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Review paper

Acute phase proteins as biomarkers of pre-slaughter stress in pigs

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ABSTRACT

Pre-slaughter handling, which include transportation, housing, social stress, heat, and dietary changes, is one of the main causes that produces stress in pigs. The appropriate biomarkers and objective laboratory criteria to evaluate pre-slaughter stress are lacking. Behavioral and physiological markers are commonly used for this reason, but these parameters may increase for reasons unrelated to stress. Acute phase proteins are considered to be markers of inflammation that have been proposed as indicators for farm animal stress monitoring. The major acute phase proteins in swine are haptoglobin, serum amyloid A, c-reactive protein, and pig major acute phase protein. Serum or plasma obtained from blood are the most used matrixes for the measurement of acute phase proteins, the collection of which involves an invasive collection method that is harmful and stressing. The use of saliva and meat juice instead of blood might overcome these disadvantages, since its collection is non-invasive and stress-free. For any assay measuring acute phase proteins, adequate analytical validation must be performed, as well as harmonization and standardization of analytical procedures. The aim of this paper is to emphasize the possibilities of use of acute phase proteins as biomarkers of pre-slaughter stress, as well as to provide survey of methodologic assays and fluids that are presently available to measure acute phase proteins.

1. Introduction

In the current pig production system, increasing attention is paid to high animal welfare standards as this is seen to be an indicator for safe, healthy, and high quality food (*Klauke et al.*, 2013). Animal welfare problems are also ethically important (*Marco-Ramell et al.*, 2011). During the pre-slaughter period, pigs are exposed to many environmental stressors (*Stajkovic et al.*, 2017). An animal's response to a stressor involves a variety of adaptive physiological mechanisms designed to restore homeostasis (*Salamano et al.*, 2008). Behavioral (aggression, immobilization, exploration, etc.) and physiological (plasma levels of cortisol and catecholamines, i.e., adrenaline and noradrenaline) markers are commonly used to assess level of stress, but these parameters may increase for reasons unrelated to stress (*Levine*, 1985; *Terlow*, 2005). Hence, the selection of suitable biomarker is a basic necessity in order to objectively and rapidly evaluate animal stress at any given time. Acute phase proteins (APPs) are a group of species-specific plasma proteins which respond to infection, inflammation and/or trauma and have been proposed as indicators for farm animal stress monitoring (*Diack et al.*, 2011; *Petersen et al.*, 2004; *Murata,* 2007).

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The objective of this study was to evaluate the effect of pre-slaughter stress on changes on concentration of acute phase proteins in pigs and to evaluate methods and fluids for APP determination.

2. Pre-slaughter stress

Stress is a general term used to describe environmental factors soliciting adaptation mechanisms and the response to these challenges (*Mormède et al*., 2007). Pre-slaughter stress induced by transport, housing, and slaughter include psychological stimuli (exposure to new social group, personnel, smells, and noises, or any other change to the familiar situation) and physical stimuli (food and water restriction, extreme thermal conditions, fatigue due to movements of the lorry, etc.) that might be aversive for the animals. Stress levels of the animal depend indirectly on the situation, and directly on the animal's evaluation of the situation, but they can only be indirectly assessed, using behavioral and physiological measurements (*Terlow*, 2005).

3. Acute phase proteins

APP are primarily synthesized by hepatocytes as part of the acute phase response (APR) which is part of the early-defense or innate immune system triggered by stress, infection, trauma, neoplasia, and inflammation (*Cray et al*., 2009). The APR is a complex reaction mediated by proinflammatory cytokines, such as interleukins-1β and –6 (IL-1β and IL-6), and tumor necrosis factor-α (TNF-α). During the APR, the serum concentration of the APP changes dramatically and can be increased or decreased after a triggering event. APPs are classified as positive (major, moderate, or minor) or negative. Depending on the magnitude of increase during the APR, major proteins increase 10- to 100-fold (pig major acute phase protein (pig-MAP), haptoglobin (Hp), serum amyloid A (SAA), c-reactive protein (CRP)), moderate proteins increase 2- to 10-fold (α-1-acid glycoprotein (AGP)), and minor proteins undergo only a slight increase (fibrinogen (Fb)) (*Ceron et al*., 2005; *Saco and Bassols*, 2023). Albumin is the major negative APP in swine which, during the APR, decreases in blood concentration, as does apolipoprotein A1 (Apo-A1) (*Ceron et al*., 2005; *Saco and Bassols*, 2023). The specific effect of pre-slaughter stress on APP concentrations is difficult to evaluate, since the handling of animals for slaughter consists of a series of procedures (transport, lairage, stunning, etc.) that are unusual for them and, therefore, stressful. Also, there are concurrent subclinical infections and traumatic lesions inherent in the crowding of animals. The kinetics of the APR should also be taken into account. The serum concentration of the rapid reacting first-line APPs (such as SAA and CRP) increases within four hours (*Petersen*, 2004). Second-line APPs are Hp, CRP and Pig-MAP (*Weschenfelder et al.*, 2012; *Salamano et al*., 2008). Also, in order to adequately determine the impact of stress on concentration of APP, the range of concentration of APP in healthy and sick animals must be established, as well as differences in their concentration between sex, and age.

