



## The contribution of total and free iodothyronines to welfare maintenance and management stress coping in Ruminants and Equines: Physiological ranges and reference values



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### ABSTRACT

In order to acquire a pattern of thyroid involvement in welfare maintenance in Ruminants and Equines, this review summarizes data concerning the reference values of total and free iodothyronines and their modifications in physiological conditions and in different management conditions (pregnancy, lactation, weaning, growth, isolation, restraint, shearing, confinement and transportation). Thyroidal and extrathyroidal tissues efficiently respond to management practices, giving a differentiated contribution to circulating iodothyronine changes. The hormonal response could be mainly attributed to the intracellular deiodination of  $T_4$  to  $T_3$ . Triiodothyronine ( $T_3$ ) and free iodothyronines ( $fT_3$  and  $fT_4$ ) result more responsive to management stress, showing different pattern with species and to various conditions, as to environmental conditions in which activities are performed. Intrinsic seasonal changes of iodothyronines and a significant pregnancy effect for  $T_3$  were recorded in mares. Higher, although not significant,  $T_3$  and  $T_4$  concentrations in barren than pregnant mares were observed in donkeys. A positive significant correlation between  $T_3$  and  $T_4$  was described only in pregnant donkeys. Moreover, a significant effect of season on  $T_3$  and  $fT_3$  changes was observed both in pregnant and barren donkeys. A significant lactating effect compared with nonlactating stage for  $T_3$  and  $T_4$  was recorded in mares. In growing foals, body weight (BW) and age were positively correlated with  $T_3$  and negatively correlated with  $T_4$ ,  $fT_4$  and  $fT_3$ . Weaning effects were shown for  $T_3$  and  $fT_4$  concentrations, indicating that weaning represents a severe stress and the presence of conspecific does not reduce psychological stress in this phase. Lambs showed significant decreased  $T_3$  and elevated  $T_4$  concentrations two weeks after weaning, with higher concentrations in both males and females compared to 24 h. Significant positive correlations were observed between BW and  $T_4$ ,  $fT_3$  and  $fT_4$  concentrations in lambs. A  $T_3$  decrease was detected after isolation, such as induced by confinement and weaning in lambs. Higher  $T_3$  concentration after restraint and shearing than after isolation and significant increases in  $T_4$ ,  $fT_3$  and  $fT_4$  values after restraint and shearing were recorded. The basal concentrations of  $fT_3$  in both the inexperienced and experienced transported horses were significantly higher than in untransported experienced horses. Moreover, increases of  $T_3$ ,  $T_4$  and  $fT_4$  after short road transportation, and significant correlations between  $T_3$  and rectal temperature (RT), body weight (BW) and heart rate (HR), confirmed their important role in coping strategy. Thyroid responsiveness to short transport is similar in domestic donkeys and horses, with a preferential release of  $T_3$  in horses. A greatest and constant release of  $T_3$  and  $T_4$ , although differentiated, after simulated transportation and after conventional transport of horses confirmed that the degree of stress induced by confinement and additional stressful stimuli associated to road transportation could differently influence the iodothyronine release. Temperamental Limousin young beef bulls showed lower  $T_4$  and  $fT_4$  concentrations after prolonged transportation than calm subjects, and a concomitant decrease of circulating ACTH, cortisol,  $T_3$  and  $fT_3$  concentrations, probably induced by down regulation of HPA axis and cortisol negative feedback. These data reinforce the importance of taking into account the evaluation of iodothyronines, and notably of  $T_3$ , as markers of welfare and stress and their role in ensuring energy homeostasis and productive and reproductive performances in Ruminants and Equines.

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## 1. Introduction

Welfare is the result of appropriate integration of the animal with its own means and with the environment; i.e. the ability to satisfy its essential needs, hierarchically organized. A generalized physiological activation of organism (arousal) to positive or negative stimuli, behavioral manifestations and biochemical and functional adaptations normally aim at maintenance of functional and emotional homeostasis. Then, stress in animals is the consequence of adverse effects of environment or management systems which force changes in an animal's physiology or behavior to avoid physiological malfunctioning, thus assisting the animal to cope with its environment.

Animals are considered “sentient beings” and can show affect, emotion, feelings and suffering. This implies that the animal must be guaranteed the possibility of expressing its functional and emotional homeostasis also in situations potentially producing pain, suffering and distress. Nevertheless, to extract information about animal emotions, their cognitive component has not been sufficiently considered so far, although the link between cognition and emotion has been extensively investigated in humans as indicating cognitive processes play an important role in generating the emotional state. The emotional state, in turn, does influence cognitive function by inducing a change in the processes of attention and memory assessment of environmental stimuli (Paul et al., 2005; Lesimple et al., 2011), detectable through the analysis of behavioral manifestations of animal cognitive biases (Mendl et al., 2009). All domestic species exhibit cognitive capacities, but with varying degrees of awareness, and emotional responses (emotion) which, depending on the degree of species-specific and individual awareness, can vary in terms of valence and intensity, creating a conscious emotion or not (feeling), that can change the behavior or act as reinforcement in learning processes (Veissier et al., 1998; Broom, 2008). This “allostatic capability” of an animal is expressed through dynamic mechanisms of physiological adaptation (coping) with species-specific and individual characteristics as a function of multiple “collative” factors affecting activation modules of stressful conditions (e.g., degree of novelty, social support, etc.), making use of specific neuro-functional mechanisms and biomodulator of nervous function and metabolic, functional and immunitary processes.

