

Full Length Research Paper

Concentration of fecal corticosterone metabolites in dominant versus subordinate buffalo heifers

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The objective of this work was to evaluate the concentration of fecal metabolites of corticosterone and to verify if there are differences between dominant and subordinate heifers. The feces of 18 buffalo heifers were collected in the estrous period, to quantify the corticosterone concentrations. The heifers were separated into three groups (G1, G2 and G3) and synchronized. The observations of the social and sexual behaviors were recorded and, from these results, the sociometric matrix was constructed to establish the social index and determine the hierarchic positions of the buffalo heifers as low, moderate and high. The fecal concentrations of corticosterone were higher in animals with high hierarchic position on day zero and describe alterations in the dominant females before synchronization, suggesting that there is an energy cost for the females in the highest position to be able to maintain their dominance status.

Key words: Estrus, hormones, social status, non-invasive technique.

INTRODUCTION

Several studies have been conducted linking glucocorticoids to social and sexual behavior of animals. Glucocorticoids (cortisol and corticosterone) have been widely researched. Among the main functions of these hormones can be highlighted as the role in the adaptive response that occurs during the stress process. For the

buffalo species, there are some studies addressing glucocorticoid hormones (Prakash and Madan, 1984; Napolitano et al., 2004; Khan et al., 2011; Titaporn Khongdel et al., 2011).

The animals that live in social groups establish dominance-subordination relations through agonistic and

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Table 1. Groups of buffalo heifers used in the social and sexual behavior test.

Group	Animal	Month of synchronization
G1	4, 6, 8, 10, 11, 14 and 24	April
G2	5, 7, 13, 15, 16, 18 and 20	May
G3	3, 9, 21 and 22	June

Table 2. Sexual and social behavior standards of buffalo heifers and the bull.

Behavior	Exhibited feature
Sexual behavior	Head with head, homosexual behavior mating with penetration, mating without penetration, attempt to mount, head in the haunch, follow the female and conduct flehmen
Social behavior	
Agonistic reactions	Push, expel
Non-agonistic reactions	Smell, lick and rub.

submission meetings that provide an increase in the release of glucocorticoids (Solano et al., 2004). Hasegawa et al. (1997) and Solano et al. (2004) verified that the concentration of cortisol influences the social behavior of cattle and detected differences in the concentrations of cortisol in plasma in relation to the hierarchic positions. However, monitoring of the concentration of glucocorticoids in the plasma can be influenced by the high level of stress caused during the blood sampling. For this interference not to occur, the non-invasive monitoring technique using feces is used, so as not to cause any stress during collection of samples. The technique of non-invasive monitoring has enabled the measurement of fecal metabolites of steroids with significant correlations with the concentrations of these hormones in the plasma and milk. It has been used mainly for researches that are addressing behavioral and reproductive including investigations of diurnal and seasonal patterns of cortisol level, social and dominance interactions, the impacts of habitat degradation, transport stress, predator-pre interactions and the effects of maternal stress (Schwarzenberger et al., 1996; Möstl and Palme, 2002; Sheriff et al., 2009).

Thus, the aim of this work was to evaluate the concentration of fecal metabolites of corticosterone and to verify if there are differences between dominant and subordinate between buffalo heifers.

MATERIALS AND METHODS

Experiment locale

The experimental work was conducted in the Cattle Rearing Sector of Instituto Federal do Espírito Santo, Campus de Alegre, Espírito Santo State, Brazil. The Campus de Alegre is located in south latitude 20°45'29" and west longitude 41°27'32", at an altitude of

approximately 120 m.

Animals

A total of 18 heifers crossbred Murrah and Mediterranean animals were included in the study. The female were 20 months old and weighed an average of 300 kg. The experiment started in the month of April during the mating season for the buffalo species in the southeastern region of Brazil. The females were synchronized using the following protocol: On day zero, the animals received a Progesterone device (CIDR - controlled internal drug release intravaginal implant containing 1.9 g of progesterone) and 2 ml of Estradiol Benzoate was applied and, nine days later, the implant was removed and 2 ml of Prostaglandin and 400 IU of eCG (Equine chorionic gonadotropin) was applied

Sexual and social behavior

The heifers were divided into three groups (Table 1), being selected according to the preliminary observations of social behavior conducted in the pasture (Madella-Oliveira et al., 2012). The social and sexual behavior observations occurred during the induced estrus on day 9 of the synchronization, in the presence of the small bull. All social and sexual behavioral interactions were recorded continuously for five consecutive days, 24 h a day. Four observers worked in shifts of 6 h/day, equipped with binoculars and recorders, totaling 360 h. During the day, the observations were made in the pasture and at night inside the pen to facilitate recording of observations. The following behaviors, shown in Table 2, were recorded.

