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Changes in Plasma GH, LH and Progesterone and Blood Metabolites Following Long-term Exogenous Somatoliberin Administration in Growing Buffaloes (*Bubalus bubalis*)

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Abstract: To investigate the effects of long-term somatoliberin on changes in plasma GH, LH and progesterone and blood metabolites in growing female buffalo calves, twelve calves within the age group of 6 to 8 months of age were divided into two groups (treatment and control groups) of six each in such a way that their mean body weight did not differ ($P>0.01$) and were fed as per Kearl standard (1982) for growing buffaloes. Treatment group buffaloes were administered i.v. with synthetic bGRF [somatoliberin; bGRF (1-44)-NH_2] @ 10μg/100 Kg. body weight at fortnight interval till 18 injections were completed (9 months). Blood samples were collected at fifteen days interval one month prior to and two month post-treatment for estimation of plasma GH, LH and progesterone and blood metabolites viz. non-esterified fatty acids (NEFA), alpha amino nitrogen and glucose. During pre-treatment period, plasma GH, LH and progesterone and blood metabolites did not differ ($P>0.05$) between the groups. Post-somatoliberin administration at the end of first month, the plasma GH and LH showed a higher trend ($P<0.001$) in somatoliberin treated buffaloes over untreated controls and this trend continued even in post-treatment period. Increasing trend ($P<0.01$) of plasma LH with advancing age of the calves was also recorded in both the groups. Unlike untreated controls, a definite up and rise trend of plasma progesterone was recorded in somatoliberin treated group during treatment period. Plasma NEFA, alpha amino nitrogen and glucose were found to be higher ($P<0.05$) in treatment than control group. Results indicate that long-term exogenous somatoliberin administration sustained a higher level of plasma GH even after cessation of treatment without showing any sign of refractoriness in this species. The buffaloes treated with somatoliberin may approach puberty earlier as they would reach the 'critical' body mass earlier, is also reflected by higher plasma progesterone and LH concentrations. However, detailed studies are required to ascertain the hypothesis.

Key words: Buffalo-endocrinology; somatoliberin; GH; LH; progesterone and blood metabolites

Introduction

Somatic growth is the outcome of interactions between genetic drive to grow, environmental factor and the supply of substrates to the organism. The endocrine system can be envisaged as the mechanism by which these interactions are coordinated and cellular replication and growth are modulated. The endocrine control of growth involves the complex interactions of several hormones and growth factors, acting both systemically and locally. Although many of these interactions are poorly defined, pituitary growth hormone and the insulin-like growth factors through which growth hormone generally acts, are essential for the normal postnatal growth of mammals (Nalbandov, 1963; Breier and Gluckman, 1991). Thus, important factor regulating growth of the animal is the growth hormone, which being anabolic in function (Machlin, 1976) increases body weight and enhances feed conversion efficiency in farm animals (Lapierre *et al.*, 1992; Sejrsen *et al.*, 1996). However, repeated direct exogenous administration of growth hormone to maintain sustained increased level of plasma growth hormone has often met with limited success. Such treatments with repeated growth hormone administration have resulted in increased serum IGF-I concentrations from liver, which has been thought to inhibit endogenous growth hormone secretion through negative feedback effects on both the hypothalamus and pituitary gland (Berelowitz *et al.*, 1981; Yamashitu *et al.*, 1986), thus causing refractoriness to growth hormone treatment with no positive beneficial effect.

As an alternative measure scientists are, therefore, beginning to explore the possibility of stimulating endogenous secretion of growth hormone by administration of a neurohormone, namely, growth hormone-releasing factor (GRF / Somatoliberin), which stimulates growth

hormone release from anterior pituitary. Long-term treatment with somatoliberin has been reported to enhance the responsiveness to subsequent administration of somatoliberin in humans and rodents (Heiman *et al.*, 1984). Exogenous somatoliberin is, therefore, advantageous to maintain sustained increase level of growth hormone secretion and its chronic repeated administration continues to induce and even enhances growth hormone release without any sign of refractoriness (Lapierre *et al.*, 1988). In heifers, the pituitary LH concentration increases four fold from birth to 12 months of age (Desjardins and Hafs, 1968) and serum concentration increases from 6 months of age through puberty (Gonzalez-Padilla *et al.*, 1975a). The progesterone level showed an increasing trend from birth through puberty in buffalo heifers (Jain and Pandey, 1983, 1985). But effects of long-term exogenous somatoliberin administration on plasma LH and progesterone has yet to be ascertained. Several short-term treatment of somatoliberin found to increase plasma non-esterified fatty acids (Johnson *et al.*, 1990; Enright *et al.*, 1989; Dahl *et al.*, 1990,1991), alpha amino nitrogen (Reynold *et al.*, 1992) and plasma glucose (Etherton *et al.*, 1986; Johnson *et al.*, 1990;Dubreuil *et al.*, 1990a; Enright *et al.*, 1989; Reynolds *et al.*, 1992), but the effects of the same for longer term is remained to be cleared.

The changes in plasma GH, LH and progesterone and subsequent changes in plasma metabolites after long-term somatoliberin administration in Indian livestock, buffaloes in particular, have not been documented so far. The objectives of the present investigation were, therefore, to determine the effects of a 9-month treatment of once in fortnight iv injection with somatoliberin on plasma GH, LH and progesterone and blood metabolites.

Materials and Methods

Animals: Twelve growing female Murrah buffalo calves within the age group of 6 to 8 months of age were selected for the study from the National Dairy Research Institute Farm, Karnal, India. After selection, the experimental animals were sent to individual pens and kept there for 30 days period to be adapted there. The animals were divided into two groups of six each (treatment and control groups) on the basis of their body weights, so that average body weights of the groups did not differ significantly ($P>0.01$) at the beginning of the experiment (66.33±6.73 and 66.25±5.39 Kg. for control and treatment group, respectively). The buffalo calves were fed on a ration consisting of concentrate mixture (CP=19.05% and TDN=67.55%) containing maize grain, groundnut cake, mustard cake, wheat bran, mineral mixture and salt and roughage (either berseem, maize or oat fodder as per availability in the farm). The calculated amount of concentrate mixture (which was given @0.5 Kg./animal and thereafter increased to 1.0Kg/animal) was fed twice a day. The roughage was provided twice a day at 9.30 A.M. in the morning and 3.00 P.M. in the afternoon. The animals were fed as per Kearl (1982) standard for growing buffaloes (targeted growth rate 500g/day) to meet the energy and protein requirement of the animals. For this purpose, concentrate mixture and roughage were calculated on dry matter basis once a week as per body weight of the animals and regular weekly adjustment of feed requirement was carried out. The animals having maximum body weight in the group was taken as standard. A free choice of fresh tap water was available throughout the day to all animals.

Treatment: Out of the twelve buffalo calves, six were kept as control group without giving any treatment. Another six calves (treatment group) were treated with somatoliberin (GRF) at an interval of fifteen days at the dose rate of 10 μg/100 Kg. body weight, i.v. till 18 injections were completed (9 months).

