

Action of exogenous oxytocin on stress modulation in crossbred Red Angus cows

Ação da ocitocina exógena na modulação do estresse em vacas mestiças Red Angus

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Abstract

Cattle (*Bos taurus* and *Bos indicus*) are organised on the basis of leadership and dominance in such a manner that a disturbance by an external stressor causes negative effects on their health, productivity, well-being, and behaviour. One of these effects is the excessive release of glucocorticoids, which results in increased alertness. We evaluated the action of exogenous oxytocin (OT) on serum cortisol levels in crossbred Red Angus heifers. Twelve Red Angus crossbred heifers were moved daily from the pasture to the corral in weeks 1 and 2 for adaptation to human contact and handling in the cattle crush. In weeks 3 and 4, they were divided into two groups of six (T1 and T2). The T1 group was administered 20 UI (2 mL) of OT via intramuscular injection and the T2 group was administered 2 mL of saline solution 0.85% (SS). In weeks 5 and 6, they were only contained in the cattle crush for evaluation. On days 01, 07, 14, 21, 28, 35, and 42, blood samples were collected by jugular venepuncture in vacuum tubes without anticoagulants. Then, serum cortisol levels were measured using a radioimmunoassay. In the period of adaptation, during weeks 1 and 2, serum cortisol levels decreased in both the groups, with higher levels in the SS group; the same result was obtained in weeks 5 and 6. During treatment, however, there was a significant difference between the two groups in week 4, with a reduction in cortisol levels in the OT group. This result suggests a modulator effect of OT on neuroendocrine response to stress.

Key words: Animal welfare. Behaviour. Cattle. Cortisol. Handling. Hormone.

Resumo

Os bovinos se organizam baseados na liderança e na dominância que quando alterados por um agente estressor externo pode ocasionar efeitos negativos na saúde, produtividade, bem-estar e comportamento. Sendo um dos efeitos a liberação de glicocorticoides de forma explosiva colocando o animal em alerta. Dessa forma, foi avaliada a ação da ocitocina (OT) exógena sobre o cortisol sérico em novilhas mestiças Red Angus. Doze novilhas mestiças Red Angus foram conduzidas diariamente ao curral nas semanas 1 e 2 para adaptação ao contato humano e manejo no tronco, nas semanas 3 e 4 divididas em dois grupos de seis submetidas aos seguintes tratamentos: T1 - 20 UI (2 mL) de OT; T2 - 2 mL de solução fisiológica 0,85% (SF), IM e nas semanas 5 e 6 somente contidas no tronco para avaliação. Nos dias 01, 07, 14, 21, 28, 35 e 42 amostras de sangue foram colhidas por venopunção da jugular em tubos à vácuo sem anticoagulante. No soro obtido mensurou-se o cortisol sérico por radioimunoensaio. No período

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de adaptação S1 e S2 houve redução do cortisol sérico nos dois grupos, com níveis maiores no grupo SF o mesmo ocorreu nas semanas 5 e 6. Durante o tratamento ocorreu uma diferença significativa entre os dois grupos na semana 4 com redução dos valores de cortisol no grupo OT. Com isso, sugerindo um efeito modulador da OT na resposta neuroendócrina ao estresse.

Palavras-chave: Bem-estar animal. Bovino. Comportamento. Cortisol. Hormônio. Manejo.

Cattle (*Bos taurus* and *Bos indicus*) are gregarious animals that develop social organisation mechanisms within groups on the basis of familiarity and competition, which result in leadership and dominance hierarchy as forms of social control (PARANHOS DA COSTA; NASCIMENTO JÚNIOR, 1986). Many factors influence animal behaviour within rearing systems. Stressful situations are characterised by an anticipated or actual state of threat to body equilibrium and the resultant response of the body. This response aids in the re-establishment of equilibrium through a complex set of physiological and behavioural responses. Stressful situations are common in dairy cows and have serious effects on their health, productivity, well-being, and behaviour. Responses to stress are mediated by the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, with widespread effects throughout the body (KIERSZENVBAUM; TRES, 2012).

