonkezelés megszűnte után rövid idővel az értékek csökkentek és visszatértek a kontroll szintjére (12,6 mmol/l és 2,6 mmol/l). A triglicerid- (16,3 g/l) és összfehérje- (60,0 g/l) tartalom

lassabban kezdett növekedni, de lassabban is tért vissza a kezelés után az alapszintre (13,5 g/l és 51,8 g/l). Az eredmények felhasználhatók arra, hogy a sárga magyar állományban referencia értékként szolgáljanak a tartós stresszhatás felismerésében.
(Kulcsszavak: stressz, sárga magyar tojótyúk, szérumanalitika, ACTH)

INTRODUCTION

Stress as a term describes the way when an organism reacts to environmental stimuli (*Harvey et al.,* 1984). A variety of stressors such as hot and cold, hunger and thirst, climatic conditions, diseases, handlings, high population density, transport, etc. have been used to study stress responses in birds or specifically in poultry species (*Siegel*, 1995). In response to environmental stimuli, hypothalamus-pituitary system regulates the secretion of adrenocorticotropin (ACTH) hormone. Upon entering to blood stream, ACTH induces an increase in corticosterone secretion from the adrenal glands. The hypothalamus-pituitary-adrenal (HPA) axis can cope with acute and chronic stress effects. However, a number of additional hormones that are not directly part of HPA-system is also involved (*Berczi*, 1986).

Administration of exogenous ACTH is a model proposed by *Thorn et al.* (1948) for studying stress in poultry. It has been shown by several studies that both the stressors and exogenous administration of ACTH lead to changes of the constituents in circulating blood through activation of the HPA-axis. Corticosterone causes a reduction in lymphocyte number and elevates heterophil/lymphocyte ratio (*Thaxton et al.,* 1982*; Siegel,* 1995; *Mitchell and Kettlewell,* 1998; *Goldstein and McEven,* 2002). In a study, *Vitinger* (1996) reported similar changes of white blood cells and differential leucocyte counts in Yellow Hungarian laying hens.

An animal adjusts continually to changes in environmental condition or stress. Metabolic changes represent a homeostatic response, which alters glucose metabolism, increases protein oxidation and fat deposition and elevates plasma cholesterol. Muscle is the major source of protein and amino acids. Chronic stress is responsible for glucose production from non-carbohydrate sources, principally protein. Metabolic changes developed by repeated administration of ACTH are presented by several authors (*Gould and Siegel,* 1985; *Klasing and Jarrell,* 1985; *Compton et al.,* 1991; *Latour et al.,* 1993; 1996).

Since 1950, strains of the native Yellow Hungarian chicken have been maintaining as a gene reserve at the Faculty of Agriculture and Food Sciences University of West Hungary in Mosonmagyaróvár. This breed is the only pure-blooded stock beside the breed of the Institute of Small Animal Research in Gödöllő. However, that stock originally descended from the breed of Mosonmagyaróvár. Inbreeding depression may play a large role determining population persistence by reducing the viability of small populations (*Sewalem et al.,* 1999). Changes of blood parameters and chronology of stress response by administration of ACTH have been employed to categorize the adaptive changes and metabolic status of this unique breeding line. Besides, studying of valuable characteristics of this species will contribute the maintenance of the breed and preserve its genetic stock.

Stress in avian species does not constitute a uniform set of adaptive responses that always occurs in a predictable temporal pattern (*Puvadolpirod and Thaxton,* 2000). It is notable that the majority of the previous studies concerned mainly juvenile male and sometimes female chickens. The aim of this study was to investigate the changes of blood glucose, cholesterol, triglyceride and total protein concentration in adult chickens

in response to repeated ACTH treatment. Besides, Thorn test can be used as an early indicator of stress and indicates the immunosuppressive effect of stressors.

MATERIALS AND METHODS

Yellow Hungarian laying hens about 56 weeks of age were used in these experiments. Laying hens were kept on litter in a naturally lightened broiler house. Chickens were fed ad libitum with commercial mash produced by Lajta Hanság Ltd. Water was given ad libitum as well. Blood samples (5 cm^3) were collected from the brachial vein using Na_2EDTA as an anticoagulant (*Nickel et al.,* 1977).

