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Increasing skeletal growth in the dry season to enhance compensatory growth in the wet season

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Abstract

This project investigated the effect of diet energy and protein on skeletal elongation rate (SER) in cattle and sheep undergoing periods of nutritional restriction and re-alimentation. One of the major aims of this project was to determine if it is possible to “un-couple” growth in skeletal height with liveweight (LW) gain in growing steers and drive additional frame size in cattle in the dry season to enhance compensatory LW gain in the wet season. Gene expression of growth plate tissue and plasma hormone analysis indicates that insulin like growth factor-1 (IGF-1) is central to the cellular pathways responsible for controlling changes in skeletal elongation in response to nutrition. When growing steers were fed diets resulting in minimal LWG, metabolisable energy (ME) was the primary nutritional component that controlled growth, skeletal elongation, bone formation and hormonal and growth plate gene expression response to the diet. These low ME diets also led to reduced bone volume and rib cortical thinning because of reduced bone formation. Adding crude protein or exogenous bovine somatatropin (bST) to a low ME diet was not able to drive additional hip height (HH) gain or compensatory LWG. However, the LW:HH relationship appears to be central to growth and strategies to create growth paths where animals diverge further from the normal LW:HH are likely to yield more effective compensatory LWG.

Executive summary

Improving growth rates of cattle in northern Australia would increase turnoff weight and reduce turnoff age as well as improving fertility in maiden and first-calf heifers. In northern Australian growing systems cattle undergo periods of restricted nutrition and minimal weight gain during the dry season, followed by more rapid growth with a high plane of nutrition during and immediately after the wet season. This ability to achieve “catch up” LWG is described as compensatory gain. Any cost benefit of dry season supplementation aimed at maintaining LW gain is lost if compensatory growth during the wet season achieves the same eventual weight. Exploiting compensatory gain will allow a targeted and cost-effective supplement strategy to maximise eventual turnoff. This project :

1. Identified the important growth factors and cellular signalling mediators within the epiphyseal growth plate (EGP).
2. Determined the effect of crude protein (CP) content or level of metabolisable energy (ME) intake on skeletal growth, subsequent compensatory growth and bone gene expression in *Bos indicus* crossbred and Holstein steers.
3. Examined the effect of bovine somatotrophic hormone (BST) on skeletal growth and bone gene expression in *Bos indicus* cross steers.
4. Identified possible strategies and options to increase dry season skeletal elongation so as to maximise compensatory growth in the wet season and potentially reduce or change dry season supplementation.
5. Developed recommendations for subsequent research to capture the benefits of this approach under commercial conditions.

One of the major aims of this project was to determine if it is possible to “un-couple” growth in skeletal height with LW gain in growing steers and drive additional frame size in cattle in the dry season to enhance compensatory LW gain in the wet season. This project examined the practical significance of changes in skeletal elongation rate (as measured by changes in hip height) in response to levels of nutrition. Bones elongate via the process of endochondral ossification at the growth plate. This is regulated by a number of hormones and local growth factors. The GH/IGF-1 axis and thyroid hormones are most likely to respond to nutritional influences.

The hormonal and growth plate-level responses to nutritional interventions were examined using a number of different strategies in 3 pen experiments. Experiment 1 (Exp1) examined the effect of high (H) or low (L) dietary crude protein (CP) content or metabolisable energy (ME) intake on skeletal growth, subsequent compensatory LWG and bone gene expression in different genotype steers; *bos indicus* cross (BX) and Holstein/Frisian (HF). Diets consisted of Lucerne chaff ad libitum (HCP-HME), Lucerne chaff restricted to an equivalent ME intake as LCP-LME steers (HCP-LME) and Mitchell grass hay ad libitum (LCP-LME). Experiment 2 (Exp2) employed similar nutritional interventions to Experiment 1 but used a single strain of *bos indicus* cross steers and imposed an additional treatment of growth hormone/bovine somatatropin (bST). In Experiment 3, fasting and re-feeding in lambs was used as nutritional model of dramatic, acute changes of bone growth. The aim of this experiment was to further elucidate the important signalling pathways in the growth plate in response to altered nutrition. Across the 3 experiments, analysis included plasma biochemistry (glucose, total protein, NEEFA, urea, calcium and phosphorus), plasma hormones (IGF-1, triiodothyronine, thyroxine, insulin, adiponectin, parathyroid hormone and leptin), plasma bone biomarkers, growth plate and bone histology and growth plate gene expression. In both Exp1 and Exp2 the experimental design was successful in creating 2 diets

with matched low ME and differing CP content but the same LWG in both cattle. Experiment 3 (Exp3) was successful in creating dramatic and reversible slowing of skeletal elongation at the growth plate in lambs.

- In growing steers, skeletal elongation and thus gain in HH progresses in the face of low ME diets and minimal LWG. This leads to a divergence of the normal LW:HH relationship during growth and this difference between expected LW and actual LW contributes to the potential for compensatory LWG.
- When growing steers were fed diets resulting in minimal LWG, ME was the primary nutritional component that controlled growth, skeletal elongation, bone formation and hormonal and growth plate gene expression response to the diet. Under these low ME nutritional conditions it may not be possible to drive additional HH gain or divergence from the LW:HH relationship to achieve additional compensatory LWG.
- Skeletal elongation at the growth plate is very responsive to additional diet ME and can slow down and rapidly speed up in response to nutrition. Growth in HH was able to return to a faster velocity than unrestricted age-matched animals of the same size suggesting restricted animals have a reduced “bone age” following restriction that explains observed “catch-up” growth in HH. While HH gain more rapid than age-matched control animals is possible following restriction, HH growth verolity greater than the growth curve of unrestricted animals of the same age does not appear to be possible in growing steers.
- Gene expresison of growth plate tissue and plasma hormone analysis indicates that IGF-1 is central to the cellular pathways responsibe for controlling changes in skeletal elongation in response to nutrition. Changes in thyroid hormones also appeared to be associated with changes in velocity of skeletal elongation. The GH/IGF-1 axis is complex and difficult to manipulate when there are extremes of growth. Steer on low ME diets were not able to respond to exogenous bST treatment which is likely to be due to lack of substrates for growth, inhibition of IGF-1 pathways at the growth plate and possible GH resistance. Steers on high ME and CP diets responded to exogenous bST with increased LWG but not skeletal elongation. This is likely to be due to the differences in maximal LWG and HH gain that are possible over the timeframe studiied.
- Growing steers on low ME diets and minimal LWG have reduced bone formation leading to cortical thinning and reduced bone volume. This could contribute to poor bone quality when superimposed on P deficiency.
- In these experiments the strategy of adding CP to a low ME diet has not delivered sufficiently increased HH during the ME restricted period to generate gains in compensatory growth during re-alimentation. However, the ability to manipulate catch-up or compensatory growth is still likely to be critical to maximising cost-effective gains in growth rates in northern Australia.
- Previous studies have demonstrated that IGF-1 concentrations are responsive to additional protein when dietary ME is higher. Supplementation strategies aimed at providing slightly higher ME diets that are sufficient to be able to exploit benefits of additional protein might be more successful in creating a LW:HH that diverges sufficiently from the normal growth path to generate a greater compensatory LWG and more cost effective improvements in growth rate.

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1 Background

Improving growth rates of cattle in northern Australia would help drive meat quality by increasing turnoff weight and reducing turnoff age as well as improving fertility in maiden and first-calf heifers. Profitability depends on maximising growth using available pasture and strategic use of supplements. In northern Australian growing systems cattle usually undergo periods of restricted nutrition and minimal weight gain during the dry season, followed by more rapid growth when a high plane of nutrition is restored during the wet season. This ability to achieve “catch up” LWG is described as compensatory gain. Any cost benefit of dry season supplementation aimed at maintaining LW gain is lost if compensatory growth during the wet season achieves the same eventual weight. Exploiting this compensatory effect should allow the most targeted and cost-effective supplement strategy to maximise eventual turnoff LW (Mclennan, 2014).

There is evidence that animals that attain a larger frame size with lower LW during a restricted period of nutrition will achieve greater subsequent compensatory LWG when returned to a higher plane of nutrition (Kidd and McLennan, 1998). There appears to be an allometic relationship between LW and HH under normal growth conditions. However, during nutritional restriction *bos indicus* steers will continue to gain height, albeit at a lower rate, even when LW gain is minimal or there is even LW loss (Mclennan, 2014, McLennan and Poppi, 2011). When nutritional restriction ends, LW is initially gained at a more rapid rate that height. This appears to occur until a more “standard” LW: HH relationship is restored. This relationship between liveweight and skeletal size may be central to compensatory gain in liveweight. If it was possible to improve skeletal growth and thus frame size during periods of restricted nutrition, improved LWG in the wet season should be achieved. The question being asked in this project is “could targeted nutritional supplementation selectively drive enhanced continued bone elongation in the face of nutrient restriction?” And would this lead to increased LW gain on a bigger frame by stimulating increased muscle mass due to more stretch stimulus on muscles from longer bones.

Previous work in growing steers fed diets varying in P content has shown that P deficient diets reduce bone elongation, measured as change in hip height (HH). This effect was at least in part, due to the decreased DM intake caused by the low P diets but with adequate energy and protein. Subsequent P supplementation rapidly increases skeletal elongation to rates similar to previously unrestricted steers. Interestingly, this increase in HH gain does not appear to be able to increase beyond that of unrestricted younger animals at the same height (Quigley, 2013). This implies that there is an upper limit to skeletal elongation rates and that no further gains in HH could be achieved in the wet season. Therefore, increasing skeletal growth during the dry season would be the most effective strategy to manipulate skeletal growth to enhance subsequent compensatory LWG.

The main hormonal regulator of linear growth in mammals is growth hormone (GH), termed bovine somatatrophin (bST) in cattle. GH acts directly on the growth plate as well as through the actions of insulin like growth factor-1 (IGF-1). IGF-1 is mainly produced in the liver as well as by many tissues and it regulates bone elongation by stimulating chondrocyte cell proliferation and differentiation within the EPG. Growth hormone and IGF-1 have been shown to be responsive to nutritional intake and IGF-1 is reduced when nutrition is limited or animals are fasted. In times of adequate nutrition, this axis is also responsible for the co-

ordinated allometric growth of all tissues such that muscle, fat and bone growing at allometric rates in proportion to each other (Schorr and Miller, 2016, Velayudhan et al., 2007). Fasting dramatically reduces the rate of longitudinal bone growth at the EPG in rodents (Farnum et al., 2003, Finkielstain et al., 2013, Gat-Yablonski et al., 2013) and malnutrition is well recognised to cause reduced linear growth and stunting in humans (de Onis and Branca, 2016). What is less well understood is the role that individual nutrients of the diet play in linear bone growth.

Bones elongate via the process of endochondral ossification (EO) at the epiphyseal growth plate (EGP). EO is a highly complex process that involves progressive proliferation and hypertrophy of chondrocytes within a cartilage template that eventually becomes vascularised and remodelled into bone. This process has been well described and it is clear that general malnutrition can cause stunting of skeletal growth. However the role of different dietary components in EO and skeletal development is still poorly understood. It is known that skeletal elongation rate increases with increasing level of nutrition (Searle et al., 1989), is increased to a limited extent with steroidal hormones (Peralta et al., 1994) and is decreased with the effects of internal parasites. The effect of energy and protein malnutrition on human growth is seen in the disease anorexia nervosa (AN). Self-imposed semi-starvation in adolescents leads to weight loss and reduced growth in height. In addition to disturbences in the GH/IGF-1 axis, hormonal changes that have been associated with skeletal growth retardation in AN include reduced thyroid hormones thyroxine (T4) and triiodothyronine (T3), elevated cortisol, and reduced sex steroids (Modan-Moses et al., 2012). Leptin is a hormone produced by fat tissue that influences appetite and energy metabolism. It regulates GH and IGF-1 actions and has direct effects on the EGP to enhance cartilage proliferation and differentiation (Gat-Yablonski et al., 2004, Gat-Yablonski et al., 2013). Leptin levels are low in AN patients and increase during treatment with associated weight gain and increased height. The process of EO at the EGP has been shown to be tightly controlled by hormonal and cellular signalling (Young, 1988, Mackie et al., 2011). but the nutritional manipulation of skeletal growth has not been well described in cattle nor has its control by endogenous hormones as a consequence of nutrient manipulation. By measuring circulating concentrations of impotant metabolic hormones and metabolites, the physiologic response to dietary manipulation can be determined.

A small number of studies in other species have demonstrated that supplementation of specific nutrients can encourage improved bone elongation rather than weight gain in the face of ongoing poor nutrition. For example, Zinc supplementation improved linear growth in stunted, malnourished Vietnamese children in the absence of any other nutritional supplementation (Ninh *et al.* 1996). This increase in linear growth with Zn supplementation was also associated with increased circulating levels of IGF-1 in the children suggesting that single nutrient administration is capable of stimulating growth factor production with influences on the EGP.

When steers are fed a diet that differs only in P content, HH gain is responsive to P levels in the diet. But this response appears to be directly related to P driving increased intake and thus ME and CP intake are likely to be the reasons for more rapid growth (Quigley 2013). During the dry season, P is not the limiting nutrient and this project is designed to determine the response of the skeleton to the nutritional factors of ME and CP when mineral availability is sufficient for growth.

Nutritional approaches that maximise for example, leptin production or the GH/IGF-1 axis response in the face of poor feed availability may provide a cost-effective method to improve live weight gain in Northern Australian production systems.

Manipulating the dietary CP was chosen as the initial nutritional approach to try to drive increased height gain in the face or restricted energy. The effect of dietary CP on the endocrine response to energy restriction has not been well examined in cattle. In humans, adequate protein intake is important for bone growth and for preventing bone loss from osteoporosis (Bonjour, 2016) but the effect of added protein in the face of a low enerergy diet has not been well examined. CP has a role in the BST/IGF-1 axis but it is not known if dietary CP alone has the ability to change skeletal elongation in cattle. This project will determine if it is possible to provide strategic supplementation of CP that would allow continued linear growth of bones without diverting nutrients to overall weight gain that cannot be supported during the dry season. Such knowledge and approach has the potential to lead to novel methods of increasing live weight gain in northern Australia.

Bone biopsies have been developed by this group to monitor bone structure and quality as well as measure any gain or loss in bone tissue in response to nutritional treatments. Previous analysis of bone biopsies and radiographs have shown that P deficiency leads to thinner cortices of long bones and ribs, reduced hip trabecular bone volume and deficits in mineralisation (Quigley et al 2013). Some of these effects are due to mineral deficiency alone and some may be due to the malnutrition induced by reduced feed intake caused by P deficiency. The effect that low dietary ME and CP have on bone quality independent of P deficiency have not been well examined in cattle but can have serious consequences. Malnutrition due to disease or AN in humans leads to loss of cortical and trabecular bone mass (osteoporosis) in humans (Robinson et al., 2016). In a case series of fractures of the humerus in New Zealand dairy heifers, bone histology revealed that the likely cause was osteoporosis induced by periods of malnutrition (Dittmer et al., 2016). Total nutrient restriction of weaner sheep has resulted in shorter, thinner bones, narrowing of growth plates and altered mineral content of forelimb bones (Cake et al., 2006,). It is possible that a similar response occurs in growing cattle during the dry season across northern Australia. These are issues which need to be more widely studied and manipulated. Bone quality is important in northern Australian cattle as P deficiency superimposed on malnutrition may contribute to sufficient bone loss to cause reduced production and fertility as well as lameness and fractures.

In order to manipulate skeletal growth we need to understand the factors that control skeletal growth. Biopsies of the tuber coxae also contain a growth plate with morphology similar to that of other long bones. Analysing repeated bone and growth plate biopsies from cattle at different nutritionally imposed rates of skeletal elongation has not been reported previously but offers an ideal method to examine to investigate the cellular response to different nutrients within the growth plate. Measuring differential gene expression (levels of messenger RNA) between treatment groups allows insight into the way cells in the growth plate are responding to imposed nutritional and hormonal treatments at different growth velocities. This information will be combined with measurement of plasma concentrations of markers of bone formation and resorption to create a comprehensive picture of cellular activity at the growth plate and the systemic factors affecting skeletal growth.

This study was undertaken as part of a call for transformational approaches that may improve northern Australian cattle production. As such it was somewhat novel and speculative but with the potential to transform how we approach supplementation to achieve high ***annual*** rates of gain. If this approach was successful then with minimal dry season supplementation we could achieve much higher and more efficient rates of LWG in the wet season to achieve an annual LWG similar to or greater than traditionally implemented high dry season supplementation regimes (Mclennan, 2014).

2 Project objectives

1. Identified the important growth factors and cellular signalling mediators within the epiphyseal growth plate (EGP).
2. Determined the effect of CP content or level of ME intake on skeletal growth, subsequent compensatory growth and bone gene expression in Bos indicus crossbred and Holstein genotype steers.
3. Examined the effect of bovine somatotrophic hormone (BST) on skeletal growth and bone gene expression in Bos indicus cross steers.
4. Identified possible strategies and options to increase dry season skeletal elongation so as to maximise compensatory growth in the wet season and potentially reduce or change dry season supplementation.
5. Developed recommendations for subsequent research to capture the benefits of this approach under commercial conditions.

3 Methodology

3.1 Experiment 1. The effect of crude protein content or level of metabolisable energy intake on skeletal growth, subsequent compensatory growth and bone gene expression in Bos indicus crossbred and Holstein genotype steers

3.1.1 Experiment introduction

This experiment used growing steers to examine the effects of crude protein (CP) and metabolisable energy (ME) intake on skeletal growth and subsequent compensatory LWG in in cattle of two different genotypes. Holstein steers were used as they are a genotype for which a lot of information about the growth and metabolism of skeletal tissue is known and they have the highest recorded HH changes recorded in the literature. *Bos indicus* steers were used as they are the predominant cattle breed across northern Australia and there is relatively little known regarding their skeletal development.

3.1.2 Experimental design, animals, diets and feeding

Fifteen Bos indicus (Brahman cross-bred, BX) steers and fifteen Bos taurus (Holstein-Friesian, HF) steers were fed a Rhodes (Choris gayana) grass and Dolichos lablab (Lablab

purpureus) mixed hay (**Table 1**) ad libitum in group pens for 23 days and in individual pens for 7 days prior to the commencement of the experiment. All steers were treated for internal and external parasites [Cydectin (5 g Moxidectin/L), Fort Dodge; Baulkham Hills, NSW, Australia) and vaccinated for bovine ephemeral fever (Webster BEF, Zoetis; West Ryde, NSW, Australia) and clostridial diseases (Ultravac 5-in-1, Zoetis; West Ryde, NSW, Australia)

At the commencement of the experiment (day 1) the HF steers [230 ± 34 kg liveweight (LW); mean ± standard deviation (SD)] and BX steers (178 ± 6 kg) were ranked and blocked on LW from heaviest to lightest within each genotype and allocated to one of five blocks of adjacent individual pens (n=6 pens/block). Three steers from the same LW ranking block within each genotype were randomly allocated to individual pens within each block of pens. Within each block of pens the steers from each genotype were then randomly allocated one of three nutritional treatments, high CP and high ME intake (HCP-HME), high CP and low ME intake (HCP-LME) and low CP and low ME intake (LCP-LME). The individual steer was considered the experimental unit.

The experiment consisted of two phases. During Phase 1 (93 days duration) the steers were offered lucerne chaff ad libitum (HCP-HME), lucerne chaff restricted to an equivalent ME intake as LCP-LME steers (HCP-LME) and Mitchell grass hay ad libitum (LCP-LME). The Mitchell grass hay was chopped to approximately 50 mm in length (Jaylor 4350 Feed Grinder, McIntosh and Son; Dalby, QLD, Australia) prior to feeding (**Table 1**).

Table 1. Organic matter (OM, g/kg DM), ash-free neutral detergent fiber (NDF, g/kg DM), ash-free acid detergent fibre (ADF, g/kg DM), crude protein (CP, g/kg DM), and mineral content of ingredients used in formulating cattle diets.

Nutrition	Mitchell grass (*Astrebla* spp)	Lucerne (*Medicago sativa*)	Rhodes grass (*Choris gayana*)/ Dolichos lablab (Lablab purpureus) hay	Cotton seed meal
OM	901	896	905	924
NDF	631	356	644	237
CP	38	200	88	428
Ca	4.7	12.2	7.9	2.5
P	1.8	3.2	3.9	14.6

Steers allocated to the LCP-LME treatment were offered 50 g cottonseed meal (CSM)/kg Mitchell grass hay as fed from day 42 until the end of Phase 1. The addition of CSM was adopted in order to stabilize the LW loss trend in this treatment. From day 1 to 42 the LWG was -0.21 and -0.17 kg for BX and HF respectively. Steers allocated to the HCP-LME treatment were offered 84 mg mono-sodium phosphate (MSP; 240 g P/kg DM)/kg LW.day such that the animals were provided with adequate dietary P required to achieve an equivalent average daily gain (ADG) to that achieved by the HCP-HME steers.

Steers offered the lucerne chaff and Mitchell grass hay ad libitum were offered the previous day's intake plus 10% and 20% respectively on an as fed basis. The feed allowance for steers offered the restricted amount of lucerne chaff was calculated from the mean ME intake of all steers within the corresponding genotype fed Mitchell grass during the previous week (MJ ME/kg LW.day).

During Phase 2 (103 days duration) all steers were offered lucerne chaff ad libitum. The steers remained in the same pens (Figure 1) throughout the experiment and had access to drinking water at all times.

Feed residues were collected and weighed at 0730 h each day and steers were offered feed at approximately 0800 h each day. Sub-samples of feed offered were collected at feeding each day and bulked over seven consecutive days. Feed residues were weighed daily, bulked over seven consecutive days for each steer. Duplicate sub-samples of feed offered and feed residues for each steer were collected at the end of each seven days period.

