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Seasonal variations in immunoreactive cortisol and fecal immunoglobulin levels in Sichuan golden monkey (*Rhinopithecus roxellana*)

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Abstract: The Sichuan golden monkey is deserving of conservation and the welfare of this species is receiving increasing attention. However, it is difficult to identify physiological parameters for assessing its conservation status. In the present study, we employed noninvasive sampling methods and immunochemical assays to determine if measurements of cortisol and immunoglobulin in feces allowed monitoring of physiological status. The results indicated that the cortisol and immunoglobulin concentrations in feces could serve as biomarkers for monitoring stress and immune status, and that there was a relationship between stress and the immune response. A tendency toward seasonal fluctuation in immunoreactive cortisol and fecal immunoglobulin concentrations emerged, indicating that these indices could be useful tools for monitoring the welfare of the monkey and could be used in the field of wildlife research.

Key words: Noninvasive sampling, immunoreactive cortisol, fecal immunoglobulin, animal welfare, Sichuan golden monkey (*Rhinopithecus roxellana*)

1. Introduction

Animal welfare is becoming prominent not only in the field of animal science but also in the public eye. However, assessment of animal welfare is very complex. If many physiological, endocrinological, and behavioral parameters are simultaneously measured, misinterpretation can be obviated to some extent (Clark et al., 1997). A potential indicator of animal welfare is the absence of stress (Hofer and East, 1998). Assessment of animal stress levels aids in welfare monitoring and improves our understanding of animal biology (Young and Hallford, 2013). It is known that stress usually enhances glucocorticoid production and hormonal output from the adrenal cortex (Axelrod and Reisine, 1984). Physiological stressing of animals caused by disturbance to their environment is adaptive in the short term; however, if glucocorticoid concentrations remain elevated for longer periods of time, chronic stress develops, and this compromises many physiological processes critical in terms of individual health and survival, including immune and reproductive functions and resistance to diseases (Sapolsky et al., 2000; Wingfield and Sapolsky, 2003; Muehlenbein, 2009).

In recent years, several studies have demonstrated the importance of noninvasive sampling methods now widely used in research on animal physiology (Heistermann, 2010). These methods allow physiological data to be collected without stressing the animal (Möstl and Palme, 2002). Analysis of fecal samples can be instructive; the data yield insights into the internal milieu of an animal (Ganswindt et al., 2003). Such analysis may even be applied retrospectively (Aggarwal and Upadhyay, 2013). These types of methods have been used in research into the physiological effects of stress in general (Möstl and Palme, 2002) and the effects of stress on immunity in particular (Paramastri et al., 2007); both managed and protected species have been investigated.

The Sichuan golden monkey (*Rhinopithecus roxellana*) is an arboreal nonhuman primate in China. Wild *R. roxellana* populations live in mountainous regions more than 1400–3000 m above sea level. The population of Sichuan golden monkey is about 14,000 (Wang et al., 1998). In recent decades, the protection and study of the monkey has received much attention, especially regarding its evolution and distribution, and ecology pertinent to the conservation of this species (Kirkpatrick, 1995; Kirkpatrick and Grueter, 2010; Sterck, 2012). However, to date, few reports have explored the physiology of stress and immunophysiology in monkeys living in seminatural conditions.

Our objectives were to explore whether immunoreactive cortisol and fecal immunoglobulin levels could be used to measure physiological status in Sichuan golden monkeys

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and to define (if possible) a correlation between measures of stress physiology and immunophysiology in Sichuan golden monkeys.

2. Materials and methods

2.1. Animals

Six adult Sichuan golden monkeys (3 males: M1, M2, M3; 3 females: F1, F2, F3) living in the Shanghai Wildlife Zoo, Shanghai, China (30°40′N–31°53′N, 120°52′E–122°12′E) were studied. The monkeys were housed together in an enclosure with an indoor nighttime resting room measuring approximately 60 m^2 in area, with access to an outdoor yard of about 900 m^2. The indoor enclosure did not have heating or air conditioning; the temperature in the indoor enclosure was the same as the ambient temperature. The ambient temperatures of the 4 seasons are shown in Table 1. All animals were healthy at all times during the experiment and were fed with their normal diet of fresh leaves of privet, mulberry, and *Pittosporum* (about 40% of total food weight) supplemented with eggs, steamed cornbread, and vegetables. Water was available ad libitum. All experiments were carried out in accordance with the National Institutes of Health guidelines. Our research also adhered to the "Principles for the Ethical Treatment of Non-Human Primates" of the American Society of Primatologists.

