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STRESS RESPONSE DYNAMICS IN ACTH AND FORMALIN TREATED CHICKENS

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Abstract

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This study was conducted with fifteen chickens (White Plymouth rock - pure initial line from the National Genetic Resource) at the age of seven weeks which were randomly allocated into three groups: I experimental group- injected (i.m.) with 1-24 ACTH (Synacthen Depot)., 8 $\mu g.kg^{-1}$B.W.; II experimental group- injected with 0.5 ml formalin (15% formaldehyde); III group (control) - injected with 0.5 ml 0.9% NaCl. They were raised in cages (5 chicks per cage). The chickens were injected at 9h a.m after 2 h period of fasting. Blood samples were obtained by venipuncture of wing vein at 1h, 3h, 5h and 24h after the injection. The objective of this study was to establish: 1).Is ACTH the main secretagogue of the adrenal glands in chickens and 2).The effect of formalin on adrenal glands in chickens. Plama corticosterone (CS) level increased 7-fold in ACTH-treated birds ($P<0.05$) at 1h after the injection while in formalin treated birds it increased only 2-fold. At 3h after the injection there was a sharp drop in corticosterone level in ACTH treated group, ($P<0.05$) , but in formalin treated chickens the values were higher ($P<0.05$) compared to those in ACTH treated chickens. Heterophil-to-lymphocyte (H:L) ratio rose at 3h ($P<0.01$) and 5h ($P<0.05$) in ACTH treated chickens, while in formalin treated chickens tended to be higher ($P>0.05$) at 5h following the injection. Hematocrit (at 1h) and erythrocyte numbers (at 3h) following the injection were elevated ($P<0.05$) in both groups. Leukocyte numbers increased significantly ($P<0.05$) at 1h in formalin treated chickens and tended to be higher ($P>0.05$) in ACTH treated birds. ACTH did not cause any significant elevation in Il-1α concentration in both groups. However, Interleukin-1α level in the formalin treated chickens tended to be higher at 1h ($P>0.05$) and was significantly lower at 24 following the injection. Plasma cholesterol levels increased significantly at 1h and returned to baseline levels at 3h after the injection of ACTH. Formalin treatment caused insignificant decline of cholesterol levels at 3h and 5h after the injection. Urea levels were significantly elevated in ACTH treated chickens at 1h ($P<0.01$), 3h ($P<0.01$) and 5h ($P<0.05$) and in formalin treated chickens at 24h following the injection. Plasma glucose levels were significantly elevated both in ACTH ($P<0.001$) and formalin ($P<0.001$) treated chickens throughout the experimental period. In conclusion: Adrenal responsiveness to ACTH and the negative feedback in chickens is similar to that in mammals. Formalin is less potent stimulator of adrenal glands than ACTH and exerts a definite effect on leukocyte numbers and Il-1α level.

*Key words***:** ACTH, formalin, stress, H: L ratio, IL-1α, white blood cells, corticosterone, chickens)

Introduction

Adrenocorticotropic hormone (ACTH, corticotrophin) is a 39 amino acid peptide with a common 1-24 N-terminal sequence in both mammals and birds, which is sufficient to elicit its biological activity.ACTH is secreted from the pituitary corticotrope cells upon excision by specific endoprotease from a multifunctional precursor protein, the propiomelanocortin (POMC), which primary structure has been established in chickens (Gerets et al., 2000). The secretion of ACTH into the general circulation results in the biosynthesis and release of corticosteroids within a few minutes in the adrenal glands. Despite a direct relationship between ACTH and corticosterone release, adrenal steroidogenesis is also under direct and indirect negative feedback and may be partially under extra-hypophyseal controls (Mormede et al., 2007). Hypophysectomy does not result in decrease in plasma levels of corticosterone in turkey (Brown, 1960) or causes partial one in quails (Bayle and Assenmacher, 1969). Puvadolpirod and Thaxton (2000) have demonstrated a delayed effect of ACTH on H/L ratio in birds as compared to that in mammals.Various physical stressors generally elicit adrenal responses, with large variability between species in terms of responses to comparable physical stressors and to ACTH. There is no accepted route, dosage or duration of treatment for administration of ACTH to evoke a given set of stress response in a predictable temporal pattern in poultry species.