Collection and extraction of fecal samples for hormone dosage

The feces of 18 buffalo heifers were collected for analysis of corticosterone metabolites. The days of collection are shown in Table 3. The feces of the animals were collected fresh, and placed in previously identified hermetic plastic bags (animal number, date and time). After collection, the material was refrigerated at 4°C in Styrofoam containing recyclable ice and later frozen (-20°C). The

Table 3. Collections of feces for quantification of the fecal metabolite concentrations of corticosterone of buffalo heifers.

Parameter	Days of Collection	Time of collection (h)
Synchronized heat	Day zero*	8 to 10 am
	Day of estrus observation	At the time of heat identification.
	Day after estrus	12 to 24 h after collection on the day of heat observation.
	Fifth after estrus	8 to 10 am

Table 4. Means and respective standard deviations of the concentrations of fecal metabolites of corticosterone (ng/g) in relation to the groups (G1, G2 and G3) on day zero (D0), on the day of estrus (DESTRUS), on the day after heat (DAESTRUS) and on the fifth day after heat (FDAESTRUS).

Hormone	Group	Mean \pm Standard deviation			
		D0	DESTRUS	DAESTRUS	FDAESTRUS
Corticosterone	G1	2,332.42 \pm 2,138.33	1,629.57 \pm 715.54	1,060.42 \pm 547.57	2,629.85 \pm 2,051.50
	G2	3,583.00 \pm 3,514.76	2,182.00 \pm 1,353.36	1,243.50 \pm 447.26	2,370.85 \pm 2,338.46
	G3	1,716.25 \pm 239.73	2,908.00 \pm 3,233.93	893.66 \pm 217.78	2,723.50 \pm 1,764.99

samples were kept at this temperature until processing of its extract. The procedure used to extract the fecal metabolites of corticosterone was conducted according to Graham et al. (2001). Aliquots of approximately 0.5 g (0.48 to 0.52 g) of feces were placed in previously identified glass test tubes (16 x 125 mm), to which 5 ml of 80% methanol was added. Later, the tubes were agitated for 30 s by vortexing followed by 12 h in a blood homogenizer. All the tubes were centrifuged at 1500 rpm for 15 min, and the supernatants were transferred to 1.5 mL Eppendorf tubes. The extracts were kept until quantification of the fecal corticosterone. ImmuChemTM corticosterone double antibody RIA kit (MP Biomedicals, LLC, Diagnostics Division, NY, USA) was used to quantify fecal corticosterone metabolites.

For the corticosterone analyses, the samples were diluted in proportions that varied from 1/10 to 1/40 in a steroid diluter of the kit (Phosphosaline gelatin buffer - pH 7.0, containing gamma globulin of rats), and hormone quantification was then conducted by RIA, in a Gamma radiation counter (Packard Cobra Auto-Gamma[®]), verifying the number of counts per minute (cpm). The results were obtained in ng/mL (nanogram per milliliter). The final metabolite values for corticosterone were converted to the weight and dilution used, through the formula below, to be expressed in ng/g (nanogram per gram of feces).

$$X = (C \times V \times D) / W$$

Where:

C = is the concentration in ng/mL provided by the test;

V = is the total volume of the extract, that is, quantity of solvent that was used to make the extraction (5 mL);

D = dilution of the extract that was used for the test;

W = is the weight of feces used in the extraction (usually between 0.1 and 0.5 g).

Calculation of the sociometric measures and statistical analysis

The results of the social behavior were transformed into a sociometric matrix, initiators of agonistic behavior are put in lines and receivers of these behaviors are put in columns. The social index was calculated according to Orihuela and Galina (1997). From the results of the social index, the heifers were classified into

three categories according to the social index (SI) values: low (SI = 0.0 to 0.33), moderate (SI = 0.33 to 0.66) and high hierarchy (SI = 0.67). To evaluate the means of the concentrations of corticosterone metabolites, the GLM process was used (SAS, 2001) and the means were compared by the SNK test, 5% probability.

RESULTS

The mean concentrations of the fecal metabolites of corticosterone of the buffalo heifers were 2,681.83 \pm 2566.72; 2,128.50 \pm 1708.62; 1,097.81 \pm 458.71; 2,549.94 \pm 1,997.03 in ng/g of feces for day zero, on the day of estrus, on the day after estrus and on the fifth day after estrus, respectively. Table 4 shows that the means and respective standard deviations of the concentrations of the fecal metabolites of corticosterone among the groups (G1, G2 and G3) do not present differences. The corticosterone concentrations in relation to the hierarchic positions showed in Figure 1, differ statistically ($P < 0.05$). The animals with high hierarchic position presented means higher than those of the other hierarchic positions (moderate and low), in relation to day zero (start day of synchronization). However, on the day of estrus, on the day after estrus and on the fifth day after estrus, no differences were found ($P > 0.05$) in the concentrations of fecal metabolites of corticosterone among the hierarchic positions of the buffalo heifers.

