Research Article

Edu-Ecotourism Concept of Equine Cortisol Metabolites and Tri/Tetra-Iodothyronine Ratio

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Abstract | The welfare factor of riding horses is often forgotten when visitors overflow during peak season at minizoo rides. We aimed to determine how many tourists and horse riders affect their cortisol levels and the tri and tetraiodothyronine (T3/T4) ratio. We employed three riding horses at the Jogja Exotarium (JE), Yogyakarta. Fecal samples were collected in the morning, afternoon, and evening for seven days, during low and high numbers of visitors. The collected fecal samples were freeze-dried for 72 hours, and 80% methanol extraction followed by Enzyme Immuno Assay (EIA) analysis on cortisol, T3 and T4 levels carried out. Other data, such as the number of visitors, mileage, and number of horse riders, are also recorded. The average daily cortisol level during the low season is 392.41 ng/g dry feces, with a total visitor count of 21 without tourists riding horses. Meanwhile, the peak session reached 413.56 ng/g dry feces with 759 visitors and 12 tourists riding horses. The average cortisol levels during low sessions in the morning, afternoon, and evening are 344.41 ng/g dry feces, 425.7 ng/gr dry feces, and 407.27 ng/g dry feces, respectively. The mean cortisol levels during peak sessions in the morning, afternoon, and evening are 462.26 ng/g dry feces, 401.64 ng/gr dry feces, and 376.98 ng/gr dry feces. The highest T3/T4 ratio was 0.58 ± 0.056 in the afternoon at peak session, while the highest low session ratio in the morning was 0.57 ± 0.068. However, both sessions' T3/T4 ratios decrease in the afternoon. There is a significant ($P<0.05$) difference in the average daily cortisol levels and morning cortisol levels during the low session and peak session. The number of tourists and the number of tourists riding horses is correlated positively with cortisol levels but not significantly. The mean cortisol levels in the afternoon and evening are not significantly different during both low and peak sessions. The T3/T4 hormone ratio did not differ significantly between the low and peak sessions in the morning, afternoon, or evening. To conclude, the number of tourists affects cortisol levels; however, the levels decline in the evening in both low and peak sessions, while the T3/T4 ratio will return to the basal value in the afternoon. This indicates that the horses are treated according to animal welfare even though the number of tourists increases.

Keywords | Animal welfare, Dry feces, Freeze-dried, Low session, Peak session, Visitor

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INTRODUCTION

The horse has natural superiority in muscle strength to support or pull weights. This superiority then functioned as a support, weight puller, as well as tourist vehicle. Back to nature concept of tourism has become highly demanding since more farmland conversion into housing. The horse has become a perfect icon to bring a rural atmosphere into tourism, because tourists are highly concerned with the appeal of a tourist attraction (Fitriani, *et al*., 2021). The edu-ecotourism has attracted visitors for its offer of animal direct interaction such as riding a horse. Zoo managers or keepers tend to neglect animal welfare concepts during peak season. According to Hall *et al*. (2018), it is very important to consider working horse welfare. Assessing the emotional component of behavior can be used as a basis for identifying fear-provoking situations which will help reduce human risk and enhance horse learning and can be used for improvements in horse management. Today animal welfare is not only highlighting the five freedoms scope, but also positive emotional experience. A crowd of visitors or inexperience and nervous rider could contribute to creating potentially dangerous situations for the visitor and the horse (Silver, 2002). Animals under physical stressors or emotions depend on complicated interactions between different biological systems (Behavior, autonomy, neuroendocrine, and/or immunity) for stress to respond (Pawluski *et al*., 2017). Increased heart rate and blood and saliva cortisol are strongly associated with acute stress (Koolhaas *et al*., 2011). Various studies have shown how horses anticipate potential stressful events such as loading transport vehicles (Schmidt *et al*., 2010), racing competitions compared to regular training (Bohák *et al*., 2018); trekking courses (Ono *et al*., 2017), or when horses take part in festivals (Maneu *et al*., 2023). Elevated cortisol levels can affect behavior (Aggressiveness, movement, and grooming) and physiological function (Depression, hypertension, immune system suppression, gastrointestinal ulceration, electrolyte imbalance, calcium loss, reduced bone mass, and growth inhibition (Birke, 2002; Breazil, 1987; Steward, 2003).

