

# Are faecal hormone levels linked to winter progression, diet quality and social rank in young ungulates ? An experiment with white-tailed deer (*Odocoileus virginianus*) fawns

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**Abstract** Hormones play a central role in the physiology and behaviour of animals. The recent development of noninvasive techniques has increased information on physical and social states of individuals through hormone measurements. The relationships among hormones, life history traits and behaviours are, however, still poorly known. For the first time, we evaluated natural winter glucocorticoid and testosterone levels in young ungulates in relation to winter progression, diet quality and social rank. Overwinter, levels of glucocorticoid and testosterone decreased, possibly due to the decline of fawns' body mass. The relationships between hormone levels and diet quality were surprising: Fawns fed the control diet presented higher glucocorticoid and lower testosterone levels than fawns fed the poor diet, suggesting that control fawns faced a higher nutritional stress than those on the poor diet. Similarly to other studies on social mammals, we found no relationship between faecal glucocorticoid levels and social rank, suggesting that social stress was similar for dominant and subordinate fawns during winter. Testosterone levels were not correlated to social rank as found previously in groups of individuals forming stable social hierarchies and maintaining stable dominance relationships. The simultaneous suppression of glucocorticoid and testosterone levels suggests for the first time that young ungulates present a

hormonal strategy to prevent fast depletion of limited proteins and fat resources during winter.

**Keywords** Diet quality · Glucocorticoids · Social rank · Testosterone · White-tailed deer

Hormones play a central role in the physiology and behaviour of individuals, influencing growth, body condition, reproductive status, social behaviour and survival (Boonstra 2005; Creel 2005). Monitoring hormonal levels can be a useful tool to obtain information on current physical and social states of organisms (Wikelski et al. 2004; Reeder and Kramer 2005). Nonetheless, assessing the relationships among hormones, life history traits and behaviours in wild animals is difficult, as captures and manipulations can greatly modify the behaviour and the basic hormonal levels of individuals (Monfort et al. 1997). Recently, scientists have developed noninvasive techniques that prevent animal disturbance and facilitate hormone measurements by using urine and faecal samples in free-ranging vertebrates (Millspaugh et al. 2001; Goymann et al. 2003). Faecal hormone assays have been privileged as an integrative measurement of hormonal levels (Wasser et al. 2000; Millspaugh et al. 2001) and have been used in a variety of disciplines such as behavioural ecology and conservation biology of birds and mammals (Whitten et al. 1998; Kitaysky et al. 1999a, b; Creel 2001).

Several factors such as seasonal environmental variations and foraging conditions have been found to influence faecal levels of hormones like, for example, glucocorticoids (Boonstra 2005; Creel 2005). Increased secretion of glucocorticoids is a response to stress (Sapolsky et al. 2000; Boonstra 2005; Reeder and Kramer 2005), and the magnitude of the increase of glucocorticoids may reflect the

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severity of the stressor (Hennessy et al. 1979). Low forage quality, in energetically stressed individuals (Kitaysky et al. 1999a), and food deprivation (Saltz and White 1991; Foley et al. 2001) have been observed to result in increased glucocorticoid levels. In temperate regions, energetic constraints faced during winter, such as poor forage conditions and high-energy expenditures, may induce a stress response in wild animals using their body reserves and muscle proteins to survive (Jensen et al. 1999). Monitoring faecal glucocorticoid levels could thus be a useful tool to evaluate the stress associated with changes in the physiological condition of individuals facing constraining environmental and foraging conditions.

In social groups, increased glucocorticoid secretions can also result from the establishment of dominance relationships through aggressive interactions (Wingfield et al. 1990; Sapolsky 1992; Creel 2001). Social stress may either be induced by the initiation of aggressive interactions, which may also involve fights, or from receiving aggressive interactions (Creel 2005). It is therefore difficult to predict whether being dominant or subordinate should be more stressful (Creel 2005). Indeed, faecal glucocorticoid levels have been found to be higher in both dominants (Creel 2001; Creel and Sands 2003) and subordinates (van Schaik et al. 1991; Sapolsky 1992), while other studies found no relationship between social rank and glucocorticoid levels (Creel et al. 1996; Goymann et al. 2003; Sousa et al. 2005).