Then, stress is completely defined as the combination of mental and biological responses of an animal to novel and threatening physical and psychological stimuli and refers to situations where there is failure to cope and when the individual is having difficulty in coping (Broom, 2008). The different cognitive ability and awareness of species can have a large influence on coping systems (Broom, 2010).

The individual response to stress is, ultimately, the function of individual “allostatic load”, i.e. the capacity of perception of stress and its control. With this in mind, stress responses must be mainly regarded as adaptive responses that enable animals to respond to environmental changes (McEwen, 2000, 2007; McEwen and Wingfield, 2010).

It is well known that physiological activities and management practices of livestock can induce associated coping responses to increased required metabolic or mental tasks, which can interfere in welfare maintenance.

On these bases, the application of the principle of “refinement”, with respect to the functional and behavioral needs of the species, elicits the “enrichment” of the environmental conditions in which animals live, and, especially, the prevention of mental distress and discomfort. Aware medical and technical acts -from animal manipulation techniques to the attention of management procedures, with a focus on “ethological needs” of the subjects - could affect positively the cognitive assessment of potential stress conditions, promoting the adoption of physiological coping strategies.

In fact, European Union (EU) policy states that animal welfare is an essential part of farming practice and, recently, Animal Welfare Strategy 2012–2015 focused the steps to improve standards, ensuring that they serve their target, the animals, on an outcome based animal

welfare indicators. Moreover, the “Animal Welfare Action Plan 2006–2010” pointed out that an assessment of welfare requires objective, reproducible data.

Adverse states in animals can be assessed primarily by observation of their behavior and by measuring physiological and biochemical changes. On the other hand, physiological measurements of stress are dependent on the interaction of many systems and some stress responses can be measured through functionality of the primary system involved.

So far, the capacity to express the specific coping responses to different physical and/or mental stressors has not been extensively explained in domestic animal species. In fact, it has been underlined that the level of complexity of the function of the animal should be taken into account when affording management practices and husbandry systems for the species to allow welfare (Broom, 2010).

To assess the impact of different stressors into animal welfare, in order to indicate whether a stimulation could be considered as beneficial (eustress) or not, needs to ascertain the correct involvement of aspecific or specific biological coping systems. Then, the definition of specific implications of different systems involved in coping responses to different stressors could be of valuable importance to assess the sustainability of the livestock management's procedures.

Animals kept in intensive housing systems are exposed to different stress conditions and they must afford the potentially aversive stimuli exhibiting coping responses, which can be either aspecific or specific, according to the nature and intensity of stressors. Animals perceive specific features in their physical and social environment. Changes in temperature and humidity, inadequate ventilation, deprivation of food and water, handling, restraint and isolation, overcrowding are significant stress conditions. Environmental stressors (heat, cold, humidity, etc.), pain or diseases are considered physiological stressors. Restraint, isolation or maternal separation can be regarded as mental or psychogenic stressors. In farm animals, recently, the role of positive social support in enhancing stress coping abilities and welfare in comparison to negative social behavior has received growing interest (Rault, 2012). Management and animal production procedures, such as shearing, breeding, milking, milk yield, transport, as well as their quality, can have a great impact on health and welfare of farm animals (Broom, 2010; Oltenacu and Broom, 2010; Broom et al., 2013; Ferlazzo et al., 2017). Age, sex and physiological conditions also affect the behavior of animals. In all these conditions, the response to stressors is dependent on collative factors' influence, such as novelty, previous experience, presence of co-specific, etc. (Fazio et al., 2011; Fazio et al., 2015a, 2015b).

Adaptive mechanisms rely on executive structures (hypothalamus, basal nuclei of the prosencephalon, mesencephalic tegment) and numerous central and peripheral biomodulators (cytokines, endorphins, dopamine, serotonin, prostaglandins). These biomodulators provide functional and behavioral adaptations to adequate levels of physical and emotional stress tested.

Physiological stressors involve neuronal circuits that decode stimuli at the level of the brain stem; previous experiences of mental stressors involve limbic areas, such as amygdala, hippocampus and the frontal cortex. All stimuli converge at the paraventricular nucleus (PVN) and activate the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis, inducing release of catecholamines, corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol.

There is an extensive literature on behavioral changes induced by stress in different farm animal species (Boissy and le Neindre, 1997; Veissier and le Neindre, 1992; Jensen et al., 1999; Jensen, 2001; Pedersen et al., 2002; da Costa et al., 2004; Mounier et al., 2006). Physiological measurements, i.e., increased heart rate (HR), increased respiratory rate (RR), adrenal activity, adrenal activity following ACTH challenge, or reduced immunological response following a challenge, can all indicate that welfare is poor (Broom, 1998).

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