BACTRIAN CAMEL FECAL STEROID ANALYSIS

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Abstract: Recent evidence suggests that wild Bactrian camels (*Camelus bactrianus ferus*) numbers are declining and that recruitment is low. Low camel recruitment may be due to high juvenile mortality rates or poor reproductive performance. Steroid fecal analyses offer non-intrusive methods of analyzing reproductive physiology in many animals. We conducted a preliminary study to develop and refine steroid fecal analyses for Bactrian camels. Fecal samples were collected before and after parturition from 2 domestic Bactrian camels being held at the Denver Zoo. A third, non-pregnant camel was used as a control. We extracted and measured estrogen (E1C), progestin (PdG), and progesterone (P4) hormone metabolites from these fecal samples. The E1C metabolite appeared to be the best indicator for detecting a pregnancy, demonstrating an approximate 20-fold increase in E1C in a pregnant vs. non-pregnant camel at 1 week pre-parturition. The data from this pilot study suggest that fecal steroid metabolite analysis may be a useful tool for identifying pregnant, free-ranging camels.

Introduction

Wild Bactrian camels (*Camelus bactrianus ferus*) are critically endangered, surviving in remnant portions of their original range in China and Mongolia (Hare 1997, Schaller 1998, Reading et al. 1999). Recent evidence from limited field research and ground surveys suggests that camel numbers are declining and that recruitment is low (i.e., a small number of young have been observed) (Zhirnov and Ilyinsky 1986, Tulgat and Schaller 1992, Xiaoming and Schaller 1996, Reading et al. 1999, McCarthy 2000). Low

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camel recruitment may be due to high juvenile mortality rates or poor reproductive performance. Poor reproduction, in turn, could be due a variety of problems, including low conception rates or inability to carry fetuses to term.

Steroid fecal analyses offer non-intrusive methods of analyzing reproductive physiology in many animals (Lasley and Kirkpatrick 1991). Traces of steroid metabolites on and within fecal material can be extracted to assess reproductive states (Kirkpatrick et al. 1991a, 1996). Thus, simply through the collection of fecal samples from known individuals we are theoretically able to track reproductive performance in individual animals by assessing changes in levels of hormonal metabolites (estrone conjugate for estrogen, pregnandiol-3-glucuronide for progestin, and progestrone) associated with pregnancy (Shideler et al. 1994). Such analyses would permit us to determine if low camel recruitment was related to physiological problems associated with reproduction.

Our goal is to develop and refine steroid fecal analyses on domestic Bactrian camels (*C. b. bactrianus*) and then transfer that technology to the field to assess reproduction rates (e.g., pregnancy rates, parturition rates) of wild Bactrian camels in the field. Consequently a preliminary study was carried out with domestic camels being held and bred at the Denver Zoo.

Methods

Fecal Collection

Fecal samples were collected from 3 domestic Bactrian camels being held at the Denver Zoological Gardens. Two camels, Shirley and Betty, were sampled from 13 and 37 days, respectively, before parturition, and for 7 and 179 days, respectively, following parturition. A third camel, Nadine, did not become pregnant and therefore was used as a control. Samples were collected opportunistically from keepers only when individual camels were observed defecating to insure that individuals could be matched to fecal samples. Samples were labeled (date and individual) and stored in a zip-locked storage bag in a freezer (<-5 °C).

Fecal Extraction

Fecal samples were thawed at room temperature for 2-3 hours. After samples were thawed, 0.5 gm of fecal material was weighed and placed into glass scintillation vials. Samples were solubilized to extract steroid metabolites. For each 0.1 gm of fecal material 1.0 ml of 20% methanol in modified enzyme immunoassay (EIA) phosphate buffer was added (Stoops et al. 1999). Samples were then shaken at room temperature for 18-24 hours and then centrifuged at 2,500 rpm for 10 minutes. The supernate was carefully removed for hormone metabolite testing. Remaining residue was subsequently dried to determine the sample's water content.

Enzyme Immunoassays

Fecal steroid concentrations were measured using EIA techniques described by Shideler et al. (1994) for estrone conjugates (E1C), an estrogen metabolite; pregnandiol-3-glucuronide (PdG), a progestin metabolite; and progesterone (P4). Results were expressed in ng steroid metabolite/g dry feces.

Results and Discussion

Fecal hormone analysis of E1C, PdG, and P4 were compared between two pregnant (Shirley and Betty) and one non-pregnant camel (Nadia) (Figure 1, Appendix). Pregnancies were confirmed by birth. Among the three analytes tested, the E1C metabolite appears to be the best indicator for detecting a pregnancy. There was an approximate 20-fold increase in E1C in a pregnant vs. non-pregnant camel at 1 week pre-parturition and a 2-3 fold difference in progesterone. PdG however, was non-discriminatory in differentiating the pregnant animals from the non-pregnant animal. However, after parturition there was a noticeable drop in both PdG and P4 in both of the pregnant Bactrian camels studied.