Another hormone, testosterone, has been traditionally studied in male vertebrates and linked to social rank, aggressiveness and reproductive status of individuals (Miller et al. 1987; Boissou 1995). In mammals, testosterone levels have been found to be positively correlated with aggressiveness (Creel et al. 1997; Muller and Wrangham 2004) and social rank (Sapolsky 1993; Pelletier et al. 2003). A positive relation between testosterone and social rank is expected in unstable social groups where aggressive interactions are frequent or when social rank is positively related to aggressiveness (Wingfield et al. 1990; Sapolsky 1993). High levels of testosterone may involve significant costs to individuals, especially during limiting seasons. In wild birds, during winter, individuals with high testosterone levels had reduced body condition and survival probability compared with individuals showing lower testosterone levels (Ketterson et al. 1991; Ketterson and Nolan 1992; Wingfield and Romero 2001). In primates, Sapolsky et al. (1986) hypothesised that individuals could decrease aggressiveness, and consequently testosterone secretion, to adopt an energy-minimising strategy during food shortages. Testosterone levels, however, have rarely been linked to environmental variations and changes in foraging conditions in mammals.

In ungulates, many studies have looked at the possible relation between dominance status, environmental conditions and glucocorticoid and testosterone levels (Li et al. 2001;

Patton et al. 2001; Pelletier et al. 2003). The majority of these studies, however, have only focused on the hormonal levels of adult males during the rut or individuals injected with hormones. Until now, no study has addressed the relation between unmanipulated hormone levels, social status and overwinter decrease of body condition in young ungulates. Our study addresses this question by using an experimental approach with groups of young ungulates fed different diet qualities.

The general aim of our study was to experimentally simulate the long-term deterioration of winter forage quality caused by a high-density deer population to evaluate the influence of winter progression, diet quality and social rank on both glucocorticoid and testosterone levels found in faeces of white-tailed deer (*Odocoileus virginianus*) fawns. Specifically, we examined whether changes in faecal glucocorticoid and testosterone levels reflected a seasonal change in the physiological condition of individuals due to (1) winter progression and (2) food quality (i.e. we compared a control and a poor diet). On one hand, we predicted that faecal glucocorticoid levels should increase during winter in response to environmental and nutritional stresses. Faecal testosterone levels, on the other hand, should decrease during winter in response to the reduction of energy allocated to social and aggressive behaviours. In addition, we predicted that the increase of glucocorticoids and the decrease of testosterone levels during winter should be greater for fawns fed the poor diet quality than for the controls. Finally, we tested the influence of individual social rank on both faecal glucocorticoid and faecal testosterone levels. We predicted that dominant and aggressive individuals would present higher glucocorticoid and testosterone levels irrespective of diet quality.

## Materials and methods

### Study area

Anticosti is a 7,943-km<sup>2</sup> island located in the Gulf of St.-Lawrence, Québec, Canada. The sub-boreal climate is characterised by cool summers and long winters (Huot 1982). Vegetation is classified as subarctic, and the main tree species found on the island are balsam fir (*Abies balsamea*), white spruce (*Picea glauca*) and black spruce (*Picea mariana*; Potvin et al. 2003). No indigenous large herbivores were present on the island before the introduction of about 220 white-tailed deer in the late 1800s (Côté 2005). In the absence of natural predators, the population increased rapidly, and local densities now reach 20 deer/km<sup>2</sup> (Potvin and Breton 2005). The high deer density on the island has had major impacts on the native flora, mainly on herb and shrub layers, and there is very little winter browse available

(Potvin et al. 2003). The current alternative preferred forage in winter is balsam fir, but because of overbrowsing by deer, almost no balsam fir stands have regenerated since the 1930s (Potvin et al. 2003). White spruce stands are rapidly replacing balsam fir stands on the island, and therefore, deer are likely to include a higher proportion of white spruce in their diet in the near future (currently 17%, Lefort et al. 2007), although white spruce is normally avoided by deer (Halls 1984; Sauvé and Côté 2007). White spruce is a lower quality forage than fir, as it contains greater concentrations of fibres and tannins (Sauvé and Côté 2007), but it is the only alternative browse species available on Anticosti Island (Tremblay et al. 2005). All deciduous shrubs and trees have been eliminated or severely reduced by deer browsing (Côté 2005; Tremblay et al. 2005). A diet containing a higher proportion of white spruce would therefore be of lower quality than the current diet based mainly on fir and could have detrimental effects on several behavioural parameters and life history traits of deer.

### Captures

We captured 13 white-tailed deer fawns (6 to 7 months old) in late fall 2003 in the western part of Anticosti Island. We only captured fawns because they represent the most vulnerable segment of the population to winter conditions (Dumont et al. 2000) and because forage quality during the first winter of life can have long-term impacts on body condition and life history traits (Stewart et al. 1999). We used physical and chemical immobilisation techniques to capture fawns (see Taillon et al. 2006). Fawns were sexed and weighed to the nearest 0.5 kg with a spring or an electronic scale. We marked all fawns individually with plastic ear tags and released them into a large semi-natural outdoor enclosure (see below).