3.1.3 Faecal, urine and rumen fluid collection

Steers were divided into two cohorts of 15 steers based on allocation blocks for collection of faecal and urine output. The two cohorts of steers were moved into individual metabolism crates on days 19 and 33 of Phase 1 of the experiment in the same sequence as their individual pens (Figure 1). Each cohort remained in the metabolism crates for 9 consecutive days, which included a 2 day adaptation period and a 7 day collection period. Total faecal and urine output and feed residues were collected and weighed daily. Daily urine output of each steer was acidified to pH 3 with 5% sulphuric acid and a 5% sub-sample was collected each day, placed at 4^{o}C and bulked over the collection period. At the end of the collection period the bulked urine was mixed well and aliquots were collected into 5 mL tubes and stored at -20^{o}C for subsequent analysis. A 10% sub-sample of faecal output was collected each day, placed at 4^{o}C and bulked over the collection period. At the end of the collection period the bulked faeces was mixed well and triplicate sub-samples were collected for subsequent analysis. Triplicate sub-samples of feed offered and residues were also collected for subsequent analysis.

Upon the completion of each faecal and urine collection period the steers were returned to their individual pens and prior to feeding rumen fluid was collected per *os* using a stomach tube attached to a hand pump. Rumen fluid sub-samples were acidified with 20% sulphuric acid or 20% metaphosphoric acid with internal standard (i.e. 4 methyl n- valeric acid) and stored at -20°C for subsequent analysis of NH_3N and volatile fatty acids (VFA) respectively.

Figure 1. Individual pens (left) and metabolism crates (right).

3.1.4 Liveweight and body dimension measurements

Liveweight was measured prior to feeding every seven days throughout the experiment. Hip height (HH) was measured using a measuring stick on days -10, 7, 35, 57, 70, 85, 103, 112, 125, 140, 154, 168, 182, 196 and 203 of the experiment. Height was measured from the ground to the highest point of the sacrum with the steer standing square in his back feet. Point of shoulder to point of olecranon (SLD-OLC), point of olecranon to accessory carpal bone (OLC-ACB), tuber coxae to tuber isheii (TC-TI), point of shoulder to tuber coxae (SLD-TC) and tuber coxae to tuber coxae (TC-TC) measurements were taken using a measuring tape on the same days as HH measurements except for day 112 when no other measurements were taken (Figure 2). Average daily gain and body measurement growth rates were calculated by regressing each measurement over time within each phase of the experiment.

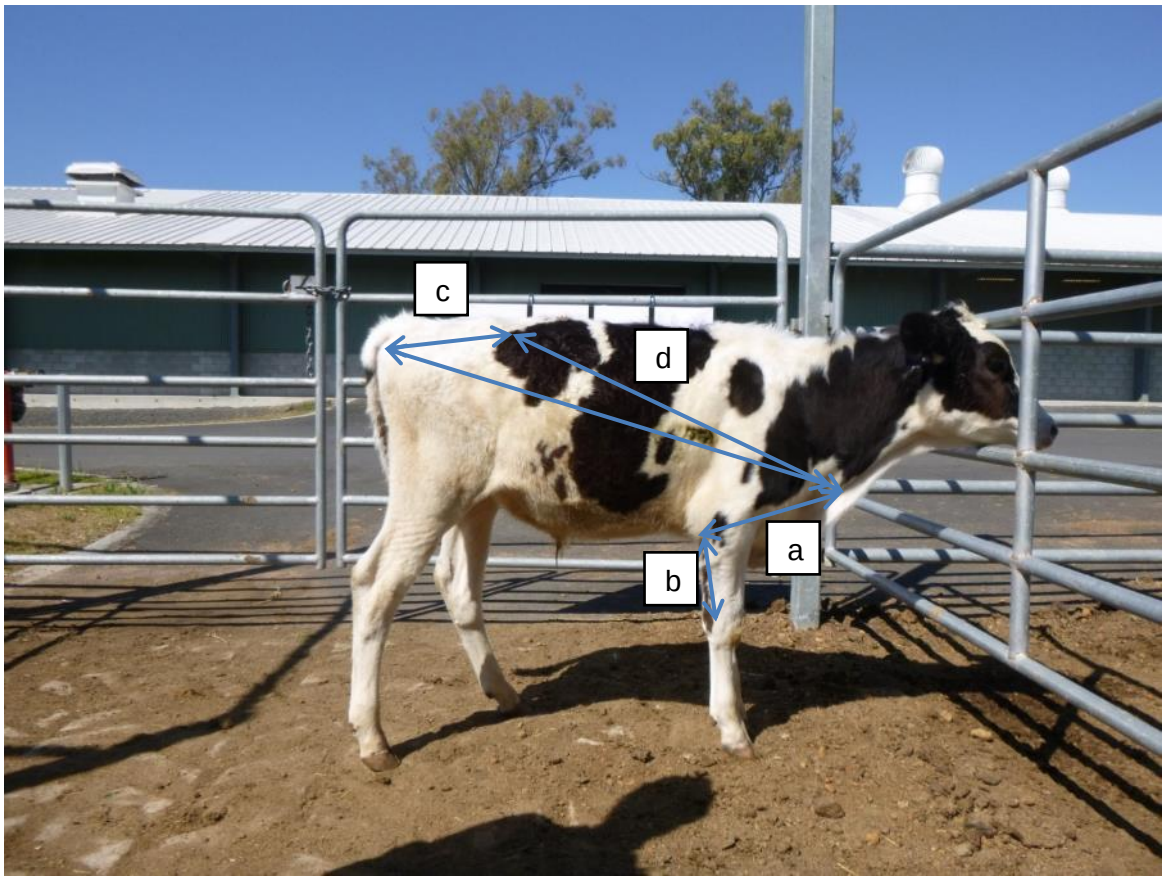

Figure 2. Body measurements

Measuring sites of the following body dimensions: (a) Point of shoulder to point of olecranon (SLD-OLC), (b) point of olecranon to accessory carpal bone (OLC-ACB), (c) tuber coxae to tuber isheii (TC-TI) and (d) point of shoulder to tuber coxae (SLD-TC)

3.1.5 Blood samples

Blood samples were collected from the jugular vein into lithium heparin coated vacutainers (Becton Dickinson; Franklin Lakes, NJ, USA) on days -10, 78, 103, 175 and 203 of the experiment. After collection the vacutainers were slowly inverted 6 to 8 times and then

placed on ice for approximately 30 min prior to centrifugation at 1700 *g* for 10 min at 4°C, plasma was collected and stored at -20°C until analysed. The concentration of insulin, leptin, IGF1, adiponectin, T4, tT3, PYD, total deoxypyridinoline (tDPD), OCN and BAP inorganic were measured in plasma samples collected on days -10, 103 and 203 of the experiment. The concentration of glucose, calcium, inorganic phosphorus, urea and total protein were measured in plasma samples collected on days 78 and 175 of the experiment.

3.1.6 Bone biopsy samples

Surgical bone biopsies were collected from the tuber coxae on the left, right and left side of each steer on days -9, 104 and 207 of the experiment respectively. The biopsy collected on day 207 was conducted on the same side as the first biopsy. The analysis of the biopsies collected on day 207 showed that growth plate structure and morphology was completely recovered after the first biopsy taken from the same side of the animal. In addition, all comparisons between growths plates were made within collection date, which means that all treatments would have been equally affected by any carry-over effects of the first biopsy, if they existed.

With steers standing in the crush, the biopsy site was clipped and scrubbed with chlorhexidine surgical scrub (Perrigo; Balcatta, WA, Australia) and wiped clean with Chlor-hex C (Jurox; Rutherford, NSW, Australia) in methylated spirits (Recochem; Lytton, QLD, Australia). The skin and deeper tissue over the tuber coxae were infiltrated with 35 to 40 mL of lignocaine hydrochloride (20 mg lignocaine hydrochloride/mL; Troy laboratories; Glendenning, NSW, Australia) and left for 5 min for effect. An incision approximately 80 mm in length was made and skin and any overlying muscle were retracted. A single biopsy 10-20 mm deep was obtained from the most central part of the tuber coxae of the ilium. A cordless drill (18v, 10mm cordless drill) and a 16 mm bone trephine (Sontec instruments, Centennial CO, 80112 U.S.A) was used to start the biopsy and then a 16 mm metal hole saw, also attached to a cordless drill, was used to obtain a deeper sample. An elevator was used to separate the sample from the parent bone. The resultant biopsy contained a thick layer of cartilage, the growth plate and trabecular bone (Figure 3).

Overlying muscle was sutured with monofilament absorbable sutures (2/0 PDS) and the external incision closed with skin staples, the surgical site was then cleaned and sprayed with Chloromide antiseptic spray (Troy laboratories; Glendenning, NSW, Australia). The bone cores were divided longitudinally using a scalpel blade and sub-samples were fixed in 10% neutral buffered formalin (NBF) and 70% ethanol and stored in fixative at 4°C for until processed.

3.1.7 Statistical analysis

All statistical analyses were conducted using the open-source software R version (R Core Team, 2013) and the linear mixed models procedure of the package “nlme” (Pinheiro et al., 2013). Prior to analysis all data was checked for normality and homoscedasticity and if necessary data was transformed according to the box-cox procedure (Osborne, 2010). Average daily gain and rate of change of body measurements were calculated by regressing each measurement over time for each phase separately. For these parameters the initial LW and HH of each phase was used as covariate when significant ($P > 0.05$). All the other

parameters were compared within the time-point at which they were collected. Nutrition and genotype treatments and their interaction were included in the model as fixed effects and animal within block were included as random factors. When analysis of variance showed a significant effect of nutrition treatment or the interaction between nutrition treatment and genotype, a tukey test post hoc was performed to explore the differences between groups. If the interaction was not significant (P>0.05), it was removed from the model and data was reanalysed including only main factors.

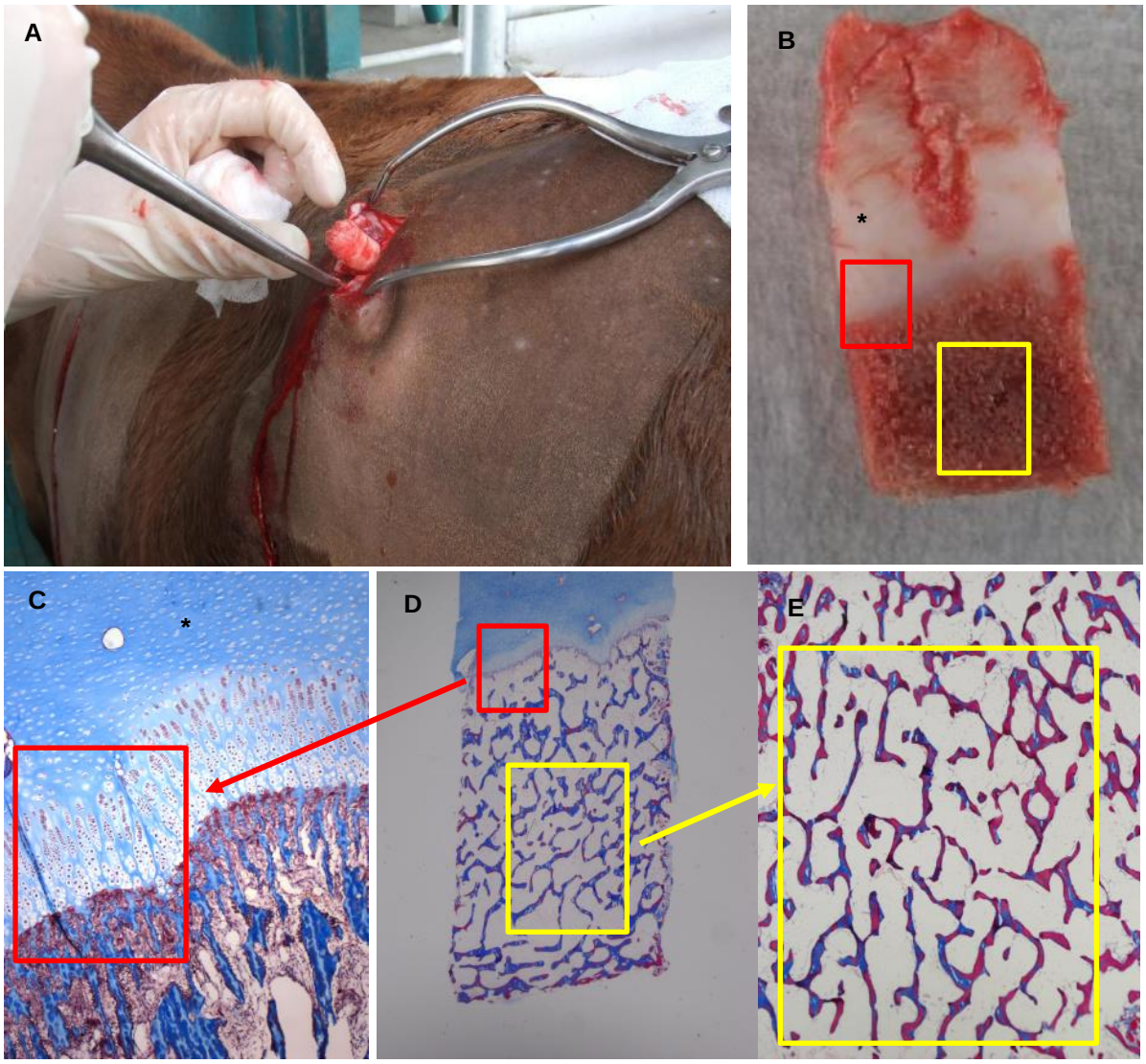

Figure 3.

Surgical bone biopsies were taken from the tuber coxae (hip bone)(A). Biopsies contained cartilage (*), a growth plate (red rectangle, B, C, D) and trabecular bone (yellow rectangle, D, E). Images show histological sections stained with Masson's trichrome stain. Scale is 200um.

3.2 Experiment 2. The effect of bovine somatotropin hormone (bST) on skeletal growth and bone gene expression in Bos indicus cross steers.

3.2.1 Experiment introduction

The molecular and cellular pathways that drive maximal skeletal growth were investigated using bST treatment in *Bos indicus* steers. The use of bST in this experiment was not to test the efficacy of this treatment for commercial use, but rather to use hormonal stimulation of growth to try to drive maximal growth rates possible and to enhance any differences between dietary treatments. Current hormonal growth promotants (HGPs) have shown no effect on skeletal growth in the dry season (McLennan, 2011) and the nutrition x bST and IGF-1 axis is a key control mechanism of growth. The inclusion of bST treatment in this experiment was as a means of elucidating mechanisms controlling skeletal growth and thus possible nutritional or hormonal pathways to increase skeletal growth. There is a lot of information about the effect and role of bST in skeletal growth of Holstein heifers under high CP diets but not with low CP diets. This experiment targeted the dry season skeletal growth and to obtain the detailed measurements of bone and other tissues the animals were slaughtered at the end of the feeding treatment period.

3.2.2 Experimental design, animals, diets and feeding

Thirty Bos indicus (Brahman cross-bred, BX) steers were fed a Rhodes grass (Choris gayana) hay ad libitum (Table 2) in group pens for 14 days and in individual pens for 7 days prior to the commencement of the experiment. All steers were treated for internal and external parasites [Cydectin (5 g Moxidectin/L), Fort Dodge; Baulkham Hills, NSW, Australia) and vaccinated against bovine ephemeral fever (Webster BEF, Zoetis; West Ryde, NSW, Australia) and clostridial diseases (Ultravac 7-in-1, Zoetis; West Ryde, NSW, Australia).

At the commencement of the experiment (day 1) the 30 BX steers (187±9.68 kg LW, mean ± SD) were ranked and blocked on LW and HH from largest to smallest and allocated to one of five blocks of adjacent individual pens (n=6 pens/block) and steers were randomly allocated to individual pens within each block of pens. Three steers from the same LW/HH ranking block were randomly allocated to receive growth hormone [bovine somatatrophin (bST); 500 mg: Sometribove zinc suspension, Elanco Animal Health] (+bST) and 3 to saline control (–bST). Within each block of pens the steers from each treatment were then randomly allocated one of three nutritional treatments, high CP and high ME intake (HCP-HME), high CP and low ME intake (HCP-LME) and low CP and low ME intake (LCP-LME). These nutritional treatments were very similar to those in experiment 1. The individual steer was considered the experimental unit.

The experiment consisted of a a 100 day period during which the steers were offered Mitchell grass hay ad libitum (LCP-LME), lucerne chaff ad libitum (HCP-HME) or lucerne chaff restricted to an equivalent ME intake as LCP-LME steers (HCP-LME)(Table 2). Steers allocated to the LCP-LME treatment were offered 50 g CSM /kg Mitchell grass hay as fed throughout the experiment. Steers allocated to the HCP-LME treatment were offered 84 mg mono-sodium phosphate (MSP; 240 g P/kg DM)/kg LW.day such that the animals were

provided with adequate dietary P required to achieve an equivalent average daily gain (ADG) to that achieved by the HCP-HME steers. By adding MSP, minerals should not have been a limiting factor in bone growth and it was expected that the HCP-LME diet contained adequate CP. The objective was to have ME limiting but other factors which affect bone growth would be adequate or not limiting to the extent of the LCP-LME diet. This would simulate a supplement of protein meal to cattle in the dry season and examine the extent to which SER could be manipulated in cattle in the dry season. Steers offered the lucerne chaff and Mitchell grass hay ad libitum were offered the previous day's intake plus 10% and 20% respectively on an as fed basis. The feed allowance for steers offered the restricted amount of lucerne chaff was calculated from the mean ME intake of all steers within the corresponding bST treatment within the same block fed Mitchell grass during the previous week (MJ ME/kg LW.day). Steers received subcutaneous injections of bST or saline every 2 weeks on the same day and LW measurments on days 1, 14, 28, 43, 56, 70, 84, and 98. The steers remained in the same pens throughout the experiment and had access to drinking water at all times.

Table 2. Organic matter (OM, g/kg DM), ash-free neutral detergent fiber (NDF, g/kg DM), ash-free acid detergent fibre (ADF, g/kg DM), crude protein (CP, g/kg DM), and mineral content of ingredients used in formulating cattle diets.

Nutrition	Mitchell grass (*Astrebla* spp)	Lucerne (*Medicago sativa*)	Rhodes grass (*Choris gayana*)	Cotton seed meal
OM	910	901	924	924
NDF	678	420	690	290
ADF	445	305	378	193
CP	40	200	63	485
Ca	4.2	12.7	4.3	2.8
P	1.5	3.3	3.6	14.2
Mg	0.9	3.3	2.3	7.5
K	4.7	29.2	10.0	18.0
Na	0.8	1.3	7.5	2.3
S	1.5	2.5	1.7	4.7

Feed residues were collected and weighed at 0730 h each day and steers were offered feed at approximately 0800 h each day. Sub-samples of feed offered were collected at feeding each day and bulked over seven consecutive days. Feed residues were weighed daily, bulked over seven consecutive days for each steer. Duplicate sub-samples of feed offered and feed residues for each steer were collected at the end of each seven days period.

3.2.3 Faecal collections

The digestibility of DM and OM were calculated from feed intake and faecal data from days 28 to 35. A 10% sub-sample of total faecal output was collected each day, placed at 4^{o}C and bulked over the collection period. At the end of the collection period the bulked faeces was mixed well and triplicate sub-samples were collected for subsequent analysis. Triplicate sub-samples of feed offered and residues were also collected for subsequent analysis.

3.2.4 Liveweight and body dimension measurements

Liveweight was measured prior to feeding every seven days throughout the experiment. HH and TC-TC measurements were taken every 2 weeks on the same day as LW measurements. The hip height measurements are commonly used in northern Australia to avoid the variable influence of hump size in Bos indicus animals and its influence on wither height which has been used in some studies in the literature. Body condition score was assessed by the same person every fourteen days using a 1 to 5 score system (score 1 indicated emaciated and 5 was obese) (Wildman et al. 1982; Edmonson et al. 1989).

3.2.5 Blood samples

Plasma samples were collected before feeding at the same time as weighing every 21 days for measurement of insulin, IGF-1, leptin, PTH, TT3, T4, bone markers and blood metabolites. Blood was collected from the jugular vein into lithium heparin coated vacutainers (Becton Dickinson; Franklin Lakes, NJ, USA) on the same day as LW measurements. After collection the vacutainers were slowly inverted 6 to 8 times and then placed on ice for approximately 30 min prior to centrifugation at 1700 *g* for 10 min at 4^{o}C, plasma was collected and stored at -20°C until analysed. The concentration of insulin, leptin, IGF1, adiponectin, T4, tT3, parathyroid hormone, CTX-1 and BAP inorganic were measured in plasma samples collected on days 0, 56 and 98. Additional samples for measurement of IGF-1 were obtained on days 28 and 84. The concentration of glucose, calcium, inorganic phosphorus, urea and total protein were measured in plasma samples collected on days 0, 56 and 98 of the experiment.

3.2.6 Bone post mortem specimnes

At the conclusion of the trial steers were euthanised and multiple tissue samples were obtained at post mortem. Segments of bone that included the growth plate as well as physeal and metaphyseal bone were cut from the tuber coxae, distal femur, distal radius and distal cannon bone (Figure 4). One centimetre transvere segments of the right 11th and 12th ribs were obtained at 25%, 50% and 75% of the lenth of the rib. At each site 3 separate samples were obtained and immediately placed into 70% ethanol, 10% NBF or snap frozen in liquid nitrogen. Fixed samples were stored at 4°C and frozen samples at -80^{0}C until processed.

