Analysis of Behavior and Cortisol Levels Of a Captive Wolf in Solitaire Versus Captive Wolves in a Pack

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By

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Wolf Credo
Respect the elders, Teach the young
Cooperate with the pack
Play when you can, Hunt when you must
Rest in between
Share your affections, Voice your feelings
Leave your mark
- Del Goetz, 1988

Introduction

Since the 19th century, the wolf population in the wild has been considered to be endangered at different times. Due to the hunting of wolves that was sponsored by the federal government between the 1800s and the mid-1960s, the wolf population became dangerously low.

Once the wolf population in the wild was severely reduced, organizations came to the aid of wolves. With the help of conservation centers, zoos, and other wildlife facilities, the remaining 300 to 400 wolves in the lower forty-eight states, most found in Minnesota, were captured and bred in captivity until the wolf populations were once again stable enough to be released into the wild. Today, there are over 3,000 wild wolves living in Minnesota alone, and the population continues to grow each year as new pups are born, and as more wolves are released into the wild. Wolves are found in more diverse settings today due to the Species Survival Plan and organizations that work to promote wolves in the wild. The Wolf Conservation Center is one of many organizations that aids in the Species Survival Plan (SSP). The SSP works to help increase the wolf population in the wild by selectively breeding wolves in captivity and then releasing the wolf packs into the wild. At the wolf conservation center, the goal is to educate humans about wolves and inform them about and their importance. The center uses four wolves known as the "Ambassador Wolves" to help educate the public. The four ambassador wolves consist of three neutered males and one spayed female. The center greatly appreciates the wonderful education that these four wolves provide, but they are also hopeful that

most wolves will not have to live in captivity, and will have the chance to live in the wild. Wolves have mainly been put in captivity in order to help with the growth of the wolf population. One of the ambassador wolves is known as "Atka," and he travels to schools, museums, libraries, and conferences where he is a fabulous teacher to people around the world. Unfortunately, Atka's behavior toward other wolves is not as exceptional, and he is currently living in solitaire due to previous conflicts with his former pack members, the three other ambassador wolves. The education that Atka has provided for so many people is extraordinary, but discourages the idea of introducing more wolves into a life in captivity.

As more research is completed, more information about wolf behavior is revealed. In each wolf pack there is generally an alpha pair, which leads the pack and works together to make decisions for the pack regarding feeding, hunting, mating, and teaching. The alpha pair is usually the only breeding pair within the pack, although there can be exceptions. Breeding season generally lasts from January until the pups are born in either April or May. During the one oestrus cycle of the female wolves, in February or March, wolves prepare for mating by attempting to attract their mate. Some behaviors displayed during breeding season include; aggression, tension, courtship, parallel walking, and eventually copulation, which can last between twenty minutes and two hours (1). The entire pack will work together to raise the pups, with litter sizes generally ranging from one pup to ten pups. The pups will live with their parents for the first two to three years of their life until they are ready to disperse and find their own pack to either create or join.

The study of *Canis lupus* is relatively uncharted, but new research is projecting further probes into the reasons for the behaviors observed among these fascinating animals. Within the pack, the lowest ranked wolf is the beta wolf, and his or her role is to initiate play, and prevent

any conflicts between other members of the pack. Each member of the pack helps with hunting and raising the pups. The wolf is often known for its howl (Figure 1), which can be sounded for many different reasons. Some of the known reasons for wolf howling include food captured, to gather the pack, to command the other pack members, and to warn other pack members of danger. The common howl for danger, however, is different from the normal howl, and it is known as the "bark-howl," which involves barking and howling. Wolves howl often, but they also use many different forms of body language to communicate with one another.