The concentration of Pig-MAP and Hp increases in pigs confronted with stressful situations and compromising animal welfare, such as longer transport, crowding, mixing unfamiliar pigs, or inadequate handling of feed (*Marco-Ramell et al.*, 2011; *Piñeiro et al.*, 2009; *Piñeiro et al.*, 2004; *Piñeiro et al.,* 2007a; *Piñeiro et al.,* 2007b). Extreme hot temperature elevated the concentration of Hp. Pig-MAP was the only APP whose concentration differed in pigs housed at different stocking densities. High-density pens had higher pig-MAP concentrations (*Marco-Ramell et al.*, 2011). The pig-MAP biomarker has the advantage of relatively low variability in its normal state compared to Hp and other APPs (*Diack, et al.*, 2011; *Piñeiro et al.,* 2009). CRP and SAA concentrations increase after shorter transport, probably because they are first-line APPs (*Saco and Bassols*, 2023). Higher concentrations of CRP may be also induced by stressful situations, such as alterations in feeding patterns and access to water and food (*Piñeiro et al.*, 2007a). Correlations between Hp, Pig-MAP, and CRP and stress status were found in research on mixing stress and human-animal relationships (*Valent et al.,* 2017). Stressors, such as social isolation and short road transport elevated levels of saliva SAA (*Soler et al.,* 2013).

4. Sample types and main methodologic techniques for quantification of APP

The most commonly used matrixes for the measurement of APP are serum or plasma obtained from blood, since blood can reflect the overall picture of the biochemical changes occurring in the body (*Franco-Martinez et al.*, 2020; *Saco and Bassols*, 2023). Determination of APP in blood can be used for monitoring animal health and welfare on farms. The blood collection is highly stressful and painful, both for the animal and the staff in charge of the sam-

pling (*Cerón et al.,* 2022). APP measurements in other fluids, such as meat juice and saliva, can be practical. The use of such non-invasive samples can offer various advantages compared to blood because they are in most cases pain and stress-free, they are faster and easier to obtain, and do not need specialized staff for their collection (*Franco-Martinez et al.*, 2020). However, it is important to point out the question of whether the concentrations of APPs always reliably reflect the concentrations of these molecules in blood. Meat juice can be easily obtained at slaughter, and with a standardized meat juice extraction protocol, which includes harmonization of muscle type and size, the results of APPs might be universally comparable. Concentrations of Pig-MAP and Hp in meat juice are closely correlated with those in plasma. These results open new possibilities for the assessment of animal health in pig production, with implications for food safety and meat quality (*Piñeiro et al.*, 2009). Saliva can be obtained by easy procedures, and repeated specimens can be obtained anytime and anywhere, leading to the possibility of more frequent analysis and better control of health and welfare. Saliva could substitute for blood in some cases, such as for measuring Hp and SAA, indicators of the health status of farms and some stress conditions such as transport, housing, isolation, and restraint. There is a good agreement between APPs quantified in saliva and serum (*Saco et al.,* 2023). Some salivary APPs can be influenced by sex and by circadian patterns (*Gutiérrez et al.,* 2013).

