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A NON-INVASIVE METHOD TO MONITOR THE SMOOTH MUSCLE ACTIVITY OF DIGESTIVE ORGANS IN GROWING PIGS

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ABSTRACT

The negative impact of stress on physiological processes has been generally known for a long time, but few studies address the decrease in the motor activity of digestion because of stress. Electromyography, a non-invasive method used in humans for nearly 100 years, is suitable for measuring smooth muscle activity. In recent years, the method has also been adapted for pigs. The aim of our preliminary study was to determine whether ACTH induction affects the smooth muscle activity of the digestive organs in pigs and whether this can be demonstrated using electromyographic measurements. Measurements were performed on 30 kg Topigs x Duroc barrows (n = 4) under normal and stress conditions. Stress induction was carried out by intravenous administration of ACTH injection at a dose of 10 µg/kg body weight, which dose corresponds to a mild stress effect. Data were recorded continuously for 8 hours, segmented into 30-minute intervals during data processing, and analyzed using Fast Fourier Transformation and expressed as Power Spectrum maximum measure of value. Our results showed significant decrease in smooth muscle activity in stomach and a tendency for reduce in small intestine post ACTH induction, which may influence digestive processes through reduced motor activity.

ÖSSZEFOGLALÁS

A stressz élettani folyamatokra gyakorolt negatív hatása régóta ismert, de kevés kutatás foglalkozik az emésztés okozta stressz miatti motorikus aktivitásának csökkenésével. Az elektromiográfia, egy humán vonatkozásban közel 100 éve alkalmazott non-invazív mérési módszer, alkalmas a simaizmok aktivitásának mérésére. Néhány éve sertésekre is megtörtént a módszer adaptációja. Vizsgálatunk célja annak megállapítása volt, hogy enyhe, indukált stresszhatás befolyásolja-e a sertések emésztőszerveinek simaizom aktivitását, és ez elektromiográfiás mérésekkel igazolható-e. 30 kg-os Topigs x Duroc genotípusú ártányokon (n = 4) végeztünk elektromiográfiás méréseket normál és stressz körülmények között. A stresszindukció 10 µg/testtömegkilogramm ACTH injekció intravénás alkalmazásával történt, amely dózis enyhe stresszhatásnak felel meg. Az elektromiográfiás jeleket 8 órán át rögzítettük, az adatfeldolgozás során 30 perces szakaszokra bontva és Fast Fourier Transzformációval elemezve, Power Spectrum maximum értékmérővel meghatározva. Eredményeink szerint az ACTH injekció beadása szignifikáns aktivitáscsökkenést eredményezett a gyomorban és tendenciózus aktivitáscsökkenést a vékonybélben, ami a motorikus aktivitás változása miatt befolyásolhatja az emésztési folyamatokat.

INTRODUCTION

The German Animal Welfare Act states: "No one may cause pain, suffering or harm to an animal without reasonable cause". The criteria include health, natural behaviour and well-being of the individual animal (Martinez and von Nolting, 2023). These are the European principles nowadays, which relate to animal experiments. The 3R principles – Reduction, Refinement, and Replacement – are essential in modern in vivo experimental animal research to ensure ethical treatment of animals. Under Refinement, non-invasive methods that avoid harm or stress are prioritized, including advanced sampling techniques. One such method for monitoring gastrointestinal tract (GIT) activity is electromyography (EMG). This technique records the electrical activity of smooth muscles, offering a stress-free and harm-free alternative for assessing motility and function, aligning with ethical and scientific standards. The measurements are based on the electrical signals generated by the interstitial cells of Cajal. These cells generate the action potential, slow waves that regulate the rhythm of smooth muscle contractions in the gastrointestinal tract (Sanders et al., 2006).

Electromyographic measurements have a history of nearly 100 years in human terms (Alvarez, 1922). A few years ago, experiments were conducted with pigs also, in which the electromyographic method was used (Nagy et al., 2021; Roszkos, 2022). Research on stress is particularly important nowadays since stress negatively affects the performance and well-being of farm animals (Martínez-Miró et al., 2016). The goal of the research aiming to explore the effect of stress is to develop new, more effective strategies for preventing stress and alleviating its negative effects. Although studies with electromyographic measurements appear in preclinical studies mostly with rats (Szűcs et al., 2018), in a stress model with awake, free-moving pigs there was no example of it, yet.

The aim of our preliminary study was to determine whether adrenocorticotropic hormone (ACTH) induction affects the smooth muscle activity of the digestive organs in pigs and whether this can be demonstrated using electromyographic measurements.

