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EFFECTS OF DIET AND BODY CONDITION ON FECAL PROGESTAGEN EXCRETION IN ELK

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ABSTRACT: Recent research demonstrated the utility of fecal progestagens (P_4) for detecting pregnancy in elk (*Cervus elaphus*) during mid- to late gestation. Several factors, however, may influence fecal P_4 excretion and limit its use in free-ranging animals. We investigated the effects of nutrition and body condition (percent ingesta-free body fat) on fecal P_4 concentrations and incidence of abortion. During mid-gestation (late December 1997 through early March 1998), 40 gravid cow elk varying in body condition were placed on three diets (high, medium, and low) in which the amount of food offered varied. Feces were collected periodically and analyzed for P_4 via radioimmunoassay. We found no significant effect of dietary treatment on P_4 concentrations, but as body condition declined, P_4 concentrations declined significantly. This decline did not impede the ability to detect pregnancy based on previously reported criteria, even for elk in such poor condition that they aborted. However, fecal P_4 concentrations in 10% (4/39) of samples collected from 13 non-pregnant animals maintained on a high plane of nutrition were false-positive for pregnancy. We suggest alternate criteria for determining pregnancy in elk using fecal P_4 values: >1.25 μg/g feces as pregnant, <1.0 μg/g feces as non-pregnant, and 1.0–1.25 μg/g feces as inconclusive. Finally, two cows that aborted did not abort until weeks after being classified as emaciated and near death, suggesting that nutrition-associated abortion in elk may not occur during mid-gestation except under extremely harsh conditions.

Key words: Abortion, *Cervus elaphus*, condition, elk, nutrition, pregnancy detection, progesterone, reproduction.

INTRODUCTION

Assessing reproductive activity or status using non-invasive fecal steroid monitoring has become routine in both captive and wild ungulate research (Lasley and Kirkpatrick, 1991; Hodges, 1996; Brown et al., 1997). One recent application of this technique is use of radioimmunoassay of fecal progestagens (P_4) (Garrott et al., 1998) or enzyme immunoassay of fecal progesterone metabolites (PdG) (Stoops et al., 1999) to assess pregnancy status in elk (*Cervus elaphus*) during mid- to late gestation. The benefits of such a technique are considerable to biologists assessing pregnancy rates and calf survival. However, there may be confounding factors influencing fecal steroid metabolite concentrations that could limit use of this technique (Wasser et al., 1993; Garrott et al., 1998; Berger et al., 1999). Some of these potential factors are dietary constituents (e.g., fiber content or digestibility and energy levels) (Goldin et al., 1981; Wasser et al., 1993), body condition (Cook et al., 2001c), stress (Plotka et al., 1983), or variations in lab technique (e.g., different immunoassays, different radioimmunoassay antiserum, different extraction techniques) (Garrott et al., 1998). Influences of any of these factors may have important implications for reproduction studies that utilize absolute fecal steroid concentrations to document reproductive activity or status.

We investigated the effects of nutrition and body condition on fecal P_4 levels and incidence of abortion to assess potential for these factors to alter criteria for determining pregnancy status as presented by Garrott et al. (1998): >1.0 μg/g feces as pregnant, <0.9 μg/g feces as non-preg-

TABLE 1. Dry matter (g of DM/kg $BM^{0.75}$) and digestible energy (kcal of DE/kg $BM^{0.75}$) feeding levels for three nutrition treatment (trt) groups (high, medium, and low) of cow elk during winter 1997–1998.[a] Feeding data also are presented for 13 non-gravid cow elk fed to provide DE moderately greater than maintenance levels.

