

Is Hair Cortisol Useful for Animal Welfare Assessment? Review of Studies in Captive Chimpanzees

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Abstract

The objective assessment of animal welfare is important for building consensus among people concerned with animals and for advancing our understanding of animals in our care. The analysis of physiological stress response using glucocorticoids is one of the major approaches for animal welfare assessment. Recently, cortisol accumulated in hair or other keratinous materials of animals has been considered as an indicator of the long-term activation of the hypothalamus-pituitary-adrenal (HPA) axis. Particularly, there is evidence that hair cortisol (HC) can be used as a measure of the long-term activation of the HPA axis in various mammals. This review discusses the utility and limitations of HC analysis for welfare assessment, mainly based on findings from captive chimpanzees living in a sanctuary in Japan. First, the methodological and physiological perspectives of how to obtain reliable results from HC analysis will be discussed; and second, the potential of using HC analysis for welfare assessment will be reviewed. A series of studies on captive chimpanzees show that HC is useful for monitoring the effects of social management on the long-term stress levels in captive chimpanzees. Although further studies are needed to clarify the extent to which HC monitoring can help us understand and improve animal welfare, application of HC analysis might be useful in investigating similar issues in marine mammals.

Key Words: chimpanzee, *Pan troglodytes*, hair cortisol, animal welfare, welfare assessment, social management

Introduction

Modern society requires ethical consideration for animals in our care. The objective assessment of animal welfare is important for building consensus among people concerned with animals and for advancing our understanding of animals in our care

(Appleby & Hughes, 1997). Various methodologies exist to assess animal welfare status, including the monitoring of behavior and measurement of physiological parameters related to health and emotional states (Fraser, 2008). Among these, a major approach is the analysis of physiological stress response using glucocorticoids, the levels of which often increase when animals face a stressor and which are known to affect animal health in several ways (Sapolsky, 2005; Squires, 2010). However, from a welfare viewpoint, interpreting acute and mild stress varies among situations and researchers. Acute stress arising from procedures inflicting physical pain, separation from conspecifics, and deprivation of food or essential environmental factors for the species concerned are considered negative for animal welfare. Nevertheless, mild stress, which is often observed when animals face appropriate and solvable challenges in their own environment, is considered important in improving animal welfare (Meehan & Mench, 2007; Clark, 2017). There is greater consensus on how to interpret long-term chronic stress, something that is commonly considered negative for animal welfare (Broom & Johnson, 1993). Not only is it challenging for animals to experience distress over a long period, but also the long-term activation of the HPA axis can result in health deterioration such as neuronal cell death, insulin resistance, muscle and bone atrophy, poor wound healing, hypertension, and even collapse of the immune system to the point of death (Reeder & Kramer, 2005). Therefore, understanding factors related to long-term stress is important for animal welfare, and the challenge is efficient assessment of long-term stress to achieve this goal.

Recently, cortisol levels accumulated in keratinous materials have been considered an indicator of the long-term activation of the HPA axis (Russell et al., 2012). Studies have analyzed cortisol level in the hair of mammals (e.g., Macbeth et al., 2010; Fourie et al., 2015; Carlitz et al., 2016; Hamel et al., 2016; Mesarcova et al., 2017), feathers of birds (Bortolotti et al., 2008), shed skins of snakes

(Berkvens et al., 2013), nails of humans (Izawa et al., 2015), skin of porpoises (Bechshoft et al., 2015), and baleen of whales (Hunt et al., 2014). Among these studies, several provide direct and indirect evidence that cortisol that is accumulated in the hair of mammals represents the accumulation of cortisol over a certain period of time. For example, Davenport et al. (2006) found that hair cortisol (HC) levels in rhesus macaques (*Macaca mulatta*) are positively correlated with salivary cortisol levels, and HC showed an increase after relocation. The induction of adrenocorticotropin releasing hormone (ACTH) challenge resulted in increased HC in chipmunks (*Tamias striatus*; Mastromonaco et al., 2014), Canadian lynx (*Lynx canadensis*; Terwissen et al., 2013), and dairy cattle (del Rosario et al., 2011). HC levels in dogs and cats were correlated with fecal glucocorticoid metabolites (Accorsi et al., 2008). These studies indicate that HC can indeed reflect the activation of the HPA axis over certain periods of time. An additional benefit of using HC as a measure of the activation of HPA is that HC reflects free cortisol levels in blood without being affected by changes in cortisol binding globulin (Russell et al., 2012). However, some technical and theoretical issues to the use of HC measurement for welfare assessment remain. This review discusses the utility and limitations of HC analysis for welfare assessment based mainly on findings from captive chimpanzees (Yamanashi et al., 2013, 2016a, 2016b, in press). Advances in HC measurement in terrestrial mammals may have implications for hormone measurement in hair and skin of marine mammals, and it may also play a role in their welfare assessment.