Thyroid hormones significantly influence metabolism, heart rate, blood pressure, nutritional physiology, brain development, and thermo-regulation independent of muscle activity. Thyroid hormones will also be responsive to nutrient deficits, lowering metabolism, and allowing the body to conserve energy during nutritional emergencies (Silva, 2002). Glucocorticoid hormones (GC) in circulation and feces can indicate a response to psychological and nutritional stress. According to Airin *et al*. (2020), when the body experiences stress and an increase in cortisol hormone, it can affect the levels of thyroid hormones, particularly T4. Inadequate energy intake caused by stress can also impact the process of gluconeogenesis, which increases by converting T4 into T3 to aid the body's metabolic process. This suggests that the ratio of T3/T4 can serve as an indicator to identify changes in an animal's physiological state. Thyroid hormones and GC hormones will respond when food availability or disruption impact can be used to design practical mitigation efforts to adapt (Wasser *et al*., 2010). The hypothalamic-pituitary-adrenocortical axis (HPA) and sympathetic adrenomedullary system (SAM) are considered the key to the stress response. They are known to have a major role in the mobilization and redistribution of oxygen and nutrients as energy for active organs and tissues. Therefore, the HPA and SAM carry important functions to prepare metabolism and cardiovascular behavior (Sapolsky *et al*., 2000). Stress levels are commonly measured by blood cortisol using enzyme-linked immunosorbent assay (ELISA) technique (Gholib, 2021). However, invasive sample collection can induce stress on the animal during handling and restraint resulting in a bias in the analysis (Sheriff *et al*., 2011; Rain *et al*., 2022). Recently non-invasive techniques for monitoring hormone levels using fecal samples have become a preferred tool for field researchers (Airin *et al*., 2020, 2022; Laura *et al*., 2020; Yuniarti *et al*., 2022; Arif *et al*., 2024). The excretion rate of metabolites is correlated to hormone levels in circulation. Hormone measurement from excreta reflects the hormone level of the body. A fecal sample is routinely excreted by animals thus minimizing contact and avoiding data bias. In addition, feces also can be used to determine reproductive cyclicity, time of ovulation, pregnancy, impending parturition, and adrenocortical (stress) status (Kersey and Dehnhard, 2014).

Research on the influence of visitors on the welfare of zoo animals has been intensified since the 1970s. Numerous studies have indicated the presence of visitors, density, activity, and position characteristics may affect zoo animal behavior and physiology. However, the variation in the results has indicated better tolerance in some zoo animals to large numbers of visitors, and some are even not impacted. Animals placed in artificial habitats are faced with a variety of potentially provocative environmental challenges, one of which is interaction with humans (Morgan and Tromborg, 2007). This research will evaluate the cortisol level and ratio T3/T4 of the horse during direct and indirect contact with zoo visitors, so that it can be used as reference data for zoo management that carries edu ecotourism concept.

MATERIALS AND METHODS

Animal ethics

All stages of sampling in this study have received approval from the Ethics Commission of the Integrated Research and Testing Laboratory of Universitas Gadjah Mada No 00049/04/LPPT/IX/2023.

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Horse husbandry

Horses are housed individually in enclosed stalls with adequate ventilation. Each stall is equipped with a grassy area with short grass and trees. The stalls are bedded with clean bedding every day. Veterinarians conduct horse examinations once every two months. Horses are fed manually based on their body weight and provided with ad libitum access to drinking water. The temperature in the stalls is maintained at 25-29°C.

Sample collection

Fecal samples (n=80) were collected in the morning (07.00-08.00), afternoon (12.00-13.00), and evening (16.00-17.00) for seven days during low and high numbers of visitors. The classification of low and peak is based on the research of Rajagopal *et al*. (2011) modified according to the conditions of the minizoo. Low visitor density is a minizoo condition during weekdays instead of holidays, while peak sessions are on weekends (holidays). The sample was kept cold during transportation to the Physiology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University then stored at -20 °C until processed.

Visitor data

Data on the number of visitors was obtained from ticket booth records. The number of visitors riding horses was included, as well as horse trail distance.

Sample preparation

Frozen fecal samples were subjected to freeze-drying for 72 hours, this process was removing water content by lyophilization until dry. The dried feces were finely grounded and 0.5 g was transferred to a test tube for methanol extraction. The extraction was carried out by adding 5 ml of 80% methanol to the sample then vortexed for 10 minutes followed by a centrifuge (PLC series) at 3000 rpm for 10 minutes. The supernatant was collected and stored in a microtube in the freezer before analysis.

Measurement of cortisol levels

Cortisol analysis protocols were according to EIA Cortisol KIT Batch LOT 96680006100323. Standard solutions, fecal extract samples, and sample tests were loaded at 50 µl into the wells. Fifty microliters of HRP conjugate cortisol in the ratio of 1:50 were then added followed by incubation for 60 minutes. The wells were then washed three times and 100 µl of Tetramethylbenzidine (TMB) were added. After 20 minutes of incubation at RT under minimum light intensity, 50 µl of stop solution was added and read at 450 nm (Zenix-320). Cortisol levels in ng/ml units were converted to ng/gr dry feces by considering diluent volume and feces weight.

Measurement of thyroid levels

Thyroid hormone analysis is performed using the KIT EIA T3 and T4 manual with LOT numbers t3g6560 and T4s6559. A standard solution of 25 µl is put into each designated well in two different microplates. A sample of fecal extract was taken using a 25 µl micropipette and inserted into every other well available in each. A working solution containing thyroxine-enzyme conjugates and 50 µl triiodothyronine-enzyme conjugates was added to each microplate well. On the triiodothyronine plate, Biotin was added as much as 50 µl. The plate is placed on the machine mixer for 20-30 seconds and incubated for 60 minutes at room temperature. Wells are washed three times using 300 µl deionized water for each sump, then add 100 µl 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) to each well and incubate under closed conditions for 30 minutes at room temperature. The stop solution is inserted as much as 50 µl, then read using an EIA machine reader with a wavelength of 450 nm. The OD value obtained is converted into a grade using a formula generated by the standard standard curve. The levels obtained in units of ng/ml are then converted into ng/gr dry feces, while the results of the ratio of T3/ T4 are obtained by dividing the levels of hormone T3 with T4.

