

Relation between the level of self-mutilation and the concentration of fecal metabolites of glucocorticoids in captive chimpanzees (*Pan troglodytes*)¹

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ABSTRACT- Pizzutto C.S., Sgai M.G.F.G., Lopes D.A., Pessutti C., Nunes A., Furtado P.V., Oliveira C.A. & Guimarães M.A.B.V. 2015. **Relation between the level of self-mutilation and the concentration of fecal metabolites of glucocorticoids in captive chimpanzees (*Pan troglodytes*)**. *Pesquisa Veterinária Brasileira* 35(1):62-66. Shape Brasil, Rua Dr. Miranda de Azevedo 779, Apartamento 104, Pompéia, São Paulo, SP 05027-000, Brazil. E-mail: cspizzutto@yahoo.com.br

The influence of stress in an environment, according with the behavioral and endocrine variables of primates, are increasingly being studied by a diversity of authors, and have shown that abnormal behaviors associated with increased glucocorticoids may be directly related with the impairment of their well-being. In this work were used 22 adult chimpanzees (*Pan troglodytes*), 11 males and 11 females, kept in captivity in three different institutions. All animals had their behavior registered by focal session using a 30 seconds sample interval, during six months, totaling 4,800 registries per each animal. During this period, fecal samples were collected 3 times a week for the extraction and measurement of the concentration of fecal metabolites of glucocorticoid by radioimmunoassay. Of the total observed, stereotypical behaviors represented $13,45 \pm 2.76\%$, and among them, self-mutilation represented $38.28 \pm 3.98\%$. The animals were classified into three different scores, according with the percentage of body surface with alopecia due to self-mutilation. It was found a positive correlation of high intensity between the scores of alopecia due to the observed mutilation and the average concentrations of fecal metabolites of glucocorticoids. This result strongly suggests that this measurement of self-mutilation in a chimpanzee can be used as an important auxiliary tool to evaluate de conditions of adaptation of an animal in captivity, functioning as a direct indicator of the presence of chronic stress.

INDEX TERMS: Chimpanzee, *Pan troglodytes*, self-mutilation, glucocorticoid fecal metabolites, captivity.

RESUMO.- [Relação do grau de automutilação com as concentrações de metabólitos fecais de glicocorticoides em chimpanzés (*Pan troglodytes*).] A influência do

estresse de um ambiente nas variáveis endócrino-comportamentais de primatas vem sendo cada vez mais estudada por diversos autores, e mostram que comportamentos anormais associados a aumentos de glicocorticóides podem estar diretamente relacionados ao comprometimento do bem-estar. Neste trabalho foram utilizados 22 chimpanzés (*Pan troglodytes*) adultos, sendo 11 machos e 11 fêmeas mantidos em cativeiro de três instituições diferentes. Todos os animais tiveram seus comportamentos registrados pelo método de amostragem focal por intervalo de tempo, durante seis meses, totalizando 4800 registros para cada animal. Amostras fecais foram coletadas três vezes por semana, durante este período, para extração e dosagem de metabólitos

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fecais de glicocorticoides por radioimunoensaio. Os comportamentos estereotipados representaram $13,45 \pm 2,76\%$ do total observado, sendo que dentre estes comportamentos a automutilação representou $38,28 \pm 3,98\%$. Os animais foram classificados em três graus diferentes, de acordo com o percentual da superfície corpórea com alopecia decorrente da automutilação. Foi encontrada uma correlação positiva de intensidade forte entre os graus de alopecia decorrentes de mutilação observados e as médias de concentrações de metabólitos fecais de glicocorticoides. Este resultado sugere fortemente que esta graduação de automutilação de um chimpanzé possa ser utilizada como uma ferramenta auxiliar importante nas avaliações das condições de adaptação do animal ao cativeiro, atuando como um indicador indireto da presença de estresse crônico.

TERMOS DE INDEXAÇÃO: Chimpanzé, *Pan troglodytes*, automutilação, metabólitos fecais de glicocorticóides, cativeiro.

INTRODUCTION

For decades the concern about the effects of the interaction between the environment and the organisms has held the attention of researchers Worldwide. The question of how to define and quantify animal welfare is still under constant debate.

