The biology of stress and its application to livestock housing and transportation assessment[1]

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ABSTRACT: *Stress* is a broad term that implies a threat to which the body needs to adjust. Stress can be classified as physical, psychological, or interoceptive in nature but usually contains components of all three classifications. The adjustment to stress induces a broad range of physiological and behavioral changes that allow for a rapid recovery or adaptation to the change. In the past, housing systems and handling procedures for farm animals were mainly assessed by descriptive behavioral studies using indicators presumed to be related to stress (i.e., stereotypic behaviors). Physiological indicators included endocrine changes of the pituitary–adrenal–axis by measuring ACTH, corticosteroids, and catecholamines. The neuroendocrine and immune systems have been studied in relation to stress effects at the cellular or neural level during the last decade. All these studies were often conducted in an isolated manner without considering that the neuroendocrine and immune systems are communicating with each other and are ultimately influenced by the animals' individual perception of a stressor. Transportation is considered a major stressor for farm animals and might have deleterious effects on health, well-being, performance, and, ultimately, product quality. Studies on the assessment of stress during animal transportation require noninvasive methods because classical approaches of data collection with direct human interference (i.e., for blood collection and heart-rate measurement) might directly alter the stress response. Telemetric devices for measuring heart and respiration rate, body temperature, and blood pressure are useful tools to obtain undisturbed responses. Also, noninvasive measurements of stress indicating metabolites in saliva, feces, or urine have been developed and validated. Parallel to behavioral observations (via video recordings), these physiological measurements provide valuable information on how livestock handling and transportation can be improved in the near future.

Key Words: Behavior, Farm Animal, Housing, Physiology, Stress, Transportation

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Introduction

Stress is defined as a condition in an animal that results from the action of one or more stressors that may be of either external or internal origin. Whether a stressor can be considered as harmful depends on the way an organism is able to cope with a threatening situation as it regains a state of homeostasis. In that way, stress can be measured and monitored in terms of behavioral and physiological alterations that might be indicative for the individual's state of well-being. The current biological view is that severe stress invariably implies poor well-being. However, stress per se may not negatively affect well-being, and the well-being of an individual might be impaired even when signs of stress are not obviously visible.

The scope of this article is to briefly review the historical development in stress research, to emphasize the current understanding of the neurobiological integration of stress, and to summarize some of the approaches to measure and interpret stress in farm animals during housing and transportation.

History of stress research

The effort of the body to maintain a stable internal environment to challenges from widely variable environments was first described by Claude Bernard (1878) and later referred to as homeostasis (Cannon, 1932). The concept and the related definitions of stress were given by Walter Cannon (1914) and Hans Selye (1936). They discovered that diverse detrimental stimuli (stressors) such as pain, hunger, thirst, severe climatic conditions, or noxious agents cause physiological

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changes in the animal that might lead to a pathological state in the long run. The nonspecific response to stressors such as the release of adrenal hormones was defined as a state of stress. Some of the long-term pathological consequences of stress are hypertrophy of the adrenals, atrophy of the thymolymphatic system and stomach ulceration. Cannon (1914) emphasized the emergency function of the adrenal medulla during stress. The hypothalamic-adrenal medullary system involves the hypothalamus, pituitary gland, the sympathetic neural pathways to the adrenal medulla, and the release of epinephrine by the adrenal gland. This short-acting stress-response was referred to as the fight-flight syndrome (**FFS**). The hypothalamic-pituitary-adrenocortical (**HPA**) stress-response system represents a longer-term, sustained response to stressors and was conceptualized by Hans Selye (general adaptation syndrome; **GAS**; Selye, 1946). The major adrenal cortical hormones are corticosteroids and aldosterone. Selye distinguished three phases of the stress response that are described as 1) alarm (Cannon's emergency concept), followed by 2) resistance (i.e., by release of corticosteroids), which if unsuccessful results in 3) exhaustion of the response system (prepathological state) and eventually death. The theory of nonspecificity of stressors was later questioned by pychophysiologists, such as Mason (1971). The activation of the HPA-axis is mainly dependent on the emotional involvement of the animal; stressors do not necessarily activate the HPA system when the animal does not perceive the situation as stressful. Based on this research, the new disciplines of psychoneuroendocrinology and psychoneuroimmunology, which evolved two decades ago, were substantially influenced by the work of Robert Ader. Numerous studies indicate that psychosocial stress can have profound effects on the immune system (Dantzer and Kelley, 1989, Ader et al., 1990). The term "stress" is used today in a much broader sense. As for the definition of "animal welfare" (Broom, 1996a), stress could refer to the coping of the animal with the environment, describing the animal's state when it is challenged beyond its behavioral and physiological capacities to adapt to its environment (Terlouw et al., 1997). The term "distress" relates to the emotional content of noxious experiences and the resulting emotional state of an animal (Hurnik et al., 1985; Mellor et al., 2000). In the context of animal well-being, this term has been used to differentiate between nonthreatening and threatening stress responses. Distress refers to a biological state in which the stress response to a threat (stressor) has a deleterious effect on the individual's well-being (Moberg, 2000). Some decades ago, Selye (1979) already distinguished between good (eustress) and bad (distress) stressors. Others argue that such a distinction between stressors becomes unnecessary in that any stressor can have a detrimental effect on the organism if it occurs often enough, and it would be hard to determine when stress becomes distress (Ladewig, 2000).

