SHORT COMMUNICATION

Hair Cortisol Concentration And Body Weight In Moose (*Alces alces*) Infested With Deer Keds (*Lipoptena cervi)*

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ABSTRACT

The deer ked (*Lipoptena cervi*), a hematophagous ectoparasite of cervids, is currently spreading in Scandinavia and the moose (*Alces alces*) is the main host. However, the impact of deer keds on moose is poorly elucidated. Therefore, hair cortisol concentration (HCC) from 262 harvested moose was analyzed in relation to age class, gender, body weight (BW) and deer ked intensity. BW was analyzed in relation to age class, gender and infestation intensity. HCC decreased with increasing deer ked intensity at low ked intensities and increased at higher ked intensities. HCC was greater in males than females and lower in yearlings compared to calves and adults. Failure to find any association between BW and deer ked intensity can indicate a negligible impact of deer ked infestation on moose foraging and metabolism at the level of infestation observed early in the infestation, but does not exclude an effect later in winter. Our findings suggest that moose generally tolerate parasitism by keds at low and moderate intensities, at least during the fall period. However, the increase in HCC at higher ked intensities suggests that the tolerance strategy might possibly be disrupted with further increases in intensities beyond the current observation range and affect animal health and welfare negatively. Examination of HCC later in the development of the keds in moose is warranted and may provide additional insights.

Keywords: Body weight, chronic stress, deer ked, hair cortisol, moose, parasitism, welfare

The deer ked (*Lipoptena cervi*) is a hematophagous louse fly (Hipposcidae) of wild cervids, and are found on moose (*Alces alces*), red deer (*Cervus elaphus*) and other species of deer in Europe, Asia and North-America (Bequaert 1942). In many areas, the swarming keds constitute a major obstacle for human outdoor activities, but the parasite has been regarded as harmless for cervids (Allan 2001; Paakkonen et al. 2012). However, Madslien et al. (2011) reported an outbreak of severe alopecia in moose associated with massive infestation of deer keds in Norway and Kynkäänniemi et al. (2014) stated that reindeer (*Rangifer tarandus tarandus)* showed signs of distress and pruritus after infestation with keds.

Hair analysis is becoming increasingly popular as a method to examine stress and reproduction hormone levels in wild mammals (Koren et al. 2019). Glucocorticoids (GC) are regarded as stress hormones and have long been employed as physiological indices of the relative condition or health of individuals and populations (Bonier et al. 2009). However, the exact mechanism(s) of GC integration in hair are still unknown. Hair may accumulate GC hormones over weeks to months, and although HCC is thought to be insensitive to the impact of acute stress, some evidence suggests that capture method may influence HCC (Cattet et al. 2014). Koren et al. (2019) found correlation between hair and serum cortisol in samples from moose (*Alces alces*). Ewacha (2016) found higher hair cortisol concentrations in moose killed by wolves than in moose harvested by humans, and suggested that chronic stress in moose is linked to poor body condition and increased vulnerability to wolf predation.

We aimed 1) to evaluate hair cortisol concentration (HCC) in moose infested with deer keds and 2) to test whether there was a negative association between deer ked infestation and moose body weight (BW). Our hypothesis was that increasing deer ked infestation intensities should be associated with increased chronic stress evident as increased HCC and a decline in body weight.

Skin samples were collected from 262 moose; 36 calves (16 males/20 females), 92 yearlings (56 males/36 females) and 134 adults (67 males/67 females) in Hedmark and Akershus county, in south-eastern Norway, during the first week of the hunting season (October 5^{th} – 12^{th} 2010). Hunters recorded gender and age class (calf, yearling, adult) of the moose, measured the carcass weight (~50% of full body weight (Wallin et al. 1996) and collected a standardized skin sample from the neck area (see (Madslien et al. 2012) for details).