Methodological and Physiological Issues

Hair Incorporation

Most previous studies have indicated that HC accumulates from blood during the growth phase in follicles (Gow et al., 2010; Russell et al., 2012) and, thus, can act as a retrospective marker of the activation of the HPA axis during the hair growth period. This hypothesis is based on findings showing the positive correlation between HC and cortisol measures from other sources (e.g., blood, saliva, urine, and feces) and on the above-mentioned experimental studies testing the effects of ACTH challenge on HC levels. In addition, several studies have also reported that factors known to increase cortisol, in general, also increase HC levels in various animals, including humans. These factors include relocation (Davenport et al., 2006; Dettmer et al., 2014), ecological disturbance (Fourie et al., 2015), and pregnancy (Kirschbaum et al., 2009). In my previous study (Yamanashi et al., in press), hair growth rate

in male chimpanzees (*Pan troglodytes*) was 1.33 ± 0.06 cm/mo ($N = 5$). Another study in chimpanzees (Yamanashi et al., 2013) reported hair length of 5.86 ± 1.83 cm (mean \pm SD). Therefore, for chimpanzees, the accumulation of cortisol over several months can be detected using HC as a measure, although individual variations may exist.

Some studies have reported the possibility of local production of cortisol in hair follicles. Ito et al. (2005) suggested that hair follicles include a functional equivalent of the HPA axis and can synthesize cortisol by stimulation of the corticotrophin-releasing hormone. Further, a later study found that keratinocyte can synthesize and activate cortisol in humans (Cirillo & Prime, 2011). Keckeis et al. (2012) also suggested the possibility of local production of cortisol in hair follicles of guinea pigs (*Cavia porcellus*) because only a small amount of systematically administered radioactive glucocorticoids in blood were detected in guinea pig hair. Sharpley et al. (2009) showed a rapid increase of HC in arm hairs in three men who underwent a 1-min cold stress test. Salaberger et al. (2016) showed that extensive brushing or dexamethasone fluid administration on the skin surface resulted in the increase of cortisol in sheep hairs.

Some studies also suggested the possibilities of contamination from external sources. For example, Russell et al. (2014) analyzed cortisol concentration in human sweat and found levels comparable to those in saliva. They also found that conventional washing methodology may not be effective in decreasing the levels. However, more recently, Grass et al. (2015), studying the effects of sweat on HC levels in humans, found that sweat-inducing challenges did not increase cortisol levels in human hair. Non-human mammals have fewer eccrine sweat glands in comparison to humans, but some species have the gland (Whitford, 1976; Folk & Semken, 1991); thus, sweat might have some influences on HC. Macbeth et al. (2010) found that contaminants, such as blood, urine, and feces that could affect HC measurement in grizzly bears (*Ursus arctos*), can be removed by washing. My earlier studies, in agreement with most studies, utilized alcohol solvent (e.g., isopropanol) for the removal of the external confounding substances (Kroshko et al., 2017). This type of procedure is imperative to get reliable results. In summary, although the usefulness of HC as a measure of the long-term activation of HPA axis is being accepted, further studies are needed to confirm sources of cortisol in hair other than accumulation from blood.