2.2. Sampling

We recognized facial features, coat color, and other physical characteristics of Sichuan golden monkeys in order to confirm the target animals. To understand the rhythm of defecation, we observed the target animals over time and collected fresh feces soon after defecation from March 2012 to February 2013. We determined the 4 seasons according to weather conditions and reports over decades: spring (from 24 March to 4 June), summer (from 5 June to 28 September), autumn (from 29 September to 1 December), and winter (from 2 December to 23 March). Fecal samples were collected between 0700 and 0900 hours for 30 days in every season. A fixed time was chosen for collection because cortisol levels in primates fluctuate daily.

Table 1. Ambient temperatures for each of the 4 seasons at the study site.

The ambient temperatures (°C)	Maximum	Minimum	Mean
Sp	21.14	14.98	17.80
Su	30.18	25.11	27.24
Au	19.08	12.38	15.43
Wi	7.18	3.18	4.99

Sp: Spring, Su: summer, Au: autumn, Wi: winter.

All samples of each season were averaged for each male and female, and these averaged values were used in the analyses. All fecal samples were labeled, placed in plastic zip-lock bags, and stored at −20 °C prior to processing.

2.3. Cortisol extraction

Cortisol in feces was extracted using a previously described technique (Yu et al., 2011). Briefly, each frozen sample was thawed and thoroughly homogenized. Approximately 10 g of each sample was desiccated in a vacuum lyophilizer (−60 °C, 24 h). Ten-milliliter amounts of 90% (v/v) ethanol were add to 0.5-g amounts of desiccated ground feces, followed by thorough extraction (using oscillation) for 20 min; each sample was next centrifuged at 500 × *g* for 20 min. Supernatants were evaporated to dryness at 60 °C. Each residue was dissolved in 1.5 mL of methanol, and this solution was transferred to a 2-mL sterile microcentrifuge tube and stored at −20 °C prior to testing.

2.4. Fecal immunoglobulin extraction

Fecal immunoglobulin was extracted via modification of previously described methods (Peters et al., 2004). Fecal samples of approximately 10 g in weight were thawed, homogenized, and desiccated using a vacuum lyophilizer (−60 °C, 24 h). Ten-milliliter amounts of dilution buffer (PBS with 0.5% [v/v] Tween, pH 7.2) were added to 0.5-g aliquots of desiccated ground feces, followed by thorough oscillation at room temperature for 20 min; centrifugation at 1500 × *g* for 20 min followed. Supernatants were subsequently centrifuged for 10 min at 10,000 × *g* at 4 °C. The resulting supernatants were transferred to 2-mL sterile microcentrifuge tubes and stored at −20 °C prior to testing.

2.5. Quantification of cortisol and immunoglobulin levels in feces

The concentrations of cortisol, IgM, IgG, and IgA in feces were assessed using enzyme-linked immunosorbent assays (ELISAs). The ELISA method has very high sensitivity and specificity. Sensitivity is the minimum concentration that can be detected. Replicability is normally expressed as coefficient of variation (CV). The CV refers to the ratio of standard deviations of individual values to averages. Cross-reaction is generally used to express specificity. The parameters of Table 2 were obtained by calculation of the standard curve and data in our work. The units of measurement were ng/g of dried feces for cortisol and µg/g dried feces for IgM, IgG, and IgA. Commercial cortisol and immunoglobulin diagnostic assay kits were purchased from the Shanghai Lianshuo Biological Technology Co., Ltd., Shanghai, China. All assays were performed according to the manufacturer's instructions.

2.6. Data analysis

All analyses were conducted using SPSS 17.0 and Microsoft Excel 2003. All data were tested for normal distribution by

Table 2. Sensitivity parameters of cortisol and immunoglobulin.