Subcutaneous injection of formalin is an excellent tool to investigate pain-evoked stress response (Abbott et al., 1997). A small volume (0.015–0.2 ml/100 g body weight in rats) of 1–5% formalin injected subcutaneously into the paw elevates plasma ACTH, corticosterone, and catecholamine levels immediately and induces marked CRH expression in the PVN (Pacak et al., 1998; Taylor et al., 1998). Some data (Aloisi et al., 1996) indicate that in rats formalin stimulates ACTH, but not corticosterone, while in human it stimulates both. Formalin administration induces an acute phase of nociception (up to 1–5 min) followed by a second stage, termed "tonic "(inflammatory), which lasts for about 1 h (Dickenson and Sullivan, 1987) A full fledged systemic inflammatory reaction results in stimulation of four major programs- the acute phase, the sickness syndrome, the pain program and the stress response, mediated by hypothalamic pituitary-adrenal axis and the sympathetic nervous system (Elenkov et al., 2005). Pain produced by the injection of formalin results from persistent tissue damage and, thus, more closely resembles clinical pain conditions (Tjolsen et al., 1992). It is still not clear whether the nociceptive component of the formalin stimulus (independent of other components such as arousal, the stress associated with handling, and stimulation of high-threshold primary somatosensory afferents) is an adequate stimulus of the pituitary-adrenal system. Despite all available data, however it is not clear whether stress response in birds is similar to that in mammals.

The objective of this study was to establish: 1). Is ACTH the main secretagogue of the adrenal glands in chickens and 2).The effect of formalin on adrenal glands in chickens.

Material and Methods

The experiment was conducted with fifteen chickens (White Plymouth rock - pure initial line from the National Genetic Resource) at the age of seven weeks which were randomly allocated into three groups: I experimental group- injected (i.m.) with 1-24 ACTH (Synacthen Depot)., 8 $\mu g.kg^{-1}$B.W.; II experimental group- injected with 0.5 ml formalin (15% formaldehyde); III group (control)- injected with 0.5 ml 0.9% NaCl, and were raised in cages (5 chicks per cage). All chickens were allowed 1 week to adapt to their experimental conditions before experimentation began. Both control and experimental chickens were fed *ad libitum* on a diet adequate to support chickens growth. During the preliminary period the chickens were raised in stress free conditions. ACTH, formalin and NaCl were injected at 9h a.m after 2 h period of fasting. Blood samples from all birds were obtained by venipuncture of wing vein at 1h, 3h, 5h and 24h after the injection.

Plasma glucose level was determined by the method of Ceriotti as modified by Profirov (1990) and plasma total cholesterol and urea levels were measured by the methods of Watson (1960) and Rerat et al. (1979), respectively.

Plasma corticosterone and Intelreukin-1α were determined using enzyme immunoassay kits (IBL, Gesellschaft fur immunchemie und immunbiologie, MBH, D 22335 Hamburg, Germany).

Hematocrit, leukocytes and erythrocyte numbers were determined by the classical methods of Ibrishimov and Lalov (1984). Peripheral blood leukocytes subpopulations were counted microscopically in smears (Giemsa-Romanovsky-stain) made immediately after each venipuncture at 1h, 3h, 5h and 24 aftrer the injections.

