Effects of long-term GH-releasing factor administration on patterns of GH and LH secretion in growing female buffaloes (*Bubalus bubalis*)

M Mondal and B S Prakash

Animal Reproduction Laboratory, Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal-132 001 (Haryana), India

Correspondence should be addressed to M Mondal, National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland-797 106, India; Email: mohan_mondal@rediffmail.com

Abstract

To investigate the effects of long-term GH-releasing factor (GRF) administration on the patterns of GH and LH secretion in growing female Murrah buffalo (*Bubalus bubalis*) calves, 12 buffaloes of 6–8 months of age were divided into two groups (treatment and control groups) of six each in such a way that average body weight between the groups did not differ significantly ($P > 0.05$). Both the groups were administered i.v. with either synthetic bovine GRF (bGRF(1–44)-NH₂) at 10 µg/100 kg body weight (treatment group) or an equal volume of normal saline (control group) at intervals of 15 days until 18 injections had been completed (9 months). Blood samples collected prior to and after the first and last injection of GRF at –60, –45, –30, –15, –10, –5 min and +5, +10, +15, +30 min, and thereafter at intervals of 15 min up to 8 h post-injection, were assayed for plasma GH and LH. Plasma progesterone was also estimated in twice-a-week samples to assess whether either group had begun ovarian cyclicity. The body weight of all animals was recorded twice a week. In all animals, a peak of GH was recorded within 5–20 min and 5–30 min after the first and last GRF injections and post-injection mean values for plasma GH were significantly ($P < 0.01$) higher compared with the control group of animals. Although peak GH values after the first and last GRF injection did not differ ($P > 0.05$), GH levels were maintained at a higher level for a longer time after the last GRF injection compared with the first (240 vs 150 min). The area under the GH response curve after the last GRF injection was found to be significantly ($P < 0.01$) higher than after the first injection (9344 ± 199.7 vs 7763 ± 112.4 ng/ml $\times$ min). The mean post-injection plasma LH levels of the treatment group were significantly ($P < 0.01$) higher after both the first and last GRF injections than in the control group of animals. Interestingly, compared with the first GRF injection, the pre-injection plasma LH level was found to be significantly higher ($P < 0.01$) at the last injection. The plasma LH concentrations around the last injection of GRF were significantly higher ($P < 0.01$) than those recorded at the time of the first injection in treated buffaloes. Correspondingly, the plasma LH concentrations in controls were also higher ($P < 0.01$) around the last injection of GRF vis-à-vis the first injection. The hormone concentration exhibited a higher pulsatility with greater amplitude after the last injection as compared with that recorded after the first injection. Although pulses of LH were also recorded in controls following the last injection, these were fewer and of lower magnitude than those seen in treated animals. No animal from either group reached puberty. GRF-treated buffaloes attained higher ($P < 0.001$) body weight than the controls. In conclusion, long-term administration of GRF induces and even enhances GH release without any sign of refractoriness, and significantly increases plasma LH also. Hence, long-term treatment with GRF may be used to maintain a sustained increased level of plasma GH in buffaloes and it may assist the animals of this species to grow faster.

Reproduction (2004) 127 45–55

Introduction

In tropical countries, unlike other dairy animals the buffalo presents the farmer with problems of growth and late maturity, which reduces their total life-time productivity. The exotic buffalo heifers have been reported to reach puberty at 15–16 months of age (at around 350 kg body

weight) and 15–17 months of age (body weight between 260 and 290 kg) in Italy and Egypt respectively (Seren *et al.* 1991, Salama *et al.* 1994). In contrast, available reports indicate that our indigenous buffalo breeds, namely, Nili-Ravi, Surti and Mehsana reach puberty at an age of 26, 28.2 and 30.1 months respectively (Govindaiah & Rai 1987, Singh 1992, CIRB Annual Report 1999–2000).

Murrah buffaloes exhibit delayed maturity even more, which limits their productive life span. Even in a well-managed farm, the age at sexual maturity for female Murrah buffaloes has been recorded to be as high as 33.1 months (NDRI Annual Report 1995–1996). The slow growth rate and thus late maturity of dairy cattle and buffaloes leads to a great economic loss at organized as well as unorganized dairy farms in India. The postnatal growth, which determines the total life-time productivity of animals, is often regarded as an important economic trait. Heifers that grow faster become sexually mature at an earlier age, as sexual maturity is directly related to growth of an animal rather than age (El-Nouty 1971). The faster-growing animals show a short prepubertal period and calve at a younger age, and have greater life-time productive efficiency with higher fecundity and lower cost of rearing than those exhibiting slow growth rate, a long prepubertal period and calving at an older age (Short & Belows 1971, Hoffman & Funk 1992).

Growth hormone (GH) is a major regulator of growth and development during postnatal life, by influencing key metabolic pathways of intermediary metabolism (Nalbandov 1963, Etherton & Kensinger 1984, Breier & Gluckman 1991). Exogenous GH for enhancement of growth had been tried in farm animals by many workers in a wide variety of species, but repeated exogenous GH has met with limited success due to refractoriness of GH synthesis and release from adenohypophysis because of negative feedback effects of insulin-like growth factor-I released from the liver in response to exogenous GH (Berelowitz *et al.* 1981, Yamashita *et al.* 1986). A direct administration of a neurohormone, namely GH-releasing factor (GRF), in its synthetic or recombinant form, took forefront as an alternative measure for enhancement of growth because of being active in a wide range of species, and thus GRF treatment could potentially be used to accelerate growth of animals of commercial importance (Gelato & Merriam 1986). Research in this direction has been carried out on a short-term basis by many workers in recent years (Lapierre *et al.* 1992, Enright *et al.* 1993, Ringuet *et al.* 1994, Binelli *et al.* 1995, Kazmer *et al.* 2000).

