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Secretion Patterns of Growth Hormone in Growing Captive Mithuns (*Bos frontalis*)

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ABSTRACT—A study was conducted in May 2003 to characterize plasma growth hormone (GH) pattern in growing mithuns (*Bos frontalis*), a rare semi-wild ruminant. Six mithun calves averaging 235 day of age and 124 kg were maintained in semi-intensive system and group-fed once daily. Animals gained at a mean rate of 0.54 kg/day, with individuals ranging from 0.34 to 0.66 kg/day. Blood samples collected at 15-minute intervals starting from 0600h for nine-hour period were assayed for plasma GH. Growth hormone patterns consisted of frequent pulses of varying amplitude. Growth hormone pulses occurred at an average frequency of 0.69/h, the rate did not differ markedly among mithuns nor hour of day. The magnitude of GH secretory pulses varied significantly among mithuns. Growth hormone peaks averaged 95.0 and 45.2 ng/ml in mithuns having the highest and lowest GH peaks, respectively. Peak and mean GH levels were associated positively (r=0.98, P<0.001) and both were associated negatively (r=–0.97 and –0.98, respectively; P<0.01) with rates of gain. Results from the study show that 1) GH peaks occur at frequent intervals throughout the sampling period and 2) alteration in GH levels and patterns are elicited more by pulse amplitude than frequency modulation.

Key words: Mithun-endocrinology, patterns, somatotropin, rare, semi-wild

INTRODUCTION

Mithun (*Bos frontalis*) is a rare semi-wild bovine species, found mainly in the North-Eastern Hills region (NEHR) of India. This unique livestock species is also found, though less in population, in Bhutan, Myanmar, Bangladesh, China and Malaysia. This prized hill animal of NEHR has an important role in the economic, social, cultural, and religious life of the local tribal population under the undulating topography and adverse climatic conditions at moderately high altitude (300 to 3000 m mean sea level). The multifarious utility of mithun is well recognized. It acts as a potential source of meat. Recently, the milk production potentiality of mithun has also been explored with the ability to produce superior quality milk. Due to remoteness of their habitats and other ecological and socio-political factors, mithuns remain one of the most neglected ungulates.

The role of long-term synthetic bovine growth hormone-releasing factor (GRF) on growth enhancement and endogenous growth hormone (GH) release has been reported recently (Mondal and Prakash, 2003, 2004). In planning to evaluate GRF analogues for growth enhancement in young mithun calves, we wanted to know when during the day (from 0600h to 1500h) GRF analogues could be administered with minimal confounding induced with spontaneous endogenous GH peaks.

No information on GH patterns throughout the day time (from 0600h to 1500h) in mithun is available. Pituitary GH is secreted in a short-term highly episodic pulsatile manner in almost all species in which it has been examined (Anfinson *et al.*, 1975; Wheaton *et al.*, 1986). The present report describes GH patterns in six growing mithun calves. Of particular interest to us was whether GH pulses occurred sporadically, or were grouped into secretory episodes occurring more frequently at some times than others.

MATERIALS AND METHODS

Animal care and management

Six growing mithuns averaging 235 days in age (range=220 to 251 days) were used in a study conducted on May 3 and May 4, 2003. Body weights on May 4 are shown in Table 1. Mithuns were maintained in semi-intensive condition. These animals were fed with mixture of locally available green grasses and tree leaves at

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Table 1. Body weights, average daily gains and plasma growth hormone (GH) characteristics in growing mithuns

Mithun No.	Body weight Kg	Rate of gain Kg/day	Mean GH level ng/ml (±SEM)	Peak level ng/ml (±SEM)	Pulse frequency pulses/h
01	110	0.49	52.43±1.38	64.28±3.52[ab]	0.67
02	160	0.66	25.89±1.15	36.11±2.37[c]	0.67
03	100	0.49	52.23±1.84	64.19±4.06[ad]	0.78
04	118	0.63	29.02±1.51	43.10±3.06[c]	0.67
05	130	0.34	70.86±3.0	94.95±1.69[e]	0.67
06	126	0.61	42.14±1.62	56.56±2.96[bd]	0.67
Mean	**124±8.45**	**0.54±0.05**	**45.43±6.84**	**59.87±8.42**	**0.69**

[a,b,c,d,e]Means without a common superscript differ (P<0.05).

Table 2. Composition (on dry matter basis in percentage) of green grasses and/or tree leaves fed to the mithun calves.