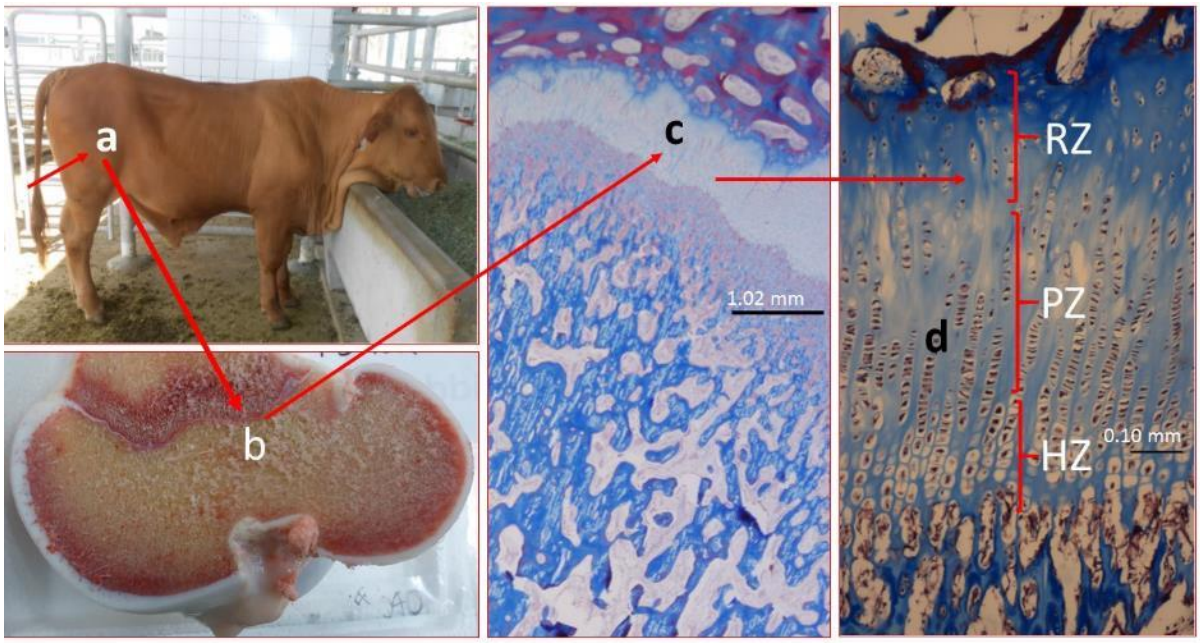

Figure 4.

Samples of growth plate and adjacent physeal and metaphyseal bone were collected at post mortem from multiple sites including distal femur (a and b). Samples were processed, sectioned and microscope images photographed at low (10x) magnification (c) and high (100x) magnification (d). Both c and d are stained with Masson's trichrome stain. RZ: resting zone, PZ: proliferative zone and HZ: hypertrophic zone of the distal femoral growth plate.

3.2.7 Radiographic measurement of long bones

At post mortem the cannon bone, humerus, combined radius/ulna, tibia 11th rib and 12th rib were dissected free of soft tissue, wrapped in plastic and stored at -20^{0}C. Radiographs were obtained using computerised digital radiology. Images were stored in a diccom format with inbuilt calibration for distance. An aluminium step wedge of a known length was used incorporated in the image when it was taken and was used to correct for any magnification of the bone due to distance from the plate receiver. The images were then measured at standard anatomical landmarks to obtain a total length of the bone.

3.2.8 Statistical analysis

The SAS software program (SAS Inst. Inc., Cary, NC) with the Mixed Model procedure (PROC MIXED) was used to analyse the data. PROC MIXED was able to handle repeated measures over time (Wang and Goonewardene, 2004). The effects of block, bST injection (hormone), diet, day and all of the interactions were used as the model. A $\log_{10}$ transformation was applied before the analysis. The level of significance was obtained from performing the type 3 test of fixed effect, this process enables us to get the P value of the treatments and their interactions.

The tests of Effect Slices were done to obtain the P value of the interaction of day effect to some combinations of treatments in the output. The data in the Least Square Means

including standard error for each treatment and some interactions were then back-transformed from log_{10} to the real values.

3.3 Experiment 3. The effect of changes in feed intake on the molecular regulation of bone elongation in sheep

3.3.1 Experiment introduction

The aim of this experiment was to investigate key molecular signals involved in regulating changes in long bone growth plates during extremes of growth in ruminants. Fasting and re-feeding in lambs were used as nutritional models of dramatic changes in growth within the EGP. These treatments were designed to drive rapid skeletal growth, followed by growth stasis and rapid growth again during re-alimentation. To develop strategies to manipulate skeletal elongation, an understanding of the underlying biology of these responses within the skeleton and muscle is required. Upregulation of Ribo Nucleic Acid (RNA) production, or gene expression, is how cells convert DNA information into the production of proteins in reponse to a myriad of stimuli and insults. This experiment was designed to measure differences in gene expression between rapidly elongating EGP and EPG with markedly reduced elongation. By comparing the histological structure and response to fasting of typical long bone EGPs with the tuber coxae EGP, this experiment was also used to validate the use of the tuber coxae biopsy samples in steers in experiments 2 and 3.

3.3.1 Experimental design, animals, diets and feeding

Twenty seven merino crossbred male lambs [five months of age and 32.5 ± 4.0. (LW, mean ± SD)] were housed in individual pens. Twenty six of the lambs were castrated males (weathers) while 1 was an uncastrated male. This animal was in the fasting group. Results within this groups were analysed and the results from this male were not found to be significanlty different to the remaining animals in the cohort. Results from this animal were therefore included in the study. Following a five day acclimatisation period the lambs were allocated to three slaughter cohorts. The 21 day experiment consisted of consecutive phases consisting of feeding (Phase 1), fasting (Phase 2) and re-feeding (Phase 3) with each phase 7 days in duration. The lambs were offered lucerne chaff (855 g OM, 157 g CP, 265 g ash-free NDF, 2.5 g P and 17.7 g Ca/kg DM) *ad libitum* during the feeding (AdLib) and re-feeding (ReFeed) phases and 100 g of Mitchell grass (856 g OM, 34 g CP, 689 g ash-free NDF, 1.5 g P and 3.8 g Ca/kg DM) hay/day during the fasting (Fast) phase. At the end of each phase nine wethers constituting the allocated treatment group were euthanized and samples collected post-mortem.

3.3.2 Liveweight and body dimension measurements

Lambs were weighed on the first day of the experiment and then on the final day of each phase of the experiment (days 1, 7, 21 and 27).

3.3.3 Blood samples

Blood samples were collected from the jugular vein into lithium heparin coated vacutainers (Becton Dickinson; Franklin Lakes, NJ, USA) from lambs on the first day of the experiment and then on the final day of each phase of the experiment (days 1, 7, 21 and 27). After collection the vacutainers were slowly inverted 6 to 8 times and then placed on ice for approximately 30 min prior to centrifugation at 1700 *g* for 10 min at 4^{o}C, plasma was collected and stored at -20°C until analysed.

3.3.4 Bone post mortem samples

At the end of each phase nine wethers were euthanized and samples collected post-mortem. Segments of bone that included the growth plate as well as physeal and metaphyseal bone were cut from the tuber coxae, distal femur and the distal radius. At each site 3 separate samples were obtained and immediately placed into 70% ethanol, 10% NBF or snap frozen in liquid nitrogen. Fixed samples were stored at 4°C and frozen samples at -80^{0}C until processed (see laboratory analysis).

3.3.5 Statistical analysis

Statistical analysis of growth plate histomorphometry measurements were conducted using the open-source software R version (R Core Team, 2013). Prior to analysis all data was checked for normality and homoscedasticity. When analysis of variance showed a significant effect of dietary treatment a turkey test post hoc was performed to explore the differences between groups.

3.4 Laboratory analysis of samples Experiments 1-3

3.4.1 Feed and faeces

Sub-samples of feed offered, feed residues and faeces were dried to a constant weight at 65^{o}C for DM determination. Samples were then ground through a 1 mm screen (Retsch ZM 200; Haan, Germany), dried for 24 h at 105°C to determine residual DM content and then combusted for 8 h at 550°C to determine OM content. Metabolisable energy in each nutritional treatment was estimated by the equation M/D = 0.172DMD – 1.707 (R^2 = 0.92; s.e. = 0.527) described in CSIRO (2007). The N content of feeds offered was measured by the Kjeldahl method using an auto-digestor (Tecator 2520, FOSS; Hillerød, Denmark) and a N analyser (Kjeltec 8400, FOSS; Hillerød, Denmark) following the manufacturers guidelines (Foss, 2003). Crude protein content was calculated using the conversion factor 6.25 x N. The content of ash-free NDF in feeds offered were measured following the procedure described by Van Soest et al. (1991) using a fiber analyzer (A200, Ankom; Macedon, NY, USA). The mineral content of feeds offered was determined on an ICP-OES spectrometer (Optima 7300 DV, PerkinElmer; Waltham, MA, USA) after a nitric-perchloric acid digestion.

3.4.2 Urine

Thawed urine sub-samples were filtered through a 0.2 µm syringe filter (Phenex-RC, Phenomenex, Torrance, CA, USA) and the concentration of purine derivatives was determined using a high-performance liquid chromatograph method described by Balcells et al. (1992). Microbial crude protein production was then estimated using the method of Chen and Gomes (1995) endogenous purine derivative values for *Bos indicus* and *Bos taurus* cattle described by Bowen et al. (2006).

3.4.3 Rumen fluid

The concentration of NH_3-N in the rumen fluid was measured by titration with 0.01 *M* HCl using a TIM 840 Titration workstation manager (Radiometer Analytical SAS; Villeubanne Cedex, France) after distillation using a semi-automatic distillation unit (UDK 139, Velp Scientifica; Usmate, MB, Italy). The concentration of individual VFA's in the rumen fluid were analysed by gas liquid chromatography (GC-2010, Shimadzu; Kyoto, Honshu, Japan) fitted with a polar capillary column (ZB-FFAP, Phenomenex; Lane Cove, NSW, Australia).

3.4.4 Plasma

The concentration of glucose, calcium, inorganic phosphorus, urea and total protein were measured in plasma samples using an Olympus AU400 auto-analyser (Beckman Coulter Diagnostic Systems Division; Melville, NY, USA) following the manufacturer's guidelines. The concentration of non-esterified fatty acids (NEFA) was measured in the same samples using an enzymatic method (Wako NEFA-C, Wako Chemicals; Osaka, Japan).

Insulin-like growth factor-1 (IGF-1) was measured with a commercial immunoradiometric assay kit (Beckman Coulter, Inc.) with extraction. Insulin was determined with the DIAsource INS-Irma which is an immonoradiometric assay based on coated tube separation. Leptin concentration was determined using Radioimmunoassay (RIA) with the use of Millipore's Multi-Species Leptin XL-85K kit. Radioimmunoassay (IMMUNOTECH, A Beckman Coulter Company) for the in vitro determination in human serum and plasma were used in the determination of PTH. Total triiodothyronine was determined using a radioimmunoassay for the in vitro in human serum and plasma (Beckman Coulter, Inc.). These procedures were done as per the manufacturer's protocol. The bone resorption biomarker C-terminal telopeptides of Type-1 collagen one (CTX-1) was measured using serum CrossLaps® (CTX-1) ELISA kit (Immunodiagnosticsystems). Bone alkaline phosphatase (BAP) as a bone formation marker was measured using MicroVue BAP kit which is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the samples. Osteocalcin was measured using an enzyme immunoassay for the quantitation of intact osteocalcin in plasma and serum (Microvue, Quidel ® Corporation). The procedures were performed using the manufacturer guidelines. All of the assay results (IGF-1, insulin, thyroid, PTH and leptin) were counted in an automatic gamma radiation counter (2470 Wizard2; Perkin Elmer, Waltham, MA, USA). The data output of counts per minute (cpm) for TC, calibrators, QC and unknown samples were processed using the software "Assay Zap" (Biosoft; Cambridge, UK) to interpolate hormone concentrations from the average cpm from each duplicate sample and the QC. All samples were analysed in

duplicate for each assay. The intra- and inter-assay co-efficient of variation (CV) were less than 10% for all assays.

3.4.5 Bone histomorphometry

All bone samples were fixed in 10% NBF and then decalcified in 10% EDTA (pH 7.0) for 6-12 weeks. Samples were individually weighed each week and decalcification was determined to be complete when samples stopped losing weight. After decalcification the samples were embedded in paraffin and multiple 5 µm thick sections were taken parallel to the longitudinal direction of the bone axis. The sections were stained with Masson trichrome and the growth plate photographed in 2 separate regions with a 40X objective and trabecular bone photographed with a 10X objective using a Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA) equipped with a digital camera Q-Imaging camera (Qimaging Corporation; Surrey, BC, Canada). All bone histomorphometry measurements were undertaken using the open-source NIH software ImageJ (Schneider et al., 2012) and the plugin BoneJ (Doube et al., 2010).

For growth plate histomorphometry 5 measurements were taken per picture for determination of the height of the proliferative zone (PZ, µm) and hypertrophic zone (HZ, µm), number of hypertrophic cells per column (cells/ µm) and diameter (µm) or area (μm^2) of terminal hypertrophic cells (**Figure 5.**). The measurements sites were evenly distributed across each zone and were taken by a single blinded observer to treatment group. Height and diameter measurements were taken parallel to the long axis of the bone and the mean of ten measurements per animal was utilized for analysis.

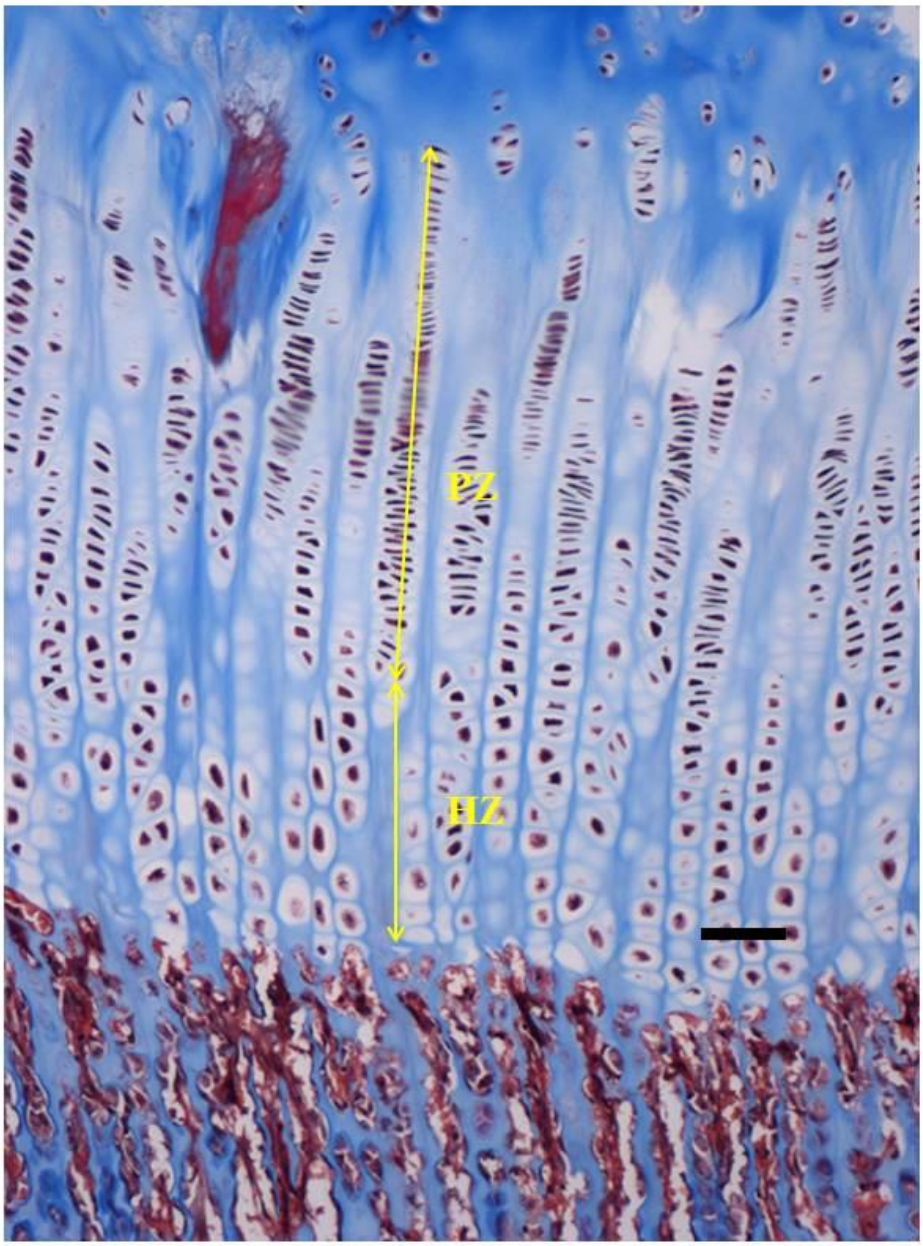

Figure 5.

Light micrograph of steer's growth plate of tuber coxae bone at 4x magnification. PZ: proliferative zone, HZ : Hypertrophic zone. The bar indicated 0.02 mm.

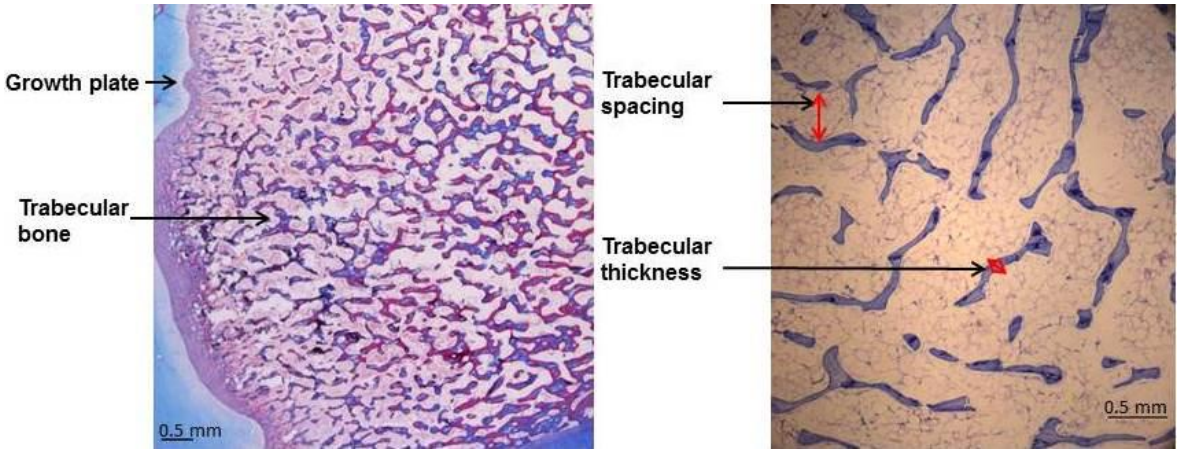

Figure 6.

Light micrograph of steer's trabecular bone of tuber coxae at 1x (left-hand side) and 2x (right-hand-side) magnification. The scale bar indicates 0.5 mm.

One representative section for each animal was selected for measures of trabecular bone parameters. The pictures were taken at 10X magnification at a standardised distance from the growth plate (300 µm). This magnification maximised the area of bone available for analysis and allowed the complete section to be analysed in one image (Figure 6). Trabecular bone measurements obtained were bone volume (BV/TV, %), average trabecular thickness (Tb.Th, µm) and average trabecular separation (Tb.Sp, µm).

3.4.6 Bone gene expression using next generation (transcriptomic) sequencing (RNA-Seq)

Bone gene expression was performed using next generation (transcriptomic) sequencing (RNA-Seq). Samples obtained at biopsy or post mortem were immediately snap frozen in liquid nitrogen and stored at -80^{0}C until processed. A section of tissue sample weighing about 300µg was used for RNA extraction. The sample of tissue was both physeal cartilage and adjacent bone with the sample centred on the cartilage/bone junction or on the physis (**Figure 5**). The sample was pulverised into a very fine powder with a mortar and pestle in liquid nitrogen. RNA was extracted using the guanidium-thiocyanatephenol-chloroform technique. The bone powder was added to 2 mls of Trizol (Life Technologies, Mulgrave, Australia) and incubated at room temperature for 5 minutes. Following centrifugation at 12,000×g for 10 minutes at 4°C, 400 µl chloroform was added to the supernatant, mixed and incubated at room temperature for 10 minutes. The aqueous phase was then precipitated following centrifugation at 12,000×g for 10 minutes at 4°C using 70% ethanol. RNA was purified using RNeasy spin columns (Qiagen, Chadstone Centre, Australia) used with on-column DNase treatment (Qiagen) to remove residual gDNA according to the manufacturer's instructions. RNA was quantified using a Nanodrop ND-100 spectrophotometer and assessed for purity by ultraviolet absorbance measurements at 260 nm and 280 nm.

Total RNA samples were sent to the Australian Genome Research Facility Ltd (AGRF, Parkville, VIC). Following quality control of the samples, sequencing was performed using the Illumina TruSeq RNA v2 sample preparation protocol for processing samples. This

protocol included cDNA Synthesis, purification of mRNA (via oligo(dT) beads), fragmentation of mRNA with divalent cations and heat, 1st strand cDNA synthesis (randomly primed) and 2nd strand cDNA synthesis. Library preparation of cDNA included DNA fragment end repair (blunt ending of DNA fragments), 3' Adenylation of DNA fragments, sequencing adapter ligation (utilizing T-A pairing of adapter and DNA fragments) and amplification of library via PCR.

Initial bioinformatics was performed by AGRF. The primary bioinformatics analysis involved demultiplexing and quality control (QC). The data was then processed through RNA-seq expression analysis workflow, which included alignment, transcript assembly, quantification and normalisation. Differential expression analysis was then performed and sent to us in spreadsheet format. Comparisons were made between treatment groups to determine genes that were significantly increased or decreased in one group over another. This difference was expressed as a negative or positive fold change.

We then performed gene ontology and pathway analysis. The functional analysis and clustering tool from the Database for Annotation, Visualisation, and Integrated Discovery (DAVID bioinformatics resources 6.7) was used (Nature Protocols 2009; 4(1):44 & Nucleic Acids Res. 2009;37(1):1). Lists of genes that were significantly increased or decreased in one group over another were uploaded into the DAVID program and cellular, metabolic or molecular pathways and pathway clusters were generated and recorded.