The practical applicability of long-term GRF administration for growth enhancement in animals depends on its potential to maintain sustained increased levels of plasma GH. The potential of exogenous long-term GRF administration on the level of plasma GH and subsequent changes of plasma luteinizing hormone (LH), if any, has not been documented so far in Indian livestock in general, and buffaloes in particular. The objective of the present study was, therefore, to assess the patterns of changes of GH and LH release after long-term GRF administration in Murrah buffaloes.

Materials and Methods

Animals

Twelve growing female Murrah buffalo (*Bubalus bubalis*) calves of 6–8 months of age were purchased from nearby villages of the National Dairy Research Institute Farm, Karnal, India. After being purchased, these animals were kept in the Institute's farm for 1 month to be acclimatized to the environment and feeding systems of the farm. Thereafter, the experimental animals were sent to individual pens and kept there for another 15 day period to be adapted there. The animals were divided into two groups (control and treatment groups) of six each on the basis of their body weights, so that average body weights of the groups did not differ significantly ($P > 0.05$) at the beginning of the experiment (66 ± 6 and 66 ± 5 kg (means $\pm$ S.E.M.) for control and treatment groups respectively). The buffalo calves were fed on a ration consisting of concentrate mixture (crude protein = 19.8% and total digestible nutrient = 69.4%) containing maize grain 27%, groundnut cake 25%, mustard cake 25%, wheat bran 21%, mineral mixture 1% and salt 1% and roughage (either berseem, maize or oat fodder as per availability in the farm). The calculated amount of concentrate mixture (which was given at 1.0 kg/animal per day) was fed twice a day. The roughage was provided twice a day at 0930 and 1500 h. The animals were fed as per the Kearn (1982) standard for growing buffaloes (targeted growth rate of 500 g/day) to meet the energy and protein requirement of the animals. For this purpose, concentrate mixture and roughage were calculated on a dry matter basis once a week as per the body weight of the animals and regular weekly adjustment of feed requirement was carried out. Fresh tap water was freely available throughout the day to all animals.

Measurement of body weight

All the animals were weighed twice a week at 3–4 day intervals on a platform of an electronic balance at 0800 h before any feed was offered. The average body weight of the two observations in a specified week was used for the calculations.

Selection of GRF dosage

Dosages greater than 10 μ g/100 kg body weight, when administered i.v., may represent supraphysiological concentrations in cattle, as several studies have failed to demonstrate increasing GH response when using such dosages (Kazmer & Zinn 1998). Lapierre *et al.* (1990) also suggested that the maximal response of GRF was achieved at comparatively reduced dosages. We, therefore, used the dose of 10 μ g/100 kg body weight i.v. in buffalo calves.

Preparation of GRF solution

Synthetic bovine GRF (bGRF(1–44)-NH₂; Product code #G0644, Sigma-Aldrich Co., St Louis, MO, USA) was purchased as a formulated lyophilized substrate. For the experiment, GRF solution was prepared by dissolving GRF in sterile distilled water at 4°C. The amount of GRF solution required was calculated a day prior to injection by taking body weights of individual animals of the treatment group, as these animals were to be administered at a dose rate of 10 µg GRF/100 kg body weight i.v.

Treatment

Control and treatment group animals were administered i.v. with either normal saline or an equal volume of GRF solution containing 10 µg GRF/100 kg body weight at an interval of 15 days until 18 injections were completed (9 months).

Blood sampling

Blood samples (3.5 ml) were collected by means of an indwelling jugular catheter prior to and after the first and last injection of GRF at –60, –45, –30, –15, –10, –5 min and +5, +10, +15, +30, +45, +60 min, and thereafter at intervals of 15 min up to 8 h post-injection, in heparinized tubes (20 IU heparin/ml of blood). Blood sampling began at 0600 h on each day. The tubes were put in an ice bucket and carried back to the laboratory immediately after collection. Blood samples were also collected twice a week (at 3–4 day intervals) from all animals by means of jugular venipuncture at 0830 h after taking the body weight throughout the experiment for plasma progesterone monitoring to assess whether either group had begun ovarian cycles. All the samples were centrifuged within 30 min of collection at 500 × g for 30 min and plasma was separated. The plasma samples thus obtained were properly labelled and stored at –20°C until hormone analysis. All experimental protocols and animal care met Institutional Animal Care and Use Committee (IACUC) regulations. Before catheterization, local anaesthesia (Xylocaine) was given and after removal of catheters the animals were treated with antibiotic (oxy-tetracycline) for 3 days.

Hormone assays

GH assay

GH was assayed by a highly sensitive enzyme immunoassay (EIA) using a second-antibody technique as described by Prakash *et al.* (2003). The lowest GH detection limit significantly from zero concentration was 50 pg/100 µl plasma, which corresponded to 0.5 ng GH/ml plasma. Intra- and inter-assay coefficients of variation 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.