A total of 36 laying hens were divided into two groups. Control group (9 hens) were given 0.2 cm^3 saline and experimental group (27 hens) were given 20 IU/kg ACTH in 0.2 cm^3 sterile saline for four consecutive days (Day 1, 2, 3 and 4) into the thigh muscle. Every day, six hours after the injection, blood samples were collected into tubes using Na_2EDTA as an anticoagulant. On Day 5, 7, 9 and 11 saline or ACTH injection were not given to hens but blood samples were obtained from the control and experimental groups at the same time as during the ACTH or saline treatment. The measured blood parameters were glucose, cholesterol, triglyceride and total protein content of plasma. The biochemical parameters were evaluated within short time as possible using commercial kits. Parameters and methods utilized for determining of blood chemistries:

Total protein by biuret method (Hitachi-912 automated analyzer) according to *Weichselbaum,* 1948; glucose analysis by GOD-POD enzymatic diagnostic kit (REANAL, Budapest, Hungary) according to *Trinder,* 1969; triglyceride by GPO-PAP test (Boehringer Mannheim) according to *Werner et al*., 1981; cholesterol by CHOD-PAP test (Boehringer Mannheim) according to *Allain et al*., 1974.

Mean, standard deviation and coefficient of variation (CV%) were calculated from levels detected in each individual, for each parameter investigated. Parameters of control and ACTH treated groups were compared using paired t-test. Also, paired t-test was used to determine time course changes of stress responses followed by repeated ACTH treatment. The level of significance was reported at $P<0.05$ (*Sváb,* 1981).

RESULTS AND DISCUSSION

Avian blood glucose values are much higher than those of mammals. There are species differences in the way in which birds regulate blood glucose. The insulin content is about 1/6 than that of mammalian, while glucagon content is about 2 to 5 times higher (*Lumeij,* 1997). Absorption of glucose from the small intestine is more intensive to that of mammals (*Husvéth,* 2000). Normal blood glucose concentration of bird ranges between 11 to 25 mmol/l (*Lumeij and Overduin,* 1990). Diabetes mellitus caused by hyperglycemia is rarely observed in birds but blood glucose levels below 8.3 mmol/l should be considered serious and life threatening.

Changes in plasma glucose concentration and significant differences between control and ACTH treated groups after repeated administration of ACTH are shown in *Table 1.* Glucose levels of control hens exhibited a small increase between Day 3 and 5 (17.0 mmol/l). It was probably caused by the stressor of consecutive handlings (twice a day). ACTH treatment exhibited elevated glucose level on Day 1, six hours after the injection (19.8 mmol/l), which differed significantly ($P<0.001$) from the control group. Between Day 2 and 5, the difference increased consistently (25.4-26.7 mmol/l) at $P<0.001$

differences between control and experimental groups. From Day 5 glucose levels began to decrease and gradually returned to the range of control group. On Day 11 glucose level of experimental group was already lower (12.6 mmol/l) compared to control group (14.5 mmol/l).

Table 1

Changes in plasma glucose concentration (mmol/l) in response to repeated administration of ACTH hormone

Time of sampling (1)	Control (2)		ACTH (3)	
	Mean (4)	SD (5)	Mean	SD
Day 0 (7)	13.7	1.0	13.2	0.9
Day 1	13.2	1.1	19.8***	2.0
Day 2	14.5	0.7	21.7***	3.1
Day 3	15.0	1.1	26.7***	5.3
Day 4	16.2	1.5	25.4***	4.0
Day 5	17.0	1.4	23.8***	4.4
Day 7	14.3	1.2	18.4**	2.4
Day 9	13.6	0.8	15.3*	1.6
Day 11	14.5	0.9	12.6	1.8

Significant differences between groups ***P<0.001; **P<0.01; *P<0.05 ⟷: ACTH treatment

1. táblázat: A vérplazma glükóztartalmának változása többszöri ACTH adagolás hatására

A mintavétel ideje(1), Kontroll(2), ACTH-val kezelt(3), Átlag(4), Szórás(5), Variációs koefficiens(6), 0. nap(7)

Time course changes and chronology (day by day) in plasma glucose, cholesterol, triglyceride and protein concentration in ACTH treated group after repeated administration of ACTH are shown in *Table 2.*

Evaluating the obtained results between the consecutive sampling times, glucose level exhibited a sharp increase after the first ACTH injection (P<0.001) on Day 1. From Day 1 to Day 2 the modest increase showed no significant differences. Another sharp increase could be seen from Day 2 to Day 3 which decreased to Day 4 with no significant differences. From Day 4 glucose concentrations decreased consistently, the levels of decline and significant differences between sampling times are shown in *Table 2.*

Plasma has got a mixture of lipoproteins that are classified according to the ratio of protein/lipid content or in terms of density. Subgroups of lipoproteins contain triglycerides, cholesterol, phospholipids and other lipid-like compounds.