Synthetic bovine growth hormone-releasing factor [bGRF(1-44)-NH_2; Product code# G0644, M/s Sigma-Aldrich Co., USA], which is identical to endogenous hypothalamic GRF, was purchased as a formulated lyophilized substrate. For the experiment, the vials containing 100μg or 1000μg GRF were taken and was dissolved in sterile distilled water just before 12 hours of injection at 4^0 C. The amount of GRF solution required was calculated a day prior to injection by taking body weights of the animals of the treatment group as the animals were to be administered at dose rate of 10μg GRF/100 Kg body weight, iv in a total volume of 2-3 ml.

Blood sampling: Blood samples were collected twice a month from all animals by means of jugular venipuncture one month prior to and up to two month post-treatment. The blood samples were collected in heparinised tubes (20 IU heparin/ml of blood), put in the ice bucket and carried back to the laboratory immediately. Once in the laboratory, all the blood samples were centrifuged at 3000 rpm for 30 minutes at 4° C and plasma was separated and the plasma thus obtained was kept in the labeled storage vials of

2 ml capacity and stored at -20° C till analysis for GH, LH and progesterone and blood metabolites, viz. NEFA, alpha amino nitrogen and glucose. All experimental protocols and animal care met IACUC regulations.

Hormone assays

GH assay: GH was assayed by a highly sensitive enzymeimmunoassay using second antibody technique as described by Prakash *et al.* (2003). The lowest GH detection limit significantly from zero concentration was 50pg/100μl plasma, which corresponded to 0.5 ng/ml plasma. Intra- and inter-assay coeffient of variations were determined using pooled plasma containing 2.0 and 64.0 ng/ml were found to be 2.62 and 0.75% and 3.83 and 4.12%, respectively from 85 assays.

LH assay: Quantification of plasma LH was carried out by EIA developed and validated in our laboratory. The sensitivity of the assay for LH in plasma at minimum detection limit was 6.25pg/well/20μl or 0.31ng/ml plasma. The intra- and inter-assay coefficients of variation of plasma LH were 4.0 and 9.7%, respectively.

Progesterone assay

Plasma progesterone was estimated in ether-extracted samples in duplicate by a RIA procedure developed in our laboratory conditions as detailed by Prakash and Madan (1986) with slight modification. Hundred microlitre plasma samples were taken for ether extraction. The sensitivity of the assay for progesterone by extraction procedure at minimum detection limit was 4pg/tube and the 50% binding limit being 70pg/tube. The intra- and inter-assay coefficients of variation of plasma progesterone were 6.7 and 11.1%, respectively. Extraction efficiency of the plasma and assay buffer were 98.2 and 98.8%, respectively.

Estimation of Blood Metabolites: Plasma non-esterified fatty acids (NEFA) were estimated by using the copper soap extraction method modified by Shipe *et al.* (1980). The estimation of plasma alpha amino nitrogen was carried out following the procedure of Goodwin (1970) and plasma glucose was measured by GOD/POD method using commercial kits (Span Diagnostics Ltd., India; Product code # 25940)

Statistical Analysis: Paired t' test was employed to test the difference between plasma GH, LH and progesterone in both groups separately for three different periods, viz. pre-treatment, treatment and post-treatment. Overall differences of plasma metabolites, viz. NEFA, alpha amino nitrogen and glucose for 12-month experimental period were assessed by using paired t-test. To test the effects of treatment and experimental period on hormonal parameters, analysis of variance technique was used in the following statistical model:

$$Y_{ijk} = \mu + m_i + n_j + e_{ijk}$$

Where,

Y_{ijk}	=	The dependent variable,
μ	=	Overall population mean,
m_i	=	The effect of i^{th} treatment,
n_j	=	The effect of j^{th} month of experimental period, and
e_{ijk}	=	Random error associated with k^{th} individual, normally and independently distributed with mean 0 and variance σ^2.

Results

During the pre-treatment period, plasma GH, LH and progesterone and blood metabolites were found to be statistically non-significant ($P>0.05$) between the treatment and untreated control group of buffaloes. Post-somatoliberin administration at the end of first month, the plasma GH levels started to show a higher trend in somatoliberin treated animals over untreated controls and this trend continued throughout the experimentation with minor changes (Fig. 1). During treatment (10.75±0.736 vs. 7.12±0.542 ng/ml for treatment and control group, respectively) as well as post-treatment period (9.69±0.553 vs. 6.63±0.244 ng/ml for treatment and control group, respectively), the mean plasma GH levels of treatment group was significantly ($P<0.001$) higher than untreated controls and mean GH concentration differed significantly ($P<0.01$) on different months of the experimentation (Fig. 1). The plasma GH concentration following first somatoliberin administration started increasing and on month 3 onwards it showed a declining trend being lowest at month 7 (6.63±0.814 ng/ml) and there was an increasing trend thereafter, the value being highest at month 10 (13.78±3.647 ng/ml). No such trend of plasma GH was recorded in untreated controls.

The plasma LH levels in treatment group increased significantly ($P<0.001$) following the first somatoliberin administration (Fig. 2). During treatment (0.516±0.025 vs. 0.360±0.0142 ng/ml for treatment and control group, respectively) as well as post-treatment period (0.788±0.087 vs. 0.570±0.055 ng/ml for treatment and control

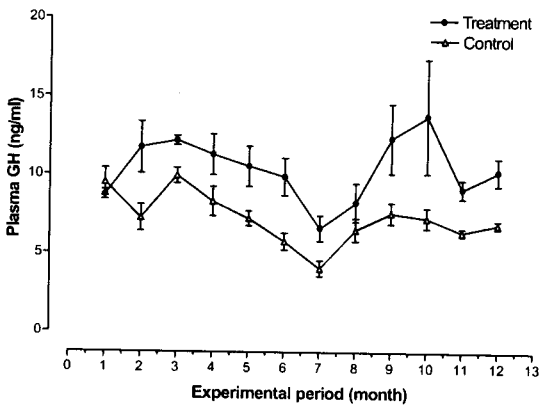

Fig. 1: Plasma GH (Mean±SEM) profile of treatment and control group of buffalo calves during 12-month of experimentation.

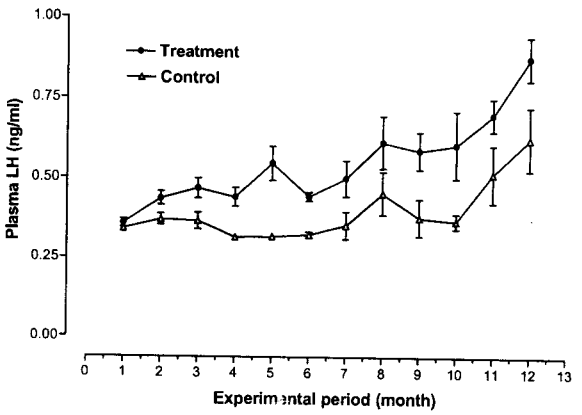

Fig. 2: Plasma LH (Mean±SEM) profile of treatment and control group of buffalo calves during 12-month of experimentation.

group, respectively) plasma LH levels were found to be significantly ($P<0.001$) higher in somatoliberin treated animals in comparison to control group of animals. When we consider the plasma LH levels in respect to age of the animals, it was found that there was an increasing trend ($P<0.01$) in plasma LH levels with the increasing age of the animals in both the groups (Fig.2). The mean values of LH at month 1 and month 12 for treatment and untreated controls were recorded to be 0.360±0.013 and 0.340±0.019 ng/ml and 0.880±0.067 and 0.630±0.199 ng/ml,

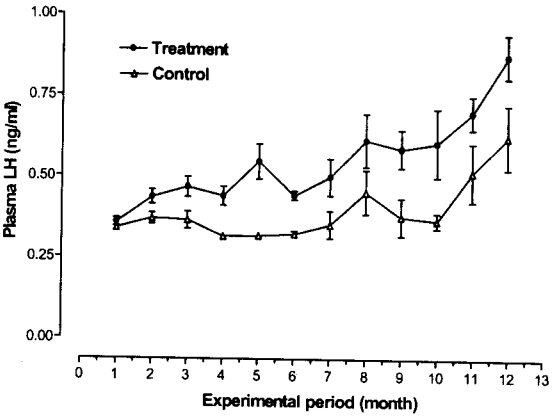

Fig. 3: Plasma progesterone (Mean±SEM) profile of treatment and control group of buffalo calves during 12-month of experimentation.