Date	Feeding strategy	High		Medium		Low		Non-gravid[b]	
		DM[c]	DE[d]	DM	DE	DM	DE	DM	DE
13 Nov	Maintenance	82	195	82	195	82	195	100	240
27 Dec	Begin trt	51	121	44	105	36	84	100	240
28 Jan	Adjust trt	47	112	40	95	33	79	100	240
19 Feb	Adjust trt	37	87	31	74	26	61	100	240
27 Feb	Adjust trt	47	112	40	95	33	79	100	240
06 Mar	End trt	67	159	67	159	67	159	100	240
18 Mar	Ad libitum	90	212	90	212	90	212	100	240
05 Apr	Ad libitum	100	308	100	308	100	308	100	240
13 Apr	Ad libitum	105	325	105	325	105	325	100	325

[a] Winter diets consisted of a low energy pellet (2.19 kcal of DE/g of DM and 14.8% crude protein) and alfalfa hay (2.57 kcal of DE/g and 15.3% crude protein); spring diets beginning 15 April consisted of a high energy pellet (3.49 kcal of DE/g of DM, 15.9% crude protein) and alfalfa hay. Crude protein, gross energy, and in vitro digestibility of solid feed were determined for each food type by the Habitat Analysis Laboratory at Washington State University (Pullman, Washington, USA). Crude protein content was determined by macro-Kjeldahl analysis, gross energy by bomb calorimetry, digestibility by 2-stage in vitro trials (Horwitz, 1980), and DE was calculated as the product of digestibility and gross energy content (Robbins, 1993).

[b] Non-gravid cows were not included in any of the nutrition treatments.

[c] DM = dry matter.

[d] DE = digestible energy.

nant, and 0.9–1.0 μg/g feces as inconclusive. Forty gravid cows of varying ingesta-free body fat were maintained on three diets varying in amount of food offered. We also assessed potential for misclassification of non-pregnant cows by collecting samples from 13 non-gravid cow elk maintained on a high plane of nutrition.

MATERIALS AND METHODS

Elk were housed in four 1-ha pens near La Grande, Oregon (45°30′N, 118°20′W; see Cook et al., 1996 for study site details). Each pen was devoid of vegetation and had a barn containing nine to 12 stalls designed for individual feeding of pellets and collection of blood, urine, and fecal samples. Facility design permitted researchers to track reproductive activity, body mass, and body condition and manipulate food rations.

Forty captive, 4 and 6 yr old gravid elk were used in this study. During autumn 1997, approximately half of these cows were lactating and had been used in a nutrition study described by Cook et al. (2001c). That study resulted in a wide variety of body condition levels among cows by mid-autumn. The remainder of the study group were maintained on high quality rations that resulted in moderate to excellent body condition by mid-autumn. All gravid cows were switched to a maintenance ration from mid-November until 27 December 1997, to ensure that their body condition would remain approximately constant until the beginning of winter.

Beginning 27 December, we placed the cows on one of three nutritional treatments. Lactating cows involved in the autumn reproduction study were placed in the high nutritional treatment ($n=19$), and the other cows were randomly assigned to the medium ($n=11$) and low nutritional treatments ($n=10$). Rations were constructed using variable proportions of low and high digestible energy (DE) pellet and alfalfa hay (Table 1) and were designed to induce variable degrees of body mass loss (Table 1). The "high" diet was set to induce 8–10% mass loss. Cows in the "medium" and "low" treatments received 85% and 70% of the food ration fed to the high treatment group. Feeding levels were gradually reduced until late February to simulate diminished forage availability that might be expected as winter progresses. Feeding levels were gradually increased during a brief transition period (27 February to 6 March); all cows had ad libitum access to identical rations thereafter (Table 1).

Elk were fed twice daily throughout the study. Each morning, cows were placed in individual stalls and offered pelleted food; where-

as hay was fed communally in the afternoon in hay mangers that were widely spaced to prevent animal exclusion. Orts of both pellets and hay were recorded daily (although no orts were left prior to ad libitum feeding in the spring). Two fecal samples per cow were collected while nutritional treatments were being implemented (on 25 February 1998 and 3 March 1998), and three fecal samples per cow were collected once treatments were terminated and all animals had been placed on identical diets (17 March 1998, 31 March 1998, and 21 April 1998). Fecal pellets from cows that aborted ($n=2$) were collected weekly. All fecal samples were stored frozen (−20 C) until analysis.