For all the above comparisons the FDR-adjusted corrected p-value (Benjamini-Hochberg correction for multiple-testing statistic) was used to determine significance of individual gene differential expression and the significance of subsequent pathway analysis.

Differential gene expression analysis was performed on 3 types of tissue samples: tuber coxae growth plate with a small amount of adjacent bone and cartilage (experiments 1 and 3), distal radius growth plate with some adjacent bone (experiment 2) and tuber coxae growth plate and cartilage with adjacent bone removed (experiment 2).

3.4.7 Ash-free NDF

The ash-free NDF content of the dry samples were determined by the method of Van Soest and Wine (1967) using a fibre extraction unit (ANKOM 220). A 0.5 g ground sample was weighed into a filter bag that was labelled using a solvent resistant marker. The bag was then put into the Bag Suspender Trays and placed into digestion vessel and anhydrous sodium sulfite (0.5 g/50 mL of neutral detergent solution (NDS)) was added followed by the NDS (1900-2000 mL or 100mL/bag) and 4 mL of alpha-amylase. The solution in the vessel was then boiled for 75 minutes. The extraction solution was then drained and the residue was rinsed 3 times with hot water 70-90°C (4 mL alpha-amylase added to the first 2 washes) followed twice by acetone. The filter bag with residues was air-dried before oven drying at 60°C for 24 h. The filter bag was weighed and ashed in a furnace at 500 °C for 4.5 h. The ash content was determined, and ash-free NDF content was then calculated. The procedure for ADF analysis was the same as for NDF determination except that acid detergent reagent was used instead of neutral detergent reagent. However, sodium sulfite was not used in ADF analysis. Mineral content of the samples was determined by digesting approximately 0.3 g of oven dried sample in 6 mL nitric acid and 2 mL perchloric acid then made up to 20 mL with

RO water. The digested samples were analysed using an inductively coupled plasma atomic emission spectrometer (Optima 7300 DV, Perkin Elmer; Wellesley, MA, USA).

4 Results

4.1 Experiment 1. The effect of crude protein content or level of metabolisable energy intake on skeletal growth, subsequent compensatory growth and bone gene expression in Bos indicus crossbred and Holstein genotype steers

4.1.1 Dry matter and metabolisable energy intake, liveweight gain, hip height gain, feed conversion ratio, liveweight:hip height and rate of change in body measurements

The appearance of experimental steers at the end of Phase 1 is shown in Figure 7. During Phase 1, steers offered the HCP-HME treatment had higher DM and ME intake than steers offered the HCP-LME and LCP-LME treatments (Table 3) while steers offered the LCP-LME treatment had higher DM intake but comparable ME and lower CP intake to steers offered the HCP-LME treatment, as planned. During Phase 2, steers that had a low ME intake during Phase 1 (HCP-LME and LCP-LME) had higher DM intake than steers fed HCP-HME during Phase 1 (Table 3). When offered ad libitum HCP-HME diet, the previously ME restricted steers showed a transitory greater ME intake when compared to control groups at the same LW and genotype (Figure 8). The pattern of ME intake over the experimental period showed a decrease as steers increased LW in both genotypes, but HF had a markedly higher ME intake than BX during the whole period (Figure 8).

Liveweight gain of HCP-HME steers was higher in Phase 1 and lower in Phase 2 compared with HCP-LME and LCP-LME steers, with no difference in LWG between HCP-LME and LCP-LME steers in either phase (Table 3 and Figure 9). Holstein-Friesian steers had higher LWG than BX steers in Phase 1 but not Phase 2 of the experiment Table 3 Dry matter and metabolisable energy intake, liveweight gain, hip height change, feed conversion ratio, liveweight:hip height and rate of change in body measurements (Table 3). There were significant interactions between nutritional and treatment genotype in both phases of the experiment. (Figure 10 and Figure 11). HF steers consuming lucerne chaff ad libitum grew faster than BX on the same nutritional treatment. HF steers fed LCP-LME during Phase 1 showed a tendency (P=0.07) for greater LWG than HCP-LME during Phase 2 Figure 9.This was the only group that showed greater (1.3 *vs* 1.5; P<0.05) LWG during Phase 2 when compared to HCP-HME steers during Phase 1. Steers fed ME restricted diets (HCP-LME and LCP-LME) had a much less efficient feed conversion compared to HCP-HME during Phase 1. During Phase 2, the previously restricted steers converted DM into LW more efficiently than steers fed HCP-HME throughout the experiment.

The rate of skeletal growth for all dimensions measured was higher in HCP-HME steers than HCP-LME and LCP-LME steers during Phase 1 (Table 3 and Figure 10 and Figure 9). Hip height gain was higher in HCP-HME but also higher in HCP-LME steers compared with LCP-LME steers but no other differences in rate of change of body dimensions were observed in

response to the additional CP intake by these steers during Phase 1. A significant interaction between nutritional treatment and genotype was found in Phase 1, the differences followed the same pattern as that of LWG (Figure 10). During Phase 2 the rate of HH gain was greater in HCP-LME and LCP-LME steers compared to HCP-HME steers, with changes in TC-TC, TC-TI and TC-SLD reflecting changes in HH (Table 3). The HH gain of restricted steers (i.e. HCP-LME and LCP-LME) during Phase 2 was not significantly different from HCP-HME steers during Phase 1. Hip height gain was higher in HF steers compared with BX steers in both phases of the experiment but differences in rate of change of other body dimensions was generally similar between genotypes during both phases.

Table 3 Dry matter and metabolisable energy intake, liveweight gain, hip height change, feed conversion ratio, liveweight:hip height and rate of change in body measurements. Dry matter (DM) intake (DMI; g/kg LW.day), metabolisable energy intake (MEI; MJ/kg LW.day), crude protein (CP) intake (CPI; g CP/kg LW.day), feed conversion ratio (kg DMI/kg LWG), LWG (kg/d), LWG (kg), change in hip height (HH change; mm/100 day), liveweight:hip height (LW:HH; kg/cm) and distance between other points on the body (TC, OLC, ACB, SLD and TI; mm/100 day) of *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments[1] (Phase 1) and undergoing re-alimentation (Phase 2)[2,3]

	Nutrition (N)					Genotype (G)				N x G	
	HCP-HME	HCP-LME	LCP-LME	sem	P	HF	BX	sem	P	sem	P
					Phase 1						
DMI	31.5 [c]	12.1 [a]	20.3 [b]	0.60	<0.001	23.6	18.9	0.6	<0.001	0.90	0.06
MEI	0.30 [b]	0.12 [a]	0.11 [a]	0.05	<0.001	0.19	0.16	0.01	<0.05	0.01	0.46
CPI	6.13 [c]	2.00 [b]	0.84 [a]	0.27	<0.001	3.15	2.82	0.22	0.15	0.22	0.06
FCR	6.3 [a]	30.2 [b]	47.2 [b]	10.1	<0.001	29.3	27.0	9.0	.032	11.3	0.10
LWG	1.27 [b]	0.16 [a]	0.14 [a]	0.03	<0.001	0.58	0.47	0.04	<0.05	0.06	0.02
HH Change	104.7 [c]	41.8 [b]	30.3 [a]	3.10	<0.001	63	54.4	2.53	<0.05	6.20	0.01
LW:HH[4]	2.1 [c]	1.5 [a]	1.7 [b]	0.04	<0.001	1.96	1.65	0.08	<0.001	0.15	0.41
TC-TC[5]	8.4 [b]	1.7 [a]	1.6 [a]	0.3	<0.001	4.5	3.3	0.3	0.58	0.4	0.29
OLC-ACB	1.82 [b]	0.48 [a]	0.04 [a]	0.3	<0.001	0.8	0.8	0.2	0.97	0.4	0.67
SLD-TC	18.6 [b]	4.5 [a]	3.5 [a]	2.4	<0.001	10.1	7.6	1.9	0.37	3.4	0.41
TC-TI	5.0 [b]	0.9 [a]	0.6 [a]	0.2	<0.001	3.0	1.4	0.2	<0.001	0.4	0.44
SLD-OLC	3.6 [b]	1.5 [a]	2.1 [ab]	0.4	<0.05	2.6	2.2	0.4	0.45	0.7	0.42
					Phase 2						
DMI	26.6 [a]	32.6 [b]	32.2 [b]	0.5	<0.001	32.1	28.8	0.4	<0.001	0.6	0.27
MEI	0.25 [a]	0.31 [b]	0.30 [b]	0.03	<0.001	0.30	0.27	0.01	<0.001	0.02	0.20
CPI	4.41	5.39	5.34	0.12	<0.001	5.33	4.77	0.10	<0.001	0.17	0.24
FCR	11.2 [a]	6.8 [b]	6.1 [b]	0.56	<0.001	9.2	6.8	0.36	<0.001	0.49	0.74
LWG	0.93 [a]	1.34 [b]	1.45 [b]	0.04	<0.001	1.3	1.2	0.04	0.08	0.07	0.04
HH Change	75 [a]	89 [b]	89 [b]	5.76	<0.05	92	77.1	7.06	<0.05	7.0	0.38
LW:HH	2.9 [b]	2.1 [a]	2.2 [a]	0.07	<0.001	2.6	2.2	0.06	<0.001	0.07	0.32
TC-TC	7.7 [a]	9.1 [ab]	9.8 [b]	0.5	<0.05	9.3	8.5	0.4	0.24	0.7	0.63
OLC-ACB	2.2	2.9	2.6	0.4	0.42	2.6	2.6	0.3	0.94	0.5	0.80
SLD-TC	4.0 [a]	10.8 [b]	13.4 [b]	1.5	<0.001	8.0	10.8	1.3	0.16	3.3	0.89
TC-TI	4.2 [a]	5.8 [b]	6.4 [b]	0.5	<0.001	5.6	5.3	0.3	0.44	0.5	<0.05
SLD-OLC	3.0	3.4	3.3	0.6	0.93	2.82	3.62	0.5	0.31	0.9	0.22

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Data are least squares means, with standard error of the mean (SEM)

[3]Means within row with different superscripts differ ($P < 0.05$)

[4]LW:HH at end of each Phase

[5]Tuber coxae (TC), Tuber isheii (TI), Olecranon (OLC), Accessory carpal bone (ACB) and point of shoulder (SLD)

Figure 7.

Appearance of *Bos indicus* (**a**, **b** and **c**) and *Bos taurus* (**d**, **e** and **f**) steers at the end of Phase 1, after approximately 100 days being fed HCP-HME (a. and d.), HCP-LME (b. and e.) and LCP-LME respectively (c. and f.).

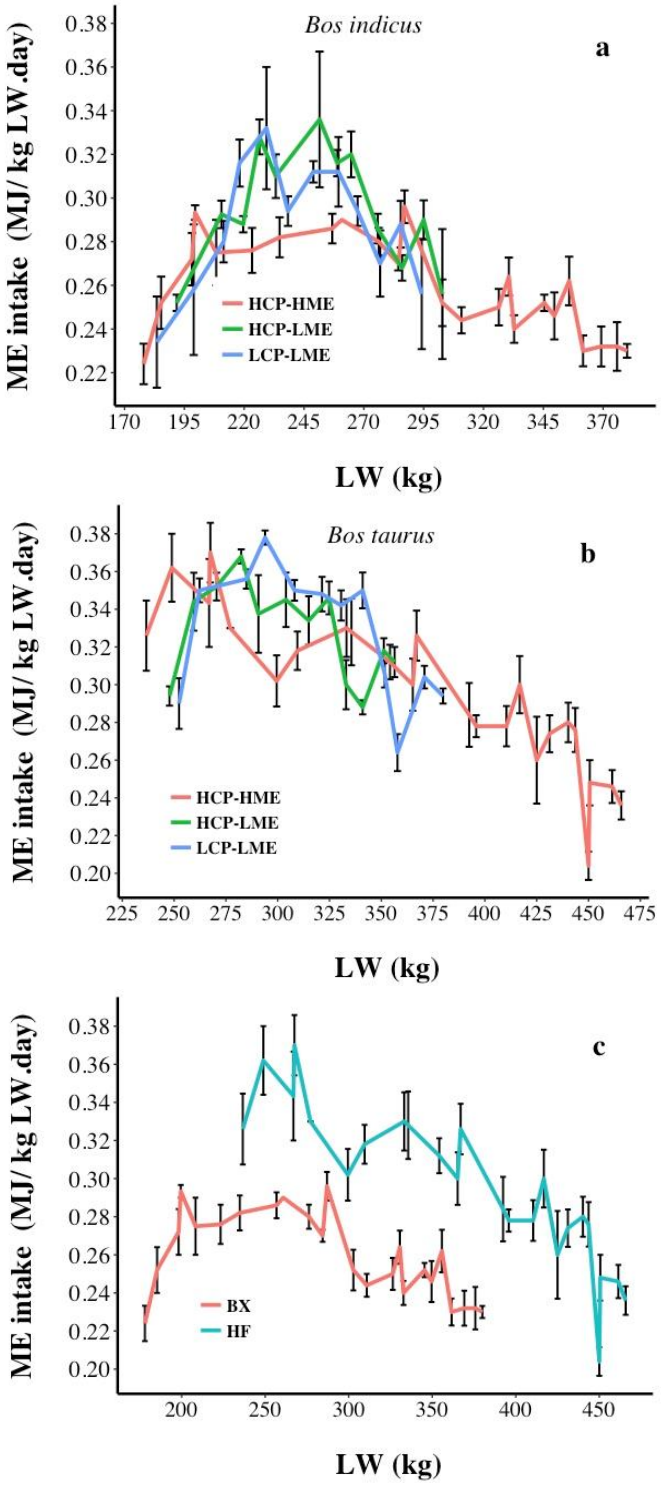

Figure 8 Relationship between metabolizable energy (ME) intake and liveweight (LW) of Bos indicus (a) and Bos taurus (b) steers fed different nutritional treatments in Phase 1[1] and HCP-HME in Phase 2, and the relationship between ME intake of Bos indicus (BX) and Bos taurus (HF) fed HCP-HME throughout the whole experimental period (c).

[1]See materials and methods for description of nutritional treatments [high CP intake and high ME intake (HCP-HME), high CP intake and low ME intake (HCP-LME) and low CP intake and low ME intake (LCP-LME)]. Each point is the arithmetic mean of 5 steers with standard error of the mean.

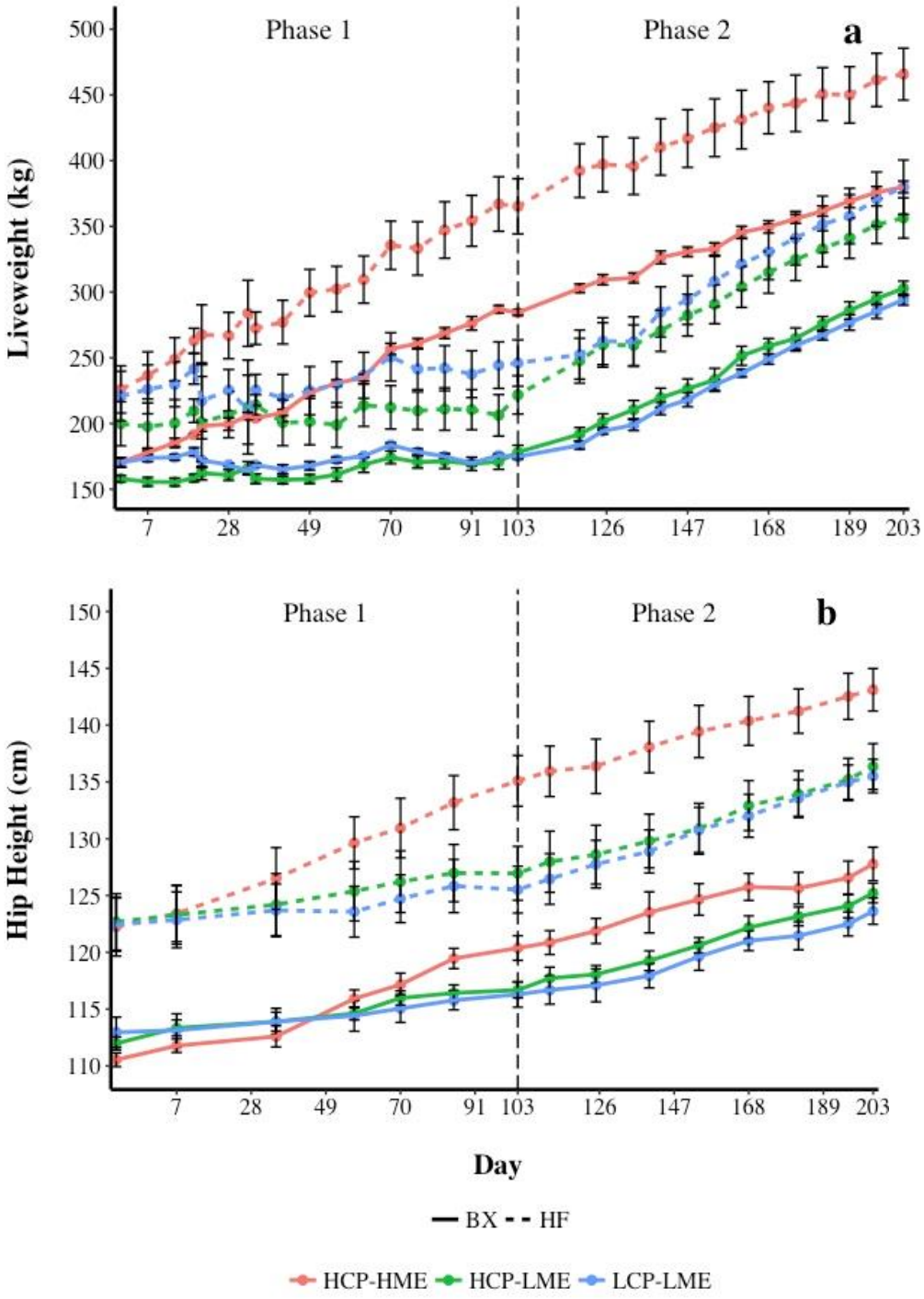

Figure 9 Change in liveweight weight and hip height

Change in liveweight (**a**) and hip height (**b**) of *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments[1] (Phase 1) and undergoing re-alimentation (Phase2).

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]. Each point is the arithmetic mean of 5 steers with standard error of the mean.

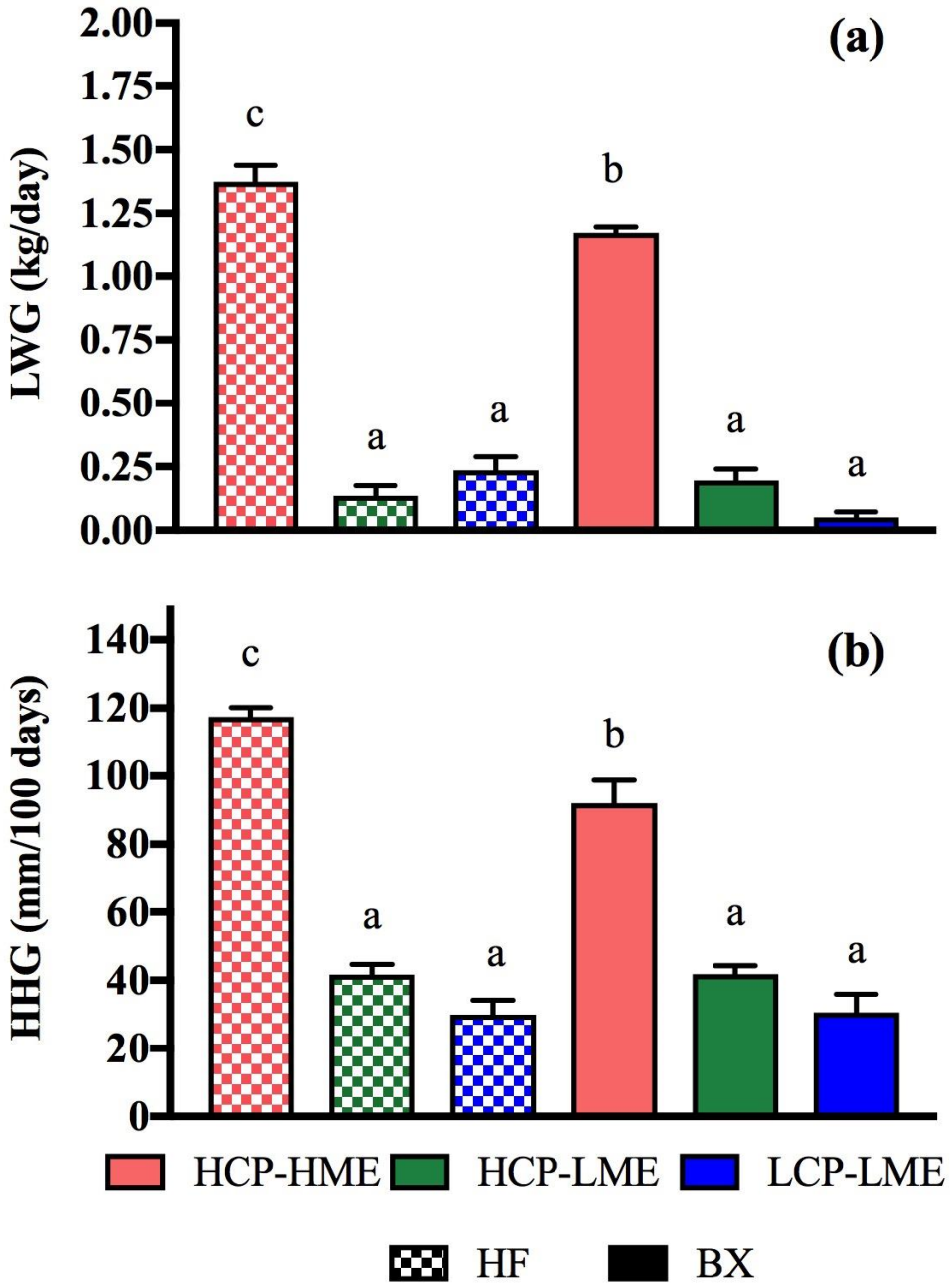

Figure 10 Liveweight gain and hip height gain in Phase 1

Liveweight gain (LWG; a) and hip height gain (HHG; b) of Bos taurus (HF) and Bos indicus (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± SEM. Means with different letters are significantly different (Tukey, $P<0.05$)1.

