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Measuring the Stress Response

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Here at the University of Minnesota College of Veterinary Medicine, we have taken a strong interest in dairy stockmanship and the interactions that occur between people and cows. The daily management of a dairy operation requires personnel to interact with the cattle many times a day. Dairy stockmanship is the implementation of low-stress cow handling techniques to improve the outcomes for both the people and the cattle.

In discussions on low-stress cattle handling, people frequently refer to flight zone of an animal. It is useful to think about the flight zone in a conceptual manner. As you approach the flight zone of an animal, they will begin to feel pressure from your presence and respond. The exact response distance will constantly be changing depending on the various factors influencing the animal. By closely observing the response of the animal as you approach her, you will be able to see when you are exerting pressure. If you move further away, you can release some or all of the pressure. The key point is that we can move animals by exerting pressure and varying the amount of pressure. The amount of pressure the animal feels depends on the distance and angle of the pressure. It is important not to over-pressure or to apply pressure in an unpredictable manner to the animal. Extreme examples of over-pressure are shouting, arm waiving, and hitting animals or using electric prods to get them to move. Cows do not respond positively when over-pressured, they exhibit agitation and may run away from the over-pressure potentially leading to slip and falls. These examples of over-pressure would be called high-stress cow handling techniques.

Skilled stockmanship actions take advantage of the basic, natural movement tendencies of cattle. It is logical to assume that proper stockmanship should result in animal handling that is low-stress for the animal. Remember that cattle are a prey species, dictating their perception of the world around them. Applying pressure properly allows us to create, change and stop movement in cattle all in a low-stress manner. Cattle want to continue to move in the direction they are going, want to follow other animals, want to see what is pressuring them, and want to return in the direction from which they came. Walking in the same direction as cattle will tend to slow them down and/or stop them. Walking in the opposite direction will tend to speed up their movement.

The general concept of low-stress handling is being widely discussed in the dairy industry today. The National Dairy FARM Program: Farmers Assuring Responsible Management ^{SM}, created by the National Milk Producers Federation (NMPF) with support from Dairy Management Inc. (DMI), specifically states as a best management practice “Employees should be properly trained to handle animals with a minimum of stress to the animal, and the consequences of inhumane handling should be known and enforced.” The National Dairy FARM Program is designed to demonstrate that U.S. milk producers are committed to providing the highest standards of animal care and quality assurance. This voluntary program, available to all producers, provides a consistent on-farm animal well-being program that includes education, on-farm evaluations and third-party verification.

Whether it be dairy stockmanship training or a program like FARM, the increased usage of the term “low-stress cattle handling techniques” has raised the questions of what exactly is stress, and how do we determine if it is “low” or “high”?

If you ask twelve people to define “stress” you would likely get 12 different answers. This creates an interesting challenge for us if we are going to attempt to determine the level of animal stress on a particular farm and whether the stress level is “low” or “high”. If we struggle to define stress, how can we measure it? The purpose of this paper is to introduce the reader to the scientific study of stress biology and to suggest that veterinarians and animal scientists can utilize this understanding to assist in the evaluation whether cow handling stress is “low” or “high” on a dairy operation. A lengthy list of references is provided at the end of the paper for those interested in researching further into the concepts of animal stress biology.

A brief history of stress research pioneers will be helpful to understand how the term came into such widespread use. Hans Seyle (1907-1982) is generally recognized for being the first researcher to demonstrate the existence of biological stress. In 1936 Seyle defined stress as "the non-specific response of the body to any demand for change." Seyle demonstrated in his research that a wide variety of noxious stimuli caused a very consistent set of pathologic changes in laboratory rats. Seyle’s work created much interest and discussion in the scientific community.

The work of Robert Sapolsky is also useful in understanding the concept of biological stress. Sapolsky suggests a very useful approach by differentiating a “stressor” from the body’s “stress response”. Sapolsky defined a stressor as anything that disrupts physiological balance. A stress response is defined as the body’s adaptations designed to re-establish the balance. Discussions at the 2011 Trends in Stress Biology course taught at Aarhus University suggested some slight refinements to the definitions.

- Stressor = event threatening or potentially threatening the homeostatic balance
- Stress Response = the bodies attempt to re-establish the homeostasis after encountering a stressor.

Stressor’s can be described by their characteristics such as: duration, frequency, intensity, predictability, and ability to be controlled. It is important to note that while stressors can be physical things (heat, cold, starvation, etc.) psychological factors can also trigger the stress response in an animal in the absence of anything physically threatening to an animal.