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Results and Discussion

Plama corticosterone (CS) level increased 7 fold in ACTH-treated birds ($P<0.05$) at 1h after the injection compared to control group, while in formalin treated bird the increase was 2-fold (Figure 1). These results indicate that ACTH in chickens is as powerful secretagogue of adrenal glands as in mammals. The present ACTH test results agree with previous data showing that continuous delivery of ACTH (8 UI/kg of BW per d for 7 d) at a rate of 1 μL/h through a miniosmotic pump stimulated CS release (Puvadolpirod and Thaxton, 2000a,b,c,d; Olanrewaju et al., 2006; Lin et al., 2007). The maximal response levels to pharmacological challenge with 1-24 ACTH (Immediate Synacthen, 5-10 $\mu g.kg^{-1}$ BW) was reported to vary according to the applied dose from 20 to 350 ng/ mL plasma (Puvadolpirod and Thaxton, 2000 a,b,c; Mormede et al., 2007). It is north worthy that plasma corticosterone levels declined sharply by the 3th and 5th h following the ACTH injection to values that were lower than baseline levels which could be explained with the existence of strong negative feedback exerted by corticosterone at the level of pituitary or hypothalamus. These results indicate that corticosterone feedback in chickens is similar to that in mammals. Our results are consistent with those of Wilckens (1995) and show that the increased plasma corticosterone levels by 1h after the injection exerted steadfast negative feedback on corticosterone secretagogues by 3h and 5h following the injection. Formalin caused slight but significant ($P<0.05$) increase in plasma corticosterone level by 1h followed by a decline at 3h and maintenance of corticosterone level within the normal range by 5h and 24h after the injection. Formalin treatment unlike ACTH treatment caused slight increase in plasma corticosterone level and therefore it is reasonable to assume that the negative feedback effect of corticosterone on its secretagogues was less pronounced than in ACTH treated chickens. This interpretation is in agreement with the higher corticosterone levels by 3h and 5h in formalin treated chickens. The extremely high level of corticosterone by 1h in ACTH treated chickens may have caused exhaustion of adrenal glands which combined with the negative feedback could explain the observed corticosterone decline by 3h and 5h after the injection. Formalin unlike ACTH exerts its effect on adrenal glands through its stimulatory effect on hypothalamic neurons, which release corticotrophin releasing hormone. Therefore the lack of sizable adrenal response in formalin treated chickens could be due either to insignificant stimulation of CRH release or to diminished response of pituitary corticotrophs. Re-

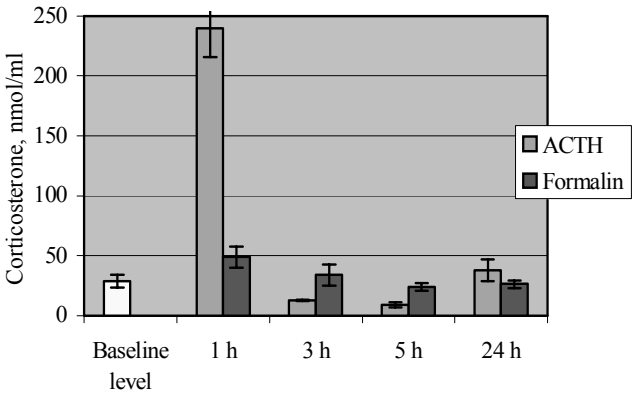

Fig. 1. Dynamics of plasma corticosterone levels in ACTH and formalin treated chickens

cently Liu et al. (2007) reported rapid inhibitory effect of corticosterone on histamine release from rat peritoneal mast cells. Thus higher corticosterone level in ACTH treated chickens might have prevented the release of histamine. However, histamine release in formalin treated chickens was probably not inhibited because of the lower plasma corticosterone level. Recently it was demonstrated that brain histamine in mice with formalin-induced pain response produces antinociception (Tamaddonfard and Rahmi, 2004).

Heterophil-to-lymphocyte ratio, which is widely used as a reliable criterion of adrenal activity, is in agreement with the observed corticosterone levels in ACTH and formalin treated chickens respectively (Figure 2). Our findings correspond to those reported by Puvadolpirod and Thaxton (2000) who found an increase of H: L ratio in ACTH infused chicks. Formalin injection is known to cause release of histamine, which is one of the major mediators of acute inflammation and allergic reactions (Elenkov et al., 2005). Formalin evoked pain and inflammation could activate the stress axis by increasing leukocytes production of cytokines. Probably Interleukins (Figure 6) stimulated the realease of CRH in order to suppress the immune system overshooting.

Hematocrit (Figure 3) levels were elevated ($P<0.05$) in both groups of chickens at 1h after the injection. This was accompanied with insignificant increase of erythrocyte numbers by 1h and further enhancement ($P<0.05$) by 3h after the injection relative to control values (Figure 4). The observed higher hematocrit values (Figure 3) could occur as a result of increased metabolism, leading to an increase in erythropoiesis (Figure 4) as a compensatory reaction to the lack of sufficient oxygen in the tissues (Olanrevaju et al., 2007), possibly because of an impaired oxygen-carrying capacity in the blood.

Leukocyte numbers in formalin treated unlike ACTH treated chickens increased significantly ($P<0.05$) at 1h following the injection relative to control level (Figure 5). It can be explained by the fact that inflammation (edema) is a prominent feature accompanying the painful response and that in general leukocyte migration to inflammatory area is a usual component of inflammation following the formalin injection and contributes to it. It is obvious that the increase of leukocyte numbers in formalin treated chickens was not due to corticosterone and was most probably mediated by histamine mediated recruitment of some substrates (SP, NK A, and NK B, PAF) on the inflammatory site. The very fact that the increased leukocyte numbers at 1h coincided with elevation of interleukin-1α gives further support to our assumption that the stimulatory effect of formalin on adrenal activity was mediated by Il-1α stimulated release of CRH.