Typically, for vertebrates, the *habitats* are not static and the animals have to adapt to situations predicted by physiological, morphological and behavioral changes. Components that are not predictable promote the so-called “state of emergency”, which results in changes in the endocrine and metabolic parameters of an organism (Möstl & Palme 2002).

Captive environments show commonly restricted and invariant stimuli (Paquete & Prescott 1988) and are largely responsible for the development of chronic stress (characterized by prolonged periods of high concentrations of glucocorticoids) or intermittent (Carlstead et al. 1992). This condition can have high costs for the animals, such as decreased individual fitness by immune suppression and tissue atrophy, decreased reproductive function (Gronli et al. 2005, Peel et al. 2005) and promote behavioral changes, also known as stereotypies (Mason 1991, Carlstead et al. 1992, McBride & Cuddelford 2001).

Several aspects may predispose stereotypic behavior, such as a genetic component (Schoenecker & Heller 2000, Schwaibold & Pillay 2001) and separation or inadequate maternal care (Lutz et al. 2003, Novak 2003, Novak et al. 2006, Latham & Mason 2008). The most commonly cited etiology is associated with the type of enclosure (Marriner & Drickmer 1994, Nash, Fritz & Alford 1999, Mallapur & Chellan 2002), such as the size and complexity of the environment (Macedonia 1987, Lyons, Young & Deag 1997), lack of stimuli (Meehan, Garner & Mench 2004, Swaisgood & Shepherdson 2006) or lack of proper socialization (Bellanca & Crockett 2002, Lutz et al. 2003, Novak 2003).

The stereotypical behaviors may manifest themselves differently depending on the species or the individual (Colemann & Maier 2010, Würbel et al. 2006). Shepherdson (1998) stated that stereotypies can be quantitative, such as hyperactivity, or qualitative. As examples, one can cite

the behaviors that the individual would not show in nature, such as walking aimless, false chewing, drinking water excessively, foraging even after feeding (Mason 1991), masturbation, food regurgitation followed by re-ingestion (Gould & Bress 1986, Dickie 1998), coprophagy (Akers & Schildkraut 1985), pacing (Boorer 1972) and self-addressed, such as self-mutilation.

Self-mutilation has been widely studied as an abnormal behavior in chimpanzees (Birkett & Fisher-Newton 2011, Ferdowsian 2011) and quantitative studies of these behaviors can demonstrate the effects of captivity among this specie (Birkett & Fisher-Newton 2011).

The association of behavioral and endocrine methods of study enables greater efficiency for the evaluation of stress intensity (Sgai et al. 2010). The concentration of glucocorticoids in plasma is widely used as one indicator of the presence of stressors. In light of the fact that blood sampling may also be dangerous or even impossible in some zoo and wildlife species, noninvasive methods for measuring glucocorticoids or their metabolites seem desirable for assessing adrenocortical function in animals (Palme 2005a) and has been used with primates (Bahr et al. 2000, Murray et al. 2013).

Noninvasive methods of measuring fecal steroid metabolites to assess an animal's endocrine status are now widely used to investigate hormone-behavior relationships, as well as questions in the fields of reproduction, animal welfare, ecology, conservation biology, and biomedicine (Palme 2005 b).

This way, the objective of this study was to analyze a possible relation between the level of self-mutilation and the concentration of fecal metabolites of glucocorticoids in captive chimpanzees.

MATERIALS AND METHODS

In this study 22 adult chimpanzees (*Pan troglodytes*) were used, 11 males and 11 females, kept in captivity, They came from three different institutions, two zoos and one private facility, the environmental enrichment program of which was not followed in the routine management of the animals. At the zoos the animals were kept in pairs, in areas of about 100m², and at the private facility, they were kept in individual enclosures, with areas of 25m². The diet at those three institutions was based on fruits, vegetables and protein items, offered twice a day, every day.

All chimpanzees had their behaviors recorded by focal session using a 30 seconds intersample interval (Altmann 1974, Martin & Bateson 1993), with recordings every 30 seconds in 30-minute sessions, in the morning (8:00 – 11:00 a.m.), time of greatest activity of the animals (determined by implementation of peak activity), totalizing 40 hours for each animal with 4,800 records during six months. All recordings were made in spreadsheets by a single observer, during exposure of the animal to the public, three times a week.

