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RESPONSE OF URINARY HYDROXYPROLINE TO DIETARY PROTEIN AND FASTING IN WHITE-TAILED DEER

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ABSTRACT: The effects of dietary protein, fasting, and refeeding on urinary hydroxyproline of nine captive female white-tailed deer (*Odocoileus virginianus*) were examined from 23 February to 3 May 1984 in northern Minnesota. In the fasted group, mean hydroxyproline : creatinine (OHP:C) was greater ($P < 0.05$) at week 4 compared to baseline at week 0. Between fasted deer and deer fed high protein-high energy (HPHE) and low protein-high energy (LPHE) diets, no difference in OHP:C ratios was detected during the initial 4 wk of the study. Urinary OHP:C ratios were significantly ($P < 0.05$) greater in the fasted group during refeeding, concomitant with greater feed consumption and weight gain. There was also a significant ($P < 0.02$) time effect in the fasted-refed group; OHP:C ratios increased during these two phases of the study. There was no difference between the HPHE and LPHE fed deer in renal OHP excretion. However, mean OHP:C ratios in yearlings (16.8 ± 2.2) were greater ($P < 0.001$) than in the adults (7.5 ± 1.2) of those groups, indicating a higher collagen turnover rate. Urinary OHP:C shows potential as an indicator of growth and starvation, and the data presented may serve as reference values.

Key words: Deer, fasting, hydroxyproline, nutrition, *Odocoileus virginianus*, refeeding, urine, experimental study.

INTRODUCTION

Hydroxyproline is a hydroxylated amino acid predominantly found in collagen associated with bone and connective tissue and is excreted in urine primarily in peptide form (Jasin et al., 1962; Grant and Kachmar, 1982). Urinary hydroxyproline reflects daily collagen turnover and in humans has shown great potential as an index of nutritional status and growth rate (Whitehead, 1965; Howells et al., 1967). It also has generated interest, although little research, by workers seeking such indices for wildlife (McCullagh, 1969). Greater urinary hydroxyproline is usually indicative of an elevated nutritional plane and increased growth rate (Lindstedt and Prockop, 1961; Jasin and Ziff, 1962; Jasin et al., 1962; Howells et al., 1967; McCullagh, 1969). The objective of our study was to determine the effects of dietary crude protein, fasting, and refeeding on urinary hydroxyproline in white-tailed deer (*Odocoileus virginianus*).

METHODS

Nine nonpregnant, female, captive white-tailed deer (seven adults, two yearlings) were maintained separately in outdoor enclosures near Grand Rapids, Minnesota (DelGiudice et al., 1987a). Three treatment groups each consisted of three deer. Two groups were fed pelleted commercial diets ad libitum from 23 February through 3 May 1984. One diet contained high protein-high energy (HPHE; 13.0% crude protein, 2,406 kcal digestible energy (DE)/kg) and the other low protein-high energy (LPHE; 6.9% crude protein, 2,406 kcal DE/kg). Each of these groups included a yearling. The third group was fasted from 27 February to 22 March 1984 (24 days), then refed the HPHE diet ad libitum for the remainder of the study (DelGiudice et al., 1987a). Deer were dependent upon snow for water intake until 1 April 1984, whereupon water was provided ad libitum.

Deer were anesthetized and sampled every 2 wk between 0800 and 1200 hr from 23 February to 3 May 1984. They were chemically immobilized with a combination of 75 mg of xylazine hydrochloride and 300 mg of ketamine hydrochloride (Mech et al., 1985), weighed, blood sampled, and catheterized for urine as part of a more comprehensive nutritional study (DelGiudice et al., 1987a, b). Urine samples were stored frozen at −20 C. Thawed urine samples were assayed for OHP by the spectrophotometric method of Prockop and Udenfriend (1960) as systematized by Howells and Whitehead (1967). Creatinine concentrations also were determined by spectrophotometry (Jaffé, 1886) with modifications and reagents recommended by Abbott Laboratories (1984). Comparisons of renal OHP excretion between groups were made as OHP:C ratios to control for excretion variability associated with single, random urine samples (DelGiudice et al., 1987c).

Data comparisons between experimental groups were conducted via one-way analysis of variance (ANOVA) with repeated measures (Hintze, 1982). Time effects within treatment groups were measured by two-way ANOVA (time versus deer). Data are presented in the text as means ± standard error of the means.

RESULTS AND DISCUSSION

There was no difference in mean urinary OHP:C ratios between the HPHE and LPHE fed deer during the 10-wk study, so these data were pooled (HE fed group) for comparisons with the fasted group (Fig. 1). Furthermore, no effects on OHP:C over time were detected in the HPHE or LPHE fed deer. Mean OHP:C (×10,000) ratios ranged from 5.7 ± 0.7 to 13.3 ± 7.3 in the HPHE fed deer and from 6.5 ± 3.5 to 15.7 ± 6.2 in the LPHE fed deer.