Each cow was weighed twice per week; the two mass estimates (kg) were averaged, and mass loss percentages were calculated from this average. Body condition also was determined for each cow in mid-February and mid-March by measuring maximum rump fat thickness via ultrasonography and a body condition score (Cook et al., 2001a,b). These measurements were used to estimate percent ingesta-free body fat (IFBF) by the equation: $\text{IFBF} = -9.8863584 + 9.1871285x - 1.3831754x^2 + 0.083951218x^3$ where x is LIVINDEX, an arithmetic combination of subcutaneous rump fat thickness and body condition score (Cook et al., 2001a, b).

In addition, 13 non-gravid animals housed in a separate 1 ha pen were fed a diet moderately above maintenance (240 kcal DE/kg $BM^{0.75}$, where BM is body mass) of pellets and alfalfa hay (Table 1). Two to five fecal samples were collected from each non-gravid elk between 1 March and 31 April 1998. These non-gravid controls were used to compare pregnant versus non-pregnant fecal P_4 concentrations during the second trimester of pregnancy.

Fecal P_4 was assayed at the Conservation and Research Center (Front Royal, Virginia, USA) using fecal hormone extraction and radioimmunoassay procedures described previously (Brown et al., 1994; Wasser et al., 1994) and validated for elk (Garrott et al., 1998). Because extraction efficiency (% recovery) was consistently high (95.6±1.0%) (±SE), hormone concentrations were not adjusted for recoveries. Inter-assay coefficients of variation for two separate internal controls were 8.1% and 15.4%. Intra-assay coefficients of variation were <10% and assay sensitivity was 3.75 pg/100 μl. All hormone concentrations are expressed as mass units of hormone excreted per gram of dry feces.

Differences in P_4 concentrations across the three nutritional treatments were analyzed with fixed-effects, repeated measures analysis of variance (ANOVA) using the multivariate mode of PROC GLM (SAS Institute, 1988). For this repeated measures analysis, the assumption of sphericity (SAS Institute, 1988:605) was violated and the Huynh-Feldt adjustment was applied to the degrees of freedom (SAS Institute, 1988:605).

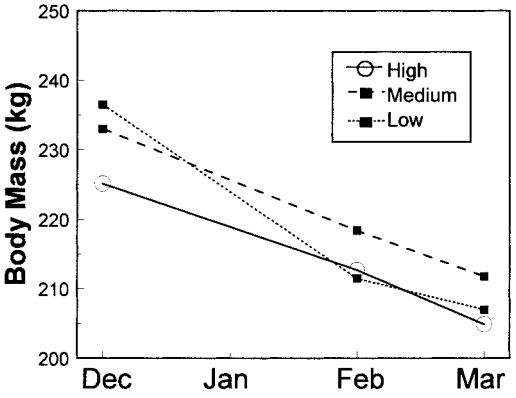

FIGURE 1. Average change in body mass (kg) for cow elk maintained on three dietary treatments (high, medium, and low) varying in quantity of ration fed. Dietary treatments were implemented in December and terminated in March.

We used an ANCOVA (PROC GLM, SAS Institute, 1988) separately for each collection period to assess the effects of IFBF (body condition), dietary treatment, and the possible interaction between the two on fecal P_4 concentrations. For the first two time periods (25 February 1998, 3 March 1998), we used February IFBF estimates. For the remaining time periods, March IFBF estimates were used. *P*-values were adjusted for sequential tests that used the same condition score (Johnson, 1998).

RESULTS

Body mass change (%) was similar between dietary treatment groups. Elk lost about 9% in the high and medium treatments and 12% in the low treatment (Fig. 1). Body condition of elk prior to the study (late autumn) varied markedly (IFBF ranged from 4.8–25.0%). By February, IFBF for the high treatment averaged 8.8±0.9% (±SE) (range 2.0–17.1%), the medium treatment averaged 12.0±0.7% (range 6.7–15.0%), and the low treatment averaged 8.8±1.0% (range 3.3–12.0%). In March, IFBF for the high treatment animals averaged 7.0±0.9% (range: −0.9–14.4%, negative values indicated severe muscle catabolism), medium treatment av-