The most common body language is seen during interaction with differently ranked members of the pack. The alpha wolf or wolves will generally have their tail(s) and ear(s) positioned the highest in the pack. In addition, only the alpha wolf or wolves will raise their leg while urinating, while all other members of the pack must squat while urinating. Also, the other members of the pack will show their respect for the alpha by lowering their ears and tails, licking or nipping at his or her muzzle, and rolling on their back to expose themselves submissively. Since wolves are such pack oriented animals, most of their behaviors are seen when in contact with other pack members. Males and females are not necessarily ranked according to gender, and often the alpha pair consists of a male wolf and a female wolf. The wolves are expected to provide for their pack by fulfilling their role, throughout most of their lives, with the exception of very young and very old wolves.

Male Wolf #1 (Atka) Howling at the Wolf Conservation Center

Figure 1: This is a photograph of "Atka" howling. He is currently living in solitaire, at the Wolf Conservation Center, adjacent to his old pack members.

Animals in captivity are often thought to have more stress because they are not living in their natural environments, and often have unrealistic human interactions. In order to test the

stress levels of wolves, cortisol is often used because it can be extracted from feces, creating a non-invasive and non-stressful method of testing. Cortisol is a hormone that is made by the adrenal glands. Cortisol levels increase as the pituitary gland releases the adrenocorticotropic hormone (ACTH) (3). Cortisol levels are tested in many animals in the wild as well as in captivity to show stress levels in a noninvasive way. The measurement of glucocorticoid metabolites in feces is often used for noninvasive evaluation of cortical activity. Research in most zoos and wildlife facilities in North America have become quite advanced, and the push for conservation programs and noninvasive research has grown tremendously since the late 1970s (5). There are multiple ways of testing for cortical levels in fecal material. The testing of both enzyme immunoassay (EIA) and radioimmunoassay (RIA) are used to compare cortisol results to corticosterone results.

The increased levels of glucocorticoids are a result of chronic stress that may cause depression, hypertension, gastrointestinal ulceration, electrolyte imbalances, calcium loss, bone reduction, and inhibition of growth (6). The glucocorticoids indicate whether physiological reactions by giving a quantitative value that can be interpreted to determine an animal's reaction to stress in its environment. Glucocorticoids do not cause the stress, but they are important indicators of stress. Therefore, cortisol testing is very important to the physical and mental health of animals in their natural environment and in captivity. Without the cortical testing, these undomesticated animals could be highly stressed and in serious danger of health problems. Glucocorticoids have also been found to reduce phobic reactions. In a study conducted at the University of Zurich, Switzerland, forty subjects with social phobias were given 25 milligrams of cortisone or a placebo. The cortisone treatment showed significant reduction in the fear of these individuals (8). Fecal cortical analysis can be done by collecting the subject's feces regularly

over a period of time. Feces can be assayed for the corticoids, and then the corticoid levels are analyzed and compared to the behavior of the subjects during that time (10).

The cortisol testing can also be done by a blood test, which can indicate increased levels of cortisol during stressful times, such as mating season. The cortisol tends to show similar increases and decreases as the growth hormone due to the pituitary gland releasing ACTH that triggers the adrenal gland to release cortisol (2). Cortisol is often tested along with testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin because it is useful to see how these hormones correlate to the cortisol during mating season. It is interesting to see that in some studies, the cortisol does not show any real correlations with any other hormones, which could be a result of many different things. After assessing the cortisol levels in various carnivores in the study performed by K.M. Young, there was varying evidence of cortisol levels correlating to other hormones (11). There could be unknown lurking variables, or unrelated factors that create more or less stress during mating season.

Goal of Project

Four captive wolves will be observed over four seasons to determine whether wolves in solitaire are more stressed than wolves in packs. The four wolves will include one male in solitaire and two males and a female in a pack. The wolves were separated approximately one year ago, when the wolf that is now in solitaire began to cause conflicts with the other pack members. With the separation of the wolf from his former pack, it is important to investigate the stress levels of the wolves, and compare how the stress levels vary from the wolf in solitaire to the wolves in the pack. By identifying stressful environments and comparing a wolf in solitaire to wolves in a pack, the cortical levels along with behavioral evidence may indicate which environment is more stressful for these undomesticated animals living in captivity. It is