Data analysis

The study analyzed data on the number of tourists, horsemen, cortisol metabolite levels, and T3/T4 ratio using the SPSS software. The T-test was used to determine the effect of the number of visitors on cortisol metabolite levels and the T3/T4 ratio. The correlation test was conducted to determine the relationship between the number of visitors, horsemen, cortisol levels, and the T3/T4 ratio. A significant effect is observed if $P<0.05$, while a strong correlation is indicated when R is close to 1 or -1.

RESULTS AND DISCUSSION

Average cortisol levels and the number of daily visitors (morning, afternoon, evening) during peak sessions are higher than low sessions (Table 1). The number of horsemen at peak session is more than during low session (Table 1). Horse cortisol levels in the morning during the peak session were higher than during the low session, but in the afternoon, cortisol levels in both conditions decreased (Table 2 and Figure 1).

Table 1: The average cortisol levels, number of visitors, and horse riders during low season and peak season.

	Cortisol level (ng/gr dry feces)	Number of visitors (people)	Number of visitor riding a horse (people)
Low season	392,41 ± 34,9^{a*}	21 ± 19*	0 ± 0
Peak season	413,56 ± 35,8^{b*}	759 ± 202*	12 ± 6

Note: ^{a,b} different letters in the same column indicate significant differences; *indicates a positive correlation

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Table 2: The average morning, afternoon, and evening cortisol levels of the riding horse during low dan peak season.

Horse ID	Cortisol level in low season (ng/gr dry feces)			No. of rider	Cortisol level in peak season (ng/gr dry feces)			No. of rider
	Morning	Afternoon	Evening		Morning	Afternoon	Evening	
1	257,37±75,19	259,68±114,83	256,18±100,05	0	485,38±239,30	234,61±85,43	320,56±84,51	16 ± 4
2	309,65±36,91	517,51±170,19	500,13±172,75	0	416,17±125,30	468,25±136,11	351,09±214,20	14 ± 4
3	385,64±74,31	490,16±108,2	465,49±240,52	0	539,38±159,12	527,46±91,17	492,66±199,72	7 ± 2
Ave	344,41±108,27[a]	425,7±144,1[a]	407,27±131,98[a]	0	462,26±67,21[b]	401,64±169,56[a]	376,98±103,15[a]	12 ± 6

Note: [a,b] different letters in the same column and row indicate significant differences.

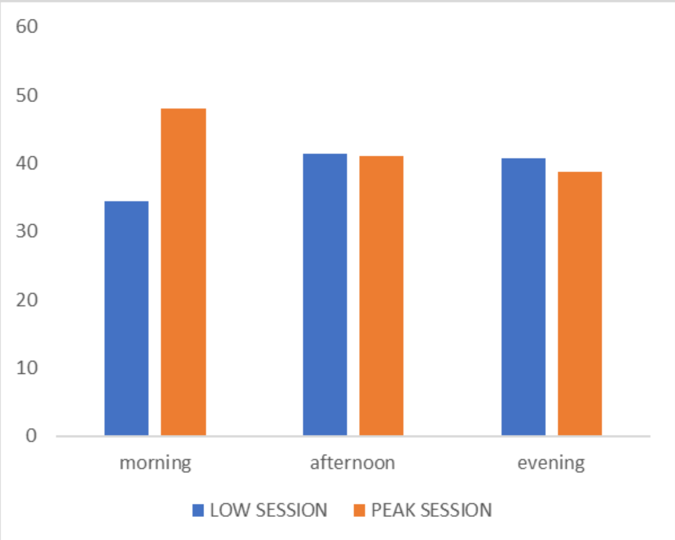

Figure 1: Cortisol level (ng/gr dry feces) comparison between low dan peak season.

Measured cortisol reflected its level condition in the body. According to (Pawluski, *et al*., 2017) there is a positive correlation between plasma cortisol levels and cortisol metabolites. The cortisol level in horses at JE during the low and peak season of visitors showed a positive correlation (r= 0.5237, r^2= 0.2838) but was not significant (Table 1). This positive, insignificant correlation indicated the increase in cortisol levels also occurred in the absence of visitor direct interaction with the horses. Various studies have shown that the presence of visitors affects wild animals in zoos and results in behavioral changes such as increased intra-group aggression or stereotyping and decreased exploration (Birke, 2002; Davis *et al*., 2005; Hosey, 2005; Sekar *et al*., 2008; Skyner *et al*., 2004). However, other researchers also reported no effect of zoo visitors on animals (Synder, 1975). Furthermore, zoo visitors even could enrich some zoo animals (Cook and Hosey, 1995).

