Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, t. 17 (2021), nr 3, 31-45
DOI: 10.5604/01.3001.0015.5024

Effect of short-term fasting on the expression of ACTH (*cMC2*) receptor in the adrenal glands of chickens (*Gallus gallus domesticus*)

Danuta Wrońska, Izabela Szpręgiel[#]

Department of Animal Physiology and Endocrinology, Faculty of Animal Sciences, University of Agriculture in Krakow, Al. Mickiewicza 24/28, Kraków, Poland

SUMMARY

The main hormone produced by the adrenal glands of hens is corticosterone, synthesized and secreted by stimulation of the HPA axis during stress. Direct activation of adrenal activity is conditioned by ACTH, which binds to the melanocortin receptor *cMC2* in the adrenals and stimulates the synthesis and release of glucocorticosteroids. One of the factors that stimulate HPA axis activity is starvation, to which chickens are very sensitive. The purpose of this study was to determine the expression of ACTH receptor *cMC2* in the adrenals of hens during short-term fasting and after restoration of the proper level of nutrition (refeeding). The results of the experiment show that 24-hour food deprivation is stressful for the hen, as indicated by increased concentrations of corticosterone in the adrenals and blood plasma. Changes in *cMC2R* expression and the level of corticosterone in the adrenals during fasting and refeeding indicate a rapid increase in HPA axis activity in response to changing levels of nutrition. The results of the experiment confirm the direct effect of ACTH on corticosterone release by avian adrenal glands.

KEY WORDS: adrenal glands, starvation, corticosterone, melanocortin receptor cMC2, chicken, cMC2

INTRODUCTION

The rate of metabolic processes in vertebrates is mainly governed by the hypothalamic-pituitary-adrenal axis, known as the HPA axis (Axelrod and Reisine, 1984). The functioning of the HPA axis in birds has been thoroughly studied and repeatedly described (Harvey et al., 1980; Harvey et al., 1984). The mechanism of its activation in response to stressors has also been described in detail (Holmes and Phillips, 1976; Harvey and Hall, 1990; Ericsson et al., 2014; Fallahsharoudi et al., 2015). The pathways for the synthesis of glucocorticosteroids, as well as the end result of activation

[#] Corresponding author e-mail: izabela.szpregiel.urk@gmail.com
Received: 06.07.2021
Received in revised form: 19.09.2021
Accepted: 20.09.2021
Published online: 29.09.2021

of this neuroendocrine system, are highly conserved among vertebrates and homologous to those described in mammals (Miller, 1988; Nebert et al., 1991). The main steroid hormone produced by the adrenal glands of birds is corticosterone (Deroos, 1961; Paster, 1991; Scanes, 2020), while the level of aldosterone synthesis is much lower (Vinson et al., 1979; Carsia et al., 1987; Carsia et al., 1988b; Collie et al., 1992). Cortisol and cortisone are also produced in this endocrine gland in birds, especially during the embryonic period and just after hatching (Kalliecharan and Hall, 1974; Idler et al., 1976; Carsia et al., 1987). Corticosterone, like the other hormones mentioned, is a glucocorticosteroid involved in the metabolism of carbohydrates and fats. Its physiological effect, like that of other glucocorticosteroids, is to increase blood glucose levels, most often in the case of HPA axis stimulation, which is the body's response to stress. For this reason these compounds are commonly called stress hormones (Harvey et al., 1980; Harvey et al., 1984; Post et al., 2003; Yuan et al., 2008; Yang et al., 2015).

The main activator of the HPA axis in terms of corticosterone secretion is adrenocorticotropic hormone (ACTH; Beuving and Vonder, 1978; Beuving and Vonder, 1986). The substance stimulating the pituitary gland to release ACTH in birds is produced by hypothalamus CRF (corticotropin-releasing factor), the equivalent of mammalian corticoliberin (CRH; Carsia et al., 1986; Romero and Wingfield, 1998). The maximum ACTH concentrations in the blood occur within 5-10 minutes from onset of the stress stimulus (Kovács and Péczely, 1991). Additionally, a time synchronization between blood concentrations of ACTH and corticosterone in response to stress has been observed (Harvey and Hall, 1990; Kovács and Péczely, 1991; Virden et al., 2007a; Virden et al., 2007b), as well as between the action of the stress factor and the release of ACTH-induced corticosterone (Beuving and Vonder, 1986; Mehaisen et al., 2017). ACTH is the most effective stimulator of corticosteroid secretion by the adrenal glands of birds. Increasing the ACTH surge in chickens has been observed to stimulate the rapid release of corticosterone from the adrenal glands, within 5 minutes (Radke et al., 1985; Beuving and Vonder, 1986), as confirmed by numerous in vitro studies (Carsia et al., 1988b; Carsia and Weber, 1988a; Collie et al., 1992).

Studies on isolated chicken adrenal cells suggest that ACTH acts through specific high- and low-affinity receptors (Carsia and Weber, 1988a). These receptors, called melanocortin receptors (MCRs), belong to the family of G-protein-coupled receptors (GPCRs) and perform a variety of physiological functions; they are responsible for the stress response, skin pigmentation, the immune response, cardiovascular regulation, and energy homeostasis (Boston and Cone, 1996; Butler and Cone, 2002). Receptor 5 MC subtypes have been identified in various human and mammalian tissues (Mountjoy et al., 1992), as well as in chickens (Takeuchi and Takahashi, 1998; Ling et al., 2004; Ling and Schiöth, 2005).