Sensitivity parameters	Cortisol	IgG	IgM	IgA
Sensitivity	≤5.0 ng/mL	<0.5 µg/mL	<5.0 µg/mL	<2.5 µg/mL
Intraassay CV	<7.0%	<8.0%	<8.0%	<6.0%
Interassay CV	<8.5%	<11.0%	<10.0%	<7.5%
Cross-reaction	No cross-reaction	<0.01%	<0.01%	<0.01%
Recovery	99%–104%	97%–104%	96%–106%	97%–102%

the Shapiro–Wilk test and for homogeneity of variances by Levene's test. If the data were normally distributed and had similar variances, then one-way analysis of variance (ANOVA) and the independent samples t-test were used. When ANOVA results were significant, multiple comparisons of means were used with S–N–K post-hoc analysis. The results are expressed as means ± SEMs. Materials of skewed distribution were tested with the Kruskal–Wallis and Mann–Whitney tests. The results are expressed as medians (quartile range, QR). We also adopted the Spearman correlation test. Statistical significance for all analyses was accepted at a level of $P < 0.05$. The variance was of high statistical significance when $P < 0.01$.

3. Results

3.1. Immunoreactive cortisol concentrations in Sichuan golden monkeys

3.1.1. Seasonal changes in immunoreactive cortisol concentrations in females

In the nonpregnant females (F1 and F2), immunoreactive cortisol concentrations in summer were significantly higher than in spring and winter ($P < 0.05$), and immunoreactive cortisol concentrations in autumn were significantly higher than in spring ($P < 0.05$); the lowest immunoreactive cortisol concentrations occurred in spring. The immunoreactive cortisol concentration of the pregnant female (F3) was significantly higher in summer than in autumn and winter ($P < 0.05$), and immunoreactive cortisol concentration in spring was significantly higher than in autumn ($P < 0.05$). The immunoreactive cortisol concentration in autumn was the lowest. In autumn, the immunoreactive cortisol concentration of nonpregnant female F2 was significantly higher than that of pregnant female F3 ($P < 0.05$) (Figure 1).

3.1.2. Seasonal changes in immunoreactive cortisol concentrations in males

The immunoreactive cortisol concentrations of males (M1, M2, and M3) were significantly higher in summer than in spring and winter ($P < 0.05$), and immunoreactive cortisol concentrations in autumn were significantly higher than in spring ($P < 0.05$); the lowest immunoreactive cortisol concentrations occurred in spring. In winter, the immunoreactive cortisol concentration of M1 was significantly lower than that of M3 ($P < 0.05$) (Figure 2).

3.1.3. Comparison of immunoreactive cortisol concentrations between nonpregnant females and males

The immunoreactive cortisol concentrations of nonpregnant females were higher than those of males in spring and summer ($P < 0.05$) (Figure 3). There was no obvious difference in immunoreactive cortisol concentrations between nonpregnant females and males throughout the year ($P > 0.05$) (Figure 4).

3.2. Fecal immunoglobulin concentrations in Sichuan golden monkeys

3.2.1. Fecal immunoglobulin concentrations in females

There was no obvious difference in fecal IgG or IgM concentrations in females in any season ($P > 0.05$). The fecal IgA concentrations of females were highest in summer ($P < 0.05$) and lower and similar to each other during all other seasons ($P > 0.05$) (Table 3).

3.2.2. Fecal immunoglobulin concentrations in males

There was no significant seasonal difference in fecal IgG or IgM concentrations in males ($P > 0.05$). In summer, the fecal IgA concentrations were significantly higher than in all other seasons ($P < 0.05$), but no significant differences were observed among the other 3 seasons ($P > 0.05$) (Table 3).

3.2.3. Comparison of fecal immunoglobulin concentrations between females and males

No significant between-sex difference in fecal immunoglobulin levels was observed in any season ($P > 0.05$) (Table 3). There was no obvious difference in fecal IgG, IgM, and IgA concentrations between nonpregnant females and males throughout the year ($P > 0.05$) (Figure 5).