### Experimental design

We established an 80×150-m enclosure surrounded by a 4-m-high game fence in a natural and mature white spruce stand where trees did not have branches lower than 3 m and the shrub layer was absent, thereby excluding any uncontrolled food input into the enclosure except litterfall. We further subdivided the enclosure in two sections of 80×50 m each, with 30–40% of the initial forest maintained as cover. We also built three basic shelters in each section to simulate wind-protected areas in the natural forest.

At the beginning of January 2004, we divided fawns in similar groups based on sex and body mass. The control group included two males and five females, whereas the poor diet group included three males and three females. There was no significant difference in initial body mass ( $\text{kg} \pm \text{SE}$ ) between the two groups of fawns (control diet,

$26.9 \pm 1.2$ ; poor diet,  $26.4 \pm 1.3$ , two-sample  $t$  test:  $t=0.24$ ,  $df=1, 11$ ,  $P=0.8$ ).

The experimental diets were composed of white spruce and a mixture of balsam fir and arboreal lichens that grow on fir branches, combined in different proportions of fresh weight. The control diet represented the actual winter diet composition of free-ranging deer on Anticosti, i.e. about 70% balsam fir, 10% lichens and 20% white spruce (Lefort et al. 2007). The poor diet was composed of 50% fir, 10% lichens and 40% spruce. Fir (with lichens) and spruce were harvested near the enclosure and shredded separately in a wood chipper (Yard Machines-5 HP wood chipper). This created a uniform mixture, which prevented fawns from selecting one of the diet components. Microhistological analyses of faeces confirmed that fawns ate the proportion of spruce and fir that we offered them (see Taillon et al. 2006 for details). Each day, 2 kg of fresh food were provided to each fawn, an amount considered sufficient to meet the basic metabolic needs of a 30-kg fawn (Huot 1982). As there was always some food left in the feeding troughs, we considered fawns to be limited only by the quality and not by the quantity of forage. Water was accessible at all times as snow.

### Behavioural observations

The two sections of the enclosure were observed simultaneously on a daily basis for approximately 6 h. From January to mid-February, observations were made during the complete daylight period, i.e. from about 0830 to 1530 hours. From mid-February to the end of March, observations were conducted alternatively in the morning (sunrise to 1200 hours) and the afternoon (1200 hours to sunset) to encompass the total daylight period. We performed 53 observation periods per enclosure for a total observation time of 616 h. The experiments ended at the end of March when snow melted.

In each section of the enclosure, we provided food in a single feeding trough (2.5 m×30 cm×30 cm), which was placed at the forest edge and was large enough to allow access to food to all individuals simultaneously. This setup favoured agonistic interactions without limiting individual access to food or impairing the ability of individuals to recognise each other, which has been shown to generate inconsistencies in dominance relationships (Côté 2000b). We used spotting scopes (15–25×) and binoculars (8×42) to conduct behavioural observations from elevated blinds situated at 15 m from the enclosure's fence.

We determined the social hierarchy of both groups of fawns from 14 January to 27 March. We used ad libitum sampling and 1-h focal observations to record aggressive interactions (Altmann 1974). The outcome of more than 99% (control diet) and 95% (poor diet) of agonistic interactions was very clear. We considered an encounter resolved when one of the opponents withdrew and, for each

dyad, we considered an individual dominant if it won more than 50% of its interactions with the other individual (Côté 2000a). We then organised interactions in a dominance matrix for each experimental group, and we tested the linearity of each matrix using a sampling process performing 10,000 randomisations (de Vries 1995). White-tailed deer fawns of both diet qualities were consistently organised in significantly linear hierarchies during winter (control diet:  $h=0.80$ ,  $h'=0.84$ ,  $P=0.05$ ; poor diet:  $h=0.86$ ,  $h'=0.94$ ,  $P=0.02$ ), and thus, we ordered fawns in hierarchies according to the methodology of de Vries (1998). We performed all procedures using Matman 1.0 for Windows (Noldus Information Technology 1998). As the number of individuals ranked varied between the experimental groups, we transformed social ranks according to the formula:  $1-(\text{rank}/N_i)$  where  $N_i$  is the number of fawns alive during period  $i$  (Côté 2000a). Social ranks therefore varied from 0 (subordinate) to 1 (dominant). When comparing diets, we considered the first three ranks as dominants and the rest as subordinates. Further descriptions of these methods can be found in Côté (2000a).

