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Use of salivary cortisol to evaluate the influence of rides in dromedary camels

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ABSTRACT

Animals in captivity and in the wild face numerous challenges, including the risk of enduring acute or chronic stress. In captivity, facilities attempt to alleviate the risk of chronic stress by providing environmental enrichment, shown to minimize behavioral disorders and stress in several species. One potential form of enrichment in zoos is training animals to provide rides for guests, however, the effect of this activity on the welfare of individual animals has never been examined. We validated the use of saliva for assessing stress in dromedary camels (*Camelus dromedarius*), an animal commonly used for rides. We then measured variation in salivary cortisol in four male camels while providing rides of differing frequency for guests at the Toronto Zoo. The camels were sampled during the ride season (June to September) using four treatments: (1) in their pasture, (2) at the ride area when not performing rides, (3) while providing a low number of rides ($n = 50$/day) and (4) while providing a high number of rides ($n = 150$/day). Furthermore, samples were taken before and after the ride season for comparison. There was a significant difference between the post-ride season treatment and the three treatments involving guest presence during the ride season (ride area, low rides, high rides). In general, cortisol concentrations were lower during the ride season and higher during the non-ride season. Based on the metrics we used, performing rides is not a stressful experience for these dromedary camels and suggests that rides may be a form of enrichment.

1. Introduction

Maintaining non-domesticated animal species in captivity can be challenging and expensive due to their unique environmental requirements. Special enclosures and exhibits must be built, and specialty diets maintained, in order to mimic the species' natural environment as well as possible to ensure its welfare (Crissey, 2005; Newberry, 1995). However, one of the challenges facing zoos is the potential stress associated with captivity, which most often results from a lack of psychological and physical stimulation (Morgan and Tromborg, 2007).

Glucocorticoids play an important role in allostasis as they are involved in regulation of the hypothalamic-pituitary adrenal (HPA) axis (regulating metabolism, immune function, sexual characteristics and homeostasis; Busch and Hayward, 2009; Romero et al., 2009; Sapolsky et al., 2000). Increased release of glucocorticoids causes reduction of non-essential activities and increased behavioral and physiological activities that allow an individual to cope, escape, or survive (McEwen and Wingfield, 2003; Wingfield and Kitaysky, 2002). Short term or acute stress involving brief rises in glucocorticoids can be beneficial when associated with routine activities, including hunting and breeding (Sapolsky et al., 2000). However, prolonged glucocorticoid elevation (chronic stress) can elicit long-term effects on various bodily functions (Martin, 2009; Romero et al., 2009). In zoo animals, these effects can raise ethical issues (Mason et al., 2007).

To minimize the risk of chronic stress, zoos incorporate various forms of enrichment into animal husbandry routines (Mason et al., 2007). Suitable enrichment is designed to increase the physical, social and temporal complexity of a captive environment to enhance the physical and psychological well-being of an animal in a positive way (Mason et al., 2007; Shepherdson, 2003). Environmental enrichment can take many forms, from simple addition of rubber mats or hiding food, to more complex climbing structures and positive reinforcement training (Clubb and Mason, 2007; Swaisgood and Shepherdson, 2005). All of which have been shown to have a positive influence on the behavior and quality of life in numerous captive species, across a variety of taxa (Mason, 2010;

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Meagher and Mason, 2012; Shyne, 2006). Although identifying forms of environmental enrichment is an important responsibility for zoos, many forms have not been assessed for their overall effect on individuals (de Azevedo et al., 2007; Swaisgood and Shepherdson, 2005).

Some zoos use human interaction, either with zoo keepers/trainers or with guests, as a form of environmental enrichment (Claxton, 2011). In the few studies that have investigated animal–human interactions, a significant decrease in negative behaviors and cortisol levels were observed in a variety of species (Claxton, 2011; Desmond and Laule, 1994; Melfi, 2013; Pomerantz and Terkel, 2009), suggesting the potential benefit of human interactions as a source of enrichment. In this sense, participation in rides could be considered a form of environmental enrichment by creating psychological stimulation through changing environments and trainer interaction. Additionally, receiving exercise may prevent boredom and obesity, two common problems associated with captivity (Meagher and Mason, 2012; Morgan and Tromborg, 2007; Newberry, 1995). Despite these potential benefits, concerns have arisen that rides may in fact be a stressor due to factors such as walking excessively, carrying a load, being close to the public for long periods of time, and being away from their enclosure (Fernandez et al., 2009; Mason et al., 2007; Morgan and Tromborg, 2007).

Glucocorticoids have been used to assess stress across multiple species and environments (Dickens and Romero, 2013; Johnstone et al., 2012). Although the use of blood plasma or serum provides a measure of circulating levels of glucocorticoids, collecting samples can be invasive and values can potentially be confounded by handling stress (Johnstone et al., 2012; Palme et al., 1999). As a result there has been interest in the development of non-invasive (e.g. feces in many species) or less invasive sampling techniques (e.g. saliva in animals conditioned to handling) for glucocorticoid analysis (Möstl and Palme, 2002). Despite the recent interest in salivary cortisol as a tool for inferring stress, validations of the technique remain largely restricted to primates (Cross et al., 2004; Heintz et al., 2011) and a handful of domesticated species (horses, pigs, cattle, sheep) (Bohák et al., 2013; Bushong et al., 2000; Chacón Pérez et al., 2004; Cooper et al., 1989). However, validation using an accepted technique, such as adrenocorticotropic hormone (ACTH) challenge, is required for new species before more comprehensive studies can be performed (Ashley et al., 2011; Hunt et al., 2004; Palme, 2012; Peeters et al., 2011; Wasser et al., 2000).