Greater renal OHP concentrations generally are associated with an elevated nutritional plane and increased growth rate (Lindstedt and Prockop, 1961; Jasin and Ziff, 1962; Jasin et al., 1962; Howells et al., 1967). We found no difference between the HPHE and LPHE groups in mean percent weight change during the study, so the absence of a difference in our OHP:C ratios parallels findings reported in the literature. Mean maximum percent weight loss in the HPHE and LPHE fed deer were 10.7 ± 1.6% and 9.3 ± 1.2% (DelGiudice et al., 1987a). Because only diets containing collagen (meat, fish, and poultry) or gelatin contain OHP, urinary OHP in herbivores is not directly affected by diet (Grant and Kachmar, 1982). McCullagh (1969) observed significant differences in OHP:C ratios between two populations of African elephants (*Loxodonta africana*) and suggested that the higher ratios reflected a faster growth rate and higher nutritional plane. Seasonal variations of OHP:C in both groups of elephants paralleled seasonal changes in rainfall, vegetation growth, and growth and resting rates of collagen turnover in all age groups.

In the fasted deer, mean OHP:C was greater ($P < 0.05$) at week 4 than at week 0. There was a significant ($P < 0.05$) correlation ($R = 0.70$, $Y = 5.073 + 0.677x$) between percent weight loss and OHP:C ratios during fasting. Between our fasted group and HE fed deer, no difference in OHP:C was found during the first 4 wk; however, mean OHP:C ratios were greater ($P < 0.05$) in the fasted group during refeeding (Fig. 1), concomitant with their greater feed consumption during the first 4 wk of refeeding (DelGiudice et al., 1987a). Percent weight gain was significantly ($P < 0.01$) correlated ($R = 0.99$, $Y = 12.640 + 0.338x$) with OHP:C in these deer. The elevated OHP:C ratios during refeeding were associated also with thyroxine concentrations that increased from 6.3 μg/dl at week 4 (22 March) to 10.4 μg/dl at week 10 (3 May) (DelGiudice et al., 1987a) and with elevated metabolic rates (Silver et al., 1969). In the fasted-refed deer, mean ratios generally increased ($P < 0.02$) during the 10-wk study (Fig. 1).

Deer 6204's OHP:C ratio increased to 20.0 at week 4, 2 days before she died of fast-related complications (DelGiudice et al., 1987a). An OHP:C of 32.0 was measured at week 10 in deer 6241 of the fasted-refed group (Fig. 1), after 2 wk of voluntary fasting when relocated to a different pen and losing 23% of her prestudy weight (DelGiudice et al., 1987a).

Although the urinary OHP levels excreted by the HE fed deer and fasted deer were comparable at weeks 2 and 4 (Fig. 1), the collagen sources of the OHP were probably different. Elevated urinary OHP observed in normally fed rats (*Rattus norvegicus*) and humans are associated predominantly with the turnover of the metabolically active, soluble collagen pool, with