important to better understand the behavioral signs of stressful environments and to see the correlation between the behavioral signs and the cortisol levels of each individual wolf. Wolves often spend months or even years in the wild on their own after dispersing from a pack and venturing to find a new pack. Since wolves have just recently become a concern for many environments, as a result of the reintroduction of wolves into the wild, wolf behavior has not yet been compared to cortical levels of wolves in contrasting environments. As wolves are reintroduced into the wild, it will be helpful for scientists to understand the stress levels and behaviors of wolves in solitaire in comparison to the wolves in packs. Similar studies have been conducted comparing the cortisol levels of male and female wolves during mating season (11). By comparing cortisol levels of a wolf in solitaire to those of wolves in packs, the study will indicate in what pack formation the wolves feel least stressed, and if their lifestyle is greatly impacted by living in solitaire or living in a pack.

Methods and Materials

Four wolves will be used as subjects in this experiment: the four wolves include three males and one female. The wolf in solitaire will be wolf #1 ("Atka"), four years old, who has been in solitaire for approximately one year, an Arctic Gray Wolf. The three wolves in the pack include: the alpha male, wolf #2 ("Apache"), nine years old, the beta male, a mixture of Arctic Gray and British Columbian Wolf, wolf #3 ("Lucas"), eight years old, a British Columbian Wolf, and the omega female, wolf #4 ("Kaila"), eleven years old, a British Columbian Wolf.

Each wolf will have their feces collected once to twice a week in order to allow for comparison of behaviors and cortisol in the four wolves over several months. Around each collection of feces, the behaviors of the wolves will be carefully observed. The wolves will be observed in the most natural conditions available, and with as little human interaction as possible

around the times of observation and collection.

The wolf pack's enclosure is three acres of fenced in wooded land where the wolves have a pond, stone caves, wooden structures, and plenty of shaded areas. The wolf in solitaire's enclosure is one acre of fenced in wooded land adjacent to the pack's enclosure with one side of the fence bordering the pack's fence line (Figure 2). The wolf in solitaire also has a pond, a wooded structure, and many shaded areas.

Enclosure for Male Wolf #1 (Atka)

Figure 2: The enclosure includes many enrichment structures for the wolf. The enclosure for the three ambassador wolves is almost identical, except for its larger size, approximately three acres. Both enclosures were used to collect feces once a week.

Food Preparation

Figure 3: The cream cheese with yellow food coloring is mixed prior to feeding. The liverwurst was fed to the wolves using the same technique with food coloring.

In order to identify the wolf feces, the wolves in the pack will be fed a food with either food coloring or bird seed to allow for identification once the test results are finalized. The wolves will have to be hand fed for this simple procedure in order to ensure the identity of each wolf's feces (Figure 3). Wolf feces will be collected once to twice a week during a routine clean-up, by removing the feces from the enclosure without disrupting the wolves. The wolf feces will be handled carefully with gloves at all times, and a shovel to get the feces into the proper containers. Once collected, the wolf feces will be stored in a freezer in veterinary fecal containers until it is taken for testing.

The extraction procedure is known as the enzyme immunoassay (EIA) sample. The

extraction can be several means: the boiling method, shaking method, or the vortexing method. In order to put the feces into extraction form, a vortexing method was used. The samples must result in an aqueous solution. First, the feces must be baked in a conventional oven until completely dried out. Next, weigh out an equal portion of each fecal sample and put into a labeled test tube. After adding 4.5 mL of ETOH and 0.5 mL of distilled water, vortex each sample for at least 5 minutes (Figure 4a). Then, centrifuge each sample for 20 minutes at 2500 rpm (Figure 4b). Pour off the extracts into a clean test tube to save. Add 4.5 mL of ETOH and 0.5 mL of distilled water to the original test tubes with vortexed feces. Vortex each sample for another 30 seconds and centrifuge again for 20 minutes at 2500 rpm. Once again, pour off extracts into test tubes with the first extracts. Next, add 3 mL of ETOH and vortex and dry again pouring off any more extracts. Add 1 mL of ETOH and vortex each sample for 1 minute. Leave samples to dry under air in a warm water bath. Restore all of the samples in the freezer after the warm water bath. The samples should all be in an aqueous form.