Extensive research on the peripheral action of oxytocin (OT) during labour and milk ejection has been published. Recent studies have also shown that OT is released from the neurohypophysis into the bloodstream and into distinct brain regions (HEINRICHS et al., 2009). The release of OT is associated with the control of stress and anxiety (WINDLE et al., 2004), and the establishment of social relations (LIM; YOUNG, 2006). Positive social behaviour has been reported to decrease hypothalamic-pituitary-adrenal axis activity, resulting in stress reduction, which suggests that OT is able to increase social interactions (CARTER, 1998).

The aim of the present study was to assess the effects of exogenous OT on serum cortisol levels in crossbred Red Angus heifers.

This study, which was conducted between October and November 2012, included 12 crossbred Zebu × Red Angus heifers (age 2-3 years) from the herd of the Universidade Federal Rural do Rio de Janeiro (UFRRJ). Prior the experiment, all animals were dewormed (albendazole base) and received topical treatment against ticks (cypermethrin base). The animals were weighed, identified and housed in a piquet. For the first 15 days (i.e., weeks 1 and 2), the heifers were moved daily to a corral in the morning (between 07h00 and 08h00). The heifers passed through a cattle crush, where they were contained for about 1 minute for adaptation to human contact and handling. Then, they were released into a fenced area and were allowed to feed on forage (chopped grass: *Panicum maximum*) and a commercial concentrate (1.5 kg animal day⁻¹).

After adaptation, the animals were randomly separated into 2 groups of 6 each. Group 1 (T1) received 20 UI (2 mL) of OT, and group 2 (T2) received 2 mL of saline solution 0.85% (SS). The treatment was carried out daily for 15 days (i.e., weeks 3 and 4). In the following 15 days (i.e., weeks 5 and 6), the animals were contained in the cattle crush for evaluation and then released to receive feeds similar to previous weeks (W).

In the morning (07h00 to 08h00) on days 01 (baseline), 07 (W1), 14 (W2), 21 (W3), 28 (W4), 35 (W5), and 42 (W6), blood samples were collected via jugular venepuncture in vacuum tubes without anticoagulants. The samples were stored in an inclined position at 4°C for 2 hours or until complete coagulation and clot retraction was observed. Next, the blood was centrifuged for 15 minutes at 1500 rpm at 4°C, and 1.0-mL aliquots of serum were stored at -80°C. These samples were

later processed using a radioimmunoassay at the UFRRJ to determine cortisol level.

In the adaptation period, the animals went from being agitated and aggressive to quiet when they were moved from the pasture to the corral and the cattle crush, which was accompanied by the decrease in serum cortisol levels in the animals in both the groups between weeks 1 and 2; however, cortisol levels were higher in the control group (SS).

When received supplementation in the trough, two heifers (one in each group) expressed aggressive behaviour, competing for space and food and blocking access to other animals throughout the experiment period. According to Paranhos da Costa and Nascimento Júnior (1986), this behaviour is a typical aspect of dominance and is common in the social organisation of the species. The other heifers did not change their behaviour during the experiment. This finding was in agreement with the report by Paranhos da Costa and Costa e Silva (2007), who showed that upon establishment of a social hierarchy in a group, the order is relatively stable and roles are respected. In such situations, disputes and disagreements are rare and order is maintained through simple threats.

Heifers in the OT group did not show changes in behaviour that could be linked to OT. Not even the heifer that displayed dominant behaviour exhibited significant behavioural changes after OT injection, represents a clear adaptation to daily handling.

The period of 6 weeks was considered sufficient for the establishment and maintenance of social order in the group, and using a number of animals suitable for the physical space helped to minimise the social stress resulting from individual space violation. In agreement with this, behavioural disorders that are typical of stress or improper handling were not observed in the heifers in the present study.