The number of tourists riding horses was also positively correlated with cortisol levels, although not significant. Evening cortisol levels tend to be lower than during the day in peak and low seasons even though the numbers of riders were varied. This shows that the distance trail and the riding load were within reasonable limits since the cortisol was decreasing in the afternoon following the circadian rhythm. The horse trail distance for a riding horse is 200 m/tourist. If the average number of tourists is 12±6 on a range of 6-20 tourists during peak sessions meaning every day the horses cover a maximum distance of 4000 m or 4 km. Based on Kentucky Equine Research (2006), stress and muscle fatigue associated with transportation are comparable to exercise. Trips of up to 90 km (50 miles) usually do not significantly affect blood profile. However, a trip of up to 900 km (540 miles) or more should ideally be followed by several days of rest before the horse is assigned for vigorous exercise.

Increasing the load on the horse can increase its metabolic rate (Wickler *et al*., 2001) and the biomechanical, physiological, biochemical, and behavioral parameters of the horse during work. According to Christensen *et al*. (2020), increasing rider weight by 15 and 25% does not have a significant effect on heart rate, salivary cortisol, behavior, and gait symmetry. Some research has shown variations in the permitted maximum load of a horse. There are variations in the load that can be given to horses, a native Japanese horse-drawn carriage (body weight 339.9±37.5 kg) is allowed to load less than 100 kg (Matsuura, 2013a, b); the maximum permitted load for a Japanese land racehorse to race is less than 70 kg (33% of body weight) (Matsuura *et al*., 2016); the permitted load carriage for Taishuh horse (Japanese small breed horse) is less than 100 kg (43 % of BW) with running speed 3.00 ms^{-1} (Matsuura *et al*., 2013a). The load received by each horse must be adjusted to its capacity and body weight. This follows Matsuura *et al*. (2013a) that to improve welfare, it is very important to know the loading capacity for each horse breed that varies in average body weight; the maximum horse to rider weight ratio is 12-23% (Christesen *et al*., 2020); or 29% of an adult horse's body weight (Matsuura *et al*., 2013a, b). In this study, the average horseman in a day during peak session was 12±6 people. The load received by each horse was different, at the moment the arrangement was based only on the length of time the horse had been used as a riding horse and adjusted to the ability of the horse by the keeper. This is supported by the results of research by Dobec *et al*. (1994) that individual variations in muscle fiber proportions can influence metabolic responses and genetics can have an important role in a horse's weight-carrying capacity (Stefánsdóttir *et al*., 2017). The highest

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cortisol levels in the study were seen in the morning samples during the peak session compared to the low session. Cortisol levels during the day were stable in both low and peak seasons (Table 2 and Figure 1).

The average morning cortisol levels during peak season are higher compared to afternoon and evening following Pawluski *et al.* (2017). Plasma cortisol concentrations were significantly lower in the evening, after a full day of work, and after one day of rest, rather than in the morning. According to Lestari *et al.* (2023), horses are more comfortable in evening on environmental temperature conditions. Horses have a circadian cortisol rhythm that reaches the maximum in the morning, and minimum in the evening (Bohak *et al.*, 2013). This is because the horse is not exposed to any disturbances that could create a false diurnal rhythm. The mean cortisol levels of horses during the afternoon and evening during both low and peak sessions were not differentially significant.

The mean cortisol levels increased during the day in the low season when the number of visitors and horse riders was lower than during the peak season. This is following Henshall *et al.* (2022), the salivary cortisol concentration of trained horses decreased during learning while the concentration of other groups (inactive group) increased. Exercise is a physical activity that leads to increased oxygen consumption, metabolic load, and as a consequence, increased cardiac output as well as various other physiological processes to support body compensation (Caspersen *et al.*, 1985). Exercise has been showing positive effects on horses and other species. Sports activities have been proven to benefit the brain for learning, memory, and cognitive function (Basso and Suzuki, 2017). Domesticated horses routinely perform a warm-up session before training or competition (Chatel and Walliams, 2021), although the intensity of the warm-up was found to be negatively correlated with subsequent performance in a steeplechase competition (Stachurska, 2018).

The analysis results showed morning cortisol levels were significantly different ($p<.05$) during low and peak season. This is strongly correlated to the number of visitors in the zoo and who made direct interactions with the horses. Venugopal and Sha (1993) have reported that behavioral problems in animals could be triggered when visitors tease the animals. Social integration decreased in captivity groups and increased behavioral abnormalities during the presence of visitors have been reported (Mitchell *et al.*, 1991). A different opinion is expressed by Carlstead and Brown (2005) that chronic exposure of captive animals to zoo visitors can reduce stress in some species. Animals are more resilient to disturbance from tourists when they are in larger groups (Newsome *et al.*, 2001) since individuals require less alert time within collective alertness in large groups (Blumstein and Daniel, 2003). Horse-human interactions have influenced the subjective emotional experience and behavioral expression of horses. This might be due to the intensive management, handling, and interactions (Hall, 2018). This study showed the highest average cortisol levels in the morning at peak season were 462.26±67.21 with the number of visitors at 759±202 and 12±6 horse riders.

