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Original Scientific Article

EFFECT OF THREE-DAY ACTH ADMINISTRATION ON CONCENTRATIONS OF CHOLESTEROL, CORTISOL, PROGESTERONE, TESTOSTERONE AND LH IN THE BOARS

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Summary: The objective of this study was to investigate effects of stress induced by ACTH on cholesterol, cortisol, progesterone, testosterone and LH concentrations in Swedish Landrace boars aged 6 to 7 months during ACTH administration and for 12 days after the cessation of treatment. ACTH treated boars (n=14) were given intravenously 10 mg/kg of ACTH for 3 days. Control group (n=14) received intramuscularly 1 mL of sterile 0.9% saline. The cortisol concentrations were significantly elevated (p<0.0001, all) in all three days of ACTH treatment and day after the last ACTH injection (p<0.01) in treated boars. In twelve days after the cessation of treatment, cortisol levels remained on physiological levels. During all three ACTH treatment days and also one day and five days after the last ACTH dosage, cholesterol concentrations were significantly decreased (p<0.05 to p<0.0001, respectively) in comparison with control boars. Progesterone and testosterone concentrations were significantly increased (p<0.001 to p<0.0001, respectively) during all three days of ACTH treatment. After the treatment there was no significant difference in progesterone and testosterone levels between treated and control boars. ACTH administration had no influence on LH levels in treated boars. Significantly reduced cholesterol concentrations in the serum of boars exposed to three-day ACTH induced stress shows its increased biotransformation, which is confirmed by the results of this paper.

Key words: boars; ACTH; stress; cortisol; hormones; cholesterol

Introduction

Attempts to define stress through the patterns of specific physiological parameters frequently yielded controversial results. In addition to its effects on changes in the secretion of pituitary gland and adrenal gland hormones, stress also affects the testicular and ovarian hormones, which are important for the animal reproduction system. It may have negative effects in certain phases of animal development and breeding and may reduce the reproduction capacities (1).

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Stressful situations such as temperature changes (2), transport (3), mixing of animals from different litters (4), and competition inside groups may affect the growth (5, 6), development of endocrine system (7, 8) and reproduction system functions in pigs (9, 10, 11). Chronic stress in females reduces the secretion of FSH and LH, and affects the absence of LH secretion at the expected time before ovulation (12) and reduction the number of gravid females (9). Acute stress situations cause an increase in progesterone concentrations in the blood of cows (13) and pigs (14), but they have no effect on LH concentrations (15). Chronic stress stimulated by increased temperatures in summer reduces hormone synthesis in ovaries and leads to lower blood concentrations of progesterone (16)

and estradiol, up to 50% compared to the winter period (17). However, although artificially stimulated ejaculation has been found to have stressful impact and to increase the cortisol concentrations, but testosterone concentrations were not changed in boars (18). In a normal ejaculation cycle in pigs including male and female contact, testosterone concentrations were increased (19).

Contemporary studies are applied stress induction by exogenous ACTH or cortisol. Acute stress stimulated by one-time ACTH application significantly increased the cortisol concentrations in 30 to 90 minutes after administration (14, 20, 21). ACTH administration during several days is used in simulations of chronic stress. Three-day stress induction by ACTH administration stimulated biochemical changes in boars during the treatment and in several-week period after its completion (22).

The objective of this study was to evaluate the effects of three-day injections of ACTH on reproduction system hormone concentrations. For that purpose, cortisol concentrations were determined as well progesterone, testosterone and LH concentrations in blood of boars before, during and after artificaly stress induction by ACTH. Also, impact on concentration of cholesterol as the main precursor for steroid hormone synthesis was monitored.

Materials and methods

Experimental animals

Boars (Swedish Landrace, n=28) aged 6 to 7 months with average weight 124.3±5.7 kg were used in the experiment. The boars were housed in individual pens on farms. The stable temperature varied between 9 and 15 °C. Water was available *ad libitum*. The animals were given 3 kg of feed concentrate *per* day containing the following components: crude protein 16.99%, crude fat 5.39%, crude fiber 5.26%, starch 37.96%, sugar 3.02% and ash 5.65%.

Treatment

The boars were randomly assigned to either control (n=14) or ACTH treated (n=14) group. Both groups of animals were handled using restraint with a snare in the procedures of administering saline and ACTH and also in the procedure of blood collection. Accordingly, both groups were introduced to the same stress conditions. Also, in order to condition the animals to handing using restraint both experimental groups were intramuscularly administered 1 mL of sterile 0.9% at 10.00 a.m. on the day before the treatment. Furthermore, on each of the three treatment days (day 2, 3 and 4), ACTH/saline was administered at 10.00 a.m. The ACTH group of boars was injected 10 mg/kg body mass of ACTH (1-39, from porcine pituitary, 80 IU/mg, Sigma-Aldrich Co., USA) into ear vein. Control group of boars were administered 1 mL of sterile 0.9% saline intramuscularly on three consecutive days as a placebo.