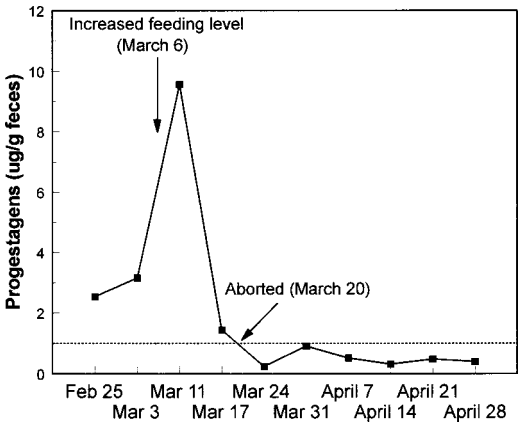

FIGURE 2. Hormonal profile for one high nutrition, poor condition (<1.0% ingesta-free body fat) elk cow that aborted. Arrows indicate the date in which the cow was put on a higher plane of nutrition and the date of abortion. The horizontal dotted line indicates the threshold value for classifying elk as pregnant (>1.0 μg/g feces from Garrott et al., 1998).

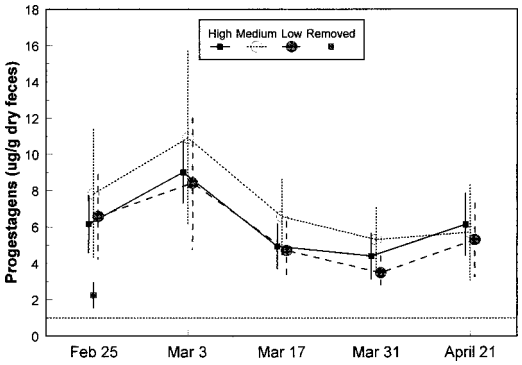

FIGURE 3. Average P_4 concentrations (μg/g feces±95% CI) for gravid cow elk maintained on three dietary treatments varying in quantity of ration fed; high (n=19), medium (n=11), and low (n=10). The gray square indicates average P_4 concentrations (μg/g feces±95% CI) for three cows before they were removed from the study due to death or rapidly deteriorating condition (one cow died and the other two had body fat levels <2.0% and aborted). The horizontal dotted line indicates the threshold value for classifying elk as pregnant (>1.0 μg/g feces from Garrott et al., 1998).

eraged 9.9±0.9% (range 4.3–13.2%), and the low treatment averaged 6.0±1.0% (range 2.0–9.9%). The extreme variation within treatment groups reflected the considerable variation in body condition that existed just prior to the beginning of the study.

One elk died from severe emaciation on 26 February, and postmortem examination revealed the presence of an apparently viable fetus. Two more elk were removed from the study between 24 and 26 February. They were in extremely poor condition (had negative estimated fat values), required veterinary treatment, and probably would have died without supportive care. These two animals aborted, but not until they had been placed on a higher diet for some time (2 wk and >6 wk after being placed on higher energy diets fed ad libitum) (Fig. 2). Three additional elk were in such poor condition (<2.0% IFBF) at that time (26 February) that we increased their ration by 20% over the following week to prevent further mortality (their data were used for all analyses). The average IFBF value for those animals removed from the study was 0.8±1.7%.

Fecal P_4 levels from pregnant cows ranged from 0.58–21.20 μg/g feces. Only one sample (0.58 μg/g feces) was misclassified as being from a non-pregnant cow (test sensitivity=0.995) and only one sample (<0.5%) from a pregnant cow fell into the inconclusive range (0.91 μg/g feces).

Four (10%) of 39 samples from non-pregnant cows were misclassified as indicating pregnancy (test specificity=0.90); their fecal P_4 concentrations ranged between 1.00 and 1.40 μg/g feces. Two other cows (2/39, 5%) excreted P_4 concentrations >0.90 but <1.00 μg/g feces, the range considered inconclusive by Garrott et al. (1998).

