THE EFFECTS OF SUPPLEMENTAL FEEDING ON STRESS HORMONE CONCENTRATIONS IN ELK

by

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TABLE OF CONTENTS

1. INTRODUCTION 1

Supplemental Feeding 2
Brucellosis 4
The Stress Response and Glucocorticoids.....7

2. METHODS 12

Project 1: Differences in Stress Hormone
Levels of Feedground and Non-Feedground Elk 12
Environmental Covariates.....13
Management Covariates.....14
Project 2: Experimental Manipulation of Feeding Density.....16
Feeding Procedures.....17
Low Density Feeding Procedures 18
Environmental Covariates.....19
Behavioral Observations.....19
Density Covariates 21
Sample Collection, Glucocorticoid Extraction
and Enzyme Immunoassay 22
Statistical Analysis 24
Fecal Glucocorticoids of Feedground vs. Non-feedgrounds Analysis 24
2008 Experimental Manipulation of Feeding Analysis 25
Stress Covariates.....25
Aggression Rates 27

3. RESULTS 28

Feedground and Non-feedground Elk Stress Hormone Concentrations 28
Low Density Feeding Treatment.....31
Stress Covariates.....33
Aggression Rates 35

4. DISCUSSION 39

Feedgrounds and the Stress Response.....40
Group Size and Density.....41
Low Density Feeding and the Stress Response.....42
Aggression Rates and the Stress Response 43

LITERATURE CITED.....45

LIST OF TABLES

Table Page

1. Description of covariates for 2007 cross-sectional study 15

2. Description of covariates for 2008 experimental study 22

3. Description of covariates used in aggression analysis.....22

4. Parameter estimates for the full model of the mean
fGC concentration for elk feedgrounds in 2007.....31

5. Mixed models developed investigate the relationship between fGC
concentration and covariates, location was included as a random effect.....33

6. AIC_c model rankings for a subset of for which snow depth was known
Models include a random effect term for location.....34

7. AIC_c model rankings for a subset of data for which feedline density
was known. Models contain a random effect for location 34

8. AIC_c table for linear regression models
developed to predict aggression rates during feeding.....38

9. Parameter estimates for the top aggression rate models selected by AIC_c 38

LIST OF FIGURES

Figure Page

1. Locations of Wyoming feedgrounds....4

2. Feedground and non-feedground locations sampled in 2007 12

3. Locations of feedgrounds included in the 2008 experimental study 17

4. Boxplots of fecal glucocorticoid concentrations at non-feedground and feedground sites in 2007 28

5. Fecal glucocorticoid hormone concentrations averaged across feedground and non-feedground locations in 2007 29

6. Mean fecal glucocorticoid concentration by year and location 30

7. Fecal glucocorticoid concentration throughout the season at control and treatment sites in 2008....32

8. Fecal glucocorticoid concentration as a function of group size and fecal glucocorticoid concentrations as a function of local density 35

9. Aggression rates as a function of time since the beginning of feeding 36

10. Mean fGC concentration as a function of aggression rate for each feedground 36

ABSTRACT

On twenty-two feedgrounds in western Wyoming, elk (*Cervus elaphus)* are provided with supplemental feed throughout the winter. Brucellosis seroprevalence of feedground elk is 26% whereas other elk in the Greater Yellowstone Ecosystem have historically had a brucellosis seroprevalence of 2-3%. The aggregation of elk during peak transmission allows brucellosis to persist in the feedground populations. In addition to creating the opportunity for disease transmission, the aggregation of elk on feedgrounds may have detrimental physiological effects. Studies have shown that chronically high stress hormone concentrations can suppress the immune system and lead to increased disease susceptibility. Potential stressors on the feedgrounds include high densities, large group sizes and aggressive social interactions. In this study I investigated how factors associated with supplemental feeding affect stress hormone levels, as indexed by fecal glucocorticoid levels, in elk on feedgrounds and elk on native winter range. I also worked with managers to experimentally alter the feeding distribution on the feedgrounds to examine how feeding density affects stress hormone levels and aggression rates. Results show that elk on feedgrounds have stress hormone levels 31% higher than elk on native winter range (Welch's $t_{27.23}$=2.39, p=0.024). Experimental reduction of feed density did not have an effect on stress hormone level or aggression rates. But note the relationship between fGCs and local densities here. Although the feeding treatments did appear to reduce local feeding densities,s this effect was not significant and was small relative to the large differences in density among sites. Regardless as to the cause of the high stress hormone levels seen in supplementally fed elk, the feedgrounds are creating an epidemiological setting for disease transmission and a physiological state that may increase susceptibility to disease. The impact of these stress hormone concentrations on disease susceptibility remains unknown, but may be an important driver of disease dynamics in these elk populations.

INTRODUCTION

Over the last century, there has been a significant increase in the number of emerging infectious diseases in humans and wildlife (Jones et al. 2008). This change as been attributed to the growth of the increasingly mobile human population (Daszak et al. 2001). Anthropogenic change can affect disease risk and burden in many different ways. For example, the loss and fragmentation of wildlife habitat may can lead to an initial increase in the density of animals (McCallum and Dobson 2002) and changes in animal behavior or changes in resource use that promote intraspecific disease transmission (Farnsworth et al. 2006, Bradley and Altizer 2007). The fragmentation of the landscape is also generally thought to have increased the interface between wildlife, livestock and humans, facilitating pathogen spill-over between different hosts (Gortazar et al. 2007). In addition, the introduction of reservoir hosts, such as livestock, have in some cases increased the density of competent hosts, contributing to the persistence of pathogens in wildlife which might not otherwise persist (Daszak et al. 2001, Gortazar et al. 2007). Anthropogenic activity has lead to disease spread through the introduction and transport of both endemic and exotic fauna and associated pathogens (Dobson and Foufopoulos 2001). Lastly, habitat degradation and pollution can stress wildlife and reduce their ability to mount an effective immune response to pathogens (Dobson and Foufopoulos 2001). In light of these trends, understanding the dynamics and drivers of wildlife disease has become increasingly important to protect human, wildlife and livestock health (Daszak et al. 2000, Jones et al. 2008).

Supplemental Feeding

Management practices are often intended to alleviate anthropogenic pressures on wildlife. One such practice is the supplemental feeding of wildlife. Effects of supplemental feeding include decreased mortality, increased reproductive success, and enhanced body condition (Peterson and Messmer 2007). However, supplemental feeding can also have adverse effects such as changed migration patterns (Peterson and Messmer 2007), increased disease transmission (Miller et al. 2003) and a high impact on surrounding habitat and wildlife (Gundersen et al. 2004, Anderson 2007). Fed wildlife species range from passerines and game birds to small mammals and big game (Grange 1937). In the northern latitudes, feeding wild ungulates is a common practice (Putman and Staines 2004) for reasons that vary from "baiting" animals for hunting, to increasing winter survival (Gundersen et al. 2004, Brown and Cooper 2006, Peterson and Messmer 2007).

The largest and most consistent ungulate feeding program occurs in Wyoming, where over 23,000 elk (*Cervus elaphus*) have been fed throughout the winter for nearly a century (Smith 2001). Supplemental feeding of elk began in 1910 to boost the shrinking elk population and to prevent wildlife damage to private property. Feeding continues today with the goals of reducing livestock-wildlife interactions for disease control and preventing wildlife damage to private property (Smith 2001).

In Wyoming, supplemental feeding of elk occurs on twenty-two feedgrounds. The Wyoming Game and Fish Department (WGFD) manages twenty-one of the feedgrounds. The U.S. Fish and Wildlife Service manages one feedground, the National

Elk Refuge (NER, Smith 2001). The feedgrounds are located in western Wyoming primarily in the foothills and valleys of the Gros Ventre, Wind River and Wyoming Mountain Ranges (Figure 1). Feedgrounds are typically located on public land and placed so elk are stopped while migrating from high elevation summer range to the low elevation winter range. The feeding areas managed by the state range in size from 0.044 to 0.39 square kilometers (WGFD unpublished data), and hold from 200 to over 1300 elk (Western EcoSystems Technology 2004). The NER, the largest of the feedgrounds, feeds over 6500 elk each winter (Smith 2001). Alfalfa or grass hay is distributed on feedgrounds daily throughout the winter by WGFD employees (for details see Methods). Feeding can begin as early as November and can continue through May. The timing and duration of feeding varies by site and depends on a number of factors including timing of snowfall, spring green-up and management decisions (Smith 2001, Cross et al. 2007).

Similar to other wildlife feeding programs there are both beneficial and adverse effects resulting from feeding elk on the Wyoming feedgrounds. For example, feedground elk have a winter mortality rate averaging less than 1.5% (Smith 2001), but the feedgrounds also facilitate intraspecific disease transmission (Smith 2001, Godfroid 2002). Historically, the seroprevalence of brucellosis in elk in the Greater Yellowstone Ecosystem (GYE) has been 2-3%, whereas seroprevalence of feedground elk is approximately 26% (Atkinson et al. 2006, Cross et al. 2007, Scurlock et al. 2007).