Adrenocorticotropic hormone had no effect on Il-1α by 1st h but inhibited Il-1α by 3h after the injection (Figure 6). Interleukin-1α in formalin treated chickens tended to be higher by 1st h in comparison

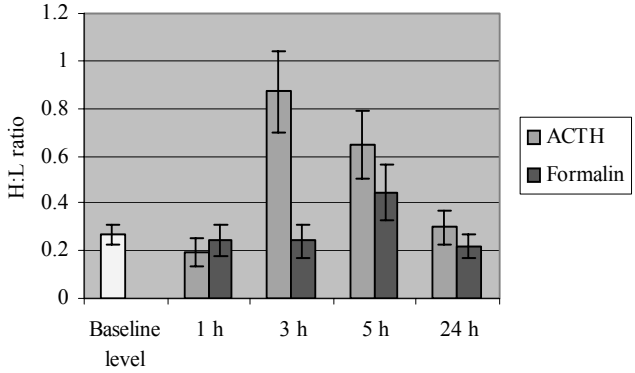

Fig. 2. Dynamics of heterophil-to-lymphocyte ratios in ACTH and formalin treated chickens

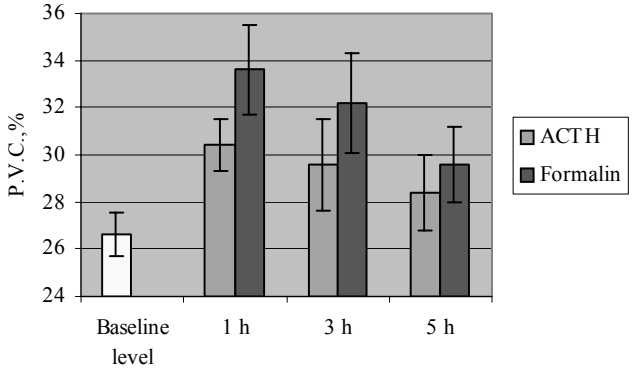

Fig. 3. Dynamics of blood P.V.C. levels in ACTH and formalin treated chickens

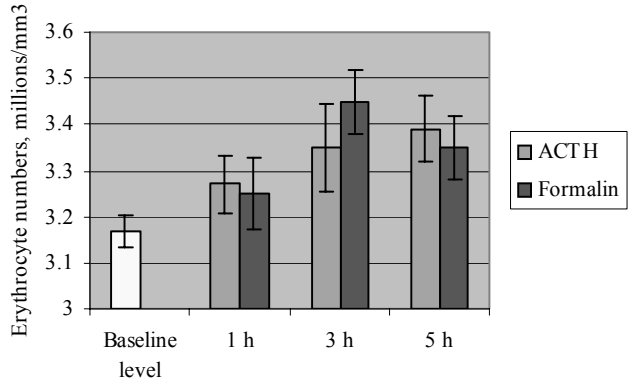

Fig. 4. Dynamics of blood erythrocyte numbers in ACTH and formalin treated chickens

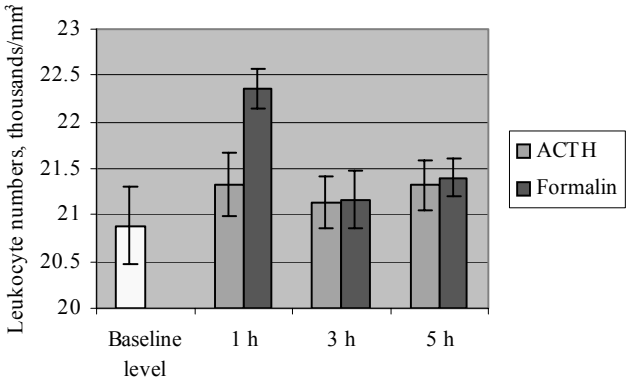

Fig. 5. Dynamics of blood leukocyte numbers in ACTH and formalin treated chickens

