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Effect of Environmental and Meteorological Conditions on Levels of Fecal Cortisol in Two Captive Species of Carnivorous

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Abstract: In this study the effect of the atmosphere was measured on the concentrations of fecal cortisol in 4 samples of Mexican Wolf and 4 samples of Mountain Cat, exposed to the environment during 4 weeks. Weekly the concentrations of fecal cortisol were measured of each one of the samples. In the samples of Mexican Wolf fickle variations were observed in the concentrations of fecal cortisol, they already stayed fluctuating between the increase and the decrease. However, according to the statistical analysis, these variations were not significant along the study ($p>0.05$). In the samples of a single Mountain Cat a sample (of 4) it showed a constant decrease from the initial until the final cortisol concentration. The other 3 samples stayed fluctuating between the increase and the decrease. In spite of this, the variations in the concentrations of fecal cortisol were not significant for the first week ($p = 0.9316$), after this week the variations were significant ($p<0.05$). In both species, the increases in the concentrations of fecal cortisol were influenced by the days of more precipitation and of lower temperatures. On the other hand, the decreases had influence for the highest temperatures and the little or no precipitation. This is due to that the precipitation provides favorable conditions for the bacterial activity, increasing the concentration of free cortisol, in the samples; on the other hand, the heat produces that the proteins of the cell clot, causing the bacterial death, that which diminishes the concentration of free cortisol. According to the statistical analysis differences were observed between the average of the concentration of fecal cortisol of the 2 species (in the Mountain Cat the concentrations of fecal cortisol were higher than in the Mexican Wolf), between the individual samples for species and between the species through the meteorological conditions, this of it owes to the differences that exist between the species, which are: The intestinal activity, the intestinal microbiota, the diet type, th metabolism of steroids, the percentage of cortisol excretion in the samples and the metabolism of bacteria after the excretion in the samples. For future studies on the evaluation of fecal cortisol as much in Mountain Cat as in Mexican Wolf is recommended to use fresh samples of not more than 3 days, since its possible to obtain sub values or over values depending on the species and of the climatic conditions of the moment.

Key words: Environment, meteorological conditions, Mexican wolf, mountain cat, fecal cortisol, cortisol levels

INTRODUCTION

Traditionally, the study of the wild fauna in freedom, from the veterinary point of view, requires of the capture of different individuals, being this not very practical and inclusive dangerous. In many cases for the animal in question or for the researcher besides that the physiologic values suffer alterations for the handling and the use of chemical contention.

Fortunately, the current laboratory techniques allow us to identify and monitor different infectious agents and physiologic values in way non invasive, being able to establish the physiologic and health state of the study animals without having to capture any individual, mainly

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for the analysis of the samples, where the animal salivates (Goymann *et al*., 1999; Möstl *et al*., 1999; Brown *et al*., 2001; Graham *et al*., 2001; Schatz an Palme, 2001; Turner *et al*., 2002; Von der Ohe and Serhveen, 2002; Young *et al*., 2004).

Between the values more used in wild animals in captivity and domestic species have been the determination of the fecal cortisol as indicator of the well-being in which are this animals, on the fact that to more stress bigger levels of fecal cortisol (Goymann *et al*., 1999; Möstl *et al*., 1999; Brückmann *et al*., 2000; Palme *et al*., 2000; Randy, 2000; Brown *et al*., 2001).

The interest exists to apply these determinations in the wild fauna in freedom, as assistants to estimate the stress grade to which could be subject of a certain population in comparison with other, between perturbed areas and conserved areas (Möstl *et al*., 1999; Brückmann *et al*., 2000; Palme *et al*., 2000; Randy, 2000; Brown *et al*., 2001; Schatz *et al*., 2001; Möstl and Palme, 2002; Turner *et al*., 2002; Von der Ohe and Serhveev, 2002). However, it is necessary to have reference of values previously determinate in species in captivity conditions. Between the values to know is important to determine the grade of variation of the fecal cortisol according the effect of atmosphere and meteorological conditions. Although in free life it is possible to identify and to gather, periodic samples of different species (overalls of mammals), they cannot always be fresh samples, for what is of vital importance to know the effect that climatic factors could have in fecal cortisol values (Möstl *et al*., 1999; Möstl and Palme, 2002; Turner *et al*., 2002).

The objective of this study is to determine the variations in the concentrations of fecal cortisol due to the influence of the atmosphere and of the meteorological conditions, in 2 species of carnivorous, Mexican Wolf (*Gypsy lupus baileyi*) and Mountain Cat (*Lynx rufus*) in the zoo of Chapultepec in Mexico City, by means of the technique of enzymatic immunoassay (EIA).

