CIRCADIAN-TIME DEPENDENCE OF PLASMA NON-ESTERIFIED FATTY ACID, GLUCOSE, AND α-AMINO NITROGEN VARIATIONS IN MITHUN (*BOS FRONTALIS*) COWS

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ABSTRACT

Metabolite transport through the plasma is an essential part of normal life, and regulation of the plasma metabolite concentration is a part of the normal pattern of metabolic regulation during feeding and fasting. Wide variation of circulating metabolites may reflect ruminant efficiency and energy status in ruminants. In the present experiments, plasma contents of α-amino nitrogen (AAN), non-esterified fatty acid (NEFA), and glucose were measured in six male and six female mithuns, every 4 h for 24-h period. These three metabolites exhibited remarkable and surprising dynamics, which would not have been detected without measurements covering the entire diurnal cycle. However, Cosinor analysis did not statistically validate any sinusoidal rhythm for either of the metabolites.Holm-Sidak multiple comparisons revealed statistically significant ($P < 0.05$) differences in glucose concentration across all the selected circadian-time points and in NEFA content at only 03:00 and 15:00 h between the two sexes. Pearson correlation revealed that plasma glucose concentration significantly correlated ($r = 0.85$, $P < 0.0001$) with AAN content but not with NEFA.

keywords: circadian-time; metabolite; mithun; nonesterified fatty acids; alpha-amino nitrogen.

INTRODUCTION

Mithun (*Bos frontalis*), a rare semi-wild bovine species, is today believed to be the domesticated gaur and is considered to have entirely and mainly descended from the race of *Bos gauras gauras* of India and Myanmar (Simoons, 1984). This ruminant species, basically used in barter trade, marriage gift, and for beef purpose, is of special significance for the tribal people of North-Eastern Hills Region (NEHR) of India. Because of their multifarious utility as a potential source of meat and superior quality milk, mithuns have an important role in the economic and social life of the local tribal population. Changes in metabolite levels within physiological limits have a significant impact on ruminant metabolism (Nikkhah, 2012). Small changes in the relative state of protein synthesis to protein degradation can markedly affect concentration of certain α-amino nitrogen (AAN) in blood pools. Glucose is the principal source of energy for life processes of the mammalian cell. All cells require a constant supply of this indispensable nutrient, and relatively small changes are tolerated without adverse effects on the health of the animal (Kaneko, 1997). Thus, endocrine regulation of glucose metabolism is of utmost importance in mammalian homeostasis and homeorhesis. Insulin, a key hormone in endocrine regulation, facilitates the movement of glucose across cell membranes, thereby regulating the concentration of blood glucose. Decreased insulin concentrations shift glucose availability to tissues that do not rely on insulin to facilitate the movement of glucose into the cell and away from the muscle and adipose tissue which respond to insulin facilitated glucose uptake (Collier et al., 1984). Decreased insulin concentrations also promote the release of non-esterified fatty acid (NEFA) by the adipose tissue (McGuire et al., 1995). Rhythmic patterns of ruminant metabolism are shown in endogenous and exogenous rhythms of blood levels of many metabolites (Piccione et al., 2003, 2004, 2005). Therefore, a potential exists to synchronize occurrences of endogenous and exogenous diurnal and circadian rhythms to manipulate nutrient utilization and partitioning, and thereby, to optimize metabolism. Thus, it is important to determine if the actual concentrations of NEFA, glucose, and α-AN, or ratios between these metabolites in blood, are reflecting different physiological states.

MATERIALS AND METHODS

The animals used in this study were six male (BW ≈ 376 ± 37 Kg, mean ± SD) and six non-lactating, non-pregnant female (BW ≈ 312 ± 62 Kg, mean ± SD) mithun (*Bos frontalis*) cows. The two sex-group samples did not statistically differ in mean age (≈ 3 years). Animals were chosen for their availability on the farm of India's National Research Centre on Mithun (NRCM) and were kept under natural conditions with the air temperature and relative humidity of 24.8-30.5 °C and 79-81 %, respectively. The experiment lasted from August 22nd to

23rd, 2012 where the times of sunrise and sunset were 04:50 h and 17:45 h, respectively, at high moderate altitude of ≈ 303 MSL, and Vapor Pressure of ≈ 19.7 (Medziphema, India). The mithuns were maintained in semi-intensive condition. Time series were analyzed for time-effect by ANOVA and for rhythm characteristics by the single cosinor fitting procedure. All the animals were born at the NRCM farm located in the Medziphema area of Nagaland State in India.