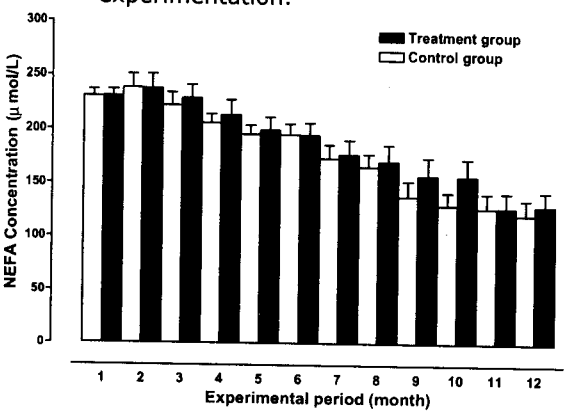

Fig. 4: Comparative monthly plasma Non-esterified fatty acid profile (μmol/L) in treatment and untreated control group of buffalo calves

respectively.

Though the plasma progesterone concentrations during the pre-treatment period (0.565±0.0143 and 0.562±0.0115 ng/ml for treatment and control group, respectively) were statistically non-significant ($P>0.01$) between the groups, but immediately following the first somatoliberin administration, the plasma progesterone levels in treated animals increased and reached a peak level of 0.675±0.024 ng/ml at month 3 declining thereafter to 0.510±0.013 ng/ml in the month 6 of the experiment (Fig. 3). There was a rise in

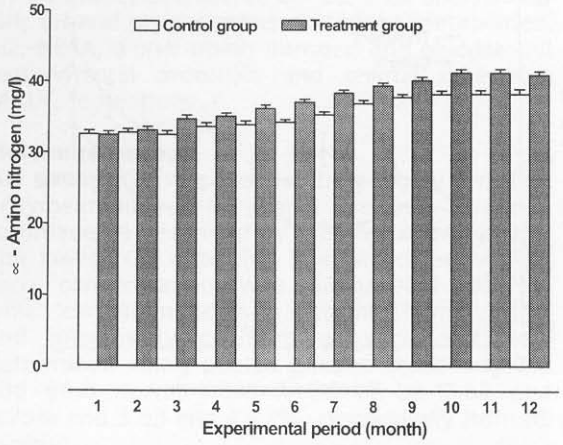

Fig. 5: Comparative monthly mean plasma ∝-amino nitrogen profile (mg/L) of treatment and untreated control group of buffalo calves

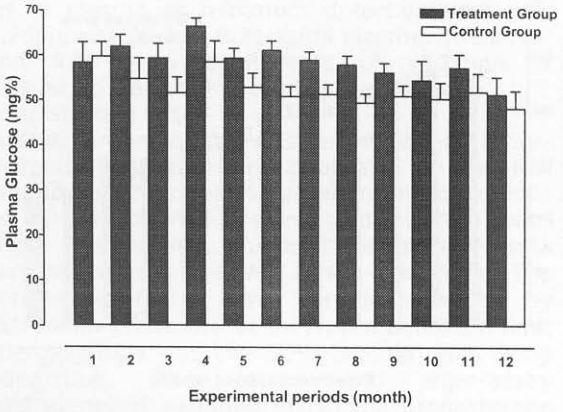

Fig. 6: Comparative monthly mean plasma Glucose concentration (mg%) between treatment and untreated control group of buffalo calves

mean progesterone levels from month 6 again reached at a peak level at month 7 (0.620±0.034 ng/ml) followed by another peak at month 10 (0.646±0.017 ng/ml) showing minor fluctuations thereafter. No distinct trend was found in plasma progesterone in untreated controls (Fig. 3). Though the plasma progesterone during post-treatment period was statistically non-significant (P>0.05), even then there was an increasing trend in somatoliberin treated buffaloes over untreated controls. In post-treatment period, the plasma progesterone levels were found to decline in both the groups. A treatment X month interaction was also detected (P<0.05) for plasma progesterone.

In the first two months of the experiment, the plasma NEFA concentrations in both the groups remained similar (P>0.01). Thereafter, NEFA concentrations showed an increasing trend (Fig. 4). The NEFA concentrations in treated animals, which were similar to those of untreated animals on 6th month of the experiment, increasing significantly (P<0.05), thereafter, till the 11th month, when the plasma NEFA concentrations again became similar with that of control group followed by another rise in concentrations on the 12th month. In this present investigation, the mean plasma NEFA concentrations of treatment group was found to be significantly higher (P<0.05) in comparison to control group of animals (185.56 ± 11.02 vs. 178.86 ± 12.14 μmol/L), and NEFA concentrations decreased significantly (P<0.01) as the age of the animal increased. Like plasma NEFA, in the first two months of the experiment, plasma α-amino nitrogen concentrations did not differ significantly (P>0.05) between the groups, but from month 2 onwards plasma α-amino nitrogen increased significantly (P<0.05) over untreated control group of animals (Fig. 5). The mean values for treatment and control group of animals were 37.52 ± 0.92 and 35.37 ± 0.69 mg/L, respectively. Unlike changes in plasma NEFA, plasma α-amino nitrogen increased significantly (P<0.01) with the advancement of age of the animals in both the groups. During first month of the experiment, mean plasma glucose levels in both the groups were similar (59.82 ± 3.21 and 59.35 ± 4.57 mg/100 ml for control and treatment group, respectively). Thereafter, the levels of plasma glucose started increasing over control and this trend continued till the end of experiment. Overall means of glucose for treatment (58.70 ± 1.10 mg/100 ml) and control groups (55.14 ± 1.01 mg/100 ml) differ significantly (P<0.05) throughout the experiment. A decreasing trend (P<0.05) of plasma glucose levels was observed in both the groups with the advancement of age.