LH assay

Quantification of plasma LH was carried out by an EIA developed and validated in our laboratory (Prakash *et al.* 2002). The sensitivity of the assay for LH in plasma at the minimum detection limit was 6.25 pg/well per 20 µl or 0.31 ng/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 an RIA procedure developed in our laboratory as detailed by Prakash & Madan (1986) with slight modification. Hundred-microlitre plasma samples were taken for ether extraction. The sensitivity of the assay for progesterone by the extraction procedure at the minimum detection limit was 4 pg/tube, the 50% binding limit being 70 pg/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.

Statistical analysis

Mean concentrations of LH and frequency of LH pulses (pulses per 9 h) were calculated for each sequential blood sample. An LH pulse was defined as an increase in LH concentration that exceeded the previous nadir by two intra-assay standard deviations (Schillo *et al.* 1988). The data for hormonal and weight-gain parameters were analysed by an ANOVA for repeated-measures technique with a post-test for linear trends to compare hormonal changes and weight gain across time using Graphpad InStat 3.0 software. To test the effects of treatment and sampling time on hormonal parameters and weight gain, an ANOVA technique was used separately for pre- and post-GRF administration and treatment × time interaction was also taken into consideration.

Area under the GH response curve (AUC) was calculated by using the Graphpad Prism 2.01 software package 1995.

Results

Plasma GH changes associated with GRF administration

In all the animals, a peak of GH was recorded within 5–20 min of the first injection and within 5–30 min of the last injection after GRF administration. In both cases, the pre-injection GH concentrations did not differ significantly ($P > 0.05$) from controls, but post-injection mean values were found to be significantly higher ($P < 0.05$) compared with the control group of animals (Figs 1 and 2). Following the first injection, GH concentrations were maintained at a higher level for 150 min post-treatment and thereafter became similar to the control group GH

Figure 1 Plasma GH (means $\pm$ S.E.M.) profiles in growing Murrah buffaloes prior to and after the first treatment with GRF (treatment group, $n = 6$) or normal saline (control group, $n = 6$). Synthetic GRF (bGRF(1–44)-NH₂) (10 μ g/100 kg body weight) or an equal volume of normal saline was administered i.v. and blood samples collected at –60, –45, –30, –15, –10, –5 min before administration, and 5, 10, 15, 30 min, and thereafter at 15 min intervals post-administration up to 8 h.

Figure 2 Plasma GH (means $\pm$ S.E.M.) profiles in growing Murrah buffaloes prior to and after the last treatment with GRF (treatment group, $n = 6$) or normal saline (control group, $n = 6$). Synthetic GRF (bGRF(1–44)-NH₂) (10 μ g/100 kg body weight) or an equal volume of normal saline was administered i.v. and blood samples were collected at –60, –45, –30, –15, –10, –5 min before administration, and 5, 10, 15, 30 min, and thereafter at 15 min intervals post-administration up to 8 h.

concentration, but GH concentrations in the last intensive bleeding samples were found to be maintained at higher levels for longer (240 min post-injection), although the peak values of the two did not differ significantly ($P > 0.05$) being 89.0 ± 5.9 and 89.1 ± 20.5 ng/ml (means $\pm$ S.E.M.) for the first and last GRF injections respectively (Figs 1 and 2).

When comparing the AUCs for the GH response curve for the treatment group in the first and last intensive bleeding samples, it was found that the AUCs for the second intensive bleeding samples were significantly ($P < 0.01$) higher than those of the first intensive bleeding samples (9344 ± 199.7 vs 7763 ± 112.4 ng/ml $\times$ min), but AUCs

did not differ significantly for the control group of animals. ANOVA of mean GH concentrations revealed a treatment $\times$ time interaction ($P < 0.01$).

Plasma LH changes associated with GRF administration

In the blood plasma samples after the first GRF injection, the mean plasma LH of the control group of animals remained basal (around 0.31 ng/ml) throughout the collection period (Fig. 3A). In the treatment group of animals also, the mean plasma LH level remained low until 285 min post-injection, rising thereafter to a peak value

Figure 3 Plasma LH (means $\pm$ S.E.M.) profiles in growing Murrah buffaloes (A) prior to and after the first treatment and (B) prior to and after the last treatment with GRF (treatment group, $n = 6$) or normal saline (control group, $n = 6$). Synthetic GRF (bGRF(1–44)-NH₂) (10 μ g/100 kg body weight) or an equal volume of normal saline was administered i.v. and blood samples were collected at –60, –45, –30, –15, –10, –5 min before administration, and 5, 10, 15, 30 min, and thereafter at 15 min intervals post-administration up to 8 h.

(0.41 ± 0.07 ng/ml) at 345 min post-injection and falling to the basal level (0.31 ng/ml) at 375 min post-injection (Fig. 3A). There was a second rise in plasma LH thereafter to a peak value of 0.49 ± 0.12 ng/ml at 435 min (Fig. 3A). When the data of post-treatment LH levels of treatment and control groups were analysed, it was found that the overall mean plasma LH level of the treatment group was significantly higher ($P < 0.01$) than the control group of animals for the first GRF injection.