Stability and Degradation of HC

I did not find any evidence of the degradation of HC according to hair shaft in a previous study because of the absence of any systematic decrease in cortisol

levels from proximal to distal parts of hair shafts (Yamanashi et al., 2013). Furthermore, in the laboratory setting, I did not find any change in the cortisol level in hair kept at ambient temperature for 2 y. These results are consistent with those of previous studies indicating the stability of HC in archaeological human hair (Webb et al., 2010) and polar bears (*Ursus maritimus*; Bechshoft et al., 2012). The stability of HC is one advantage in comparison to other media for cortisol analysis. Although my previous studies reported the stability of HC over a relatively long period, Carlitz et al. (2016) have found degradation effects in the hair of wild chimpanzees. HC level showed a systematic decrease along the hair shaft in the hair of wild chimpanzees, and hair found in their old beds showed decreased cortisol levels. However, they found only negligible differences in the hair of zoo chimpanzees (Carlitz et al., 2015). This is consistent with results of my previous study (Yamanashi et al., 2013) in which my coauthors and I analyzed the hair of captive chimpanzees and found no such degradation effect over time. Precipitation or sunlight may have caused the degradation in HC in samples from wild chimpanzees. Previous studies have suggested that washing (Hamel et al., 2011) and light radiation (Grass et al., 2016) affect HC levels. In captive settings, chimpanzees can protect themselves from strong sunlight and rain. Therefore, at least in captivity, HC levels appear to be stable for a long period of time, although caution is required here because environmental characteristics may vary in each situation.

Effects of Age and Sex

I found no age-related effects on HC in captive chimpanzees (age range 5 to 44 y; Yamanashi et al., 2016a), although some studies have found such effects in other species of primates—rhesus macaques (Dettmer et al., 2012), vervet monkeys (*Chlorocebus aethiops*; Laudenslager et al., 2012), and baboons (*Papio anubis* and *P. hamadryas*; Fourie et al., 2015a). These studies included younger individuals than those in my study (Yamanashi et al., 2016a). The fact that my study did not include infants may explain why no age-related effects were observed. Sex significantly influences HC levels in captive chimpanzees, with hair from males containing significantly more cortisol than hair from females (Yamanashi et al., 2016a).

Effects of Technical Factors: Recommendation to Obtain Reliable Results

HC analysis involves various steps prior to immunoassay such as sample collection, washing, grinding, and extracting and drying the hormone. Each of these factors may potentially contribute to variations in cortisol measurement. In my previous studies, I found that HC concentration varies depending on

the source of hair on the body, hair color, and on certain procedures (Yamanashi et al., 2013, 2016b). Hair obtained from the side of the body contained more cortisol than that obtained from the arm in chimpanzees. HC concentration in samples obtained from similar body parts did not significantly differ. Differences in HC level based on body region have been reported in another study of chimpanzees (Carlitz et al., 2015) and some other species (Macbeth et al., 2010; Terwissen et al., 2013; Fourie et al., 2016). Nevertheless, I also found that differences of HC between proximal and distal segments of hair obtained from arm hair and that obtained from the side of the body were significantly correlated (Yamanashi et al., 2016b), suggesting that we can obtain reliable information by sampling similar body parts, even though absolute hair cortisol concentrations differ across different body regions. I also found that HC levels from white hairs were significantly higher than those from black hairs. Such differences based on hair color have also been found in dogs (Bennett & Hayssen, 2010).

In terms of technical factors, I found that the fineness to which the hair is homogenized before extraction, the extraction time, and the size of hair samples were sources of variation. In my studies, I used a homogenizer to grind the hair. The finer the hair sample, the higher the HC level obtained and that longer extraction times resulted in higher HC levels. I also found that HC levels assessed from different sample weights showed significant variation: levels obtained from small samples (< 5 mg) were significantly higher than those obtained from larger samples (> 5 mg), leading us to use hair samples of at least 5 mg (preferably > 10 mg).

From the results of the studies, I recommend that hair should be collected from a consistent location on the body, that hair of a consistent color should be selected, and that close attention should be paid to technical factors when performing HC analysis with enzyme immunoassays.

Application to Welfare Assessment

In previous studies, my colleagues and I have used HC to assess the effects of relocation and of the captive social environment on the long-term stress level of chimpanzees living in the Kumamoto Sanctuary (KS), Kyoto University, Japan. Established in 2007, KS was the first chimpanzee sanctuary in Japan, having been previously known as Chimpanzee Sanctuary Uto in 2011 when the institution was passed to Kyoto University from the Sanwa Kagaku Research Institute (for more information, read Morimura et al., 2010). KS accommodates ex-laboratory chimpanzees and chimpanzees considered surplus in Japanese zoos. It promotes the social life of chimpanzees, and three types

of social group have been formed: (1) all males, (2) single male with multiple females, and (3) multiple males with multiple females. In addition to the lifelong care of chimpanzees and bonobos at KS, non-invasive research (e.g., cognitive, behavioral, endocrinological, and genetic) is conducted.