Discussion

In the present investigation, plasma GH was found to be significantly (P<0.01) higher consistently throughout the experiment (except pre-treatment period) in somatoliberin treated animals than in controls (Fig. 1). Ringuet *et al.* (1994) subjected growing Holstein dairy heifers for 246 days twice daily with subcutaneous injections of somatoliberin, which resulted in elevated plasma GH levels throughout the trial than untreated controls and suggested that

somatoliberin could be used to induce daily growth hormone release without loss of responsiveness over an extended period of time in young dairy heifers. In the present study, despite the dosage and time intervals between two injections being different, the responsiveness of exogenous somatoliberin had not faded away, and continued even after termination of somatoliberin administration. Similarly, sustained increase level of endogenous GH was remained for 10 days to 3 months (Enright *et al.*, 1986, 1988, 1989, 1993; Lapierre *et al.*, 1988, 1990; Moseley *et al.*, 1985, 1987; Dahl *et al.*, 1990; 1991; Hongerholt *et al.*, 1992; Simpson *et al.*, 1992; Binelli *et al.*, 1995; Vanderkooi *et al.*, 1995) in cattle treated with exogenous somatoliberin. Similarly, intermittent administration of somatoliberin enhanced secretion and circulating concentrations of growth hormone in sheep (Hart *et al.*, 1985; Della-Fera *et al.*, 1986) and lambs were not refractory to exogenous somatoliberin for least 8 weeks (Beermann *et al.*, 1990). Wheaton *et al.* (1988) observed that plasma growth hormone was sustained at 4 fold higher levels than controls. Pigs treated with exogenous somatoliberin were also found to maintain sustained increase level of endogenous growth hormone (Takano *et al.*, 1985; Dubreuil *et al.*, 1990a; Johnson *et al.*, 1990). Sustained higher level of plasma GH in somatoliberin-treated growing buffaloes in the present investigation may be explained by non-refractoriness to repeated exogenous somatoliberin-induced GH synthesis and release from somatotrophs of anterior pituitary as also explained in bovines (Cella *et al.*, 1985; Ringuet *et al.*, 1994; Lapierre *et al.*, 1988).

Several studies are also available which also describe a decreased GH response to exogenous somatoliberin and this decrease was explained to be due to ageing rather than chronic treatment (Sonntag *et al.*, 1980; Ceda *et al.*, 1986; Cuttler *et al.*, 1986; Lapierre *et al.*, 1990). The long-term somatoliberin treatment in the present study enhanced the responsiveness to subsequent administration of somatoliberin as reported in humans and rodents (Heiman *et al.*, 1984). Since the treatment was restricted to a period of 9 months, the GH decline due to ageing as perceived by other workers was not seen in the present study even in untreated controls.

Serum LH concentration decreased from birth to about 15 weeks of age and then increased to 39 weeks of age (Dodson *et al.*, 1988) and episodic LH release increases gradually from birth to puberty (Schams *et al.*, 1981; Day *et al.*, 1984), while others have been unable to detect such changes (Gonzalez-Padilla *et al.*, 1975a; McLeod *et al.*, 1984). In the present study, plasma LH concentrations of both the groups (treatment and control; Fig. 2) increased as the age of the animals advanced, which is supported by the experiment of Schams *et al.* (1981) and Dodson *et al.* (1988), and this increase may be due to wave-like follicular development patterns as found in bovines (Desjardin and Hafs, 1968; Evans *et al.*, 1994). Desjardins and Hafs (1968) observed an increase in the number of large and small ovarian follicles (>5 and <5 mm diameter) from birth to 4 months of age. In heifer calves as young as 2 weeks of age, ovarian follicles grew in a wave-like fashion similar to those of adult cattle and early rise in gonadotropin secretion (between 2 to 8 weeks of age) stimulated the increase in numbers of follicles and follicle diameters, indicating an early critical step in reproductive development (Evans *et al.*, 1994). Decline in negative-feedback mechanisms of estradiol on hypothalamic centres as the animals approaches puberty (Day *et al.*, 1984) has also been reported which may permit an increase in the frequency of LH pulses (Day *et al.*, 1984, 1987; Dodson *et al.*, 1988).

The trend of LH levels found in the present investigation for control groups is essentially similar to that found in cattle (Dodson *et al.*, 1988) and in buffaloes (Jain and Pandey, 1985). However, comparatively slight higher values as observed by Jain and Pandey may be due to a) infrequent collection of samples (once from particular age group), b) different animals used for different age groups, and c) different LH assay system employed by them.

Schoppee *et al.* (1996) found that active immunization against somatoliberin in calves at 3.5 months of age extended the age at puberty due to delayed stimulation of LH. The higher plasma concentrations of LH in somatoliberin treated animals may be a pointer to the possibility of puberty being advanced in these animals. Endogenous IGF-I concentrations have also been found to increase after somatoliberin administration (Plouzek *et al.*, 1988; Lapierre *et al.*, 1992; Reynolds *et al.*, 1993; Swanchara *et al.*, 1999), which have been known to alter the ovarian functions in the pre-pubertal bovines increasing plasma LH level also. A similar mechanism as seen in bovines may also be true in the case of the present investigation. Increasing trend of plasma LH in the present investigation (Fig. 2) may also be due to increased responsiveness of pituitary to LHRH as animals become older (Schams *et al.*, 1991; Day

et al., 1987).

In the present investigation, the plasma progesterone of somatoliberin treated animals was found to be higher than untreated control group of animals (Fig. 3). Spicer and Enright (1991), who found that treatment with somatoliberin analogs increased size of large follicles and progesterone concentration in medium sized follicles in mature heifers. Similarly, somatoliberin was found to increase progesterone production by granulosa cells in rats (Moretti *et al.*, 1990). *In vitro* studies show that the rate of progesterone synthesis is regulated by GH in bovine granulosa cells, via the local production of IGF-I (Savion *et al.*, 1981; Colenbrander *et al.*, 1984). Exogenous somatoliberin increased plasma GH and IGF-I in bovines, which may also be the factors causing higher progesterone concentration in somatoliberin treated buffaloes in the present study. Schemm *et al.* (1990) also reported positive correlations between GH treatment and concentration of progesterone. Gallo and Block (1991) observed increased progesterone secretion during estrous cycles and pregnancy when rbGH was injected and speculated that these effects may have been caused by an increase in IGF-I production stimulated by the rbGH treatment (Spicer *et al.*, 1990). In both the groups, the source of progesterone is luteinized tissue within the ovary located beneath the ovarian surface (Berardinelli *et al.*, 1979) and the role of these short luteal phases in the pubertal process are unclear. The significance of increased progesterone may be two-folds as suggested by Gonzalez-Padilla *et al.* (1975a,b). Progesterone may establish a phasic pattern of release of LH because after the concentration of progesterone increased the pattern of release of LH changed from one that showed a continuous series of relatively large peaks to one that showed intermittent or phasic peaks with low concentration between peaks. Alternatively, progesterone may act to sensitize the ovaries to LH. Estradiol-17β caused release of LH in the pubertal heifers, but these heifers did not ovulate unless pretreated with progesterone (Gonzalez-Padilla *et al.*, 1975b).