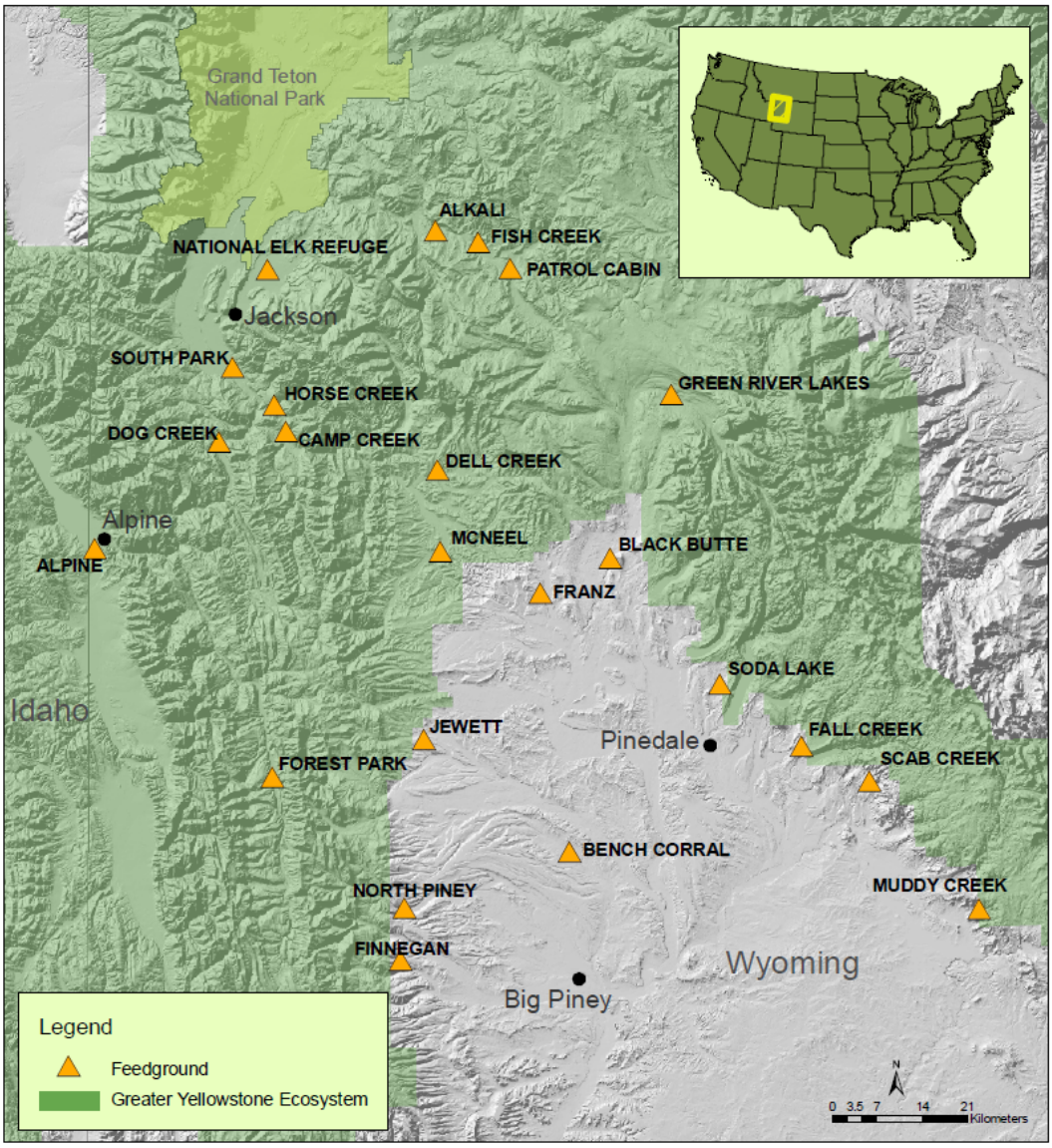

Figure 1. Locations of Wyoming feedgrounds.

Brucellosis

Brucellosis is a disease of livestock and wildlife and one of the most common zoonotic diseases in the world (Pappas et al. 2006). In the United States, brucellosis has been eradicated in livestock everywhere except within the GYE (Ragan 2002) where

cattle are periodically infected from wildlife. Brucellosis in the GYE is caused by *Brucella abortus*, a gram negative intracellular bacteria (for review see Godfroid 2002). After exposure, the bacteria localize in the reproductive organs and often cause the abortion of the first pregnancy after infection, but subsequent pregnancies are usually successful (Thorne et al. 1978a, Ragan 2002). The aborted fetus and associated birth fluids are highly infectious and contact with these materials by other animals is the primary source of transmission (Thorne et al. 1978a, Godfroid 2002). Abortion is the most obvious symptom of brucellosis, but other symptoms include inflammation of the joints, and lameness (Thorne et al. 1978a). Brucellosis first appeared in the GYE in 1917 in bison that were infected by cattle (Meagher and Meyer 1994). Brucellosis was detected in the elk populations of Yellowstone and Jackson Hole by the 1930s (Thorne et al. 1978b, Meagher and Meyer 1994).

There are many economic, cultural and political issues surrounding the feedgrounds which make them highly controversial issue in the region (Bienen and Tabor 2006). Containing the elk on the feedgrounds is a way to reduce interactions between the elk and livestock on an annual basis. This is particularly important to the cattle industry, because detection of brucellosis in livestock leads to economic consequences for the industry including increased testing costs, trade restrictions and statewide loss of brucellosis free status. The feedgrounds also reduce elk damage, such as elk eating stored hay, to private property. This is important to the property owners, but also important to WGFD because, by law, WGFD is required to compensate owners for damage done by elk (Dean et al. 2004). Hunters and outfitters also benefit from the

supplemental winter feeding of elk because it keeps the elk populations high which provides many hunting opportunities. Substantial revenue is generated for the local communities from recreation associated with wildlife including hunting and wildlife watching (Smith 2001). Although concentrating the elk during the winter helps reduce the risk of brucellosis transmission to wildlife, the feedgrounds also aggregate the animals during the peak of brucellosis transmission allowing the disease to be maintained. There is fear that if other diseases, such as CWD, are introduced on the feedgrounds there could be severe mortality within those elk populations (Bienen and Tabor 2006). The cost of feeding the elk and maintaining the feedgrounds is borne by the state and federal agencies that manage the feedgrounds. In 2004 it cost $1.36 million dollars for WGFD to operate the feedground program (Dean et al. 2004). The issues outlined above and the diverse group of stakeholders makes the purpose and effectiveness of the elk feedgrounds a point of contention in the region which is why a clear, scientific understanding of the disease dynamics on the feedgrounds is essential.

Previous research has increased the understanding of the dynamics and drivers of brucellosis on the feedgrounds. Cross et al. (2007) examined how brucellosis seroprevalence was associated with length of the feeding season. They found that the total number of days elk are fed explains 59% of the variance in seroprevalence between feedgrounds (Cross et al. 2007). The more time spent on the feedground, the more likely an elk is to encounter infected birth materials, particularly when feeding overlaps with the third trimester of pregnancy when abortions are likely to take place. Thus, the timing of

the aggregation of animals on the feedground is an important factor contributing to the persistence of the disease in these populations (Smith 2001, Cross et al. 2007).

Though the length of the feeding season explains a high proportion of the variance in seroprevalence among sites, 41% of this variation remains unexplained. It is clear that by aggregating elk during the peak transmission period the feedgrounds are creating conditions that allow brucellosis to persist. However, it is also possible that the conditions on the feedgrounds create a physiological state that makes the elk more susceptible to disease. Specifically, the large group sizes, high densities and frequent social interactions might lead to a chronic stress response. This physiological response may weaken the immune function and affect susceptibility to disease (see below). Although many studies have investigated the relationship between group size, density and disease, few studies have examined how stress could act as an alternative or additional mechanism by which density or group size affects disease.

The Stress Response and Glucocorticoids

When an animal encounters a stressor, the hypothalamic pituitary adrenocortical axis (HPA) is activated leading to a cascade of numerous hormones which results in the production of glucocorticoids (GCs) (Munck et al. 1984, Mostl and Palme 2002). The GCs circulate through the body and stimulate physiological changes, such as slowing digestion and increasing glucose availability to help deal with the stressor (Mostl and Palme 2002, Reeder and Kramer 2005). In the short-term this is adaptive, but chronically elevated GCs can have harmful effects including slowed growth, reduced reproductive

success and suppression of the immune system (Mostl and Palme 2002, Reeder and Kramer 2005). Glucocorticoids have been shown to have direct effects on immune function by decreasing natural killer cell activity, inhibiting production of cytokines such as γ-interferon, and reducing proliferation of T-cells (Munck et al. 1984, Coe 2002). Decreased immune response due to stress has been documented in many species (Barnard et al. 1994, Oppliger et al. 1998). For instance, an experiment by Oppliger et al. (1998) found that in the common lizard, *Lacerta vivipara*, parasite loads increased when they were in high stress situations.

An effective way to monitor stress hormone levels in wildlife is to measure fecal glucocorticoids (fGC). Fecal glucocorticoids provide an index to quantify the physiological stress response (Keay et al. 2006, Lane 2006) and this technique has been successfully used to assess stress hormone levels in elk. (Millspaugh et al. 2001, Creel et al. 2002). The hormone concentration in feces represents the accumulation of stress hormones from the previous 12-24 hours (Keay et al. 2006). The collection of samples is non-invasive, and it is unlikely that disturbance due to sample collection influences the concentration of the hormones, as can be a problem for studies using blood samples (Mostl and Palme 2002).

In this study I investigate how environmental and management variables associated with supplemental feeding affect stress hormone levels in elk. The conditions on the feedgrounds are very different than those found on native winter range, and elk on the feedgrounds are presented with many persistent potential stressors; including densities and group sizes much higher than those found in elk using native winter range,

unpredictable and disruptive human activity and high rates of interaction. Studies in other animal systems have shown that stressors including human disturbance (Millspaugh et al. 2001, Creel et al. 2002, Pereira et al. 2006), social competition (Creel 2001), snowpack (Creel et al. 2002, Huber et al. 2003), large group size (Barnard et al. 1994, Rogovin et al. 2003) and high density (Rogovin et al. 2003, Li et al. 2007) can initiate a stress response. Given the effects that chronically elevated stress hormones can have on immune function and the numerous potential stressors elk encounter on the feedgrounds,it is possible that increases in stress hormone concentrations influence disease dynamics on the feedgrounds. This study involved two projects: a cross sectional study of stress hormone concentrations of 21 populations of feedground elk and non-feedground elk, and an experimental manipulation of feeding density and the subsequent effects upon stress hormone concentrations.