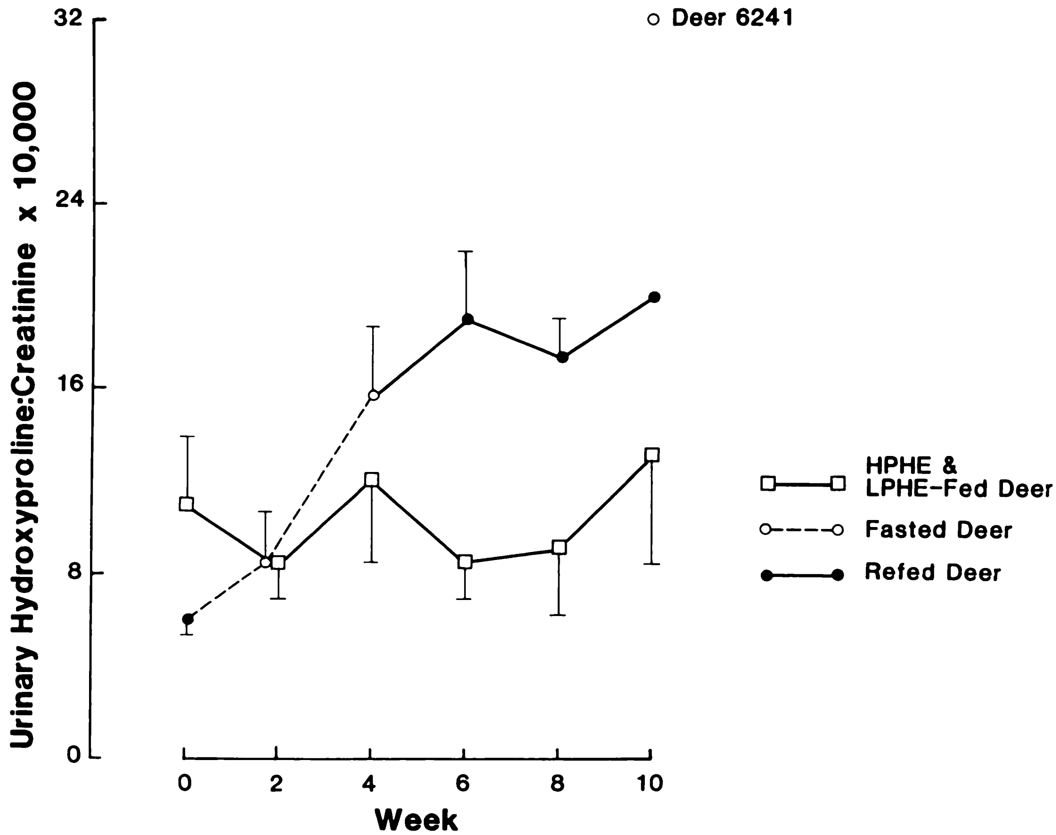

FIGURE 1. Mean (±SE) urinary hydroxyproline : creatinine ratios for white-tailed deer fed HPHE (high protein-high energy) and LPHE (low protein-high energy), fasted, and refed, 23 February to 3 May 1984, Grand Rapids, Minnesota; deer 6241 voluntarily fasted after week 8 until week 10 (see text).

greater concentrations and proportionately larger soluble pools being associated with younger growing individuals (Gross, 1958; Lindstedt and Prockop, 1961; Jasin et al., 1962; Smiley and Ziff, 1964). We measured greater ($P < 0.001$) overall mean OHP:C ratios in our two HE fed yearlings (16.8 ± 2.2) compared to the adult deer (7.5 ± 1.2) fed HE. In fact, when yearling OHP:C data were excluded from the analysis, the mean OHP:C ratio of the fasted deer was greater ($P < 0.04$) than that of the HE fed adult deer at week 4.

Our fasted deer lost proportionately more weight (18 ± 4%) during the first 4 wk of the study than did the HE fed deer (DelGiudice et al., 1987a), and the high OHP:C ratios of the former were probably more attributable to catabolism of the large pool of mature collagen (Prockop, 1962), much of which is associated with the bone matrix. Increased urinary OHP has been documented in growth-stunted progeny of female rats maintained with a restricted diet during gestation and lactation (Hsu and Chow, 1973). Evidence in that study indicated the elevated OHP was derived from increased proteolysis. Serum and urinary calcium (Ca) had gradually declined by week 4 in the two deer that survived fasting (DelGiudice et al., 1987a, b). The higher OHP:C ratios measured in fasted deer 6204 (week 4) and anorexic deer 6241 (week 10) were associated with elevated renal excretion of Ca (DelGiudice et al., 1987b), indicating increased net bone resorption (Goulding, 1981). Elevated serum and urinary OHP have been measured in postpartal lactating cows compared to prepartal nonlactating cows, indicating the importance of bone-matrix catabolism to Ca homeostasis (Black and Capen, 1971). High serum and urinary urea nitrogen in fasted deer in the present study also indicated increased proteolysis (Goodman et al., 1980).

We catheterized four captive adult does during August 1984, while they subsisted on natural vegetation supplemented by the HPHE diet, and we measured a mean OHP:C ratio of 20.8 ± 9.5. Three of the four deer had values comparable to the HE fed group during spring (week 10, Fig. 1), whereas one deer yielded a much higher ratio (49). All the ratios were notably higher than the overall mean for HE fed adults during the winter study, indicating that there is a seasonal effect and faster collagen turnover during summer compared to winter.

CONCLUSION

Urinary OHP:C ratios do not appear useful for discriminating between deer consuming diets of different crude protein content if the direction and degree of weight change do not differ between the deer. However, this study has shown that prolonged fasting is associated with increasing OHP:C ratios that are probably reflective of increased proteolysis and deteriorating condition. Deer growth rates, age, and season also influence renal OHP excretion. It is evident from this study that OHP:C data may be useful only if considered in conjunction with knowledge of the deer's age and the season of urine sample collection. Additional research is indicated for a more thorough understanding of the dynamics of OHP in relation to deer nutrition and condition.

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