I conducted HC analysis for the welfare assessment of these sanctuary-dwelling chimpanzees. Using scissors, caretakers or researchers who were familiar with the subject chimpanzees cut arm hair samples. Most hair samples were taken when the subjects were completely awake; few samples were collected during a health checkup under anesthesia. I could collect samples as described from all chimpanzees housed at the sanctuary during the study period.

Effects of Relocation

In 2012, eight chimpanzees (two males and six females) were transferred to the KS as a result of institutional closures. Arm hair samples were collected from these eight chimpanzees twice prior to and seven times after relocation, and HC levels were measured (Yamanashi et al., 2016a). I found that HC levels in the first year after relocation were significantly higher than those before relocation or in the second year after relocation. Although all chimpanzees exhibited these significant changes, there were individual differences in their reactions to relocation, possibly due to social factors. The alpha male did not show changes in HC levels, whereas another male, the subject of aggressive behavior, showed the highest increase in HC levels. The timing of the increase in these levels in the subordinate male corresponded to periods of heightened aggression. Thus, not only relocation but also social factors appear to influence changes in HC concentration.

Effects of Social Environment on Long-Term Stress Level in Captive Chimpanzees

Providing an opportunity to live in a social group is one of the most important aspects for captive care and management of chimpanzees because they are gregarious animals. Previous studies have reported the negative impact of isolation on primates (Suomi, 1997; Kalcher et al., 2013; Freeman & Ross, 2014; Kalcher-Sommersguter et al., 2015). Additionally, social interactions with bonded individuals have stress-reducing effects in wild chimpanzees (Wittig et al., 2016). However, social management is one of the most difficult aspects of captive management (Bloomsmith & Baker, 2001; Fritz & Howell, 2001; Morimura et al., 2010). Although the formation of complex social groups comparable to those of wild groups is recommended, this can sometimes result in undesirable consequences. For example, male

chimpanzees are aggressive (Wilson et al., 2014); hence, keeping several adult males in a captive environment alongside females often results in escalated aggression. As a result, surplus males emerge, and unnatural social compositions, such as all-male groups, are sometimes formed to solve the problem of “surplus” animals. Furthermore, in human and non-human animals, social relationships can have both positive and negative consequences, depending on the circumstances and quality of the relationship (Hennessy et al., 2009). Because social relationships are unpredictable and can affect animal welfare over a long period of time, social management plays a role in maximizing the positive effects while reducing the stress derived from them. However, little is known regarding the effects of social relationships on long-term stress levels in captive chimpanzees. HC assay has enabled us to investigate this link with a view to better understanding the social management of captive chimpanzees.

In my first study on this subject, my colleagues and I assessed HC levels in six male chimpanzees living in all-male groups to investigate any relationship with aggressive interactions and found that HC levels were positively correlated with the level of aggression received (Yamanashi et al., 2013). This finding was replicated in subsequent studies—one with a larger sample size of 58 chimpanzees (Yamanashi et al., 2016a) and another involving a different social group that included 11 male chimpanzees (Yamanashi et al., in press). In these studies, I also investigated any association between the rate of initiating aggression and HC levels, but the results were mixed with negative correlation or no significant correlation. I also found sex differences in the relationship between aggression and HC. In females, no significant association was found between aggression received and HC, whereas a positive correlation was found between the rate of initiating aggression and HC. Males and females might differ in the reaction to aggression because they have different sociality both in the wild and in captive situations. This might be one reason why HC levels are higher in males than in females. Other factors, such as early rearing conditions and social group formation, may also influence HC levels. Thus far, I have not found any evidence that chimpanzees in all-male groups show higher stress responses than those in mixed-sex social groups, although an all-male group formation has not been observed in the wild.