MATERIALS AND METHODS

The experiment was conducted at the Hungarian University of Agriculture and Life Sciences, Department of Farm Animal Nutrition, in December 2023. During the study, 4 Topigs x Duroc growing barrows were involved with an average body weight of 30 kg. The pigs were kept in individual pens with concrete floors without bedding. Feeding occurred 2 times a day at 8 a.m. and 3 p.m. with *ad libitum* drinking water. Considering that we investigated the effect of stress in our self-control study, the animals needed to be well prepared for the measurements in a period which lasted for about one month. The animals had to become comfortable with handling and human presence as well as wearing the west that holds the wires and the holter used for electromyographic measurements. A self-control experiment was designed, the same animals were measured on the control and on the stress-induced day. There were two following days, the first day was the control day without stress, while on the stressed day the animals got ACTH injection (Synachten 0,25 mg/1 ml) through a permanent vein catheter intravenously at a dose

of 10 µg per kilogram of body weight, in order to perform standardized stress induction. The dose stands for a mild stress effect due to a short-term increase in cortisol levels.

For electromyographic measurements, we used the data-collecting holter device from MSB-Met Kft., which is capable of gathering data using a wire connected to two electrodes placed on the surface of the skin. After shaving and cleaning the required area, two self-adhesive electrodes were applied to the skin. One electrode on the left side was placed near the heart, right behind the front left leg. The other electrode on the right side was placed on the fold of the skin connecting the pig's thigh and lower abdomen. The measurements lasted for 9 hours from which 8 hours were analysed. For data analysis, we used the EasyChart software. The measured 8 hours were divided into 30-minute intervals. From the 8-hour-long analysis we assigned the main interval: between the timepoint of the ACTH injection and 2 hours after the administration. For data analysis, we selected from the software the Power Spectrum maximum (PSmax) analysis function, which calculates with Fast Fourier Transformation the muscle work done by the organ in the selected 30-minute interval. Statistical analysis was performed with paired Student's t-test, comparing data of 30-minute intervals of the control day with data of the stress induction day of each animal's own.

RESULTS AND DISCUSSION

There was a significant difference in blood serum cortisol levels measured through the control day and the stressed day as shown in Figure 1.

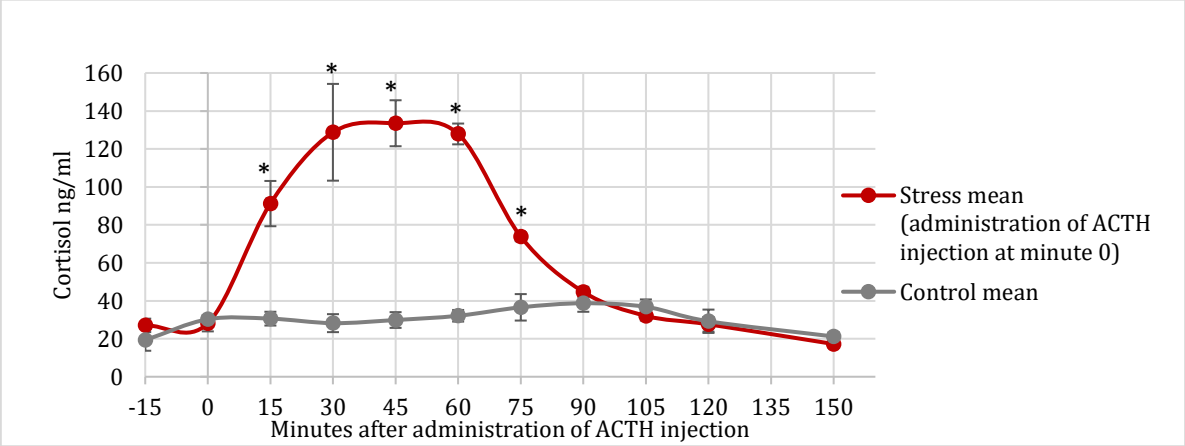

Figure 1 Blood serum total cortisol levels on the control day and stress-induced day, n = 4

Blood serum total cortisol levels were in a normal range on the control day (Plut et al., 2023). On the stress-induced day, the cortisol levels were significantly higher ($p < 0,05$) between the 15th and 75th minutes post-stress induction. This proves that the stress induction was successful. Results of the smooth muscle activity in different segments of the gastrointestinal tract right after the stress induction compared to the pairwise periods in nonstressed days are shown in Figure 2.

The smooth muscle activity of the stomach decreased significantly ($p < 0.05$) in the 60-minute interval after administration of ACTH injection. In 30-minute intervals 90 minutes after the ACTH injection the activity of the small intestine reduced slightly ($p = 0.09$) The activity of the large intestine was statistically the same on the control and stress-induced

day until the 120th and 150th-minute interval, the activity increased at that time point. The numerical decrease in smooth muscle activity is noticeable across all three digestive organs in most of the intervals post-ACTH induction.