#### Faecal sampling

We recorded from a distance the locations of faecal samples for each individual on the snow and collected the samples at the end of each daily observation periods to minimise disturbance. Time lag between deposition and the collection of samples was never longer than 6 h. Faecal samples were collected approximately every 10 days during winter for each fawn (samples per fawn:  $N=11.0\pm 0.8$ ; total:  $N=132$ ). We were unable to collect faecal samples for one fawn of the poor diet quality; this fawn was therefore excluded. Faecal samples were placed in individually labelled airtight plastic bags and kept frozen ( $-20^\circ\text{C}$ ) until assays. We also recorded time of defecation, as it may affect hormone concentrations. We observed no difference, however, in faecal glucocorticoids and testosterone concentrations of samples collected at different hours of the day throughout the winter (REG: glucocorticoids,  $F=0.88$ ,  $df=1$ , 63,  $P=0.4$ ; testosterone,  $F=3.25$ ,  $df=1$ , 63,  $P=0.08$ ).

#### Hormone analyses

In the laboratory, faecal samples ( $0.50\pm 0.02$  g) were placed into extraction tubes with 5.0 ml of 80% methanol and shaken mechanically overnight ( $\leq 12$  h) for extraction. The contents were allowed to settle and samples were then centrifuged ( $780\times g$ , 15 min,  $25^\circ\text{C}$ ). The supernatant was transferred into clean tubes and extracts were stored at minus  $20^\circ\text{C}$  until assay.

For assay purposes, extracts were diluted 1:16 for the corticosterone radioimmunoassay (RIA) and 1:9 for the

testosterone enzyme-immunoassay (EIA). Extracts were diluted in assay buffers [RIA: RIA buffer (ICN  $^{125}\text{I}$ -corticosterone RIA kit) and EIA: phosphate-buffered saline, pH 7.0]. All hormone measurements were carried out in duplicate. Concentrations were determined as nanogram per millilitre and then divided by the mass of faeces extracted to express the results as nanogram per gram of wet faeces (Wasser et al. 2000; Möstl et al. 2002; Möstl and Palme 2002). Wet weights of faeces are normally used for ruminants, unless it is obvious that there are variations in faecal consistency (i.e. loose stool, diarrhea; Möstl et al. 2002). If there are no differences in consistency, drying or lyophilising samples does not affect the concentration of hormone metabolites (Möstl and Palme 2002). None of the samples in our study presented differences in faecal consistency. In addition, we oven-dried samples of faeces and found no difference in the water content of faecal samples from the control diet ( $24.4\pm 1.2\%$ ;  $N=14$ ) and the poor diet ( $26.3\pm 1.2\%$ ;  $N=15$ ;  $t=1.12$ ,  $P=0.3$ ), indicating that using dry weight of faeces would not change the differences in hormone concentration observed between diets.

For glucocorticoid assays, we used ICN  $^{125}\text{I}$ -corticosterone radioimmunoassay kits (Cat# 07-120102, ICN Biomedicals, Inc., Costa Mesa, CA) that were previously validated to quantify faecal glucocorticoid metabolite concentrations in white-tailed deer (Millsbaugh et al. 2002). Sensitivity of the assay was 0.06 ng/ml (the lowest concentration detectable). Intra-assay coefficient of variation was  $<10\%$  ( $N=2$  replicates per sample).

Testosterone metabolites were extracted from faeces according to the method described by Walker et al. (2002). The testosterone EIA utilises anti-testosterone R156/7 antibody (C. Munro, U.C. Davis), testosterone-horseradish peroxidase (C. Munro, U.C. Davis), and  $17\beta$ -hydroxy-4-androsten-3-one for standard preparation (Steraloids, cat#A6950). Sensitivity of the assay was 4.8 ng/g. Specific data provided by the manufacturer include cross-reactivity as follows: testosterone 100%,  $5\alpha$ -dihydrotestosterone 57.4%, androstenedione 0.27%, androsterone 0.04%, dehydroepiandrosterone 0.04%,  $\beta$ -estradiol 0.02%, progesterone  $<0.02\%$ , pregnenolone  $<0.02\%$  and hydrocortisone  $<0.02\%$ . It is possible, therefore, that androgen metabolites other than the compounds tested were present in the faeces and contributed to the assayed result.

The testosterone assay was validated for faecal extracts of white-tailed deer fawns by demonstrating parallelism between serial dilutions of faecal extracts (from neat to 1:128) and a standard curve (4.8 to 1250 pg/50  $\mu\text{l}$ ). To assess the physiological relevance of immunoreactive faecal testosterone, samples were collected, in spring 2004, from free-ranging animals, which were divided in two groups:

adult males ( $N=28$ ) and adult females ( $N=27$ ). The concentrations of testosterone for the groups were for adult males:  $210\pm 17$  ng/g, adult females  $178\pm 18$  ng/g and fawns for the experiment  $138\pm 5$  ng/g ( $N=132$ ). Concentrations were higher in adults than in fawns ( $t=5.86$ ;  $df=1, 184$ ,  $P<0.0001$ ). Parallelism was verified by demonstrating that slopes produced from serially diluted samples were not different from those generated from standard curves ( $r=0.98$ ,  $P<0.0001$  for males;  $r=0.97$ ,  $P<0.0001$  for females; and  $r=0.99$ ,  $P<0.0001$  for fawns). The intra and inter-assay coefficients of variation for white-tailed deer control samples were  $<10$  and  $<15\%$ , respectively. The minimum testosterone level measured for fawns was  $56.8$  ng/g and the maximum  $235.1$  ng/g.