3.3. Correlation between immunoreactive cortisol and fecal immunoglobulin levels

The correlations between immunoreactive cortisol and fecal immunoglobulin levels in both females and males were assessed via Spearman correlation analysis (Table 4). Cortisol concentration was highly significantly and

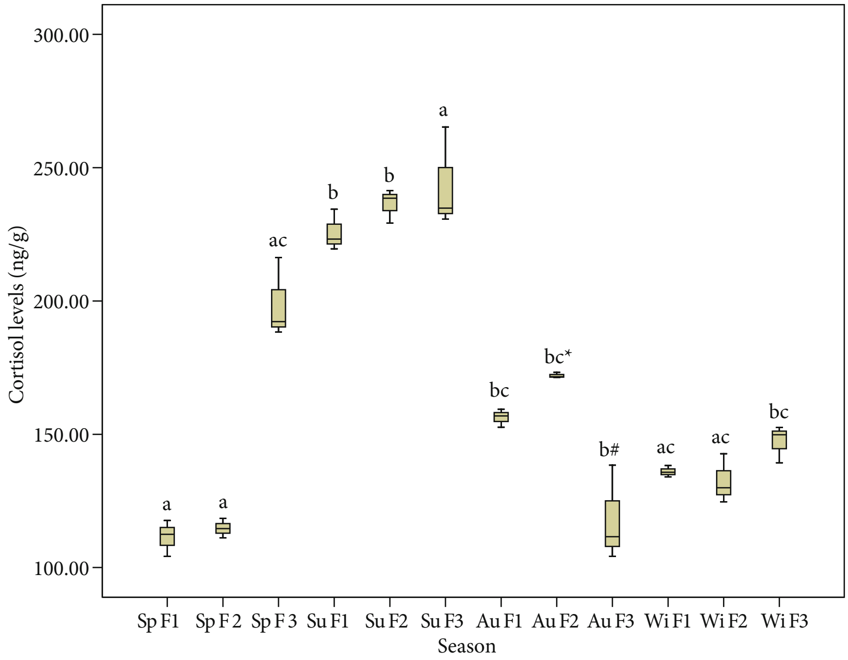

Figure 1. Immunoreactive cortisol concentrations in nonpregnant (F1 and F2) and pregnant (F3) females within seasons (ng/g). Sp: spring; Su: summer; Au: autumn; Wi: winter. [a,b,c,d]: Histograms that share the same letters do not differ from each other, whereas histograms with different letters are different at $P < 0.05$. [*,#]: $P < 0.05$, bar with * was significantly higher than bar with #.

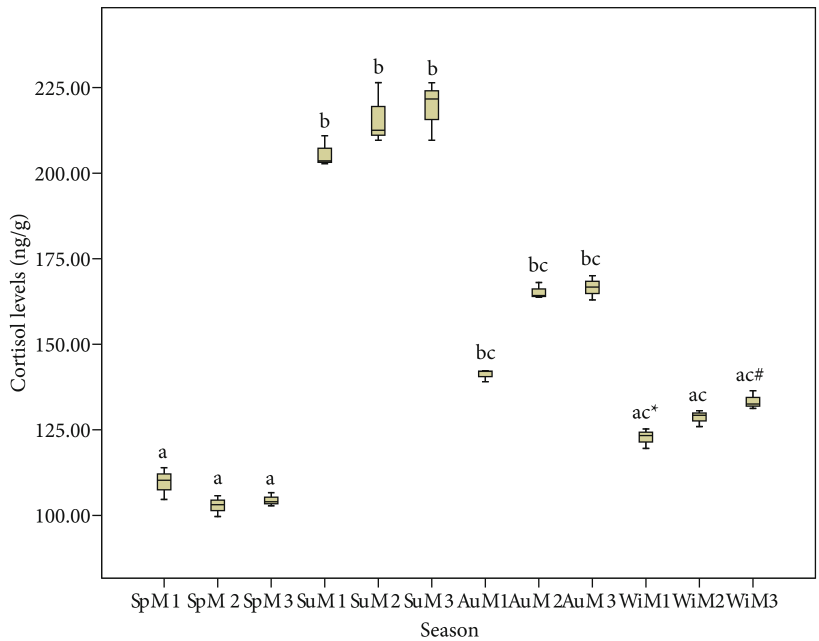

Figure 2. Immunoreactive cortisol concentrations in males (M1 was the dominant male; M2 and M3 were all-male units) within seasons (ng/g). Sp: Spring; Su: summer; Au: autumn; Wi: winter. [a,b,c,d]: Histograms that share the same letters do not differ from each other, whereas histograms with different letters are different at $P < 0.05$. [*,#]: $P < 0.05$, bar with # was significantly higher than bar with *.