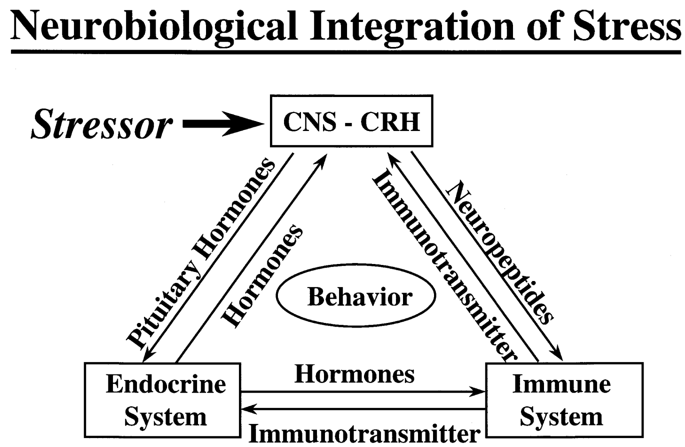

Figure 1. Communication between central nervous system (CNS), endocrine system, and immune system. CRH = corticotropin-releasing hormone.

Neurobiological Integration of Stress

The response to stressors requires a progression of events beginning with sensing and signaling an animal's various biological mechanisms that a threat exists. These events are followed by activation of neurophysiological mechanisms to mount a biological effort to resist and prevent major damage (Ewing et al. 1999). The various sensory detectors not only receive the information but also transform that information into neural signals that are transmitted to either or both cognitive and non-cognitive centers of the nervous system to generate a coordinated response to the challenge. The central nervous system (**CNS**), endocrine system, and immune system interact, respond to stressful stimuli in a coordinated manner, and influence the behavior of an animal (Figure 1). The presence of hormones, neurotransmitters, and receptors common to all three systems supports the view that communication exists between these systems (DeSouza, 1993).

The parasympathetic nervous system maintains homeostasis and is mainly responsible for energy conservation and relaxation during stress (Porges, 1995). Parasympathetic activities are antagonized by sympathetic activities that mobilize energy during stress. The secretion of catecholamines (epinephrine and norepinephrine) from the adrenal medulla is characteristic of the FFS, preparing the body for an active coping response to the stressor (i.e., by increasing heart rate and blood pressure). Studies on different individual coping strategies have shown that proactive animals (removing themselves from the source of stress or removing the source itself) seemed to be predominated by the sympathetic nervous system, whereas the reactive animals (aimed to reduce the emotional impact of stress) seemed to be predominated by the parasympathetic nervous system (Koolhaas et al., 1999). The ratio between sympathetic and parasympathetic activity

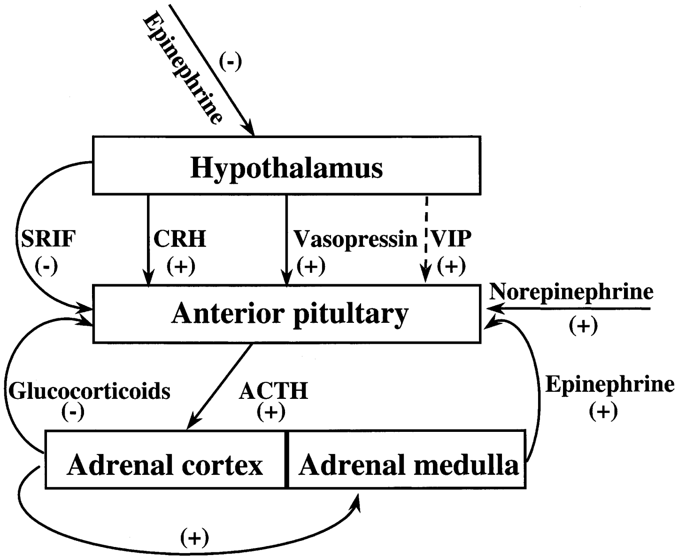

Figure 2. Regulation of glucocorticoid secretion (modified after Axelrod and Reisine, 1984). SRIF = somatotropin-releasing inhibitor factor (somatostatin); CRH = corticotropin-releasing hormone; VIP = vasodilatory intestinal peptide.

determines whether an animal is in a state of physical or psychological arousal or relaxation.