In ruminants, measuring salivary cortisol may present a particular challenge as individuals regurgitate both food (bolus) and ruminal fluid (secreted from their rumen sac) between meals or while grazing, all of which is mixed with saliva in the mouth (Aschenbach et al., 2011; McDougall, 1948). Typically, rumen has a pH of 4.0, whereas saliva has a basic pH (>7.0) due to its high sodium bicarbonate concentration and role as a buffer (Aschenbach et al., 2011). The effect of rumen and food on salivary cortisol levels is not yet known, although dramatic changes in color and consistency of saliva throughout the day are observed (Majchrzak, personal observation). Changes in color may provide information regarding saliva composition (i.e. amount of saliva vs rumen), especially if correlated with pH. Changes in salivary pH associated with mastication are largely based on food type consumed (Owens et al., 1998). Although some studies have successfully measured salivary cortisol in domestic ruminants (Bushong et al., 2000; Palme, 2012), only one study has been performed on wild ruminants (white tailed deer, *Odocoileus virginianus*), which ingest a more diverse diet (Millspaugh et al., 2002).

Dromedary camels are considered pseudo-ruminants, due to their three-chambered stomachs, but they maintain digestive processes characteristic of true ruminants (Dehority, 2002). No validation of salivary cortisol exists for pseudo-ruminants (Anderson et al., 2004), which could differ from true ruminants due to their smaller parotid glands and different saliva composition (Kay and Maloiy, 1989).

With the exception of human studies, which have shown saliva to be stable for up to a week at room temperature (Chen et al., 1992), there is very limited information on proper handling and storage of saliva samples (Kalbitzer and Heistermann, 2013). Proper storage is particularly important for pseudo- and true ruminants given the fluctuating pH and composition of saliva, as well as substantial microbial levels in saliva (Aschenbach et al., 2011).

We studied an exotic ruminant, the dromedary camel (*Camelus dromedarius*), a species that is found in the majority of zoos in North America, and used commonly for rides. Outside of zoos, camels have global significance, and are increasingly used for milk and meat production, racing, and as companion animals (Kurtu, 2004; Rahman et al., 2009). The objectives of our study were to: (1) validate the enzyme immunoassay (EIA) used for salivary cortisol detection with an ACTH challenge; (2) evaluate the stability of salivary cortisol under different storage conditions (freezing vs. refrigeration and multiple freeze–thaw cycles; and (3) investigate the effects of rides on salivary cortisol and the welfare of the camels. We hypothesize that rides are a form of enrichment for camels and therefore, we predict that salivary cortisol will be lower in individuals when they are providing rides than when they are not providing rides.

2. Materials and methods

2.1. General husbandry and saliva collection

2.1.1. Camels and husbandry

For the assay validation, data were collected in February, 2011, from three sexually mature, castrated male camels aged 4, 8, and 16 years (weighing between 450 and 900 kg) housed at the Bowmanville Zoological Park, Bowmanville, Canada. Our sample size ($n = 3$) was comparable to ACTH validations in other species (e.g. white-tailed deer ($n = 3$), Millspaugh et al., 2002; Canada lynx ($n = 3$), Terwissen et al., 2013). Each camel was accustomed to being handled on a regular basis by the veterinary and husbandry staff at the zoo. During the sampling days, camels were kept together indoors in their regular enclosure. Each individual received approximately 6.8 kg of hay daily; distributed throughout the day. Water was withheld for 30 min prior to sample collection, but was provided after each sample was collected. No supplies, such as tubes or vials, were reused to avoid potential contamination of samples. All saliva samples were collected by the same person (YNM).

To test for the influence of performing rides on salivary cortisol levels, saliva samples were collected from four, castrated, male camels aged 4, 8, 12, 16 years (weighing between 450 and 900 kg), between May and November 2011 (three of these were also involved in the validation, above). Our sample size ($n = 4$) was comparable to other studies evaluating the effectiveness of enrichments in various species (e.g. maned wolf ($n = 3$), Coelho et al., 2012; collared anteater ($n = 5$), Eguizábal et al., 2013; rhinoceros ($n = 2$), elephants ($n = 6$), Menargues et al., 2008). The camels were first sampled at their home location, Bowmanville Zoological Park (BZP), in May (and later in November) to establish non-ride season cortisol values, and at their summer location, Toronto Zoo (TZ), where they provided rides for guests (June–September; ride season). Each camel was accustomed to spending the summer at TZ, and had done so previously at least once. All four camels had been performing rides for at least 2 years. Throughout the study, camels were fed a standard amount of hay, grain, and horse crunch treats (dry square pellets made from hay, designed for horses). The