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Relationship between Sociability and Faecal Cortisol in Captive Alpine Musk Deer *Moschus chrysogaster* Hodgson, 1839 (Mammalia: Moschidae)

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Abstract: Sociability indicates the variances of affinity and solitary among animals and it is strongly correlated with cortisol levels. This study was conducted in 2020, from September 16th to October 15th, at the Zhuanglang Musk Deer Farm in Gansu Province of China. We recorded behaviours of 41 captive Alpine musk deer (*Moschus chrysogaster*) (in 12 enclosures) using the integrated methods of focal sampling and all occurrence recording for sociability analysis, while non-invasive sampling methods and radioimmunoassay were used to collect and measure faecal cortisol level. The relationship between the sociability of captive Alpine musk deer and faecal cortisol hormone was analysed. The results showed that the sociability of captive Alpine musk deer was 14.754% (± 3.026%) (n=41). Individuals in high-density enclosures (19.090% ± 4.497%, n=26) were significantly more sociable as compared to those in low-density enclosures (7.979% ± 2.640%, n=15; *P*=0.011). The cortisol level of Alpine musk deer was 109.215 (± 5.349) ng/g (n=41). The cortisol level of female individuals (120.419 ± 11.103 ng/g, n=14) was significantly higher than that of male individuals (103.406 ± 5.575 ng/g, n=27; *P*=0.008). The male musk deer in high sociability (44.114% ± 9.364%, n=8) had lower faecal cortisol levels (102.415 ± 14.885 ng/g, n=8) than those in low sociability (5.518% ± 1.369%, n=19) (103.823 ± 5.243 ng/g, n=19), however these differences were not significant (*P*=0.911). The female musk deer in high sociability (20.556% ± 2.174%, n=3) had significantly lower (*P*=0.035) faecal cortisol levels (77.018 ± 17.036 ng/g, n=3) than those in low sociability (7.773% ± 1.595%, n=11) (132.256 ± 11.079 ng/g, n=11), which could be related to maternal care and weaning period. It is suggested that the enclosing density should be increased to improve captive Alpine musk deer's (especially female musk deer) sociability in the domestication of musk deer.

Key words: artificial social environment, behavioural-endocrine stress response, small solitary forest ruminant, in captivity, sociability, faecal cortisol hormone

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Introduction

Sociability is a fundamental characteristic among animals, reflecting the diverse range of affinities and tendencies toward solitude that exist among them (Wilson 2000). The expression intensity of an individual animal's social affinity behaviour can be used to measure the strength of its sociability (Capitanio et al. 2004). The sociability of animals is correlated with sex, age, population density, dominance rank, kinship. For example, adult rhesus monkeys (*Macaca mulatta*) scored higher sociability than individuals aged one to five years (Stevenson et al. 1978). As density increased, the sociability of wild elk (*Cervus canadensis*) living in national parks in the Canadian Rocky Mountains also increased (Found et al. 2019). Research has demonstrated that kinship was significantly positively correlated to social bond strength, whereas rank distance was significantly negatively correlated to social bonds in Tibetan macaques (*Macaca thibetana*) (Gu et al. 2019). Individual variations in sociability can also be observed in many captive animals, such as chicks, cattle, sheep and brushtail possums (*Trichosurus vulpecula*) (Gartland et al. 2022, Jenny et al. 2010).

Environmental stress can trigger a behavioural-endocrine stress response in animals by activating the hypothalamic–pituitary–adrenocortical (HPA) axis, as well as the release of glucocorticoids, such as cortisol (COR), facilitates self-protection. Cortisol levels can be used as an indicator of stress in animals and many non-injury-based tests have found that higher cortisol levels are often associated with greater stress in animals (Capezzuto et al. 2008, Jurke et al. 2015, Qin et al. 2020, Salas et al. 2016, Wooddell et al. 2017). The cortisol levels of animals are correlated with their sex, enclosure density, mating season and social rank. For example, the cortisol level of free-living striped mice (*Rhabdomys pumilio*) was significantly lower (about half) during the mating season (Schradin et al. 2008). In male rock hyraxes (*Procavia capensis*), cortisol levels were associated with social rank, with dominants showing the highest levels (Koren et al. 2008). In forest musk deer (*Moschus berezovskii*), females had significantly higher cortisol levels than males (He et al. 2014).