Stress experienced by animals can be known through changes in levels of the hormone T3 *(triiodothyronine)* and hormone T4 (*thyroxine*) in addition to the hormone cortisol. Both hormones are hormones produced by the thyroid gland. Generally, T3 and T4 regulate basal metabolic rate, oxygen use, cellular metabolism, average tissue growth, and differentiation (Cochran, 2011). In this study, the ratio of T3/ T4 in the morning during the peak session was lower than during the intense session, while during the day, the ratio in the peak session was higher than in the joint session. This shows that when cortisol is high, it will impact a low T3/T4 ratio; conversely, when cortisol decreases, there will be an increase in the T3/T4 ratio again. A high T3/T4 ratio can be caused by the absence of conversion of T4 into a more active T3 hormone, so there will be an increase in the value of the T3/T4 ratio. According to Ferlazzo *et al.* (2018), increased levels of the hormone T3 are positively correlated with the hormone cortisol. The sudden increase in the T3 hormone is the body's physiological response in carrying out metabolic adaptations in dealing with changes that occur (Fazio *et al.*, 2022). In horses that exercise, there will be a sudden increase in T3; this phenomenon is most likely a way for horses to adjust to the exercise given (Gonzales *et al.*, 2010). Based on Figure 2 and Table 3 the high ratio of T3/T4 during the day during the peak session is the body's response after an increase in morning cortisol. The body's ability to convert T4 into T3 is an indicator of the body's recovery response, as happened in this study; the value of the T3/T4 ratio changes in the afternoon shows that horses used as riding horses can adjust metabolism in the recovery process from exercise carried out in a day.

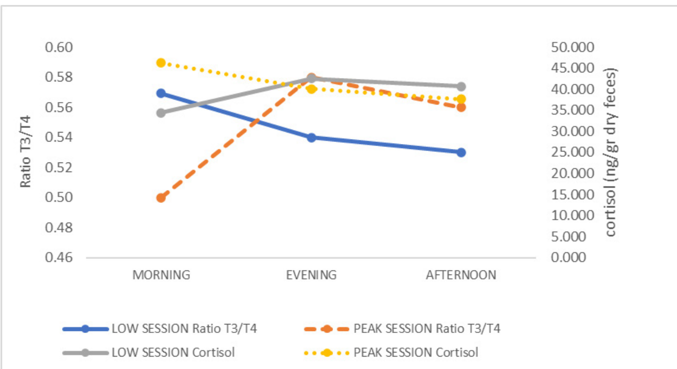

Figure 2: Comparison of T3/T4 ratio and cortisol levels (ng/g dry feces) during morning, afternoon, and evening riding horse between low and peak seasons.

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Table 3: The average morning, afternoon, and evening T3/T4 ratio of the riding horse during low dan peak season.

	Ratio T3/T4		
	Morning	**Afternoon**	**Evening**
Low session	0.57 ± 0.068	0.55 ± 0.047	0.52 ± 0.014
Peak session	0.50 ± 0.061	0.58 ± 0.056	0.55 ± 0.19

The occurrence of fluctuations in fecal cortisol metabolites indicates the functioning of HPA axis regulation of cortisol, and chronic stress does not occur that changes the regulation (Starr *et al*., 2019). According to Ferlazzo *et al*. (2018), there is an interaction between the HPA and HPT axes; their research stated that horse exercise can cause an endocrine response and the role of iodothyronine in overcoming the stimulation. Spiga *et al*. (2014) state that the hypothalamic-pituitary-adrenal (HPA) axis regulates circulating glucocorticoid hormone levels. The central neuroendocrine system in mammals provides a quick response and defense against stress. According to Ewen (2007), glucocorticoids (cortisol in humans and corticosterone in rodents) are vital hormones that regulate many physiological functions, including glucose, fat, and protein metabolism. In basal conditions without pressure, glucocorticoids are released with a pronounced circadian rhythm, characterized by peak levels of glucocorticoids during the active phase (Spiga *et al*., 2014).

The reduction of average cortisol levels in the afternoon at both seasons shows the normal circadian rhythm of the riding horse in the JE mini zoo. The hypothalamic-pituitary-adrenal (HPA) axis regulates circulating glucocorticoid hormone levels and is the primary neuroendocrine system in mammals that provides rapid response and defense against stress; when unpressurized basal conditions, glucocorticoids are released with circadian rhythms (Spiga *et al*., 2014). Cortisol production has an independent daily circadian rhythm with the lowest concentration at night (Ljubijanke *et al*., 2008). According to Pawluski *et al*. (2017) cortisol levels can be used as an indicator of animal welfare. Horses kept in a welfare-supporting environment will have low plasma and fecal (FCM) cortisol levels.

CONCLUSIONS AND RECOMMENDATIONS

The cortisol will reach its highest level in the morning and then decrease in the afternoon and evening during both low and peak sessions. The number of tourists and the number of horse riders are positively correlated with cortisol levels elevation but not significantly different. The T3/T4 ratio is highest during the day at peak session but will return to basal levels in the afternoon. This shows that the concept of edu ecotourism in minizoos has considered animal welfare.