### Statistical analyses

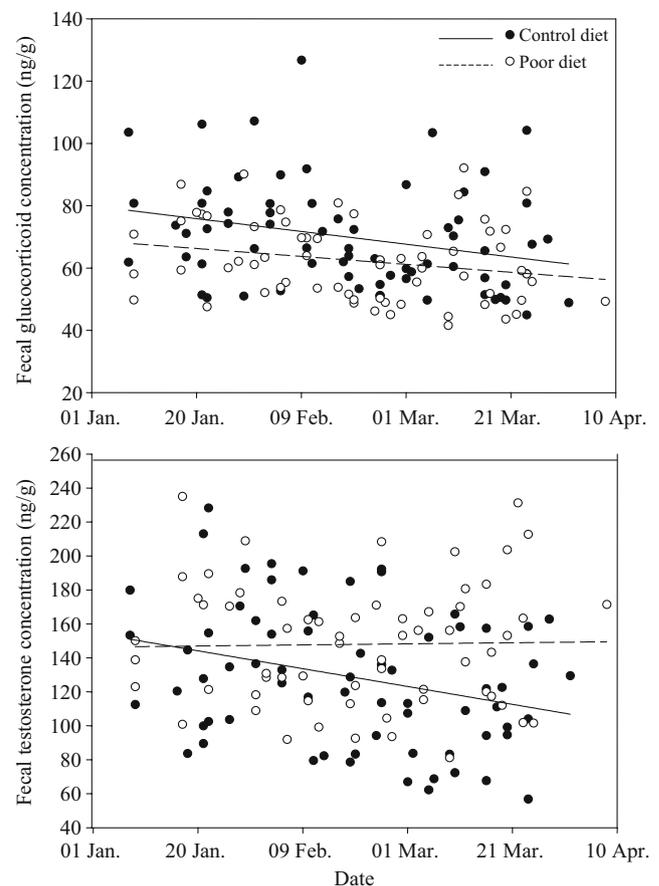
We used a simple correlation to relate glucocorticoid and testosterone concentrations within individuals (Corr procedure, SAS Institute 8.0, Cary, NC, USA). We used linear mixed models to test the effects of diet quality, social rank, winter progression (Julian day) and all second-degree interactions on hormone concentrations of fawns (Mixed procedure, SAS Institute 8.0). We used mixed models with random coefficients to avoid pseudo-replication, as our sampling design included repeated observations of the same individuals (Littell et al. 1996). Our models were sensitive to multiple data arising from independent subjects and consisted of analyses of covariance with each subject treated as a co-variable (Littell et al. 1996). In considering the subject as a random factor and repeated observations, we obtained linear models describing glucocorticoid and testosterone variations for each individual. We applied a square root transformation to glucocorticoid and testosterone concentrations to meet the assumptions of normality and homogeneity of variance. All data are presented as means  $\pm$  SE and  $\alpha=0.05$ .

### Results

Overwinter faecal glucocorticoid and testosterone concentrations were positively correlated for both diet qualities (control diet:  $N=69$ ,  $R=0.30$ ;  $P=0.01$ ; poor diet:  $N=63$ ,  $R=0.53$ ;  $P<0.0001$ ). For glucocorticoid, the final model explained 60% of the variance and included an effect of diet quality ( $F=7.45$ ,  $df=1, 9$ ,  $P=0.02$ ) and winter progression ( $F=7.81$ ,  $df=1, 9$ ,  $P=0.02$ ), but not of social rank ( $F=0.01$ ,  $df=1, 9$ ,  $P=0.9$ ). The average faecal glucocorticoid concentrations were higher for fawns fed the control diet quality than for fawns fed the poor diet (control diet,  $69.8\pm 1.8$  ng/g,  $N=69$ ; poor diet,  $62.3\pm 1.9$  ng/g,  $N=63$ ). These concentrations were similar to levels found for

white-tailed deer in the literature (baseline levels of faecal glucocorticoids in adult white-tailed deer females  $<75$  ng/g; Millsbaugh and Washburn 2003). We also detected a significant decrease of faecal glucocorticoid concentrations during winter (Fig. 1). The decline was similar for fawns of both diets (interaction day  $\times$  diet;  $F=0.01$ ,  $df=1, 9$ ,  $P=0.9$ , Fig. 1) and for dominant and subordinate fawns (interaction day  $\times$  rank;  $F=2.77$ ,  $df=1, 9$ ,  $P=0.1$ ). For fawns that died during winter, faecal glucocorticoid concentration within a week of death did not vary compared to other times during winter (average concentration during winter:  $67.2\pm 1.9$  ng/g,  $N=42$ ; average concentration before death:  $64.1\pm 4.4$  ng/g,  $t=0.65$ ,  $df=1, 8$ ,  $P=0.5$ ).