Blood sampling

All boars were frequently handled using restraint with a snare and habituated to blood collection procedure to be performed *via* jugular vein on the day before the experimental period and on each day of the experiment 90 min after drug administration (ACTH or saline). Also, blood samples were collected on days 1, 5, 8 and 12 after the treatment (day 5, 9, 12 and 16). Approximately 15 mL of blood was obtained using a sterile syringe (Becton Dickinson S.A., Spain). Blood samples were immediately transferred to tubes for serum samples (SST, DB VacutainerÒ, Preanalytical Solutions Belliver Industrial Estate, UK). Blood samples were subsequently centrifuged at 750 x *g* for 10 min, separated and stored at -20 °C until the analysis.

Hormone analysis

Serum cortisol was determined by radioimmunoassay serum using commercially available radioimmunoassay Coat-A-Count Kit (Diagnostic Products Corp., USA) according to the manufacturer's instructions. The antibodies used against cortisol had the following specificity: cortisol 100%; progesterone 0.02% and aldosterone 0.03%. Samples were quantified in two assays, with average intra- and interassay coefficients of variation of 7.5% and 12.0%, respectively. The detection limit of assay was 0.1 nmol/L.

Serum progesterone was measured by a solid phase radioimmunoassay Coat-A-Count Progesterone (Diagnostic Products Corp., USA), used according to the manufacturer's recommendations. The antibodies used against progesterone

had the following specificity: progesterone 100%; 5α-pregnan-3,20-dione 9.0%; 5α hydroxyprogesterone 3.4%; cortisol 0.03% and testosterone 0.1%. Samples were quantified in two assays, with average intra- and interassay coefficients of variation of 9.2% and 14.0%, respectively. The detection limit of assay was 0.1 nmol/L.

Serum testosterone levels were determined using a Coat-a-Count kit (Diagnostic Products Corp., USA) with a detection limit of 0.15 nmol/L. All samples were analyzed in a two assay with intra- and interassay coefficients of variation of 7.0% and 10.0%, respectively.

Serum LH was measured by homologous double antybody RIA using specific antiserum prepared in rabbits (23). Samples were quantified in two assays, with intra-assay coefficients of variation of 5.3 % and the detection limit of assay was 0.1 μg/L.

Cholesterol analysis

In serum samples all blood chemical measures were analyzed on a Technicon RA-1000 System Spectrophotometer (Technicon® Instruments Corporation, Tarrytown, New York). Blood serum cholesterol levels were measured by the enzymatic endpoint method using a commercial kit (Randox Laboratories Ltd.), yielding intra- and inter-assay coefficients of variation of 4.8% and 7.6%, respectively.

Statistical analysis

The statistical analyses were performed using statistical package 6.1 Statistica® software (StatSoft® Inc., USA). The observations were analyzed by the analysis of variance. The statistical model included the effects of treatment, period, and interaction between period and treatment. Results were expressed as mean ± SE. Differences in cholesterol and hormone concentration between control and ACTH groups of animals were analyzed by Student's *t*-test for independent pairs. The repeated measurements of cortisol, testosterone, progesteron and cholesterol concentration across the time were analyzed using a two-way repeated measures analysis of variance, with the Greenhouse-Geisser adjustment (ε value) to the *p* values to account for any violation of the sphericity assumption. The repeated measures factor was time (day of checking) and the between groups factor was ACTH administration. Probability values ≤0.05 were considered to be statistically significant.

Results

Cholesterol

During all three ACTH treatment days, cholesterol concentrations were significantly decreased ($p<0.01$ to $p<0.0001$, respectively) in treated boars (Fig. 1). The mean concentrations of cholesterol during treatment days were 1.84 ± 0.041 to 1.86 ± 0.042 mmol/L in ACTH group, whereas in control group they were 2.06 ± 0.03 to 2.13 ± 0.06 mmol/L. Also, one day (day 5) and five days (day 9) after the last ACTH dosage, concentrations of cholesterol were significantly lower ($p<0.05$, respectively) in comparison with control animals. However, there were no significant differences in cholesterol concentrations during treatment in control and also in ACTH group by repeated analysis of variance.