Cognitive brain centers such as the cerebral cortex perceive external threats and act to initiate response mechanisms via nervous signals that activate corticotropin-releasing hormone (**CRH**) producing neurons, mainly in the paraventricular nucleus of the hypothalamus (Johnson et al., 1992). Corticotropin-releasing hormone is released by axon terminals that project to the region of the median eminence and is transported by the hypophyseal portal blood system to the anterior pituitary, where it increases the synthesis and secretion of ACTH, β-endorphin, β-lipotropin, and α-melanocyte-stimulating hormone/α-melanotropin (Axelrod and Reisine, 1984). Corticotropin-releasing hormone not only activates the HPA axis, it also has a neurotransmitter function in the brain. For example, the intracerebroventricular administration of CRH activates the sympathetic and adrenomedullary system, resulting in elevations of plasma catecholamine concentrations and increases in arterial pressure and heart rate (Fisher et al., 1982). Increasing glucocorticoid concentrations in the blood may inhibit ACTH secretion from the pituitary gland (feedback control; Figure 2). However, the release of ACTH is dependent on the intensity of the stressor and modulated by a steady-state sensitive feedback mechanism. Mild stressors might, therefore, be gradually inhibited by a glucococorticoid feedback, whereas severe stressors are not inhibited (Axelrod and Reisine, 1984).

Corticotropin-releasing hormone receptors have been identified in several brain regions including areas involved in cognitive function as well as in limbic areas involved in emotion (DeSouza et al., 1991). This explains why centrally administered CRH causes emotional responses in animals (Dunn and Berridge, 1990; Johnson et al., 1994). The limbic system of the brain includes structures such as the hypothalamus (the endocrine control center), thalamic nuclei, amygdala, and hippocampus (Figure 3). Henry and Stephens (1977) hypothesized that the amygdala and hippocampus are involved in two different coping processes that enable an animal to respond to threatening and frustrating stimuli (coping/predictability concept). Several studies have confirmed that unpredictable or uncontrollable stimuli will activate the hippocampal pathway and the HPA axis, leading to depression of behavior. The ability to adjust to some stressors (controllability), however, seems to be under the control of the amygdala through activation of the sympathetic nervous system and prepares the animal for fight and flight responses (see Figure 4). The activation of one or the other system depends on many factors such as genetic disposition, early experience and cognitive ability, and on the stressor quality and quantity (Ladewig, 1994). Psychological stressors perceived as threats may be equally important as those of a physical nature in challenging coping mechanisms. Situations of uncertainty, social pressure and fear are potent stressors with relevance for the well-being of animals, leading to severe damage to specific target organs and tissues or even to death in some species (von Holst, 1997).

Correlative evidence supports a link between stress and disease susceptibility. Both physical and psychological stressors have been demonstrated to suppress T- and B-lymphocyte blastogenesis, natural killer cell activity, and cytokine production (interleukin-2 [**IL-2**] and interferon-γ [**IFN-γ**]; Cunnick et al., 1988; Sonnenfeld et al., 1992). Cytokines (i.e., IL-1, IL-6, tumor-necrosis factor-α [**TNF-α**], and IFN-γ are considered as mediators of the immunological and pathological responses to stress and infection (Kelley et al., 1994; Warren et al., 1997). The cytokine IL-1 not only induces fever and reduces food intake, it also stimulates the HPA axis and inhibits the hypothalamic-pituitary-gonadal axis (Dantzer and Kelley, 1989). The release of stress hormones does, however, not only suppress immune competence. Glucocorticoids are also known to increase the nonspecific resistance toward stressors and infections (Larson et al., 1985). If glucocorticoids are not present when an animal is severely stressed, there is no sustained metabolic response and the animal will be less able to cope with the stressor (Ewing et al., 1999). This explains why animals with inadequate function of the adrenal glands are more likely to die from stress than others with intact glands (von Faber and Haid, 1995).