The sociability of animals helps to reduce the physiological stress response of animals, which finally reduces cortisol levels (Grand et al. 2012). For example, dogs (*Canis familiaris*) with more social connections had significantly lower cortisol levels (Ottenheimer et al. 2013). The adult female rhesus macaques (*Macaca mulatta*), which initiated higher levels of social affiliation, had significantly lower levels of cortisol concentrations (Wooddell et al. 2017). Capitanio (2004) found that the low-sociable male rhesus monkeys (*Macaca mulatta*) had higher cortisol levels compared to high-sociable individuals. Yamanashi (2018) found that captive male chimpanzees (*Pan troglodytes*) which rarely received grooming behaviours from other individuals, may have chronically elevated cortisol levels. Grand (2012) reported that captive adult female African elephants (*Loxodonta africana*), which exhibited more social behaviours, had relatively lower cortisol levels. So far, most of the studies on the relationship between sociability and cortisol levels have focused on group living mammals with strong sociability, while there is a lack of relevant studies on solitary animals under social environmental stress.

Alpine musk deer *Moschus chrysogaster* Hodgson, 1839, a small solitary ruminant distributed throughout Qinghai-Tibet plateau and surrounding areas, is critically endangered due to overutilisation and habitat destruction. Musk deer farming has become an important tool to conserve Alpine musk deer. Alpine musk deer are highly territorial and solitary in the wild, but modern *ex-situ* conservation measures of musk deer's domestication are based on the artificial social environment. Currently, there is a scarcity of research focusing on the "social" behavioural endocrinology of musk deer within a social environment. Therefore, the results of this study can deepen the understanding of the sociability of animals, improving sociability to reduce stress and can provide an important reference for *ex-situ* conservation of endangered musk deer.

Materials and Methods

Study site

The study was conducted at the Gansu Zhuang Lang Fengchun Musk Deer Breeding Farm (hereinafter referred to as musk deer farm), which is located in the east of the Qilian Mountain to the Yumei Region and the west foot of the Liupan Mountain. The site is located at 1800 m a.s.l., with four distinct seasons. Annual temperature averages 8.1°C, with the highest average temperature in July and the lowest in January. Annual precipitation is 489 mm (mostly concentrated in July, August and September) and the annual frost-free period is about 142 days.

Zhuang Lang Breeding Centre of Alpine musk deer was built in 1990 with an area of 0.1 km^2 and contains more than 250 enclosures. The enclosures are divided by brick walls and linked through wooden gates, with six to 12 enclosures positioned ad-

Fig. 1. The overall condition of the enclosure

jacently to create a breeding area. Each enclosure encompasses an outdoor yard measuring 200 m^2, accompanied by 5–8 adjoining rooms arranged on one side. Within each enclosure, there are typically two to seven musk deer. These rooms were open and were used to improve environmental heterogeneity and provide shelter for animals avoiding adverse weather. In the middle of the outdoor yard, there is a shed measuring 2 × 3 × 2 m for animals to rest. The overall view of the enclosure is shown in Fig. 1 below.

Experimental animals

Animals were fed twice a day (at 05:30 and 16:30 in summer and at 08:30 and 17:00 in winter), mainly with fresh leaves (in summer and autumn) or dried leaves (in winter and spring), which were collected from the natural habitats of wild Alpine musk deer. Furthermore, the deer were provided with supplemental artificial food (mainly consisting of flour, wheat bran and some seasonal vegetables) and water. Apart from feeding and cleaning the enclosure, people rarely approached the musk deer for the rest of the day. Each Alpine musk deer wore a plastic ear tag to be individually identified.