In the laboratory, guard hairs for HCC analysis were collected by shaving a skin area of 2 x 2 cm and stored in paper envelopes at room temperature until analysis. Finally, deer ked infestation intensity were calculated from all skin samples (Madslien et al. 2012). The cortisol analysis was performed at the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, Canada). Only guard hairs with the follicles removed were used to determine HCC. Surface contamination was removed by washing hairs with methanol (five 3 min washes). Between 100 and 200 mg of washed and dried hair were ground to a fine powder using a ball mill, and then weighed. Extraction of cortisol from hair and cortisol concentration (pg/mg) was determined as described by Macbeth et al. (2010).

Generalized additive modeling (GAM) with Gamma distribution and inverse link was run to analyze the effect of ked intensity on HCC. Ked intensity was ln-transformed to normalize and stabilize the variance. Age class, sex and body weight were then added to the baseline model (i.e., forward selection), using the small-sample-corrected Akaike Information Criterion (AICc). We used generalized linear regression to analyze the effect of age class, sex, and ked intensity on moose body weight. Statistical analyses were performed using the R statistical software (R version 2.14.1) and the GAM was run in the R package mgcv.

Average ked infestation intensity (average number of keds/cm^2) in skin samples and HCC varied among sex and age classes (Table 1). HCC first decreased at low ked intensity and increased at higher ked intensities (Figure 1a). Yearlings had lower HCC than adults ($p=0.001$)

and males had higher HCC than females (p= 0.001) (Table 2). When analyzing moose with deer ked intensities greater or equal to the mean, we found a slight increase in HCC with increasing ked intensities (Table 2b, Figure 2). Variation in body weight was best explained by age, class and gender (Figure 1b, Supplementary Table 1).

In studies evaluating the relationship between parasite load and GCs, varying results have been found (Carlsson et al. 2016). We found that males have higher HCC than females and that HCC was positively associated with body weights larger than ~ 150 kg (i.e. adults, Figure 1b). Similarly, Di Francesco et al. (2017) found significantly higher qiviut (hair) cortisol levels in muskoxen males than females. Body weight was found to be negatively associated with HCC for body weights less than ~125 kg (i.e. yearlings, Figure 1b). This is in accordance with Mislan et al. (2016) that found polar bears in poorer body condition to have higher levels of HCC.

According to the 'cort-fitness hypothesis', high baseline GC levels indicate an individual or population in poor condition and with low relative fitness (Bonier et al. 2009). However, the baseline or stress-induced GC levels in plasma from laboratory and wild animals were not found to change in a consistent manner in response to chronic stress DickensRomero (2013). Bonier et al. (2009) also found inconsistent relationships between plasma GCs and fitness. Pawluski et al. (2017) suggested that the reported variability of GC levels under chronic stress may be due to the species studied, type of stressor or welfare measure, duration of stress, and techniques used to analyze GC levels.

Most moose had low to moderate deer ked intensities, and only a few had high ked intensities. Paakkonen et al. (2012) found only minor effects of even high infestation levels of deer ked on a range of systemic physiological measures, including plasma cortisol concentrations. The slight increase in HCC at higher ked intensities may indicate that HCC provide a more reliable picture of chronic stress than plasma cortisol. HCC may remain less affected by GC secretion

in response to acute stress caused by hunting, as performed in both studies, compared to plasma cortisol.

Several studies have suggested that local cortisol production may contribute to glucocorticoid integration in hair, due to a parallel, but peripheral, stress axis within the hair follicles (Ito et al. 2005; Keckeis et al. 2012). This does not imply that steroids measured in hair reflect local production only, but that this process is likely influenced by both the central- and peripheral-HPA-axes (Cattet et al. 2014). Salaberger et al. (2016) found that mechanical irritation significantly increased HCC in sheep. Skin irritation caused by deer keds may have induced local GC production and/or metabolism in hair follicle cells regulated by locally-expressed HPA mediators, which could result in localized effects (Stubsjøen et al. 2015).

The lack of association between deer ked intensity and body weight suggests that keds may have minimal impact on moose foraging and metabolism early in the infestation period. However, we cannot rule out the possibility that deer keds may significantly impact moose during late winter when food is limited, the weather is harsh, and the parasites have exploited the host for a longer period of time.