Stress in Livestock during Housing and Transportation

Studies on stress responses in farm animals are often conducted on the basis of single physiological alter-

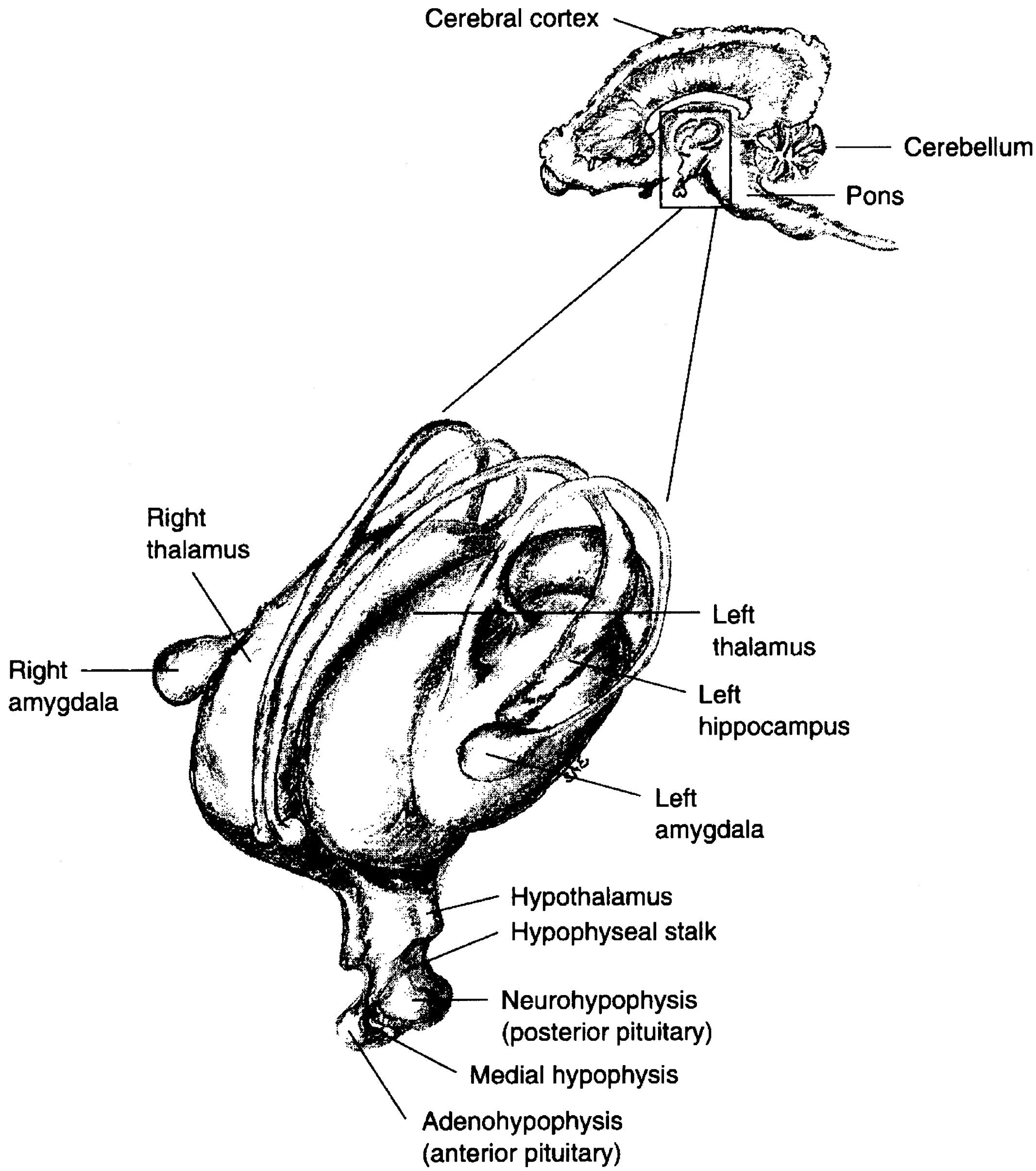

Figure 3. Limbic system (from Ewing et al., 1999, p 38).