For testosterone, the final model explained 83% of the variance and included an effect of diet quality ( $F=9.11$ ,  $df=1, 9$ ,  $P=0.01$ ), a marginal influence of winter progression ( $F=4.43$ ,  $df=1, 9$ ,  $P=0.06$ ) and a significant interaction between winter progression and social rank ( $F=8.97$ ,  $df=1, 9$ ,  $P=0.02$ ), whereas social rank as a main factor had no effect ( $F=0.60$ ,  $df=1, 9$ ,  $P=0.5$ ). Faecal testosterone concentrations were higher for fawns fed the poor diet than



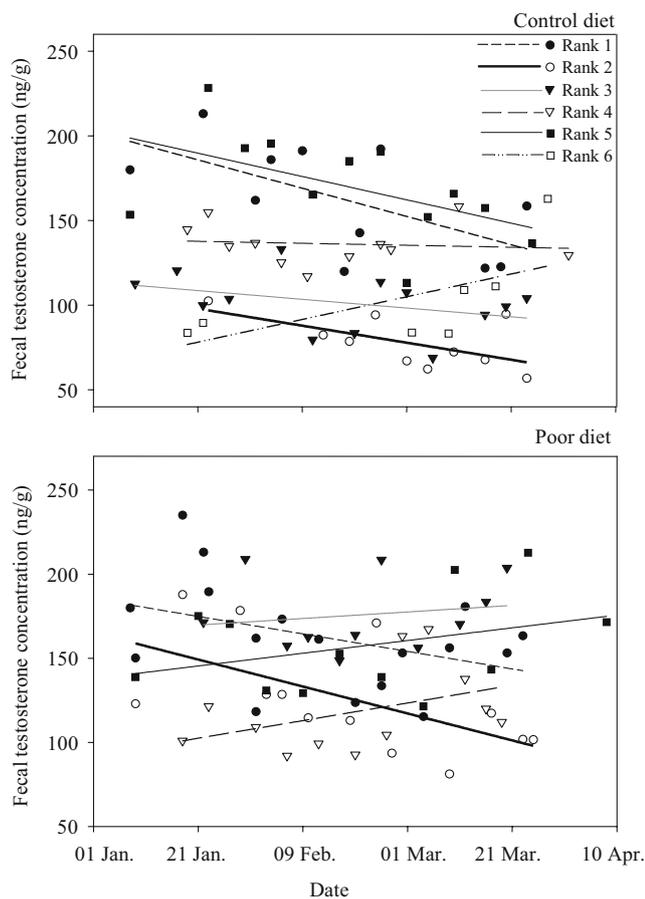
**Fig. 1** Variation in faecal glucocorticoid and testosterone concentrations during the winter of 2004 for captive white-tailed deer fawns fed two different diet qualities on Anticosti Island, Québec, Canada

for fawns fed the control diet (control diet  $130.4 \pm 4.8$  ng/g,  $N=69$ ; poor diet,  $148.0 \pm 5.0$  ng/g,  $N=63$ ). Faecal testosterone concentration tended to decrease during the winter (Fig. 1). However, variations in faecal testosterone concentrations during winter differed according to the social rank of fawns: five of six dominant fawns showed a decrease of faecal testosterone concentrations during winter, whereas three of the five subordinates showed an increase of testosterone concentrations during winter (Fig. 2). Finally, fawns that died overwinter showed a sharp increase in faecal testosterone concentrations just before death (average concentration during winter,  $138.6 \pm 5.6$ ,  $N=42$ ; average concentration within a week of death,  $220.3 \pm 12.8$ ,  $t=5.9$ ,  $df=1, 8$ ,  $P<0.0001$ ).

## Discussion

Our study is the first to evaluate natural winter glucocorticoid and testosterone levels in young ungulates in relation

to diet quality and social rank and also to validate the use of faecal testosterone EIA for white-tailed deer. Contrary to our predictions, glucocorticoid levels decreased during winter. Fawns may have reduced glucocorticoids secretion and suppressed the stress response during the winter when their body condition was too low to fuel a full stress response. We also observed a simultaneous decline of testosterone levels during winter that could be associated with the decline of body mass. We found no relationship between faecal glucocorticoid levels and social rank, suggesting that social stress was similar for dominant and subordinate fawns during winter. Testosterone levels and social rank were not correlated, as previously observed in social groups of individuals that maintained stable dominance relationships and social hierarchies (Wingfield et al. 1990; van Schaik et al. 1991). Our results revealed that the relationships between faecal hormone levels and behaviours are not straightforward, but that young mammals may suppress the stress response (i.e. glucocorticoids secretion) and the secretion of testosterone to withstand harsh winter conditions.