The mean plasma LH levels in animals of the treatment group were found to be always higher in the second intensive bleeding samples throughout the 9 h collection period (Fig. 3B). In the pre-treatment period, the mean LH

levels of animals of the treatment group were significantly higher ($P < 0.01$) than those of the control group of animals (Fig. 3B). Three distinct peaks of LH were found after GRF administration in the treatment group of animals and the peak values were 0.97 ± 0.22 ng/ml at 150 min, 0.98 ± 0.36 ng/ml at 300 min and 0.94 ± 0.31 ng/ml at 420 min post-injection. No such peaks of plasma LH were observed in controls, in which the plasma LH remained almost at basal level throughout the collection period, and the value ranged from 0.31 ± 0.00 to 0.45 ± 0.05 ng/ml (Fig. 3B).

The mean plasma LH concentrations around the last injection of GRF administration were significantly higher

($P < 0.01$) than those recorded at the time of the first injection in GRF-treated buffaloes (Fig. 3A and B). Correspondingly, the plasma LH concentrations in controls were also higher ($P < 0.01$) around the last injection vis-à-vis the first injection (Fig. 3A and B). The hormone concentration exhibited a higher pulsatility with greater amplitude after the last injection as compared with that recorded after the first injection (Fig. 3A and B). Although pulses of LH were recorded also in controls following the last injection, these were fewer and of lower magnitude than those seen in GRF-treated animals (Fig. 3B).

Representative examples of individual buffalo plasma LH concentration patterns from control and treatment groups for the first and last GRF injections are presented for two buffaloes from each group in Figs 4 and 5 respectively. No LH pulse was recorded in controls but the frequency of LH pulses ranged between one and three per 9 h sampling period in GRF-treated animals during the first GRF injection (Fig. 4). During the last GRF injection, the frequency of LH pulses ranged between one and three, and three and seven per 9 h sampling period for control and treatment animals respectively (Fig. 5). Statistical analysis of LH pulse frequencies indicated that there was a treatment $\times$ time interaction ($P < 0.01$).

Figure 4 Plasma LH profiles for individual representative buffaloes, two each from the control (a, b) and treatment group (c, d) during the first GRF injection. Treatment and control group buffaloes were treated with either synthetic GRF (bGRF(1–44)-NH₂) (10 µg/100 kg body weight) or an equal volume of normal saline i.v. and blood samples were collected at –60, –45, –30, –15, –10, –5 min before administration, and 5, 10, 15, 30 min, and thereafter at 15 min intervals post-administration up to 8 h.

Weekly mean plasma progesterone profiles throughout the experiment

The weekly mean plasma progesterone level estimated in twice-a-week samples (3–4 day intervals) is presented in Fig. 6. From week 2 onwards plasma progesterone levels in treated animals increased and reached a peak level of 0.74 ± 0.03 ng/ml at week 6 declining thereafter to 0.48 ± 0.03 ng/ml at the 19th week of experimentation. There was a rise in mean progesterone levels from week 19, again to a peak of 0.69 ± 0.06 ng/ml at week 23, showing minor fluctuations thereafter. No distinct trend was found in plasma progesterone in controls, where progesterone levels were found to be within the range of 0.40 ± 0.05 to 0.61 ± 0.05 ng/ml from week 1 onwards until the end of the last intensive sampling. Overall, mean progesterone of the treatment group (0.60 ± 0.01 ng/ml) was found to be significantly higher ($P < 0.01$) than control group (0.52 ± 0.01 ng/ml). No individual animal from either group had plasma progesterone concentrations ≥ 1.0 ng/ml for at least two subsequent samples collected at 3–4 day intervals.

Body weight

The changes in body weight for the treatment and control group of animals are presented in Fig. 7. At week 1, the

Figure 5 Plasma LH profiles for individual representative buffaloes, two each from the control (a, b) and treatment group (c, d) during the last GRF injection. Treatment and control group buffaloes were treated either with synthetic GRF (bGRF(1–44)-NH₂) (10 µg/100 kg body weight) or an equal volume of normal saline i.v. and blood samples were collected at –60, –45, –30, –15, –10, –5 min before administration, and 5, 10, 15, 30 min, and thereafter at 15 min intervals up to 8 h.

body weights of the treatment and control groups of animals did not differ significantly ($P > 0.01$), but the body weight of the treatment group showed a significantly increasing trend ($P < 0.01$) as GRF injections started in the first week of the experiment compared with the control group, and this trend continued until the last injection of GRF.

Discussion

Plasma GH changes associated with GRF administration

Peaks of GH similar to those recorded in the present study were also found in the study of Moseley *et al.* (1984) after GRF i.v. at 100, 300 and 1000 µg/312 kg body weight within 20 min in Holstein heifers. A biphasic release of GH after the highest dose of GRF (1000 µg) was observed by Moseley *et al.* (1984), which may be due to release of endogenous GRF or reduction in secretion of endogenous somatostatin. Such a biphasic GH release was not observed in the present investigation and this may be due to the comparatively low dose (10 µg/100 kg body weight) used in the present study. With a lower dose rate of GRF

administration of 10, 25, 50 and 100 µg/352 kg body weight a GRF-induced GH peak was registered within 5–15 min and GH concentrations declined by 20 min after treatment in Holstein steers (Moseley *et al.* 1984), which is similar to the results of the present investigation. Similar peak values of GH were found to occur within 5–20 min post-injection in dairy calves and heifers (Gluckman *et al.* 1987, Enright *et al.* 1989, 1993, Lapierre *et al.* 1990, Simpson *et al.* 1992, Hongerholt *et al.* 1992, Ringuet *et al.* 1994, Kazmer & Zinn 1998, Kazmer *et al.* 2000, Wiener *et al.* 2000), which further supports the present investigation.