Cortisol

Cortisol concentrations were significantly elevated ($p<0.0001$, all) in all three days of treatment in treated boars (Fig. 2). The mean concentrations of cortisol during treatment days were 335.5 ± 25.48 to 412.5 ± 24.74 nmol/L in ACTH group and 36.9 ± 3.49 to 39.2 ± 3.06 nmol/L in control group. One day after the last ACTH dosage (day 5), concentration of cortisol remained elevate (58.2 ± 3.99 nmol/L) and it was significantly higher ($p<0.01$) in comparison with control animals. There was no significant difference on days 9, 12 and 16 after the treatment. In control animals, the cortisol response remained stable throughout the experimental period. During treatment significant differences by repeated analysis of variance for cortisol concentrations in ACTH group were obtained ($\varepsilon = 0.404$, $p<0.001$).

Progesterone

The progesterone pattern of ACTH boars was similar to cortisol pattern (Fig. 3). The concentrations were significantly increased ($p<0.001$ to $p<0.0001$, respectively) during all three days of treatment in ACTH group of boars. The mean con-

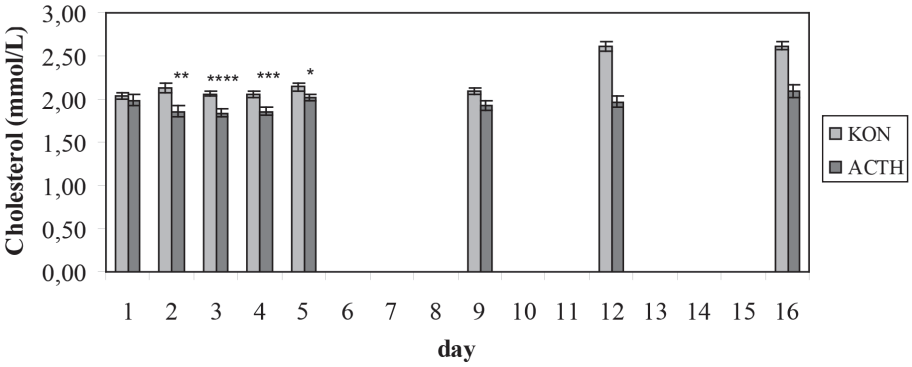

Figure 1: Serum concentrations of cholesterol (mmol/L; mean ± SE) during and after treatment with ACTH in ACTH (n = 14) and control group (KON; n = 14) of boars. Significant differences between groups: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

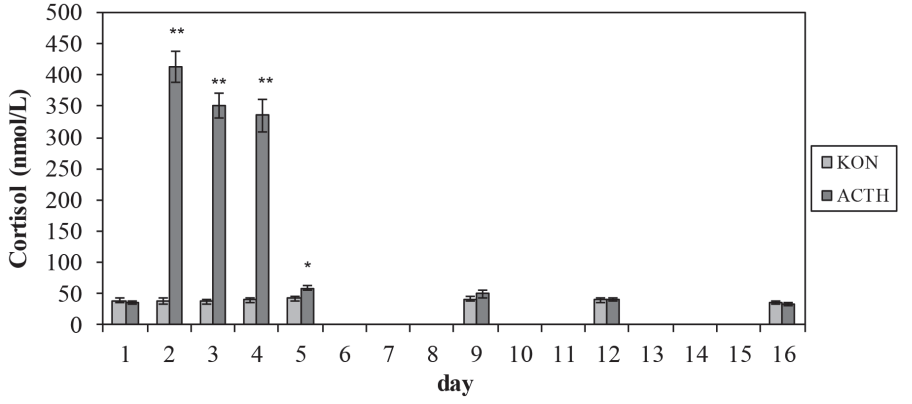

Figure 2: Serum concentrations of cortisol (nmol/L; mean ± SE) during and after treatment with ACTH in ACTH (n = 14) and control group (KON; n = 14) of boars. Significant differences between groups: * $p<0.01$, ** $p<0.0001$

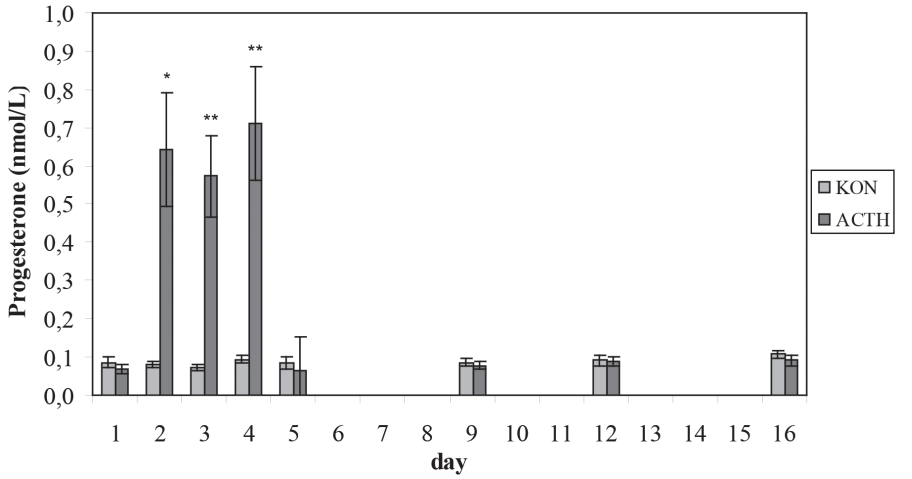

Figure 3: Serum concentrations of progesterone (nmol/L; mean ± SE) during and after treatment with ACTH in ACTH (n = 14) and control group (KON; n = 14) of boars. Significant differences between groups: * $p<0.001$, ** $p<0.0001$