Extraction Process: Vortexing and Centrifuging

a) **b)**

Figure 4: (a) Shows the vortexer on the left being used to mix up each sample containing feces, ETOH, and distilled water, before it is placed in the centrifuge. (b) Shows the centrifuge filled with 6 samples at a time running for 20 minutes at 2500 rpm.

The feces in extraction form are then tested for cortisol levels using an Immulite 2000 machine. The cortical testing will reveal stress levels for each individual sample. Then, the cortisol levels are compared, between the three wolves in the pack and the one wolf in solitaire.

The cortisol levels alone cannot indicate more or less stress; the results must be compared to behaviors as well. The Immulite 2000 assays each sample of approximately 0.5 mL. The samples were tested in trays filled with individual test tubes, each tray holding fifteen numbered test tubes. The trays ran the samples through a cortisol test that allowed for numerical cortisol level results.

Additionally, the behavior will be observed for use as additional evidence to enrich the cortical testing results. The behavioral analysis will include an equal amount of time observing the pack of wolves and the wolf in solitary to see if their behaviors reveal stress or lack of stress. The wolves in the pack will show a lot more interactive behaviors often reflecting their rank within the pack. Behaviors such as tail position, ear position, licking of the muzzle, rolling over, biting at the face, and posture all are commonly seen among the pack members. In contrast, the wolf in solitary will not show these same interactive behaviors, but instead he will conduct his own behavior and activity. The wolf in solitaire often displays behaviors of various roles of the members of the pack, all the way from alpha to omega because he is in a transition stage, and he is in the process of deciding which role best fits his environment. The wolves in the pack are not entirely separated from the wolf in solitary because of their adjacent enclosures. Often, when the wolves in the pack are closer to the enclosure of the solitaire wolf, the wolf in solitary shows more signs of a lower ranked wolf. However, when the wolf in solitaire goes farther away from the pack of wolves, he often shows signs of a higher ranked wolf. Some common behaviors to look for include tail position, ear position, howling, interaction with other members of the pack (only for the pack wolves), and reaction to their environment and surroundings.

Results

There were a total of 42 samples collected and tested for cortisol levels. Of these 42

samples, 12 were from the wolf in solitaire and 30 were from the wolves in the pack (Figure 5). Since the samples from the pack were not always individually identifiable, the data is a generalization of the pack compared to the solitaire wolf.

Data Table: Cortisol Levels of Wolves by Date

Date	Atka	Amb	Amb	Amb	Amb Averages	Without Outliers
1- 2/22/2007	3.52	1.21				
2- 3/15/2007		12.2				
3- 3/22/2007		<1.00				
4- 4/13/2007		4.24				
5- 5/2/2007	13.7	5.83	3.07		4.45	4.45
6- 5/10/2007	8.81	9.65	4.99	10.5	8.38	10.075
7- 5/17/2007	20.7	9.27	5.25	9.29	7.937	9.28
8- 5/25/2007	9.87	5.84	9.43	22.5	12.59	7.635
9- 6/8/2007	11	12.6	11.5	19	14.3667	12.05
10- 7/5/2007	8.67	3.27	12.7	19.5	11.8233	11.8233
11- 7/19/2007	7.94	1.47	1.29	4.62	2.46	1.38
12- 8/3/2007	2.55	11.8			11.8	11.8
13- 8/7/2007	3.16	17	1.77		9.385	1.77
14- 8/9/2007	3.03					
15- 8/16/2007	3.1	1.71	2.09	24.5	9.433	1.9

Key: Atka = Wolf in Solitaire Amb = Ambassador Wolves (pack wolves)

Atka's cortisol levels < Ambassador Cortisol Levels
Atka's cortisol levels > Ambassador Cortisol Levels (with outliers)
Atka's cortisol levels > Ambassador Cortisol Levels (without outliers)

Figure 5: The data table categorizes the data from each category (pack/solitaire) and shows the averages of the pack's cortisol levels from each date. The data table distinguishes which dates show higher cortisol levels for the pack wolves and which dates show higher cortisol levels for the wolf in solitaire. Since many of the samples showed cortisol levels that were much higher or lower than the other samples, it was necessary that the averages were calculated without these cortisol levels. Once the extreme cortisol levels were removed, new means were calculated, showing a more accurate average for the pack wolves.