The present study showed that higher plasma GH levels in GRF-treated animals were maintained for 150 min post-first injection and 240 min post-last injection compared with controls (Figs 1 and 2). Similar results on the duration of post-GRF elevated plasma GH treatment were obtained in bovines by Enright *et al.* (1989, 1993). Moseley *et al.* (1984) observed that GH levels declined to baseline within 120–240 min in bovines post-injection with different doses of GRF (100, 300 and 1000 µg/312 kg body weight). In contrast, Simpson *et al.* (1992) found an elevated GH concentration until 5 h post-treatment in

Figure 6 Weekly plasma progesterone profiles (means $\pm$ S.E.M.) of treatment and control group of buffaloes for 9 months. Treatment and control groups of buffaloes were administered with either synthetic GRF (bGRF(1–44)-NH₂) (10 μ g/100 kg body weight) or an equal volume of normal saline i.v. at 15 day intervals until 18 injections were completed (9 months) and blood samples were collected twice a week (at 3–4 day intervals) by jugular venipuncture at 0830 h from week 1 until the last injection of GRF.

Figure 7 Weekly mean body weight of treatment and control groups of buffaloes for 9 months. Treatment and control groups were administered with either synthetic GRF (bGRF(1–44)-NH₂) (10 μ g/100 kg body weight) or an equal volume of normal saline i.v. at 15 day intervals until 18 injections were completed (9 months) and all animals were weighed twice a week (at 3–4 days interval) at 0800 h from week 1 until the last injection of GRF.

primiparous heifers treated with 12.5 μ g/kg s.c. injections of GRF.

AUCs for GH response curves increased in GRF-treated bovines (Moseley *et al.* 1984, Petitclerc *et al.* 1987, Enright *et al.* 1989, 1993, Lapierre *et al.* 1990, Simpson *et al.* 1992, Ringuet *et al.* 1994, Kazmer & Zinn 1998, Kazmer *et al.* 2000, Wiener *et al.* 2000), ovines (Hart *et al.* 1985, Della-Fera *et al.* 1986, Kensinger *et al.* 1987, Wheaton *et al.* 1988, Byrem *et al.* 1989, Beermann *et al.*

1990, Godfredson *et al.* 1990), and swine (Takano *et al.* 1985, Dubreuil *et al.* 1990) over placebo. The present study revealed similar observations in GRF-administered growing Murrah buffaloes. An interesting finding of the present investigation is that although the experimental animals became older from the first through to the 18th GRF injection (9 months of treatment), still responsiveness to repeated administration of GRF was not affected, but rather became higher in terms of the GH response AUC.

Long-term treatment with GRF was found to enhance the responsiveness to subsequent administration of GRF in humans and rodents (Heiman *et al.* 1984). The results of the present investigation also agree with those of Hangerholt *et al.* (1992), who found that the time of the GH peak and the height of the peak in response to exogenous GRF was not affected by chronic GRF administration in bovines. In another study (Ringuet *et al.* 1994), twice daily s.c. administration of GRF for 246 days at 5 µg/kg body weight in Holstein dairy heifers resulted in an increase in GH concentrations throughout the trial and all heifers responded to GRF until the last day of the experiment, suggesting that GRF may be used to induce daily GH release without loss of responsiveness over an extended period of time in young dairy heifers. Similarly, exogenous intermittent administration of GRF enhanced secretion and circulating concentrations of GH in ovines without them becoming refractory to GRF (Hart *et al.* 1985, Della-Fera *et al.* 1986, Kensinger *et al.* 1987, Wheaton *et al.* 1988, Byrem *et al.* 1989, Beermann *et al.* 1990, Godfredson *et al.* 1990). Chronic repeated administration of GRF in growing pigs did not induce desensitization of somatotroph cells. On the contrary, an increased responsiveness was observed as the days of treatment advanced (Takano *et al.* 1985, Dubreuil *et al.* 1990) and this could be the result of stimulation of GH synthesis and release by GRF (Cella *et al.* 1985). In contrast, Lapierre *et al.* (1990) observed decreased ($P < 0.01$) GH responsiveness with days of treatment in dairy calves, but this decrease was due to ageing rather than the chronic treatment period. On all the days of blood sampling a peak of endogenous GH was always registered, suggesting chronic GRF injections for 3 months did not cause any refractoriness of endogenous GH release. The GRF-induced GH response was also found to decrease with age in rats (Sonntag *et al.* 1983, Ceda *et al.* 1986, Cuttler *et al.* 1986) and cattle (Johke *et al.* 1984), and this decrease could be due to a decrease in sensitivity of somatotrophs to GRF with ageing (Ceda *et al.* 1986), an increase in sensitivity of somatotrophs to somatostatin with ageing (Cuttler *et al.* 1986) and/or to an age-related accumulation of somatostatin in somatotrophs (Brazeau *et al.* 1986).