Our results suggest that moderately intense deer ked infestations do not cause major chronic stress or loss of body weight during the early infestation period of the deer ked. However, the increase in HCC at higher ked intensities even during this early parasitic phase of the infestation may be indicative of an increasingly negative response later in the infestation, affecting animal welfare negatively.

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TABLES

Table 1. Summary statistics for deer ked intensity, hair cortisol concentration (HCC) and weight of moose infested with deer keds in south-eastern Norway.

	Ked intensity	*Range*	*SD*	**HCC**	*Range*	*SD*	**Weight**	*Range*	*SD*	**n**
Calf	0.26	0.052-0.700	0.17	2.04	0.17-9.90	1.96	72	42-97	13	36
Female	0.31	0.065-0.700	0.18	1.57	0.17-3.40	0.87	68	42-88	12	20
Male	0.21	0.052-0.441	0.12	2.63	0.35-9.90	2.71	76	60-97	13	16
Yearling	0.34	0.004-1.405	0.28	1.35	0.14-3.97	0.70	131	75-185	20	92
Female	0.33	0.004-1.321	0.27	1.24	0.14-3.16	0.61	126	92-160	16	36
Male	0.34	0.033-1.405	0.29	1.41	0.19-3.97	0.75	134	75-185	22	56
Adult	0.25	0.007-1.167	0.22	1.78	0.22-9.21	1.43	191	129-320	35	134
Female	0.22	0.024-0.598	0.16	1.48	0.23-8.23	1.06	177	129-271	25	67
Male	0.28	0.007-1.167	0.26	2.09	0.22-9.21	1.68	206	130-320	38	67
Total	0.28	0.004-1.405	0.24	1.66	0.14-9.90	1.34	154	42-320	51	262

Ked intensity = keds/cm²

HCC (Hair Cortisol Concentration) = pg/mg

Weight = kilogram (kg)

n = sample size

Table 2a, b. Hair cortisol concentration (HCC, pg/mg) as a function of deer ked intensity using gamma distribution with inverse link. Due to the inverse link of the gamma-distribution, a significant negative estimate refers to a positive association with HCC. □AICc refers to the change in the Akaike Information Criterion corrected for small sample sizes (AICc) when the corresponding variable is excluded from the model.

	Estimate	Std. Error	t value	Pr(>|t|)	□AICc
Intercept	0.734	0.0499	17.4	<0.001	
Age Calf	-0.089	0.051	-1.74	0.08	15.3
Age Yearling	0.178	0.053	3.33	0.001	
Gender male	-0.154	0.045	-3.43	0.001	10.6
factor(agePine < 75)	-0.232	0.045	-5.12	<0.001	21.0
	edf	Ref.df	F	p-value	
s(ln Intensity)	2.88	2.99	11.1	<0.001	26.2

s = spline function

edf = estimated degree of freedom for the spline function

Deer ked intensity is ln transformed, centered around mean, and scaled to variance 1.

The reference level (Intercept) is adult females at average ln deer ked intensity, from a location with age of pine forest > 75 years.

Table 2b. HCC as a function of deer ked intensity for individuals with higher or equal to mean intensity.

	Estimate	Std. Error	t value	Pr(>|t|)	□AICc
Intercept	0.682	0.035	19.5	<0.001	
ln Intensity	-0.097	0.029	-3.3	0.001	6.9

Deer ked intensity is ln transformed, centered around mean, and scaled to variance 1.

SUPPLEMENTARY MATERIAL

Supplementary Table 1 Body weight modelled as a spline of ln deer ked intensity using gamma-distribution with a log-link. When accounting for sex and age class, there was no significant association with body weight and deer ked intensity.

	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	5.145	0.032	161.6	<0.001
AgeCalf	-0.902	0.052	-17.3	<0.001
AgeYearling	-0.343	0.039	-8.8	<0.001
GenMale	0.125	0.036	3.5	<0.001
	edf	Ref.df	F	p-value
s(ln Intensity)	1	1.0	2.2	0.15

edf = estimated degree of freedom for the spline function
Deer ked intensity is ln transformed, centered around mean and scaled to variance 1.
The reference level (Intercept) is female adults at population average of ln deer ked intensity.