Cholesterol is a precursor of several steroids, such as corticosteroids, bile acids and vitamin D. Administration of exogenous ACTH or the various stressors lead to the activation of HPA-axis, release of adrenal steroids and an increased level of cholesterol (*Trout and Mashaly,* 1994).

About 20% of plasma cholesterol can be obtained from the diet. The other part (80%) is synthesized by the liver or small intestine from acetyl-CoA. There is a tremendous increase in the amount of cholesterol in plasma during the egg-laying period. Cholesterol content of egg has many and varied uses in the developing embryo. Blood

cholesterol of laying hens ranges between 2,07-2,98 mmol/l 80 to 115 mg/dl (*Shafey et al.,* 2003).

Changes of plasma cholesterol levels after ACTH treatment are presented in *Table 3.*

Table 2

Time course changes in blood parameters in response to repeated administration of ACTH hormone

Time of sampling (1)	Glucose (2)	Cholesterol (3)	Triglyceride (4)	Protein (5)
Day 0 (6)	-	-	-	-
Day 1	***	**	NS	NS
Day 2	NS	*	NS	NS
Day 3	***	NS	*	**
Day 4	NS	NS	**	NS
Day 5	NS	*	NS	**
Day 7	***	NS	*	**
Day 9	***	*	NS	NS
Day 11	***	*	*	*

Significant differences between groups: ***P<0.001; **P<0.01; *P<0.05, NS: no significancy

2. táblázat: A vérparaméterek koncentrációjának változása az idő függvényében többszöri ACTH adagolás hatására

A mintavétel ideje(1), Glükóz(2), Koleszterein(3), Triglicerid(4), Fehérje(5), 0. nap(6), NS: nem szignifikáns

ACTH caused similar changes in plasma cholesterol content compared with the changing of glucose levels. On Day 4 and 5 a subtle increase was obtained in control group (3.4 mmol/l) comparing with the initial value on the first day (2.7 mmol/l). Values of standard deviation and coefficient of variation also exhibited a small increase. These slight changes were probably caused by the consecutive handlings (injections, blood sample collections) as birds could not get accustomed to the repeated handlings (*Freeman et al.,* 1975). Cholesterol value of ACTH treated group elevated on Day 2 (from 2.7 mmol/l to 3.6 mmol/l) at P<0.05 differences between control and experimental groups. There was a further increase on Day 3 and 4 (3.7 mmol/l and 4.1 mmol/l) at P<0.01 and P<0.001 significances. On Day 5 after cessation of ACTH treatment, cholesterol level began to decrease (3.6 mmol/l, P<0.05) and on Day 7 it returned to the normal range. However, at Day 11 results were lower than those of the control.

Time course changes in plasma cholesterol concentration in ACTH treated group are shown in *Table 2.* Results were significantly increased on the first day after ACTH treatment (P<0.01), and this increase continued to Day 4 A consistent significant reduction began from Day 5 as it could be seen in *Table 2.*

Triglyceride concentration of the blood is about 10 times greater during the egg laying period under the control of estrogen. In a special way, triglyceride is transported directly to the developing oocyte by VLDL. Triglyceride values of plasma ranges between 10.8 and 18.2 g/l (*Mori et al.,* 2000, *Shafey et al.,* 2003).