DISCUSSION

In response to synchronization of the estrus cycle, all the buffalo heifers showed symptoms characterizing estrus, as the females started displaying estrus behavior 12 to 72 h after removal of the CIDR. The results showed that the concentration of fecal metabolites of corticosterone was low one day after estrus, which could suggest that

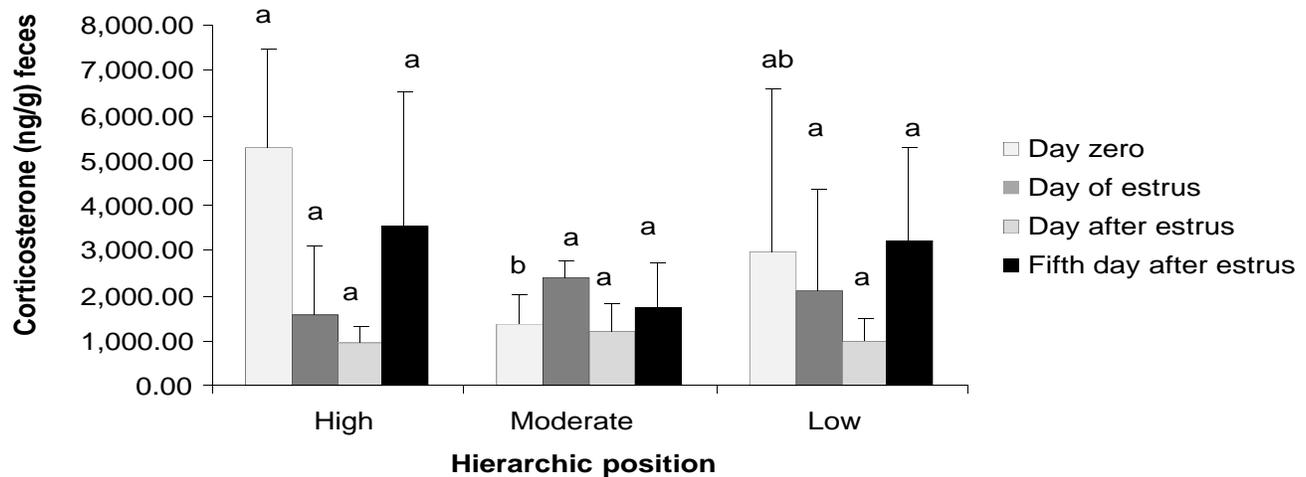


Figure 1. Mean and respective standard deviation of the concentrations of fecal corticosterone metabolites (ng/g) in relation to hierarchic positions of buffalo heifers on day zero, the day of estrus, the day after the heat and on the fifth day after estrus.

the bull's presence reduces the level of stress of the buffalo heifers. On day zero, when the heifers were not in contact with the bull and which was before the induced estrus, higher and significant values of corticosterone concentrations were detected in the heifers with higher hierarchic position, indicating that in the bull's absence, the animals with high hierarchic position showed a higher level of excitability. Making a comparison with the results of the plasma concentrations of cortisol in cattle, Solano et al. (2004) verified that cortisol influences the hierarchic behavior of cows and observed that the animals with greater dominance showed higher levels of cortisol, which would agree with results of the concentrations of fecal metabolites of corticosterone in the buffalo heifers. Encarnação (1983) and Hasegawa et al. (1997) observed low concentrations of glucocorticoids in the blood of dominant animals and higher concentrations as the position dropped in the social scale of the herd, in which the last animal had the highest stress.

Considering the results of this work, we verify the need for more studies on the social and sexual behavior of heifers, as well as determination of the fecal metabolite concentrations of glucocorticoids hormones in this species. There is no data published in the literature consulted that relates to fecal corticosteroid metabolites in heifers and social behavior, thus this study probably pioneers work of this kind in this species.

Conclusion

The results found for corticosterone indicate that only on the day before estrus was the stress level increasing, especially in the animals of greater dominance, in relation to the hierarchic position, suggesting that despite all the benefits of a dominant animal in relation to the subordinates, there are differential energy cost between

high and moderate. The male's presence could indicate a reduction in the level of stress in the buffalo heifers.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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