More than 800 Alpine musk deer were domesticated at the musk deer farm. This study involved 41 Alpine musk deer (27 males and 14 females) in 12 group enclosures, of which three females were nursing and rearing their young. According to age, the Alpine musk deer were divided into sub-adult (≤2 years old: 3 individuals, 2 females and 1 male) and adult groups (all 3 years old: 38 individuals, 12 females and 26 males). According to the captive breeding method, the 12 Alpine musk deer enclosures were divided into an all-male group (23 musk deer in 7 groups), the all-female group (2 musk deer in 1 group) and a mixed group (16 musk deer in 4 groups, of which 2 groups were of 1 male with 2 female musk deer and 2 groups were 1 male with 4 females). Given the typical practice of housing 4 musk deer per enclosure in musk deer farms, different enclosures can be categorised into a low-density group (≤ 4 individuals: 7 enclosures, 15 individuals) and a high-density group (> 4 individuals: 5 enclosures, 26 individuals), based on the number of individuals within each enclosure.

Behavioural samplings and sociability determination

All 41 individuals of captive Alpine musk deer were sampled. To ensure the effectiveness, comprehensiveness and representativeness of behavioural sampling, observations were conducted daily from 16th September to 15th October, 2019, once in the mornings from 06:00 to 10:00 and once in the evenings from 16:00 to 20:00. This is because musk deer activity peaked within the time period mentioned above (Meng et al. 2002). The duration of social behaviours was recorded using continuous focal sampling and all occurrence records (Altmann 1974). Behavioural sampling was assisted through using binoculars (16° x 50°). Each observed enclosure was monitored for one hour in both the morning and

the afternoon. Observations were performed by one observer for a total of 120 h (10-min-long sessions each sampling unit).

Since this study occurred during the non-mating season, the behavioural observations excluded mating behaviours. Based on long-term observations of captive horse musk by Meng et al. (2007), the affiliative behaviours of captive Alpine musk deer mainly included mutual grooming (two individuals grooming each other, licking the fur, etc.) and touching (non-aggressive touching of body parts such as head and shoulder between individuals).

The sociability was represented by the ratio of the duration of affinity behaviour to the sampling time of behaviour. According to the mean value of sociability in captive Alpine musk deer, the sample animals were divided into high sociability group (greater than or equal to the mean value) and low sociability group (less than the mean value) (Yuan et al. 2021).

Faecal cortisol determination

Alpine musk deer is alert and timid, collecting blood could lead to a strong stress response and induce the secretion of adrenal glucocorticoid, which may interfere with the level of cortisol, so it is not suitable to collect blood samples to detect cortisol. Capezzuto (2008) stated that the profiles of cortisol faecal metabolites reflect the serum concentrations of the same hormone in pregnant goats, an ungulate species. Therefore, in this study, non-invasive sampling was used to collect fresh faecal samples of Alpine musk deer in the experimental period and determine faecal cortisol metabolite levels (He et al. 2014, Lang et al. 2012).

Fresh faecal samples were collected twice a week, frozen immediately and stored at –20 °C. Eight faecal samples were collected from each musk deer during the whole experimental period. Faecal cortisol was extracted using the ethanol-based procedure as described by Blumstein (2006) with slight modifications. 0.2 g of faeces was mixed with 5 ml of 90% ethanol in a 15 ml glass tube and extracted using a "water bath" at 80°C for 20 min. All tubes were then centrifuged at 1500 rpm for 20 min and the supernatant was recovered. An additional 5 ml of 90% ethanol was added to the faecal pellet, which was then vortexed for 1 min and centrifuged at 1500 rpm for 20 min. The supernatant was combined to make the sample to be measured.