Effect of ACTH on plasma levels of triglyceride is summarized in *Table 4.*

Table 3

Changes in plasma cholesterol concentration (mmol/l) in response to repeated administration of ACTH hormone

Time of sampling (1)	Contol (2)		ACTH (3)	
	Mean (4)	SD (5)	Mean	SD
Day 0 (7)	2.7	0.5	2.7	0.4
Day 1	3.0	0.4	3.2	0.5
Day 2	3.2	0.5	3.6*	0.7
Day 3	3.1	0.3	3.7**	0.9
Day 4	3.1	0.6	4.1***	0.8
Day 5	3.4	0.5	3.6*	0.6
Day 7	3.1	0.4	3.3	0.5
Day 9	2.9	0.4	3.0	0.4
Day 11	2.9	0.4	2.6	0.5

Significant differences between groups*** P<0,001; ** P<0,01; * P<0,05, ⟷: ACTH treatment

3. táblázat: A vérplazma koleszterin tartalmának változása többszöri ACTH adagolás hatására

A mintavétel ideje(1), kontroll(2), ACTH-val kezelt(3), átlag(4),szórás (5), variációs koefficiens(6), 0. nap(7)

Table 4

Changes in plasma triglyceride concentration (g/l) in response to repeated administration of ACTH hormone

Time of Sampling (1)	Contol (2)		ACTH (3)	
	Mean (4)	SD (5)	Mean	SD
Day 0 (7)	12.8	2.1	13.1	2.5
Day 1	12.8	2.5	13.0	2.3
Day 2	12.6	2.8	12.9	3.2
Day 3	13.0	3.1	13.5*	3.3
Day 4	13.4	3.0	16.3***	3.5
Day 5	13.1	2.6	15.9***	3.4
Day 7	12.9	2.3	14.1**	3.3
Day 9	12.9	2.8	13.7*	3.4
Day 11	13.0	2.5	13.5	3.1

Significant differences between groups: ***P<0,001; **P<0,01; *P<0,05, ⟷: ACTH treatment

4. táblázat: A vérplazma triglicerid tartalmának változása többszöri ACTH adagolás hatására

A mintavétel ideje(1), kontroll(2), ACTH-val kezelt(3), átlag(4), szórás(5), variációs koefficiens(6), 0. nap(7)

Triglyceride value of ACTH treated group began to increase only on Day 3 (13.5g/l, P<0.05) compared to control birds. On Day 4 and 5 there were further increases at higher significant differences (15.9 g/l and 16.3 g/l, P<0.001). Then a slow, gradual decline was observed but triglyceride content was even higher on Day 11 compared to that of control group. Triglyceride mobilization started after draining glycogen reservoir in the liver. *Gould and Siegel* (1985) observed the changes of VLDL and triglyceride content after administration of ACTH. There were no triglyceride secretions even 16-18 hours after ACTH treatment. Therefore, triglyceride content could not be used as an early indicator of stress.

Levels of triglyceride concentration and significant differences between sampling times in ACTH treated hens are shown in *Table 2.* Triglyceride concentrations were not affected significantly to Day 3 when the first significant difference (P<0.05) was obtained. Elevation continued at Day 4 (P<0.01) than a slow decline began and plasma triglyceride concentration reached the minimum level on Day 11. However, at Day 11 results were still higher than those of the control.

Blood plasma contains a complex mixture of proteins with different structures and properties (transthiretin, albumin, α-, β- and γ-globulins). Albumins, α-, β-globulins are synthesized by the liver. The main metabolic roles of these groups are the general transport functions. γ-globulins are synthesized by the B-lymphocytes as a response to antigenic stimuli. In general, plasma of birds contains about 30-60 g/l protein. (*Campbell,* 1997):

Changes of plasma total protein levels are shown in *Table 5.*

Table 5

Changes in plasma total protein concentration in response to repeated administration of ACTH hormone (g/l)

Time of Sampling (1)	Control (2)		ACTH (3)	
	Mean (4)	SD (5)	Mean	SD
Day 0 (7)	49.6	5.6	51.1	6.0
Day 1	50.1	6.2	51.8	4.9
Day 2	51.6	4.9	53.2*	5.1
Day 3	52.5	6.4	60.0**	7.8
Day 4	51.8	3.8	59.6**	8.2
Day 5	48.2	7.1	52.5*	5.7
Day 7	51.2	5.1	48.1	4.3
Day 9	49.4	4.8	47.2	4.6
Day 11	51.3	3.7	51.8	4.8

Significant differences between groups** P<0,01; * P<0,05, ⟷: ACTH treatment

5. táblázat. A vérplazma összfehérje tartalmának változása többszöri ACTH adagolás hatására

A mintavétel ideje(1), kontroll(2), ACTH-val kezelt(3), átlag(4),szórás (5), variációs koefficiens(6), 0. nap(7)

Total protein content of plasma showed the less notable changing in ACTH treated group. The first slight significant difference between control and experimental groups was observed on Day 2 (53.2 g/l, P<0.05). Differences enhanced on Day 3 and 4 (60 g/l,

P<0.01) but on Day 5 plasma protein showed a tendency to decline (52.5 g/l, P<0.05) and on Day 7 and 9 values were lower compared with the control value.