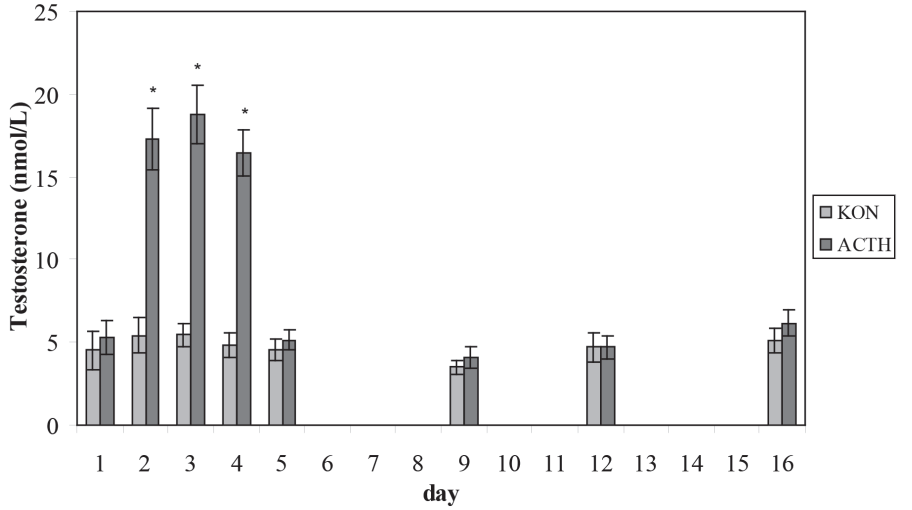

Figure 4: Serum concentrations of testosterone (nmol/L; mean ± SE) during and after treatment with ACTH in ACTH (n = 14) and control group (KON; n = 14) of boars. Significant differences between groups: *$p<0.0001$

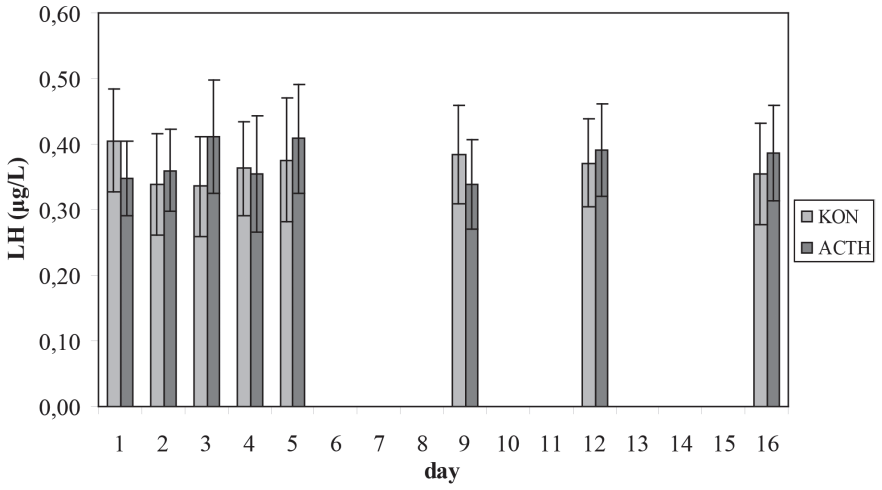

Figure 5: Serum concentrations of LH (mg/L; mean ± SE) during and after treatment with ACTH in ACTH (n = 14) and control group (KON; n = 14) of boars

centrations of progesterone during treatment were 0.57 ± 0.106 to 0.71 ± 0.149 nmol/L in treated boars and 0.072 ± 0.009 to 0.094 ± 0.008 nmol/L in the control group. After the treatment there was no significant difference between the experimental groups of animals. Repeated analysis of variance shown significant differences for progesterone concentrations in group treated with ACTH ($\varepsilon = 0.338$, $p<0.0002$).

Testosterone

Also, testosterone pattern of ACTH boars was similar to cortisol pattern during tretmant, so the concentrations were significantly increased ($p<0.0001$, all) in treated boars (Fig. 4). The mean concentrations of testosterone rose during the treatment from basal levels to the range of 16.5 ± 1.41 to 18.8 ± 1.80 nmol/L and stayed in the range 4.5 ± 1.15 to 5.5 ± 0.69 nmol/L in the control group. After the treatment there was no significant difference between treated and control boars. Repeated analysis of variance shown significant differences for testosterone levels in ACTH group during treatment ($\varepsilon = 0.604$, $p<0.001$).