The MC1, MC3, MC4 and MC5 receptors bind to melanotropin (MSH), which has a high affinity for them and performs its function through them. The MC2 receptor is the only receptor in this family to bind only to ACTH. MC2R is mostly expressed in the adrenal cortex and at low levels in adipocytes (Mountjoy et al., 1992; Boston and Cone, 1996; Butler and Cone, 2002). Accurate data on avian melanocortin receptors are lacking, but chicken melanocortin (cMC) receptors appear to be orthologues of mammalian MCRs and perform similar functions (Ling et al., 2004; Ling and Schiöth, 2005). The mRNA of the cMC2 receptor has been detected in the adrenal glands of chickens, indicating that this receptor is the counterpart of mammalian MC2 (Takeuchi et al., 1998; Ling et al., 2004; Ling and Schiöth, 2005). The cMC2 receptor has the ability to bind to glucocorticosteroids,

which may be important because glucocorticosteroids induce a significant increase in MC2R expression, as demonstrated by studies in cattle (Darbeida and Durand, 1990; Dores, 2013).

One of the factors that can stimulate the HPA axis is starvation. The specific structure of the digestive system of birds causes these animals to digest very quickly. Food may pass from the goitre to the cloaca after 2-4 hours, depending on its type and the physiological state of the animal (Kaupp and Ivey, 1923). For this reason, birds, especially domestic chickens, are extremely sensitive to the stress of starvation. In light of the above, it can be assumed that released corticosterone as well as changes in the activity of the cMC2 receptor may modulate the hormonal activity of the adrenal glands in birds when they are deprived of food. The aim of the research was to determine the in vivo expression of melanocortin (cMC) receptor mRNA and the degree of corticosterone secretion from the adrenal glands of chickens during short-term (24 h) deprivation of feed and after restoration of the proper level of nutrition (refeeding).

MATERIALS AND METHODS

Chemicals

The chemicals were purchased from the following companies: Corticosterone Double Antibody RIA Kit (Catalog No. MP07120102, MP Biomedicals, LLC Diagnostic Division, NY, USA); Eukaryotic 18S rRNA Endogenous Control, TaqMan Gene Expression Assays and Master Mix, the High-Capacity cDNA Reverse Transcription Kit, and TRI-reagent (Applied Biosystems/ThermoFisher Scientific, Foster City, CA, USA). All other reagents were obtained from ICN Biomedicals (Aurora, IL, USA), Sigma (St. Louis, MO, USA) or POCH (Gliwice, Poland).

Animals and experimental design

The experiment was conducted on 15 immature Hy-Line Brown hens (average body weight 1,28 ± 0,06 kg) at the age of 15 weeks, purchased from the commercial farm H&P2 (Czarków, Poland). The animals were housed in an experimental building belonging to the Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow. The hens were housed in individual cages in compliance with principles governing the rearing of commercial flocks of laying hens, in a hall equipped with forced ventilation, lighting 14L:10D (lights-on at 0800h and off at 2200h), with free access to commercial food (17,5 MJ•kg^{-1}, 15% protein; DKMII) and water. Before the experiment, birds were divided randomly into three groups (n = 5 in each group), as follows: (i) with feed and water *ad libitum* (control); (ii) fasted for 24 h (short fasting); and (iii) fasted for 24 h and then allowed access to food for 24 h (refeeding). The experiment was conducted according to a research protocol approved by the Local Animal Ethics Committee in Krakow (No. 49/OP/2004).

On the first day of the experiment blood was collected from the wing vein of all hens in the amount of 2 ml into heparinized tubes. After 24 h of feed deprivation, blood was collected from the animals in group 2, followed by decapitation. Blood samples were collected from the birds in group 3 at 24 h after the return to normal feeding, followed by decapitation. Blood samples from each collection date were centrifuged for 10 minutes (8000 rpm), and the plasma was immediately frozen at –20°C until corticosterone determinations by radioimmunoassay (RIA). After decapitation of the control and experimental animals, the recovered adrenal glands were placed in Petri dishes on ice in a physiological saline solution. Then the adrenal glands of each chicken were cut into smaller sections of similar weight (about 50 mg). Selected fragments of tissue were weighed and then homogenized in liquid nitrogen. On the scheduled day of assay the homogenates were diluted in 0,5 mL of

phosphate buffer (pH = 7,5). The homogenates were collected and kept at –20°C until corticosterone determinations in tissue by RIA. The sections of the adrenal glands were placed in 1 ml of RNAlater (Stabilization Solution for Tissue; Sigma). The samples prepared in this way were placed in a freezer at –80°C until the analyses.

Steroid hormone determination in tissue

The amount of corticosterone was measured using the Corticosterone Double Antibody RIA Kit (MP Biomedicals, LLC Diagnostic Division, NY, USA) in accordance with the manufacturer's instructions. Radioactivity was measured in a Wizard gamma counter (LKB, Austria). All samples and standards were tested in duplicate. The sensitivity of the method was 0,70 ng/ml; intra- and interassay coefficients of variation were 5,5% and 8,0%. The hormone concentration in the incubation medium was calculated per mg of adrenal medulla tissue.

Total RNA isolation and RT-PCR analysis

Total RNA from adrenal tissue was isolated with TRI Reagent (ThermoFisher Scientific) according to the manufacturer's instructions. RNA density and quality were evaluated by measuring probe extinction at 260 and 280 nm. Total RNA (2 µg) was used as a template in cDNA synthesis. Reverse transcription (RT) was performed using a Master-cycler (Eppendorf, USA) with the High-Capacity cDNA Reverse Transcription Kit according to the following profile: (i) 25°C, 10 min; (ii) 37°C, 120 min and (iii) 85°C, 5 min. The first strand of cDNA was stored at –20°C and after dilution (5x) used for qPCR amplification based on 50 nuclease chemistry using TaqManTM MGB (minor groove binder) probes. Multiplex qPCR was performed in a 96-well thermocycler (StepOne Plus, Applied Biosystems, USA). Assay-on-Demand, TaqMan MGB Gene Expression Kits with specific TaqMan MGB probes designed by Applied Biosystems/ThermoFisher Scientific were used for cMC2 analysis of mRNA expression in the adrenal tissue (accession number: NM_00103515.1); amplicon size: 107 bp. 18S rRNA was used as a reference gene (Eukaryotic 18S rRNA Endogenous Control, GenBank AF173612.1; amplicon size: 187 bp; Applied Biosystems/ThermoFisher Scientific: cat # 4310893E). All reactions were performed in duplicate. The data were calculated according to the ΔΔCt method previously described by Livak and Schmittgen (2001), using the expression in the control group of adrenal tissue as the calibrator (RQ = 1), and are presented as RQ ± SEM.