Analytical techniques for the determination of APP in swine include colorimetric (Hp), ELISA (Hp, CRP, Pig-MAP, SAA), immunoturbidimetric (Hp, CRP, Pig-MAP), radial immunodiffusion (RID) (Hp, SAA), and point-of-care type of analysis (Hp, CRP, SAA). Other technologies and biosensors have been adapted more recently (*Saco and Bassols*, 2023). All those methodologic assays have advantages and disadvantages and are still being improved to increase their specificity, sensitivity, economic availability, and user-friendliness. All of them, except the colorimetric method, are species-specific. When a large number of samples have to be assayed, automated techniques such as colorimetric and immunoturbidimetric are advisable.

For adequate interpretation of the results obtained by these methods, accurate reference intervals (RIs) of APP are necessary. Calculation of RIs has to take into account the influence of variables such as age, sex, breed, and type of housing. Also, an adequate analytical validation must be performed in order to assure analytical test specificity, reliability, accuracy, and repeatability, as well as to ensure harmonization and standardization of analytical procedures.

5. Conclusion

APP concentrations change in pigs confronted with situations causing stress and compromising animal welfare, such as transport, crowding, mixing with other pigs, or inadequate handling of feed. The use of APPs as biomarkers of pre-slaughter stress needs to be recognized and further explored. The most commonly used matrixes for the determination of APP are serum or plasma. Other sample types, saliva and meat juice, show potential uses. Reference materials for the calibration of reagents are necessary to expand the use of analytical techniques to determine APPs. The main problem in considering APPs as valid to assess pre-slaughter stress is the lack of standard basal values from healthy animals from different farm conditions, ages, and sex.

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References

Ceron, J. J., Eckersall, P. D. & Martynez-Subiela, S. (2005). Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Veterinary Clinical Pathology*, 34, 85–99, https://doi.org/10.1111/j.1939-165x.2005.tb00019.x

Cerón, J. J., Contreras-Aguilar, M.D, Escribano, D., Martínez-Miró, S., López-Martínez, M. J., Ortín-Bustillo, A., Franco-Martínez, L., Rubio, C. P., Muñoz-Prieto, A., Tvarijonaviciute, A., López-Arjona, M., Martínez-Subiela S. & Tecles F. (2022). Basics for the potential use of saliva to evaluate stress, inflammation, immune system, and redox homeostasis in pigs. *BMC Veterinary Research*, 18(1), 81, https://doi.org/10.1186/S12917-022-03176-W

Cray, C., Zaias J. & Altman, N. H. (2009). Acute phase response in animals: a review. *Comparative Medicine*, 59(6), 517–526.

Diack, A. B., Gladney, C. D., Mellencamp, M. A., Stear, M. J., & Eckersall, P. D. (2011). Characterisation of plasma acute phase protein concentrations in a high health boar herd. *Veterinary Immunology and Immunopathology*, 139, 107–112, https://doi.org/10.1016/j.vetimm.2010.09.003

Franco-Martinez, L., Martinez-Subiela, S., Ceron, J. J., Tecles, F., Eckersa, P. D., Oravcov, K. & Tvarijonaviciute A. (2020). Biomarkers of health and welfare: A One Health perspective from the laboratory side. *Research in Veterinary Science*, 128, 299–307, https://doi.org/10.1016/j.rvsc.2019.12.012

Gutiérrez, A. M., Escribano, D., Fuentes, M. & Cerón, J. J. (2013). Circadian pattern of acute phase proteins in the saliva of growing pigs. *The Veterinary Journal*, 196(2), 167–170, https://doi.org/10.1016/j.tvjl.2012.10.003

Klauke, T. N., Piñeiro, M., Schulze-Geisthövel, S., Plattes, S., Selhorst, T. & Petersen, B. (2013). Coherence of animal health, welfare and carcass quality in pork production chains. *Meat Science*, 95(3), 704–711, https://doi.org/10.1016/j.meatsci.2013.03.022

Levine, S. (1985). A definition of stress. In: Moberg, G.P. (Ed.), Animal Stress. Waverly Press, Baltimore, MA, USA, pp. 51–69.