Sapolsky in his writings proposes that the stress response evolved as adaptive survival mechanism for animals. It is now increasingly recognized that the consequences of the stress response can be maladaptive and that there is a “biological cost” to the animal for mounting a stress response. It is actually incorrect to state that stress makes an animal sick. To be correct, one should state that the stress response makes you more likely to get diseases that make you sick.

There is no single litmus test for stress because of the multiple ways the body responds to stressors. Since stressors will result in both behavioral responses and physiological responses on the part of the animal proper assessment of an animal’s stress response requires one look both. One cannot interpret physiological test results without knowing the behavior.

An understanding of stockmanship principles will help one to be aware of behavior responses in animals. The physiological components of the stress response are significantly influenced by the endocrine system. Broadly speaking, all stressors provoke some degree of cortisol secretion as well as a multitude of other physiologic responses. The exact orchestration the many hormones involved will vary depending on the stressor. In this way, different stressors have a different “stress signature” that describes the overall stress response. Work in this area is very interesting and in the future will most certainly allow us to improve and refine our evaluation of the physiological response to stress.

It is still our present understanding that glucocorticoids (cortisol) and catecholamine’s (adrenalin) together mediate most of the changes that form the stress response. Today, measuring cortisol remains the gold standard to evaluate the physiologic response to stressors. Researchers are actively engaged in searching for additional physiologic measures, but it is clear that cortisol does play an important role. Understanding the Hypothalamic-Pituitary-Adrenal axis (HPA axis) is critical to understanding the physiology of the stress response.

Blood sampling has been the traditional measure used to evaluate the cortisol level in an animal. However, plasma cortisol evaluation is not without issues. For example, obtaining a blood sample in itself can be stressful, especially in wildlife or zoo animals. Dr. Rupert Palme (Dept. Biomed. Sciences/Biochemistry,University of Veterinary Medicine, Vienna) and other researchers have been actively looking into alternatives to blood sampling. Cortisol is metabolized in the liver and cortisol metabolites are excreted in the urine and feces. Measuring cortisol metabolites in the feces (FCM’s) has received a significant amount of attention. Since 1997, over 130 publications have used the measurement of FCM’s on a wide variety of animal species, including dairy cattle.

In 2011, the University of Minnesota Veterinary Diagnostic Lab (VDL) completed a validation study using a commercially available Radio-Immuno-Assay (MP Biomedicals, Diagnostic Division, 13 Mountain View Avenue, Orangeburg, NY 109062) to measure FCM’s in bovine feces at our VDL. We anticipate the ability to measure FCM’s will be an important additional tool complementing behavior analysis study of dairy cattle. Measuring FCM’s hopefully will assist dairy research projects that are designed to evaluate whether a particular handling technique can be considered low-stress animal handling.

References

Brown RE. 1994. An introduction to neuroendocrinology, chapters 1-4, Cambridge University Press, p. 1-55.

Cohen S, Kessler R, Gordon LU. *Measuring Stress: A Guide for Health and Social Scientists*. (1997, Oxford University Press) ISBN 978-0-19-512120-9

Hansen SW, Malmkvist J, Palme R, Damgaard BM. 2007. Do double cages and access to occupational material improve the welfare of farmed mink? Anim. Welf. 16, 63-76

Hayward LS, Wingfield (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. Gen. Comp. Endocrinol. 135, 365-371.

Herskin MS, Jensen KH. 2002. Effects of open field testing and associated handling v. handling alone on the adrenocortical reactivity of piglets around weaning. J. Anim. Sci. 74, 485-491.

Jarvis S, Moinard C, Robson SK, Baxter E, Ormandy E, Douglas AJ, Seckl JR, Russell JA, Lawrence AB. 2006. Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. Horm. Behav. 49: 68-80.

Jensen P, Toates FM. 1997. Stress as a state of motivational systems. Appl. Anim. Behav. Sci. 53, 145-156.

Jensen P, Andersson L. 2005. Genomics meets ethology: A new route to understanding domestication, behavior, and substainability in animal breeding. Ambio 34, 320-324.

Joëls M, Karst H, DeRijk R, de Kloet ER. 2007. The coming out of the brain mineralocorticoid receptor. Trends Neurosci. 31, 1-7.

Laviola G, Hannan AJ, Macrí S, Solinas M, Jaber M. 2008. Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol. Disease 31, 159-168.

Macrí S, Mason GJ, Würbel H. 2004. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur. J. Neurosci. 20, 1017-1024.