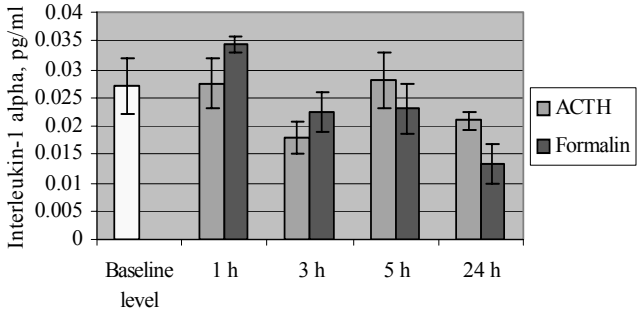

Fig. 6. Dynamics of plasma Interleukin-1α in ACTH and formalin treated chickens

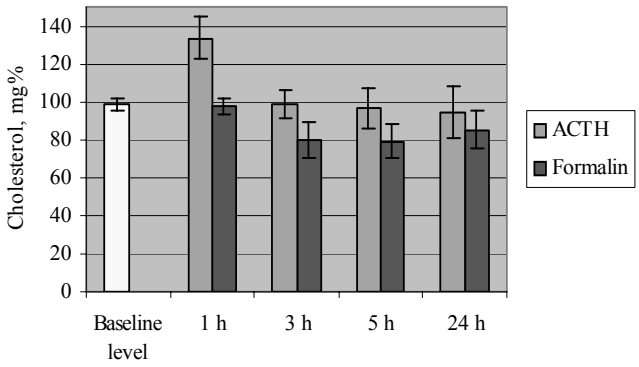

Fig.7. Dynamics of plasma cholesterol levels in ACTH and formalin treated chickens

with baseline level. The observed elevation of Il-1α was probably due to histamine produced at the injection sites and its stimulatory effect on proinflamatory cytokines. Cytokines are known to stimulate hypothalamic CRH release, which in turn leads to pituitary adrenocorticotrophic (ACTH) hormone secretion into peripheral circulation (O'Connor et al., 2000). This adrenocorticosteroid response controls the immune response by inhibiting further cytokine production in experimental chickens.

Cholesterol level in ACTH treated chickens increased at 1h ($P<0.05$) and then returned to the baseline level at 3h after the injection (Figure 7). Cholesterol was accepted like a promising indicator of stress in chickens (Puvadolpirod and Thaxton, 2000b). Plasma cholesterol level in formalin treated chickens was not influenced at 1h following the injection but tended to be lower during the rest of the experimental period. It is important to note that the elevated cholesterol level in ACTH treated chickens at 1h after the injection coincided with the significantly elevated corticosterone level.

Urea levels in ACTH treated chickens (Figure 8) were elevated at 1h ($P<0.01$), 3h ($P<0.01$) and 5h ($P<0.05$) and tended to be higher at 24 h following the injection. Plasma urea levels in formalin treated chickens were even higher ($P>0.05$) and reached significant values at 24h after the injection relative to the baseline level. This finding is consistant with the results of Til et al. (1989) and Tobe et al. (1989) who found high blood urea and creatinine in rats given forlamlehyde containing water to drink. Another study conducted in Fresian heifers given casein or soybean meals treated with formalin revealed also an increased plasma concentration of urea (Oddham et. al., 1982). Khan et al. (2006) recently reported that urea level