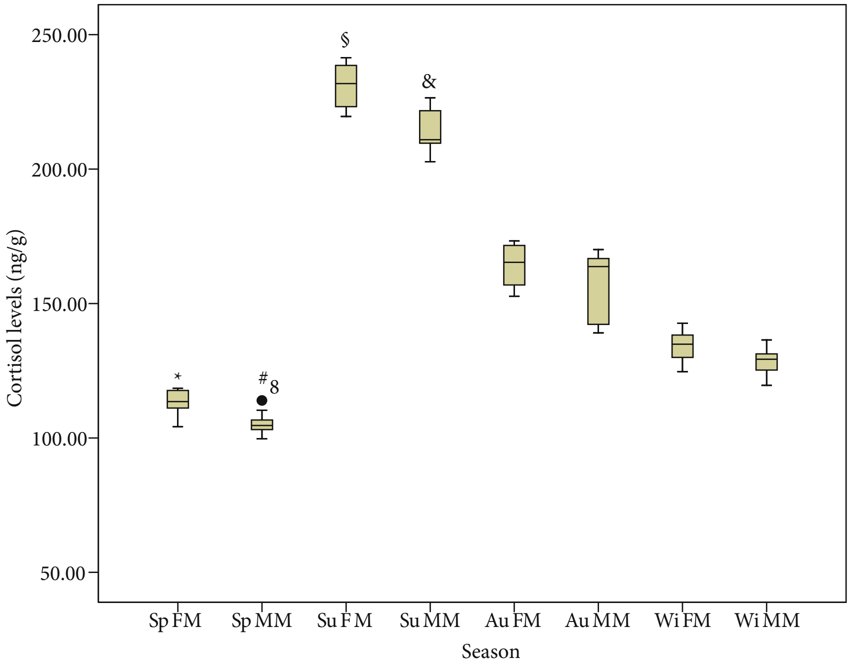

Figure 3. The immunoreactive cortisol concentrations of Sichuan golden monkeys within seasons (ng/g). Sp: Spring; Su: summer; Au: autumn; Wi: winter. FM refers to the mean of nonpregnant females (F1 and F2); MM refers to the mean of males (M1, M2, and M3). *,#, §,&: $P < 0.05$, bar with * was significantly higher than bar with #, and bar with § was significantly higher than bar with &.

positively correlated with the level of IgA, but not with the levels of IgG or IgM.

4. Discussion

4.1. Association between cortisol concentration and stress in Sichuan golden monkeys

Adverse situations stimulate adrenal activity, resulting in increases in glucocorticoid and/or catecholamine secretion. In most mammals, the primary glucocorticoid released is cortisol (Nigel, 2012). Many studies have demonstrated the relationship between cortisol concentration and seasonal variation (Bubenik et al., 1983; Schiml et al., 1996). In the present study, immunoreactive cortisol concentrations in Sichuan golden monkeys tended to fluctuate seasonally.

In the nonpregnant females, the immunoreactive cortisol concentration was highest in summer because of heat stress. The Sichuan golden monkey is a seasonal breeder and matings peak between September and December (Zhang et al., 2000). Estrus is a physiological phenomenon caused by follicular development of the ovaries, and it is subject to the hypothalamus–pituitary–ovary axial system. In the mating peak period, estrus of nonpregnant females is frequent. Sexual behavior is more common. In addition, there is mating competition among females in one-male, multiple-female units. These factors may cause stress responses in the body; therefore, cortisol concentrations of females were elevated in autumn. Females are responsible for 95% of all sexual solicitations (proceptive behavior); the typical pattern is prostration, and the females compete for sex with the dominant male. Fecal cortisol measures in free-ranging female ring-tailed lemurs (*Lemur catta*) were significantly correlated with dominance indices:

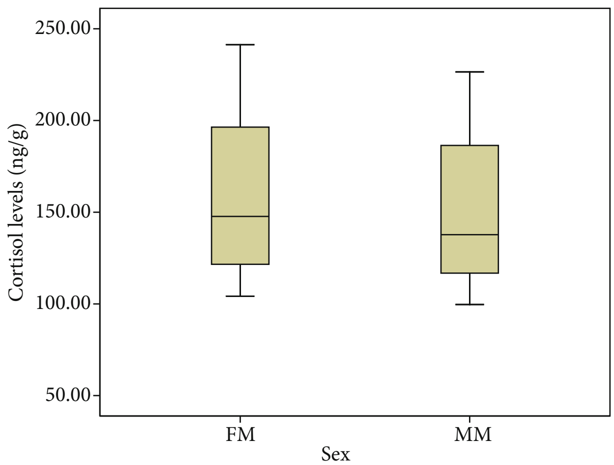

Figure 4. The immunoreactive cortisol concentrations of Sichuan golden monkeys over the year (ng/g). FM refers to mean of nonpregnant females (F1 and F2); MM refers to mean of males (M1, M2, and M3).