1See materials and methods for description of nutritional treatments [high CP intake and high ME intake (HCP-HME), high CP intake and low ME intake (HCP-LME) and low CP intake and low ME intake (LCP-LME)].

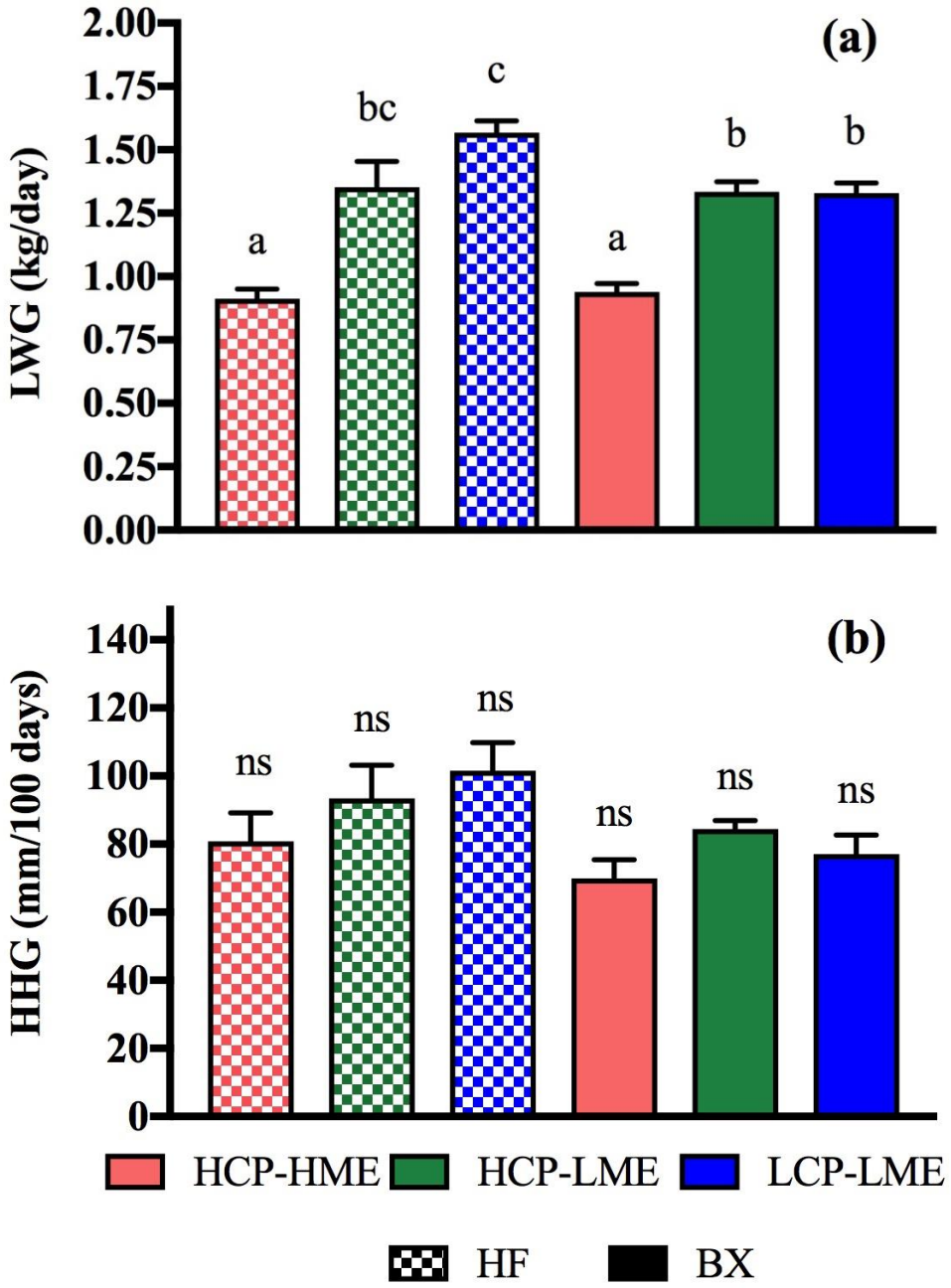

Figure 11 Liveweight gain and hip height gain Phase 2

Liveweight gain (LWG; a) and hip height gain (HHG; b) of Bos taurus (HF) and Bos indicus (BX) steers undergoing re-alimentation (Phase 2). Data are expressed as means ± SEM. Means with different superscripts are significantly different ($P \leq 0.05$)[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

4.1.2 Rumen parameters and microbial protein production

The digestibility of the lucerne chaff did not differ with intake in the current experiment [65.6% and 67.2% at ad libitum (HCP-HME) and restricted intakes (HCP-LME)] but was significantly higher ($P<0.01$) than the digestibility of the Mitchell grass hay (40.1%) consumed by steers offered the LCP-LME treatment. Steers fed the Mitchell grass hay based diet (LCP-LME) had a lower concentration of NH_3N in the rumen and produced less MCP and had a lower EMCP than steers fed the lucerne chaff based diet (HCP-HME and HCP-LME) regardless of DM and ME intake (Table 4). HF steers fed the LME-LCP treatment diet had a lower concentration of NH3N in the rumen than BX steers fed the same nutritional treatment (Figure 12). Microbial protein production but not the EMCP was greater in steers fed lucerne chaff *ad libitum* (HCP-HME) compared with steers fed a restricted amount of lucerne chaff (HCP-LME). Steers with restricted DM intake (HCP-LME) had a lower concentration of VFA in the rumen than steers fed ad libitum (HCP-HME and LCP-LME) regardless of the CP content of the diet. The interaction between genotype and nutritional treatments showed that this effect was only observed in HF steers (Figure 12).

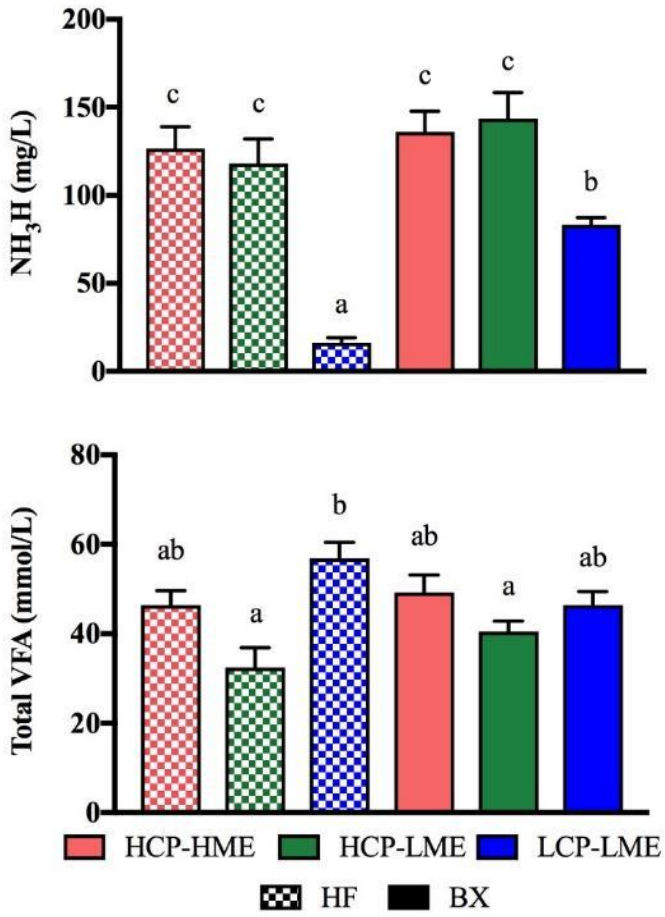

Figure 12. Concentration of rumen ammonia and total volatile fatty acids

Concentration of rumen ammonia (NH_3H; **a**) and total volatile fatty acids (VFA; **b**) in *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± sem. Means with different letters are significantly different (Tukey, P <0.05) [1].

[1] See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

Table 4 Metabolic parameters experiment 1

The dry matter digestibility (DMD; %), pH, concentration of ammonia (NH_3N; mg/L) and volatile fatty acids (VFA, mmol/L) and the molar percentage of acetic, butyric and propionic acids in the rumen fluid and the microbial protein (MCP; g/ kg LW.day) production and the efficiency of MCP production (EMCP; g MCP/kg DOMI) of Bos taurus (HF) and Bos indicus (BX) steers fed different nutritional treatments[1] during Phase 1 of the experiment

	Nutrition (N)					Genotype (G)			
	HCP-HME	HCP-LME	LCP-LME	sem	P	HF	BX	sem	P
DMD	65.6 [b]	67.2 [b]	40.1 [a]	1.3	<0.001	56.7	58.6	1.6	0.11
pH	7.20 [b]	7.27 [b]	6.90 [a]	0.1	<0.001	7.14	7.11	0.1	0.65
NH_3N	131.4 [b]	130.9 [b]	49.7 [a]	11.0	<0.001	87	121	6.4	<0.005
MCP prod	2.17 [c]	0.62 [b]	0.32 [a]	0.1	<0.001	1.15	0.92	0.1	0.77
EMCP	91.0 [b]	72.6 [b]	32.2 [a]	9.7	<0.001	65.8	64.7	5.4	0.88
Total VFA	47.8 [b]	36.5 [a]	51.7 [b]	2.5	<0.001	45.2	45.4	2.0	0.96
Acetic	74.1 [a]	74.2 [a]	78.1 [b]	0.5	<0.001	76.7	74.2	0.5	<0.001
Propionic	13.0 [a]	12.3 [a]	14.4 [b]	0.3	<0.001	13.2	13.3	0.2	0.76
Butyric	8.4 [c]	4.15 [b]	2.5 [a]	0.6	<0.001	6.5	3.4	0.5	<0.001

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Data are least squares means, with standard error of the mean (sem)

[3]Means within row with different superscripts differ ($P < 0.05$)

4.1.1 Plasma metabolites, hormones and bone metabolism markers

The concentration of blood metabolites, hormones and bone metabolism markers were statistically analysed within collection date for the effect of nutritional treatments, genotype as well as the interaction between both factors. All main factors are shown in separate Figures as well as the significant interactions.

The plasma concentration of glucose and Ca were both affected by nutrition during Phase 1 of the experiment (Table 5), which were higher in steers consuming HCP-HME diet. Urea-N concentration was higher in the plasma of steers fed high CP treatment regardless of the ME intake. Nutritional treatment had no effect on the concentration of total protein, NEFA or inorganic P in the plasma during Phase 1 of the experiment. There were no significant differences in any of the metabolites analysed during Phase 2 of the experiment when steers were consuming the same diet. The concentration of NEFA and PUN was higher in the plasma of BX steers compared with HF steers at the end of both phases of the experiment.

In Phase 1 the concentration of insulin and IGF-1 were higher (P<0.0001) in the plasma of steers fed the HCP-HME diet compared with steers fed the LME diets (Figure 13), with no difference in concentration in steers offered the HCP-LME and LCP-LME diets. Leptin

concentration was not affected by the nutritional treatments. BX had initially higher concentration of leptin than HF but no differences were observed between two genotypes after the imposition of the nutritional treatments. During Phase 1, the plasma T3 hormone showed an additive response to dietary CP and ME intake (Figure 14). It was highest in steers fed HCP-HME followed by HCP-LME and lowest in LCP-LME group. No significant differences were found when comparing the concentration of T3 between the genotypes (2.3 vs 2.0 nmol/L for HF and BX respectively; P=0.82; results not shown). The adiponectin concentration was not different between genotypes throughout the experiment. Interestingly, the nutritional restriction treatments had no effect on the concentration of adiponectin by the end of the Phase 1, but there was a significant higher (P=0.02) adiponectin concentration in steers that consumed HCP-HME diet compared to HCP-LME by the end of Phase 2. Adponectin was the only hormone that showed significant different response to the nutritional treatments imposed during Phase 1. There was also no genotype effect on any hormone at the end of either phases of the experiment (Figure 15). However, a significant interaction between genotype and nutritional treatments at the end of Phase 1 was found for T4, with HF steers having significantly (P=0.004) lower concentrations of T4 when submitted to reduced intake of CP and ME while BX steers were not affected (Figure 16). The decrease in T4 in HF fed low CP and ME diets led to a rise in T3:T4 ratio which was significantly higher than BX and HF steers fed high protein low ME and also BX steers fed low CP and low ME diet (Figure 17).

Table 5. The concentration of glucose (mmol/L), total protein (g/L), non-esterified fatty acids (NEFA; mEq/L), plasma urea nitrogen (PUN, mmol/L), inorganic P (mmol/L) and total calcium (Ca; mmol/L) in the plasma of Bos taurus (HF) and Bos indicus (BX) steers fed different nutritional treatments[1] (Phase 1) and undergoing re-alimentation (Phase2)[2,3,4] . Results are from blood samples collected at the end of treatment phase.

	Nutrition (N)					Genotype (G)				N x G	
	HCP-HME	HCP-LME	LCP-LME	SEM	P	HF	BX	sem	P	SEM	P
			Phase 1								
Glucose	5.2 [b]	3.9 [a]	3.7 [a]	0.09	<0.001	4.2	4.3	0.07	0.82	0.14	0.36
Total Protein	65.7	66.1	64.7	1.4	0.62	67	64	1.2	0.05	1.8	0.46
NEFA	0.20	0.16	0.11	0.03	0.24	0.10	0.22	0.02	0.001	0.04	0.45
PUN	7.1 [b]	7.5 [b]	1.2 [a]	0.2	<0.001	4.8	5.8	0.2	0.007	0.4	0.92
P	2.3	2.1	2.3	0.09	0.22	2.2	2.3	0.08	0.62	0.14	0.85
Ca	2.4 [b]	2.1 [a]	2.1 [a]	0.03	<0.001	2.2	2.2	0.02	0.90	0.04	0.28
			Phase 2								
Glucose	4.8	4.9	5.0	0.1	0.19	4.9	4.8	0.08	0.28	0.14	0.63
Total Protein	67.2	65.0	65.9	1.2	0.52	67.4	64.6	1.0	0.06	1.8	0.35
NEFA	0.14	0.16	0.13	0.03	0.74	0.09	0.19	0.02	0.007	0.04	0.35
PUN	7.7	7.8	7.4	0.2	0.64	7.0	8.3	0.2	<0.001	0.3	0.04
P	2.2	2.3	2.3	0.08	0.70	2.3	2.3	0.07	0.81	0.12	0.84
Ca	2.0	2.1	2.2	0.05	0.23	2.1	2.0	0.04	0.17	0.07	0.61

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Data are least squares means, with standard error of the mean (sem)

[3]Means within row with different superscripts differ ($P < 0.05$)

[4]The only significant N x G interaction was PUN (P=0.04)

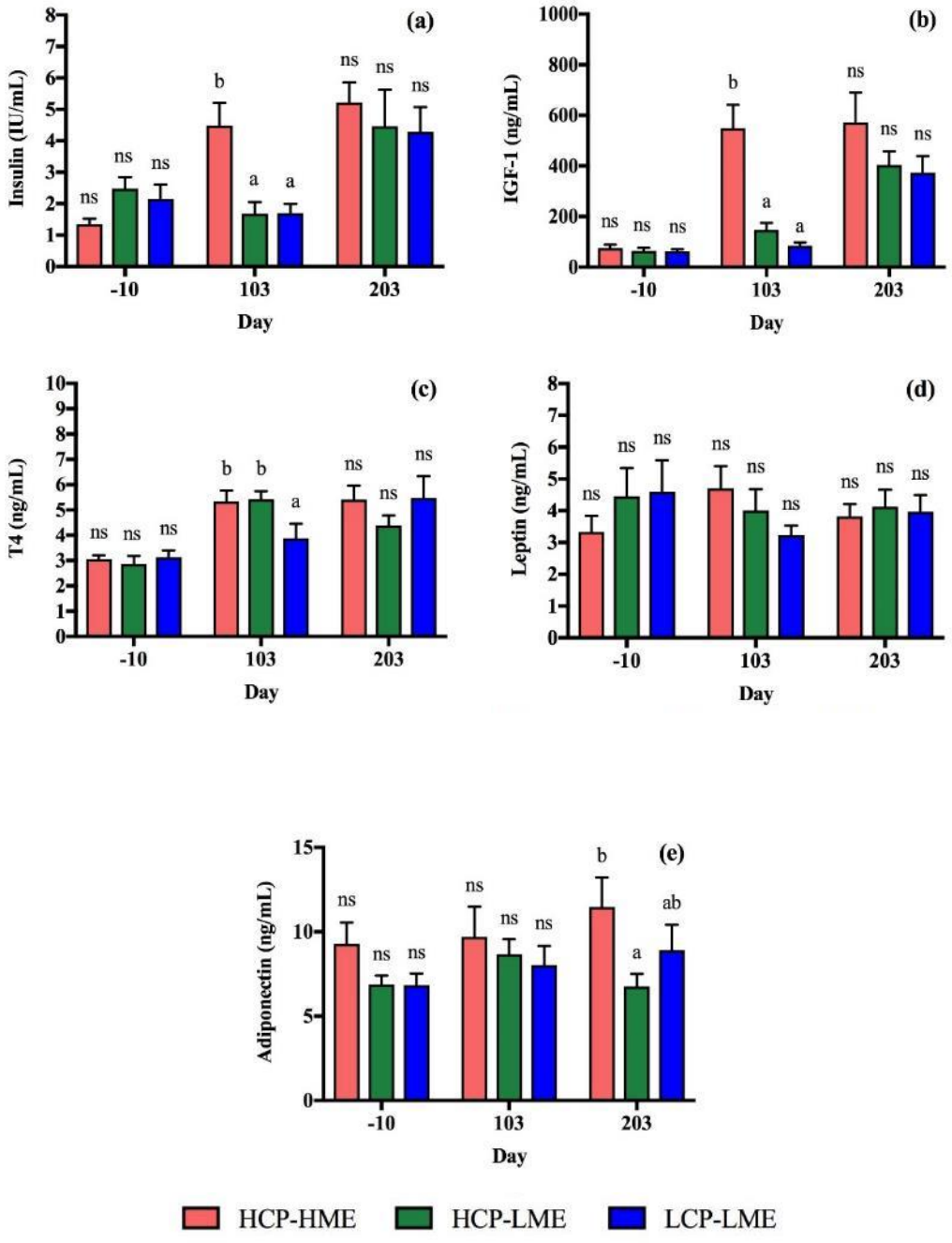

Figure 13 Plasma concnetrations of hormones diet effect

The plasma concentration of insulin (IU/mL; **a**), insulin-like growth factor-1 (IGF-1; ng/mL; **b**), thyroxine (T4; ng/mL; **c**), leptin (ng/mL; **d**) and of adiponectin (ng/mL; **e**) in steers fed different nutritional treatments prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± SEM. Means with different superscripts on given days are significantly different (Tukey, *P* <0.05) and ns is non significant [1].