Since the samples were randomly sampled, it is hard to determine why some of the cortisol levels were so far from most of the cortisol levels. Generally, the cortisol levels are not drastically different between the wolf in solitaire and the wolf in the pack. The average cortisol level of the wolf in solitaire was less than the average cortisol level of the wolves in a pack, but

only by approximately 0.599. Without the outliers, the wolf in solitaire's cortisol levels were greater than those of the wolves in the pack by almost half 8:15. Slightly more than half the time, the wolf in solitaire showed higher cortisol levels than the wolves in the pack. This data suggests that the wolf in solitaire may be not significantly more stressed than the wolves in the pack. Though the data does not show that the wolf in solitaire is more stressed than the wolves in the pack, the data demonstrates that the wolves have fairly similar cortisol levels and approximately the same stress levels.

Statistical Analysis of Data

QC OnCall

Summary Data Report

Panel:	DPC-3A				
Lab:	261394	Lot:	0021	Expires:	3/30/2008
Lab Name:	Montefiore Hospital Med Center	Control Name:	CON-6		
Department:	Pathology	System:	Serum		
Contact:	Irene Ostrowsky	Manufacturer:	DPC		
Data for	7/2007	Printed	8/31/2007	/ Page	2
Range	6/1/2007 through 8/31/2007				

			Level 1		Level 2		Level 3	
			Month	Cumulative	Month	Cumulative	Month	Cumulative
Analyte:	Cortisol	Mean	3.43	3.61	12.19	12.08	32.25	31.77
Method:	Chemiluminescence	SD	0.25	0.39	0.68	0.91	1.67	1.87
Instrument:	DPC IMMULITE 2000	CV	7.30	10.67	5.14	7.54	5.19	5.90
Reagent:	Dedicated Reagent	# Points	31	358	31	360	31	357
Unit:	µg/dL							
Temperature:	No Temperature							

Figure 6: This summary data report can be used to figure out the means, standard deviations, and margins of error. (I.e. A cortisol level of 3.52 could be written as 3.52 ± 10.67% = 3.52 ± .375584 = a cortisol level between 3.144416 and 3.895584) By figuring out the range for each data point, the cortisol levels become more accurate with a smaller margin of error.

The summary data report (Figure 6) gives the cortisol means, standard deviations, coefficients of error, and number of points at three different levels. This table can be used to