Table 3. The concentrations of immunoglobulin in feces of Sichuan golden monkeys (μg/g).

		F1	F2	FM	M1	M2	M3	MM
Sp	IgG	287.95 ± 5.30	293.45 ± 7.09	290.70 ± 4.15	291.81 ± 5.88	272.59 ± 20.80	277.49 ± 14.98	280.63 ± 8.12
	IgM	654.43 ± 4.87	649.77 ± 7.33	652.10 ± 4.07	659.56 ± 4.50	646.18 ± 9.69	640.82 ± 18.68	648.85 ± 6.81
	IgA	1372.28 ± 43.72[b]	1364.85 ± 10.21[b]	1368.57 ± 20.15[b]	1398.73 ± 6.63[b]	1351.66 ± 26.91[b]	1344.86 ± 39.76[b]	1365.09 ± 16.36[b]
Su	IgG	300.98 ± 2.40	312.16 ± 10.33	306.57 ± 5.36	297.86 ± 5.77	301.59 ± 11.26	300.61 ± 8.58	299.99 ± 4.45
	IgM	677.34 ± 8.21	670.52 ± 14.84	673.93 ± 7.73	675.43 ± 9.02	681.18 ± 11.96	659.58 ± 16.69	672.29 ± 7.27
	IgA	1594.47 ± 7.21[a]	1513.88 ± 5.17[a]	1554.17 ± 18.45[a]	1545.99 ± 16.53[a]	1520.91 ± 13.53[a]	1553.60 ± 14.99[a]	1540.17 ± 9.01[a]
Au	IgG	291.55 ± 8.40	297.63 ± 9.83	294.59 ± 5.94	289.57 ± 1.45	291.34 ± 14.56	306.98 ± 8.24	295.96 ± 5.58
	IgM	638.59 ± 21.46	657.94 ± 2.97	648.27 ± 10.61	628.97 ± 23.56	628.79 ± 27.30	672.22 ± 10.83	643.32 ± 13.05
	IgA	1440.67 ± 40.26[b]	1402.90 ± 15.74[b]	1421.79 ± 21.10[b]	1408.98 ± 18.24[b]	1400.99 ± 11.69[b]	1405.38 ± 14.32[b]	1405.12 ± 7.59[b]
Wi	IgG	301.91 ± 2.25	294.25 ± 6.67	298.08 ± 3.58	300.49 ± 3.37	281.35 ± 11.21	270.91 ± 27.06	284.25 ± 9.55
	IgM	644.64 ± 5.42	667.88 ± 10.87	656.25 ± 7.52	654.31 ± 9.31	662.31 ± 6.93	643.53 ± 5.90	653.39 ± 4.64
	IgA	1364.35 ± 16.80[b]	1357.12 ± 32.62[b]	1360.74 ± 16.49[b]	1418.20 ± 14.11[b]	1321.88 ± 46.47[b]	1338.13 ± 41.06[b]	1359.40 ± 23.63[b]
M	IgG	295.60 ± 2.87	299.37 ± 4.33	297.49 ± 2.57	294.93 ± 2.34	286.69 ± 7.17	289.00 ± 8.41	290.21 ± 3.70
	IgM	653.75 ± 6.80	661.53 ± 4.94	657.64 ± 4.19	654.57 ± 7.69	654.78 ± 9.05	654.04 ± 7.08	654.46 ± 4.47
	IgA	1442.94 ± 30.85	1409.69 ± 20.54	1426.32 ± 18.45	1442.98 ± 19.09	1398.86 ± 25.88	1410.49 ± 29.17	1417.44 ± 14.40