LH

There was no significant difference in LH concentrations between controls and treated boars during and after ACTH induction (Fig. 5). In both groups of boars mean concentrations were in the range 0.34 ± 0.076 to 0.41 ± 0.086 µg/L. During treatment repeated measures ANOVA showed no significant differences for LH concentrations in both experimental groups.

Discussion

Various experimental models have been applied to explain the effects and consequences of stress on the animals. Different stressful conditions and their duration cause changes in cortisol secretion in pigs (2, 24). Adrenal gland response to ACTH stimulation depends on the intensity and duration of ACTH effects and there is a difference between the acute effects within a minute, and chronic effects which may take hours or days (25). Thus for instance, the levels of cortisol in pig serum is increased by low ambient temperature and reduced by high ambient temperature (2). Repetition of acute stressful situations reduced ACTH and cortisol levels, however at the same time the adrenalin level and heart rate remain increased (26). Chronic stress is changing the basic daily rhythm of ACTH and cortisol secretion in pig serum, which is characterized with an increase between 1.00 a.m. and 7.00 a.m. and fall of concentrations almost by half around 7.00 p.m. (27, 28, 29).

In this study, the application of three-day ACTH stress induction model reduced the individual differences in adrenal gland response in animals during the treatment. During three days of ACTH administration, a significant increase in serum cortisol concentration was observed 90 min after injection (from 34.7 to 415.5 nmol/L). This is consistent with previous reports in female pigs (7, 14, 30) and boars (22, 31). According to the previous results, cortisol concentration remains increased for 24 hours after the last administration of ACTH (22). High cortisol concentrations in serum of young boars were found in stressful situations such as territorial conflict (4) and sexual excitement (18).

This investigation monitored the effects of ACTH induced stress on steroid hormone concentrations and the main precursor of synthesis of steroid hormones of cholesterol. Endocytosis through cell membrane transported the cholesterol from blood into the cellular cytosol and the internal mitochondrial membrane (25). ACTH induces the transfer of cholesterol into the interior of adrenal gland cell mitohondrion, increasing the number of receptors for linking of LDL and HDL lipoproteins on cell membrane and providing the intake of lipoprotein through endocytosis into the cells (32). It also incrases the activity of cholesterol estherase in lisosomes, and cholesterol esthers from LDL or lipid drops are hydrolised into free cholesterol. This increases the quantity of free intracellular cholesterol and reduces cholesterol concentration in circulation (25). Also, ACTH stimulates the desmolase activity, starting the process of steroidogenesys by side-chain cleavage on C_{21} atom, where cholesterol is converted into pregnenolon (25, 33).

In this research, stress induction with ACTH reduced the cholesterol concentrations in boar serum during all three days of treatment. These results show a stimulated synthesis of cortisol and other steroid hormones, which is proven by the results of this study. Cholesterol concentrations in this experiement remained low in the week after ACTH induced stress, while steroid hormone concentrations back to control level. In acute stressful situations, such as temperature change, insignificant changes in cholesterol and cortisol levels were observed in the blood of pigs (2).

Also, in this study, stress induction with ACTH stimulated the synthesis of progesterone and testosterone in boars during ACTH administration. However, the applied stress induction model had no long-term effects on the concentrations of these hormones and by termination of ACTH administration, hormone concentrations returned to their pre-treatment levels. The observed concentrations of progesterone and testosterone in the blood of control group boars were in compliance with the reference values in previous studies (19, 34, 35). However, one-time ACTH administration caused an increase in progesterone concentrations in the serum of male guinea-pigs (36), pigs (14, 20, 37) and castrated boars (38). As in addition to its synthesis by ovaries, progesterone is also synthesized by the adrenal gland, it is deemed that progesterone may represent a significant factor in the interpretation of hormone profile in the animal plasma during stress (14, 39, 40). Namely, in adrenalectomized pigs, there was no increased progesterone synthesis, which shows that progesterone secreted after the ACTH administration originates from the adrenal gland (39).