Individual ethograms were prepared, however for this work, just information related to self-mutilation were used. The degree of self-mutilation was classified into three scores, according to the percentage of alopecia along the body surface, ranging from 0 to 2 (Fig.1):

- Score 0: Absence of areas without hair;
- Score 1: Areas with alopecia in up to approximately 30% of body

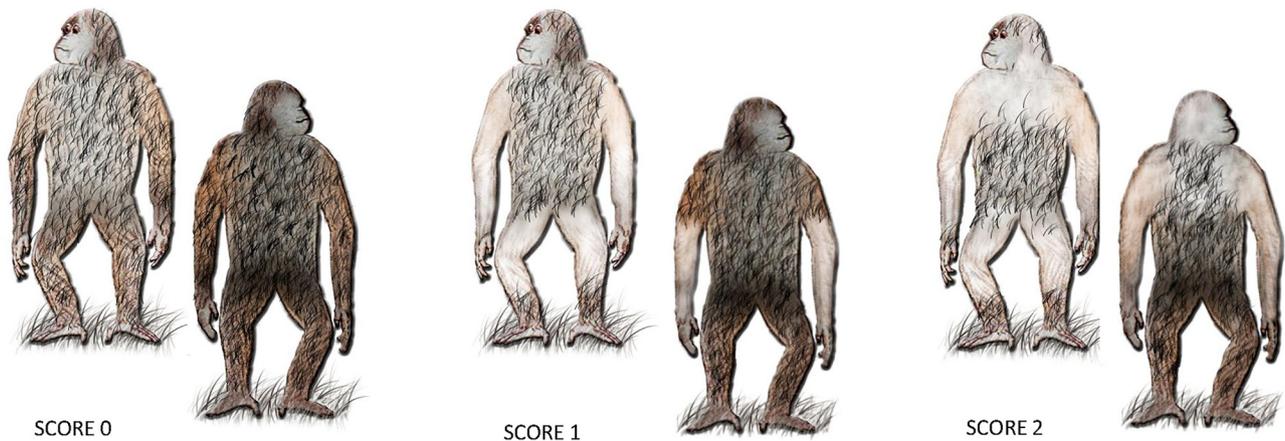


Fig.1. Schematic figure showing different levels of self-mutilation (Score 0 = Absence of areas without hair; Score1 = Areas with alopecia in up to approximately 30% of body surface, reaching upper limbs, scapular region and lower limbs; Score 2 = Areas with alopecia in more than 30% of body surface, reaching upper limbs, scapular region, lower limbs, chest, dorsum, cervical area and head) of chimpanzees (*Pan troglodytes*), front and dorsal view.

surface, reaching upper limbs, scapular region and lower limbs;
- Score 2: Areas with alopecia in more than 30% of body surface, reaching upper limbs, scapular region, lower limbs, chest, dorsum, cervical area and head.

All chimpanzees had fecal samples collected during six months. The samples were collected three times a week, in early morning (between 8:00 and 10:00 am), homogenized and packed in plastic bags like "Ziploc". After identification with date and time of harvesting, samples were then kept in a freezer at -20°C until processed at the Laboratory of Hormonal Dosages of the Department of Animal Reproduction, Faculty of Veterinary Medicine, University of São Paulo.

The extraction of metabolites was performed according to the recommended technique by Whitten et al. (1998) for chimpanzees (*Pan troglodytes*) feces. For the dosing of the glucocorticoids metabolites, it was used the commercial kit for measurement of corticosterone of double antibody (Corticosterone[®] MP Biomedical - New York), previously validated by Pizzutto et al. (2008).

Statistical analysis

The frequency of occurrence (%) of self-mutilation behavior was calculated in relation to the total number of behavioral records for each animal, as well as the frequencies for each degree of self-mutilation. There were calculated the means and the standard deviations of the concentration of fecal glucocorticoid metabolites of all animals within each degree of mutilation. The results were tested for normality using the method of Kolmogorov-Smirnov, and for homogeneity of variances using the method of Bartlett, in order to characterize them as parametric. Means were then compared by the ANOVA test, complemented by Tukey Test, both with $p < 0.05$. The concentrations of the hormone metabolites were correlated with the degree of mutilation using the Pearson's correlation test, with $p < 0.05$.