Feed stuffs	Dry matter	Organic matter	Ash	Ether extract	Crude protein	Acid detergent fibre
Albizzia sp (21%)	44.5	95.3	4.7	2.4	15.2	31.9
Borrena hirticulata (9%)	12.1	81.6	18.4	0.6	17.8	18.1
Thysaenolaena maxima (13%)	27.0	94.7	8.3	1.9	12.3	37.9
Curculigo recusvata (27%)	14.7	84.5	15.5	3.0	19.9	34.2
Ficus hirta (20%)	26.7	83.4	16.6	1.4	14.3	32.0

1100h as shown in Table 2. These animals were also fed with concentrate mixture at the rate of 1.0 kg/day consisting of crushed maize grain (30%), wheat bran (15%), rice polish (20%), mustard cake (33%), mineral mixture (1%) and salt (1%). The chemical composition of the concentrate mixture was organic matter (92.74%), ash (7.26%), crude protein (19.00%), ether extract (6.68%), crude fibre (4.92%), nitrogen free extract (62.14%), total carbohydrate (69.90%) and acid detergent fibre (9.61%). Calves were born from September to October in 2002 at National Research Centre on Mithun Farm located at Medziphema area of Nagaland state of India. Rates of gain presented in Table 1 were calculated from differences between February and April weightings. Average daily gains calculated from March and April were similar.

Blood Sampling

Blood samples (3 ml) were collected from all animals by means of an indwelling jugular catheter starting from 0600h on each day at 15 minutes interval for 9 hours in heparinised tubes (20 IU heparin/ml of blood). Blood samples were centrifuged immediately and plasma stored at −20°C until assayed for GH. All experimental protocols and animal care met the regulations of Institutional Animal Care and Use Committee (IACUC). Before catheterization, local anesthesia (Xylocaine®) was given and after removal of catheter the animal was treated with antibiotic (Oxytetracycline®) for seven days.
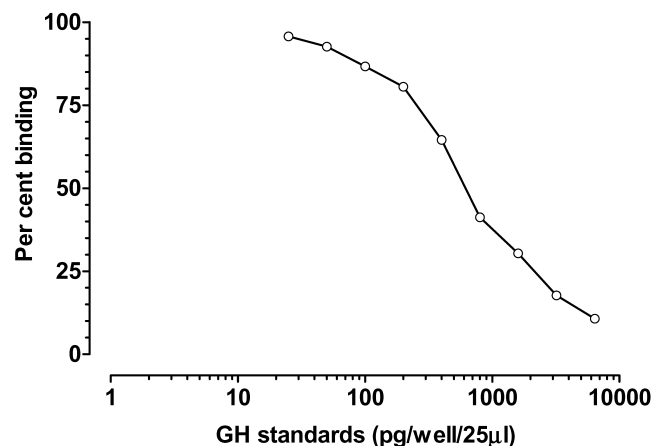
Growth Hormone Assay

GH was estimated by an enzyme immunoassay developed in our laboratory condition using second antibody technique as detailed below:

1. The first coating was performed by adding 0.63 μg of goat IgG antirabbit IgG dissolved in 100 μl of coating buffer (15 mM Na$_2$CO$_3$, 35 mM NaHCO$_3$, pH 9.6; Sigma-Aldrich corporation, St. Louis, Missouri, USA) per well of the microtiterplate (Greiner labotrechnik, Germany). The plates were subsequently incubated overnight at 4°C.
2. For blocking the remaining binding sites, 300 μl of 1%BSA

in phosphate buffer was added to all the wells and incubated for 40 to 50 minutes at room temperature under constant shaking.
3. The coated plates were washed twice with 350 μl/well of washing solution (0.05% Tween 20) using an automated microtiterplate washer (Model: EL 50 8MS, USA).
4. Duplicates of 25 μl of unknown plasma or bovine GH standards (USDA-bGH-B-1) prepared in charcoal treated plasma ranging from 25 pg/25 μl/well to 12,800 pg/25 μl/well were simultaneously pipetted into respective wells along with 100 μl of GH antibody diluted 1:40,000 in assay buffer (50 mM NaPO$_4$, 0.15 M NaCl, 0.02% thiomersal; pH 7.4; Sigma-Aldrich corporation, St. Louis, Missouri, USA) with the aid of a dilutor-dispensor. Plates were incubated overnight at room temperature after 30 minutes constant agitation. They were then decanted and washed two times with washing solution

Fig. 1. A typical standard curve for growth hormone in mithun plasma. Optical density was measured at 450 nm.

before addition of 100 μl of biotinyl-GH conjugate per well diluted 1:6,000 in assay buffer. The plates were further incubated for 30 minutes with constant agitation, decanted and washed four times. Then, 20 ng streptavidin-peroxidase (Sigma-Aldrich corporation, St. Louis, Missouri, USA) in 100 μl of assay buffer was added to all the wells and the plates now wrapped in aluminium foils were incubated for a further 30 minutes under constant agitation. All steps were performed at room temperature.