However, stressful situations with increased cortisol and progesterone also increased the testosterone concentrations in the blood of boars (19, 41) and horses (42). Also, administration of a single ACTH dose in rats (43), rabbits, guinea-pigs (36) and boars (41) at first stimulated the testosterone synthesis. However, several hours after ACTH administration, cortisol concentrations in blood were very high and testosterone concentrations were lower than normal physiological values (36, 41). Double effects of ACTH on testosterone level have not been explained. The assumption is that the initial testosterone secretion increase, found also in this study, is a result of ACTH effects on increased arterial blood flow in the adrenal gland or testicles (44). Also, ACTH administration had no effects on testosterone synthesis if the boars were adrenalectomised (45). The assumption is that the effects on testosterone synthesis are mediated by the adrenal gland, i.e. cortisol acts directly on testicles, by a mechanism independent from hypothalamus-pituitary gland control system (19, 41). Investigations in rats and guinea pig showed that glucocorticoids had a direct inhibition effect on the synthesis of testosterone in Leydig cells (36, 43, 46, 47).

Application of three-day ACTH stress induction model in this study had no changed of LH concentrations in the blood of boars. The results were in compliance with the previous studies of acute stress. Administration of single dose of ACTH in guinea-pigs (36), rats (43, 46) and pigs (12, 15) also had no changed LH serum concentrations. Measured LH concentrations in this research were similar to values determined in previous studies (21, 48). In chronic stress situations caused by ACTH administration during 7 or more days in pigs or rats, the secreted glucocorticoids caused a reduction of LH, FSH and testosterone concentrations in blood (39, 46, 49). It was found that a seven-day ACTH treatment inhibited the increase in basal LH concentration after overiectomy in female pigs only when the adrenal gland was not removed (39).

In conclusion, in boars exposed to three-day ACTH stimulated stress significant reduction of cholesterol concentrations were observed witch is

explained by its increased biotransformation and as result elevated serum concentration of cortisol and also progesterone and testosterone were measured.

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References

1. Grandin T. Transferring results of behavioral research to industry to improve animal welfare on the farm, ranch and slaughter plant. Appl Anim Behav Sci 2003; 81: 215–28.

2. Hicks TA, McGlone JJ, Whisnant CS, Kattesh HG, Norman RL. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J Anim Sci 1998; 76: 474–83.

3. Perremans S, Randall JM, Rombouts G, Decuypere E, Geers R. Effect of whole-body vibration in the vertical axis on cortisol and adrenocorticotropic hormone levels in piglets. J Anim Sci 2001; 79: 975–81.

4. Deguchi E, Akuzawa M. Effects of fighting after grouping on plasma cortisol concentration and lymphocyte blastogenesis of peripheral blood mononuclear cells induced by mitogens in piglets. J Vet Med Sci 1998; 60: 149–53.

5. Collin A, van Milgen J, Le Dividich J. Modelling the effects of high temperature on food intake in young growing pigs. Anim Sci 2000; 72: 519–27.

6. Henessey DP, Stelmasiak T, Johnson NE. Consistent capacity for adrenocortical response to ACTH administration in pigs. Am J Vet Res 1988; 49: 1276–83.

7. Haussmann MF, Caroll JA, Weesner GD, Daniels MJ, Matteri RL, Lay DCJr. Administration of ACTH to restrained, pregnant sows alters their pigs' hypothalamic-pituitary-adrenal (HPA) axis. J Anim Sci 2000; 78: 2399–2411.

8. Williams MT, Davis HN, McCrea AE, Hennessy MB. Stress during pregnancy alters the offspring hypothalamic, pituitary, adrenal, and testicular response to isolation on the day of weaning. Neurotoxicol Teratol 1999; 21: 653–59.

9. Barnett JL, Hemsworth PH. The effects of individual and group housing on sexual behaviour and pregnancy in pigs. Anim Reprod Sci 1991; 25: 265–73.

10. Einarsson S, Madej A, Tsuma V. The influence of stress on early pregnancy in the pig. Anim Reprod Sci 1996; 42: 165–72.

11. Hemsworth PH, Cronin GM, Hansen C, Winfield CG. The effects of two oestrus detection procedures and intense boar stimulation near the time of oestrus on mating efficiency. Appl Anim Behav Sci 1984; 12: 339–47.

12. Ziecik AJ, Zieminska A. Effect of transport stress and hydrocortisone on the oestrogen-induced luteinizing hormone surge in pigs. Anim Reprod Sci 1995; 37: 337–47.

13. Copper C, Evans ACO, Cook S, Rawlings NC. Cortisol, progesterone and β-endorphin response to stress in calves. Can J Anim Sci 1995; 75: 197–201.

14. Tsuma VT, Einarsson S, Madaj A, Forsberg M, Lundeheim N. Plasma levels of progesterone and cortisol after ACTH administration in lactating primiparous sows. Acta Vet Scand 1998; 39: 71–76.

15. Turner AI, Hemsworth PH, Canny BJ, Tilbrook AJ. Sustained but not repeated acute elevation of cortisol impaired the luteinizing hormone surge, estrus, and ovulation in gilts. Biol Reprod 1999; 61: 614–20.