The exact mechanisms by which stress influences gastrointestinal motility are primarily explored in the literature through review articles emphasizing hormonal interactions and invasive experiments conducted on rats. These studies typically confirm the occurrence of motility reduction without elucidating its precise pathways. To date, the impact of stress on digestive motility remains unclear (Pluske et al., 2019), particularly in swine, where the literature is notably sparse.

Some sources (Heymann-Mönnikes et al., 1991; Lewis et al., 2002) attribute this reduction to the effects of corticotropin-releasing factor (CRF). However, the process is considerably more complex in the body, involving a biochemical cascade where ACTH appears after CRF release, followed by cortisol production.

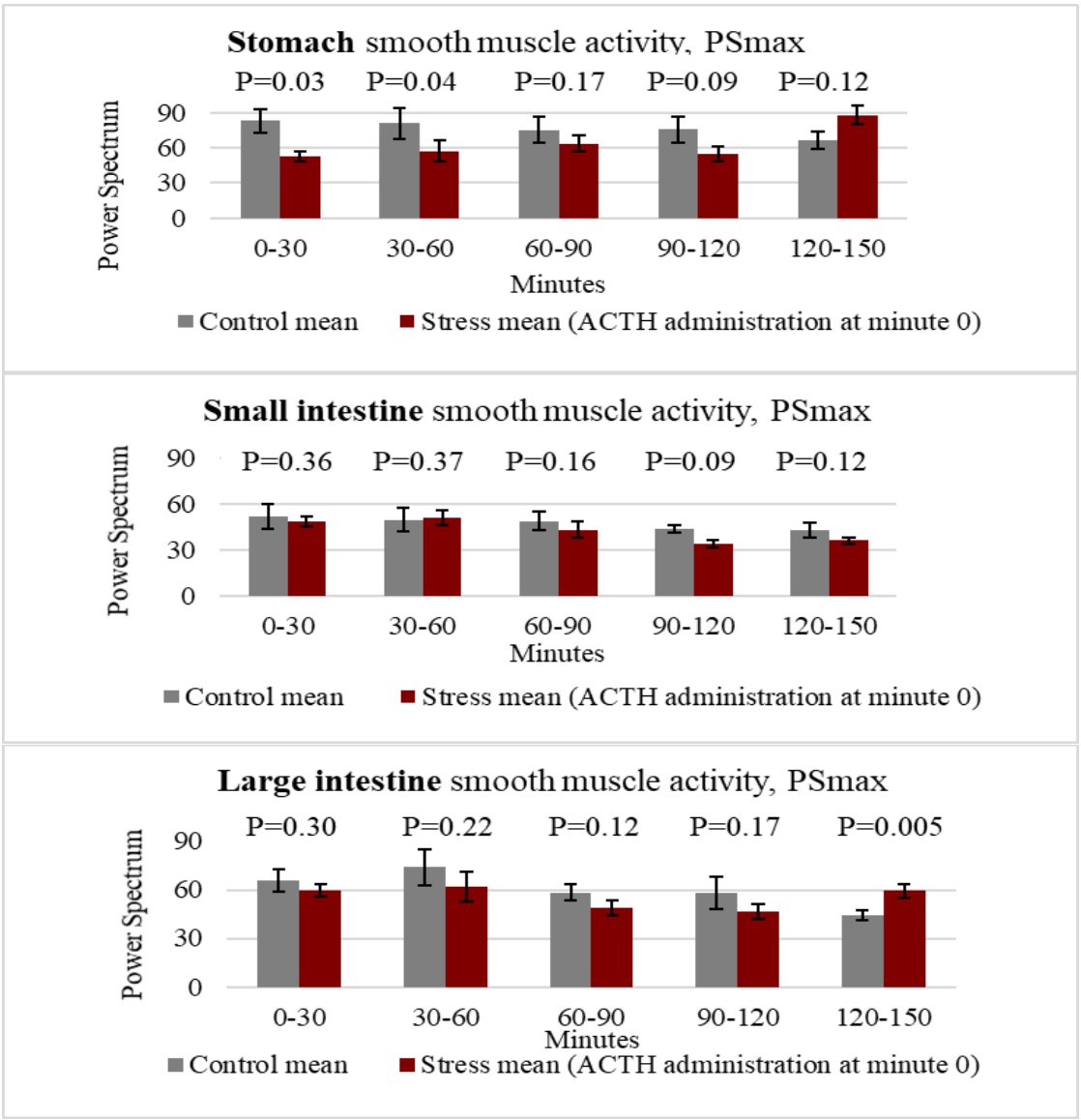

Figure 2 Means with standard errors of smooth muscle activity of the stomach, small intestine, and large intestine in each 30-minute interval post-stress induction

Other studies suggest that Prolactin-releasing peptide (PrRP) may regulate gastric motor activity within the dorsal vagal complex by modulating excitatory synaptic inputs from the nucleus tractus solitarius to vagus motor neurons projecting to the stomach (Grabauskas et al., 2004). Despite these findings, no literature addresses the time-domain reduction in the activity of digestive organs' smooth muscle, post-stress, but the answers are likely to be found in the hormonal background activity affecting the entire organism.