Slightly higher values of plasma progesterone in the present study in buffalo calves than reported by Jain and Pandey (1985) may be due to more frequent blood samplings and using the same animals for the whole study, whereas, Jain and Pandey (1985) collected single blood sample from different animals belonging to different age groups of buffaloes. Salama *et al.* (1994) reported that progesterone concentration throughout 4 weeks before puberty remained <0.5 ng/ml in Egyptian buffalo heifers, averaging 0.4 ± 0.8 ng/ml, which is comparable to our study. Similarly, throughout the 3 weeks prior to puberty, progesterone concentration ranged between non-detectable values (<0.1 ng/ml) and 1.7 ng/ml with an average 0.3 ± 0.03 ng/ml and the overall mean of progesterone concentration showed a early increase from third week before puberty (0.18 ± 0.05 ng/ml) to the third day pre-puberty (0.58 ± 0.12 ng/ml) in Egyptian buffaloes.

The result of the present investigation revealed significantly ($P<0.05$) higher mean plasma NEFA in somatoliberin treated animals over control though at 6th and 11th month of the experiment, there was no significant difference of plasma NEFA between the groups (Fig. 4). This result is found to be essentially similar to the observations of Dahl *et al.* (1990, 1991), who observed consistently greater plasma NEFA at 1, 30 and 59 days of somatoliberin treatment than in untreated cows, but differences were significant ($P<0.01$) only at 30 days of infusion. Similar results were also found by Lapierre *et al.* (1988) in lactating multiparous dairy cows and Binelli *et al.* (1995) in primiparous lactating cows. The plasma NEFA concentrations found in the present observation is within the range prescribed by Adler and Wertheimer (1962) in cattle, and by Singh (1992) in cows and buffaloes. The increased plasma NEFA in the present study in somatoliberin treated growing buffaloes may be due to the following reasons :

* Mobilization of lipid store to cope-up with the higher growth rate (Gluckman *et al.*, 1987).
* To meet the demand of energy required for higher growth (Eisemann *et al.*, 1986; Kartiarso *et al.*, 1989) as influenced by higher plasma GH, which being lipolytic in action increases lipolysis (Barbano *et al.*, 1992).
* Due to negative energy balance caused by higher growth rate in the somatoliberin-treated animals (Dahl *et al.*, 1990, 1991; Binelli *et al.*, 1995).
* Increased number of β-adrenergic receptors and greater sensitivity of lipolysis (Vernon and Flint, 1989) in response to increased GH as influenced by somatoliberin administration.
* Due to decreased liver removal of NEFA (Reynolds *et al.*, 1992).

The significant decrease of plasma NEFA with the advancement of the age of animals in both the groups found in the present study is in accordance with the result of Hornick *et al.*

(1998) found in growing bovines.

In contrast, Simpson *et al.* (1992) found no significant (P>0.01) difference of plasma NEFA in somatoliberin-treated and untreated control prepartum beef heifers due to the fact that all the animals were in positive energy balance. Similarly in pigs, Dubreuil *et al.* (1990b) observed no significant difference (P>0.05) of plasma NEFA by somatoliberin treatment, whereas, Johnson *et al.* (1990) found an increased serum NEFA in pigs treated with somatoliberin. On the other hand, Dubreuil *et al.* (1990a) found that a decrease in body fat occurred without an increase in sera free fatty acids in the pigs treated with somatoliberin analog, which could indicate that the molecular form of GH released by somatoliberin is not lipolytic in this species. These results are also indicative of a species specific response of NEFA production under somatoliberin treatment.

Hornick *et al.* (1998) reported that rapidly growing beef cattle had more plasma α-amino nitrogen, which is in agreement with the present investigation, where somatoliberin-treated buffaloes exhibiting higher growth rates (data not shown) also have higher (P<0.01) plasma α-amino nitrogen. The significant increase (P<0.01) in plasma α-amino nitrogen with advancement of age in both the groups is in close conformity with the result of Hornick *et al.* (1996, 1998) and this may be due to a higher tissue release or a decrease of tissue catch-up of amino acids from plasma, because the muscle protein degradation to muscle protein synthesis ratio varies with age (Simon, 1989).

Consistently higher (P<0.01) level of plasma glucose in somatoliberin-treated animals than seen in untreated controls in the present investigation (Fig. 6) is in agreement with the results of earlier investigations in cattle (Enright *et al.*, 1989; Reynolds *et al.*, 1992), in sheep (Hart *et al.*, 1985), and in pigs (Etherton *et al.*, 1986; Dubreuil *et al.*, 1990a) treated with somatoliberin for a comparatively shorter period of time. In contrast, reports are also available that somatoliberin administration did not show any significant change in plasma glucose in cattle (Dahl *et al.*, 1990, 1991; Lapierre *et al.*, 1991; Hongerholt *et al.*, 1992; Simpson *et al.*, 1992; Enright *et al.*, 1993) and in pigs (Dubreuil *et al.*, 1990b).

The significantly higher (P<0.01) level of glucose in somatoliberin-treated growing female buffalo calves in the present study may be due to higher endogenous GH-induced insulin-resistant condition in insulin dependent tissues resulting in reduced cellular uptake of glucose (Etherton *et al.*, 1986; Walton and Etherton, 1986) as well as increased hepatic glucose output coupled with impaired glucose clearance (Gopinath and Etherton, 1989a,b). Dubreuil *et al.* (1990a), however, concluded that increase in serum glucose in response to exogenous chronic administration of somatoliberin may not be due to hepatic glucose release, because the GH-induced hepatic glucose lasts only 30 min in dogs (Vaitkus *et al.*, 1984), and the increased glucose level probably is the result of the antagonistic action between GH and insulin on adipocytes (Walton and Etherton, 1986; Walton *et al.*, 1987). The plasma glucose though increased in somatoliberin-treated animals in the present investigation, were found to be within the normal range (40-80 mg/dl) for cattle (Swenson, 1984).

The declining trend of plasma glucose in both the groups with the advancement of age is in agreement with the observations by Bide *et al.* (1973), Blum *et al.* (1985) and Hornick *et al.* (1998) in cattle.

In conclusion, repeated exogenous somatoliberin administration for longer period of time in growing buffaloes sustained a higher level of plasma GH even after cessation of treatment without showing any sign of refractoriness. Its long-term administration also increased plasma metabolites viz. NEFA, alpha amino nitrogen and glucose. The buffaloes treated with somatoliberin may approach puberty earlier as they would reach the 'critical' body mass earlier. The other indications for early puberty is also reflected by higher plasma progesterone and LH concentrations than untreated control groups. However, detailed studies are required in peripubertal buffaloes to ascertain the hypothesis.

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References

Adler, J.H. and E. Wertheimer, 1962. Reciprocal relationship between blood glucose and plasma FFA in dairy cows. Bull. Res. Council, Israel, 10E: 97.

Barbano, D.M., J. M. Lynch, D. E. Bauman, G. F. Hartnell, R. L. Hintz and M. A. Nemeth, 1992. Effect of a prolonged-release formulation of N-methionyl bovine somatotropin (sometribove) on milk composition. J. Dairy Sci., 75: 1775.

Beermann, D.H., Hogue, D.E., Fishell, V.K., Aronica, S., Dickson, H.W. and Schricker, B.R. 1990. Exogenous human growth hormone-releasing factor and ovine somatotropin improve growth performance and composition of gain in lambs. J. Anim. Sci., 68: 4122-4133.

Berardinelli, J.G., Daily, R.A., Butcher, R.L., Inskeep, E.K. 1979. Source of progesterone prior to puberty in heifers. J. Anim. Sci., 49: 1276-1280.