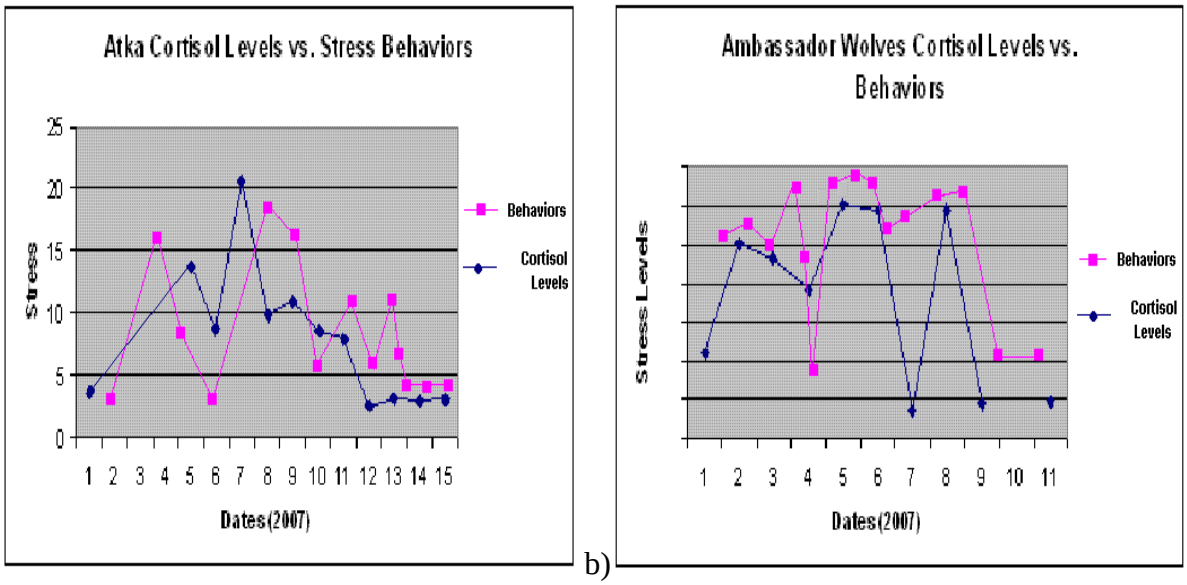
determine a margin of error for each cortisol level by showing a range instead of an exact cortisol level. (I.e. A cortisol level of 3.52 could be written as 3.52 ± 10.67% = 3.52 ± .375584 = a cortisol level between 3.144416 and 3.895584) By figuring out the range for each data point, the cortisol levels become more accurate with a smaller margin of error.

In addition to the cortisol levels, the behaviors observed appeared to correlate directly with the cortisol levels. The behavior of the pack varied throughout the study. For about a month of the study, June to July, the two male wolves in the pack showed dominant and aggressive behavior toward the female in the pack. The male wolves chased her into a cave structure and showed aggressive behavior when she tried to leave it. The male wolves did not let the female wolf near the fence and were particularly aggressive during feeding times. The pack conflict may have led to some high cortisol levels for the female wolf because of her lack of freedom and difficulties with the other pack members. The pack conflict appeared to subside with time and soon after the female wolf was seen more frequently outside of the cave and freely coming up to the fence. The reasons for the conflict were unknown and surprising for the season, as aggressive behavior would have been more probable in the breeding season of February and March, when hormones often result in aggression and other pack conflicts.

Other than the temporary conflict between the pack wolves, the behaviors seen within the pack appeared to be very civilized and cooperative. When feeding, the alpha wolf ate first, and then the beta wolf ate after the alpha gave his permission. The beta wolf was much more interested in the food, and as a result the alpha often gave up his food quickly to the beta wolf. The omega wolf did not usually get the food until after the alpha and beta wolves had taken their share. There was some aggression and pack behavior seen during the feeding with ear position and tail position indicating pack rank. In addition, the alpha wolf often growled or pushed the

other wolves out of his way to show his authority. The beta and omega wolves often showed submissive behavior toward the alpha wolf such as licking at his muzzle, rolling over on their backs, and standing lower to the ground than the alpha. All of these behaviors appeared to be appropriate and common pack conduct. The wolves have tremendous respect for one another and cooperate with each other constantly. The wolves also howl at one another to call, warn, or respond to each other. The alpha wolf generally howled first, followed by the beta and then the omega, but the omega sometimes did initiate the howling, as part of her role in the pack.

Cortisol Levels vs. Stress Behavior Graphs for Wolf in Solitaire and Wolves in a Pack



a) b)

Figure 7: The numbers on the x-axis indicate the dates from the chart above (Figure 5). The line graphs both show many points that increase and decrease on similar dates. The general increases and decreases in the stress appear to be relevant in both the behavioral analysis and the cortisol levels. The behavioral graph was created from an estimated interpretation of the behavioral stress signs, which was done using a quantitative method of determining behaviors that indicate stress.