Progestagen concentrations of pregnant elk changed throughout the winter study ($F_{4,140}$=23.47, P≤0.001), but the dietary treatment ($F_{2,35}$=1.14, P=0.33) and the time by diet interaction effect ($F_{8,140}$=0.63, P=0.72) were insignificant (Fig. 3). Fecal P_4 concentrations in the three cows that either died or subsequently aborted were lower (Fig. 3, P=0.018) than the remaining females on 25 February (the last collection time before animals were removed from the study). However,

TABLE 2. Analysis of covariance results testing effects of diet, percent ingesta-free body fat (IFBF), and the interaction of the two variables on fecal protestagen concentrations.

	P-values		
Date	Diet	IFBF	Interaction
25 Feb[a]	0.234	≤0.001[b]	0.190
3 Mar[a,c]	0.757	0.038[b]	0.727
17 Mar[c,d]	0.612	0.006[b]	0.421
31 Mar[c,d]	0.344	0.015[b]	0.184
21 Apr[c,d]	0.755	0.257	0.737

[a] Body fat estimates used were from mid-February.
[b] Indicates significant results based on an adjustment of the *P*-value for sequential testing using the same condition data.
[c] Does not include one elk that had died, and two elk that were removed from the study due to rapidly deteriorating condition.
[d] Body fat estimates used were from mid-March.

fecal P_4 concentrations in these three cows exceeded the criteria of 1.0 μg/g feces indicating pregnancy (Garrott et al., 1998); the lowest value was 1.44 μg/g feces.

Analysis of covariance indicated that for every collection period except the last (13 April), IFBF was significantly related to P_4 ($P<0.05$), while dietary treatment or the interaction of treatment and condition remained insignificant (Table 2).

DISCUSSION

One concern with using absolute values for fecal P_4 to identify pregnancy status relates to the potential impact of dietary change on steroid excretion. For example, elk may experience dramatic seasonal shifts in forage quality and quantity which could potentially influence steroid excretion rates, thereby confounding and limiting the accuracy of fecal P_4 for detecting pregnancy during mid- to late winter.

Nutrition may influence steroid excretion in at least two ways: 1) levels of digestible energy in the diet may affect luteal progesterone production (e.g., domestic livestock; Spitzer et al., 1978; Imakawa et al., 1983), directly or indirectly influencing fecal steroid concentrations; or 2) dietary fiber or indigestible matter may affect excretion rates and steroid concentrations by altering gastrointestinal retention time, gut-fill, food intake, or total fecal output (Goldin et al., 1981; Wasser et al., 1993). We did not detect an effect of daily digestible energy and food intake levels during mid-gestation despite diets that caused starvation-related mortality. Cook et al. (2001c) also reported no influence of diet on fecal P_4 excretion in elk during the breeding period using similar techniques and a greater range in diet levels.

Discrepancies between our findings using fecal concentrations in elk and findings in the livestock using plasma concentrations are not clear. However, diet may interfere with excretion patterns such that a change in plasma progesterone does not necessarily translate to identical changes in fecal P_4. Plasma progesterone concentrations reflect a balance between luteal hormone synthesis and metabolic clearance by the liver and kidneys (Ganong, 1979). While fecal P_4 concentrations are typically related to plasma concentrations, fecal P_4 also may be influenced by blood flow to the gut and liver, metabolic clearance rate, or total fecal output. Hepatic blood flow and progesterone turnover increased as feed intake increased in gilts (Prime et al., 1988). Similarly, increased feed intake was associated with decreased plasma progesterone and increased metabolic clearance of progesterone in sheep (Parr et al., 1993). In general, better nutrition appears to increase plasma progesterone turnover, and better diets produce less feces; both effects should increase serum and fecal P_4 concentrations as diet improves. Conversely, fecal P_4 should decline with diet quality. For example, fecal steroid concentrations declined in the monogastric baboon (Wasser et al., 1993) as dietary fiber was increased incrementally from 5% to 15%. These authors suggested that the excretion of unconjugated steroids was directly or indirectly related to both gastrointestinal (GI) passage time and the fiber-induced increase in total fecal bulk. Feed intake was not discussed.