**Fig. 2** Variation of faecal testosterone concentration of captive white-tailed deer fawns of different social ranks during the winter of 2004 on Anticosti Island, Québec, Canada. Social ranks vary from 1 (dominant) to 6 (subordinate)

## Faecal glucocorticoid levels

During winter, northern ungulates have to deal with low forage abundance and quality (Gray and Servello 1995; Tremblay et al. 2005) and high energy expenditures caused by the demands for thermoregulation and locomotion in deep snow (Moen 1976). When body condition is declining, herbivores use body reserves and muscle proteins to survive (DelGiudice et al. 1998; Jensen et al. 1999). This is commonly associated with increased glucocorticoid secretion (Kitaysky et al. 1999b; Nelson 1999). We detected, however, a significant decrease of faecal glucocorticoid levels throughout winter that was similar for fawns on both diets and for different social ranks. Our previous work, using the same experimental design (Taillon et al. 2006; Taillon and Côté 2007), revealed that body mass decreased for all fawns during winter with no effect of either diet quality or social rank. Thus, the decrease of faecal glucocorticoid levels observed during winter may be related to the decline of fawns' body mass. Studies on Arctic birds have suggested that the stress response, based on glucocorticoid levels, could be blunted during fall and winter (Romero et al. 1997, 1998) and that glucocorticoid release may thus be modulated by the physiological state of the animals (Wingfield et al. 1994). Individuals may greatly reduce glucocorticoids secretion and suppress the stress response during chronic food shortages or during the winter when their body condition is too low to fuel a full stress response (Romero et al. 1997; Kitaysky et al. 1999b; Boonstra 2005). The suppression of the stress response may therefore prevent fast depletion of limited protein resources (Le Ninan et al. 1988; Boonstra 2005). As

deer on Anticosti Island can withstand extreme body condition deterioration (Taillon et al. 2006), we suggest that a similar compromise could explain the decrease of glucocorticoid levels in fawns during winter. Such suppression of the stress response could also explain why fawns did not present an increase of glucocorticoid levels a few days before death when body mass declined to a critical level.

Increased glucocorticoid levels may result from nutritional stressors such as low forage quality (Kitaysky et al. 1999a) and food deprivation (Saltz and White 1991; Foley et al. 2001). Our results indicated, however, that fawns fed the control diet showed higher glucocorticoid levels than fawns fed the poor diet quality, suggesting that control fawns faced a higher stress than those on the poor diet. A multitude of factors such as environmental variations and social status may influence glucocorticoid levels (Nelson 1999). In a previous study on the same groups of fawns (Taillon and Côté 2007), we found that fawns fed the control diet showed a slight increase or maintenance of aggressiveness during winter, whereas fawns fed the poor diet quality showed a decrease of aggressiveness throughout winter. The increase or maintenance of aggressiveness throughout winter could partly explain the higher glucocorticoid levels observed for fawns on the control diet. We contend, however, that it is difficult to identify precisely which factors affected the stress level of fawns under restrictive foraging conditions.

In social groups, variations in the stability of dominance relationships and in the relation between social rank and aggressiveness may influence faecal glucocorticoid levels of individuals of different social ranks (Sapolsky 1993; Whitten et al. 1998; Creel 2005). Similarly to other studies on social mammals (Creel et al. 1996; Goymann et al. 2003; Sousa et al. 2005), we found no relationship between faecal glucocorticoid levels and social rank of fawns. Our previous study (Taillon and Côté 2006) showed that fawns were consistently organised in linear hierarchies during winter and that aggressive interactions among fawns were highly directional. These two characteristics, which contributed to the long-term stability of the social hierarchies (Thompson 1993; Côté 2000a), combined with a possible suppression of the stress response of fawns during winter, may explain the absence of a relationship between social rank and faecal glucocorticoid levels. Thus, based on faecal glucocorticoid levels, social stress appeared to have been similar for dominant and subordinate fawns during our study.