CONCLUSIONS

Electromyographic measurements can be performed on awake, freely moving pigs in a stress model. Using this method, the activity of the three measured digestive organs was detectable. Even mild induced stress (e.g., a low dose of ACTH) reduced the activity of the stomach and small intestine; in the stomach post-ACTH induction, this reduction was significant even with a low animal number.

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REFERENCES

Alvarez, W. C. (1922): The electrogastrogram and what it shows. J. Am. Med. Assoc., 78, 1116–1118. DOI: https://doi.org/10.1001/jama.1922.02640680020008

Grabauskas, G., Zhou, S. Y., Das, S., Lu, Y., Owyang, C., & Moises, H. C. (2004): Prolactin-releasing peptide affects gastric motor function in rat by modulating synaptic transmission in the dorsal vagal complex. The Journal of Physiology, 561(Pt 3), 821–839. DOI: https://doi.org/10.1113/jphysiol.2004.072736

Heymann-Mönnikes, I., Taché, Y., Trauner, M., Weiner, H., & Garrick, T. (1991): CRF microinjected into the dorsal vagal complex inhibits TRH analog- and kainic acid-stimulated gastric contractility in rats. Brain Research, 554(1–2), 139–144. DOI: https://doi.org/10.1016/0006-8993(91)90181-t

Lewis, M. W., Hermann, G. E., Rogers, R. C., & Travagli, R. A. (2002): In vitro and in vivo ana-lysis of the effects of corticotropin releasing factor on rat dorsal vagal complex. The Journal of Physiology, 543(1), 135–146. DOI: https://doi.org/10.1113/jphysiol.2002.019281

Martinez, J., & von Nolting, C. (2023): Review: "Animal welfare" – A European concept. Animal: an international journal of animal bioscience, 17 Suppl 4, 100839. DOI: https://doi.org/10.1016/j.animal.2023.100839

Martínez-Miró, S., Tecles, F., Ramón, M., Escribano, D., Hernández, F., Madrid, J., Orengo, J., Martínez-Subiela, S., Manteca, X., & Cerón, J. J. (2016): Causes, consequences and biomarkers of stress in swine: an update. BMC veterinary research, 12(1), 171. DOI: https://doi.org/10.1186/s12917-016-0791-8

Nagy, K., Fébel, H., Bazar, G., Grosz, G., Gáspár, R., Ferenc Szűcs, K., & Tóth, T. (2021): Non-invasive smooth muscle electromyography (SMEMG) as a novel monitoring technology of the gastrointestinal tract of awake, free-moving pigs – A pilot study. Plos one, 16(9), e0257311. DOI: https://doi.org/10.1371/journal.pone.0257311

Pluske, J. R., Miller, D. W., Sterndale, S. O., & Turpin, D. L. (2019): Associations between gast-rointestinal-tract function and the stress response after weaning in pigs. Animal Production Science, 59(11), 2015–2022. DOI: https://doi.org/10.1071/AN19279

Plut, J., Snoj, T., Golinar Oven, I., & Štukelj, M. (2023): The Combination of Serum and Oral Fluid Cortisol Levels and Welfare Quality Protocol® for Assessment of Pig Welfare on Intensive Farms. Agriculture, 13(2), 351. DOI: https://doi.org/10.3390/agriculture13020351

Roszkos, R. (2022): The evolution of electromyography and the possibilities of its use in the reproduction biological processes of sows. Állattenyésztés és Takarmányozás, 71(2), 88–104. Retrieved from https://www.szaktudas.hu

Sanders, K. M., Koh, S. D., & Ward, S. M. (2006): Interstitial cells of cajal as pacemakers in the gastrointestinal tract. Annu. Rev. Physiol., 68, 307–343. DOI: https://doi.org/10.1146/annurev.physiol.68.040504.094718

Szűcs, K. F., Grosz, G., Süle, M., Sztojkov-Ivanov, A., Ducza, E., Márki, A., Kothencz, A., Balogh, L., & Gáspár, R. (2018): Detection of stress and the effects of central nervous system depressants by gastrointestinal smooth muscle electromyography in wakeful rats. Life sciences, 205, 1–8. DOI: https://doi.org/10.1016/j.lfs.2018.05.015