Statistical analysis

The results were subjected to one-way ANOVA followed by the Tukey post hoc test. Differences between values are presented as means ± SEM and considered to be significant at $P < 0,05$. Calculations were performed using Statistica v.13.1 software (StatSoft, Inc, Tulsa, OK, USA). Figures were prepared using Grapher 12 (Golden Software Inc., USA).

RESULTS

In the group of control hens, the concentration of corticosterone in the blood plasma was estimated at 4,28 ± 0,56 ng/mg (Fig. 1). The lack of feed for 24 hours resulted in a significant increase in the concentration of the hormone, to 7,65 ± 0,45 ng/mg ($P < 0,05$), while 24 h after restoration of normal nutrition, its concentration decreased compared to the values recorded during fasting, but were still significantly higher than in the control group (5,66 ± 0,65 ng/mg; $P < 0,05$; Fig. 1).

The corticosterone concentration of 22,2 ± 3,2 ng/mg in the adrenal tissue in the control group did not change significantly after short-term fasting (24,2 ± 6,1 ng/mg; $P > 0,05$). At 24 h after normal

nutrition was restored, it decreased significantly compared to the values in the other two groups (15,9 ± 1,8 ng/mg; $P < 0,05$; Fig. 2).

RQ PCR analysis showed a significant increase in the expression of the cMC2R receptor for ACTH in the adrenal glands of fasted chickens in relation to the values found in the group of control birds ($P < 0,05$), while at 24 h after restoration of normal nutrition, the value did not differ statistically from the value in the control group ($P < 0,05$; Fig. 3).

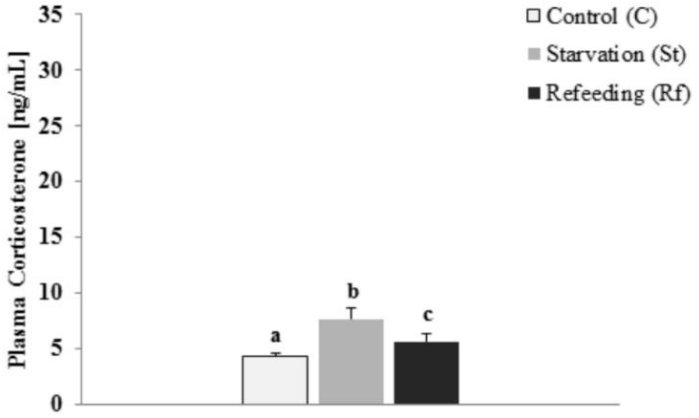

Fig. 1. The effect of starvation and refeeding on corticosterone concentration in chicken blood plasma (one-way analysis of variance; different superscript letters a, b, c indicate significant differences between experimental groups at $P \leq 0,05$)

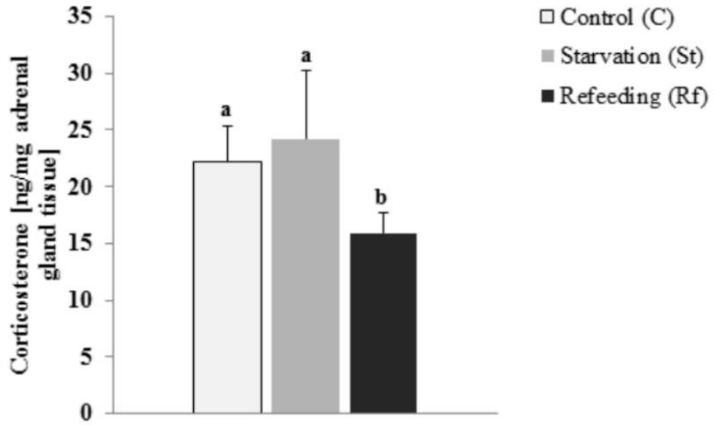

Fig. 2. The effect of starvation and refeeding on corticosterone concentrations in chicken adrenal tissue (one-way analysis of variance; different superscript letters a, b, c indicate significant differences between experimental groups at $P \leq 0,05$)

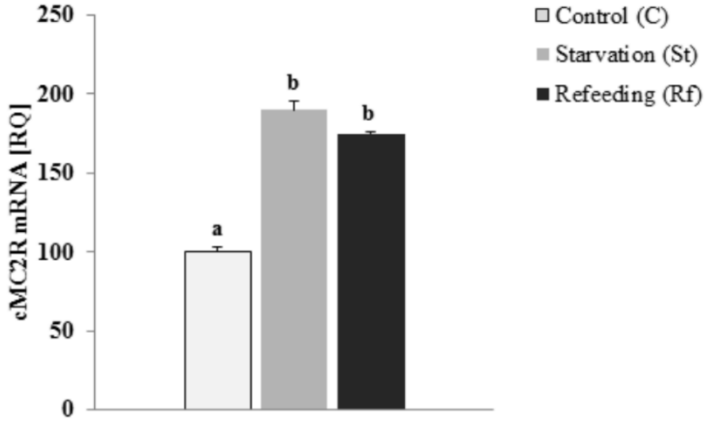

Fig. 3. Relative expression of cMC2R mRNA in chicken adrenal tissue after starvation and refeeding (one-way analysis of variance; different superscript letters a, b, c indicate significant differences between experimental groups at $P \leq 0,05$)