A radioimmunoassay was developed and validated to measure corticoid metabolite concentrations in faeces. Faecal cortisol quantitative diagnostic kits were obtained from Beijing Beifang Biological Technology Co., Ltd., Beijing, China. The assays were performed according to the manufacturer's directions. Sensitivity parameters are as follows, sensitivity ≤ 5.0 ug/ml, intra-assay CV < 10% and the inter-assay CV < 15%, no cross-reaction. For each faeces, water content was calculated (dry in the oven at 65°C for 8 h) and was used to adjust the final faecal cortisol concentrations (FCC).

Statistical analysis

The Kolmogorov-Smirnov Test was used to test for normality of the data. The Generalised Linear Model (GLM) was used to explore the main effect of gender, age and enclosure density on sociability and cortisol and the interaction among variables. The Pearson correlation and Linear regression analyses were used to explore the relationship between sociability and cortisol levels. An independent-samples T-test was used to analyse sociability rank effects. The Spearman correlation and Mann-Whitney U test were used to determine relationships between cortisol levels and sociability when the original data and standardised data were not normally distributed. Statistical analyses were conducted using SPSS 22.0 and all reported statistical probabilities were two-tailed at $\alpha = 0.05$. The results were expressed as the mean ± standard deviation and the significance level was set at $P=0.05$.

Results

Sociability and cortisol of Alpine musk deer

Over a 150-minute sampling period, a single musk deer individual displayed social behaviour for an average of 22 min 7.86 s ± 4 min 32.34 s (n=41). The sociability of Alpine musk deer was 14.754% ± 3.026% (n=41). As shown in Fig. 2, the distribution of sociability data was normal after square root standardisation (Kolmogorov–Smirnov Test: $Z=0.104$, $P=0.200$). The skewness coefficient of the normal curve was 2.605, indicating that the data of sociability were distributed near the low sociability area, while the kurtosis coefficient was 7.908, indicating that the sociability distribution was more concentrated, so captive Alpine musk deer's sociability was generally low. The sociability of most individuals (78.05%) was less than 20% and only 4.88% of individuals' sociability was higher than 70%. According to the mean value of sociability in captive Alpine musk deer (14.754%), the individuals were divided into high sociability (≥ 14.754%, n=11) and low sociability (< 14.754%, n=30).

The mean cortisol level of Alpine musk deer was 109.215 ± 5.349 ng/g (n=41). As shown in Fig.

2, the distribution of cortisol level data was normal (Kolmogorov–Smirnov Test: Z=0.084, *P*=0.200). The skewness coefficient of the normal curve was 0.424, indicating that the data of cortisol level were distributed near the average cortisol subject and the kurtosis coefficient was 0.297, indicating that cortisol distribution was more dispersed. Thus, the cortisol levels of captive Alpine musk deer were within the average range. The cortisol levels of most individuals (53.66%) fell between 80 and 120 ng/g, with only 21.95% having cortisol levels exceeding 140 ng/g.

Relationship between sociability and age, sex and enclosure density

The results of the generalised linear model (GLM) showed that individuals' sociability was significantly correlated with enclosure density (*P*=0.01). We found that individuals in high-density enclosures (19.090% ± 4.497%, n=26) were significantly more sociable as compared to those in low-density enclosures (7.979% ± 2.640%, n=15) ($F_{(1,39)}$=7.165, *P*=0.011).

There were no significant effects of gender ($F_{(1,39)}$=0.292, *P*=0.592), age ($F_{(1,39)}$ =0.358, *P*=0.702) and interactions among variables (gender * age, $F_{(1,39)}$=0.276, *P*=0.761; gender * enclosure density, $F_{(1,39)}$=1.790, *P*=0.183; age * enclosure density, $F_{(1,39)}$=2.508, *P*=0.097; gender * age * enclosure density, $F_{(1,39)}$=0.210, *P*=0.811) on sociability.

Relationship between cortisol and age, sex and enclosure density

The results of the GLM showed that individual cortisol levels correlated with the sex of animals. The cortisol level (120.419 ± 11.103 ng/g, n=14) of female Alpine musk deer was significantly higher than that of male individuals (103.406 ± 5.575 ng/g, n=27) ($F_{(1,39)}$=8.112, *P*=0.008).