[1] See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

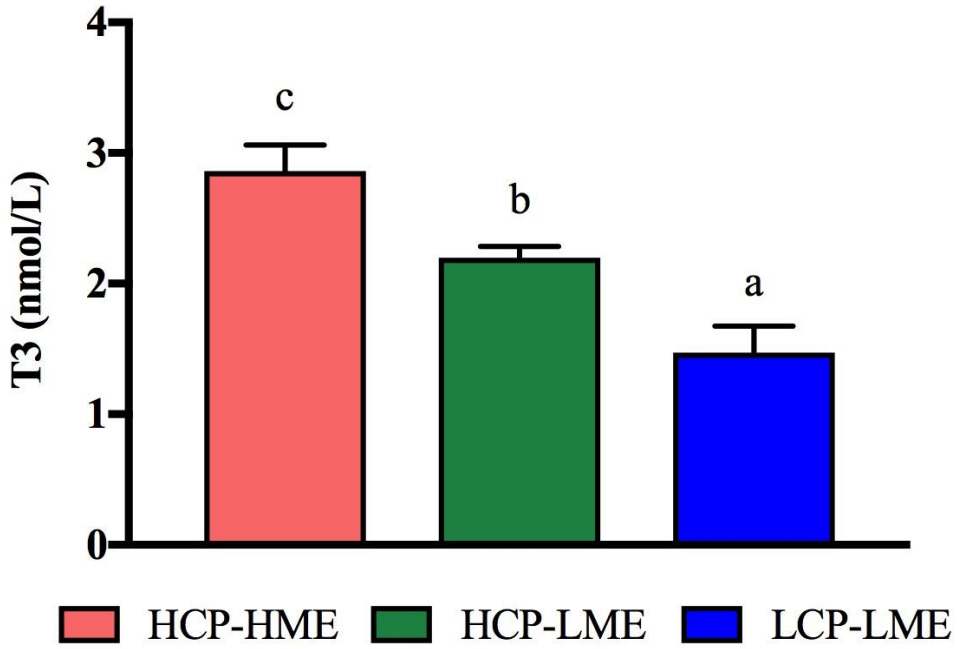

Figure 14. The plasma concentration of triiodothyronine (T3; nmol/L) of steers fed different nutritional treatments at the end of Phase 1 (day 103). Data are expressed as means ± SEM. Means with different superscripts on given days are significantly different (Tukey, P<0.05)[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

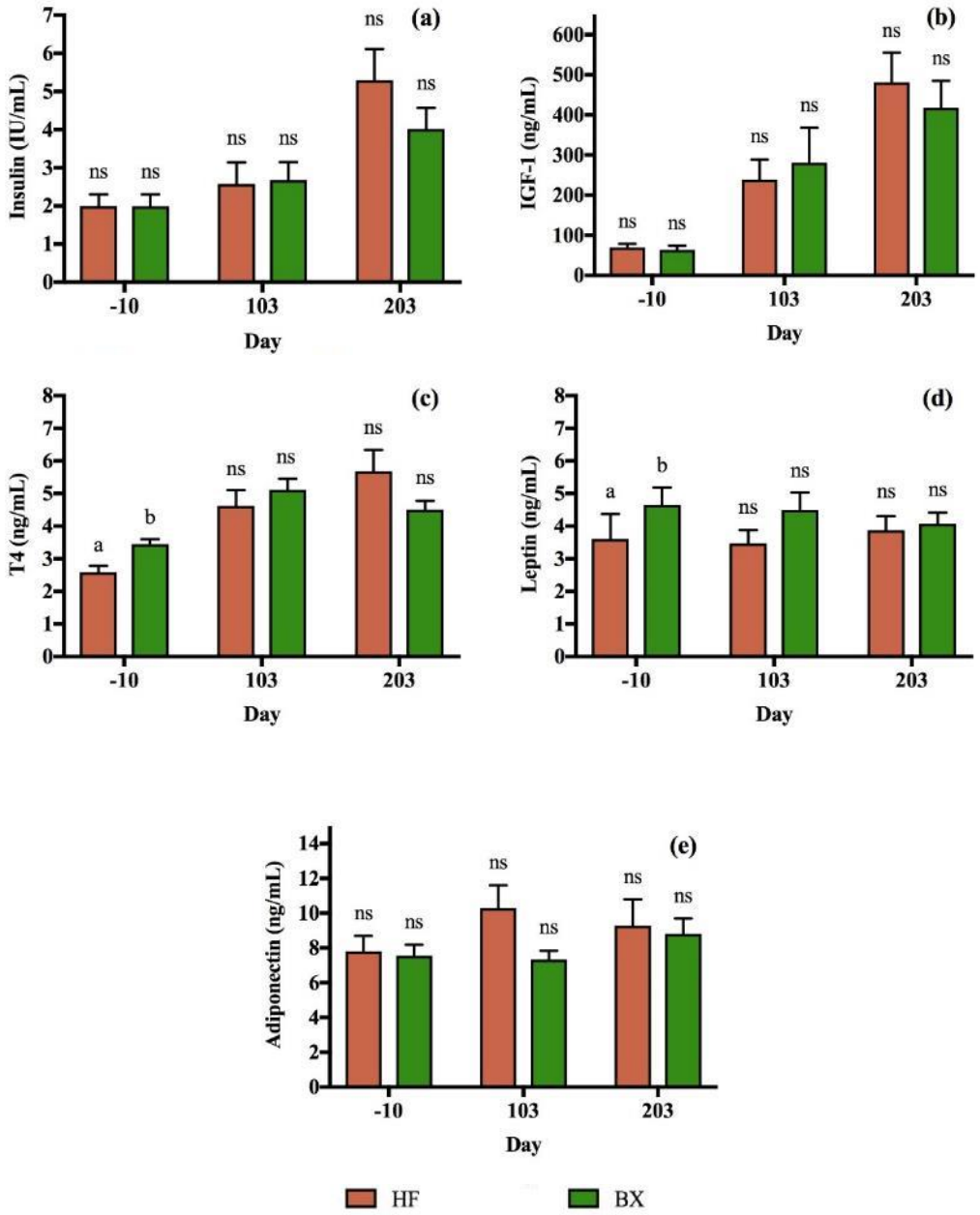

Figure 15 Plasma concnetrations of hormones genotype effect

The plasma concentration of insulin (IU/mL; **a**), insulin-like growth factor-1 (IGF-1; ng/mL; **b**), thyroxine (T4; ng/mL; **c**), leptin (ng/mL; **d**) and adiponectin (ng/mL; **e**) in *Bos taurus* (HF) and *Bos indicus* (BX) steers prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± SEM. Means with different superscripts on given days are significantly different (Tukey, P<0.05) and ns is non-significant[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

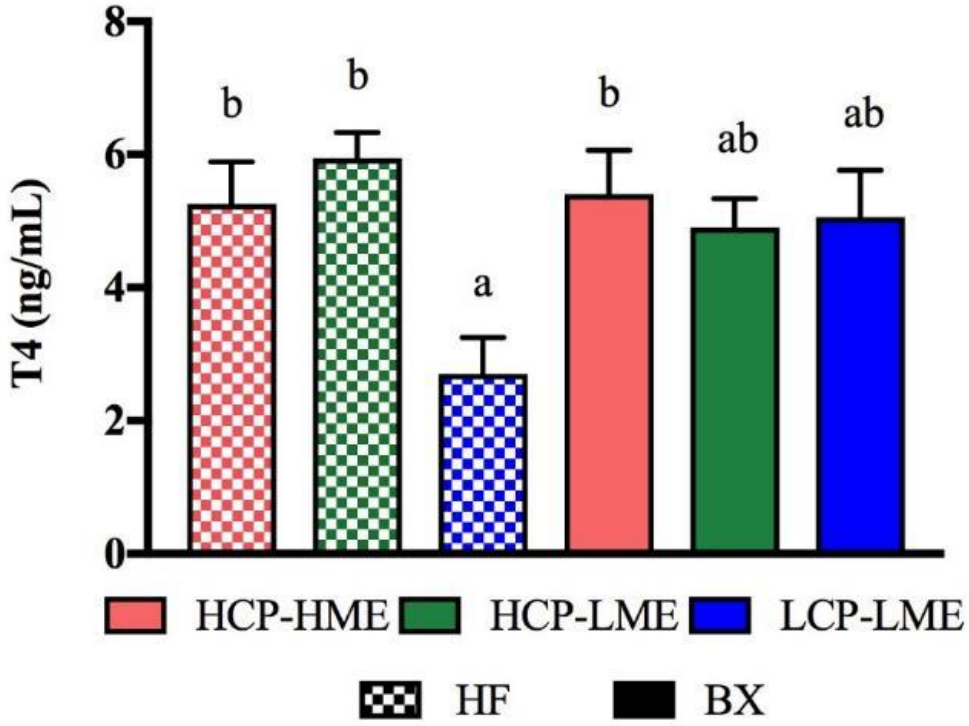

Figure 16 Concentration of thyroxine

Concentration of thyroxine (T4) in the plasma of *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± sem. Means with different letters are significantly different (Tukey, P <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

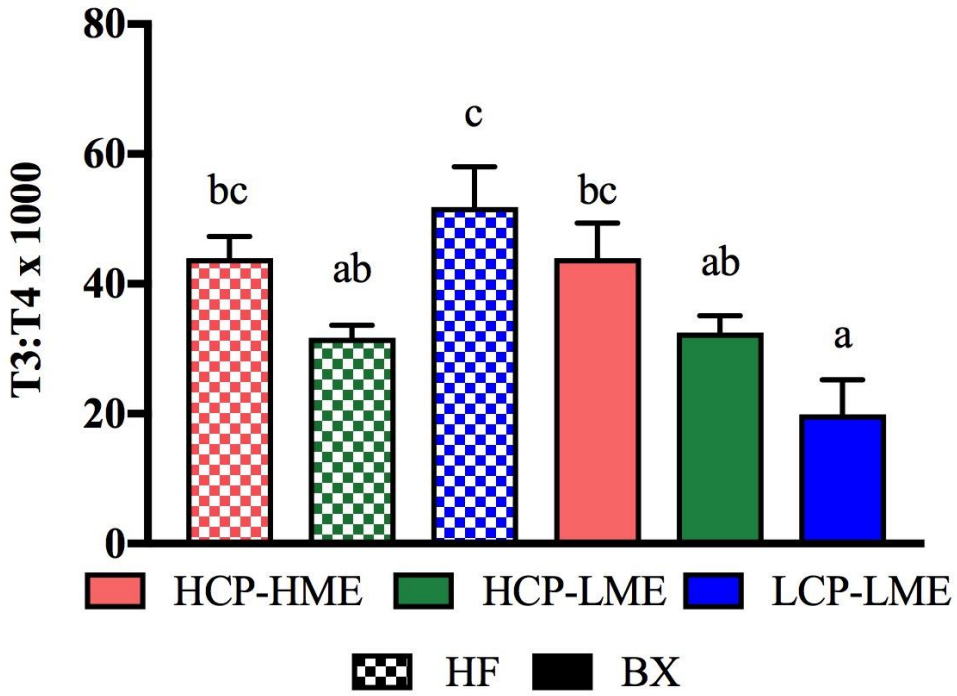

Figure 17. Triiodothyronine (T3) and thyroxine (T4) ratio in the plasma of Bos taurus (HF) and Bos indicus (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± SEM. Means with different letters are significantly different (Tukey, P<0.05)[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

During Phase 1, BAP concentrations increased markedly in HCP-HME steers compared to steers consuming low energy diets (HCP-LME and LCP-LME) (Figure 18). In contrast, no significant nutritional effects on plasma OCN concentrations were found. For HCP-HME steers, tDPD was increased at the end of Phase 1, being significantly (P<0.05) different from HCP-LME steers, but not LCP-LME steers. Further, a significant interaction between nutritional and genotype effects was found for tDPD concentration. There was lower plasma tDPD concentrations in HCP-LME compared to LCP-LME, at the end of Phase 1, and this effect was observed in HF but not BX steers (Figure 20and Figure 19). For PYD, LCP-LME steers had significantly (P<0.05) higher PYD than HCP-LME steers, but HCP-HME steers were not different from either other group. Overall there was no significant effect of genotype on bone biomarker concentrations in Phase 1, but at the end of Phase 2 (day 203) HF had higher plasma PYD and OCN but lower concentration of tDPD concentrations than BX steers (Figure 19). In general nutritional treatments applied in Phase 1 had no effect on the concentration of most of the bone metabolism markers by the end of the re-alimentation phase (Phase 2 except Bos taurus steers fed LCP-LME during Phase 1 showed a higher concentration of BAP on Phase 2 than steers of the same genotype offered HCP-LME (Figure 21).

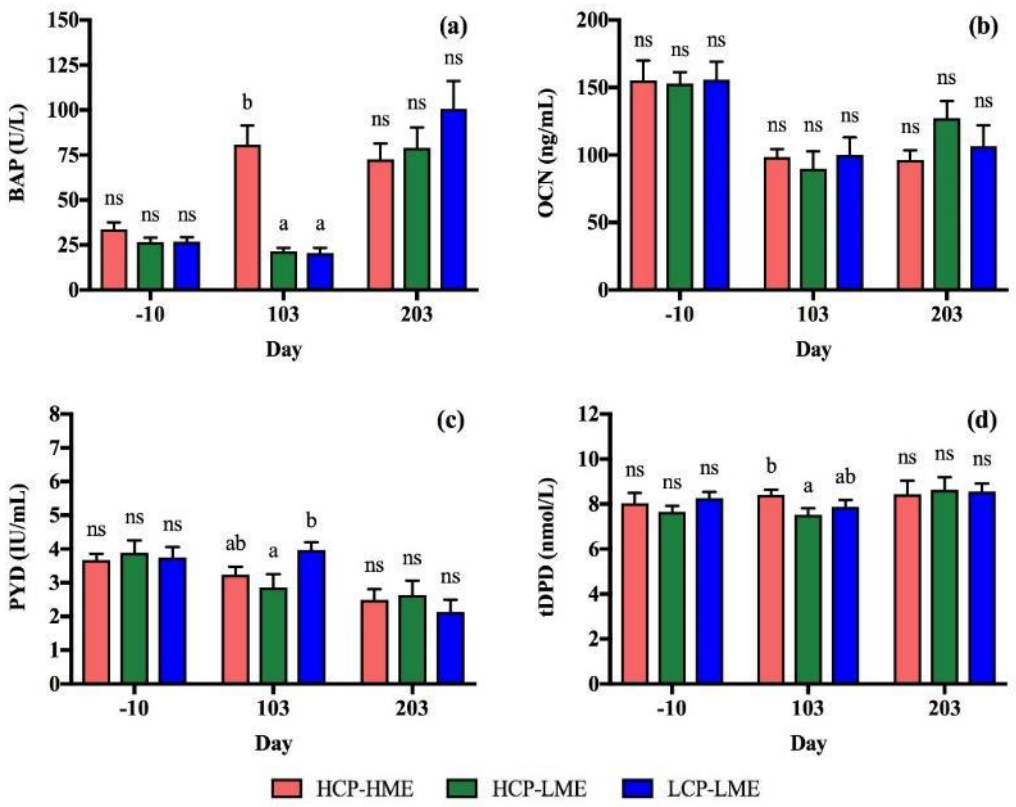

Figure 18. The plasma concentration of bone alkaline phosphatase (BAP; U/L; a), osteocalcin (OCN; ng/mL; b), pyridinoline (PYD; nmol/L; c) and total deoxypyridinoline (tDPD; nmol/L; d) in the plasma of steers prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (Tukey, *P* <0.05) and ns is non-significant[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

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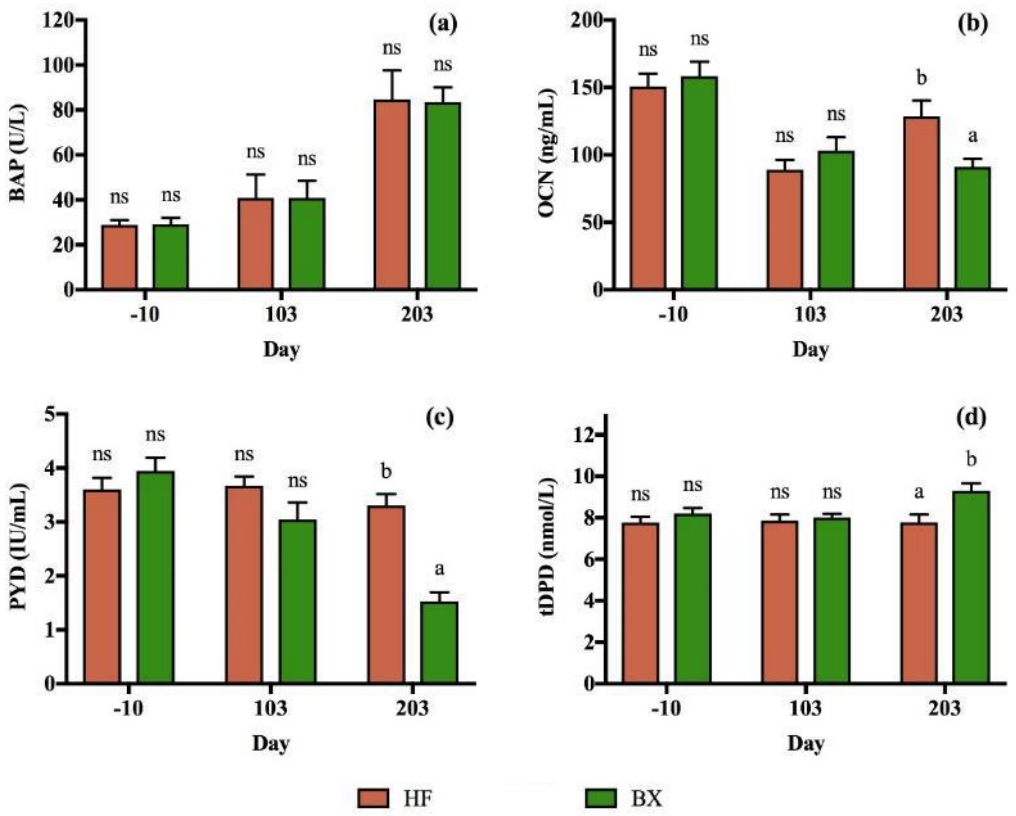

Figure 19. The concentration of bone alkaline phosphatase (BAP; U/L; a), osteocalcin (OCN; ng/mL; b), pyridinoline (PYD; nmol/L; c) and total deoxypyridinoline (tDPD; nmol/L; d) in the plasma of *Bos taurus* (HF) and *Bos indicus* (BX) steers prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (Tukey, *P* <0.05) and ns is non-significant[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

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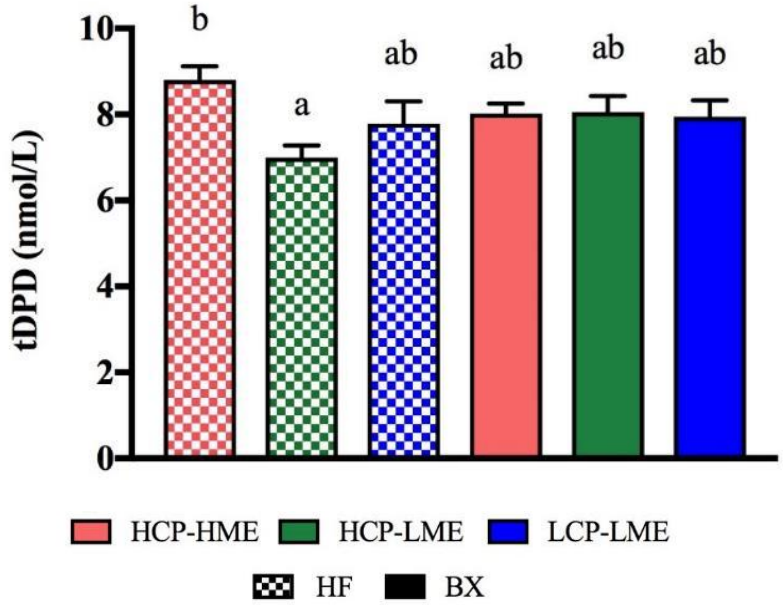

Figure 20. Concentration of total deoxypyridinoline (tDPD) in the plasma of *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± sem. Means with different letters are significantly different (Tukey, *P* <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

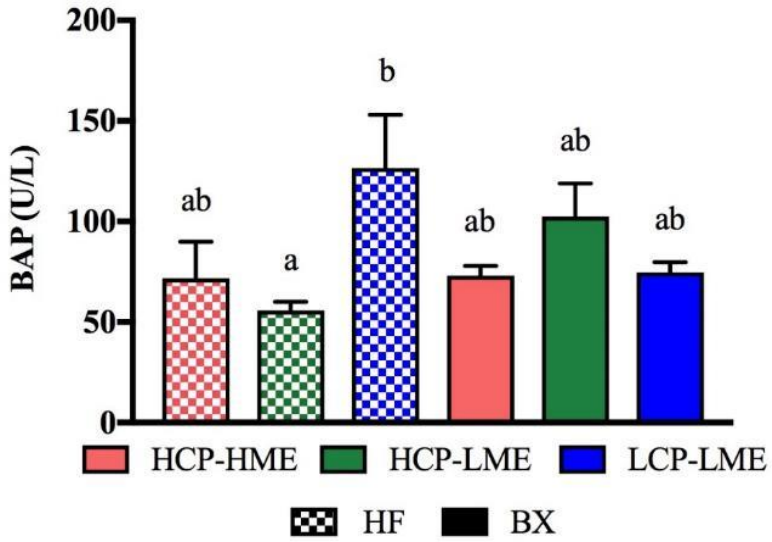

Figure 21. Concentration of bone alkaline phosphatase (BAP) in the plasma of *Bos taurus* (HF) and *Bos indicus* (BX) steers undergoing re-alimentation (Phase 2). Data are expressed as means ± sem. Means with different letters are significantly different (Tukey, *P* <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

4.1.1 Growth plate and trabecular bone histomorphometry

Bone histological changes in trabecular bone were monitored in biopsies taken from the tuber coxae bone and bone slices examined histologically to determine proliferative and hypertrophic zones and their characteristics. Reduced ME intake decreased the height of the hypertrophic zone of the growth plate independent of the level of CP intake (Figure 22 and Figure 23). Conversely, height of the proliferative phase was only affected in *Bos taurus* but not in *Bos indicus* steers (Figure 24). The diameter of terminal hypertrophic chondrocytes showed an additive effect for CP and ME intake. There were more chondrocytes per column at the hypertrophic zone of HCP-HME than HCP-LME but no differences were found between LME treatments. At the end of Phase 2, the proliferative zone of steers fed LME in Phase 1 was higher than HCP-HME. In addition, there was a tendency (P = 0.07) for the hypertrophic zone to be significantly thicker in these treatments. In all collection dates, proliferative and hypertrophic zones were thicker in HX steers (Figure 25). The number of hypertrophic chondrocytes per column and diameter of terminal hypertrophic chondrocyte were also greater in the *Bos taurus* steers but only on the first collection date.

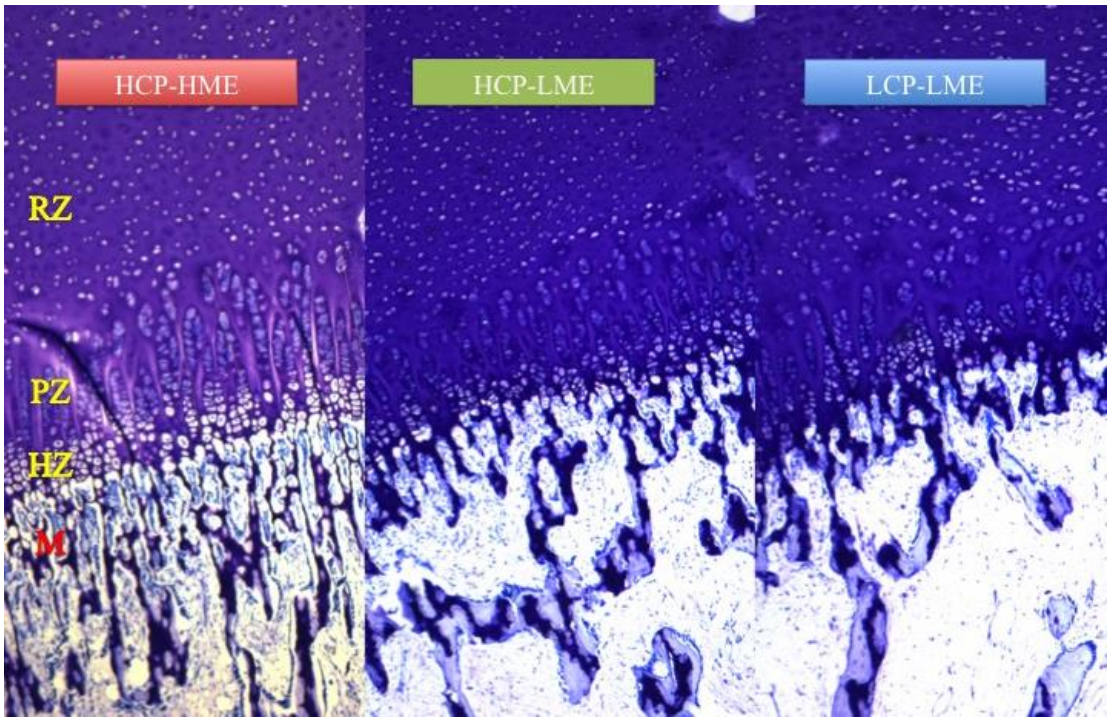

Figure 22. Tuber coxae growth plate structure of steers fed HCP-HME, HCP-LME and LCP-LME respectively at the end of Phase 1. Sections were stained with toluidine blue and photographed at 4X using an Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA). Resting, proliferative, and hypertrophic zones and metaphysis are assigned as: RZ, PZ, HZ and M respectively.