The present study was approved by the Bioethics Commission of the School of Veterinary Medicine and Animal Science, São Paulo University (FMVZ-USP), Brazil under procedure number 413/2004.

RESULTS

From the 4,800 behavioral records for each chimpanzee, a repertoire was obtained of 19 different behaviors. The ab-

normal behaviors observed had a frequency of occurrence of $13.45 \pm 2.76\%$, and among them, self-mutilation represented $38.28 \pm 3.98\%$ of those abnormal behaviors.

From the 22 chimpanzees studied, 20 presented some level of self-mutilation and were classified as: 2 animals with score 0; 15 animals with score 1; and 5 animals with score 2.

The quality control of the assays performed to measure the concentrations of glucocorticoid fecal metabolites showed the following results: coefficients of variation inter-assay: 12.75%, intra-assay: 2.21% and minimum sensitivity detected: 2.55ng/dl.

Mean concentrations of glucocorticoid fecal metabolites found in three different scores of self-mutilation were: 34.65 ± 9.35 , 90.34 ± 29.08 and 138.82 ± 38.45 ng/g of dry feces for scores 0, 1 and 2 respectively, showing significant differences (Fig.2).

It was found a positive correlation of high intensity (0.82), $p < 0.0001$ between the means of glucocorticoid fecal metabolites concentrations and the different scores of self-mutilation.

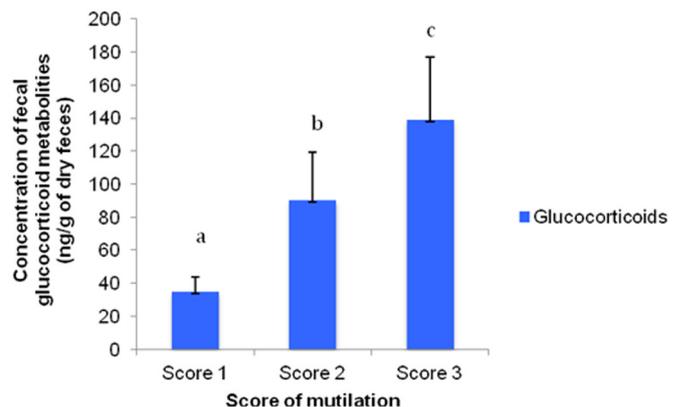


Fig.2. Means and SD of concentrations of fecal metabolites of glucocorticoids in different scores of self-mutilation in captive chimpanzees (*Pan troglodytes*). (*Different superscript letters denote significance at $P < 0.05$).

DISCUSSION

Self-mutilation presented the highest frequency of occurrence of abnormal behaviors (mean 38.28% \pm 3.98), in contrast with the findings of Ferdowsian (2011), in which coprophagy was the abnormal behavior with a higher prevalence. The frequency of occurrence of abnormal behaviors (13.45% \pm 2.76), can also be considered higher comparing to the findings by Bloomsmith and Lambeth (1995) and Ferdowsian (2011) however, no work had previously related the occurrence of abnormal behaviors with concentrations of glucocorticoids. The high positive correlation found between the means of concentrations of glucocorticoid fecal metabolites and the different scores of self-mutilation, suggested that this kind of lesion can be associated to a chronic stress situation and consequent dysfunctional behavior. These results showed that chimpanzees subjected to captive environments with poor quality stimuli may develop a pattern of qualitative stereotypical behavior as described by Swaisgood and Shepherdson (2006) and this can be directly related to the endocrine-physiological response of the animal against a condition of difficult adaptation. It could represent the specie adaptive-biological-cost due to the conditions of captivity.

In this study, it was not compared the different levels of mutilation according to age, sex or animal individual history, based in Birkett & Fisher-Newton (2011) when he found no difference between these variables for the study of abnormal behaviors in chimpanzees. To our knowledge, this is the first study establishing a relationship between cutaneous lesions, stereotypical behavior and concentrations of glucocorticoid fecal metabolites in chimpanzees, suggesting a chronic stress captive related situation.

CONCLUSIONS

In this study was found a relationship between the means of concentrations of glucocorticoid fecal metabolites and the different scores of self-mutilation.

These results strongly suggest that the level of self-mutilation can be used as a tool to help important evaluations of the conditions for animal adaptation in captivity, acting as an indicator of chronic stress in chimpanzees.

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