There were no significant effects on the sociability of enclosure density ($F_{(1,39)}$=0.151, *P*=0.700), age ($F_{(1,39)}$=0.400, *P*=0.535) and interactions among variables (gender * age, $F_{(1,39)}$=0.101, *P*=0.767, gender * enclosure density, $F_{(1,39)}$=0.840, *P*=0.367, age * enclosure density, $F_{(1,39)}$=0.760, *P*=0.391, gender * age * enclosure density, $F_{(1,39)}$=0.841, *P*=0.367).

Relationship between sociability and cortisol levels of Alpine musk deer

Since sex has a significant effect on cortisol levels, sex differentiation can also influence the relationship between sociability and cortisol levels.

As seen in Fig. 4, linear regression showed that there was no relationship between cortisol levels and the sociability of male individuals (Pearson: *r*=–0.112, *P*=0.580, n=27), female individuals (Pearson: *r*=–0.319, *P*=0.266, n=14) or individuals regardless of sex (Pearson: *r*=–0.133, *P*=0.409, n=41). The link between cortisol and the sociability of male musk deer, the sociability of females and the sociability of individuals can be approximated by the equations y=-0.149x+105.94 (R^2=0.014, *P*=0.553); y=-1.816x+139.51 (R^2=0.102, *P*=0.266); and y=-0.290x+113.492 (R^2=0.027, *P*=0.306).

As shown in Fig. 5 and Table 2, the male musk deer with high sociability (44.114% ± 9.364%, n=8) had lower faecal cortisol levels (102.415 ± 14.885 ng/g, n=8) than those with low sociability (5.518% ± 1.369%, n=19) (103.823 ± 5.243 ng/g, n=19) but that association was not significant (*P*=0.911).