The objectives of the first project, the cross-sectional study, were: 1) to determine if stress hormone concentrations differed between elk on feedgrounds and elk on native winter range and 2) to investigate what environmental and management factors were associated with stress hormone concentrations among feedgrounds. Elk on native winter range could have higher stress levels due to limited forage and harsh winter snow conditions (Sweeney and Sweeney 1984, Huber et al. 2003). Alternatively, feedground elk could have higher stress levels due to the unnatural conditions and stressors on the feedgrounds. Previous studies have shown that density (Li et al. 2007) and snow depth (Creel et al. 2002, Huber et al. 2003) are correlated with stress hormone levels in

ungulates, and I expected them to play an important role in fGC concentrations in feedground elk as well.

The feedgrounds provided a unique opportunity to conduct a large scale field experiment. In the second project I took advantage of this opportunity and worked with managers to experimentally manipulate of feed density. I used a before-after-control-impact design to study the effect of this manipulation on stress hormone concentrations. On control sites, standard feeding procedures were used and hay was distributed in a few, long, continuous feedlines (see Methods). On treatment sites low density feeding procedures were used and hay was distributed in a grid-like pattern across a broader region of the feedground than in control site. The intent of low density feeding was to create conditions more similar to those found on native winter range by encouraging elk to spread out into smaller groups while feeding. However, even during low density feeding elk densities remained higher than densities typical of elk on native winter range.

I hypothesized that by reducing the density of elk during feeding, the number of interactions between elk would decrease. I was specifically interested in how aggressive interactions changed in response to low density feeding because aggressive interactions might lead to increased GC concentrations. I used behavioral observations to investigate if interaction rates were affected by low density feeding and if the interactions could be the mechanism increasing stress hormone concentrations on the feedgrounds.

The objectives of the second project, the experimental manipulation of feed density, were to 1) evaluate if low density feeding reduced stress hormone levels, 2) evaluate if rates of behavioral interaction (and particularly aggressive interaction) were

affected by the type of feeding 3) investigate whether aggression was associated with stress hormone levels and 4) examine how stress hormone concentrations were associated with environmental and management factors such as group size, density and snow depth.

METHODS

Project 1: Differences in Stress Hormone Concentrations of Feedground and Non-Feedground Elk

I collected fecal samples at sixteen feedground sites and from five native winter range sites between March 5th, and 18th, 2007 to investigate how stress hormone concentrations differ between feedground elk and non-feedground elk. Feedgrounds I sampled included: Alpine, Black Butte, Camp Creek, Dell Creek, Dog Creek, Finnegan, Forest Park, Franz, Horse Creek, Jewett, McNeel, Muddy Creek, Patrol Cabin, Soda Lake, South Park, and the National Elk Refuge. Native winter range samples were collected in the Northern Range of Yellowstone National Park, Buffalo Valley near Moran WY, in northeastern Utah, Miller Mountain in the Wyoming Range and at Cokeville Meadows National Wildlife refuge in Wyoming (Figure 2).

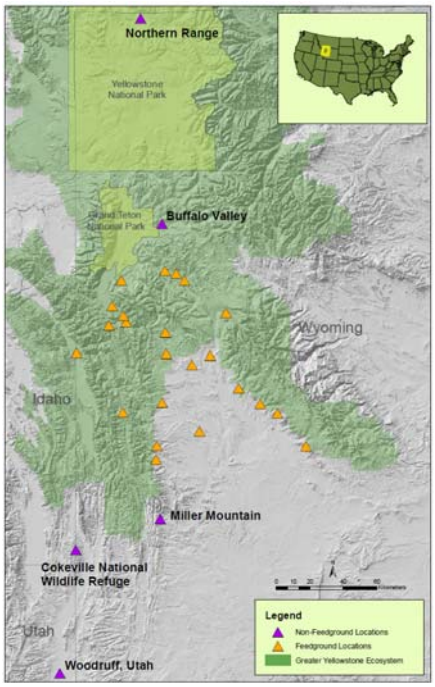

Figure 2. Feedground (orange) and non-feedground (purple) locations sampled in 2007.

In addition to the fecal samples collected in 2007, there were data available on fGC concentrations for elk elsewhere in the GYE for the years 2003-2008. Non-feedground samples included (years and sample size in parentheses); Madison-Firehole (2003, n=145), Wall Creek (2003, n=31; 2004, n=81, 2005 n=60), Blacktail WMA (2003, n=21; 2004, n=81; 2005, n=75), Gallatin (2004, n=135), Dome Mountain (2005, n=30), Sun Ranch (2006, n=19) and Buffalo Valley (2006, n=33). The additional feedground sites sampled in 2006 included Bench Corral (n=27), Dell Creek (n=5), Franz (n=10), Alpine (n=29), Muddy Creek (n=9), Scab Creek (n=13), Soda Lake (n=50), and South Park (n=21). Feedgrounds sampled in 2008 included Bench Corral (n=85), Camp Creek (n=81), Dog Creek (n=98), Fall Creek (n=60), Forest Park (n=8), Franz (n=82), and Soda Lake (n=84). These data provided more information on the mean fGC levels and amount of variation expected in fGCs at native winter range sites.

Environmental Covariates

In 2007 I collected data on group size, snow pack, elk density and management activities at each site in addition to the fecal samples (Table 1). At feedgrounds, group size is defined as the number of elk on the feedground, this number was based on annual counts conducted by WGFD. These counts were conducted on the ground by WGFD employees in the middle of the feeding season when elk attendance was at a maximum. At non-feedground sites, I determined group size by counting the number of visible individuals in the group from a vantage point on the ground prior to collecting the fecal sample.

The feeding area at each feedground site was determined by WGFD. The feeding area is based on polygons that were digitizing using 2002 orthophotos in GIS. The polygons were ground-truthed by feedground managers (WGFD personal communication). I used this measure of feedground area to determine feedground density, which I defined as group size divided by feedground area (elk/ km^2). Density was not determined for native winter range sites.

I measured snow depth by digging through the snow until the ground was reached and measuring the depth to the nearest centimeter. Snow depth was measured on the feeding area and in an area of unpacked snow adjacent to the feeding area. At many feedgrounds the snow on the feeding area was compacted and icy, allowing the animals to walk on top of it. At most feedgrounds elk rely predominately on the supplemental feed that is provided and seldom forage off the feedground. Therefore, it is possible that snowpack on the feedground does not influence stress hormone concentrations and that snow depth adjacent to the feedground is a better indication about the snow conditions an elk would encounter if it moved away from the feeding area. I used snow depth adjacent to the feedground in the analysis.

Management Covariates

Throughout the season management activities include vaccination and trapping on the feedgrounds. This unpredictable human activity may be disruptive and induce a stress response in the elk. Vaccination occurs yearly on each feedground (except Dell Creek) during the season and can take from one to several days. During vaccination, calf elk are shot with a Strain 19 vaccine biobullet and marked with a paint ball to indicate that they

have been vaccinated. In 2007 vaccination coincided with sample collection. Some feedgrounds had not been vaccinated prior to sample collection while others had been vaccinated as recently as two days prior to sample collection. I included a categorical variable for whether vaccination had occurred prior to sample collection (before sample collection=1, no vaccination or after sample collection=0). The type and intensity of trapping events can be highly variable among feedgrounds. Capture efforts are usually focused on 4-6 feedgrounds annually and trapping events can take place on one or several occasions. Trapping methods include corral traps and chemical immobilization. In addition to the trapping event there may be disturbance associated trap preparations such as shoveling snow out of the corral trap. I accounted for trapping activity with a categorical variable that denoted if trapping had occurred (i.e. yes=1, no=0) on the feedground during the season.

Table 1: Description of covariates used in 2007 cross sectional study.

Covariate Name	**Description**
Snow Depth	Depth of snow adjacent to feedground (cm)
Group Size	Number of elk on the feedground
Feedground Density	Group Size / Area delineated as feeding area (elk/ km^2)
Trapping Activity	Trapping activity occurred at that location (Yes=1, No=0)
Vaccination	Vaccination occurred prior to sample collection (Yes=1, No=0)

Project 2: Experimental Manipulation of Feeding Density

I used a before-after-control-impact (BACI) design to assess how fGC concentrations changed in response to changes in the feeding regime. In the BACI design several measurements are made before the impact so that natural variability can be estimated (Stewart-Oaten et al. 1986). After the impact, the control sites continue to provide information on variation due to random influence (Stewart-Oaten et al. 1986) and the treatment sites provide information on the effect of the impact. The measurements from before and after are pooled by site. Then the treatment effect can be evaluated by taking the difference between the before and after measurements and comparing these differences across control and treatment groups using a t-test (Stewart-Oaten et al. 1986, Conner et al. 2007). If the impact had an effect, one would expect the difference (before-after) of the treatment sites to be different that of the control sites.

This project was conducted from January through March of 2008. The four treatment, or low density (LD), feedgrounds were chosen by WGFD because they were perceived as low risk for wildlife-livestock interactions (Scurlock et al. 2007). Low density feeding treatment sites included Bench Corral, Fall Creek, Forest Park and Soda Lake. Standard feeding procedures were used throughout the season on three control feedgrounds. Control sites were chosen for accessibility and willingness of feeders to work with the researcher. Control sites were Camp Creek, Dog Creek and Franz feedgrounds (Figure 3).