5. The plates were washed five times with washing solution and incubated further in the dark for 40 minutes after addition of 150 μl of substrate solution per well (Substrate buffer: 0.05M citric acid, 0.11M Na_2HPO_4, 0.05% ureum peroxide;

pH 4.0 adjusted with 5N HCl; substrate solution: 17 ml substrate buffer plus 340 μl 3,3′, 5,5′-tetramethyl benzidene; 12.5 mg/ml dimethyl sulfoxide; Sigma-Aldrich corporation, St. Louis, Missouri, USA). The reaction was stopped by the addition of 50 μl 4N H_2SO_4 and the colour measured at 450 nm with a 12-channel microtiter plate reader (Model: ECIL, Microscan, India).

A typical standard curve for GH in mithun plasma is shown in Fig. 1. The lowest GH detection limit significantly from zero concentration was 25 pg/25 μl plasma, which corresponded to 1.0 ng/ml plasma. The 50% relative binding (B/B0) sensitivity was 900 pg/25 μl plasma/well, which corresponded to 36.0 ng GH/ml plasma.

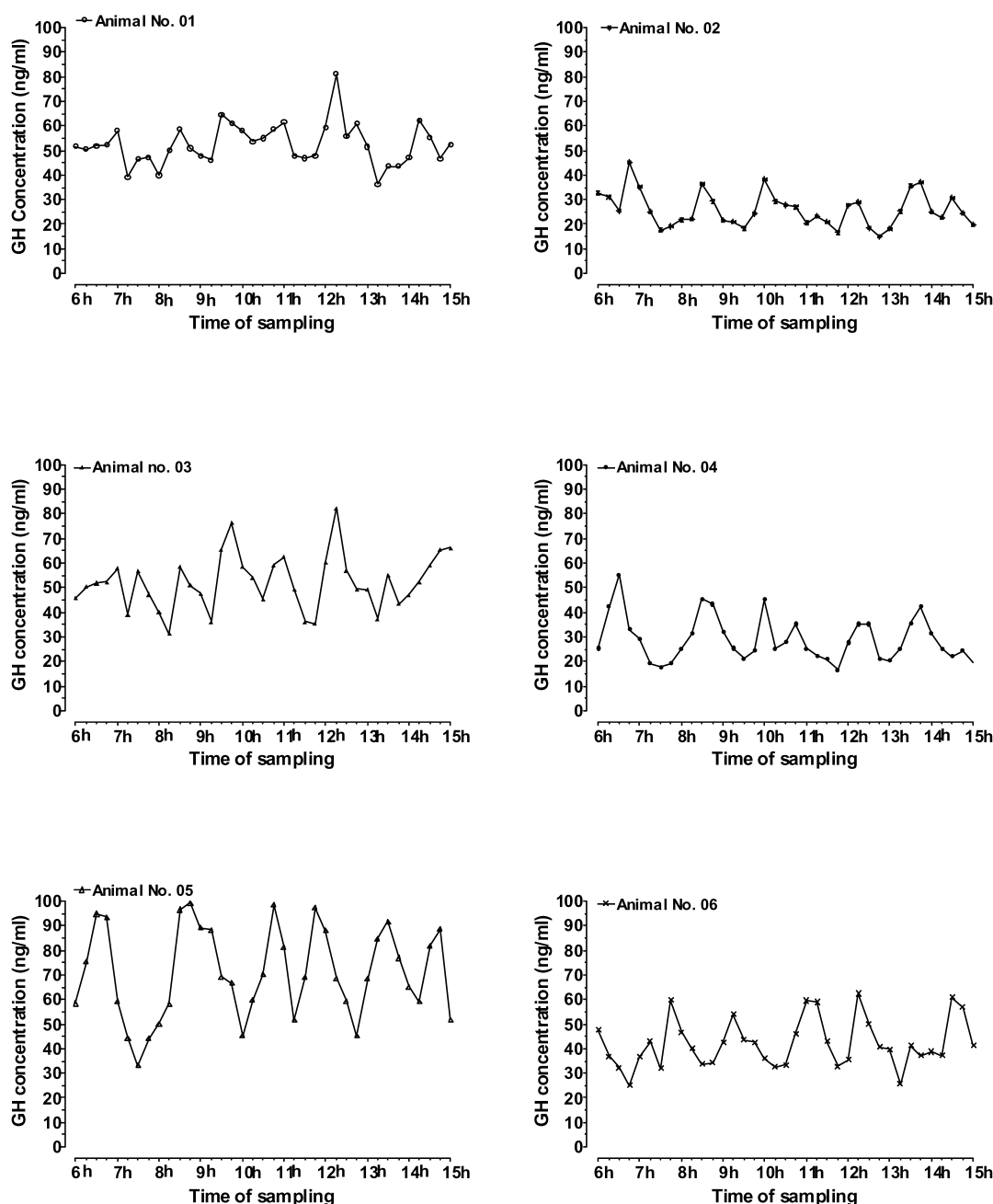Intra- and inter- assay coefficients of variations were deter-