DISCUSSION

The results of the in vivo experiment described in the present study showed a change in the concentration of corticosterone and in mRNA expression of the cMC2 receptor in the adrenal glands of starving hens. High-intensity environmental, metabolic or psychological stimuli that pose a threat to homeostasis to which the body must adapt are perceived as stressors. Stressors are factors that activate the HPA axis and ultimately lead to the secretion of glucocorticosteroids from the adrenal glands. Stress during growth and development is well known to have a major impact on a number of phenotypic traits later in life. Repeated exposure to adverse factors during the postnatal development of birds can significantly alter the physiological stress response. The latest research on birds suggests that long-term exposure to stress factors alters the activity of many neuroendocrine systems that determine the ability to respond to changing environmental factors and enable survival. Chronic stress exposure has also been shown to permanently change the activity of the HPA axis (Spencer et al., 2009). As described in the introduction to the study, ACTH is responsible for the activation of the HPA axis in birds and its final effect: the synthesis and secretion of corticosterone. This hormone, secreted by the pituitary in response to stress, acts on the adrenal glands through the cMC2 receptor expressed in the adrenal glands. In response to stress, this receptor binds to ACTH, which stimulates the synthesis and secretion of corticosterone by the adrenal glands of birds. The adrenal glands of domestic chickens contain about 1600 ng ACTH/gland, while its concentration in the blood ranges from 20 to 150 pg/ml (Castro et al., 1986), and may increase more than twofold in response to various stressors (Harvey and Hall, 1990; Kovács and Péczely, 1991).

In birds, the HPA axis is activated in response to a stressor through the central nervous system (Harvey and Hall, 1990). The stress response may be general (non-specific) or specific for a given stressor. The non-specific stress response is always accompanied by activation of the adrenal cortex and secretion of corticosterone (Harvey et al., 1984). The experiment showed that after 24 hours, the expression of the receptor in stressed birds doubled in comparison with the control group. This increase translates into a potential increase in the concentration of corticosterone in the adrenal glands of stressed birds, which was also observed. The amount of the hormone in the adrenal glands of fasted chickens is similar to that noted in the group of control birds, but the value in the blood plasma is almost twice as high as in the control (Fig. 2). Expression of the cMC2 receptor in the adrenal glands of chickens after feeding was reintroduced proved to be slightly lower than in the group of control birds (Fig. 3). Also, the concentration of corticosterone in the adrenal glands of these birds was lower than in the control animals (Fig. 2), while its concentration in the blood plasma remained higher than in the control group (Fig. 3). Since corticosterone is released from the adrenal glands in response to ACTH, the results confirm the role of the cMC2 receptor in regulating the hormonal activity of the adrenal glands and indicate its importance in the stress response.

The results indicate that despite the removal of the stressor and the return to normal nutrition, homeostasis was not restored. The lower corticosterone concentration in the adrenal glands of birds during refeeding may be due to the depletion of reserves of this hormone in these glands. During starvation, the adrenal glands secrete large amounts of corticosterone, thus depleting its stores. The body takes a few days to restore the amount of corticosterone in the adrenal glands to a baseline level. Corticosterone, like other glucocorticosteroid hormones, increases blood glucose levels and accelerates gluconeogenesis, which initially mobilizes the organism. However, a long-term surplus of corticosterone in the blood causes enormous havoc in the body: protein breakdown, inhibition of

fat metabolism, slowing of lipolysis, a reduction in the rate of glucose consumption by skeletal muscles, and acceleration of the breakdown of fatty acids into ketone bodies. The higher plasma level of corticosterone in our own research indicates that the time elapsed since the removal of the stress factor was insufficient to judge whether the birds will return to homeostasis. Despite the withdrawal of the stressor, the blood corticosterone level remains elevated, so it can be assumed that the animal is still under stress (Jimeno et al., 2018; Neuman-Lee et al., 2020).

ACTH secreted from the pituitary is well known to control the release of corticosterone from the adrenal glands of birds and is itself controlled by CRF produced in the hypothalamus, as well as by vasotocin (AVT) and mesotocin (MT) (Castro et al., 1986; Mikami, 1986; Ball et al., 1989). Studies conducted in the 1980s on pituitary glands isolated from ducklings and chickens confirmed that these factors stimulate ACTH secretion and thus affect corticosterone secretion by the adrenal glands (Hayashi et al., 1991; Jurkevich et al., 2008). The ability of ACTH to act directly on adrenal cells is due to the presence of specific receptors for this tropic hormone. As mentioned above, the action of ACTH is conditioned by the presence and activity of the MC2 receptor (Schiöth, 2001), which is more specific than other receptors from this family, as it binds only to ACTH and has no affinity for other melanocortins, while other MCRs can be activated by both ACTH and MSH (Abdel-Malek, 2001). All MC receptors were cloned as early as the 1990s, including the specific ACTH receptor MC2, which in chickens is expressed both in the adrenal glands and in the spleen (Mountjoy et al., 1992; Takeuchi et al., 1998). Before this period, only the physiological effects of ACTH were known, as well as those of MSH, which performs its functions through the MC1 and MC3-MC5 receptors.

As mentioned in the introduction, birds are especially sensitive to stress-related to changes in nutrition (Bigot et al., 2003; Mahmoud and Edens, 2012; Nelson et al., 2020; Wang et al., 2020; Abo-Al-Ela et al., 2021). Fasting is a particularly strong stressor (Kontecka et al., 1999; Kitaysky et al., 2001). The plasma concentration of corticosterone increases after 24 hours of fasting, which was confirmed in our experiment and in earlier studies conducted both in immature birds (Nir et al., 1975; Harvey et al., 1983; Harvey et al., 1984) and adults (Scanes et al., 1980a; Scanes et al., 1980b; Scanes, 2016). On the other hand, an increase in the corticosterone concentration may also be a factor stimulating food intake in birds, as shown by recent studies in Japanese quail (Hull et al., 2007; Hazard et al., 2008; Wall and Cockrem, 2009; Wall and Cockrem, 2010).