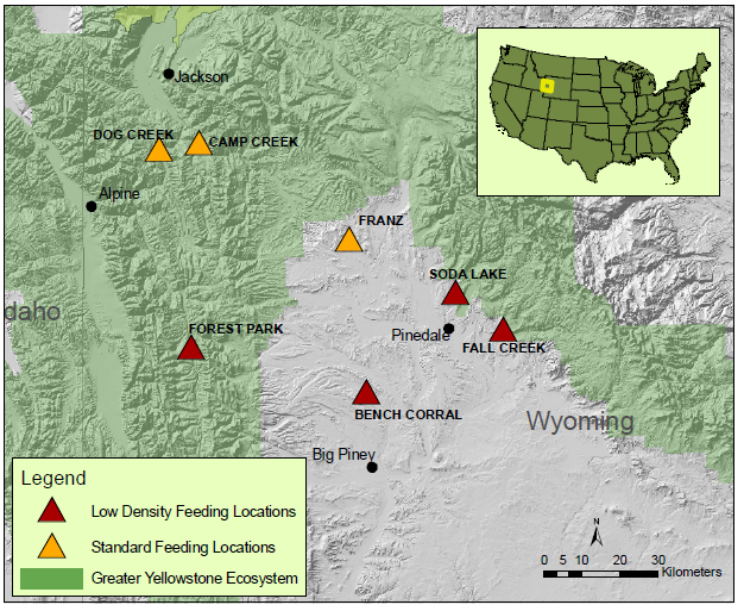

Figure 3. Locations of feedgrounds included in the 2008 low density feeding experiment. Treatment (low density feeding) locations are in red and control (standard feeding) locations are in orange.

Feeding Procedures

Elk at state-operated feedgrounds are fed grass or alfalfa hay. The daily amount of feed is typically 3.6 to 4.5 kilograms of hay per elk (Smith 2001). The hay is loaded onto a horse-drawn sleigh or flatbed truck and distributed by hand. Typically, there is one feeder at each feedground who feeds the elk everyday throughout the season. The time of day that elk are fed is fairly consistent within a feedground, but varies from 8:00 am and 2:00 pm among feedgrounds. Distribution of the feed can take one-half hour to several hours depending on how much hay is fed, how much help the feeder has, and the feedground conditions (ie. snow depth). During standard feeding the hay is distributed in long, continuous feedlines. When feeding begins, elk are usually bedded or standing on the feedground and they approach the feed quickly, sometimes even surrounding the feed

sleigh. Feeding typically requires several sleigh-loads of hay, and while the feeder is reloading elk are already eating the previous load of hay. When the next load of hay is distributed, elk usually move to the newest hay within minutes, leading to high feeding densities at the beginning of each new feedline.

Low Density Feeding Procedures

Low density feeding was initiated on treatment sites at least three weeks (22-61 days) after elk arrived at the feedground giving them time to adjust to standard feeding practices. Low density feeding was implemented by supplying the same amount of hay per elk, but utilizing more of the feedground by distributing the hay in smaller piles and a more grid-like pattern. The start date of low density feeding was staggered beginning February 1st to allow consistent sampling of each site. At each of the seven sites, I collected fecal samples three times during standard feeding and at least three times after low density feeding was initiated. Fecal samples were collected on days 3, 10 and 30 after the initiation of LD feeding from each treatment site. Control sites were sampled at similar staggered intervals. After low density feeding began at a site, it was to continue for the duration of the feeding season. However, the implementation of low density feeding was a logistically more challenging task than anticipated due to deep snow and lack of feeder cooperation. Deep snow at one site inhibited the feeder from cutting new feedline tracks because the horses could not plow through the snow. One feeder did not agree that low density feeding may be useful and refused to implement low density feeding on a regular basis because it required more time. As a result, low density feeding was carried out intermittently rather than continuously at two feedgrounds. At these

sites, an effort was made to ensure that low density feeding was continuously implemented for several days prior to sample collection and during observations. At the site with deep snow, low density feeding was implemented when the snow packed and the feeder was able to plow new feedlines. At the other site a WFGD biologist went to the feedground for the 3-4 days prior sample collection and enforced LD feeding procedures each of those days. I scheduled my observations such that I would observe on the 3rd or 4th day of LD feeding, and after observations collect fecal samples.

Environmental Covariates

During each sampling occasion I also collected information on group size, snow depth, and feedline length (Table 2). I defined group size as a count of all elk on the feedground. Feeders regularly count the number of elk on the feedground to determine how much feed to supply, so I used the most current count done by the feeders for the measure of group size. I determined snow depth by digging through the snow until I reached solid ground, then I measured distance from the top of the snowpack to the ground. I measured feedline length during feeding using a GPS and recorded the length to the nearest 100 meters. The mean feedline length was 2.2 km (SE±0.25km) and ranged from 0.6 km to 6.4 km.

Behavioral Observations

I used focal subgroup sampling (Altmann 1974) to conduct observations of aggressive behaviors on the feedgrounds. I conducted observations on each feedground once during standard feeding and once after low density feeding was initiated. At two

feedgrounds (Bench Corral and Corral Dog Creek) I conducted observations three times during standard feeding and two (Bench Corral) or three (Dog Creek) times after low density was initiated to get determine daily variability in aggression rates.

I used a Nikon field scope to observe elk at distances of 49 to 615 (mean=254) meters. During observations I was positioned either in the haystack on the feedground or on a hill at least 150 meters away so that elk were not disturbed by my presence. I began my observation session when feeding began because that was when interactions between elk were most likely to occur. Each observation session consisted of ten, 10-minute observation periods and ended 2.5-3 hours after the start of feeding. I selected a new focal subgroup for each 10-minute period. A focal subgroup consisted of the individuals on the feedline that fell within the field of view of the scope. I selected a focal subgroup based on the criteria that observations were always on the area of the feedline where hay had most recently been laid, and there were at least three elk in the field of view at the beginning of the observation. I recorded the number of elk in the focal subgroup at the beginning and end of the observation period and calculated the mean number of elk per observation (mean=17.18, max=76, min=0). I used cow elk body length to estimate the length of the feedline within the field of view during observations; this measure was converted to meters using the conversion of one elk body length equals 2.5 meters (WGFD personal communication).

I used a hand-held digital recorder that automatically logged the time of the observation to record aggressive behaviors. The aggressive behaviors I recorded included: rear and flail, spar, head-up grimace, bite, kick, charge, chase and displacement

(Weckerly 2001). I used only the most overt aggressive behaviors: rear and flail (standing on hind legs and 'boxing'), spar (males interlock antlers) and head up grimace (nose up, ears back), in analysis because these events could be recorded reliably even while observing several animals. I also recorded the age/sex category (adult male, adult female, calf) of the initiator and recipient of aggressive interactions. I report aggression rates as the number of aggressive interactions per individual per hour.

Density Covariates

Density may be biologically important to elk at different scales, so I examined three potentially important measures of density; local density, feedline density and feedground density. I measured local feeding density during observations and therefore I only collected it in 2008. Local density is defined as the average number of elk in the focal subgroup divided by the observed length of feedline (number of elk/km) within view. This is a measure of density that captures very localized changes in density as feeding progresses. The second measure of density I used is feedline density. Feedline density is the number of elk on the feedground divided by the length of the feedline which was measured using a GPS (elk per kilometer of feedline). The last measure of density I used was feedground density which reflects the general crowding on feedground. Feedground density is the number of elk on the feedground divided by the feeding area (number of elk/km^2). Feedground area is based on polygons delineated in GIS by WGFD as the feeding area on the feedground (WGFD unpublished data). To account for the high densities due to the behavioral response to feeding I included the number of minutes since the most recent load of hay as a covariate in the analysis.

Table 2. Description of covariates used in analysis of stress hormone concentrations for the 2008 experimental manipulation of feed density.

Covariate Name	**Description**
Location	Feedground name (7 feedground locations)
Feedline Density	Group Size / Total length of the feedline measured by GPS (elk/km)
Feedground Density	Group Size / Area delineated as feeding area (elk/ km^2)
Snow Depth	Depth of snow adjacent to feedground (cm)
Cumulative Days	Number of days elk have been fed prior to the sampling date
Date	Number of days since January 1st
Group Size	Number of elk on the feedground
Aggression Rate	Mean aggression rate for a Location (interactions/elk/hour)

Table 3. Description of covariates used in analysis of aggression rates for the 2008 experimental manipulation of feed density study.

Covariate Name	**Description**
Location	Feedground name (7 feedground locations)
Local Density	Density at which elk were observed to be at feeding (#elk/km)
Load Minutes	Minutes since the most recent load of hay was distributed
Minutes	Minutes since feeding began
Feeding Type	Standard or Low Density feeding protocol (Std or LD)

Sample Collection, Glucocorticoid Extraction and Enzyme Immunoassay

On each sampling occasion, I collected 10-20 random fecal samples from unique individuals off of the ground. Each sample was consisted of three to sixteen fecal pellets from a single, unknown individual. I determined that pellets were from a single individual based on distance between samples, size of pellets and temperature of pellets. If I suspected a pile of feces to contain pellets from more than one individual I did not collect it. To minimize the effects of time and environmental variables on the degradation of hormones only fresh fecal samples, those not yet frozen, were collected. With the temperatures typical of winter on the feedgrounds, these criteria were likely to

provide samples less than a few hours old. Fecal samples were stored in 50 ml polypropylene centrifuge tubes and frozen in conventional freezer until they were transferred to a -20° C freezer where they remained until extraction. In 2007, I sampled feces from 301 individuals at 21 feedground and non-feedground sites. In 2008 I collected nearly 650 fecal samples from the seven feedgrounds included in the experiment.