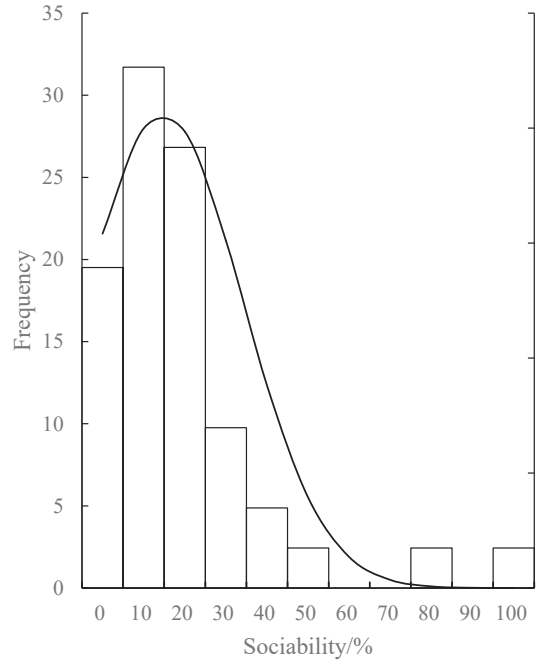

Fig. 2. Distribution of sociability in captive Alpine musk deer

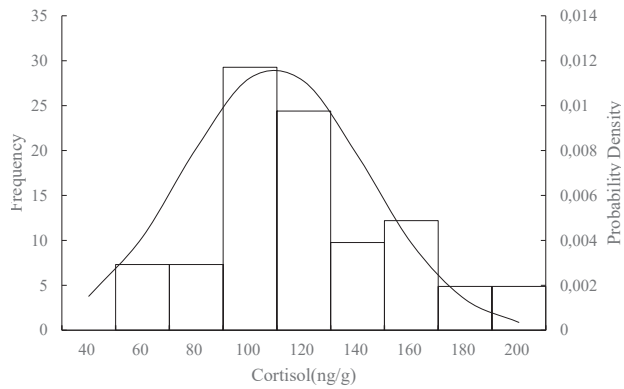

Fig. 3. Distribution of cortisol levels in captive Alpine musk deer

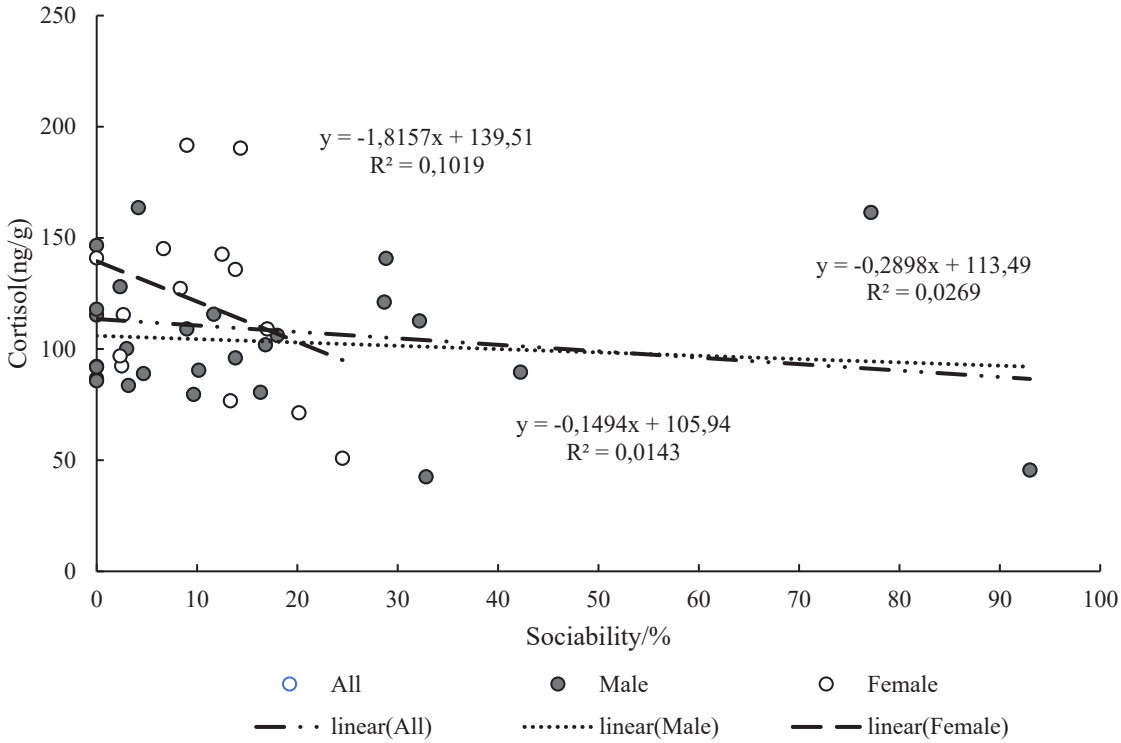

Fig. 4. The relationship between faecal cortisol levels and sociability of musk deer

Table 1. Comparison of cortisol levels in musk deer of different sociality groups and different sexes, using the independent-samples T-test. * – significant associations.

	Low sociability (<14.754%)	High sociability (≥14.754%)	P	All COR (ng/g)
Female	132.256±11.079 (n=11)	77.018±17.036 (n=3)	0.035*	120.419±11.103 (n=14)
Male	103.823±5.243 (n=19)	102.415±14.885 (n=8)	0.911	103.406±5.575 (n=27)
Sig	0.006*	0.052		0.008*
All	115.899±5.994 (n=30)	94.819±10.069 (n=11)	0.066	109.215±5.349 (n=41)

The female musk deer in high sociability (20.556% ± 2.174%, n=3) had significantly lower faecal cortisol levels (77.018 ± 17.036 ng/g, n=3) than those in low sociability (7.773% ± 1.595%, n=11) (132.256 ± 11.079 ng/g, n=11) (T-test: *P*=0.035).