Macrí S, Pasquali P, Bonsignore LC, Pieretti S, Cirulli F, Chiarotti F, Laviola G. 2007. Moderate neonatal stress decreases within-group variation in behavioural, immune and HPA responses in adult mice. PLoS ONE 2(10): e1015. doi:10.1371/journal.pone.0001015., 1-9

Macrí S, Würbel H. 2006. Developmental plasticity of HPA and fear responses in rats: A critical review of the maternal mediation hypothesis. Horm. Behav. 50, 667-680.

Malmkvist J, Damgaard BM, Pedersen LJ, Jørgensen E, Thodberg K, Chaloupkova H, Bruckmaier, RM. 2009. Effects of thermal environment on HPA-axis hormones, oxytocin and behavioral activity in periparturient sows. J. Anim. Sci. 87, 2796-2805.

Malmkvist J, Palme R. 2008. Periparturient nest building: implications for parturition, kit survival, maternal stress and behaviour in farmed mink (Mustela vison). Appl. Anim. Behav. Sci. 114, 270-283.

Mateo JM, Cavigelli SA. 2005. A validation for extraction methods for noninvasive sampling of glucocorticoids in free-living ground squirrels. Physiol. Biochem. Zool. 78, 1069-1084.

Mellor DJ, Cook CJ, Stafford KJ. 2000. Quantifying some responses to pain as a stressor. Chap. 9 in GP Moberg and JA Mench (eds.), The biology of animal stress, CAB international, 171-198.

Herskin MS, Munksgaard L, Ladewig J. 2004. Effects of acute stressors on nociception, adrenocortical responses and behaviour of dairy cows. Physiol. Behav. 83, 411-420.

Heistermann M, Palme R, Ganswindt A. *Comparison of Different Enzymeimmunoassays for Assessment of Adrenocortical Activity in Primates Based on Fecal Analysis.* American Journal of Primatology 68:257–273 (2006)

Moberg GP. 2000. Biological response to stress: Implications for animal welfare. Chapter 1 in GP Moberg and JA Mench (eds), The biology of Animal Stress, CAB international, p. 1-21.

Mormede P, Andanson S, Auperin B, Beerda B, Guemene D, Malmkvist J, Manteca X, Manteuffel G,Prunet P, van Reenen CG, Richard S, Veissier I. 2007. Exploration of the hypothalamic-pituitaryadrenal function as a tool to evaluate animal welfare. Physiol. Beh. 92, 317-339.

Morrow CJ, Kolver ES, Verkerk GA, Matthews LR. *Fecal Glucocorticoid Metabolites as a Measure of Adrenal Activity in Dairy Cattle.* General and Comparative Endocrinology 126, 229-241 (2002)

Möstl E, Rettenbacher S, Palme R. 2005. Measurement of corticosterone metabolites in birds' droppings: An analytical approach. Annals New York Acad. Sci. 1046, 17-34.
Palme R. 2005. Measuring fecal steroids: Guidelines for practical application. Annals New York Acad. Sci. 1046, 75-80.

Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E. 2005. Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion and noninvasive measurement in fecal samples. Trends in Comparative Endocrinology and Neurobiology. Annals New York Acad. Sci. 1040**,** 162-171.

Palme R, Robia C, Baumgartner W, Mostl E. *Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations.* Veterinary Record (2000) 146, 108-109

Parker KJ, Maestripieri D. 2010. Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. Neurosci. Biobehav. Rev. In press, doi: 10.1016/j.neubiorev.2010.09.003. 18pp

Sapolsky RM. 2002. Endocrinology of the Stress-Response. Chapter 11 in J. B. Becker, M. Breedlove, D. Crews, M. M. McCarty, Behavioral Endocrinology. 2nd edition. MIT Press, London, UK.

Sapolsky, R. *Why Zebras Don't Get Ulcers* (1994, Holt/Owl 3rd Rep. Ed. 2004) ISBN 978-0-8050-7369-0

Touma C, Palme R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. Annals New York Acad. Sci. 1046, 54-74.

Selye H. 1936. A syndrome produced by diverse nocuous agents. Nature 138, 32.

Weis JM. 1968. Effects of coping responses on stress. J. Comp. Phsyiol. Psychol. 65, 251-260.

Weis JM. 1971. Effect of coping behaviour in different warning signal conditions on stress pathology in rats. J. Comp. Physiol. Psychol. 77, 1-13.

Wolfer DP, Litvin O, Morf S, Nitsch RM, Lipp H-P, Würbel H. 2004. Cage enrichment and mouse behaviour. Nature 432, 821-822