MATERIALS AND METHODS

During 2 weeks there were carried out observations in the housing of each species in the Zoological of Chapultepec in Mexico City, from 9 to noon, to register the hour and the place in that it happened the defecation, besides interviewing the guard-animals of each species; with this, it was determined that the best hour in gathering the fecal samples, it would be between 7 and 9am.

Taking of samples: There were gathered the samples of each species during 4 serial Monday. Each sample was homogenized and it was placed in a specific place, being exposed to the climatic effects (solar light, temperature, wind, relative humidity, precipitation). Every Monday a portion of the sample (2-3 g) was taken, homogenized (this repeated until completing 4 weeks for sample). It was placed in a flask with alcohol to 70% and it was stored so far in the laboratory for the extraction of steroids.

The prosecution of the samples was carried out in the Laboratory of Reproduction "MVZ Juan Téllez Girón" of the Zoological of Chapultepec.

Total extraction of steroids: About 0.5 g was weighed of each sample, to be subjected to the total extraction of steroids (ETE) with the help of dietilic ether.

Analysis of samples: Each sample was analyzed by copy, being a total of 40 tests for each species.

The quantification of fecal cortisol was carried out by means of the test of Enzymatic Immunoassay (EIA), with a reader EL×800, Bio-Tek Instruments, Inc and the program Ridawin of Bio-pharm. Kits was used (Cortisol EIA DSL-10-2000, Diagnostic Systems Laboratories) and he/she was carried out the technique according to the laboratory indications), modifying the concentration of the standard curve.

Registration of the meteorological conditions: The data of the National Meteorological System were used (SMN) for the region of Tacubaya in Mexico City. To gather the data, we went to the offices of the Meteorological System.

Results: Because the used kit is designed to give the concentration of the sample in mg/dl it was necessary to carry out the conversion from the units to ng g^{-1} of samples.

Statistical analysis: The descriptive statistic of the variables low study was calculated, for the general population (2 species) and for each one of the species.

To know the effect of meteorological conditions and the differences between the species on the fecal cortisol concentration along the 4 weeks of the study, it was carried out a variance analysis using mixed models. The pattern included the fixed effects of average of the concentrations of fecal cortisol for species, meteorological conditions and its interaction, as well as the random effect of the individual samples of each species.

To compare the variations of the cortisol concentrations weekly, the 4 samples of each species were averaged (per week) and with the obtained stockings the analysis was made.

RESULTS

Mexican wolf: The results, in raw, of the concentrations of fecal cortisol of Mexican Wolf are shown in the Table 1.

In the Table 2, we can observe the meteorological conditions of temperature and precipitation, presented during the meteorological conditions of exhibition from the samples to the atmosphere.

In the Table 1 of the concentrations of fecal cortisol in Mexican Wolf the following aspects are observed.

In three of the 4 samples (it shows 1, 2 and 3) its registered an increase with regard to the initial concentration, during the first 2 weeks (to exception of the sample 3, which diminished toward the second week), it stops later to show a decrease that was only continuous in a sample (it shows 2), in the remaining ones after this decrease a new increase was observed, without reaching the biggest pick, to finish diminishing again (to exception of the sample 1).

The sample 4 had a behavior different to the other ones, being observed a notorious decrease the first 2 weeks, increase the following week again, staying in this concentration.

Under the meteorological conditions of study (Table 2) it stands out the following:

As for the temperature (T°), the week in which registered the highest was in the 4th (22°C). The weeks in which the T° was lower were the 5th and the 6th (19°C).

In the seventh week it was registered the biggest precipitation (1.017 mm) and in the week 4 registered no rain (0 mm).

Even if fluctuating changes are observed in the concentrations of fecal cortisol accumulated in the samples of Mexican Wolf along the 4 weeks (Table 3), these, according to the statistical analysis, didn't have significant differences during the first week ($p = 0.2425$), but they presented significant differences in week 4 where precipitation was low and ($p = 0$).

Table 1: Concentrations (raw) of fecal cortisol in Mexican wolf per week

Week	Date	Concentration of fecal cortisol (ng g^{-1}) sample 1	Concentration of fecal cortisol (ng g^{-1}) sample 2	Concentration of fecal cortisol (ng g^{-1}) sample 3	Concentration of fecal cortisol fecal (ng g^{-1}) sample 4
Week 0	04/04/05	367			
Week 1	11/04/05	600	200		
Week 2	18/04/05	960	1233	167	
Week 3	25/04/05	240	1760	320	280
Week 4	02/05/05	633	500	160	160
Week 5	09/05/05		240	267	40
Week 6	16/05/05			67	120
Week 7	23/05/05				120