FIGURE LEGENDS

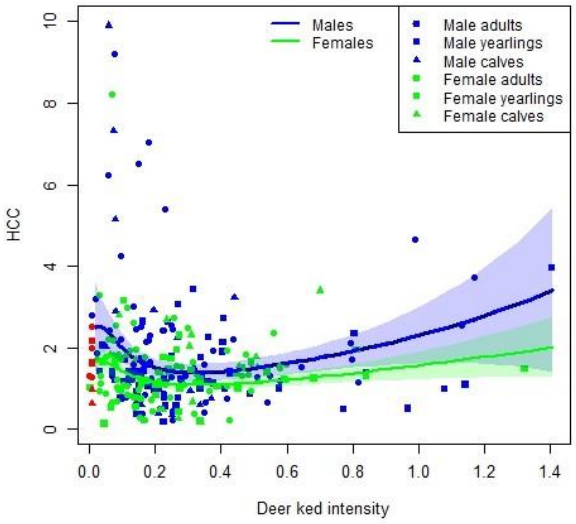

Figure 1a

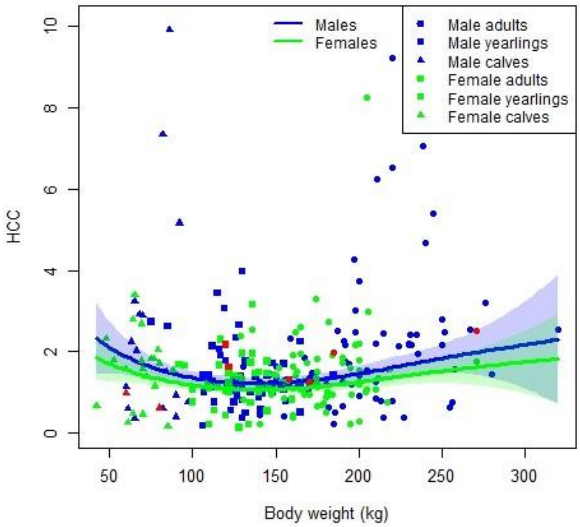

Figure 1b

Figure 1 Prediction of hair cortisol concentration (HCC, pg/mg) shown as a spline function of deer ked intensity (a) and body weight (b). Prediction lines together with 95% confidence envelopes are shown for males (blue) and females (green) at the level of (a) overall mean of deer ked intensity, and (b) mean body weight of adult females and adult males, respectively. Raw data of deer ked intensity (keds/cm^2) and corresponding cortisol concentration (HCC, pg/mg) are imposed as points of various symbols for the three age classes.

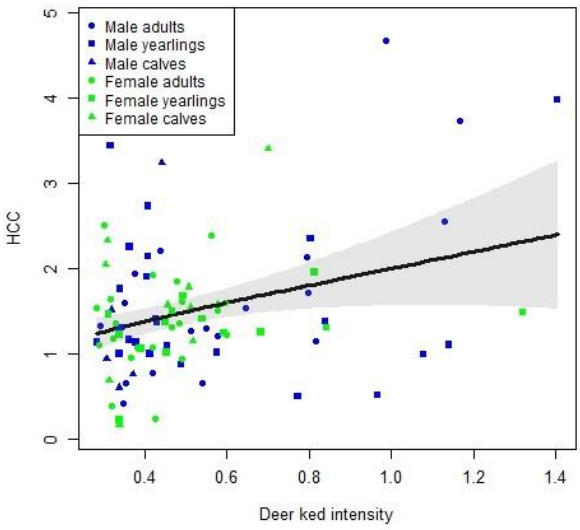

Figure 2 Prediction of HCC (pg/mg) shown as a function of deer ked intensity for individuals with above mean infestation (prediction lines together with 95% confidence envelopes).