Fecal samples were dried and steroids were extracted using the procedure outlined in Creel et al. (2002). Briefly, samples were mixed using a modified bit with a low-speed power drill, and then 1-2 grams of each sample were weighed (to 0.001 g with a Mettler analytical balance), dried using a rotary evaporator and weighed again. Next, 0.2 grams of the dried sample was boiled in ethanol for 20 minutes then this solution was centrifuged for 15 minutes. The supernatant was transferred into a new test tube and dried under air. The remaining residue was then rinsed with ethanol and dried again. Samples were reconstituted with 1 mL of methanol, extracts were stored at -20° C until assayed (Creel et al. 2002).

The concentration of glucocorticoids in each extract was determined using a cortisol EIA kit from Assay Designs. Extracts were diluted with assay buffer to produce extract dilutions of 1:31, which yielded binding at the steepest portion of the standard curve. I assayed all samples in duplicate and if the intra-assay coefficient of variation (CV) for a sample was over 11% I re-ran the sample up to two times.

All samples included in analysis for project one had a CV under 10.95% with a mean CV of 5.19% (±0.029 SE). In 2007 there were 53 samples from non-feedground

sites and 171 samples from feedground sites. For the 2008 project, 578 samples were included in the analysis. All samples in project two had a CV under 10.75% (mean CV =4.42%±0.12 SE). The concentration of fGCs is reported in ng GC/g dried feces.

Statistical Analysis

Fecal Glucocorticoids of Feedground vs. Non-feedgrounds Analysis

I performed all statistical analysis using R 2.8.0 (R Core Development Team 2008). I collected fecal samples randomly off the ground from unknown individuals. Therefore, I collapsed the fGC concentrations into a mean value for each sampling occasion resulting in 21 observations (16 feedground, 5 non-feedground sites). I used the mean fGC concentration from each site to pool the data from 2007 into feedground (n=16) and non-feedground (n=5) groups and I used Welch's t-test to compare the fGC concentrations between the two groups. I also used the fGC data available from all sites (n=30) and years (2003-2008) to examine how fGC concentrations differed between feedground and non-feedground sites. For this analysis I used the mean fGC concentration for each site-year combination as an observation (e.g. Madison-Firehole 2003). Again, I used a Welch's t-test to compare the fGC concentrations between feedground (n=31) and non-feedground elk (n=16).

I used multiple linear regression to determine the association between fGC concentration and snow depth, group size, feedground density, and management activity at feedground sites. The NER was excluded from this analysis because it is very different then the other feedgrounds (ie. the group size is 6 times larger than any other feedground

and fed alfalfa pellets instead of hay). The assumptions of constant variance, linearity and normally distributed residuals were met.

2008 Experimental Manipulation of Feeding Analysis

To examine whether switching from standard feeding to low density feeding had an effect on fGC concentration, I pooled the data for each site into before and after groups. I took the difference between the before and after measurements, then compared the differences across control and treatment sites using Welch's t-test (Welch 1947, Stewart-Oaten et al. 1986) which adjusts for different variances and sample sizes in the compared groups.

Stress Covariates I used linear mixed models to examine the association between fGC concentration and date, cumulative days of feeding, feedground density, and group size (n=47, Table 2). I included location as a random effect. I did not include cumulative days of feeding and date in the same models because they were highly correlated (r=0.87). For the same reason I did not include feedground density and group size (r=0.77) in the same models. I compared these models using AIC_c. There was no evidence of non-linearity, and the residuals appeared normally distributed.

I had substantial missing data for two covariates, snow depth and feedline density, so I developed two additional model suites and within these suites used a subset of the data (n=18 for snow depth, n=37 for feedline density) to examine these covariates. These two model suites contained the top ranked models from the full data set model

suite, and additional models that included the covariate of interest (snow depth or feedline density). I compared the models within these two suites using AIC_c.

I predicted a positive correlation between fGC concentration and the two measures of density, feedground density and feedline density, because studies in other animal systems have found increases in fGC concentrations with density (Li et al. 2007). I also expected a positive correlation between fGC concentration and group size. I included cumulative days of feeding to account for the amount of time the elk had been on the feedground. I had two alternative hypotheses for how this variable might be related to fGC concentration. A positive relationship between fGC concentration and cumulative days would indicate that the stress response increased as elk spent more time on the feedgrounds. Alternatively, if elk became habituated to the conditions on the feedground throughout the season I would expect to see fGC concentrations fall throughout the feeding season. I expected a positive correlation between fGC concentration and snow depth because it has been shown to be an important predictor of fGCs in other parts of the GYE (Creel et al. 2002).

I was also interested in how aggression rate and local density were associated with fGC concentration. These covariates were collected during the behavioral observations and were not necessarily collected at the same time (or day before) as fecal samples. Observational data were only collected on two occasions, once before and once after low density feeding was initiated at most feedgrounds. Low density feeding did not have an effect on local density or aggression rate (see results). Therefore, I pooled the

data for these covariates and the fGC concentration for each site to investigate any relationships.

Aggression Rates I developed linear regression models for the response variable aggression rate to investigate how the covariates location, load minutes, local density, feeding type, and minutes since feeding began were associated with aggression rate (Table 3). I predicted that aggression rate would increase as local density increased because high densities would drive competition for the feed. The goal of low density feeding was to reduce elk density during feeding, and I predicted that aggression rates would be lower during low density feeding. I predicted a negative correlation between aggression rate and minutes since feeding began because competition for food is likely to be the highest immediately after feeding begins, and subside as more feed is distributed and as elk become satiated. During my observations of elk on the feedgrounds, I noted that elk usually moved to the newest load of hay shortly (within minutes) after it was distributed leading to high densities at the beginning of each load. Therefore, I predicted that aggression rate would be inversely related to load minutes. I compared models using AIC_c (Burnham and Andersen 2002).

I examined whether there was an association between fGCs and aggression rate. Data on aggression rates were collected during the behavioral observations and were not necessarily collected on the same day as fecal samples. I pooled the data by site for fGC concentration and aggression rate to investigate any relationships.

RESULTS

Feedground and Non-Feedground Elk Stress Hormone Concentrations

As expected, analysis of the 2007 cross sectional data revealed a large amount of variation in fGC concentrations within and among sites (Figure 4). Individual fGC levels ranged over an order of magnitude, from 28 to 366 ng/g of dried feces. There was a statistically significant difference in the mean fGC concentration of feedground elk and non-feedground elk ($t_{8.585}$=2.303, p=0.048). Feedground elk had fGC concentrations 43% higher than non-feedground elk (123.6 ±9.4 SE, compared to 86.4±13.1 SE, Figure 5).

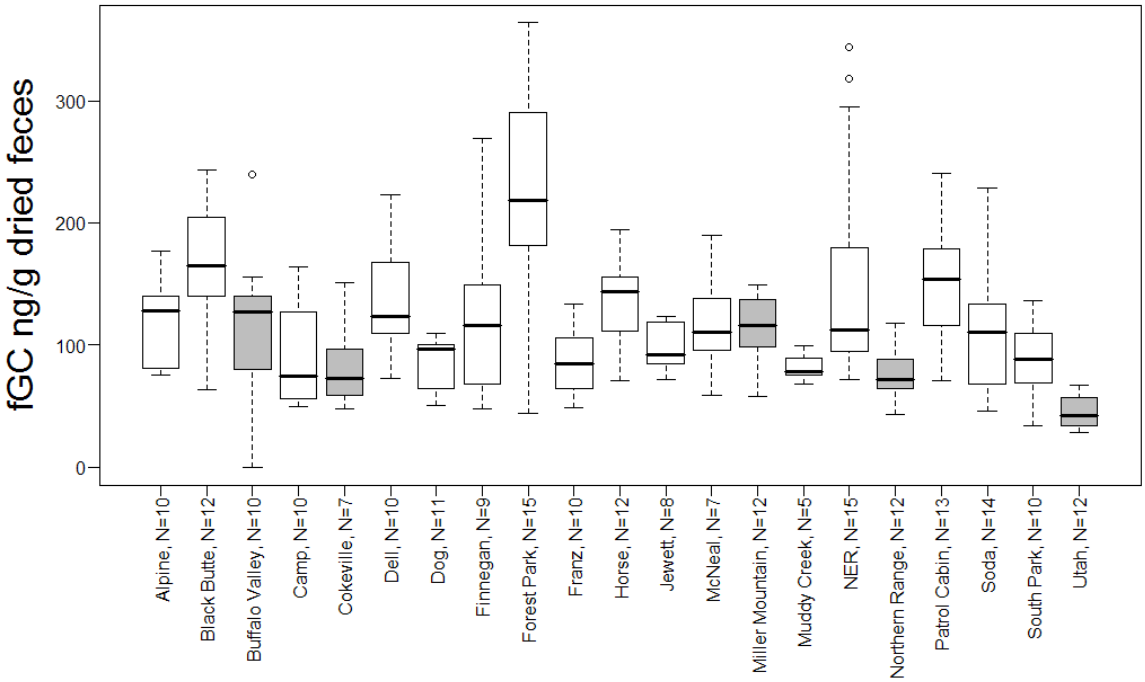

Figure 4. Boxplots of fecal glucocorticoids (fGC) concentrations at non-feedground (gray boxes) and feedground sites (white boxes). The dark line shows the median fGC concentration. The bottom and top of the box are the 25th and 75th percentiles of the data. The whiskers show the 1.5 times the interquartile range. Points indicate extreme outliers.