Sp: Spring; Su: summer; Au: autumn; Wi: winter; M: mean. FM refers to the mean of nonpregnant females (F1 and F2); MM refers the mean of males (M1, M2, and M3).
[a,b]: Values in the same column that share the same letters do not differ from each other, whereas values in the same column with different letters are different at $P < 0.05$.

high-index individuals had high cortisol values and low-index individuals had low cortisol values (Cavigelli, 1999). Female sexual behavior is primarily correlated with physiological status, mate choice, and age in *Rhinopithecus roxellana* (Ren et al., 2000, 2003). In the present study, F1 was the high-index individual and F2 was the low-index individual. However, F1 was elderly; she was 21 years old. F2 was 10 years old and she was fecund. The older the female, the more inactive the sexual drive (Ren et al., 2000). As F2 had intense attraction to the opposite sex, the immunoreactive cortisol concentration of F2 was a little higher than that of F1 at the peak of matings. On the other hand, males chose the most appropriate target for mating to ensure successful reproduction.

The immunoreactive cortisol concentration of the pregnant female (F3) was highest in summer because

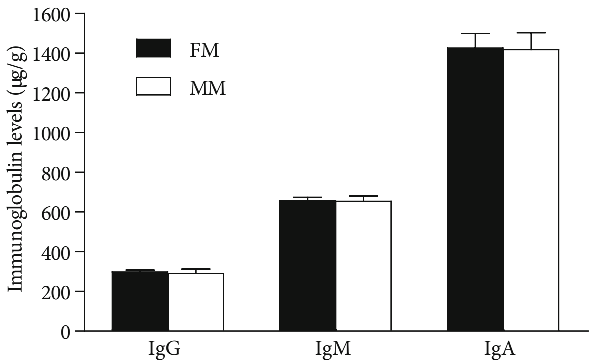

Figure 5. The fecal immunoglobulin levels of Sichuan golden monkeys over the year (ng/g). FM refers to mean of nonpregnant females (F1 and F2); MM refers to mean of males (M1, M2, and M3).

heat stress can be very pronounced. The pregnant female (F3) gave birth to a baby male monkey in spring. Thus, she experienced childbirth and associated physiological changes including uterine contraction and lactation, and she needed to protect the infant. The immunoreactive cortisol concentration of this female was therefore elevated. However, cross-reactions of the corticoid assay with those gestagen metabolites may have caused the elevated cortisol concentrations (Quillfeldt et al., 2011).

With respect to temporal factors, male immunoreactive cortisol levels peaked in summer in association with heat stress. In autumn, males also showed elevated cortisol concentrations at the peak time of mating. In snub-nosed monkeys, as in many other colobines, the most common module at the base of a multilevel society is a one-male, multiple-female unit or a "harem", with all-male units often living in the neighborhood (Kirkpatrick, 1998; Tan et al., 2007; Kirkpatrick and Grueter, 2010). In our study, the immunoreactive cortisol concentration of the dominant male (M1) was significantly lower than that of the all-male units (M2 and M3) in winter. A subordinate individual is exposed to high rates of stress (e.g., frequent aggression from higher-ranking individuals) and has little social support. This is in line with earlier findings (Abbott et al., 2003).

A comparison of immunoreactive cortisol concentrations between pregnant and nonpregnant females showed that their physiological stages may cause differences in cortisol levels. There was no significant difference in immunoreactive cortisol concentration between the nonpregnant females and the males over the year. However, the immunoreactive cortisol concentrations of the nonpregnant females were higher than those of the males in spring and summer. This may be connected with individual differences and the social structure of the species. Our results may indicate that environmental conditions, natural physiology, and social status are all stressors of Sichuan golden monkeys. Hence, we conclude that the concentration of immunoreactive cortisol reflects the stress status of the animal.