Table 2: Meteorological conditions during analysis

Week	Date	Temperature (°C)	Precipitation (mm)
Week 0	04/04/05		
Week 1	11/04/05	21	0.505
Week 2	18/04/05	20	0.588
Week 3	25/04/05	20	0.614
Week 4	02/05/05	22	0
Week 5	09/05/05	19	0.611
Week 6	16/05/05	19	0.506
Week 7	23/05/05	20	1.017

Table 3: Concentrations (medium) of fecal cortisol of Mexican Wolf calculated along, weekly

Weeks	Mexican wolf	p-value
Week 0	254	
Week 1	578	0.2425
Week 2	730	0.0915
Week 3	282	0.9178
Week 4	265	0.9665

Table 4: Concentrations (raw) of fecal cortisol of mountain cat calculated weekly

Week	Date	Concentration of fecal cortisol (ng g^{-1}) sample 1	Concentration of fecal cortisol (ng g^{-1}) sample 2	Concentration of fecal cortisol (ng g^{-1}) sample 3	Concentration of fecal cortisol fecal (ng g^{-1}) sample 4
Week 0	04/04/05	2080			
Week 1	11/04/05	1967	1333		
Week 2	18/04/05	320	1640	800	
Week 3	25/04/05	1400	333	400	133
Week 4	02/05/05	600	333	400	433
Week 5	09/05/05		400	100	360
Week 6	16/05/05			67	240
Week 7	23/05/05				200

Table 5: Concentrations (medium) of fecal cortisol of Mountain cat calculated along, weekly

Weeks	Mountain cat	p-value
Week 0	1087	
Week 1	1110	0.9316
Week 2	353	0.0123
Week 3	518	0.0467
Week 4	317	0.0090

Mountain cat: The results (raw) in the concentrations of fecal cortisol of mountain Cat they are shown in the Table 4.

In the Table 4 of the concentrations of fecal cortisol of mountain Cat.

Two of the 4 samples (it shows 1 and 3) they are similar since in both registered a decrease, based on the initial concentration, during the first 4 weeks, only in the sample 1 a notorious increase is given, without surpassing the maximum pick, in the third week, it stops later to diminish in the last week or week 4.

The samples 2 and 4 had a similar behavior, being observed an increase toward the 1st week, diminishing toward the second week. The sample 4 continues diminishing until the last week. And the sample 2 stays in that concentration, increasing at the end.

The meteorological conditions presented during the exhibition of the fecal samples of mountain Cat were the same ones that in the Mexican Wolf, because the samples of both species were exposed to the same metereological conditions.

Although, fluctuating changes are observed in the concentrations of fecal cortisol accumulated in the samples of mountain Cat along the 4 weeks (Table 5), these, according to the statistical analysis, didn't have significant differences during the first week ($p = 0.9316$), but they presented significant differences for the remaining weeks ($p<0.05$).

Differences between the 2 species: In the statistical analysis we can notice that the effect of the average of the concentrations of fecal cortisol for species was significant ($p = 0.0458$), the same as that of the individual samples for species ($p = 0.0015$) and that of the interaction between species and meteorological conditions ($p = 0.0434$), while the effect of meteorological conditions was statistically marginal ($p = 0.0619$).

Of the accumulated values we find that the stocking for Mexican Wolf was of 422 ng g^{-1} with a standard deviation of ±219 ng g^{-1}; while for the mountain Cat the stocking was of 677 ng g^{-1} and the standard deviation was of ±392 ng g^{-1}.

DISCUSSION

In the 4 samples of Mexican Wolf fluctuations were observed between the increase and the decrease of the cortisol concentrations fecal week after week. No sample was constant; however they show a tendency toward the decrease of the initial value along the study.

On the other hand, in the Mountain Cat 3 of the 4 samples stayed fluctuating between the increase and decrease of the fecal cortisol concentrations week after week. Only the sample 3 stayed in a constant decrease until the last week. The same as in the Mexican Wolf, the samples of Mountain Cat present a tendency toward the decrease of the initial value along the study.

In both species, the increases and the decreases in the concentrations of fecal cortisol were influenced by the temperature and the precipitation, presented during the analysis; since the concentrations of fecal cortisol spread to ascend during the days of more precipitation and of lower temperatures, on the contrary, they spread to diminish during the weeks of no or little precipitation and of higher temperatures. These results coincide with the opposing ones for Washburn and Millspaugh (2002), since they observed in samples of deer that the concentrations of the metabolites of fecal glucocorticoids increased in the samples exposed to rain and they diminished in those exposed to high temperatures (38°C). This can be due to that the rain the humidity increases providing favorable conditions for the growth and the bacterial population's metabolism. According to Levinson and Jawetz (1998) and Washburn and Millspaugh (2002), this increase in the bacterial activity results in an increase in the concentration of free cortisol in the samples. On the other hand, when the bacterias undergo the heat, the proteins of the cell clot causing the bacterial death (Freeman, 1989), when not having bacterial development the concentration of free cortisol in the samples it diminishes or it doesn't increase.