Compared to the enormous amount of research into the functioning of the HPA axis and the regulatory mechanisms of the functioning of this system in mammals, corresponding knowledge regarding birds remains limited, despite their importance in the course of the stress response (Harvey and Hall, 1990; Fallahsharoudi et al., 2015). Chickens and their response to stress are undeniably of great theoretical and practical importance (Barnett and Hemsworth, 2003). In domestic chickens, such stress increases the adrenal mass and the rate of steroidogenesis, and consequently the amount of ACTH and corticosterone (Carsia et al., 1988b; Carsia and Weber, 1988a; Weber et al., 1990). Conversely, in the domestic turkey, another representative of the same family, starvation stress induces changes in the structure of the adrenal glands and reduces the rate of steroidogenesis (Carsia and McIlroy, 1998). Interestingly, the same stressor may act in opposite ways in closely related species, but it has been established that HPA axis components in birds may respond differently to stress (Harvey and Hall, 1990). For example, seabirds have developed their own mechanism of adaptation to insufficient nutrition. Studies in various species of seabirds have found elevated levels of corticosterone due to starvation (Kitaysky et al., 1999, Kitaysky et al., 2000; Walker et al., 2005),

which was associated with a reduction of fat stores. It is believed that high corticosterone levels may allow these birds to alter their metabolic pathways so that protein catabolism becomes an alternative energy source (Axelrod and Reisine, 1984; Le Ninan et al., 1988), which to some extent was confirmed in the present study, in which the plasma level of corticosterone did not return to the baseline value after the normal level of nutrition was restored. In addition, Kitayski et al. (2001) observed that an increase in corticosterone secretion resulted in an increase in the supply of food to chicks by their parents, which could restore homeostasis in the young, but this was not always successful and the chick often remained hungry.

In domestic chickens, even short-term starvation stress can be unfavourable, as confirmed in the present study. A similar situation is observed in mammals (Sapolsky et al., 1986). The physiological response to starvation stress consists of three phases, distinguished by the rate of weight loss. This three-phase response to stress has also been observed in birds, including domestic chickens (Webster, 2003). In our experiment, however, we analysed starvation of much shorter duration than the entire three phases. The stress of 24-hour feed deprivation activates the HPA axis in domestic chickens, as confirmed by our own research. As a result of the 24-hour fasting, the cMC2 receptor levels doubled compared to the control, suggesting that the adrenal glands were 'preparing' for secretion of greater amounts of ACTH from the pituitary gland, which in turn increases the production and secretion of corticosterone from the adrenal glands into the blood. These results indicate that even such a short fast is a great stressor for these animals. The sensitivity of chickens to this particular stressor is due to the specific structure of their digestive system, which is relatively short – food passes very quickly from the goitre to the cloaca, in some cases after 2 hours (Kaupp and Ivey, 1923). One day after removal of the stress factor (hunger) and restoration of normal feeding, the hens were still in a state of stress, as evidenced by the persistently high plasma level of corticosterone. It is also evidence that the animals have not yet returned to homeostasis. This is also indicated by the decrease in the expression of the cMC2 receptor and the level of corticosterone in the adrenal glands. The results of our experiment clearly show that the time elapsed since the removal of the stressor was too short for the stressed organism to return to homeostasis. An additional stress stimulus, acting for a short time, would be detrimental to the animal; the body would need even more time to overcome the stress, and the functioning of physiological systems could be disturbed. Stress lasting longer than a day would be equally dangerous. In poultry farming, it is important not to expose farm birds to stress, because it adversely affects their breeding.

In conclusion, the results of the experiment show that domestic hens are extremely sensitive to the stress of starvation. Even short-term deprivation of feed disturbs the functioning of the body, which is not restored one day after removal of the stressor. Even after such a short period of stress, it takes a long time for homeostasis to be restored in the domestic hen. Any stress is a huge burden for the body that reduces the productivity of farm animals, which is why it is so important to protect them against the effects not only of hunger, but of other stressors as well.

REFERENCES

1. Abdel-Malek Z.A. (2001). Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. Cell Mol Life Sci., 58(3): 434-41, doi: 10.1007/PL00000868
2. Abo-Al-Ela H.G., El-Kassas S., El-Naggar K., Abdo SE., Jahejo A.R., Al Wakeel R.A. (2021). Stress and immunity in poultry: light management and nanotechnology as effective immune