In contrast to the pack behavior, the wolf in solitaire showed less activity, as he had no other wolves to work with within his enclosure. The wolf in solitaire did show varying tail and ear positioning while in his enclosure. When he came near the fence line that bordered his former pack, he showed non-dominant tail and ear positioning, but when he was on the opposite side of his enclosure, not bordering his former pack, he showed dominant ear and tail

positioning. The wolf in solitaire would also howl, but generally in response to other wolves howling in surrounding enclosures, often from his former pack. The wolf in solitaire would come to the fence and show excitement when visitors came near his enclosure. The wolf in solitaire reacted very differently than the Mexican wolves studied at different zoos, in another study, for cortisol levels in response to the number of human visitors. The Mexican wolves showed higher cortisol levels and behavioral signs of stress when there were more visitors (10). In contrast to the study at zoos, the wolf in solitaire at the Wolf Conservation Center would often whine, jump, and rub against the fence to attract human attention to himself. The wolf in solitaire showed more interest in human attention than the pack wolves, most likely because his lack of contact with other wolves.

Discussion

The results provide little evidence that this wolf in solitaire had higher cortisol levels and more stressful behavior than the wolves in a pack. The similar cortisol levels between the wolf in solitaire and the wolves in the pack suggest that the stress levels of all of the wolves were fairly similar during the testing time. This information helps to show that keeping animals in solitaire in captivity is not necessarily detrimental for the animal. For the wolf in solitaire, his cortisol levels were not significantly higher than the wolves in the pack, and his behaviors were observed to be similar to those of the pack members. There was a wide range in the cortisol levels, ranging from less than one to over twenty-four. Even though some of these cortisol levels may be outliers that have to be removed from the data calculations, it is interesting to see what a wide variety of cortisol levels were obtained. The cortisol levels are simply numbers that indicate the hydrocortisone levels of steroid and the glucocorticoids that are secreted by the adrenal cortex. The cortisol levels do not necessarily directly reflect stress levels, but often

fluctuate according to stress. When there is an increased secretion of glucocorticoids, it is generally a response to stress (4). The cause of the stress cannot be determined based on the cortisol levels, but the behaviors and the environmental conditions can help us analyze why stress levels might be elevated.

One major problem that changed the original research plan was the troubles that were faced with feeding the wolves food coloring and bird seed to try to identify feces. Unfortunately, the bird seed and the food coloring were only seen in the feces a few times over the course of the study. After six months of not seeing any sign of food coloring or bird seed in the feces (March through August), the feeding stopped and became just observations. Due to the natural, seasonal overgrowth in the enclosures, the feces eventually became very difficult to find, especially for any food coloring or bird seed to stand out. The feces were still collected at least once a week, but no more food coloring or bird seed was noted. When the enclosure clears out with the seasonal change into winter, feeding will continue with the food coloring. The colors yellow and green appeared to be the most prominent in the feces. The bird seed was much harder to find and identify, and therefore will not be used when feeding resumes in the winter months.

It was fascinating to see the wolves through all four seasons because their behaviors as well as their physical appearance vary so greatly. In the winter the wolves were much more active, showing behavior at almost all hours of the day. The wolves looked a lot larger due to their undercoat being fully grown in. The undercoat provides warmth and dryness for the wolves to live in temperatures as low as seventy degrees below zero. The pack of wolves enjoyed activities such as walking on their frozen pond, displaying lots of pack behavior (muzzling each other, growling, ear positions, tail position, and wrestling), and running through the snow or on the frozen ground. In addition, there was an increased amount of howling and howling on

command (howling back to human howls or other extraneous noises). The wolf in solitaire was also more active in the winter months. The solitaire wolf spent more time by the fence-line bordering the pack fence-line. He displayed less dominant behavior when he was closer to the pack wolves, and more dominant behavior when he was farther from the pack wolves. The wolf in solitaire was more mobile and also ran around the enclosure, walked across the frozen pond, and sniffed around his enclosure. The behaviors were similar for the wolves in the pack and the wolf in solitaire, but overall, the wolves in the pack were more active because they had each other to display pack behaviors with. In a previous study at Montana State University, tests were performed to observe whether varying precipitation or temperature affected glucocorticoid levels. The study found that there appeared to be no impact from the differing climates on the glucocorticoid levels (9). The climate was recorded in this study, but it was not focused on as a major cause of stress level variations.