16. Wolfenson D, Lew BJ, Thatcher WW, Graber Y, Meidan R. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim Reprod Sci 1997; 47: 9–19.

17. Badinga L, Thatcher WW, Wilcox CJ, Morris G, Entwistle K, Wolfenson D. Effect of season on follicular dynamics and plasma concentrations of oestradiol-17β, progesterone and luteinizing hormone in lactating Holstein cows. Theriogenology 1994; 42: 1263–74.

18. Bishop JD, Malven PV, Singleton WL, Weesner GD. Hormonal and behavioral correlates of emotional states in sexually trained boars. J Anim Sci 1999; 77: 3339–45.

19. Borg KE, Esbenshade KL, Johnson BH. Cortisol, growth hormone, and testosterone concentrations during mating behavior in the bull and boar. J Anim Sci 1991; 69: 3230-40.

20. Mwanza AM, Madej A, Kindahl H, Lundeheim N, Einarsson S. Plasma levels of cortisol, progesterone, oestradiol-17b and prostaglandin F2a metabolite after ACTH (Synacthen Depot®)

administration in ovariectomized gilts. J Vet Med A 2000; 47: 193–200.

21. Razdan P, Mwanza AM, Kindahl H, Rodriguez-Martinez H, Hultén F, Einarsson S. Effect of repeated ACTH-stimulation on early embryonic development and hormonal profiles in sows. Anim Reprod Sci 2002; 70: 127–37.

22. Bilandžić N, Žurić M, Lojkić M, Šimić B, Milić D, Barač I. Cortisol and immune measures in boars exposed to three-day administration of exogenous adrenocorticotropic hormone. Vet Res Commun 2006; 30: 433-44.

23. Jedlinska M, Rozewiecka L, Ziecik AJ. Effect of hypoprolactinaemia and hyperprolactinaemia on LH secretion endocrine function of testes and structure of seminiferous tubules in boars. J Reprod Fertil 1995; 103: 265-72.

24. Jensen KH, Pedersen LJ, Nielsen EK, Heller KE, Ladewig J, Jorgensen E. Intermittent stress in pigs: effect on behaviour, pituitary-adrenocortical axis, growth, and gastric ulceration. Physiol Behav 1996; 59: 741–8.

25. Orth DN, Kovacs WJ. The adrenal cortex. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. Williams textbook of endocrinology. 9th ed. Philadelphia, Pennsylvania: W.B. Saunders Company, 1998: 517–664.

26. Schrader L, Ladewig J. Temporal differences in the responses of the pituitary adrenocortical axis, the sympathoadrenomedullar axis, heart rate, and behaviour to a daily repeated stressor in domestic pigs. Physiol Behav 1999; 66: 775–83.

27. Hay M, Meunier-Salaün M-C, Brulaud F, Monnier M, Morméde P. Assessment of hypothalamic-pituitary-adrenal axis and sympathetic nervous system activity in pregnant sows through the measurement of glucocorticoids and catecholamines in urine. J Anim Sci 2000; 78: 420–8.

28. Janssens CJJG, Helmond FA, Wiegant VM. The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day. Domest Anim Endocrinol 1995; 12: 167–77.

29. Klemcke HG, Nienaber JA, Hahn GL. Plasma adrenocorticotropic hormone and cortisol in pigs: effects of time of day on basal and stressor-altered concentrations. Exp Biol Med 1989; 190: 42–53.

30. Janssens CJJG, Helmond FA, Wiegant VM. Increased cortisol response to exogenous adrenocorticotropic. J Anim Sci 1994; 72: 1771–7.

31. Liptrap RM, Raeside JI. Effect of cortisol on the response to gonadotrophin releasing hormone in the boar. J Endocrinol 1983; 97: 75-81.

32. Jefcoate C. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. J Clin Invest 2002; 110: 881–90.

33. DiBartolomeis MJ, Jefcoate CR. Characterization of the acute stimulation of steroidogenesis in primary bovine adrenal cortical cell cultures. J Biol Chem 1984; 259: 10159–167.

34. Liptrap RM, Raeside JI. A relationship between plasma concentrations of testosterone and corticosteroids during sexual and aggressive behaviour in the boar. J Endocrinol 1987; 76: 75-85.

35. Wichmann U, Wichmann G, Krause W. Serum levels of testosterone precursors, testosterone and estradiol in 10 animal species. Exp Clin Endocrinol 1984; 83: 283–90.

36. Fenske M. Role of cortisol in the ACTH-induced suppression of testicular steroidogenesis in guinea pigs. J Endocrinol 1997; 154: 407–14.

37. Brandt Y, Lang A, Madej A, Rodriguez-Martinez H, Einarsson S. Impact of ACTH administration on the oviductal sperm reservoir in sows: The local endocrine environment and distribution of spermatozoa. Anim Reprod Sci 2006; 92: 107-22.