The Alpine musk deer in high sociability (34.442% ± 6.652%, n=11) had slightly lower faecal cortisol levels (94.819 ± 10.069 ng/g, n=11) than those in low sociability (5.613% ± 0.983%, n=30) (115.899 ± 5.994 ng/g, n=30) but these differences were not significant (T-test: *P*=0.066).

Discussion

Numerous studies in animals have demonstrated that the intensity of social affinity behaviour expressed by animals is an explicit manifestation of the individual's "personality measure" and is closely related to its physiological state (Capitanio et al. 2004, Grand et al. 2012, Wooddell et al. 2017, Yamanashi et al. 2018). There are widespread social interactions among individuals living in groups of animals. Among them, the positive social relationship formed by the positive social interaction is beneficial for alleviating the physiological stress response, inhibiting the activity of the HPA axis and reducing the level of cortisol (Capitanio et al. 2004, Hennessy et al. 2009, Salas et al. 2016, Wooddell et al. 2017). Thus, the consistent association between sociability and cortisol makes them indices of captive animal management.

In this study, individuals' sociability was quantified by measuring the expression of affiliative behaviours. We found that the sociability of Alpine musk deer was 14.754% ± 3.026%, which was

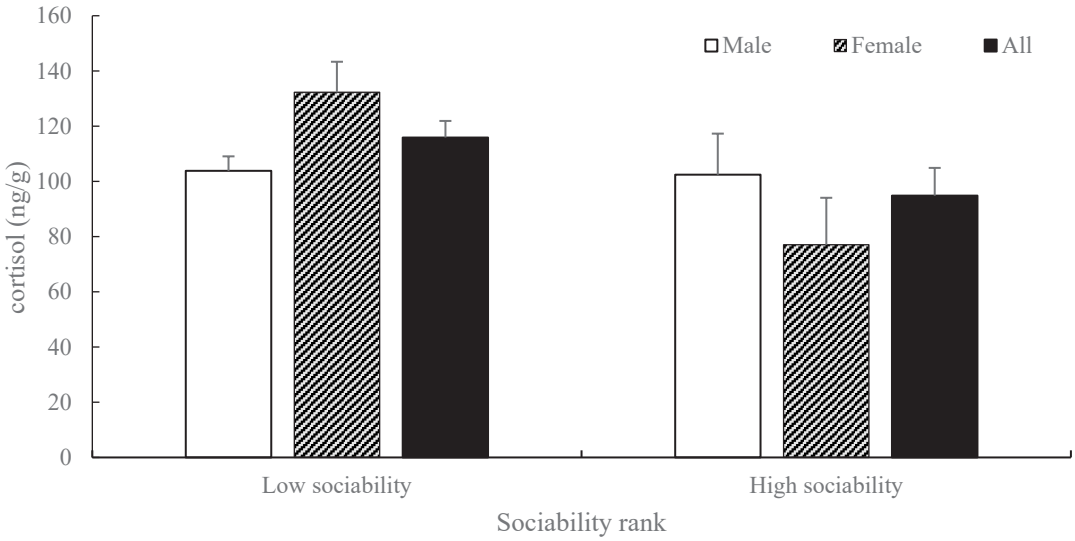

Fig. 5. Faecal cortisol levels of Alpine musk deer in low and high sociability

lower than the social affinity of captive forest musk deer (57.4%: Fang et al. 2019), indicating that the proportion of time spent on mutual grooming and touching behaviours in the artificial condition was relatively low and the expression of social affinity behaviours was weak. It further illustrates that in artificial condition, Alpine musk deer, being a typical small solitary forest ruminant (Meng et al. 2012), still retain an unsociable disposition or wild nature, owing also to the fact that Alpine musk deer are hard to domesticate (Zhang 1983). Similarly, Black (2000) found that brown brocket deer (*Mazama gouazoubira*), also a small solitary forest ruminant, exhibited low sociability in a large enclosure and tended to live alone.