Plasma LH in blood samples collected prior to and after GRF administration

In the present investigation, plasma LH was found to be significantly higher in blood samples collected around the first and last injections of GRF in treated animals than in controls. Higher pulse frequency was recorded during the last injection of GRF and the basal LH levels in treated animals were also higher. The LH levels of control animals in both the intensive bleeding samples were significantly ($P < 0.05$) lower, and plasma LH concentrations in blood samples collected around the first injection remained close to the basal level (0.31 ng/ml). The plasma LH concentrations in controls were also higher ($P < 0.01$)

around the last injection vis-à-vis the first injection. The hormone concentration exhibited a higher pulsatility with greater amplitude after the last injection as compared with that recorded after the first injection. Although pulses of LH were recorded also in controls following the last injection in individual animals, these were fewer and of lower magnitude than those seen in treated animals. The results bring out clearly a greater pituitary responsiveness to GRF treatment in terms of LH release, which may also be an indication that GRF treatment could enhance the maturity process in buffaloes in terms of ovarian steroidogenesis. From the early postnatal stimulation of gonads by the hypothalamic and pituitary hormones, progesterone plays a key role in the changes leading to puberty. Puberty is determined by monitoring plasma progesterone concentrations; when plasma progesterone becomes > 1.0 ng/ml for at least two subsequent samples collected at 3–4 day intervals it is said that the animal has attained puberty (Ringuet *et al.* 1994, Salama *et al.* 1994, Melvin *et al.* 1999). Although the plasma progesterone of GRF-treated animals was found to be always higher than the control group of buffaloes in this study, the level did not reach ≥ 1.0 ng/ml even in a single sample collected at 3–4 day intervals for any animal from either group, suggesting that no animal in this experiment had reached puberty by the time of the last injection of GRF. The GRF-treated buffaloes were advancing towards puberty, which is also indicated by their higher body weight gain (Fig. 7). In both the groups, the source of progesterone may be 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 is unclear.

The results of the present investigation are in accord with the study of Jimenez-Krassel *et al.* (1999), who also found higher serum LH in the cattle treated with GRF. Exogenous GH tended to increase LH pulse frequency over control pigs (Gilbertson *et al.* 1991), which further supports the result of the present investigation. The pulse frequency and amplitude of LH found in the present investigation showed an increasing pattern with age, which is essentially similar to that reported in bovines (Dodson *et al.* 1988).

In contrast, Moseley *et al.* (1984) reported that LH release in response to different doses of GRF in steers was not significant. Similarly, exogenous GRF administration did not affect endogenous LH release in rats (Wehrenberg & Ling 1983) and man (Thorner *et al.* 1983). Endogenous LH secretion was also not affected by exogenous somatotrophin administration (McShane *et al.* 1989, Hall *et al.* 1994). The interesting results from the present investigation may be due to (i) species differences, (ii) age at which GRF was applied and its duration, and (iii) the study being carried out in intact animals.

Conclusion

It may be concluded from the present investigation that long-term administration of GRF induces and even enhances GH release without any sign of refractoriness, and significantly increases plasma LH. GRF-treated buffaloes obtained significantly higher ($P < 0.01$) body weight than controls. Long-term treatment with GRF may, therefore, be used to maintain sustained increased levels of plasma GH in buffaloes and it could assist these animals to grow faster.

Acknowledgements

The authors wish to thank Dr Mark Hennies, Institut für Physiologie, Biochemie und Hygiene der Tiere, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany for the generous gift of highly specific bovine GH antibody. The supply of highly purified reference preparation of bovine GH, LH and bovine LH antiserum by the United States Department of Agriculture, Animal Hormone Program, Beltsville, USA is gratefully acknowledged. Financial assistance provided by National Agricultural Technology Project PSR No. 47 for this study is also duly acknowledged.