In the warmer months, spring and summer, the wolves were much more lethargic. With little movement during the day, and only some activity at dawn and dusk, the wolves' behaviors were drastically different than the behaviors seen in the colder months. The wolves in the pack were very slow and lazy during the day, often having to be called to the fence-line, even for food. The pack behavior was not seen as much, with little interaction between the wolves, even when feeding. The wolf in solitaire was also constantly resting during the day, with little movement. It would often take a long time to find him in his enclosure because he was usually looking for a shaded area. The wolves seemed to have similar behaviors in the warmer months because the activity level was not very high for both the pack wolves and the solitaire wolf.

The four wolves that were studied in this experiment allowed for important information about animals in captivity to be discovered. Many facilities including conservation centers, zoos,

aquariums, and other wildlife institutions can use this study and apply it to their facilities. This study suggests that wolves kept in solitaire and under conditions similar to those at the Wolf Conservation Center, do not have significantly higher cortisol levels than wolves kept in a pack.

Further research could be done to determine whether this is true for other animals besides wolves. Since wolves are such group-oriented pack animals, one would think that other animals would also show similar levels of cortisol between animals in solitaire and animals in groups. Animals that are less cooperative and pack-like might even show higher cortisol levels than their counterpart animals in a group and lower cortisol levels from animals in solitaire. It would also be interesting to apply this study to wolves at other facilities. Since the four wolves that were studied have been exposed to humans, their behavior and lifestyle may not be the same as wolves who have less human exposure. If this study was performed on wolves in a more natural, wild setting, the cortisol levels and behaviors might have shown different results. Testing cortisol levels and behavioral analysis can be applied to many animals in different environments, but by studying wolves, which are known to be pack animals; it is surprising to see that the wolf in solitaire did not show behaviors or cortisol levels to indicate that he was significantly more stressed than the wolves in the pack.

Conclusion

After observing the behaviors and testing the cortisol levels of four wolves, three in a pack, and one in solitaire, the results of this study show that wolves in solitaire are not necessarily more stressed than wolves in a pack. There was not significant evidence to prove that the wolf in solitaire was more stressed because the cortisol levels were not significantly higher than the pack's cortisol levels, and the solitaire wolf's behaviors did not indicate more stress than the pack behaviors. The predicted outcome of the wolf in solitaire being more

stressed than the wolves in a pack was not supported, but the study supported similar levels of stress, which is a better outcome for wildlife facilities. By finding that the wolf in solitaire had similar cortisol levels as the wolves in the pack, wolf facilities might be able to keep certain wolves in solitaire with less concern about their stress levels. The behaviors observed from the wolf in solitaire and the wolves in a pack did not show significant differences, supporting the similar cortisol levels between the wolves being studied. The correlation between the cortisol levels and the behavioral observations was fairly consistent, and the behavioral changes were mainly seasonal and temperature dependent.

Wildlife facilities can apply the findings of this study to other animals under similar circumstances. Since it has been found that the stress levels of wolves in solitaire are not significantly greater than those of wolves in a pack, it is acceptable for wolves without a pack to remain in solitaire for some time without being extremely stressed. Since the wolves that were studied are held at a facility where they are not constantly interacting with humans, their lives may be less stressful both as a pack and in solitaire. However, various facilities could use this information to further research other animals at different facilities to examine whether they have similar stress levels when they are separated from a group.

This study provides valuable information, especially with a wolf population that is just beginning to grow after years of endangerment to the species. Wolves that are being saved in species survival plans and other organizations can live alone for some time without the stress that would be expected when not among fellow pack members. With the wolf living adjacent to his former pack members, he was not in total isolation and he was still in the vicinity of other wolves. All four wolves in this study offered analysis of their lifestyle in a somewhat natural, although captive, physical environment, and two unique groupings of wolves. The cortisol levels

and behavioral analysis have presented a non-invasive study that resulted in an interesting finding that could be used to keep stress levels down for wolves and other captive wildlife species as well.

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