#### Faecal testosterone levels

Our study is the first to use noninvasive techniques to monitor testosterone levels in white-tailed deer faecal samples. Previous studies on white-tailed deer mainly used

blood samples of adult males analysed with RIA (Lincoln and Kay 1979; Miller et al. 1987; Bubenick et al. 2005). We wanted to validate a practical procedure to monitor testosterone in faecal samples using a simple extraction method and an EIA, which is more economical and requires less sophisticated laboratory equipment than RIA analysis. Our results confirmed that an EIA is applicable for white-tailed deer, as faecal testosterone levels obtained were consistent with the physiological state of individuals (i.e. comparing adults and immatures, males and females) and with levels reported for ungulates in the literature (Li et al. 2001; Patton et al. 2001; Pereira et al. 2005). Our assay results may, however, be slightly affected by cross-reactivity with other metabolites because not all testosterone-derived metabolites have been identified.

In wild animals, testosterone has been traditionally linked to social rank, aggressiveness and reproductive status of individuals (Wingfield et al. 1990; Bouissou 1995). Testosterone levels have been found to increase before the breeding season and to be positively correlated to social rank and aggressive behaviours (Pelletier et al. 2003; Li et al. 2004). As shown in birds (Wingfield et al. 1990; Ketterson and Nolan 1992), testosterone levels may also vary according to environmental variations and foraging conditions. In our experiment, we observed that faecal testosterone levels of fawns tended to decrease during winter with a similar response for both diet qualities, as our previous work revealed that body mass decreased for all fawns during winter independently of diet quality (Taillon et al. 2006; Taillon and Côté 2007). As high levels of testosterone may involve significant physiological costs to individuals (Ketterson et al. 1991; Wingfield and Romero 2001), especially during limiting seasons, faecal testosterone levels may thus decrease with the decline of fawns' body mass. It is possible that fawns during winter suppressed the secretion of testosterone, similarly to glucocorticoids, to prevent fast depletion of limited protein and fat resources. The overwinter correlation between faecal glucocorticoid and testosterone concentrations for fawns on both diet qualities supports this suggestion. The sharp increase of testosterone levels in faeces collected a few days before the death of individuals, however, is difficult to explain. Although no other study has reported such a result, we suggest that as elevated testosterone levels are associated with increased metabolic rate resulting from the loss of body mass and fat reserves (Ketterson et al. 1991; Ketterson and Nolan 1992), fawns in a critical physical state may have shown a sharp increase of testosterone levels resulting from increased metabolic activity just before death.

Foraging conditions may also influence faecal testosterone levels. We observed that faecal testosterone concentrations were higher for fawns fed the poor diet quality than for fawns fed the control diet. This result is opposite to our prediction, as

we expected fawns fed the poor diet quality to decrease aggressive behaviours because these behaviours are costly and involve higher testosterone secretion. We did not find any other example in the literature on the effect of diet quality on testosterone levels in a similar context. The higher concentration of testosterone in faeces of individuals on the poor diet quality may reflect an adverse effect of low-quality nutrition.

In mammals, testosterone levels have been found to be positively correlated with aggressiveness (Creel et al. 1997; Muller and Wrangham 2004) and social rank (Sapolsky 1993; Pelletier et al. 2003). Surprisingly, even if social rank was positively related to aggressiveness in fawns of our experiment (Taillon and Côté 2007), we found no relationship between testosterone concentration and social rank as a main factor. Wingfield et al. (1990) suggested that this could be explained by the “challenge hypothesis”, which states that testosterone levels should be low and uncorrelated with social rank once stable dominance relationships have been established. As in other species where testosterone levels and social rank were not correlated (van Schaik et al. 1991; Robbins and Czekala 1997), fawns in our study formed stable social hierarchies, and dominance relationships remained stable overwinter (Taillon and Côté 2006), supporting the “challenge hypothesis”. We found, however, that dominant fawns showed a decrease and most subordinates an increase in faecal testosterone concentrations during winter. This interaction cannot be explained by a change in aggressiveness because aggressiveness remained higher for dominant than subordinate fawns throughout the winter (Taillon and Côté 2007). We have no clear explanation for this result, but it suggests that the relationships between testosterone and behaviours may be more flexible than has been commonly assumed.

## Conclusion

The relationships between faecal hormone levels and behaviours are not straightforward. In our study, using fawns may have limited our capacities to answer questions, as fawns are still growing and may present an immature endocrine system. On the other hand, they are also more vulnerable than adults and their hormone levels may respond more quickly than adults to environmental or social variations. In any case, studies on the relationships between behaviours and hormones are scarce, and our study provides the first results on unmanipulated levels of hormones in young wild ungulates. Interestingly, our results suggest that fawns seem to present a hormonal strategy that allows them to withstand the harsh winter conditions faced on Anticosti Island. They appear to suppress the stress response (i.e. glucocorticoids secretion) and the secretion of testosterone, which are both costly to individuals. To our

knowledge, this is the first time that such a strategy is reported in ungulates.

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