Berelowitz, M., M. Szabo, L. A. Frohman, S. Firestone and R. L. Hintz, R.L. 1981. Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary Sci., 212: 1279-1281.

Bide, R.W., W. J. Dorward and M. E. Tumbleson, 1973. Clinical chemistry of grain-fed cattle. 1. A preliminary study of a basic biomedical profile. Can. J. Anim. Sci., 53: 697-707.

Binelli, M., W. K. Vanderkooi, L. T. Chapin, M. J. VandeHaar, J. D. Turner, W. M. Moseley and H. A. Tucker, H.A. 1995. Comparison of growth hormone-releasing factor and somatotropin : Body growth and lactation of primiparous cows. J. Dairy Sci., 78: 2129-2139.

Blum, J.W., W. Schnyder, P. L. Kunz, A. K. Blom, H. Bikel and A. Schurch, 1985. Reduced and compensatory growth : Endocrine and metabolic changes during feed restriction and refeeding in steers. J. Nutr., 115: 417-424.

Breier, B.H. and P. D. Gluckman, 1991. The regulation of postnatal growth : Nutritional influences on endocrine pathways and function of the somatotrophic axis. Livest. Prod. Sci., 27: 77-94.

Ceda, G.P., G. Valenti, V. Butturini and A. R. Hoffman, 1986. Diminished pituitary responsiveness to growth hormone-releasing factor in aging male rats. Endocrinology, 118: 2109.

Cella, S.G., V. Gennaro, R. Puggioni, C. Pintou and E. E. Muller, 1985. Human pancreatic growth hormone-releasing factor stimulates growth hormone synthesis and release in infant rats : An *in vitro* study. Endocrinology, 116: 574.

Colenbrander, B., G. J. Garssen, J. C. Meijer, and G. S. G. Spencer, 1984. Interaction of hormones and growth factors. Tenth International Congress of Animal Reproduction and Artificial Insemination, 10-14 June, 1994. University of Illinois at Urbana, Champaign, IL, pp: 17.

Cuttler, L., J. B. Welsh and M. Szabo, 1986. The effect of age on somatostatin suppression of basal growth hormone (GH) releasing factor-stimulated, and dibutyryl adenosine 3',5'-monophosphate-stimulated GH release from rat pituitary cells in monolayer culture. Endocrinology, 119: 152.

Dahl, G.E., L. T. Chapin, M. S. Allen, W. M. Moseley, and H. A. Tucker, 1991. Comparison of somatotropin and growth-hormone-releasing factor on milk yield, serum hormones, and energy status. J. Dairy Sci., 74: 3421-3428.

Dahl, G.E., L. T. Chapin, S. A. Zinn, W. M. Moseley, T. R. Schwartz and H. A. Tucker, 1990. Sixty-day infusions of somatotropin-releasing factor stimulates milk production in dairy cows. J. Dairy Sci., 73: 2444.

Day, M.L., K. Imakawa, M. Garcia-Winder, D. D. Zalesky, B. D. Schanbacher, R. J. Kittok and J. E. Kinder, 1984. Endocrine mechanisms of puberty in heifers: estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod., 31: 332-341.

Day, M.L., K. Imakawa, P. L. Wolfe, R. J. Kittok, J. E. Kinder, 1987. Endocrine mechanisms of puberty in heifers : Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. Biol. Reprod., 37: 1054-1065.

Della-Fera, M.A., F. C. Buonomo and C. A. Baile, 1986. Growth hormone releasing factor and secretion of growth hormone in sheep, calves and pigs. Domest. Anim. Endocrinol., 3: 165.

Desjardins, C. and H. D. Hafs, 1968. Levels of pituitary FSH and LH in heifers from birth through puberty. J. Anim. Sci., 27: 472-477.

Dodson, S.E. B. J. McLeod, W. Haresign, A. R. Peters and G. E. Lamming, 1988. Endocrine changes from birth to puberty in the heifers. J. Reprod. Fertil., 82: 527-538.

Dubreuil, P., Y. Couture, G. Pelletier, D. Petitclerc, H. Lapierre, S. Pommier, P. Gaudreau, J. Morisset and P. Brazeau, 1990b. Effects of porcine growth hormone-releasing factor (1-29) NH_2 and thyrotropin-releasing factor on pig growth performance. Can. J. Anim. Sci., 70: 459-467.

Dubreuil, P., D. Petitclerc, G. Pelletier, P. Gaudreau, C. Farmer, T. F. Mowles and P. Brazeau, 1990a. Effect of dose and frequency of administration of a potent analog of human growth hormone-releasing factor on hormone secretion and growth in pigs. J. Anim. Sci., 68: 1254-1268.

Eisemann, J.H., A. C. Hammond, D. E. Bauman, P. J. Reynolds, S. N. McCutcheon, H. F. Tyrrell, and G. L. Haaland, 1986. Effect of bovine growth hormone administration on metabolism of growing Hereford heifers : Protein and lipid metabolism and plasma concentrations of metabolites and hormones. J. Nutr., 116: 2504-2515.

Enright, W.J., L. T. Chapin, W. M. Moseley, S. A. Zinn and H. A. Tucker, 1986. Growth hormone releasing factor stimulates milk production and sustains growth hormone release in Holstein cows. J. Dairy Sci., 69: 344.

Enright, W.J., L. T. Chapin, W. M. Moseley, S. A. Zinn, M. B. Kamdav, L. F. Krabil and H. A. Tucker, 1989. Effects of infusions of various doses of bovine growth hormone-releasing factor on blood hormones and metabolites in lactating Holstein cows. J. Endocrinol., 122: 671-679.

Enright, W.J., D. J. Prendivile, L. J. Spicer, P. R. Stricker, A. P. Moloney, T. F. Mowles and R. M. Campbell, 1993. Effects of growth hormone-releasing factor and (or) thyrotropin-releasing hormone on growth, feed efficiency, carcass characteristics and blood hormones and metabolites in beef heifers. J. Anim. Sci., 71: 2395-2405.

Enright, W.J., S. A. Zinn, L. T. Chapin and H. A. Tucker, 1988. Growth hormone response of bull calves to growth hormone-releasing factor. Proc. Soc. Exp. Biol. Med., 184: 483.

Etherton, T.D. and P. E. Walton, 1986. Hormonal and metabolic regulation of lipid metabolism in domestic livestock. J. Anim. Sci., 63: 76.

Evans, A.C.O., G. P. Adams and N. C. Rawlings, 1994. Follicular and hormonal development in prepubertal heifers from 2 to 36 weeks of age. J. Reprod. Fertil., 102: 463-470.

Gallo, G.F. and E. Block, 1991. Effects of recombinant bovine somatotropin on hypophyseal and ovarian functions of lactating dairy cows. Can. J. Anim. Sci., 71: 343-353.

Gluckman, P. D., B. H. Breier and S. R. Davis, 1987. Physiology of the somatotrophic axis with particular reference to the ruminant. J. Dairy Sci., 70: 442.

Gonzalez-Padilla, E., R. Ruiz, D. Le Ferer, A. Denham and J. N. Witbank, 1975b. Puberty in beef heifers. 3. Induction of fertile estrus. J. Anim. Sci., 40: 1100-1118.