RESULTS

The data revealed that concentrations of the three studied parameters significantly (One-way ANOVA, $P < 0.05$) varied according to the sampling-time within the 24-h cycle (Table 1).

Table1. Two-way ANOVA analysis of circulating NEFA, Glucose, and α-Amino nitrogen variations in mithuns. NEFA and Glucose vary as functions of both time and gender while α-Amino nitrogen content shows significant variation only according to the time. There's a significant interaction between the two factors time and gender for each studied metabolite.

SOURCE OF VARIANCE	df	NEFA		Glucose		α-Amino Nitrogen	
		F	*P*	*F*	*P*	*F*	*P*
TIME	5	45.4	< 0.0001	5.1	0.0012	13.4	0.026
GENDER	1	14.7	< 0.0001	69.5	< 0.0001	1.07	NS
INTERACTION	5	22.6	< 0.0001	12.0	< 0.0001	26.3	0.0004

No statistically significant rhythm was detected by cosinor analysis in any of the metabolites. 24-h means of NEFA (170.7 ± 33.4 µmol/L *vs.* 52.8 ± 6.1 µmols/L) and glucose (79.1 ± 1.4 mg/dl *vs.* 58.1 ± 1.5 mg/dl) concentrations were significantly (Student *t*-test, $P < 0.001$) higher in males than in females (Figures 1 & 2).

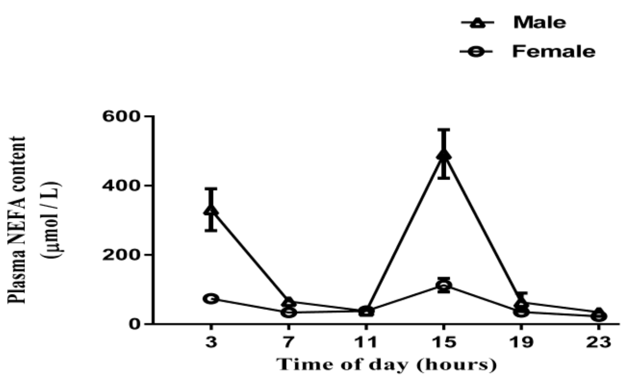

Figure 1

However, this gender-related difference in means was not significant for AAN content (Figure 3). Multiple-*t*-tests revealed significant ($P < 0.05$) gender-related differences at all the selected circadian-time points (03:00, 07:00, 11:00, 15:00, 19:00, and 23:00 h) in glucose content, but only at 03:00 and 15:00 h in NEFA concentration.

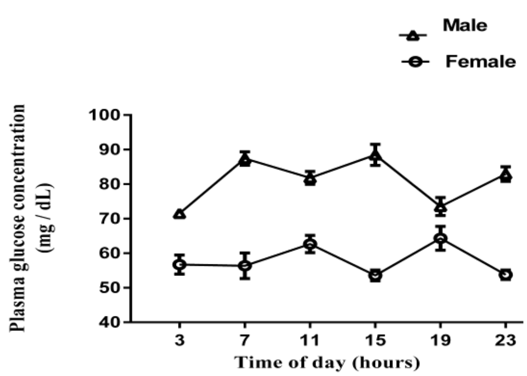

Figure 2

Glucose showed a strong positive correlation (r = 0.85, $P < 0.0001$) with AAN, but did not significantly correlate with NEFA. Statistically significant changes according to both sampling-time ($P < 0.002$) and gender ($P < 0.0001$) were revealed by two-way ANOVA in NEFA and glucose concentrations, but not in AAN.