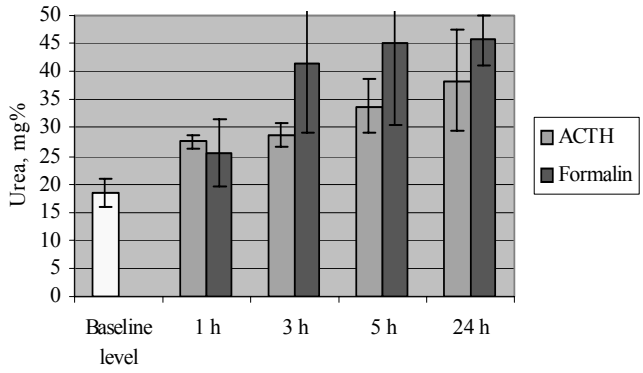

Fig. 8. Dynamics of plasma urea levels in ACTH and formalin treated chikens

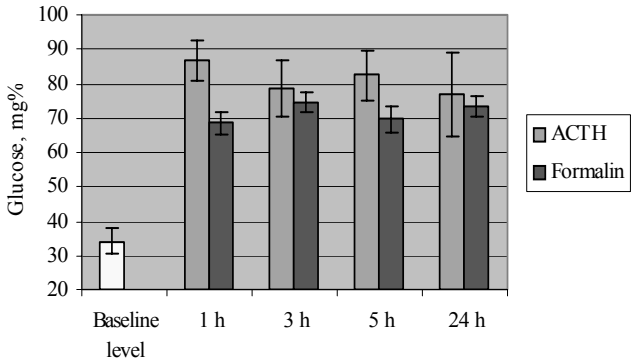

Fig. 9. Dynamics of plasma glucose levels in ACTH and formalin treated chickens

was significantly ($P<0.001$) elevated in birds (White Leghorn cockerels) fed higher formalin levels. It is clear that the elevated urea level in formalin treated chickens throughout the experimental period was not due to corticosterone since its level remained within the normal range during the same period (3-14 h). Most probably formalin-induced increase of urea was mediated by histamine produced at the site of injection.

Plasma glucose level (Figure 9) was significantly elevated in ACTH ($P<0.001$) and formalin ($P<0.001$) treated chickens compared to baseline level. Blood glucose levels increased rapidly, and remained high throughout the experimental period despite the short time elevation of corticosterone level. Consequently, the increased glucose level in our case could be due to cytokines-induced insuline resistance in liver and muscle in animals under stress (Marik and Raghavan, 2004). According to an alternative explanation presented by Black et al. (1982) stress-induced glucose mobilization from existing stores is followed by a rapid insulin resistance which inhibits its further storage.

Conclusion

- Adrenal response to ACTH and the negative feedback control exerted by corticosterone in chickens are similar to those in mammals.

- Formalin is less potent stimulator of adrenal glands than ACTH.

References

Abbott, F. V., Y. Hong and P. Blier, 1997 Persisting sensitization of the behavioural response to formalin- induced injury in the rat through activation of serotonin2A receptors. *Neuroscience,* **77**: 575–584.

Aloisi, A. M., M. E. Albonetti and G. Carli, 1996. Formalin-induced changes in adrenocorticotropic hormone and corticosterone plasma levels and hippocampal choline acetyltransferase activity in male and female rats. *Neuroscience*, **74**: 1019-1024.

Bayle, J. D. and I. Assenmacher, 1969. Hypothalamo-hypophyseal regulation of adrenal cortex and thyroid function in quail. *Bull Biol. Fr.Belg.* , **103**: 245-260.

Black, P. R., D. C. Brooks, P. Q. Bessey, R. R. Wolfe and D. W. Wilmore,1982. Mechanisms of insulin resistance following injury. *Ann. Surg.* , **196**: 420–435.

Brown, K. I., 1960. Response of turkey adrenals to ACTH and stress as measured by plasma corticosterone. *Anat. Rec.,* **137**: 344.

Brown, M., L. Fisher, J. Spiess, C. Rivier, J. Rivier and W. Vale, 1982. CRF: actions on the sympathetic nervous system and metabolism. *Endocrinology,* **111**: 928–931.

Dickenson, A. H. and A. F. Sullivan, 1987. Subcutaneous formalin-induced activity of dorsal horn

neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. *Pain,* **30**: 349–360.

Elenkov, I., D. Iezonni, A. Dally, A. Harris and G. Groussos, 2005. Cytokine dysregulation, inflammation and well-being. *Neuroimmunoregulation*, **12**: 255-269.

Gerets, H. H., K. Peetrs, L. Arckens, F. Vandesande and L. R. Bergman, 2000. Sequence and distribution of pro-opiomelanocortin in the pituitary and the brain of the chicken (*Gallus gallus*). *J. Comp. Neur*., **417**: 25-262.

Ibrishimov, N. and H. Lalov, 1984. Clinical and laboratory investigations in veterinary medicine. Publisher "*Zemizdat",* Sofia (Bg).

Khan, S., M. Hussain and Z. Khan, 2006. Effect of formalin feeding or administered into crops of White leghorn cockerels on hematological and biochemical parameters. *Poultry Science,* **85**: 1513-1519.

Liu, C., J. Zhou, L. D. Zhang, Y. X. Wang, Z. M. Kang, Y. Z.Chen and C. L. Jiang, 2007. Rapid inhibitory effect of corticosterone on histamine release from rat peritoneal mast cells. *Horm. Metab. Es.,* **39** (4): 273-277.