However, in the statistical analysis of the present study we can notice, in the Mexican Wolf that significant differences don't exist in the variations of the concentrations of fecal cortisol along the 4 weeks of study, on the other hand, in the Mountain Cat significant differences exist in the concentrations of fecal cortisol after the first week. In the study of Washburn and Millspaugh (2002), the differences in the variations of fecal cortisol according to meteorological conditions were significant ($p<0.05$). This can be influenced by the differences that exist between the species, like they are: Differences in the quantity of metabolites and of present bacterias in the samples (the bacterial enzymes produce a biodegradation after having excreted the sample), due to the diversities that exist in the metabolism of the steroids and in the intestinal microbiota, this last difference is caused by the diet (Wasser *et al*., 2000; Khan *et al*., 2002; Möstl and Palme, 2002; Turner *et al*., 2002; Washburn and Millspaugh, 2002).

According to the statistical analysis we can notice that significant differences exist between the species, the individual samples for species and between the species through the meteorological conditions, since in the one accumulated of the Mexican Wolf (Graph 4) it is observed that the concentrations of fecal cortisol spread to increase the first two weeks to finish diminishing in the subsequent weeks. On the other hand the concentrations in the one accumulated of Mountain Cat (Graph 6) they have a slight increase to the first week, showing a tendency to diminish in the following weeks.

The above mentioned can be due to diverse factors: The concentration of reflective fecal cortisol, the adrenal secretion of this hormone after a period of specific meteorological conditions for each species and for each animal (Möstl and Palme, 2002), this can be influenced by the diet type, the characteristics of the intestinal microbiota, the different metabolisms of the steroids, the state of gastrointestinal activity, which is of 16-28 h in the cat and of 20-28 in the dog and the metabolism of the bacterias after the excretion of the samples (Wasser *et al*., 2000; Schatz and Palme, 2001; Möstl and Palme, 2002; Von der Ohe and Servheen, 2002).

Also, the concentrations of fecal cortisol, on the average, were bigger in the mountain Cat than in the Mexican Wolf, this is due to that: In the domestic cat, more than 70% of the excretion of the metabolites of the cortisol it is in the samples and these they are, in their majority, in not conjugated form (Brown *et al*., 2001; Schatz and Palme, 2001), while in the dog the excretion is of less than 30% and the metabolites they are mainly in conjugated form (Schatz and Palme, 2001; Young *et al*., 2004).

The test of EIA used in this study is more reactive with the not conjugated metabolites (Schatz and Palme, 2001), that which explains, also, the one that you/they have been bigger in the determination of fecal cortisol the values in Mountain Cat that in Mexican Wolf.

CONCLUSION

In conclusion, in the samples of both species, the concentrations of fecal cortisol didn't remain constant along the meteorological conditions of exhibition of the samples to the atmosphere, showing increases in the concentrations of fecal cortisol in the days of more precipitation and of lower temperatures and, decreases in the days of higher temperatures and of little or null precipitation. There are significant differences between the concentrations of the fecal cortisol in both species. In the Mountain Cat the concentration, on the average, was higher than in the Mexican Wolf. And the variations according to the exposition to meteorological conditions, the samples, were more significant, statistically, for the Mountain Cat than for the Mexican Wolf. Both samples, in spite of the increases and decreases, spread to diminish along the meteorological conditions. The concentration of fecal cortisol accumulated during the exposition to meteorological conditions in Mexican Wolf was in a range of 203-641 ng g^{-1} and in Mountain Cat it was between 285-1069 ng g^{-1}.

RECOMMENDATIONS

Because maintaining the exposed samples to the atmosphere, they went drying off and the air dispersed the particles, in the last sample (4th week) it was very small sample, reason why, in later studies, it is advisable to take several samples and mix perfectly; this way one will have readiness of a good quantity of samples, so that the day of the last taking of samples the spare quantity is enough for its analysis.

It is possible to recommend, with this study to evaluate concentrations of fecal cortisol of Mountain Cat and of Mexican Wolf, as much in captivity as in free life, it is preferable to use fresh fecal samples from 1-3 days, having a bigger margin in the case of the Mexican Wolf.

It is advisable that similar papers with animals in free life, in parallel form, with animals in captivity, with the purpose of creating a mark of reference of changes with concentrations of fecal cortisol.

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