4.2. Fecal immunoglobulin concentration reflects the immune status of Sichuan golden monkeys

Concentrations of immunoglobulin (also termed antibodies) are maintained at levels ensuring health. Several reports have found that immunoglobulin levels profoundly affect wildlife health (Turton et al., 1997; Taylor et al., 2002). In the present study, no significant differences in fecal IgM, IgG, or IgA concentrations were evident between the sexes; the risk of infection was thus the same for all experimental monkeys. No significant seasonal differences in fecal IgM or IgG concentrations were noted; infective agents of monkeys thus did not vary significantly by season. IgA concentration was significantly higher in summer than during the other 3 seasons, when the concentrations were similar. Summer temperatures attain yearly peaks and microbial load and variety (principally in food) are highest in this season. Microbial load directly affects the immune system of Sichuan golden monkeys. Thus, summer elevations in IgA levels reflect the response of the digestive tract of the monkey to environmental microorganisms. Good management during summer is important for protection of the species; food quality, water supply, and housing all need special attention in this season. The infection rates of Sichuan golden monkeys declined with decreases in ambient temperature. The diversity and levels of microorganisms during the (cold) winter were low, and the IgA secretion levels were thus also relatively low.

Table 4. Correlation between immunoreactive cortisol and fecal immunoglobulin levels in Sichuan golden monkeys.

Correlation	Cortisol and IgG	Cortisol and IgM	Cortisol and IgA
Females	0.357	0.294	0.687**
Males	0.291	0.306	0.680**

** : $P < 0.01$, significant correlation between the cortisol and IgA concentrations; n = 2 females (F1 and F2) and n = 3 males (M1, M2, and M3).

Thus, we conclude that fecal immunoglobulin concentration can be used to monitor immune system functioning in Sichuan golden monkeys.

4.3. Correlation between stress and immune physiology in Sichuan golden monkeys

Animals experience various environmental conditions; the factors of predators and human and nutritional stressors may adversely affect health status because of neuroendocrine disruption and stress-induced immunosuppression. The immune system is used to defend against and cope with environmental stress. Stress can suppress or enhance or have a balancing effect on the immune functions of animals, enhancing susceptibility of an organism to various diseases (Smith et al., 1972; Fan et al., 1996; Aggarwal and Upadhyay, 2013). In the present study, no significant correlation was noted between immunoreactive cortisol concentration and fecal IgG or IgM concentrations in males or females, but obvious correlation was observed between immunoreactive cortisol concentrations and fecal IgA concentrations in both sexes over the seasons.

The monkeys did not suffer from any serious disease during the experimental period; basically, they were in good health. The monkeys reacted moderately to stress; no change in overall immune status was evident. This may explain why no significant association was noted between fecal IgG and IgM concentrations on the one hand and immunoreactive cortisol concentration on the other.

Several environmental and physiological factors can affect the immune responses of animals under stress, and IgA has been suggested as a potential marker of long-term stress (Royo et al., 2004). In the present study, the stress responses of Sichuan golden monkeys living in seminatural conditions, but responding to the external environment and other factors, were within the normal physiological range. Clear differences in such levels were evident between males and females. Thus, the mucosal immune response and the stress response were synchronized. However, the data do not allow us to conclude that stress per se elevated the IgA concentration. Certainly, summer heat is a common cause of elevation of fecal IgA and immunoreactive cortisol levels in Sichuan golden monkeys. Moreover, in terms of serving as a marker of immune physiology, the fecal IgA level is more sensitive than the IgG and IgM levels. Therefore, the observed correlation between stress responses and the health status of the Sichuan golden monkey allows us to evaluate the responses to physiological stressors imposed by the environment.

4.4. Evaluation of wildlife welfare by measuring of immunoreactive cortisol and fecal immunoglobulin levels

In efforts to assess animal welfare, the number of publications quantifying levels of stress-sensitive molecules in feces has increased (Möstle and Palme, 2002). Some are of the view that the levels of fecal glucocorticoid metabolites and immunoglobulins can be used to assess welfare (Rehbinder and Hau, 2006; Shutt et al., 2012). Working with Sichuan golden monkeys living in seminatural conditions, we found that it was possible to assess welfare by measuring immunoreactive cortisol and fecal immunoglobulin concentrations; such an approach has very broad applications in wildlife research.

In this study, immunoreactive cortisol and fecal IgA levels tended to fluctuate seasonally in Sichuan golden monkeys. However, the differences of IgM and IgG levels in feces were not significant with the variation of the seasons. Moreover, the stress and immune responses were related. Therefore, these responses can be used to monitor stress and immune system functioning, serving as surrogate measures of welfare. Further work is needed to explore the relationship between environmental pressure (experienced both in zoos and in the wild) and physiological responses using analysis of the type described above to assist in the conservation and management of this species and other wildlife.

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