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NOVELTY STATEMENT

The findings of this study provide evidence that incorporating riding horses in tourism, especially in Indonesia, with the concept of Edu-ecotourism, can be done while maintaining animal welfare. This can serve as a useful reference for tour managers who share the same concept.

AUTHOR'S CONTRIBUTION

All authors participate from the beginning of this study until the publications are released.

Conflict of interest

The authors declare have no conflict of interest. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

REFERENCES

Airin CM, Hana A, Sarmin, Astuti P (2020). Fecal cortisol and progesterone concentrations in post Partus of Etawah crossbreed goat. Proceeding The 1st International Conference on Veterinary, Animal, and Environmental Sciences. https://doi.org/10.1051/e3sconf/202015101031

Airin CM, Hana A, Sarmin, Astuti P (2022). Hormon progesteron feses kambing ettawah Bunting. J. Ilmu Petern. Vet., 12(1): 24-29. https://doi.org/10.46549/jipvet.v12i1.224

Arif M, Airin CM, Dratrianto DS, Yoelinda VT, Sarmin, Fibrianto YH, Astuti, P (2024). Changes in testosterone and corticosterone profiles in stone magpie birds under songbird contest condition. Adv. Anim. Vet. Sci., 12(3): 405-410. https://doi.org/10.17582/journal.aavs/2024/12.3.405.410

Basso JC, Suzuki WA (2017). The effects of acute exercise on mood, cognition, neurophysiology, and neurochemical pathways: A review. Brain Plast., 2: 127–152. https://doi.org/10.3233/BPL-160040

Bertin FR, Frank N, Breuhaus BA, Schott HC, Kritchevsky JE (2023). Diagnosis and management of thyroid disorders and thyroid hormone supplementation in adult horses and foals. Equine Vet. J., 2023: 1-10. https://doi.org/10.1111/evj.13981

Birke L (2002). Effects of browse, human visitors and noise on the behaviour of captive orangutans. Anim. Welfare, 11: 189–202. https://doi.org/10.1017/S0962728600028141

Blumstein DT, Daniel JC (2003). Foraging behaviour of three

Tasmanian macropodid marsupials in response to present and historical predation threat. Ecography, 26: 585–594. https://doi.org/10.1034/j.1600-0587.2003.03516.x

Bohák Z, Harnos A, Joó K, Szenci O. Kovács L (2018). Anticipatory response before competition in Standardbred racehorses. PLoS One, 13: e0201691. https://doi.org/10.1371/journal.pone.0201691

Breazil JE (1987). Physiological basis and consequences of distress in animals. J. Am. Vet. Med. Assoc., 191: 1212–1215.

Carlstead K, Brown JL (2005). Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Zoo Biol., 24: 215–232. Chicago, IL: The University of Chicago Press. https://doi.org/10.1002/zoo.20050

Caspersen CJ, Powell KE, Christenson GM (1985). Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. Publ. Health Rep., 100: 126–131.

Chatel MM, Williams JM (2021). What's in a warm-up? A preliminary investigation of how European dressage riders and show jumpers warm-up their horses for training and at competition. Compar. Exer. Physiol., 17: 99–108. https://doi.org/10.3920/CEP200035

Christensen JW, Bathellier S, Rhodin M, Palme R, Uldahl M (2020). Increased rider weight did not induce changes in behavior and physiological parameters in horses. Animals, 10: 95. https://doi.org/10.3390/ani10010095

Cochran PE. (2011). Veterinary Anatomy and Physiology: A clinical Laboratory Manual 2nd Edition. USA: Delmar Cengage Learning.

Cook S, Hosey GR (1995). Interaction sequences between chimpanzees and human visitors at the zoo. Zoo Biol., 14: 431–444. https://doi.org/10.1002/zoo.1430140505

Davis N, Schaffner CM, Smith DE (2005). Evidence that zoo visitors influence HPA activity in spider monkeys (*Ateles geoffroyii rufiventris*). Appl. Anim. Behav. Sci., 90: 131–141. https://doi.org/10.1016/j.applanim.2004.08.020

Dobec RL, Borger ML, Neal SM (1994). Correlation of real-time ultrasonic measures of fat and longissimus muscle area in Standardbred horses with lifetime racing record and lifetime winnings. J. Anim. Sci., 72: 44.