References

- Beermann DH, Hogue DE, Fishell VK, Aronica S, Dickson HW & Schrick BR 1990 Exogenous human growth hormone-releasing factor and ovine somatotropin improve growth performance and composition of gain in lambs. *Journal of Animal Science* **68** 4122–4133.
- Berardinelli JG, Daily RA, Butcher RL & Inskeep EK 1979 Source of progesterone prior to puberty in heifers. *Journal of Animal Science* **49** 1276–1280.
- Berelowitz M, Szabo M, Frohman LA, Firestone S & Hintz RL 1981 Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science* **212** 1279–1281.
- Binelli M, Vanderkooi WK, Chapin LT, VandeHaar MJ, Turner JD, Moseley WM *et al.* 1995 Comparison of growth hormone-releasing factor and somatotropin: body growth and lactation of primiparous cows. *Journal of Dairy Science* **78** 2129–2139.
- Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB, Briskin A *et al.* 1986 Growth hormone-releasing factor: isolation, characterization and physiology. In *Human Growth Hormone*, pp 347–366. Eds S Raiti & RA Tolman. New York: Plenum Press.
- Breier BH & Gluckman PD 1991 The regulation of postnatal growth: nutritional influences on endocrine pathways and function of the somatotrophic axis. *Livestock Production Science* **27** 77–94.
- Byrem TD, Dwyer DA, Aronica SM, Dickson HW, Schrick BR & Beermann DH 1989 Effects on continuous infusion of human growth hormone-releasing factor (hGRF) on lamb growth and composition. *FASEB Journal* **3** A938 (Abstract).
- Ceda GP, Valenti G, Butturini V & Hoffman AR 1986 Diminished pituitary responsiveness to growth hormone-releasing factor in aging male rats. *Endocrinology* **118** 2109–2114.
- Cella SG, Gennaro V, Puggioni R, Pintou C & Muller EE 1985 Human pancreatic growth hormone-releasing factor stimulates growth hormone synthesis and release in infant rats: an *in vitro* study. *Endocrinology* **116** 574–577.
- CIRB Annual Report 1999–2000. Hisar, Haryana, India: Central Institute for Research on Buffaloes.
- Cuttler L, Welsh JB & Szabo M 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** 152–158.
- Della-Fera MA, Buonomo FC & Baile CA 1986 Growth hormone releasing factor and secretion of growth hormone in sheep, calves and pigs. *Domestic Animal Endocrinology* **3** 165–176.
- Dodson SE, McLeod BJ, Haresign W, Peters AR & Lamming GE 1988 Endocrine changes from birth to puberty in heifers. *Journal of Reproduction and Fertility* **82** 527–538.
- Dubreuil P, Petitclerc D, Pelletier G, Gaudreau P, Farmer C, Mowles TF *et al.* 1990 Effect of dose and frequency of administration of a potent analog of human growth hormone-releasing factor on hormone secretion and growth in pigs. *Journal of Animal Science* **68** 1254–1268.
- El-Nouty F 1971 The effect of different systems before and after weaning on age at puberty and age at first conception in buffalo heifers. *MSc Thesis*. Ain Shams University, Egypt.
- Enright WJ, Chapin LT, Moseley WM, Zinn SA, Kamdavi MB, Krabill LF *et al.* 1989 Effects of infusions of various doses of bovine growth hormone-releasing factor on blood hormones and metabolites in lactating Holstein cows. *Journal of Endocrinology* **122** 671–679.
- Enright WJ, Prendiville DJ, Spicer LJ, Stricker PR, Moloney AP, Mowles TF *et al.* 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. *Journal of Animal Science* **71** 2395–2405.
- Etherton TD & Kensinger RS 1984 Endocrine regulation of fetal and postnatal meat animal growth. *Journal of Animal Science* **59** 511–528.
- Gelato MC & Merriam GR 1986 Growth hormone releasing hormone. *Annual Review of Physiology* **48** 569–591.
- Gilbertson J, Kirkwood RN & Thacker PA 1991 Timing of growth hormone injections and reproduction in gilts. *Canadian Journal of Animal Science* **71** 717–723.
- Gluckman PD, Breier BH & Davis SR 1987 Physiology of the somatotrophic axis with particular reference to the ruminant. *Journal of Dairy Science* **70** 442–466.
- Godfredson JA, Wheaton JE, Crooker BA, Wong EA, Campbell RM & Mowles TF 1990 Growth performance and carcass composition of lambs infused for 28 days with a growth hormone-releasing factor analogue. *Journal of Animal Science* **68** 3624–3632.
- Govindaiah MG & Rai AV 1987 Productive and reproductive traits of medium sized buffaloes. *Indian Journal of Dairy Science* **40** 333–337.
- Hall JB, Schillo KK, Fitzgerald BP & Bradley NW 1994 Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development and onset of puberty in beef heifers. *Journal of Animal Science* **72** 709–718.
- Hart IC, Chadwick PME, Coert A, James S & Simmonds AD 1985 Effect 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. *Journal of Endocrinology* **105** 113–119.
- Heiman ML, Murphy WA, Nekola MV, Lance VA & Coy HA 1984 Administration of human pancreatic growth hormone-releasing factor (GRF) analogs enhances responsiveness of culture rat pituitary cells to GRF. *Biochemical and Biophysical Research Communications* **124** 217–222.
- Hoffman PC & Funk DA 1992 Applied dynamics of dairy replacement growth and management. *Journal of Dairy Science* **75** 2504–2516.
- Hongerholt DD, Crooker BA, Wheaton JE, Carlson KM & Jorgenson DM 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. *Journal of Animal Science* **70** 1439–1448.