These studies only examined steroid metabolite concentrations from 7 to 40 days pre-parturition, thus it will be important to examine values for entire pregnancies if this approach is to prove useful in the field. In a similar study of pregnancy detection in bison, for example, discriminating urinary hormone metabolite values did not occur until the beginning of the second trimester of pregnancy (Kirkpatrick et al. 1991b), and in elk discriminating values do not appear until the third trimester of pregnancy (White et. al. 1995).

The data from this pilot study suggest that fecal steroid metabolite analysis may be a useful tool for identifying pregnant, free-ranging camels. The next obvious step in the development of this methodology will be staging a full pregnancy in captive Bactrian camels before taking these methods to the field for use on wild camels. Because of the critical status of wild Bactrian camels and their extreme shyness (flight distances are usually 2 or more kilometers; Reading et al. 1999), such non-invasive methods offer a potentially valuable tool for assessing possible causes of wild camel decline in Mongolia. These methods have been successfully applied to wild populations of feral horses (*Equus cabalus*) (Kirkpatrick et al. 1991a), bison (*Bison bison*) (Kirkpatrick et al. 1996), elk (*Cervus canadensis*) (White et al. 1995), and caribou (*Rangifer caribou*) (Messier et al. 1990). Other possible causes of camel decline, such as wolf (*Canis lupus*) predation (Tulgat and Schaller 1992, Tulgat 1995), can simultaneously be assessed. Identifying the cause(s) of wild camel decline is the first crucial step in developing effective conservation programs.

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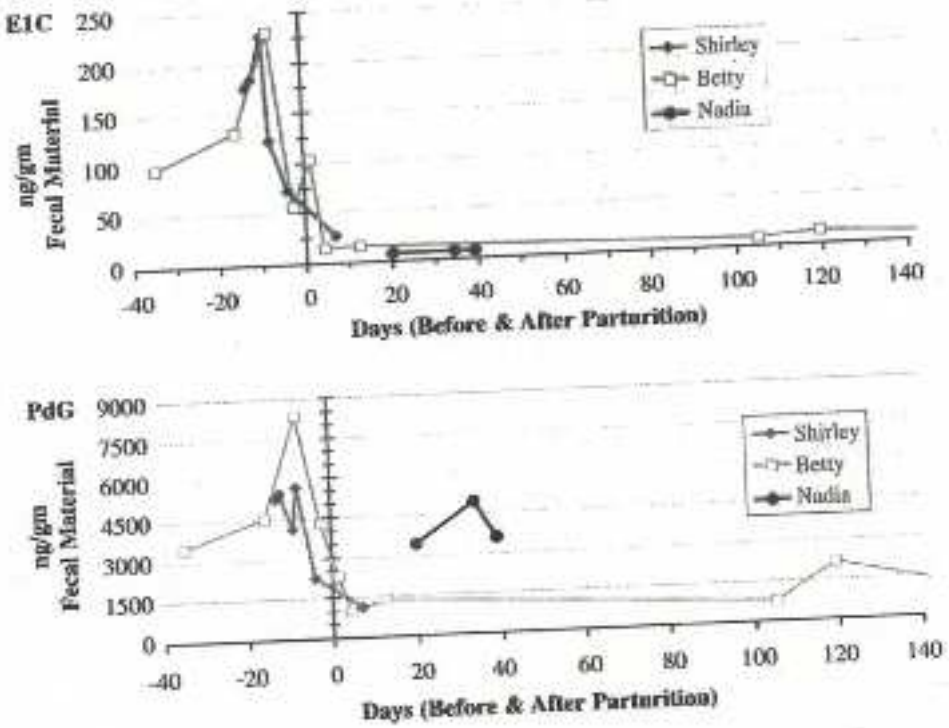

Figure 1. Estrogen (E1C) and progestrone (P4 and PdG) metabolite concentrations in the feces of 3 domestic Bactrian camels (*Camelus bactrianus*) at the Denver Zoo.

Note: Nadia was a control animal that was not impregnated.

Appendix:

Estrogen (E1C) and Progestrone (P4 and PdG) Metabolite Concentrations in the Feces of 3 Domestic Bactrian Camels (*Camelus bactrianus*) at the Denver Zoo.

Camel	Date	E1C ng/gm Dried feces	PdG ng/gm Dried feces	P4 ng/gm Dried feces
Shirley				
	3/15/97	177.3	5271	531.9
	3/16/97	184.4	5425	532.2
	3/19/97	227.1	4134	465.2
	3/20/97	123.4	5680	497.5
	3/24/97	72.7	2261	247.6
	3/28/97	parturition		
	4/4/97	26.6	1158	328.7
Nadia (non-pregnant)				
	4/11/97	7.7	3406	301.5
	4/25/97	7.3	4950	323.8
	4/30/97	7.9	3549	260.6
	5/24/97	death		
Betty				
	3/29/97	95.4	3468	317.7
	4/17/97	131.5	4494	409.1
	4/25/97	229.6	8340	798.4
	4/30/97	55.1	4261	340.8
	5/3/97	parturition		
	5/4/97	102.1	2278	389.3
	5/7/97	14.4	940	196.5
	5/15/97	14.7	1355	391
	8/16/97	7.4	759	227.4
	8/30/97	12	2140	348.8
	10/22/97	3.4	482	127.8
	10/29/97	5.7	552	163.5

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