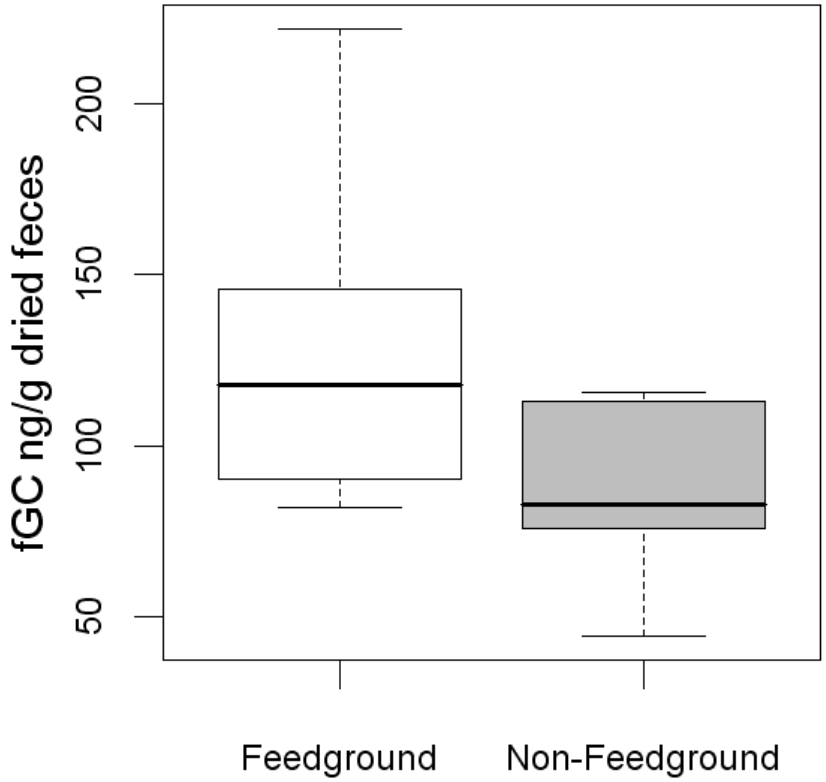

Figure 5. Fecal glucocorticoid (fGC) hormone concentrations averaged across feedground and non-feedground populations in 2007. The dark line shows the median fGC concentration. The bottom and top of the box are the 25th and 75th percentiles. The whiskers show the 1.5 times the interquartile range. Points indicate extreme outliers.

The analysis of all the site-year combinations of fGC concentration revealed similar results (Figure 6). Feedground elk had fGC concentrations that were significantly higher (Welch's $t_{27.23}$=2.39, p=0.024) than non-feedground elk. Feedground fGC concentrations were 31% higher than non-feedground fGC concentrations (125.39±6.60 SE, compared to 95.95 ±10.43 SE).

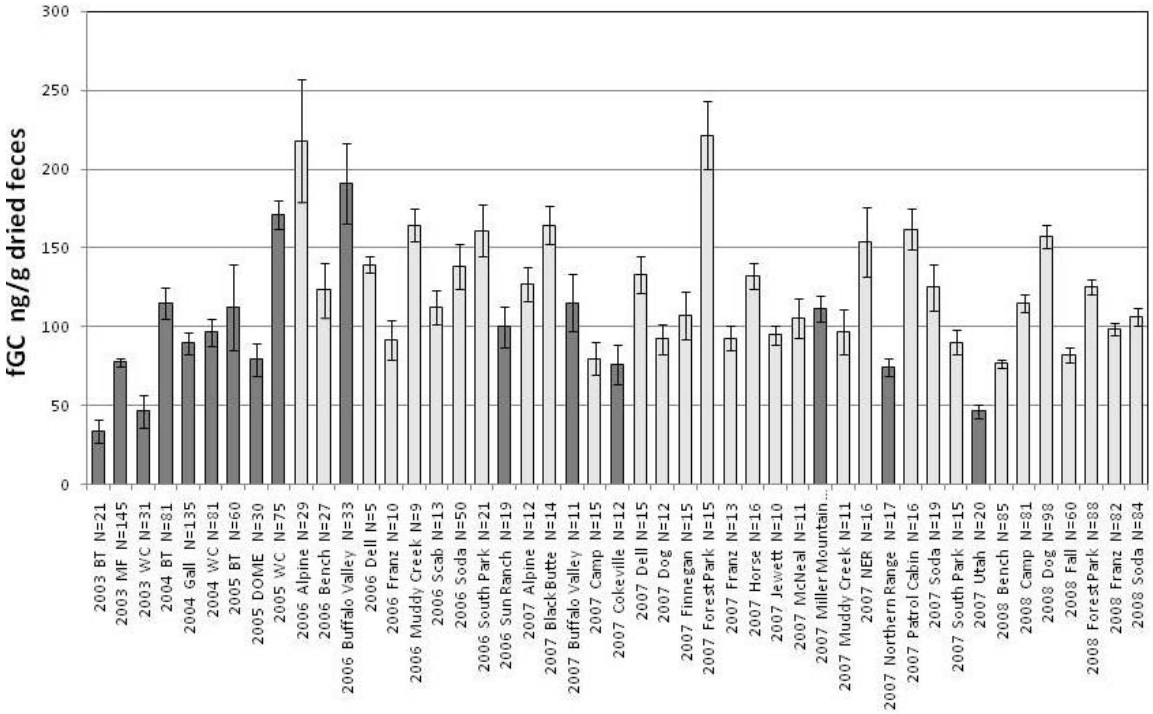

Figure 6. Mean fecal glucocorticoid concentration by year and location. Non-feedground sites are in dark gray, feedground sites are in light gray. Error bars show one standard error.

There was no evidence to suggest that group size, feedground density, or management activities were associated with fGC concentration in 2007 (Table 3). There was evidence suggestive of a positive relationship between fGC concentration and snow depth, but when I removed data for Forest Park, an outlier in snow depth, this trend was no longer apparent.

Table 4. Parameter estimates for a full model of the mean fGC concentration for elk feedground sites in 2007.

Parameter	Estimate	SE	P-value
Intercept	28.98	40.05	0.4877
Trapping Activity (Yes)	28.58	28.88	0.3482
Vaccination Before (Yes)	46.39	29.05	0.1447
Snow Depth	1.34	0.60	0.0515
Group Size	0.0052	0.03	0.8583
Feedground Density	-0.0004	0.004	0.9198

Low Density Feeding Treatment

The feedline density at treatment sites was lower during low density feeding (mean=309 elk/km) than during standard feeding procedures (mean=386 elk/km), but this was not a statistically significant reduction in density (Welch's $t_{3.54}$=0.71, p=0.52). Similar non-significant results were found for the comparison of local density during standard feeding (815 elk/km ± 92 SE) and low density feeding (627 elk/km ± 97 SE) at treatment site (Welch's $t_{3.635}$=1.21, p=0.30). There was not a significant change in fGC concentrations when treatment sites were switched from standard feeding to low density feeding ($t_{2.343}$ =-1.416, p=0.275, Figure 7).