Marco-Ramell, A., Pato, R., Peña, R., Saco, Y., Manteca, X., Ruiz de la Torre, J. L & Bassols, A. (2011). Identification of serum stress biomarkers in pigs housed at different stocking densities. *The Veterinary Journal*, 190(2), 66–71, https://doi.org/10.1016/j.tvjl.2011.01.003

Mormède, P., Andanson, S., Aupérin, B., Beerda,B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C., G., Richard, S. & Veissier, I. (2007). Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiology & Behavior*, 92, 317–339, https://doi.org/10.1016/j.physbeh.2006.12.003

Murata, H. (2007). Stress and acute phase protein response: an inconspicuous but essential linkage. *The Veterinary Journal*, 173, 473–474, https://doi.org/10.1016/j.tvjl.2006.05.008

Petersen, H. H., Nielsen, J. P. & Heegaard, P. M. (2004). Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, 35(2), 163–187, https://doi.org/10.1051/vetres:2004002

Piñeiro, C., Morales, J., Piñeiro, M., Lampreave, F. & Mateos, G. G. (2004). Effects of induced mixing and pen size on performance and serum concentration of acute phase proteins in growing pigs. *Journal of Animal Science*, 82, 505 (abstr).

Piñeiro, C., Piñeiro, M., Morales, J., Andres, M., Lorenzo, E., del Pozo, M., Alava, M. A. & Lampreave, F. (2009). Pig-MAP and haptoglobin concentration reference values in swine from commercial farms. *The Veterinary Journal*, 179, 78–84, https://doi.org/10.1016/j.tvjl.2007.08.010.

Piñeiro, C., Piñeiro, M., Morales, J., Carpintero, R., Campbell, F. M. & Eckersall, P. D., Toussaint, M. J., Alava, M. A., & Lampreave, F. (2007a). Pig Acute-Phase Protein levels after stress induced by changes in the pattern of food administration. Animal, 1(1), 133–9, https://doi.org/10.1017/S1751731107283909

Piñeiro, M., Gymnich, S., Knura, S., Piñeiro, C. & Petersen, B. (2009). Meat juice: an alternative matrix for assessing animal health by measuring acute phase proteins. Correlations of pig-MAP and haptoglobin concentrations in pig meat juice and plasma. *Research in Veterinary Science*, 87, 273–276, https://doi.org/10.1016/j.rvsc.2009.03.013

Piñeiro, M., Piñeiro, C., Carpintero, R., Morales, J., Campbell, F. M., Eckersall, P. D., Toussaint, M. J. & Lampreave, F. (2007b). Characterisation of the pig acute phase protein response to road transport. *The Veterinary Journal*, 173(3), 669–674, https://doi.org/10.1016/j.tvjl.2006.02.006

Saco, Y. & Bassols, A. (2023). Acute phase proteins in cattle and swine: A review. *Veterinary Clinical Pathology*, 52(1), 50–63, https://doi.org/10.1111/vcp.13220

Salamano, G., Mellia, E., Candiani, D., Ingravalle, F., Bruno, R, Ru G. & Doglione, L. (2008). Changes in haptoglobin, C-reactive protein and pig-MAP during a housing period following long distance transport in swine. *The Veterinary Journal*, 177(1), 110–115, https://doi.org/10.1016/j.tvjl.2007.03.015

Soler, L., Gutiérrez, A., Escribano, D., Fuentes, M. & Cerón, J. J. (2013). Response of salivary haptoglobin and serum amyloid A to social isolation and short road transport stress in pigs. *Research in Veterinary Science*, 95, 298–302, https://doi.org/10.1016/j.rvsc.2013.03.007

Stajkovic, S., Teodorovic, V., Baltic, M. & Karabasil, N (2017). Pre-slaughter stress and pork quality. *IOP Conference Series Earth and Environmental Science*, 85 012034 https://doi.org/10.1088/1755-1315/85/1/012034

Terlow, C. (2005). Stress reactions at slaughter and meat quality in pigs: genetic background and prior experience: A brief review of recent findings. *Livestock Production Science*, 94(1–2), 125–135, https://doi.org/10.1016/j.livprodsci.2004.11.032

Valent, D., Arroyo, L., Peña, R., Yu, K., Carreras, R., Mainau, E., Velarde, A. & Bassols, A. (2017). Effects on pig immunophysiology, PBMC proteome and brain neurotransmitters caused by group mixing stress and human-animal relationship. *PLoS One*, 12(5), e0176928, https://doi.org/10.1371/journal.pone.0176928

Weschenfelder A.V., Torrey, S., Devillers, N., Crowe, T., Bassols, A., Saco, Y., Piñeiro, M., Saucier, L. & Faucitano, L. (2012). Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance. *Journal of Animal Science*, 90(9), 3220–3231, https://doi.org/10.2527/jas.2012-4676