Fig. 2. Plasma growth hormone (GH) profiles in individual mithun calves. Blood samples were collected at 15-minute intervals for 9 hours starting from 0600h to 1500h. Sunrise was at 0532h and sunset at 1740h. Animals were fed at 1100h.

mined using pooled plasma containing 2.0 and 256.0 ng/ml were found to be 9.6% and 6.8% and 8.8% and 7.1%, respectively.

Statistical Analysis

Mean concentrations of GH and frequency of GH pulses (pulses/9 h) were calculated for sequential blood sample. A GH pulse was defined as an increase in GH concentration that exceeded the previous nadir by two intra-assay standard deviations (Schillo *et al.*, 1988). Number of pulses occurring each hour and their peak levels were examined statistically for differences among mithun and hour in a complete block design and with Duncan's new multiple range test (Steel and Torrie, 1960). Linear correlation was used to examine associations among GH peak levels, GH mean concentrations and rates of gain.

RESULTS

Plasma GH patterns in individual mithun calves are presented in Fig. 2. The pulsatile nature of GH secretion is evident in each profile. GH patterns appear from visual inspection to consist of almost continuous series of secretory
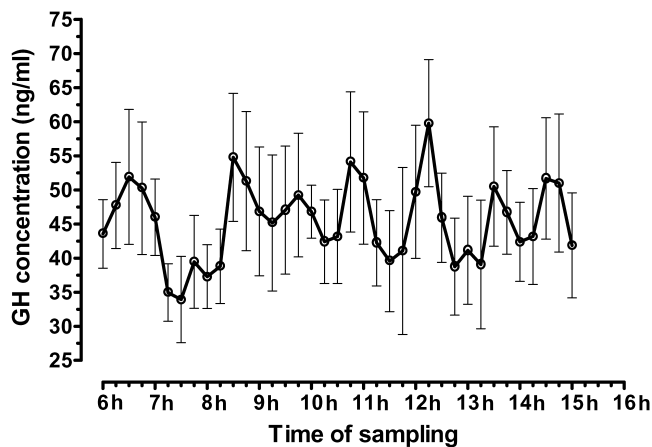

Fig. 3. Plasma growth hormone (GH) levels shown in Fig. 2 were averaged and presented in this figure with standard errors.
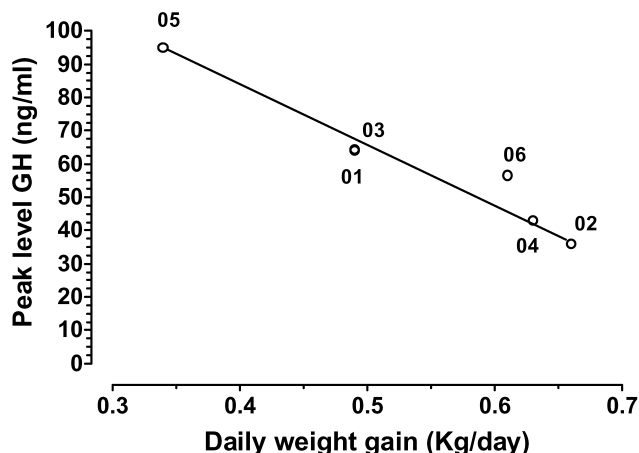

Fig. 4. Peak levels of growth hormone (GH) occurring during 9 hours sampling period in each mithun calf were averaged and plotted against the rates of gain. Rates of gain were determined for 120-day period preceding the day of blood collection. The mean GH shows negative correlation with rates of gain (r=–0.98).

bursts, separated into four or five episodes by intervening periods of decreasing GH levels. There were few occasions in which GH levels appeared to reflect basal GH secretion. Average GH peaks did not differ in respect to time of sampling (P>0.05; Fig. 3).