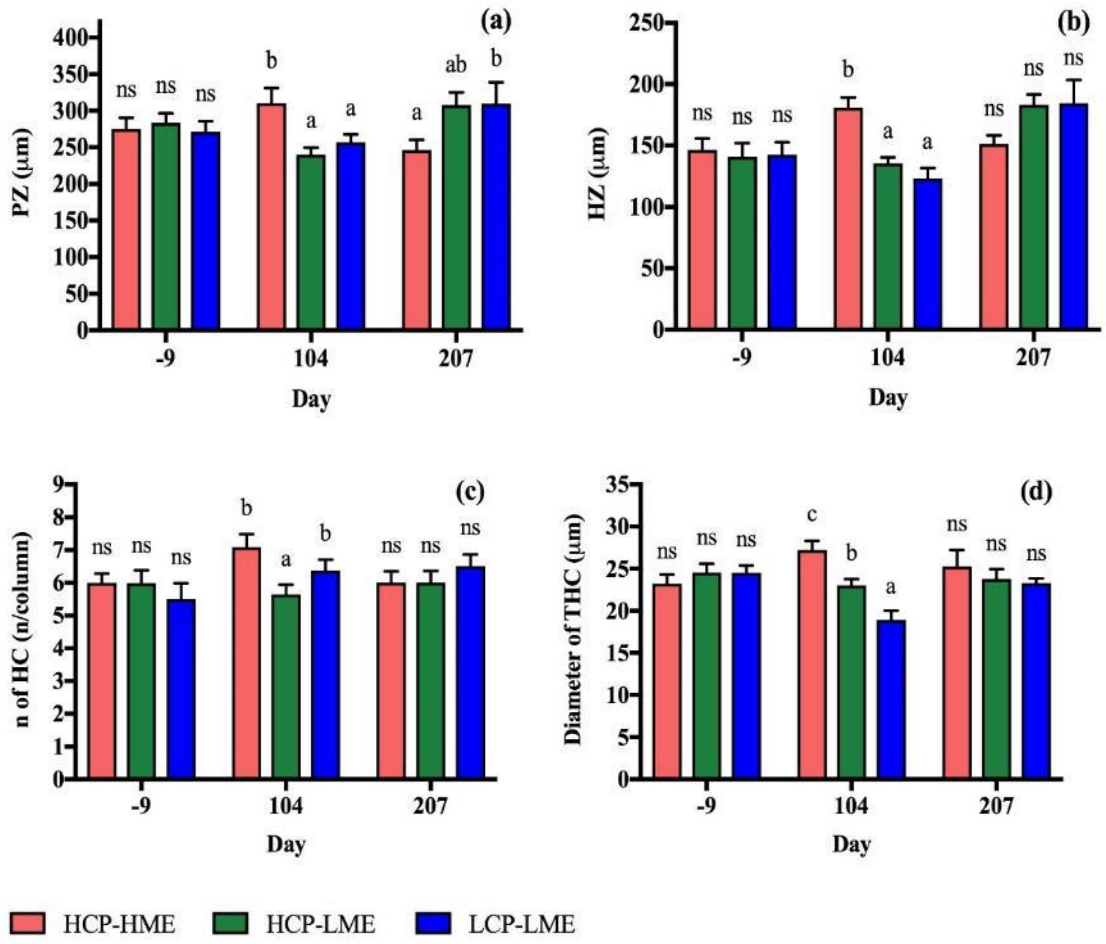

Figure 23. Height of proliferative (PZ; a) and hypertrophic zone (HZ; b), number of hypertrophic chondrocytes (HC) per column (n of HC; c), diameter of terminal HC (THC; d) of steers prior the start of the experiment (day -9), at the end of Phase 1 (day 104) and at the end of Phase 2 (day 207). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (Tukey, *P* <0.05) and ns is non-significant [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

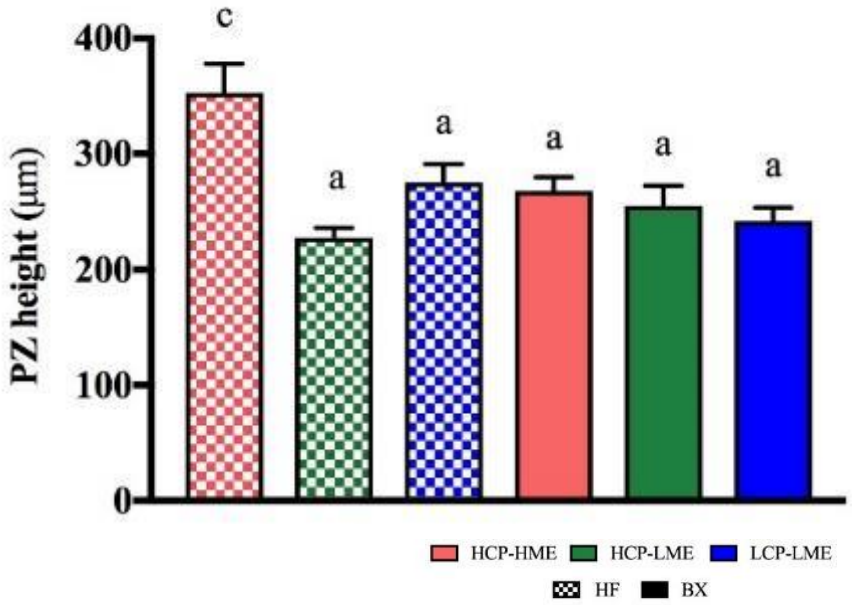

Figure 24. Height of the proliferative zone (PZ) of tuber coxae samples collected from *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± sem. Means with different letters are significantly different (Tukey, *P* <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

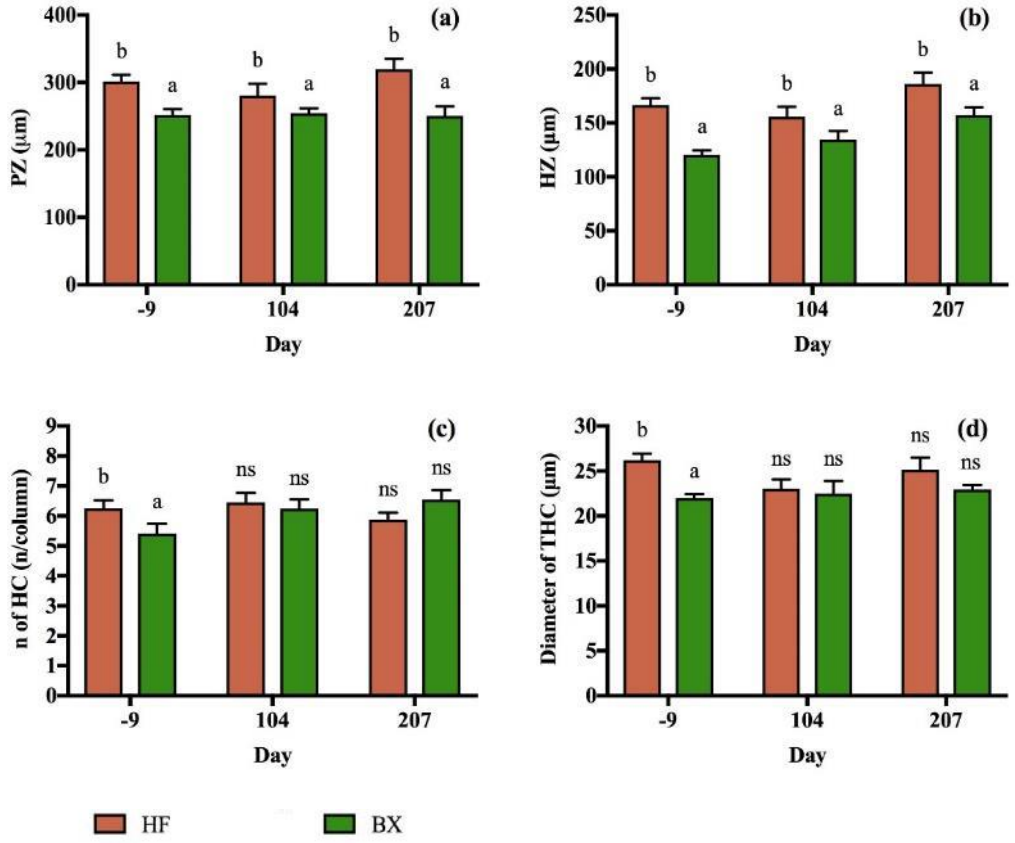

Figure 25. Height of proliferative (PZ; a) and hypertrophic zone (HZ; b), number of hypertrophic chondrocytes (HC) per column (n of HC; c), diameter of terminal HC (THC; d) of *Bos taurus* (HF) and *Bos indicus* (BX) steers prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (*P* <0.05).

A significant difference between nutritional groups was found in bone volume prior the imposition of the diets on day -9 (Figure 27). This variable was then analysed using the initial measurement as a covariate of the percentage of change during each phase (Figure 30). Prior to the start of nutritional treatments, HF steers had significantly higher bone volume and trabecular thickness than BX (Figure 28). In addition at the end of Phase 1, *Bos indicus* steers showed a larger trabecular separation and smaller bone surface than *Bos taurus* steers. Trabecular bone of steers fed HCP-HME had higher volume, surface and also smaller separation at the end of Phase 1 than steers consuming low ME (Figure 26). Only BX steers showed reductions in trabecular thickness due to reduced ME intake, but there was no effect of CP intake during ME restriction (Figure 29). Steers fed LCP-LME had significant loss of trabecular bone during Phase 1 when compared to HCP-HME (Figure 26). The differences in trabecular parameters found in Phase 1 were no longer evident at the end of Phase 2. There was a significant interaction of genotype and nutritional effects for percentage of change in trabecular bone volume during Phase 2 (Figure 30). BX steers fed LME diets during Phase 1 had a significant increase in bone volume when fed HCP-HME *ad*

libtum. Trabecular separation was bigger and bone surface smaller in BX than in HF at the end of Phase 1. But trabecular thickness was higher in BX at the end of the following phase.

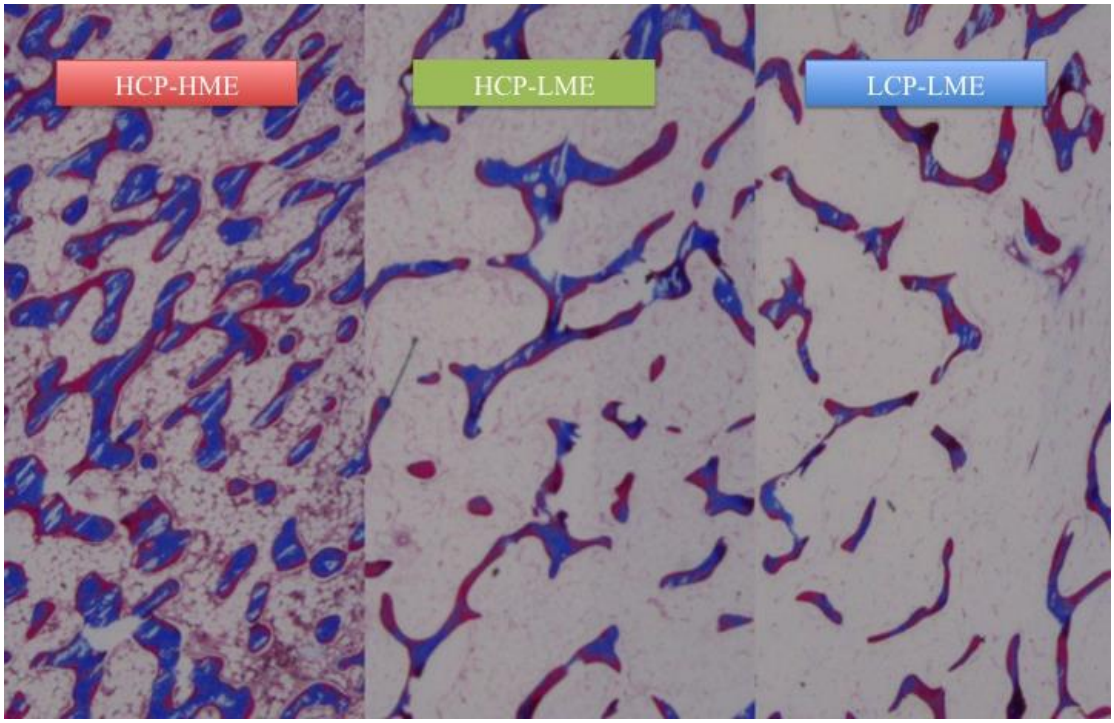

Figure 26

Trabecular bone structure of steers fed HCP-HME, HCP-LME and LCP-LME respectively at the end of Phase 1. Sections were stained with masson trichrome and photographed at 4X using an Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA).

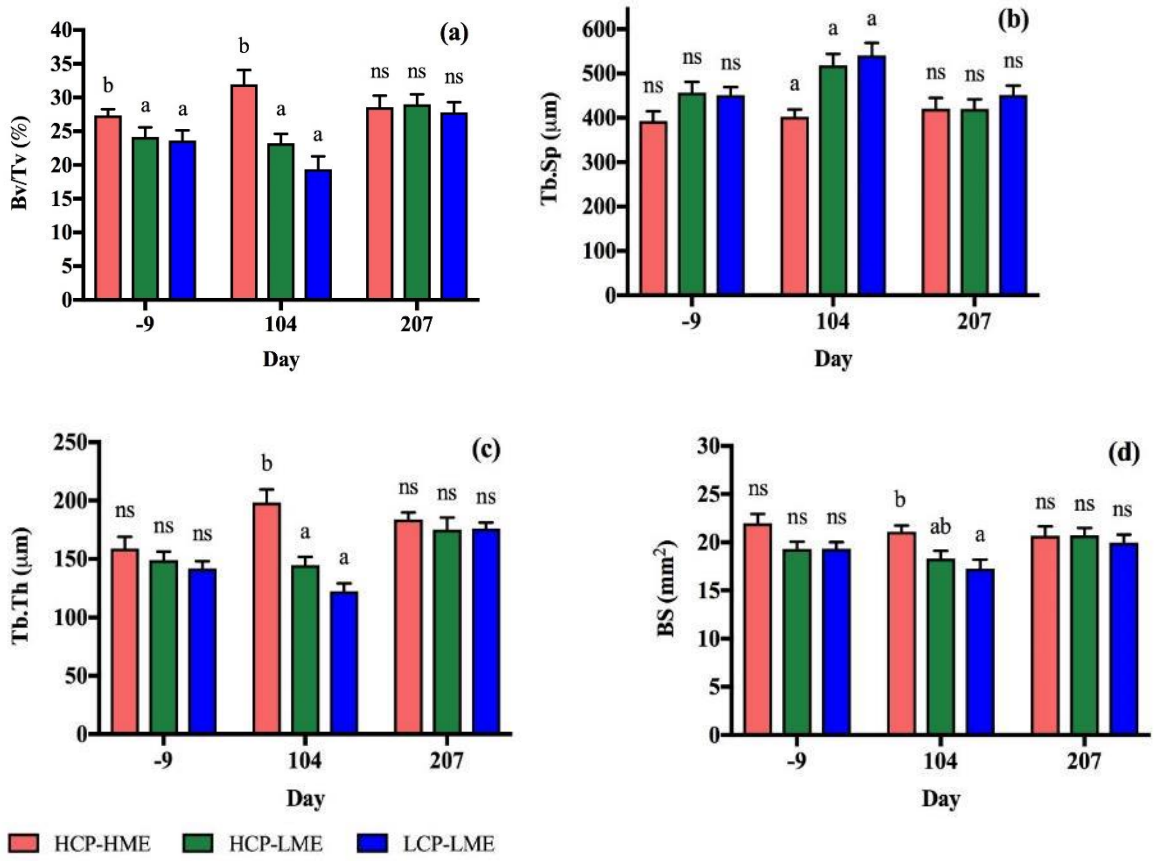

Figure 27. Bone volume (Bv/Tv; %; a), trabecular separation (Tb.Th; µm; b), trabecular thickness (Th.Sp; µm; c) and bone surface (BS; mm^2; d) of steers prior the start of the experiment (day -9), at the end of Phase 1 (day 104) and at the end of Phase 2 (day 207). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (*P* <0.05) and ns is non-significant [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

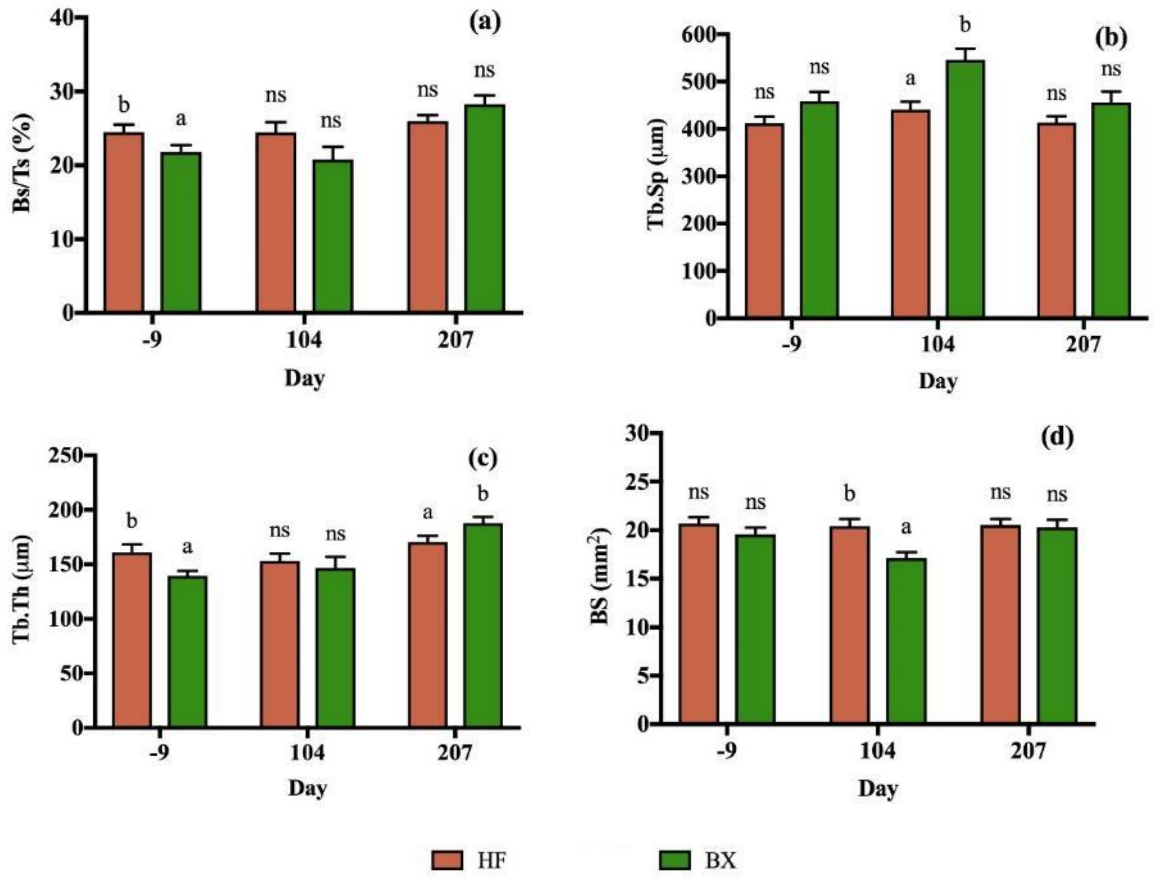

Figure 28. Bone volume (Bv/Tv; %; a), trabecular separation (Tb.Th; µm; b), trabecular thickness (Th.Sp; µm; c) and bone surface (BS; mm^2; d) of *Bos taurus* (HF) and *Bos indicus* (BX) steers prior the start of the experiment (day -10), at the end of Phase 1 (day 103) and at the end of Phase 2 (day 203). Data are expressed as means ± sem. Means with different letters within collection day are significantly different (Tukey, *P* <0.05).