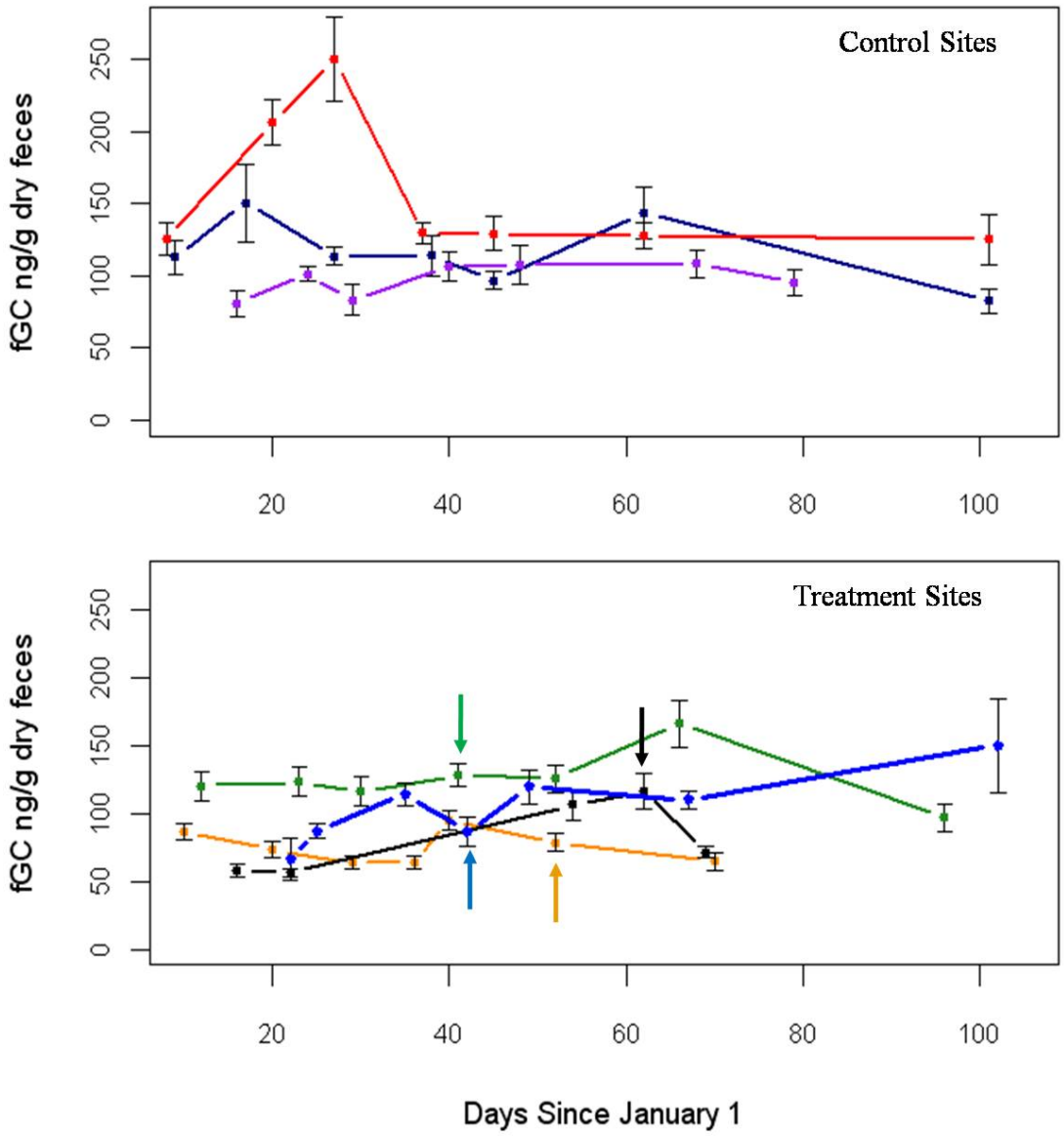

Figure 7. Fecal glucocorticoid concentration (fGC) throughout the season at control sites (top panel) and treatment sites (bottom panel). Each line represents a different site (Dark Blue=Camp Creek, Red=Dog Creek, Purple=Franz, Orange=Bench Corral, Green=Forest Park, Black=Fall Creek, Blue=Soda Lake). Points represent the mean of samples collected on that occasion. The first sample taken after low density feeding is shown by arrow.

Stress Covariates

Mixed models that included covariates thought to be important predictors of fGC concentration were compared using AIC_c (Table 5). The top ranked model was an intercept only model with site as a random effect. The parameter estimate for the intercept from this model was 108.67 ± 10.42 SE (t_{40}=10.43, p<0.0001). Based on the model rankings there is no evidence that date, cumulative days of feeding , group size or feedground density were significantly associated with fGC concentration when location was accounted for as a random effect.

Table 5. Mixed models developed investigate the relationship between fGC concentration and covariates, location was included as a random effect in all models. AIC_c was used to rank models, k is the number of parameters in each model.

Model Parameters	k	AIC_c	ΔAIC_c
Intercept	3	458.06	0.00
Date	4	462.24	4.18
Cumulative Days	4	462.27	4.21
Group Size	4	463.70	5.64
Feedground Density	4	467.73	9.67
Group Size + Date	5	467.88	9.82
Group Size + Cumulative Days	5	467.94	9.88
Feedground Density + Cumulative Days	5	471.97	13.91
Feedground Density + Date	5	472.00	13.93
Intercept only (no random effect)	2	474.85	16.79

In the snow depth model suite the intercept only model again was the top ranked model (Table 6), and models that included snow depth were greater than 2 AIC_c units from the top model. In the feedline density suite, the top model rankings remained the

same as in the original model suite, and all models that included feedline density received little support (Table 7).

Table 6. AIC_c model rankings for a subset of data for which snow depth was known (n=18). Models contain a random effect for location.

Fixed Effects	k	AIC_c	ΔAIC_c
Intercept	3	158.81	0.00
Snow	4	162.66	3.85
Date	4	163.82	5.01
Cumulative Days	4	163.89	5.08
Date + Snow	5	168.09	9.28
Cumulative Days + Snow	5	168.16	168.16
Group Size	4	168.26	168.26
Group Size+ Snow Depth	5	172.05	172.05

Table 7. AIC_c model rankings for a subset of data for which feedline density was known (n=37). Models contain a random effect for location.

Fixed Effects	k	AIC_c	ΔAIC_c
Intercept	3	290.07	0.00
Date	4	292.57	3.98
Cumulative Days	4	292.62	4.03
Group Size	4	292.89	4.30
Feedline Density	4	296.37	7.78
Cumulative Days + Feedline Density	5	299.70	11.94
Feedline Density + Date	5	299.73	11.97
Feedline Density + Group Size	5	300.31	12.55

The covariates that I thought would be important predictors of stress hormone level were not supported in the mixed models as seen by the preceding AIC tables. This lack of an association is likely because the covariates, particularly group size, are confounded with location. For this reason I pooled data for location means for fGC concentration and explored correlations with the group size and density covariates. There

was a strong positive correlation between seasonal mean fGC concentration and group size (r=0.76, Figure 8a). I also found a strong positive correlation between seasonal mean fGC concentration and mean local density (r=0.91, Figure 8b). This analysis suggests that the location effect in the mixed models is a density or group size effect.

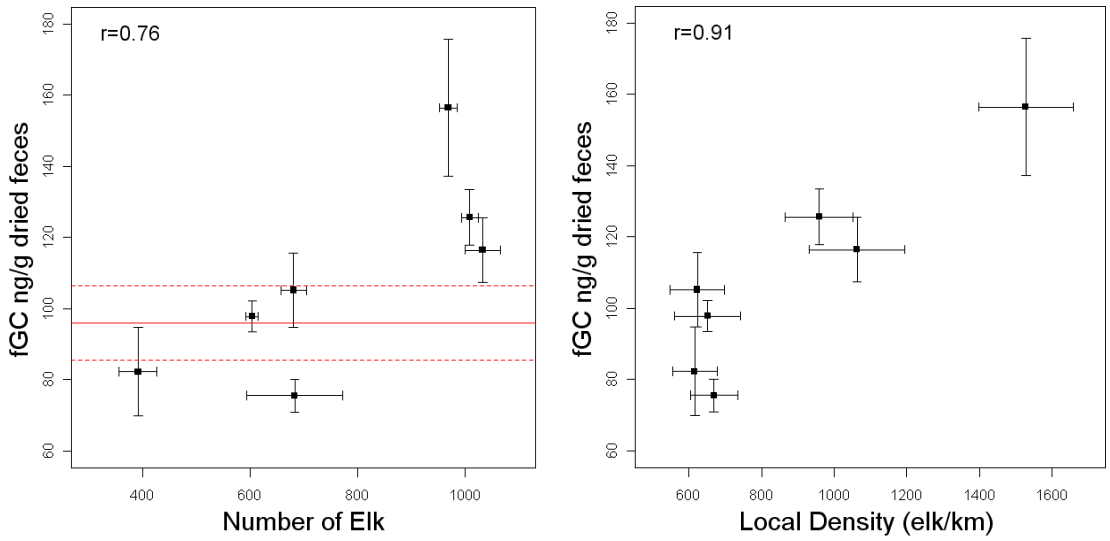

Figure 8. Fecal glucocorticoids concentration as a function of group size (a). Solid red line shows the mean fGC concentration of non-feedground elk, dotted lines show one standard error. (b) Fecal glucocorticoid concentrations as a function of local density. Points are feedground means ± SE.

Aggression Rate

Aggression rate was significantly higher during feeding (1.212 interactions/elk/hour) than in the hours prior to feeding (0.170 interactions/elk/hour, $t_{69.98}$=11.08, p<0.0001, Figure 9). Aggression rate was not significantly correlated with local density during feeding (p=0.61, t_{211}=0.0009). There was no detectable relationship between mean fGC and mean aggression rate (p=0.73, t_6=0.363, r^2=0.26, Figure 10).

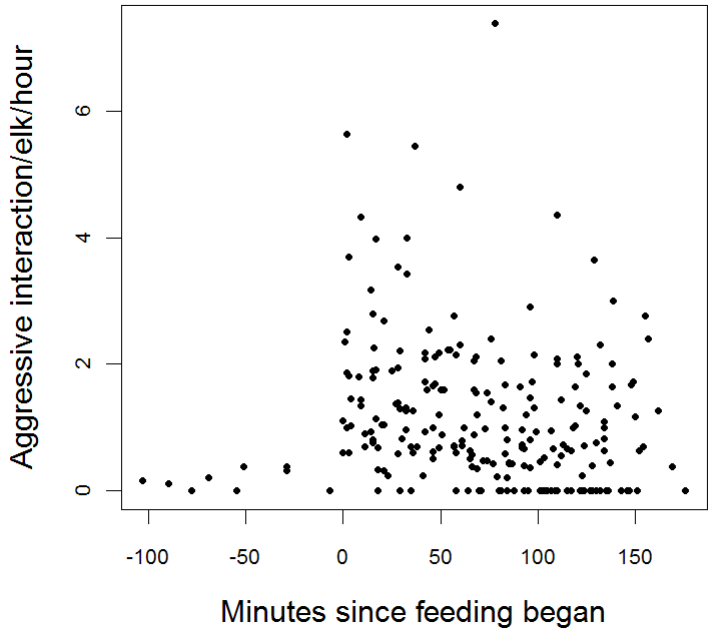

Figure 9. Aggression rates were lower during the hours prior to feeding. Each point represents one ten-minute observation.

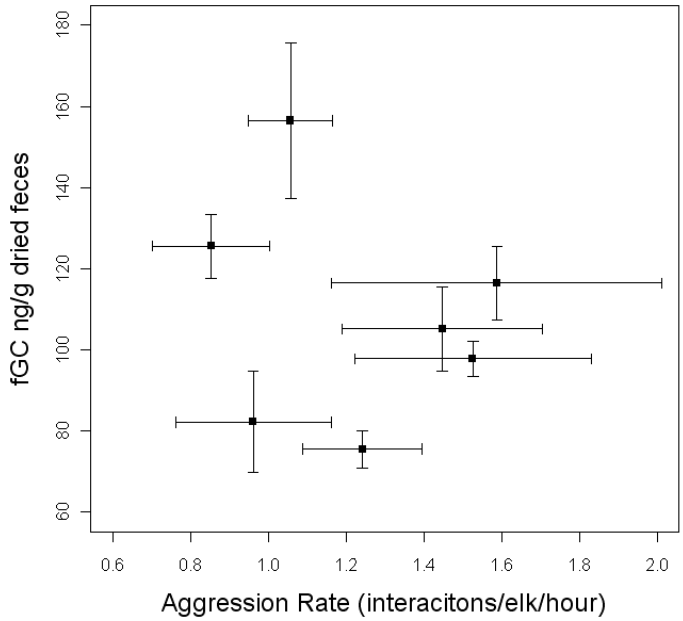

Figure 10. Mean fGC concentration as a function of aggression rate for each feedground. Data are location means ± SE.