ations or irregular behavioral phenomena that might be difficult to interpret. In the past, most physiological studies on stress were solely based on the analysis of glucocorticoids or catecholamines without considering that multiple physiological systems are altered during stress (Dantzer and Mormède, 1983). These measurements of single variables (i.e., cortisol) are of little value when not considering the context in which the substance is released and not knowing what consequences it has for an animal's well-being (Rushen, 1991). A major problem of applied stress research in livestock relates to the methodology of stress assessment or to the nonspecificity of the selected variable. The stress an animal might experience during the blood collection procedure might interfere with the effect of housing or management procedure under investigation. Vocalization analysis has been useful to obtain information on stressful procedures such as the castration of young pigs (White et al., 1995; Weary et al., 1998; Horn et al., 1999). Housing environments often do not allow continuous blood sampling (i.e., in groups of free-moving animals), which is usually a prerequisite for consideration of the natural circadian and episodic release of hormones (Ladewig and Smidt, 1989). Remotely controlled blood sampling devices (Carragher et al., 1997) and wireless heart-rate monitors (Hopster and Blokhuis, 1994; Marchant et al., 1997; Hansen and von Borell, 1998) can help to overcome some of these problems. Elevated hormone concentrations as well as an increase in heart rate does not necessarily relate to a stressful situation with negative consequences for the well-being of the animal. Physical activities (Jacobson and Cook, 1998) and presumably pleasurable events such as sexual intercourse (Lade-

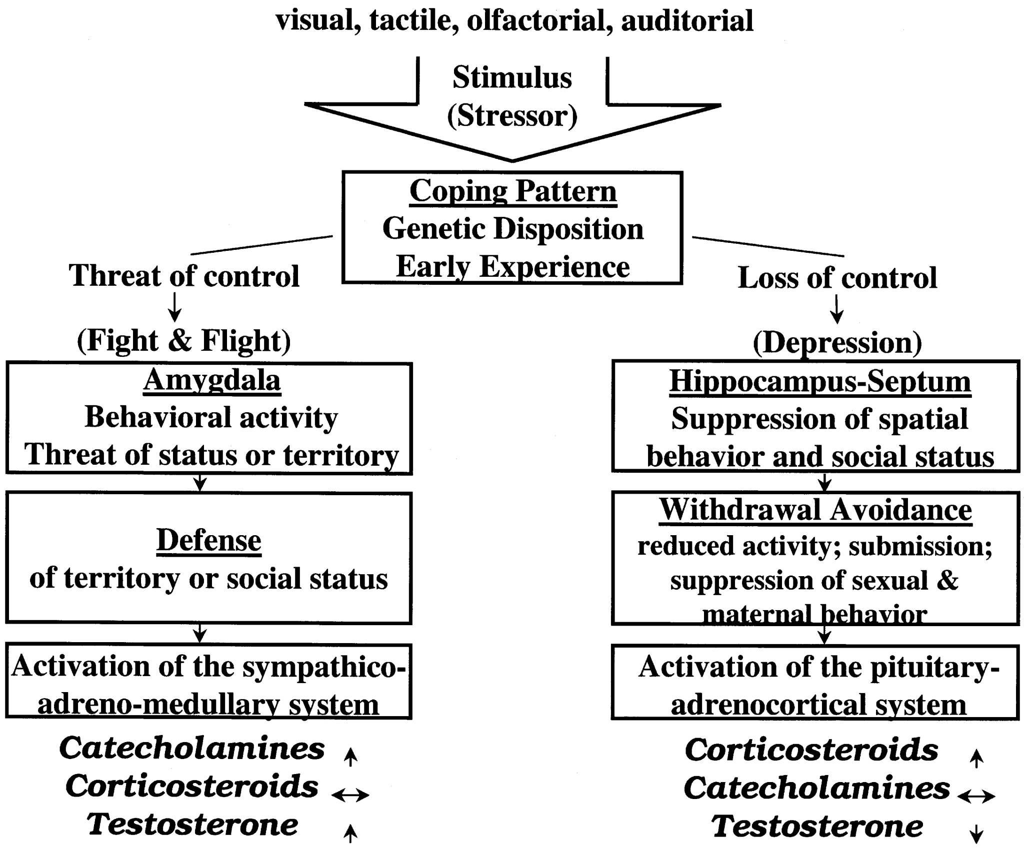

Figure 4. Coping/predictability concept (modified after Henry and Stephens, 1977).

wig, 2000) also increase these traits. Recent studies utilize a new variable deriving from heart-rate fluctuation (analysis of beat-to-beat intervals: heart-rate variability; Akselrod, 1995) with which the relative ratio between sympathetic and parasympathetic activity can be estimated (Thayer et al., 1997; Mohr et al., 2000). The method has been recently applied to pigs for the assessment of social stress and stereotypic behavior in relation to housing conditions (Hansen, 2000; Schouten et al., 2000).