Relationships between forage quality, fiber levels, indigestible plant matter, and

digestive kinetics are more complex in ruminants than in monogastrics. As DE declines, digestible and indigestible fiber in diets increases. Increasing dietary levels of either type of fiber slows the rate of particle breakdown and ingesta passage (Spalinger and Robbins, 1992), and feed intake declines (Minson and Wilson, 1994; Grey and Servello, 1995). Thus, in ruminants, declining nutritive value of diets with an associated increase in dietary fiber intake may: 1) directly affect luteal progesterone synthesis; 2) reduce blood flow to the alimentary tract, metabolism and clearance of plasma progesterone, and fecal P_4 concentrations; 3) increase gut fill due to slowed passage through the GI tract, thereby 'diluting' fecal P_4 concentrations; 4) reduce gut fill in the lower alimentary tract due to reduced food intake, with a resultant increase in fecal P_4 concentrations; or 5) have multiple interactions and compensations among all the above, thereby confounding prediction of dietary effects on fecal P_4 without indexing fecal steroid excretion rates (Wasser et al., 1993).

We conclude that more research is necessary to determine the influence of diet on fecal P_4. Indeed, almost nothing is known about the impact of extreme dietary changes, such as those that occur during winter when diets are most anomalous (e.g., sticks, bark, and pinecones can predominate diets). The efficacy of fecal P_4 measures under such extreme conditions has not been tested, and our experiments did not mimic these extreme levels of digestible and indigestible fiber. Nevertheless, taken together with previous research (Garrott et al., 1998; Cook et al., 2001c), the present data suggest that fecal P_4 as an index to pregnancy is robust even for elk subjected to considerable variation in nutrition.

However, the specificity of this one-sample pregnancy test was only 0.90 (i.e., 10% of non-pregnant samples were false positive). During mid- to late gestation, potential exists for false positives if females continue to cycle. This is because a one-sample pregnancy test cannot distinguish between luteal phase P_4 concentrations and P_4 concentrations due to pregnancy. This extended cycling would be more probable in a captive setting rather than in a wild setting due to artificially high nutrition, supplemental lighting (no lights were present at our study site), or other factors, and perhaps could account for an increased number of false positives in our non-gravid cows. However, P_4 levels of these false positive (1.0 to 1.4 μg/g) generally were below that of cycling females (1.5 to 4.4 μg/g) during a typical estrous cycle and were within the range of fecal P_4 levels found in non-cycling female elk in autumn (see Cook et al., 2001c). Thus, we have no evidence that our false positive results were due to prolonged cycling during the anestrous period of mid-winter to early spring. It is more likely that the values of these misclassified elk fall within the normal range of non-gravid elk, thereby suggesting a reconsideration of previously published criteria (Garrott et al., 1998). This may be appropriate because development of the original criteria was hampered by small sample sizes from known non-pregnant elk (Garrott et al., 1998). Our data suggest a slight modification: >1.25 μg/g feces=pregnant, <1.0 μg/g feces=non-pregnant, and 1.0–1.25 μg/g feces=inconclusive.

Fecal P_4 concentrations declined as IFBF levels declined (see also Cook et al., 2001c), but even in cows that were near death, fecal P_4 concentrations remained above the 1.0 μg/g feces criteria for pregnancy suggested by Garrott et al. (1998), and above our modified criteria of 1.25 μg/g feces. Although more work is needed to understand influences of long-term dietary restriction on P_4 dynamics, our data suggest that there has been strong selection for elk to maintain P_4 production during pregnancy, and therefore maintain pregnancy, at almost any cost. Such a mechanism may explain why an apparently viable fetus was found in a cow that died of starvation during this study. Similar observa-

tions have been made in long-term wild elk studies in Yellowstone National Park (R. A. Garrott, unpubl. data). Moreover, two abortions in our study occurred 2–6 wk after both cows had reached the point they would have died (<1% body fat) if we had not intervened with supportive care. Thus, during acute undernutrition, fetal loss, even in severely undernourished cows, may be rare during the second trimester of pregnancy. In addition, three cows in very poor condition (<2.0% body fat) maintained gestation and produced viable calves. This suggests that fetal deaths from severe undernutrition are more probable in the third trimester (e.g., stillbirths) than in the second trimester (see also Thorne et al., 1976; Verme and Ullrey, 1984).

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