Aggression rate models were compared using AIC_c (Table 8) and the parameters for the top ranked models are in Table 9. The top four models all contained the parameter load minutes. The parameter estimates for load minutes from the top ranked models indicate a negative correlation between aggression rate and load minutes, such that aggression rates are highest immediately after the hay is distributed and taper off through time. Models that did not contain load minutes received little support. Location also appears in the top four models indicating that some variation in aggression rates is due to feedground-specific attributes. The model that ranked second, which is within 2 ΔAIC_c units of the top model, had the additional predictor, local density. The parameter estimate (-0.1501 ± 0.12 SE) for local density indicates there is little evidence (t_{203}=-1.27, p=0.2039) to support an association with fGC concentration.

Table 8. Linear regression models developed to predict aggression rates during feeding. AIC_c was used in model selection, k is the number of parameters in each model.

Model Parameters	k	AIC_c	Δ AIC_c
Location+Load Minutes	9	638.23	0.00
Location+Load Minutes+Local Density	10	638.75	0.51
Location+Load Minutes+Feeding Type	10	640.41	2.18
Location+Load Minutes+Feeding Type+Local Density	11	640.97	2.74
Minutes	3	642.00	3.77
Load Minutes+Local Density	4	642.38	4.15
Location+Load Minutes+Feeding Type +Local Density+Minutes	12	643.22	4.99
Load Minutes	3	644.87	6.64
Load Minutes+Feeding Type	4	646.54	8.31
Location+Load Minutes+Location*Load Minutes	15	647.06	8.82
Location+Minutes	9	647.20	8.97
Local Density	3	656.06	17.83
Feeding Type	3	656.32	18.09
Location	8	658.13	19.90
Location+Feeding Type	9	659.98	21.75
Location+Local Density	9	660.31	22.08

Table 9. Parameter estimates for the top Aggression rate models selected by AIC_c.

Model 1: Aggression Rate ~ Location + Load Minutes

Parameter	Estimate	SE	P-value
Intercept	2.0512	0.2222	<0.0001
Location Camp	0.0643	0.2884	0.8239
Location Dog	-0.7780	0.2339	0.0010
Location Fall	-0.0431	0.2924	0.8828
Location Forest	-0.8665	0.2997	0.0043
Location Franz	-0.0412	0.2853	0.8852
Location Soda	-0.3145	0.2979	0.2923
Load Minutes	-0.0109	0.0023	<0.0001

DISCUSSION

Theoretical and empirical studies have explored the relationship between density, contacts and disease transmission (for example: (Arneberg et al. 1998, Arneberg 2001, Wright and Gompper 2005), however, few studies have examined how stress might act as an alternative mechanism by which density or group size affects disease. A common tenet of disease ecology is that there is a positive correlation between host density and contact rate, which, in the case of density-dependent transmission, leads to an increase in pathogen transmission (McCallum and Dobson 2002). Aside from an increase in contacts, an increase in group size or density might lead to increased competition over resources, and this competition may induce a stress response. Chronically elevated stress hormone levels may affect disease transmission by weakening immune function. The results of this study suggest that the aggregation of a large number of animals may increase disease prevalence not only by increasing contacts, but also by increasing stress hormone levels which may increase either susceptibility or infectiousness.

I found that the fGC concentrations of elk on feedgrounds were 31% higher than fGC concentrations of elk on native winter range. I also found a strong correlation between group size, local density and fGC concentration. The group sizes and densities of elk on feedgrounds were significantly higher than those found on elk native winter range. These unnatural conditions on the feedgrounds persist throughout the feeding season, and as my results indicate, these conditions may induce a stress response in elk.

Feedgrounds and the Stress Response

Studies have found varied results regarding the direction of the correlation between food availability or quality and stress hormone concentrations. For example, stress hormone levels were inversely correlated with food availability in a captive herd of mule deer (Saltz and White 1991) and in song sparrows (Clinchy et al. 2004). Conversely, Taillon and Cote (2008) found that stress hormone concentrations in white tailed deer were positively correlated with higher quality feed. The authors suggested that when feed quality was low there was a decreased physiological response to stressors to reduce energy expenditure (Taillon and Cote 2008). Whether the high fGC concentrations in feedground elk is a result of increased nutritional intake enabling a greater physiological response, or the direct result of conditions on the feedgrounds is unknown.

Previous research has demonstrated the wide ranging and detrimental effects of a chronic stress response on survival, reproduction and immune function (reviewed in Sapolsky 2002). The low winter mortality rates on the feedgrounds (Smith 2001) suggest that survival is not impacted by the high fGC concentrations. It is possible that the positive effects of feeding offset any detrimental effects that the high fGCs may have on survival. The impact of stress on disease dynamics could include increased susceptibility to disease through a suppressed immune system, increased intensity of disease once infected, or an increase in transmission if high GCs increase the likelihood of an abortion. The link between fGCs, immune function and the effect on disease has

not yet been investigated in feedground elk and future studies should examine this question.

Group Size and Density

In 2008 I found a significant correlation between the mean local density and the mean fGC concentration at each site. This finding indicates that local density is the scale at which density is affecting elk fGC levels. I also found a significant positive correlation between group size and fGC concentration. The findings of this analysis suggest that increases in group size or local density may not only affect disease transmission by increasing contacts, but also by affecting stress hormone levels, which in turn may affect immune function.

When I tested for effects of group size and density using mixed effects models with location as a random effect, I did not find any evidence of an association between these covariates and fGC concentration. Location itself was the single most important predictor of fGC concentration (Table 5). Group size varied little within a site during the season, so the group size and location effects are confounded. Therefore, it is possible that the location effect may be primarily driven by the group size differences among sites (Figure 8).

The importance of location in the mixed effect models indicates that there are location-specific attributes that cause the variation in fGC concentration, these attributes may include the covariates I measured but may also include attributes of the feedground that did not include in my analysis. For example, off-feedground factors such as nearby roads, snowmobile trails or activities on surrounding private property may induce a stress

response. Other characteristics, such as topography of the landscape, wolf presence, and elk movements on and around the feedground may also be important. Including these factors in future studies may further elucidate what drives the variation in stress hormone levels among sites.

Low Density Feeding and the Stress Response

The experimental manipulation of feed density did not have a detectable effect on stress hormone levels. The low density treatment did not significantly reduce elk densities within a feedground and created too little variation in density to detect a signal in the stress response. However, when local densities were compared across feedgrounds there was a much wider range of densities sampled. It was across this wide range of densities that the association between stress hormone level and local density was strong.

The lack of an association between the fGC concentration and the treatment (LD feeding) may be because the impact of low density feeding on fGC concentrations was minimal compared to the impact of other stressors associated with the feedgrounds. Control and treatment feedgrounds were not randomly assigned and although there was not a significant difference, local density, feedline density and group size were all lower at the treatment (LD) sites at the beginning of the experiment. Furthermore, stress hormone concentrations at these sites were lower, and approached the fGC concentration of non-feedground elk. If the fGC concentration observed in non-feedground elk represents a baseline fGC concentration for elk in the winter, then management changes at feedgrounds that already have low fGC concentrations may not have an effect on stress

hormone levels. Finally, on two feedgrounds low density feeding was not continuously implemented which may have reduced the effectiveness of the low density treatment.

Aggression Rates and Stress Response

Aggression rates observed during feeding were at least ten times higher than those observed in elk on native winter range (Creel, personal communication) and more than seven times higher during feeding than in the hours prior to feeding. Feedground elk may be able to expend more energy on aggressive behaviors than non-feedground elk because they have consistent access to resources (Taillon and Cote 2008). Even while elk are not being fed they are often clustered on the feedgrounds at very high densities. Despite the high density on feedgrounds while not being fed, the highest rates of aggressive behaviors were observed during feeding when there was competition for a food resource. The implementation of low density feeding did not have a detectable effect on aggression rate. This is likely because low density feeding did not necessarily spread out the elk due to elk quickly moving to the new hay as each load is distributed. This behavior kept competitive interactions among elk high even when low density feeding was in effect.

There was no detectable relationship between aggression rate and stress hormone level. Both aggression rates and stress hormone concentrations are likely to vary widely for an individual on a daily basis and it may be hard to link aggression and fGCs given the data collected. Other factors that were not accounted for in this study that may be influencing stress hormone levels such as sex, dominance hierarchy, and less overt social interactions (reviewed in Keay et al. 2006, Lane 2006), which were assumed to be randomly distributed among sites and sampling events. It is also possible that the

aggressive interactions are not necessary to induce the stress response, but that it is the general crowding on the feedgrounds is what drives the stress response.

Regardless as to the cause of the high stress hormone levels seen in supplementally fed elk, the feedgrounds are creating an epidemiological setting for disease transmission and a physiological state that may increase susceptibility to disease. The impact of these stress hormone concentrations on disease susceptibility of elk remains unknown, but may be an important driver of disease dynamics in feedground elk populations.

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