The impact of housing conditions and management procedures on physiological traits depends on the duration and intensity of the factor that acts on the animal. Short stressful events (i.e., direct handling, isolation, and transportation) are usually followed by an increase in stress hormones, whereas chronic events (i.e., long-acting heat stress or restraint) might not affect the basal hormone concentration at all or even lower the concentration in the blood (down-regulation; Ladewig, 2000). Chronic, presumably stressful, situations such as permanent tethering of animals might only be visualized by hormone profiles that are continuously recorded (Ladewig and Smidt, 1989) or by testing the function of the adrenal cortex via stimulation with ACTH (von Borell and Ladewig, 1989; ACTH challenge test). The results from these studies are often contradictory, presumably reflecting the differences in the quality, intensity, frequency, and duration of the impinging stressor (Jensen et al., 1996). For example, repeated stressors of low intensity such as a confrontation with a novel housing environment will eventually lead to a decreased response intensity, whereas repeated painful stimuli are likely to increase the response (concept of desensitization vs sensitization; as reviewed by Ladewig, 2000). Therefore, animals might adapt to long-acting housing conditions that are presumed to be perceived as stressful (i.e., tether housing) or might not respond with permanent alterations on the various stress response systems. One could hypothesize that animals under intensive housing conditions are only exhibiting chronic-intermittent stress responses occurring in situations such as standing up or lying down in inadequately designed cubicle or tether stalls (Ladewig and Smidt, 1989; Ladewig, 2000). In a recent study on the effect of repeated social isolation in pigs, ACTH and cortisol responses gradually diminished with repeated stressor exposure, whereas the epinephrine and heart-rate responses remained elevated (Schrader and Ladewig, 1999).

The response of an individual animal to a stressor also depends on genetic factors and early experiences. There is a debate among behavioral physiologists whether farm animals develop distinct coping strategies as demonstrated in laboratory animals. Recent studies with cattle and pigs support that idea (Mendl et al., 1992; Boissy and Bouissou, 1995; Ruis et al., 2000).

The interaction between the CNS and endocrine and immune systems has also been studied in farm animals. Centrally administered CRH to pigs resulted in characteristic dose-dependent endocrinological, immunological, and behavioral responses (Johnson et al., 1994). More recent studies are concerned with the role of glucocorticoid and mineralocorticoid receptors in the limbic system of pigs in relation to weaning and restraint stress (Kanitz et al., 1998, Zanella, 1999).

Handling and transportation are considered as major stressors for farm animals (as reviewed by Grandin, 1997; Knowles and Warriss, 2000) and might have deleterious effects on health, well-being, performance, and, ultimately, product quality (Lay et al., 1996; Schütte et al., 1996; Geverink et al., 1998). Most studies on farm animals were concerned with stress during animal handling and to a lesser degree on transportation stress per se. This has to do with the methodological difficulties in obtaining information from animals in an environment where space and visibility are very limited. These studies, therefore, require noninvasive methods because classical approaches to data collection with direct human interference (i.e., for blood collection and heart rate measurement) might directly alter the stress response. Telemetric devices for measuring heart and respiration rate, body temperature, and blood pressure are useful tools for obtaining undisturbed responses. Recently, noninvasive measurements of stress indicating metabolites in saliva, feces, or urine have been developed and validated (Broom, 1996b; Palme et al., 1996, 2000). A saliva collection device (Oral-Diffusion-Sink; Wade, 1992) has been recently applied to stress research in pigs (Schönreiter and Zanella, 2000). The device stays for a defined period of time (i.e., during transportation) in the mouth of an animal and collects stress hormones that have passed a membrane into a tube from where the average hormone concentration is analyzed. A combination with detailed behavioral observations (via videorecordings) is required for these physiological stress variables in order to facilitate interpretation of results for the specific situation in which they occur.

Implications

Recent stress research indicates that the endocrine, immune, and central nervous systems interact and respond to stressful stimuli in a coordinated manner. The presence of hormones, neurotransmitters, and receptors common to all three systems supports the view that communication exists among these systems. Psychological stressors perceived as threats may be equally important as those of a physical nature in challenging coping mechanisms. Situations of uncertainty, social pressure, and fear are potent stressors with relevance for the well-being of animals. Noninvasive methods for measuring stress-indicating variables have been developed in addition to classical descriptive behavioral observations, allowing an evaluation of stress by multiple criteria under different housing conditions and management procedures. These behavioral and physiological measurements provide valuable information on how livestock housing, handling, and transportation can be improved in the near future.

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