Various types of social interaction, in addition to aggression, exist among chimpanzees. A recent study of wild chimpanzees in Budongo Forest, Uganda, found that grooming with bonding partners can have a stress-reducing effect as observed in the reduction of the urinary glucocorticoids

level after grooming (Wittig et al., 2016). Another study suggested that factors such as the availability of social support possibly influence the relationship between social status and measures of stress physiology (Abbott et al., 2003). Therefore, understanding how affiliative relationships affect long-term stress responses is important. I investigated the relationship between affiliative social behaviors and HC in 11 male chimpanzees living in an all-male group and found a significant correlation between the rate of receiving aggression and the Grooming Balance Index (GBI) calculated by subtracting the rate at which grooming is given from that at which it is received. Furthermore, GBI and HC showed a significant negative correlation, indicating that individuals receiving higher levels of aggression show skewed patterns of social grooming in which they have to invest their time in giving grooming while often not receiving the same from others. Situations like these may cause long-term elevation in cortisol levels in such individuals. Although my previous study found that mutual social grooming indicates an affiliative relationship (Yamanashi et al., 2018), the rate of mutual social grooming was not related to HC levels. Neither social play nor the rate of giving and receiving social grooming showed a significant association with HC levels.

Taking the results of the above-mentioned studies as a whole, social relationships affect long-term stress levels in captive chimpanzees. Not only aggressive interactions but also overall social situations might be related to an increase in stress over a long period of time. Aggression and incompatible combinations of individuals are often inevitable in captive chimpanzee groups, particularly large groups. Therefore, decisions on group formation should be made by considering the situation of each chimpanzee to ensure that each individual can have balanced relationships with others. In the KS, staff were aware of this and rotated the members of the all-male group to prevent escalated aggression (particularly directed toward immigrant individuals) and subsequent excessive stress. The groups were occasionally provided with opportunities to split up so that individuals could maintain an appropriate distance from each other. Despite this, social situations strongly influenced stress levels, particularly in male chimpanzees. Future studies exploring additional ways to ameliorate stress deriving from the social environment are required.

General Discussion

Results of this and other relevant studies provide direct and indirect evidence that HC can be used as a measure of the long-term activation of the HPA axis in various mammals. However, some studies

have focused on the local production of cortisol in hair follicles and on changes in HC levels, and further investigation is needed to clarify the actual pathways of the incorporation of cortisol into hair. My studies in captive chimpanzees support the view that HC levels reflect the long-term activation of the HPA axis and, therefore, are useful to monitor stress in this species. I found that HC increased after the relocation and the positive correlation between HC and the rate of receiving aggression in male chimpanzees three times, so far. Additionally, my studies illustrate the contribution of social factors to the long-term accumulation of cortisol in captive chimpanzees. I believe that these are particularly useful for monitoring the effects of social management.

The marine mammals also possess complex social systems like chimpanzees. Several studies showed that their cognitive skills are comparable with those of great apes (Clark, 2013). Social life with multiple conspecifics is not only essential for gregarious animals behaviorally, physiologically, and psychologically, but could also allow them great opportunities to exert these cognitive skills. Dolphins are known to show similar sociality as chimpanzees and often benefit from living with conspecifics (Connor, 2000). For example, bottlenose dolphins (*Tursiops truncatus*), one of the most prevalent marine mammals in captivity (Clark, 2013), live in a multi-male and multi-female, fission–fusion society, where the group members often change. Chimpanzee societies also share such characteristics and utilize tactics to live in a physically and temporally complex society. Therefore, the Association of Zoos and Aquariums (AZA) recommends that the social groups be comprised of at least three adult males, five adult females, and several immature individuals (AZA Ape Taxon Advisory Group, 2010). Further, KS recommends groups of at least five chimpanzees, including multiple adult males if conditions permit (Morimura et al., 2010). Still, providing a complex social environment and opportunities for interacting with conspecifics is one of the most challenging issues in chimpanzee welfare science.

Similar to wild dolphins, captive dolphins require a complex social environment, and these social relationships may influence their welfare states. Some discussion regarding the social management of captive chimpanzees may be applicable to that of captive dolphins. As with chimpanzees, social environment could also be a source of stress in marine mammals. For example, Waples & Gales (2002) discussed the possible influence of social stress on the illness and death of bottlenose dolphins in the aquarium setting. According to them, the changes in social relationship and

interactions is a potential source of stress. Thus, it is imperative to regulate such stress to maintain the physical and mental health of animals. Monitoring stress derived from the social environment is one of the crucial steps in achieving this goal.