enhancers to fight stress. Cell Stress Chaperones, 26(3): 457-472, doi: 10.1007/s12192-021-01204-6
3. Axelrod J., Reisine T.D. (1984). Stress hormones: their interaction and regulation. Science, 224(4648): 452-9, doi: 10.1126/science.6143403
4. Ball G.F., Faris P.L., Wingfield J.C. (1989). Immunohistochemical localization of corticotropin-releasing factor in selected brain areas of the European starling (Sturnus vulgaris) and the song sparrow (Melospiza melodia). Cell Tissue Res., 257(1): 155-61, doi: 10.1007/BF00221646
5. Barnett J.L., Hemsworth P.H. (2003). Science and its application in assessing the welfare of laying hens in the egg industry. Aust Vet J., 81(10): 615-24, doi: 10.1111/j.1751-0813.2003.tb12506.x
6. Beuving G., Vonder G.M. (1978). Effect of stressing factors on corticosterone levels in the plasma of laying hens. Gen Comp Endocrinol., 35(2): 153-9, doi: 10.1016/0016-6480(78)90157-0
7. Beuving G., Vonder G.M. (1986). Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and plasma baseline levels of corticosterone. Gen Comp Endocrinol., 62(3): 353-8, doi: 10.1016/0016-6480(86)90044-4
8. Bigot K., Mignon-Grasteau S., Picard M., Tesseraud S. (2003). Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. Poultry science, 82(5), 781-788, doi: 10.1093/ps/82.5.781
9. Boston B.A., Cone R.D. (1996). Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. Endocrinology, 137(5): 2043-50, doi: 10.1210/endo.137.5.8612546
10. Butler A.A., Cone R.D. (2002). The melanocortin receptors: lessons from knockout models. Neuropeptides, 36(2-3): 77-84, doi: 10.1054/npep.2002.0890
11. Castro M.G., Estivariz F.E., Iturriza F.C. (1986). The regulation of the corticomelanotropic cell activity in Aves--II. Effect of various peptides on the release of ACTH from dispersed, perfused duck pituitary cells. Comp Biochem Physiol A Comp Physiol., 83(1): 71-5, doi: 10.1016/0300-9629(86)90090-3
12. Carsia R.V., Weber H., Perez F.M Jr. (1986). Corticotropin-releasing factor stimulates the release of adrenocorticotropin from domestic fowl pituitary cells. Endocrinology, 118(1): 143-8, doi: 10.1210/endo-118-1-143
13. Carsia R.V., Morin M.E., Rosen H.D., Weber H. (1987). Ontogenic corticosteroidogenesis of the domestic fowl: response of isolated adrenocortical cells. Proc Soc Exp Biol Med., 184(4): 436-45, doi: 10.3181/00379727-184-42498
14. Carsia R.V., Weber H. (1988a). Protein malnutrition in the domestic fowl induces alterations in adrenocortical cell adrenocorticotropin receptors. Endocrinology, 122(2): 681-8, doi: 10.1210/endo-122-2-681
15. Carsia R.V., Weber H., Lauterio T.J. (1988b). Protein malnutrition in the domestic fowl induces alterations in adrenocortical function. Endocrinology, 122(2): 673-80, doi: 10.1210/endo-122-2-673
16. Carsia R.V., McIlroy P.J. (1998). Dietary protein restriction stress in the domestic turkey (Meleagris gallopavo) induces hypofunction and remodeling of adrenal steroidogenic tissue. Gen Comp Endocrinol., 109(1): 140-53, doi: 10.1006/gcen.1997.7016

17. Collie M.A., Holmes W.N., Cronshaw J. (1992). A comparison of the responses of dispersed steroidogenic cells derived from embryonic adrenal tissue from the domestic chicken (Gallus domesticus), the domestic Pekin duck and the wild mallard duck (Anas platyrhynchos), and the domestic muscovy duck (Cairina moschata). Gen Comp Endocrinol., 88(3): 375-87, doi: 10.1016/0016-6480(92)90232-9
18. Darbeida H., Durand P. (1990). Mechanism of glucocorticoid enhancement of the responsiveness of ovine adrenocortical cells to adrenocorticotropin. Biochem Biophys Res Commun., 166(3): 1183-91, doi: 10.1016/0006-291x(90)90991-u
19. Deroos R. (1961). The corticoids of the avian adrenal gland. Gen Comp Endocrinol., 1: 494-512, doi: 10.1016/0016-6480(61)90012-0
20. Dores R.M. (2013). Observations on the evolution of the melanocortin receptor gene family: distinctive features of the melanocortin-2 receptor. Front Neurosci., 7: 28, doi: 10.3389/fnins.2013.00028
21. Ericsson M., Fallahsharoudi A., Bergquist J., Kushnir M.M., Jensen P. (2014). Domestication effects on behavioural and hormonal responses to acute stress in chickens. Physiol Behav., 133: 161-9, doi: 10.1016/j.physbeh.2014.05.024
22. Fallahsharoudi A., de Kock N., Johnsson M., Ubhayasekera S.J., Bergquist J., Wright D., Jensen P. (2015). Domestication Effects on Stress Induced Steroid Secretion and Adrenal Gene Expression in Chickens. Sci Rep., 5: 15345, doi: 10.1038/srep15345
23. Harvey S., Merry B.J., Phillips J.G. (1980). Influence of stress on the secretion of corticosterone in the duck (Anas platyrhynchos). J Endocrinol., 87(1): 161-71, doi: 10.1677/joe.0.0870161
24. Harvey S., Klandorf H., Pinchasov Y. (1983). Visual and metabolic stimuli cause adrenocortical suppression in fasted chickens during refeeding. Neuroendocrinology, 37(1): 59-63, doi: 10.1159/000123516.
25. Harvey S., Phillips J.G., Rees A., Hall T.R. (1984). Stress and adrenal function. J. Exp. Zool., 232: 633-645, doi: 10.1002/jez.1402320332
26. Harvey S., Hall T.R. (1990). Hormones and stress in birds: activation of the hypothalamo-pituitary-adrenal axis. Prog Clin Biol Res., 342: 453-60, PMID: 2200026
27. Hayashi H., Imai K., Imai K. (1991). Characterization of chicken ACTH and alpha-MSH: the primary sequence of chicken ACTH is more similar to Xenopus ACTH than to other avian ACTH. Gen Comp Endocrinol., 82(3): 434-43, doi: 10.1016/0016-6480(91)90318-z
28. Hazard D., Couty M., Richard S., Guémené D. (2008). Intensity and duration of corticosterone response to stressful situations in Japanese quail divergently selected for tonic immobility. Gen Comp Endocrinol., 155(2): 288-97, doi: 10.1016/j.ygcen.2007.05.009
29. Holmes W.N., Phillips J.G. (1976). The adrenal cortex of birds. In: Chester-Jones I., Henderson I.W. (Eds.), General, Comparative, and Clinical Endocrinologyof the Adrenal Cortex. Academic Press, London, UK, pp. 293-420
30. Hull K.L., Cockrem J.F., Bridges J.P., Candy E.J., Davidson C.M. (2007). Effects of corticosterone treatment on growth, development, and the corticosterone response to handling in young Japanese quail (Coturnix coturnix japonica). Comp Biochem Physiol A Mol Integr Physiol., 148(3): 531-43, doi: 10.1016/j.cbpa.2007.06.423