Mithun calves averaged 0.69 pulses/h and this rate did not differ significantly (P>0.05) among mithun (Table 1). Peak GH levels did differ (P<0.001) among mithuns; mithun no. 05 had the highest average peak level, 95.0 ng/ml and mithun no. 02 the lowest, 45.2 ng/ml (Table 1). Animals with higher peak GH levels also had higher mean GH concentrations and vice versa (r=0.98; P<0.001). Peak GH levels and mean GH concentrations were correlated inversely with rates of gain (r=–0.97 and –0.98, respectively; P<0.01; Fig. 4).

DISCUSSION

GH profiles of six mithun calves were characterized by frequent GH peaks of varying amplitude. Peaks occurred at an overall rate of 0.69/hour, which did not differ markedly among mithuns. There were few occasions in which GH levels appeared to result primarily from basal GH secretion. Frequent GH peaks also continues throughout the sampling period without sustained interruption (Wheaton *et al.*, 1986; Driver and Forbes, 1981). Whether the wide range of GH pulse amplitudes arise from alterations in the amount of GRF or somatostatin secreted, or from modulation of the responsiveness of somatotropin, somatostatin and GRF or from integrated changes in both hypothalamic GRF and somatostatin secretion and pituitary responsiveness is unknown.

Mithun no. 05 had six distinct pulses in 9-hour sampling period. Each two pulses were bracketed by a time gap where GH pulses were reduced in amplitude. This particular GH patterns were found in all the mithuns. Secretory episodes in all mithuns were individually specific in timing and duration. A similar situation exists in sheep in that rhythmic GH secretory patterns occur in some animals, while irregular patterns are observed in others (Driver and Forbes, 1981). GH pulses were present during each hour of the day when all the mithuns were considered; thus there is no time at which exogenous GRF could be administered without expecting induced and spontaneous GH pulses to overlap in some mithuns. However, fewest GH pulses occurred from 0700h to 0815h. Exogenous GRF may, therefore, be administered during 0700h to 0815h period with less effect of endogenous spontaneous release of GH for enhancement of growth in this species.

Plasma GH pattern reported for cattle (Wheaton *et al.*, 1986) differ qualitatively and quantitatively from those reported herein in mithun, a semi-wild rare bovine species. In their study, steers averaged 0.66 pulses/h that peaked at a mean level of 34.40 ng/ml. Mithuns in the present study averaged 0.69 pulses/h that peaked at a mean level of 59.9 ng/ml. Perhaps the contrasting patterns indicate the extent

to which factors related to species, age, weight and metabolic and behavioural states can affect GH secretion. State of domestication may, perhaps, play role on plasma GH levels, as mithun is still today a semi-wild species.

Mean GH level differed among mithuns. Mithuns having higher mean GH levels also had higher peak levels and vice versa. Differences in mean GH levels among dairy cows likewise have been attributed to the magnitude of secretory spikes (Vasilatos and Wangsness, 1981). Mean and peak GH levels were correlated negatively with rates of gain. Plasma GH concentrations and rates of gain and protein deposition have been reported to be inversely related in cattle (Purchas et al., 1971; Fox et al., 1974). Mithun calves gained an average 0.54 kg/day, but inter-animal variation was substantial, ranging from 0.34 kg/day in mithun no. 05 to 0.66 kg/day in mithun no. 02. GH peaks averaged 95.0 and 45.2 ng/ml in these mithuns, respectively. GH, which is lipolytic in ruminants and plasma GH concentrations are elevated at times when energy is being mobilized from tissue (Bassett, 1974; Vasilatos and Wangsness, 1981; Vernon 1981). A decrease in plasma non-esterified fatty acid levels stimulates GH secretion (Reynaert et al., 1975). The higher plasma GH levels in the slower-gaining mithuns suggests that these animals were in a poorer energy status than the faster-gaining mithuns. Mithuns were group-fed so it is not known whether some calves consumed more feed than the others. Although higher GH levels in slower-gaining mithuns is reconciled easily with a catabolic effect of GH on lipid metabolism, it seems paradoxical with respect to the anabolic action of GH on protein synthesis. In the context of endocrine and metabolic adjustments to negative energy balance, increased GH secretion may be of teleonomic value by increasing the efficiency of protein synthesis. This speculation stems from the improved feed efficiencies seen in animals administered exogenous GH (Moseley et al., 1982); and from more efficient utilization of energy and protein in energy-restricted cattle, demonstrated by compensatory growth following full feeding (Fox et al., 1974; Moseley et al., 1982). An accurate assessment of the relationship between GH profiles and protein metabolism awaits a better understanding of the cellular mode of GH action and the interaction of GH with other growth factors in this rare semi-wild species.

ACKNOWLEDGEMENTS

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