Ewen Mc B. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. 87(3):873-904

Fazio E, Lindner A, Cravana C, Wegener J, Medica P, Hartmann U, Ferlazzo A. (2022). Effects of Standardized Exercise Tests on Plasma Thyroid Hormones' Kinetics in Standardbred Race. J. Equine Vet. Sci.,

Ferlazzo A, Cravana C, Fazio E, Medica P. (2018). Is There an Interplay Between the Hypothalamus-Pituitary- Thyroid and the Hypothalamus-Pituitary-Adrenal Axes During Exercise-Stress Coping in Horses? J. Equine Vet. Sci., 62: 85–97

Fitriani M, Syafaruddin dan Edy JK (2021). Analisis faktor-faktor yang Mempengaruhi Minat Kunjungan Ulang Wisatawan ke Kebun Binatang Taman Rimba Provinsi Jambi. E J. Perspektif Ekonomi dan Pembangunan Daerah. 10(1). https://doi.org/10.22437/pdpd.v10i1.12496

Gholib, Sri W, Rahma M, Muslim A (2021). Evaluasi validitas human cortisol enzyme-linked immunosorbent assay (ELISA) kit dan waktu sentrifugasi sampel darah untuk pengukuran konsentrasi hormon kortisol pada kambing kacang. J. Agripet., 21(1): 97-105. https://doi.org/10.17969/agripet.v21i1.18153

González O, González E, Sánchez C, Pinto J, González I, Enríquez I, Martínez R, Filgueira G, White A. (2010). Effect Of Exercise On Erythrocyte Β-Adrenergic Receptors And Plasma Concentrations Of Catecholamines And Thyroid Hormones In Thoroughbred Horses. Equine Veterinary Journal

Hall C, Hayley R, Gemma P, Liane P, Natalie W (2018). Assessing equine emotional state. Appl. Anim. Behav. Sci., 205: 183–193. https://doi.org/10.1016/j.applanim.2018.03.006

Henshall C. Hayley R, Nidhish F, Rafeal F (2022). The effect of stress and exercise on the learning performance of horses. Sci. Rep., 12: 1918. https://doi.org/10.1038/s41598-021-03582-4

Hosey GR (2005). How does the zoo environment affect the behaviour of captive primates? Appl. Anim. Behav. Sci., 90: 107–129. https://doi.org/10.1016/j.applanim.2004.08.015

Kentucky Equine Research. (2006). Transportation of Horses. https://ker.com/equinews/transportation-of-horses/

Kersey, Dehnhard (2014). The use of non-invasive and minimally invasive methods in endocrinology for threatened mammalian species conservation. Gen. Compar. Endocrinol., 203: 296–306.

Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P, Richer-Levin G, Sgoifo A, Steimer, T, Stiedl, O, van Dijk G, Wöhr M, Fuchs E (2011). Stress revisited: A critical evaluation of the stress concept. Neurosci. Biobehav. Rev., 35: 1291e1301. https://doi.org/10.1016/j.neubiorev.2011.02.003

Laura L, Adyatama A, Indra Y, Achadri Y, Airin CM (2020). The effect of different physiological reproduction conditions on Estradiol (E2) residues in local raw milk. Pak. J. Nutr., 19(3): 127-131. https://doi.org/10.3923/pjn.2020.127.131

Lestari A, Susanti HI, Ananda SA, Rusny (2023). Manajemen pemeliharaan kuda di desa gantarang, kabupaten jeneponto. J. Anim. Husb., 2(2): 88-94. https://doi.org/10.24252/anoa.v2i2.39954

Ljubijanke N, Ranka P, Sabiha S, Aida S, Ismet T, Emin S (2008). Daily fluctuation of cortisol in the saliva and serum of healthy persons. Bosn J. Basic Med. Sci., 8(2). https://doi.org/10.17305/bjbms.2008.2962

Maneu-Sergi O, Annaïs C, Paula S, Manel L (2023). Cortisol variations to estimate the physiological stress response in horses at a traditional equestrian event. Animals, 13: 396. https://doi.org/10.3390/ani13030396

Matsuura A, Sakuma S, Irimajiri M, Hodate K (2013a). Maximum permissible load weight of a Taishuh pony at a trot. J. Anim. Sci., 91: 3989–3996. https://doi.org/10.2527/jas.2012-5540

Matsuura A, Irimajiri M, Matsuzaki K, Hiraguri Y, Nakanowatari T, Yamazaki A, Hodate K (2013b). Method for estimating maximum permissible load weight for Japanese native horses using accelerometer-based gait analysis. Anim. Sci. J., 84: 75–81. https://doi.org/10.1111/j.1740-0929.2012.01041.x

Matsuura A, Mano H, Irimajiri M, Hodate K (2016). Maximum permissible load for Yonaguni ponies (Japanese landrace horses) trotting over a short, straight course. Anim. Welf., 25: 151–156. https://doi.org/10.7120/09627286.25.1.151

Mitchell G, Obradovich SD, Herring FH, Dowd B, Tromburg C (1991). Threats to observers, keepers, visitors, and others by zoo mangabeys (*Cercocebus galeritus chrysogaster*). Primates,