In some captive situations, we have to consider the social group formation, which was not observed in wild habitats. With chimpanzees, all-male grouping is one such example, and my co-authors and I checked the effects of such group formation in the previous study. The results confirmed that there were no adverse effects present since HC levels did not increase in captive male chimpanzees living in all-male groups. Shepherdson et al. (2013) conducted a multi-institutional study for exploring the variables that could potentially be associated with the welfare of zoo-housed polar bears. One of the potential factors responsible for the reduction in the occurrence of stereotypic behaviors is the negative correlation between the number of bears housed together and the rate of stereotypy. Wild polar bears spend most of their time in solitary. However, in captivity, they may benefit from the company of other compatible individuals. The above evidence suggests the need for further research focusing on the importance of optimal social environment for marine mammals. Incorporating stress assessment using hormones in hair and skin allows researchers to explore such important welfare issues.

There are strengths and limitations to the use of HC as a measure of long-term stress (Table 1). Compared with cortisol in other sample types (e.g., blood, urine, saliva, and feces), HC is very stable, at least in captive settings, which gives hair a great advantage as a marker of long-term stress. In addition, the need to sample at the time of stress is avoided, allowing researchers to retrospectively

sample. Thus, we might be able to check the effects of husbandry changes or any other welfare-related factors after the event. Collecting other types of samples multiple times from group-living animals is often difficult, and because the collection of hair is non-invasive, the technique is less stressful to the animals concerned. However, limitations exist in estimating the exact time period of stress exposure. In humans, it is often assumed that hair has a fairly predictable growth rate; but in my studies on chimpanzees, individual variations in the rate of hair growth were observed. Additionally, a few millimeters of hair lie beneath the skin surface, and the time lag for its emergence needs to be considered (Russell et al., 2012). These factors create a difficulty in estimating the exact time period when the animals faced stressors. Furthermore, the possibility of cortisol degradation due to precipitation and sunlight must be considered as suggested from studies of wild populations of chimpanzees and *in vitro* experiments. It would be particularly important to consider these factors if this technique was applied to estimate hormones in hair or skin of marine animals because these animals are more exposed to water and possibly sunlight, dependent on species and habitats.

Although cortisol was the main target of my studies, it is also possible to quantify other types of steroid hormones from hair such as testosterone, estrogen, and progesterone. Furthermore, some studies also included stable isotope analysis to understand nutritional status (Bryan et al., 2013; Webb et al., 2014). Hair can be a useful source of information for understanding animal physiology. Some studies have used the technique to estimate the reproductive status of wild animals and investigate the underlying mechanism of the relationship between long-term stress and environment (Bryan

Table 1. Summary of the characteristic comparisons of cortisol measurement between hair and other matrices

	Other (Blood, urine, saliva, and feces)	Hair
Validity	High	Moderate (evidence accumulated but requires more studies)
Stability	Low	High (easy storage)
Duration of stress	Short	Long (several months in chimps)
Time resolution	High	Low
Variability	Low to high	High (e.g., body regions and color)
Diurnal change	Yes	No
Reproducibility	Low to high	High
External influence	Low to high	Possibly (e.g., precipitation and UV)
Age and sex	Yes	Yes

et al., 2013; Terwissen et al., 2014; Tennenhouse et al., 2017). A recent study reported variable effects of sunlight on the levels of different types of steroid hormones (Grass et al., 2016). Future studies are needed to confirm the utility of hair as a means to quantify other types of steroid hormones.

The above-mentioned points are important in answering the question, “Do HC levels indeed reflect the long-term accumulation of cortisol?” To completely answer the question, “Is HC useful for animal welfare assessment?,” we need more understanding regarding the potential link between HC and other indicators of welfare, such as fitness, in animals. Bonier et al. (2009) reviewed the available research to investigate the link between baseline glucocorticoid levels and fitness in wild animals. They found that although several studies supported the view that high cortisol levels indicate the poor condition and decreased fitness of an individual or population (Cort-fitness hypothesis), other studies indicated the opposite or no relationship between cortisol and fitness. Because HC enables the estimation of long-term stress, unlike previous studies, it might be suitable to test such a hypothesis in long-lived animals. Chimpanzees and marine mammals are long-lived, and understanding such fitness outcomes for these animals requires time. Investigations on this topic have recently begun: Rakotoniaina et al. (2017) found a negative correlation between HC and survival probability in mouse lemurs (*Microcebus murinus*) in the wild. Thus far, I have a positive impression of HC as a measure for an efficient estimation of long-term stress levels in chimpanzees. However, further research is required to examine the effectiveness of HC as a means of welfare assessment.

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