- Jimenez-Krassel F, Binelli M, Tucker HA & Ireland JJ 1999 Effect of long-term infusion with recombinant growth hormone-releasing factor and recombinant bovine somatotropin on development and function of dominant follicles and corpora lutea in Holstein cows. *Journal of Dairy Science* **82** 1917–1926.
- Johke T, Hodate K, Ohashi S, Sharaki M & Sawano S 1984 Growth hormone response to human pancreatic growth hormone releasing factor in cattle. *Endocrinologia Japonica* **31** 55–61.
- Kazmer GW & Zinn SA 1998 Serum somatotropin concentrations in Holstein heifers administered growth hormone-releasing factor and somatostatin. *Animal Science* **66** 551–556.
- Kazmer GW, Zinn SA & Strausbaugh LD 2000 Growth hormone response to somatostatin-28 and growth hormone-releasing factor in dairy heifers. *Journal of Dairy Science* **83** 2282–2284.
- Kearl LC 1982 Domestic buffalo. In *Nutrient Requirements of Ruminants in Developing Countries*, pp 89–113. Logan, UT: International Feedstuffs Institute, Utah State University.
- Kensingler RS, McMunn LM, Stover RK, Schricker BR, Maccacchini ML, Harpster HW *et al.* 1987 Plasma somatotropin response to exogenous growth hormone releasing factor in lambs. *Journal of Animal Science* **64** 1002–1009.
- Lapierre H, Petitclerc D, Pelletier G, Delorme L, Dubreuil P, Morisset J *et al.* 1990 Effect of human growth hormone-releasing factor and (or) thyrotropin-releasing factor on hormone concentrations in dairy calves. *Journal of Animal Science* **68** 2436–2449.
- Lapierre H, Tyrrell HF, Reynolds CK, Elsasser TH, Gaudreau P & Brazeau P 1992 Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: whole body energy and nitrogen metabolism. *Journal of Animal Science* **70** 764–772.
- McShane TM, Schillo KK, Boling JA, Bradley NW & Hall JB 1989 Effects of recombinant DNA-derived somatotropin and dietary energy intake on development of beef heifers: I. Growth and puberty. *Journal of Animal Science* **67** 2230–2236.
- Melvin EJ, Lindsay BR, Quintal-Franco J, Zanella E, Fike KE *et al.* 1999 Circulating concentrations of estradiol, luteinizing hormone and follicle-stimulating hormone during waves of ovarian follicular development in prepubertal cattle. *Biology of Reproduction* **60** 405–412.
- Moseley WM, Krabill LF, Friedman AR & Olsen RF 1984 Growth hormone response of steers injected with synthetic human pancreatic growth hormone-releasing factors. *Journal of Animal Science* **58** 430–435.
- Nalbandov AV 1963 Symposium on growth: endocrine causes of growth and growth stasis. *Journal of Animal Research* **22** 558–560.
- NDRI Annual Report 1995–1996. Karnal, Haryana, India: National Dairy Research Institute.
- Petitclerc D, Pelletier G, Lapierre H, Gaudreau P, Couture Y, Dubreuil P *et al.* 1987 Dose response of two synthetic human growth hormone-releasing factors on growth hormone release in heifers and pigs. *Journal of Animal Science* **65** 996–1005.
- Prakash BS & Madan ML 1986 Peripheral plasma oestradiol-17 β , progesterone and cortisol in buffaloes induced to calve with dexamethasone and vetoestrol. *Animal Reproduction Science* **11** 111–122.
- Prakash BS, Paul V & Anandlaxmi N 2002 Development and validation of a simple, sensitive, second antibody format enzyme immunoassay for LH determination in plasma. *Journal of Immunological Methods* **270** 281–290.
- Prakash BS, Mondal M & Anandlaxmi N 2003 Development and validation of a simple sensitive enzyme immunoassay for GH determination in buffalo plasma. *Journal of Immunoassay and Immunochemistry* **24** 409–420.
- Ringuet H, Pelletier G, Brazeau P, Gaudreau P, Guilbault LA, Morisset J *et al.* 1994 Long-term effects of human growth hormone-releasing hormone and photoperiod on hormone release and puberty in dairy heifers. *Journal of Animal Science* **72** 2709–2717.
- Salama MAM, Mokhless EM & Barkawi AH 1994 Pubertal performance of Egyptian buffalo heifers. *Buffalo Journal* **1** 61–66.
- Schillo KK, Green MA & Hayes SH 1988 Effects of adrenalectomy on photoperiod-induced changes in release of luteinizing hormone and prolactin in ovariectomized ewes. *Journal of Reproduction and Fertility* **83** 431–438.
- Seren E, Pameggiani A, Campanile G, Esposito L, Barile VL & Montemurro N 1991 Luteinizing hormone secretion associated with puberty in buffalo heifers. *Proceedings of Third World Buffalo Congress*, May 1991, Bulgaria. Vol 3, pp 660–665.
- Short RE & Bellows RA 1971 Relationships between weight gains, age at puberty, and reproductive performance in heifers. *Journal of Animal Science* **32** 127–131.
- Simpson RB, Armstrong JD & Harvey RW 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. *Journal of Animal Science* **70** 1478–1487.
- Singh D 1992 Breed characterization of Mehsana buffaloes and strategies for their genetic improvement. *PhD Thesis*. National Dairy Research Institute (Deemed University), Karnal, India.
- Sonntag WE, Hylka VW & Meites J 1983 Impaired ability of old male rats to secrete growth hormone *in vivo* but not *in vitro* in response to hpGRF (1-44). *Endocrinology* **113** 2305–2307.
- Takano R, Izuka NH, Shizume K, Honda N & Ling C 1985 Plasma growth hormone (GH) response to single and repetitive subcutaneous administration of GH-releasing factor (pGRF-44) in normal and GH deficient children. *Journal of Endocrinology* **117** 4211–4219.
- Thorner MO, Spices J, Vance ML, Rozol AD, Kaiser DL, Webster JD *et al.* 1983 Human pancreatic growth hormone-releasing factor selectively stimulates growth hormone secretion in man. *Lancet* **1** 24–28.
- Wehrenberg WB & Ling N 1983 The absence of an age-related change in pituitary response to growth hormone-releasing factor in rats. *Neuroendocrinology* **37** 473–475.
- Wheaton JE, Al-Raheem SN, Godfredson JA, Doru JM, Wong EA, Vale W *et al.* 1988 Use of osmotic pumps for subcutaneous infusion of growth hormone-releasing factors in steers and withers. *Journal of Animal Science* **66** 2876–2884.
- Wiener P, Angus KD, McCullough E & Woolliams JA 2000 Growth hormone response to infusion of somatostatin in dairy calves: testing its use as a diagnostic tool for genetic merit. *Animal Science* **71** 219–226.
- Yamashita S, Weiss M & Mehmed S 1986 Insulin-like growth factor-I regulates growth hormone secretion and mRNA levels in human pituitary tumor cells. *Journal of Clinical Endocrinology and Metabolism* **63** 730–735.

Received 12 June 2003

First decision 6 August 2003

Accepted 11 September 2003