38. Madej A, Mwanza AM, Kindahl H, Einarsson S. The effect of ACTH and CRH on plasma levels of cortisol and prostaglandin $F_{2\alpha}$ in cycling gilts and castrated boars. In: Proceedings of the 14th International Congress on Animal Reproduction. Stockholm, Sweden, 2000: 61.

39. Fonda ES, Rampacek GB, Kraeling RR. The effect of adrenocorticotropin or hydrocortisone on serum luteinizing hormone concentrations after adrenalectomy and/or ovariectomy in the prepuberal gilt. Endocrinology 1984; 114: 268–73.

40. Bolanos JM, Molina JR, Forsberg M. Effect of blood sampling and administration of ACTH on cortisol and progesterone levels in ovariectomized zebu cows (*Bos indictus*). Acta Vet Scand 1997; 38: 1–7.

41. Liptrap RM, Raeside JI. Increase in the plasma testosterone concentration after injection of adrenocorticotrophin in boars. J Endocrinol 1975; 66: 123-31.

42. Cox JE, Williams JH. Some aspects of the reproductive endocrinology of the stallion and cryptorchid. J Reprod Fertil 1976; 23: 75–79.

43. Mann DR, Free C, Nelson C, Strott C, Collins DC. Mutually independent effects of adrenocorticotropin on luteinizing hormone and testosterone secretion. Endocrinology 1987; 120: 1542–50.

44. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocr Rev 1998; 19: 101–43.

45. Liptrap RM, Raeside JI. Effect of corticotrophin and corticosteroids on plasma interstitial cell-stimulating hormone and urinary steroids in the boar. J Endocrinol 1968; 42: 33-43.

46. Bambino TH, Hsueh AJW. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis *in vivo* and *in vitro.* Endocrinology 1981; 108: 2142–8.

47. Marić D, Kostić T, Kovačević R. Effects of acute and chronic immobilization stress on rat Leydig cell steroidogenesis. J Ster Biochem Mol Biol 1996; 58: 351–5.

48. Wise T, Zanella EL, Lunstra DD, Ford JJ. Relationship of gonadotropins, testosterone, and cortisol in response to GnRH and GnRH antagonist in boars selected for high and low follicle-stimulating hormone levels. J Anim Sci 2000; 78: 1577–90.

49. Vreeburg JTM, De Greef WJ, Ooms MP, Van Wouw P, Weber RFA. Effects of adrenocorticotropin and corticosterone on the negative feedback action of testosterone in the adult male rat. Endocrinology 1984; 115: 977–83.

VPLIV TRIDNEVNEGA DODAWJANJA ACTH NA RAVEN HOLESTEROLA, KORTIZOLA, PROGESTERONA, TESTOSTERONA IN LH PRI MERJASCIH

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Povzetek: Z raziskavo smo želeli ugotoviti vpliv stresa, povzročenega z ACTH, na raven holesterola, kortizola, progesterona, testosterona in LH pri merjascih švedske deželne pasme, starih 6 do 7 mesecev, med samo aplikacijo ACTH in do 12 dni po prenehanju tretiranja. Živali v skupini ACTH (n = 14) so 3 dni dobivale ACTH (10 m g / kg, i.v.), živali v kontrolni skupini (n = 14) pa so prejele 1 ml sterilne fiziološke raztopine i.m. Koncentracija kortizola je bila pri tretiranih merjascih statistično značilno povišana ($p < 0,0001$) v vseh treh dneh tretiranja in še naslednji dan po zadnji injekciji ACTH ($p < 0,01$). Po 12 dneh je bila raven kortizola na fiziološki ravni. V primerjavi s kontrolnimi živalmi so imeli merjasci, tretirani z ACTH, značilno nižjo koncentracijo holesterola v vseh treh dneh tretiranja, pa tudi en dan in 5 dni po zadnjem odmerku ACTH ($p < 0,05$ do $p < 0,0001$). Koncentracije progesterona in testosterona so bile bistveno povečane ($p < 0,001$ do $p < 0,0001$) v vseh treh dneh tretiranja z ACTH, po končanem dodajanju pa ni bilo bistvenih razlik v koncentraciji progesterona in testosterona med tretiranimi in kontrolnimi merjasci. Tretiranje z ACTH ni vplivalo na raven LH. Občutno znižane koncentracije holesterola v serumu merjascev, ki so bili izpostavljeni 3-dnevnemu stresu, povzročenemu z aplikacijo ACTH, kaže na povečano biotransformacijo holesterola v steroidne hormone, kortizol, progesteron in testosteron, kar je v skladu z rezultati te raziskave.

Ključne besede: merjasci; ACTH; stres; kortizol; hormoni; holesterol