We observed that individuals residing in high-density enclosures exhibited significantly greater sociability when compared to the individuals in low-density enclosures. Wild musk deer are not completely solitary but live in a group within large distances and looseness between individuals; social behaviours are less expressed among individuals (Yang et al. 1998). Enclosures differ greatly from wild habitat, indicating restricted space and close social contact so that sociability levels increase with density (He et al. 2014). Similarly, the sociability of many ungulates increases with population density (Found 2019). He (2014) found that cortisol levels of female forest musk deer (*Moschus berezovskii*) were significantly higher when in groups of smaller size as compared to larger size groups.

Faecal cortisol level is a reliable and non-invasive physiological stress indicator for musk deer (He et al. 2014). Qin (2020) found that the cortisol of Alpine musk deer at the Musk Deer Breeding Centre of Xinglong Mountain Nature Reserve was 101.529 ± 8.553 ng/g, slightly lower than the cortisol levels of forest musk deer in the musk deer farm in Fengxian County (Lang et al. 2012) due to species differences. The sex of captive musk deer had a significant effect on cortisol levels: cortisol levels of females were significantly higher than that of males. Cortisol secretion is affected by the gonadal hormone effect, gonads regulate HPA axis activity, androgens inhibit glucocorticoid secretion from adrenal glands, while estradiol promotes adrenocorticotropin-releasing hormone (ACTH), which in turn promotes glucocorticoid secretion and there are differences in cortisol secretion between genders (Salas et al. 2016). This study took place during the non-mating season, a period when females were more alert than males during the nursing period and had higher stress physiological levels, resulting in higher cortisol levels (Lang et al. 2012), which again verified the conclusion that the nursing period may affect cortisol secretion (Baker et al. 2013). Similar results were found for the ring-tailed lemur (*Lemur catta*), red deer (*Cervus elaphus*) and forest musk deer (*M. berezovskii*) (He et al. 2014, Salas et al. 2016, Starling et al. 2010).

Our results suggested that the cortisol levels of female musk deer were significantly higher than that of males with low sociability, while no significant differences were found between the sexes with high sociability. This was mainly related to the significant changes in the cortisol levels of female musk deer. The female musk deer in high sociability had significantly lower faecal cortisol levels than those in low sociability (P=0.035), indicating that female musk deer, which engage more in affinity social behav-

iours, were less physiologically stressed in captivity and better able to adapt to environmental stress and improve individual welfare and health. Moreover, we recorded that all three female musk deer with high sociability were involved in nursing. Lactation promoted the secretion of oxytocin (OT), which at higher concentrations promoted the development of affectionate social behaviour and formed intimate social bonds between mothers and the young. Additionally, lactation inhibited the activity of the HPA axis and reduced the concentration of cortisol (Hennessy et al. 2009). This finding further validates the conclusion that the presence of young can alleviate the physiological stress response of mothers (Hennessy et al. 2009). Similarly, Wooddell (2017) found that maternal care, such as mutual gaze between mothers and young, significantly reduced cortisol levels in female rhesus monkeys (*Macaca mulatta*) three months after delivery.

Conclusions

The sociability level of the Alpine musk deer is low in enclosures and increases with the population density. Musk deer domestication can be classified into commercial domestication and *ex-situ* conservation domestication. In commercial domestication, Alpine musk deer should be farmed in sociable groups with reasonable density in order to improve the individual's sociability and domestication. Further, individuals with higher sociability can be used as mating ones, which is conducive to the improvement of its domestication and eventually achieve breeding of "high-sociality" strains. In the *ex-situ* conservation domestication, especially the basic population prepared for wild release, Alpine musk deer's solitary nature should be maintained and we should reduce its density to increase the success of wild release. In addition, during the lactation period, maternal care should be appropriately prolonged to improve the health of Alpine musk deer.

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