Links Researchers

32: 515–522. https://doi.org/10.1007/BF02381942
Morgan KN, Tromborg CT. (2007). Sources of stress in captivity. Applied Animal Behaviour Science . 102:262–3
Newsome D, Moore SA, Dowling RK (2001). Natural area tourism: Ecology, impacts and management. Channel View Publications, Sydney.
Ono A, Matsuura A, Yamazaki Y, Sakai W, Watanabe K, Nakanowatari T, Kobayashi H, Irimajiri M, Hodate K (2017). Influence of riders' skill on plasma cortisol levels of horses walking on forest and field trekking courses. Anim. Sci. J., 88: 1629–1635. https://doi.org/10.1111/asj.12801
Pasciu V, Nieddu M, Baralla E, Muzzeddu M, Porcu C, Sotgiu FD, Berlinguer F (2022). Non-invasive assay for measurement of fecal triiodothyronine (T3) metabolite levels in European mouflon (O*vis aries musimon*). Front. Vet. Sci., 9: 851794. https://doi.org/10.3389/fvets.2022.851794
Pawluski, Patrick J, Severine H, Anaelle B, Rupert P, Caroline C, Martine H (2017). Low plasma cortisol and fecal cortisol metabolite measures as indicators of compromised welfare in domestic horses (*Equus caballus*). PLoS One Health, pp. 1-18. https://doi.org/10.1371/journal.pone.0182257
Rain K, Anggoro D, Indarjulianto S, Astuti P, Airin CM (2022). Faecal triiodothyronine hormone levels in female iguana during weekday and weekend in in situ conservation. Bio Web Conf., 49(01012). https://doi.org/10.1051/bioconf/20224901012
Rajagopal T, Govindaraju A, Mahadevan S (2011). Impact of zoo visitors on the fecal cortisol levels and behavior of an Endangered species: Indian Blackbuck (*Antelope cervicapra* L.). J. Appl. Anim. Welf. Sci., 14: 18–32. https://doi.org/10.1080/10888705.2011.527598
Sapolsky RM, Romero LM, Munck AU (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev., 21: 55–89. https://doi.org/10.1210/edrv.21.1.0389
Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B, Aurich J, Faure, JM, Aurich C (2010). Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. Domest. Anim. Endocrinol., 38: 179–189. https://doi.org/10.1016/j.domaniend.2009.10.002
Sekar M, Rajagopal T, Archunan G (2008). Influence of zoo visitor presence on the behavior of captive Indian gaur (*Bos gaurus gaurus*) in a zoological park. J. Appl. Anim. Welf. Sci., 11: 352–357. https://doi.org/10.1080/10888700802330093
Sheriff MJ, Dantzer B, Delehanty B (2011). Measuring stress in wildlife: Techniques for quantifying glucocorticoids. Oecologia, 166(4): 869–887. https://doi.org/10.1007/s00442-011-1943-y
Silver J (2002). Spinal injuries resulting from horse riding accidents. Nature, 40: 264-271. https://doi.org/10.1038/sj.sc.3101280
Skyner LJ, Amory JR, Hosey GR (2004). The effects of visitors on the self-injurious behaviour of a male pileated gibbon (*Hylobates pileatus*). Der Zoologische Garten, 74: 38–41.
Spiga F, Jamie J, Walker, John R, Terry, Stafford LL (2014). HPA Axis-Rhythms. Compreh. Physiol., 4: 1273-1298. https://doi.org/10.1002/cphy.c140003
Stachurska A (2018). Effect of warm-up intensity on horse-rider dyad's performance in jumping. Ciência Rural, 48: 1. https://doi.org/10.1590/0103-8478cr20170638
Starr L, Dienes K, Irina L, Shaw YZA. (2019). Chronic stress exposure, diurnal cortisol slope, and implications for mood and fatigue: Moderation by multilocus HPA-Axis genetic variation. Phychoneuroendocrinology. 100:156-163.
Stefánsdóttir GJ, Gunnarsson V, Roepstorff L, Ragnarsson S, Jansson A (2017). The effect of rider weight and additional weight in Icelandic horses in tölt: Part I. Physiological responses. Animal, 11: 1558–1566. https://doi.org/10.1017/S17517311170005566
Steward PM (2003). The adrenal cortex. In: P.R. Larsen, H.M. Kronenberg, S. Melmed and K.S. Polonsky (Eds.), Williams textbook of endocrinology, 10th ed.,. Philadelphia, PA: Saunders. pp. 491–551.
Synder RL (1975). Behavioral stress in captive animals. In: G. Rabb (Ed.), Research in zoos and aquariums. Washington, DC: National Academy of Sciences. pp. 41–76.
Venugopal B, Sha AA (1993). Visitor behaviour at lion-tailed macaque in the Mysor zoos. Zoos Print J., 8: 45–48.
Wasser SK, Azkarate JC, Booth RK, Hayward L, Hunt K, Ayres K, Vynne C, Gobush K, Canales-Espinosa D, Rodríguez-Luna E. (2010) Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species General and Comparative Endocrinology 168: 1–7..
Wickler SJ, Hoyt DF, Cogger EA, Hall KM (2001). Effect of load on preferred speed and cost of transport. J. Appl. Physiol., 90: 1548–1551. https://doi.org/10.1152/jappl.2001.90.4.1548
Yuniarti W, Nugraheni YR, Fibrianto YH, Artama WT, Susmiati T (2022). Comparison of progesterone hormone in feces and milk of Friesian Holstein cattle at lactating period. Indones. J. Vet. Sci., 3(1): 38-40.

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