Serum cortisol levels in cattle show wide variations according to the literature. They vary according to animal breed and the conditions under

which the studies were conducted. Gatto (2007) pointed out the possibility of variation depending on the methodology used and on individual responses. Under the conditions of the present study, serum cortisol levels ranged from $0.5 \mu\text{g dL}^{-1}$ to $10.5 \mu\text{g dL}^{-1}$. Therefore, the lowest level obtained was normal and the highest was increased compared with reference values for the species (RADOSTITS et al., 2002). Individually, all animals showed elevated levels of serum cortisol at some point. The two animals with the highest variations in serum cortisol levels displayed typical dominant behaviour, especially during feeding.

According to Farwell et al. (1983), basal cortisol levels are relatively constant in any individual animal ($0.5\text{-}2.0 \mu\text{g dL}^{-1}$) and show circadian variations, with levels being higher in the morning. The values detected in our study contradict these findings because the overall and individual values varied up to 6.2-fold and 10.4-fold in the SS and OT groups, respectively, in the same week under the same conditions of handling and treatment.

The maximum, minimum, mean and the standard deviation of weekly serum cortisol levels in both the groups is shown in Table 1.

From the second week onwards, mean serum cortisol levels were always higher in the control group (saline solution). The differences were not statistically significant ($P \geq 0.05$) in the adjustment period (W1 and W2) and after treatment (W5 and W6). However, serum cortisol levels tended to be decreased in the OT group, with a statistically significant difference ($P = 0.0303$) compared to the SS group, after 14 days of treatment (W4). Lower levels in the OT group ($1.7 \pm 0.7 \mu\text{g dL}^{-1}$) suggest a modulatory effect of OT on the neuroendocrine response to stress, as proposed by Windle et al. (2004) and Neumann (2008). In the context of animal welfare, plasma cortisol levels are considered an indicator for the analysis of short-term effects of handling practices (BROOM; FRASER, 2007) and a sign of the physiological response to stress

(BOND et al., 2012). Thus, the reduction in mean serum cortisol levels observed during the adaptation period in the present study reflects the quality of handling of the animals.

Table 1. Maximum, minimum, mean and standard deviation (SD) of serum cortisol ($\mu\text{g/dL}$) in crossbred Zebu \times Red Angus heifers according to the daily treatment with 20 UI (2 mL) of oxytocin (OT) or saline (SS) administered intramuscularly during containment in the cattle crush.

Week	Group	Average \pm SD	Minimum	Maximum	P Value	Handling
W1	OT	2.7 \pm 1.5	1.2	4.9	0.8646	Adaptation-untreated
	SS	2.5 \pm 0.5	2	2.9		
W2	OT	1.6 \pm 0.6	0.7	2.4	0.2300	Adaptation-untreated
	SS	3.0 \pm 2.3	0.5	5.7		
W3	OT	1.5 \pm 0.9	0.5	2.5	0.0967	Treatment
	SS	3.1 \pm 2.2	0.5	6.9		
W4	OT	1.7 \pm 0.7	0.9	2.5	0.0303	Treatment
	SS	4.7 \pm 2.6	1.9	9.4		
W5	OT	1.8 \pm 1.0	0.6	2.9	0.0779	Evaluation-untreated
	SS	3.9 \pm 3.3	1.9	10.5		
W6	OT	2.4 \pm 1.1	1.2	3.9	0.5738	Evaluation-untreated
	SS	2.8 \pm 1.5	1.2	5		

It is worth highlighting that the blood collected to determine cortisol levels was obtained during containment and soon after the administration of either OT or SS. Previous research indicates that it takes approximately 20 min for cortisol levels to increase after exposure to acute stress and 2 h to reach its maximum level (SILANIKOVE, 2000). Thus, physiologically, the levels obtained in the present study reflect the animals' condition prior to blood collection. Hence, animal handling contributed to the effects on cortisol levels and to the expected effect of OT as indicated in the results of week 4.

Administration of exogenous OT induced stress modulation 14 days after treatment, demonstrated by reduction in serum cortisol levels in the treated heifers compared with the control group in the same week.

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