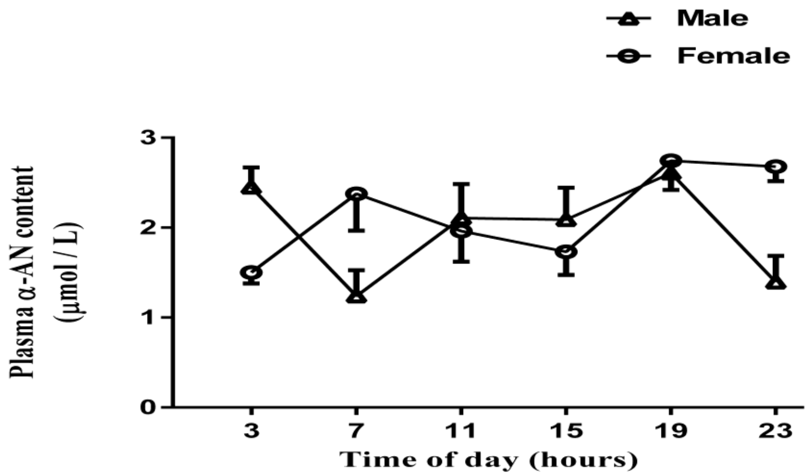

Figure 3

A statistically significant ($P < 0.0005$) interaction between the two factors time and gender was validated in all the three studied metabolites.

DISCUSSION

Our study provides new information about the effect of circadian-time on plasma concentrations of NEFA, AAN, and glucose. The major value of this study focusing on temporal patterns resides in the possibility of detecting such extreme differences as demonstrated here, which would easily be overlooked if only single time points were analyzed. Thus, differences were found in the concentrations of these metabolites that were dependent on the sampling-time. However, although the Cosinor analysis did not validate any rhythmicity in NEFA, AAN, and glucose variations, these metabolites concentrations showed marked fluctuations over a 24-h period. Hence, plasma NEFA concentration peaked early at the light- and dark-phase onsets, more precisely at ≈ 03:00 h and 15:00 h, but the NEFA peak level was five-fold lower in females than in males. Moreover, both glucose and AAN showed various peak times across the 24-h period with a marked unsimilarity between the both genders. In fact, the location of peak levels of several metabolites, including NEFA, AAN, and glucose may suggest the influence of photoperiod and lights-on-and-off times as regulators of feeding behavior and nutrient metabolism (Sehgal, 2004). Our findings showed that plasma glucose and AAN concentrations increased and that plasma NEFA decreased after eating at each of the two daily feeding times. These metabolite concentration responses were similar to those reported for growing mithun heifers (Mondal et al., 2005) and growing buffaloes (Mondal and Prakash, 2004). The increase of the plasma glucose concentration following the consumption of a meal, for instance, is known to be mainly due to the breakdown of carbohydrates supplied by the feed resources (Guyton, 1986). Furthermore, the absence of dramatic diurnal variation of plasma AAN and its postprandial elevation confirmed that feeding is the main source of the variation of this metabolite. AAN is reported to be absorbed across the ruminant tissues (30% of total net absorption) and intestinal tissues (70% of the total net absorption) in cattle (Theurer et al., 2002). These observations clearly suggest that the time from last meal, which was about 17:00 h in this study, besides the photoperiod, also influences the variations of NEFA and glucose concentrations in mithuns. Furthermore, our results revealed that both glucose and NEFA contents were higher in males than in females, suggesting that the demands of energy were greater in males. It is indubitable that in twice-daily-fed mithuns, an energy deficit occurs during the interprandial period, and that the animals respond by increasing plasma NEFA. Moreover, our findings showed that glucose content strongly correlated with AAN concentration, indicating that there is a positive relationship between the nutritional status and protein synthesis status of animals. In fact, blood glucose levels have been reported to provide an index of nutritional status (Morand-Fehr et al., 1977), whereas the plasma AAN is considered as an indicator of protein synthesis status (Hornick et al., 1998) in ruminant animals.

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