31. Idler D.R., Walsh J.M., Kalliecharan R., Hall B.K. (1976). Identification of cortisol in plasma of the embryonic chick. Gen Comp Endocrinol., 30(4): 539-40, doi: 10.1016/0016-6480(76)90126-x
32. Jimeno B., Hau M., Verhulst S. (2018). Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. Sci Rep. 8(1): 13020, doi:10.1038/s41598-018-31258-z
33. Jurkevich A., Berghman L.R., Cornett L.E., Kuenzel W.J. (2008). Immunohistochemical characterization of chicken pituitary cells containing the vasotocin VT2 receptor. Cell Tissue Res., 333(2): 253-62, doi: 10.1007/s00441-008-0636-2
34. Kalliecharan R., Hall B.K. (1974). A developmental study of the levels of progesterone, corticosterone, cortisol, and cortisone circulating in plasma of chick embryos. Gen Comp Endocrinol., 24(4): 364-72, doi: 10.1016/0016-6480(74)90149-x
35. Kaupp B.F., Ivey J.E. (1923). Time required for food to pass through the intestinal tract of fowls. J. agric. Res., 23: 721-725
36. Kitaysky A., Piatt J., Wingfield J., Romano M. (1999). The adrenocortical stress-response of Black-legged Kittiwake chicks in relation to dietary restrictions. J Comp Physiol B., 169, 303-310, doi.org/10.1007/s003600050225
37. Kitaysky A.S., Hunt Jr, G.L., Flint E.N., Rubega M.A., Decker M.B. (2000). Resource allocation in breeding seabirds: responses to fluctuations in their food supply. Marine Ecology Progress Series, 206: 283-296, doi.org/10.3354/meps206283
38. Kitaysky A.S., Kitaiskaia E.V., Wingfield J.C., Piatt J.F. (2001). Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. J Comp Physiol B., 171(8): 701-9, doi: 10.1007/s003600100230
39. Kontecka H., Książkiewicz J.M., Nogowski L. (1999). Effects of different stressors on laying rate and selected blood indices in reproductive ducks. Journal of Animal and Feed Sciences, 8(1): 63-72, doi:10.22358/jafs/68808/1999
40. Kovács K.J., Péczely P. (1991). Plasma adrenocorticotropin in domestic geese: effects of ether stress and endocrine manipulations. Gen Comp Endocrinol., 84(2): 192-8, doi: 10.1016/0016-6480(91)90042-5
41. Le Ninan F., Cherel Y., Sardet C., Le Maho Y. (1988). Plasma hormone levels in relation to lipid and protein metabolism during prolonged fasting in king penguin chicks. Gen Comp Endocrinol., 71(2): 331-7, doi: 10.1016/0016-6480(88)90261-4
42. Ling M.K., Hotta E., Kilianova Z., Haitina T., Ringholm A., Johansson L., Gallo-Payet N., Takeuchi S., Schiöth H.B. (2004). The melanocortin receptor subtypes in chicken have high preference to ACTH-derived peptides. Br J Pharmacol., 143(5): 626-37, doi: 10.1038/sj.bjp.0705900
43. Ling M.K., Schiöth H.B. (2005). Subtype-specific pharmacological properties of the melanocortin receptors in chicken. Ann N Y Acad Sci., 1040: 378-80, doi: 10.1196/annals.1327.067
44. Livak K.J., Schmittgen T. D. (2001) . Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods, Dec; 25(4): 402-408, doi: 10.1006/meth.2001.1262

45. Mahmoud K.Z., Edens F.W. (2012). Breeder age affects small intestine development of broiler chicks with immediate or delayed access to feed. British poultry science, 53(1), 32-41, doi:10.1080/00071668.2011.652596
46. Mehaisen G.M., Eshak M.G., Elkaiaty A.M., Atta A.M., Mashaly M.M., Abass A.O. (2017). Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. PLoS One, 12(2):e0172684, doi: 10.1371/journal.pone.0172684
47. Mikami S. (1986). Immunocytochemistry of the avian hypothalamus and adenohypophysis. Int Rev Cytol., 103: 189-248, doi: 10.1016/s0074-7696(08)60836-0
48. Miller W.L. (1988) Molecular biology of steroid hormone synthesis. Endocr Rev., 9(3): 295-318, doi: 10.1210/edrv-9-3-295
49. Mountjoy K.G., Robbins L.S., Mortrud M.T., Cone R.D. (1992). The cloning of a family of genes that encode the melanocortin receptors. Science, 257(5074): 1248-51, doi: 10.1126/science.1325670
50. Nebert D.W., Nelson D.R., Coon M.J., Estabrook R.W., Feyereisen R., Fujii-Kuriyama Y., Gonzalez F.J., Guengerich F.P., Gunsalus I.C., Johnson E.F. (1991). The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. DNA Cell Biol., 10(1): 1-14, doi: 10.1089/dna.1991.10.1
51. Nelson J.R., Sobotik E.B., Athrey G., Archer G.S. (2020). Effects of supplementing yeast fermentate in the feed or drinking water on stress susceptibility, plasma chemistry, cytokine levels, antioxidant status, and stress- and immune-related gene expression of broiler chickens. Poult Sci., 99(7): 3312-3318, doi: 10.1016/j.psj.2020.03.037
52. Neuman-Lee L.A., Hudson S.B., Webb A.C., French, S. S. (2020). Investigating the relationship between corticosterone and glucose in a reptile. The Journal of experimental biology, 223(2): jeb203885. https://doi.org/10.1242/jeb.203885
53. Nir I., Yam D., Perek M. (1975). Effects of stress on the corticosterone content of the blood plasma and adrenal gland of intact and bursectomized Gallus domesticus. Poult Sci., 54(6): 2101-10, doi: 10.3382/ps.0542101
54. Paster M.B. (1991). Avian reproductive endocrinology. Vet Clin North Am Small Anim Pract., 21(6): 1343-59, doi: 10.1016/s0195-5616(91)50143-1
55. Post J., Rebel J.M., ter Huurne A.A. (2003). Physiological effects of elevated plasma corticosterone concentrations in broiler chickens. An alternative means by which to assess the physiological effects of stress. Poult Sci., 82(8): 1313-8, doi: 10.1093/ps/82.8.1313
56. Radke W.J., Albasi C.M., Harvey S. (1985). Stress and ACTH stimulated aldosterone secretion in the fowl (Gallus domesticus). Comp. Biochem. Physiol., A82: 285-288, doi: 10.1016/0300-9629(85)90855-2
57. Romero L.M., Wingfield J.C. (1998). Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's white-crowned sparrows (Zonotrichia leucophrys gambelii). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol., 119(1): 31-6, doi: 10.1016/s0742-8413(97)00167-9
58. Sapolsky R.M., Krey L.C., McEwen B.S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev., 7(3): 284-301, doi: 10.1210/edrv-7-3-284
59. Scanes C.G., Harvey S., Chadwick A., Gales L., Newcomer W.S. (1980a). Diurnal variations in serum luteinizing hormone, growth hormone, and prolactin concentrations in intact and