Marik, P. E. and M. Raghavan, 2004. Stress–hyperglicemia, insulin and immunomodulation in sepsis. *Intensive Care Med*., **30**: 748-756.

Mechanick, J. I., 2006. Metabolic mechanisms of stress hyperglycemia. *Journal of Parenteral and Enteral Nutrition*, **30**: 157-163.

Mormede, P., S. Andanson, B. Auperin, B. Beerda, D. Guemene, J. Malmkvist, X. Manteka, G. Manteuffel, P. Prunet, C. G. van Reenen, S. Richard and I. Veisier, 2007. Explorations of hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiology&Behaviour, **92**: 317-339.

O'Connor, T. M., D. J. O'Halloran and F. Shanahan, 2000. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *Q.J.Med.,***93**: 323-333.

Oddham, J. D., I. C. Hart and J. A. Jines, 1982. Formaldehyde-trested proteins for dairy cows: Effect on blood hormone concentrations. *Br. J. Nutr*., **48**: 543-547.

Olanrewaju, H. A., J. P. Thaxton, W. A. Dozier and S. L. Branton, 2007. Electrolyte Diets, Stress, and Acid-Base Balance in Broiler Chickens.*Poult Sci.,* **86**: 1363-1371.

Olanrewaju, H. A., S. Wongpichet, J. P. Thaxton, W. A. Dozier and S. L. Branton, 2006. Stress and acid-base balance in chickens. *Poult. Sci*., **85**: 1266–1274.

Pacak, K., M. Palkovits, G. Yadid, R. Kvetnansky, I. J. Kopin and D. S. Goldstein, 1998. Heterogeneous neurochemical responses to different stressors: a test of Selye's doctrine of nonspecificity. *Am J Physiol.,* **275**: R1247–R1255.

Profirov, Y., 1990. Amilolytic activity in the digestive tract of lambs during the first months after birth. *Arch. Anim. Nutr.,* **40**: 232-244.

Puvadolpirod, S. and J. P. Thaxton, 2000a. Model of physiological stress in chickens. 1. Response Parameters. *Poultry Science*, **79**: 363-369.

Puvadolpirod, S. and J. P. Thaxton, 2000b. Model of physiological stress in chickens. 2. Dosimetry of adrenocorticotropin. *Poult. Sci*., **79**: 370–376.

Puvadolpirod, S. and J. P. Thaxton, 2000c. Model of physiological stress in chickens. 3. Temporal patterns of response. *Poult. Sci*., **79**: 377–382.

Puvadolpirod, S. and J. P. Thaxton, 2000d. Model of physiological stress in chickens. 4. Digestion and metabolism. *Poult. Sci*., **79:** 383–390.

Rerat, A., C. Lisoprawski and P. Vaissade, 1979. Metabolisme de l'uree dans le tube digestive du pore: donnee preliminaires et qualitatives et quantitative. *Bull. Acad. Vet*., **52**: 333-346.

Tamaddonfard, E. and S. Rahmi, 2004. Central effect of histamine and peripheral effect of histidine on the formalin-induced pain response in mice. *Clinical and Experimental Pharmacology and Physiology*, **31**: 518-522.

Taylor, B.K., S.F. Akana, M.A. Peterson, M.F. Dallman, A.I. Basbaum 1998 Pituitary-adrenocortical responses to persistent noxious stimuli in the awake rat: endogenous corticosterone does not reduce nociception in the formalin test. Endocrinology, **139**: 2407–2413

Til, H. P., R. A. Woutersen, V. J. Feron, V. H. Hollanders, H. E. Falke and J. J. Clary, 1989. Two-year drinking-water study of formaldehyde in rats. *Food Chem. Toxic.,* **27**: 77-87.

Tjolsen, A., O. G. Berge, S. Hunskaar, J. H. Rosland and K. Hole, 1992. The formalin test: an evaluation of the method. *Pain,* **51**: 5–17.

Tobe, M., K. Naito and Y. Kurokawa, 1989. Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology,* **56**: 79-86.

Watson, D., 1960. A simple method for determination of serum cholesterol. *Clin. Chem.Acta,* **5**: 637-642.

Wilckens, T., 1995. Glucocorticoids and immune function: physiological relevance and pathogenic potential of hormonal dysfunction. *Trends Pharmacol Sci*, **16**: 193–197.

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