Time course changes in blood protein content showed some similarity with triglyceride changes. The first significant difference was obtained at Day 3 (P<0.01). Blood protein content began to decrease from Day 4 in comparison to the previous sampling times (*Table 2*).

It has been shown by several studies that both the stressors and exogenous administration of ACTH lead to changes of the constituents in circulating blood. Physiological responses of poultry to the environment vary tremendously depending on what type of environmental stressor is imposed.

Darkening and feed withdrawal treatment increased serum glucose, albumin, triglyceride and cholesterol concentrations (*Sahin and Kucuk,* 2001). In contrast, *Koelkebeck and Odom* (1995) obtained no dramatic effect on glucose and total protein content of laying hens after heat stress. After a 10-day-long food and light deprivation *Baron et al.* (1999) exhibited decreasing in plasma lipoprotein concentration. *Beuving and Vonder* (1978) observed an increase in plasma corticosterone and blood parameters in young and old laying hens and roosters within short time after ACTH injection. The increase was more rapid in younger than older birds.

The response to ACTH in adult (56 weeks old) Yellow Hungarian laying hens was found rapid in blood glucose and cholesterol contents and a little slowlier in triglyceride and protein contents of treated hens.

Serum glucose was elevated across time of sampling in ACTH-treated hens compared to those of controls. Glucose concentrations found in the present study are in agreement with the findings of *Thompson et al.* (1993) and *Siegel* (1995). Changes of plasma glucose level can be an early indicator of stress condition. However, several factors, such as many hormonal events and the nutrition influence blood glucose level (*Chamblee et al.,* 1989).

Multiple ACTH treatment increased the cholesterol level of blood sharply coupled with previous findings of *Puvadolpirod and Thaxton* (2000). Other stressors such as cage density resulted lower cholesterol increases in birds *(Brake and Peebles,* 1992).
ACTH administration has been reported to increase plasma triglyceride concentrations in chickens (*Davison et al.,* 1985, *Latour et al.,* 1996). These results are coupled with the findings in Yellow Hungarian laying hens. Similar elevations can be obtained when various infections and diseases are introduced in birds (*Burnham et al.,* 2003).

Evaluations of plasma levels of total protein are in agreement with the metabolic changes associated with stress in chickens (*Siegel,* 1995). Changing of total protein content is influenced by more factors. ACTH treatment increases the level of corticosterone in blood, which results the increase of protein synthesis in the liver. Changes in protein metabolism reflect changes in the relative rates of protein synthesis and degradation. Corticosterone acts at the liver to increase glucose production and induce synthesis of enzymes involved in amino acid catabolism. Besides, corticosterone acts to amplify the actions of other hormones involved in protein metabolism. At the same time gluconeogenesis is started accompanied by an increase in the rate of protein degradation and glucogenetic amino acids serve as the starting molecules of glucose synthesis (*Harbuz and Lightman*, 1992).

In summary: due to multiple ACTH injections blood plasma glucose, cholesterol, triglyceride and total protein content increased as a response against stress factors These results are in agreement with results previously obtained by *Davison et al,.* (1983); *Latour et al.,* (1996); *Puvadolpirod and Thaxton* (2000).

CONCLUSIONS

Repeated ACTH injections resulted meaningful metabolic changes. Glucose and cholesterol level increased in short time after administration of ACTH but values returned to the range of the control after the cessation of ACTH treatment. Triglyceride and protein content increased and decreased slowlier than the previously mentioned two parameters. In conclusion, repeated administration of ACTH caused sharp metabolic changes in the level of glucose, cholesterol, triglyceride and total protein. The obtained set of results suggests that changing of blood parameters exhibited the classical increases and decreases after ACTH treatment. These results can prove that despite of the inbreeding and long-term selecting stocks, this breed possesses dependable persistence. Values obtained in the present study will contribute towards a better understanding of this unique breeding line and can serve as reference values to evaluate harmful stress effects in the future.

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