pinealectomised chickens. Gen Comp Endocrinol., 41(2): 266-9, doi: 10.1016/0016-6480(80)90153-7

60. Scanes C.G., Merrill G.F., Ford R., Mauser P., Horowitz C. (1980b). Effects of stress (hypoglycaemia, endotoxin, and ether) on the peripheral circulating concentration of corticosterone in the domestic fowl (Gallus domesticus). Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 66(2), 183-186, doi.org/10.1016/0306-4492(80)90123-9
61. Scanes C.G. (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poult Sci., 95(9): 2208-15, doi: 10.3382/ps/pew137
62. Scanes C.G. (2020). Avian Physiology: Are Birds Simply Feathered Mammals? Front Physiol., 11: 542466, doi: 10.3389/fphys.2020.542466
63. Schiöth H.B. (2001). The physiological role of melanocortin receptors. Vitam Horm., 63: 195-232, doi: 10.1016/s0083-6729(01)63007-3
64. Spencer K.A., Evans N.P., Monaghan P. (2009). Postnatal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. Endocrinology, 150(4): 1931-4, doi: 10.1210/en.2008-1471
65. Takeuchi S., Kudo T., Takahashi S. (1998). Molecular cloning of the chicken melanocortin 2 (ACTH)-receptor gene. Biochim Biophys Acta., 1403(1): 102-8, doi: 10.1016/s0167-4889(98)00022-6
66. Takeuchi S., Takahashi S. (1998). Melanocortin receptor genes in the chicken-tissue distributions. Gen Comp Endocrinol., 112(2): 220-31, doi: 10.1006/gcen.1998.7167
67. Vinson G.P., Whitehouse B.J., Goddard C., Sibley C.P. (1979). Comparative and evolutionary aspects of aldosterone secretion and zona glomerulosa function. J Endocrinol., 81(2): 5P-24P. PMID: 222871
68. Virden W.S., Thaxton J.P., Corzo A., Dozier W.A., 3rd, Kidd M.T. (2007a). Evaluation of models using corticosterone and adrenocorticotropin to induce conditions mimicking physiological stress in commercial broilers. Poult Sci., 86(12): 2485-91, doi: 10.3382/ps.2006-00215
69. Virden W.S., Lilburn M.S., Thaxton J.P., Corzo A., Hoehler D., Kidd MT. (2007b). The effect of corticosterone-induced stress on amino acid digestibility in Ross broilers. Poult Sci., 86(2): 338-42, doi: 10.1093/ps/86.2.338
70. Walker B.G., Wingfield J.C., Boersma P.D. (2005). Age and food deprivation affects expression of the glucocorticosteroid stress response in Magellanic penguin (Spheniscus magellanicus) chicks. Physiol Biochem Zool., 78(1): 78-89, doi: 10.1086/422769
71. Wall J.P., Cockrem J.F. (2009). Effects of corticosterone treatment on responses to fasting in Japanese quail. Comp Biochem Physiol A Mol Integr Physiol., 154(2): 211-5, doi: 10.1016/j.cbpa.2009.06.006
72. Wall J.P., Cockrem J.F. (2010). Effects of corticosterone treatment in laying Japanese quail. Br Poult Sci., 51(2): 278-88, doi: 10.1080/00071661003745828
73. Wang J.S., Hu H.J., Xu Y.B., Wang D.C., Jiang L., Li K.X., Wang Y.Y., Zhan X.A. (2020). Effects of posthatch feed deprivation on residual yolk absorption, macronutrients synthesis, and organ development in broiler chicks. Poult Sci., 99(11): 5587-5597, doi: 10.1016/j.psj.2020.08.032

74. Weber H., Kocsis J.F., Lauterio T.J., Carsia R.V. (1990). Dietary protein restriction stress and adrenocortical function: evidence for transient and long-term induction of enhanced cellular function. Endocrinology, 127(6): 3138-50, doi: 10.1210/endo-127-6-3138
75. Webster A.B. (2003). Physiology and behavior of the hen during induced molt. Poult Sci., 82(6): 992-1002, doi: 10.1093/ps/82.6.992
76. Yang J., Liu L., Sheikhahmadi A., Wang Y., Li C., Jiao H., Lin H., Song Z. (2015). Effects of corticosterone and dietary energy on immune function of broiler chickens. PLoS One, 10(3):e0119750, doi: 10.1371/journal.pone.0119750
77. Yuan L., Lin H., Jiang K.J., Jiao H.C., Song Z.G. (2008). Corticosterone administration and high-energy feed results in enhanced fat accumulation and insulin resistance in broiler chickens. Br Poult Sci., 49(4): 487-95, doi: 10.1080/00071660802251731