Gonzalez-Padilla, E., J. N. Wiltbank and G. D. Niswender, 1975a. Puberty in beef heifers. 1. The interrelationship between pituitary, hypothalamus and ovarian hormones. J. Anim. Sci., 40: 1091-1104.

Goodwin, J.F., 1970. Estimation of alpha-amino nitrogen in plasma. Analytical Abst., 19: 438.

Gopinath, R. and T. D. Etherton, 1989a. Effects of porcine growth hormone on glucose metabolism of pigs. 1. Acute and chronic effects on plasma glucose and insulin status. J. Anim. Sci., 67: 682.

Gopinath, R. and T. D. Etherton, 1989b. Effects of porcine growth hormone on glucose metabolism of pigs. 2. Glucose tolerance, peripheral tissue insulin sensitivity and glucose kinetics. J. Anim. Sci., 67: 689.

Hart, I.C., P. M. E. Chadwick, A. Coert, S. James and A. D. Simmonds, 1985. Effects of different growth hormone releasing factors on the concentrations of growth hormone, insulin and metabolites in the plasma of sheep maintained in positive and negative energy balance. J. Endocrinol., 105: 113.

Heiman, M.L., W. A. Murphy, M. V. Nekola, V. A. Lance and H. Coy, 1984. Administration of human pancreatic growth hormone-releasing factor (GRF) analogs enhances responsiveness of culture rat pituitary cells to GRF. Biochem. Biophys. Res. Commun., 124: 217.

Hongerholt, D.D., B. A. Crooker, J. E. Wheaton, K. M. Carlson and D. M. Jorgenson, 1992. Effects of growth hormone releasing factor analogue and an estradiol-trenbolone acetate implant on somatotropin, insulin-like growth factor-I and metabolite profiles in growing Hereford steers. J. Anim. Sci., 70: 1439.

Hornick, J.L., C. Van Eenaeme, M. Diez, V. Minet and L. Istasse, 1998. Different periods of feed restriction before compensatory growth in Belgian Blue bulls. 2. Plasma metabolites and hormones. J. Anim. Sci., 76: 260-271.

Hornick, J.L., C. Van Eenaeme, S. Gauthier, P. Baldwin and L. Istasse, 1996. Glucose, alpha-amino nitrogen, and amino acid exchange across the hind limb in young double-muscled type bulls maintained at two growth rates. Can. J. Anim. Sci., 74: 193-202.

Jain, G.C. and R. S. Pandey, 1983. Changes in the profiles of peripheral plasma oestradiol-17β and luteinizing hormone from birth to puberty following conception in buffalo heifers (*Bubalus bubalis*). Asian J. Dairy Res., 2: 227-233.

Jain, G.C. and R. S. Pandey, 1985. Influence of age, weaning, season and body weight on the levels of progesterone, estradiol-17β and luteinizing hormone in growing buffalo heifers (*Bubalus bubalis*). Anim. Reprod. Sci., 9: 213-221.

Johnson, J.L., M. T. Coffey, K. L. Esbenshade, B. R. Schricker and D. H. Pilkington, 1990. The effect of human growth hormone-releasing factor or procine somatotropin on serum hormones and metabolites, growth performance and carcass traits in swine. J. Anim. Sci., 68: 3204-3211.

Kartiarso, M., D. Martin and E. Teleni, 1989. The pattern of utilization of body fat reserves by working cattle and buffalo. DAP Project Bull., 8: 7-8.

Kearl, L.C., 1982. Domestic buffalo. In: Nutrient Requirements of Ruminants in Developing Countries. International Feedstuffs Institute, Utah State University, Utah, USA, pp: 89-113.

Lapierre, H. and C. Farmer, 1992. Effect of immunization against GRF on growth in sheep. Domest. Anim. Endocrinol., 9: 389-393.

Lapierre, H., G. Pelletier, D. Petitclere, P. Debreuil, J. Morisset, P. Gaudreau, Y. Couture and P. Brazeau, 1988. Effect on human growth hormone-releasing factor (1-29) NH_2 on growth hormone release and milk production in dairy cows. J. Dairy Sci., 71: 92-98.

Lapierre, H., G. Pelletier, D. Petitclere, P. Debreuil, J. Morisset, P. Gaudreau, Y. Couture, and Brazeau, P. 1988. Effect on human growth hormone-releasing factor (1-29) NH_2 on growth hormone release and milk production in dairy cows. J. Dairy Sci., 71: 92-98.

Lapierre, H., G. Pelletier, D. Petitclere, P. Debreuil, J. Morisset, P. Gaudreau, Y. Couture and P. Brazeau, 1991. Effect of human growth hormone-releasing factor and (or) thyrotropin-releasing factor on growth, carcass composition, diet digestibility, nutrient balance, and plasma constituents in dairy calves. J. Anim. Sci., 69: 587.

Lapierre, H., D. Petitclere, G. Pelletier, L. Delorme, P. Dubreuil, J. Morisset, P. Gaudreau, Y. Couture and P. Brazeau, 1990. Effect of human growth hormone-releasing factor and (or) thyrotropin-releasing factor on hormone concentrations in dairy calves. J. Anim. Sci., 68: 2436.

Lapierre, H., H. F. Tyrrell, C. K. Reynolds, T. H. Elsasser, P. Gaudreau and P. Brazeau, 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers : Whole body energy and nitrogen metabolism. J. Anim. Sci., 70: 764-772.

Machlin, L.J., 1976. Role of growth hormone in improving animal production. Environ. Qual. Safety, 5: 43.

McLeod, B.J., W. Haresign, A. R. Peters and G. E. Lamming, 1984. Plasma LH and FSH concentrations in prepubertal beef heifers and in response to repeated injections of low doses of GnRH. J. Reprod. Fertil., 70: 137-144.

Moretti, C.A., N. S. Bagnato, G. Frajese and K. J. Catt, 1990. Receptor mediated actions on growth hormone releasing factor on granulosa cell differentiation. EndocrinilogyI, 127: 2117-2126.

Moseley, W.M., J. Huisman and E. J. Van Weerden, 1987. Serum growth hormone and nitrogen metabolism responses in young bull calves infused with growth hormone-releasing factor for 20 days. Domest. Anim. Endocrinol., 4: 51.

Moseley, W.M., L. F. Krabill, A. R. Friedman, and R. F. Olsen, 1985. Administration of synthetic human pancreatic GRF for five days sustains raised concentrations of GH in steers. J. Endocrinol., 104: 433.

Nalbandov, A.V., 1963. Symposium on growth : endocrine causes of growth and growth stasis. J. Anim. Res., 22: 558-560.

Plouzek, C.A., W. Vale, J. Rivier, L. L. Anderson and A. Trenkle, 1988. Growth hormone-releasing factor on growth hormone secretion in prepubertal calves. Proc. Soc. Exp. Biol. Med., 188: 198-205.

Prakash, B. S. and M. L. Madan, 1986. Peripheral plasma estradiol-17β, progesterone and cortisol in buffaloes induced to calve with dexamethasone and vetoestrol. Anim. Reprod. Sci., 11: 111-122.

Prakash, B.S., M. Mondal and N. Anandlaxmi, 2003. Development and validation of a simple sensitive enzyme immunoassay for GH determination in buffalo plasma. J. Immun. Immunochem. (In Press).

Reynolds, C.K. and H. F. Tyrrell, 1993. Effects of immunization against growth hormone-releasing factor in goats. Domes. Anim. Endo., 10: 512-519.

Reynolds, C.K., H. Lapierre, H. F. Tyrrell, T. H. Elasasser, R. C. Staples, P. Gaudreau and P. Grazeau, 1992. Effects of growth-hormone-releasing factor and feed intake on energy metabolism in growing beef steers : Net nutrient metabolism by portal drained viscera and liver. J. Anim. Sci., 70: 752-763.

Ringuet, H., G. Pelletier, P. Brazeau, P. Gaudreau, L. A. Guilbault, J. Morisset, Y. Couture and D. Petitchere, 1994. Long-term effects of human growth hormone-releasing hormone and photoperiod on hormone release and puberty in dairy heifers. J. Anim. Sci., 72: 2709-2717.

Salama, M.A.M., E. M. Mokhless and A. H. Barkawi, 1994. Pubertal performance of Egyptian buffalo heifers. Buff. J., 1: 61-66.

Savion, N., G. M. Lui, R. Laherty, and D. B. Gospodarowicz, 1981. Factors controlling proliferation and progesterone production by bovine granulosa cells in serum-free medium. Endocrinology, 109: 412

Schams, D., E. Schallenberger, S. Gombe and H. Karg, 1981. Endocrine patterns associated with puberty in male and female cattle. J. Reprod. Fert., 30(Suppl.1): 103-110.

Schams, D., W. Schwab and M. Kirchgessner, 1991. Concentrations of bGH, IGF-I, insulin and NEFA in blood plasma as well as bGH and IGF-I in milk of dairy cows after application of recombinant bGH. 6. Influence of bGH on energy and protein metabolism of dairy cows at different stages of lactation. J. Anim. Physiol and Anim. Nutr., 65: 126-132.

Schemm, S.R., D.R. Deaver, L.C. Griel and L.D. Muller, 1990. Effects of recombinant bovine somatotropin on luteinizing hormone and ovarian function in lactating dairy cows. Biol. Reprod., 45: 815-821.

Schoppee, P.D., J.D. Armstrong, R.W. Harvey, M.D.Whitacre, A. Felix and R.M. Campbell, 1996. Immunization against growth hormone-releasing factor or chronic feed restriction initiated at 3.5 months of age reduces ovarian response to pulsatile administration of gonadotropin-releasing hormone at 6 months of age and delays onset of puberty in heifers. Biol. Reprod., 55: 87-98.

Sejrsen, K., N. Oksbjerg, M. Vestergaard and M.T.Sorensen, 1996. Growth hormone and related peptides as growth promoters. Proceedings of Scientific Conference on Growth Promotion in Meat Production, Brussels, Belgium, 87-119.

Shipe, W.F., G.F.Stenyk and K.B. Fountain, 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. J. Dairy Sci., 63: 193-198.

Simon, O., 1989. Metabolism of proteins and amino acids. In: Protein Metabolism in Farm Animals (H.D. Bock, B.O. Eggum, A.G. Low, O. Simon and T. Zebrowska, eds.), 273. Oxford Sci. Publ. Deutscher, Landwirtsschaftsverlag, Berlin.

Simpson, R.B., J.D. Armstrong and R.W. Harvey, 1992. Effect of prepartum administration of growth hormone-releasing factor on somatotropin, insulin-like growth factor-I, milk production and post-partum return to ovarian activity in primiparous beef heifers. J. Anim. Sci., 70: 1478.

Singh, S.V., 1992. Female buffalo and cow draught energy production and utilization in farming system. Ph.D. Thesis, National Dairy Research Institute (Deemed University), Karnal, India.

Sonntag, W.E., V.W. Hylka and J. Meites, 1983. Impaired ability of old male rats to secret growth hormone *in vivo* but not *in vitro* in response to hpGRF (1-44). Endocrinology, 113: 2305.

Spicer, L.J. and W.J. Enright, 1991. Concentrations of insulin-like growth factor-I and steroids in follicular fluid or preovulatory bovine ovarian follicles : Effect of daily injections of a growth hormone-releasing factor analog and (or) thyrotropin-releasing hormone. J. Anim. Sci., 69: 1143-1149.

Spicer, L.J., D.J. Langhout, E. Alpizar, S.K. Williams, R.M. Campbell, T.F. Mowles and W.J. Enright, 1990. Effects of growth hormone releasing factor and vasoactive intestinal peptide on proliferation and steroidogenesis of bovine granulosa cells. Mol. Cell. Endocrinol., 83: 73-78.

Swanchara, K.W., J.D. Armstrong and J.H. Britt, 1999. Effects of active immunization against growth hormone-releasing factor on puberty and reproductive development in gilts. J. Anim. Sci., 77: 1807-1814.

Swenson, M.J., 1984. Physiological properties and cellular and chemical constituents of blood. In: Duke's Physiology of Domestic Animals, 10th Edn., p.34. Cornell Univ. Press, Ithaca, NY.

Takano, R., N.H. Izuka, K. Shizume, N. Honda and C. Ling, 1985. Plasma Growth Hormone (GH) reponse to single and repetitive subcutaneous administration of GH-releasing factor (pGRF-44) in normal and GH deficient children. J. Endocrinol., 117: 4211-4219.

Vaitkus, P., A. Serek, K.H. Norwich, O.V. Sirek, R.H. Unger and V. Harris, 1984. Rapid changes in hepatic glucose output after a pulse of growth hormone in dogs. Amer. J. Physiol., 246: E14.

Vanderkooi, W.K., M.J. Vandehaar, B.K. Sharma, M. Binelli, H.A. Tucker, R.M. Akars and W.M. Moseley, 1995. Comparison of growth hormone-releasing factor and somatotropin: The sòmatotropic axis in lactating primiparous cows. J. Dairy Sci., 78: 2140-2149.

Vernon, R.G. and D.J. Flint, 1989. Role of growth hormone in the regulation of adipocyte growth and function. In: Biotechnology in Growth Regulation (R.B. Heap, C.G. Prosser and G.E. Lamming, eds.), p.57. Butterworths, London, England.

Walton, P.E and T.D. Etherton, 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: Antagonism by porcine growth hormone. J. Anim. Sci., 62: 1584.

Walton, P.E., T.D. Etherton and C.S. Chung, 1987. Exogenous pituitary and recombinant growth hormones induced insulin and insulin-like growth factor 1 resistance in pig adipose tissue. Domest. Anim. Endocrinol., 4: 183.

Wheaton, J.E., S.N. Al-Raheem, J.A. Godfredson, J.M. Doru, E.A. Wong, W.Vale, J. Revier, T.F. Mowles, E.P. Heimer and A.M. Felix, 1988. Use of osmotic pumps for subcutaneous infusion of growth hormone-releasing factors in steers and withers. J. Anim. Sci., 66: 2876.

Yamashitu, S., M.Weiss and S. Mehmed, 1986. Insulin-like growth factor-I regulates growth hormone secretion and mRNA levels in human pituitary tumor cells. J. Clin. Endo. and Metab., 63: 730-735.