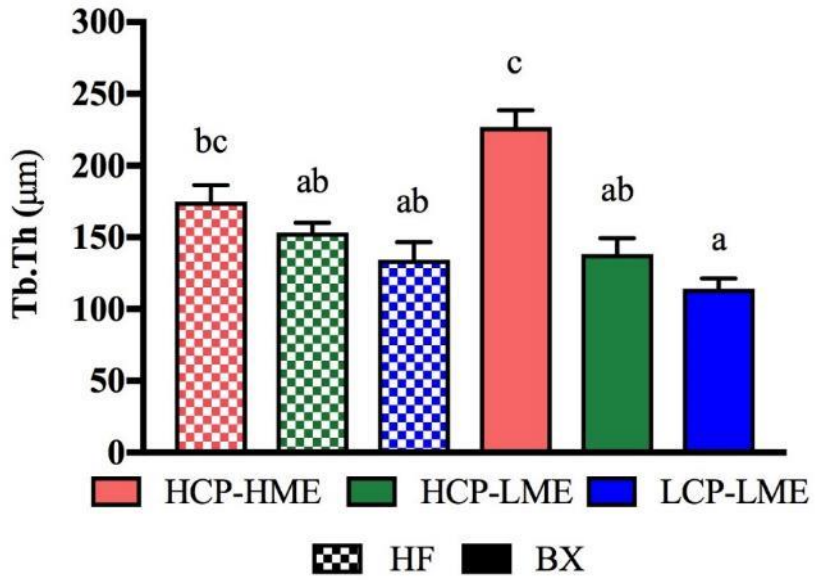

Figure 29. Trabecular thickness (Tb.Th) of tuber coxae samples collected from Bos taurus (HF) and Bos indicus (BX) steers fed different nutritional treatments (Phase 1). Data are expressed as means ± sem. Means with different letters are significantly different (P <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

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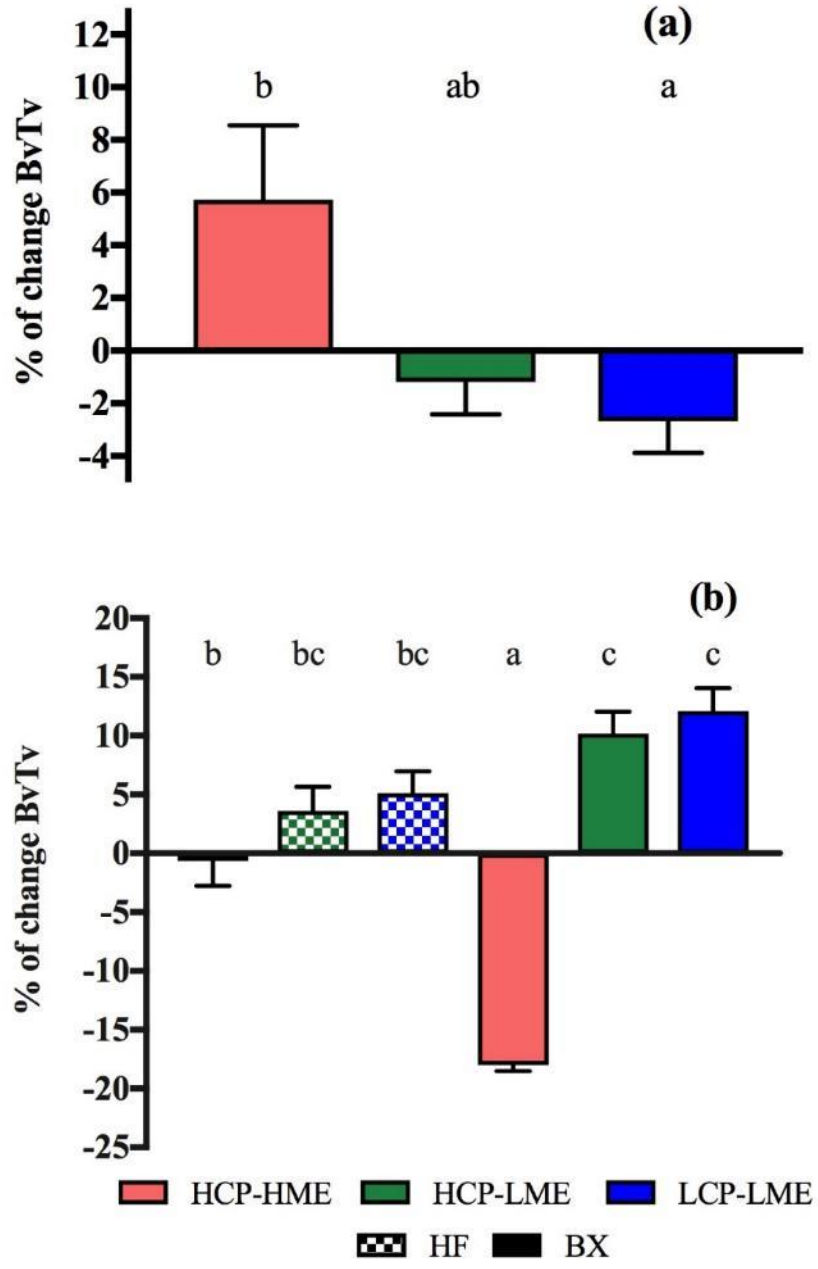


Figure 30. Percentage of change in trabecular bone volume of tuber coxae samples collected from *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments (Phase 1; a) and during re-alimentation (Phase 2; b). During Phase 1, the interaction between genotype and nutritional factors was not significant so the means of both genotypes were pulled by nutritional treatment. Data are expressed as means ± sem. Means with different letters are significantly different (*P* <0.05) [1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)].

4.2 Experiment 2. The effect of bovine somatotropin hormone (bST) on skeletal growth and bone gene expression in Bos indicus cross steers.

4.2.1 Dry matter and metabolisable energy intake, average daily gain, hip height change, feed conversion ratio, liveweight:hip height and rate of change in body measurements

The typical appearance of steers in each diet is shown in (Figure 31). Steers fed the HCP-HME diet had higher DM and ME intake than steers fed the HCP-LME and LCP-LME diets while steers offered the LCP-LME diet had higher DM intake but comparable ME intake to steers fed the HCP-LME diet, as planned (Table 6).

DM intake was not different in bST treated steers compared to saline treated controls. The dry matter and organic matter digestibility of the lucerne chaff was significantly higher in steers fed LME-HCP compared to HME-HCP and both lucerne diets were significantly higher ($P<0.01$) than the digestibility of the Mitchell grass consumed by steers offered the LCP-LME treatment. Digestibility was unaffected by bST treatment.

LWG of steers fed the HCP-HME diet was higher than those fed HCP-LME and LCP-LME diets, with no difference in LWG between HCP-LME and LCP-LME group steers (Table 6). bST treated steers had higher LWG than control steers. There were significant interactions between nutritional and bST treatments. bST steers consuming *ad libitum* lucerne chaff grew faster than saline treated steers on the same diet (Table 6).

Steers fed the HME-HCP diet had the highest skeletal growth with the rate of HH gain and HW gain significantly greater than in the HCP-LME and LCP-LME groups. There was no difference in LWG or in HH gain or HW gain between the HCP-LME and LCP-LME diet groups. Steers in the HCP-LME and LCP-LME maintained weight during the experiment and had a small increase in HH and HW. Steers in these groups also had a significantly lower BCS compared to the steers in the HCP-HME group (Table 6).

BCS of steers in the HCP-HME group increased sharply from day 0 to 40 before plateauing. In comparison, BCS of steers in the HCP-LME and LCP-LME groups did not change until day 60 and then decreased until the end of the experiment (Figure 32).

bST had no effect on any of the parameters measured except for LWG and LW: HH (Table 6). Steers injected with bST had higher LWG and were 27% heavier than steers injected with saline (Figure 33). Steers injected with bST also had a greater LW: HH ratio.

Figure 31. Typical appearance of steers offered different nutritional treatments and treated with bST(+) or saline control (-) every 14 days at the end of a 98 day experiment[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

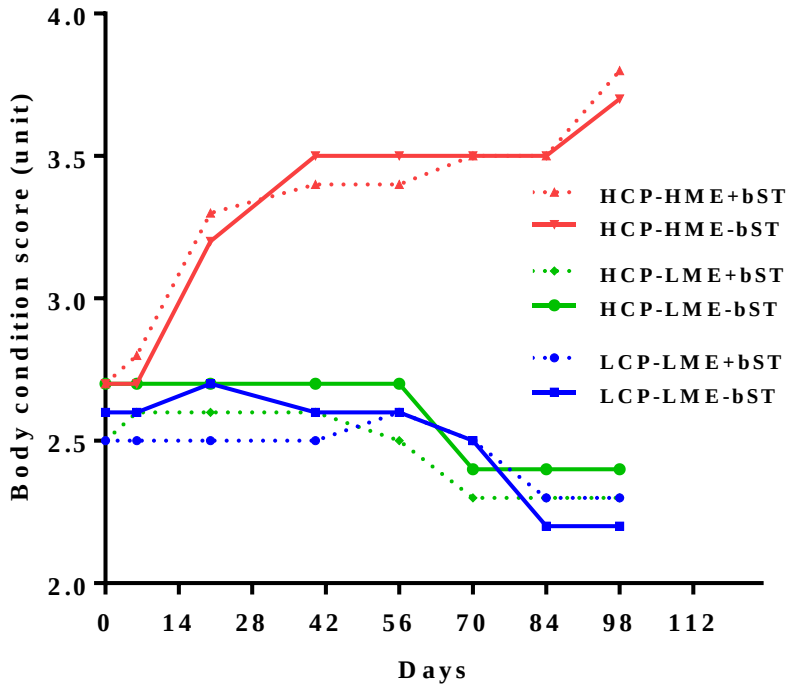


Figure 32. Body condition score (scale 1-5) of steers offered different nutritional treatments and administered with bST(+) or saline control (-) every 14 days for a 98 day treatment period[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

Table 6. Metabolisable energy intake (MEI, MJ/kg LW.day), dry matter digestibility (DMD, %), organic matter digestibility (OMD, %), liveweight gain (LWG, kg/d), intake (g/kg W. d), hip height change (mm/100 days), hip width change (mm/100 days), body condition score and ratio of liveweight to hip height (LW:HH, kg/mm) of Brahman cross steers fed different nutritional treatments[1, 5] and treated with bST[2, 5] or saline control administered subcutaneous every 14 days for a 98 day treatment period.

	HCP-HME	HCP-LME	LCP-LME	P	SEM	+bST	-bST	P	SEM
MEI	0.24 [a]	0.07 [b]	0.07 [b]	<0.001	0.004	0.13	0.13	0.66	0.003
DMD	57.9[b]	60.4 [a]	38.0 [c]	<0.001	0.85	53.00	51.30	0.09	0.69
OMD	59.5[b]	62.5[a]	43.0[c]	<0.001	0.84	55.64	54.34	0.19	0.68
LWG	1.24 [a]	0.04 [b]	-0.02 [b]	<0.001	0.03	0.47	0.37	<0.01	0.03
Intake	28.5[a]	9.7[c]	17.0[b]	<0.001	0.40	18.22	18.55	0.47	0.32
HH change	98.9 [a]	38.5 [b]	31.2 [b]	<0.001	3.46	58.68	53.76	0.23	2.83
LW:HH[3]	0.25[a]	0.15[b]	0.16[b]	<0.001	0.002	0.19	0.18	0.01	0.0002
HW change	72.4[a]	6.1[b]	1.4[b]	<0.001	1.72	26.19	27.08	0.66	1.40
BCS[4]	3.8[a]	2.4[b]	2.3[b]	<0.001	0.07	2.80	2.77	0.68	0.06

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]LW:HH at end of experiment

[4]BCS at the end of experiment.

[5]Means within row with different superscripts differ ($P < 0.05$)

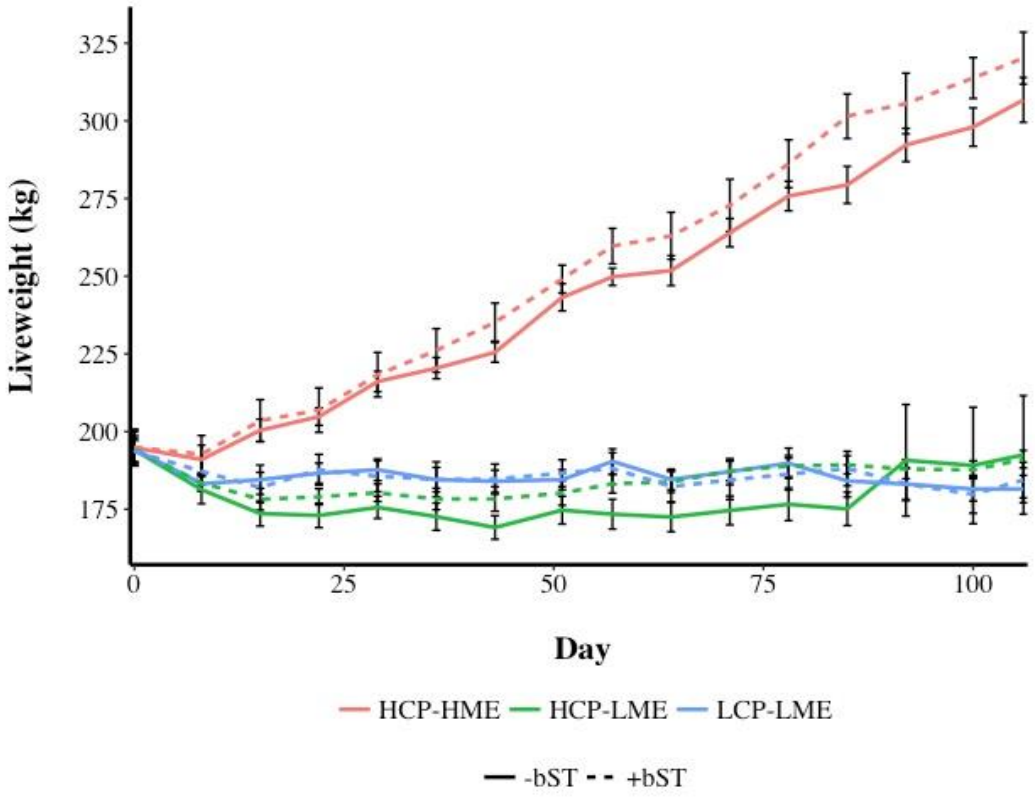


Figure 33. Change in liveweight of steers offered different nutritional treatments and treated with bST(+) or saline control (-) every 14 days for a 98 day treatment period. The bars showed standard errors[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

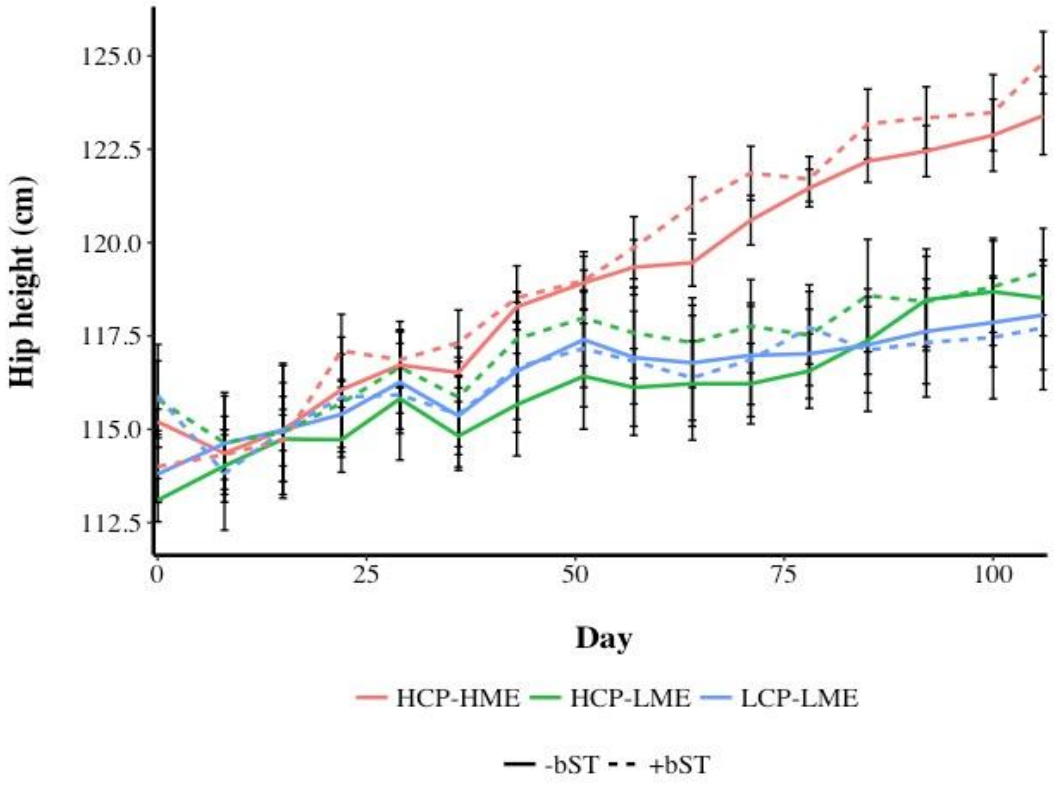


Figure 34. Change in hip height of steers under different nutritional treatments and treated with bST(+) or saline control (-) every 14 days for a 98 day treatment period. The bars showed standard errors[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

4.2.2 Metabolites, hormones and bone markers

The plasma concentrations of glucose, inorganic P, NEFA and urea nitrogen were sensitive to nutritional treatments (**Table 7**). At the end of the experiment on day 98 P and glucose concentrations were higher in HCP-HME steers. Plasma urea nitrogen concentration was higher in steers fed high CP treatment independently of the MEI and steers offered LME-LCP had a very low urea nitrogen at the end of the experiment. . Plasma NEFA was highest in steers offered the LME-HCP diet followed by HME-HCP with steers offered LME-LCP having the lowest NEFA. Nutritional treatment had no effect on the plasma concentrations of total protein or Ca.

All metabolites were different at days 56 and 98 compared to day 0 (Table 8). P, glucose and total protein were lower than on day 0 while Ca and urea nitrogen were higher than on day 0. Total protein showed a progressive decrease over time with the average concentration lower at 98 days than 56 days. Urea nitrogen showed a progressive increase in concentration over time.

There were diet x time interactions for all metabolites excpet for Ca (Figure 35 and Figure 36).

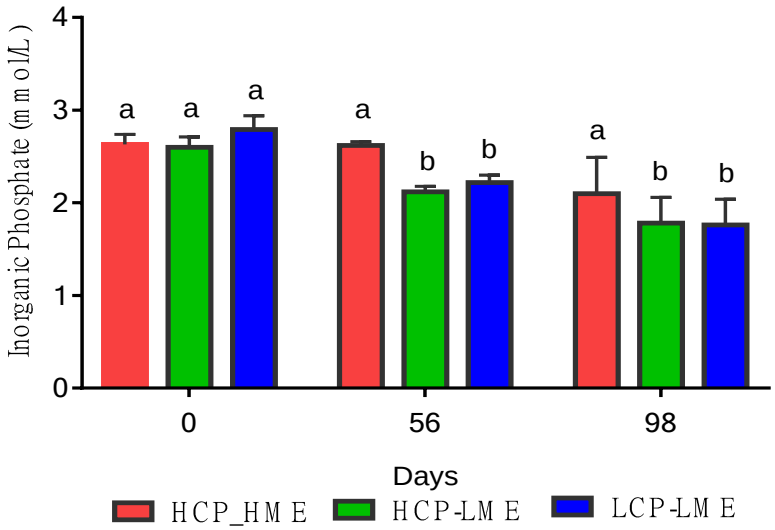


Figure 35. Mean of inorganic phosphorous in the plasma of steers under different nutritional treatments at the start of the experiment (day 0), at the middle of treatment (day 56) and at the end of the experiment (day 98) and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period. The bars showed standard errors. Means with different letters are significantly different (*P* < 0.05) [1]

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

Table 7. Plasma metabolites; diet and bST effects

Plasma inorganic P (P; mmol/L), total calcium (Ca; mmol/L), glucose (mmol/L), total protein (g/L), non-esterified fatty acids (NEFA; eq/L), urea nitrogen (PUN; mmol/L) of Brahman cross steers fed different diets (D)[1] and treated with bST[2] or saline control administered subcutaneous every every 14 days for a 98 day treatment period [3, 4]. Results are from blood samples collected at the end of treatment phase.

	Diet (D)					bST treatment (bST)			
	HCP-HME	HCP-LME	LCP-LME	SE	P	+bST	-bST	SE	P
P	2.7[a]	2.3[b]	2.5[b]	0.07	<0.01	2.4	2.5	0.06	0.09
Ca	2.25	2.12	2.18	0.04	0.13	2.21	2.16	0.03	0.30
Glucose	5.0[a]	4.0[b]	4.0[b]	0.08	<0.0001	4.3	4.3	0.06	0.71
Total Protein	68.0	68.8	65.1	1.50	0.20	67.47	67.18	1.22	0.87
NEFA	0.39[ab]	0.51[a]	0.35[b]	0.05	0.08	0.38	0.45	0.04	0.25
PUN	5.98[a]	5.63[a]	2.30[b]	0.18	<0.0001	4.60	4.70	0.15	0.65

Table 8. Plasma metabolites; time effects and interactions

Plasma inorganic P (P; mmol/L), total calcium (Ca; mmol/L), glucose (mmol/L), total protein (g/L), non-esterified fatty acids (NEFA; eq/L), urea nitrogen (PUN; mmol/L) of brahman cross steers fed different diets (D)[1] and treated with bST[2] or saline control administered subcutaneous every every 14 days for a 98 day treatment period [3, 4]. Results are from blood samples collected at the end of treatment phase.

	Times (T)[5]					D*T	D*bST	bST*T	D*bST*T
	0	56	98	SE	P				
P	2.7[a]	2.3[b]	2.4[b]	0.06	<0.0001	0.02	0.75	0.06	0.17
Ca	2.09[b]	2.21[a]	2.27[a]	0.03	<0.01	0.11	0.98	0.85	0.89
Glucose	4.4[a]	4.3[b]	4.3[b]	0.06	0.40	<0.0001	0.07	0.51	0.76
Total Protein	70.93[a]	67.30[b]	63.74[c]	1.00	<0.0001	0.04	0.92	0.74	0.71
NEFA	0.41[b]	0.58[a]	0.25[c]	0.04	<0.0001	0.008	0.32	0.29	0.73
PUN	1.86[c]	5.51[b]	6.55[a]	0.14	<0.0001	<0.0001	0.26	0.46	0.09

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Measurements at the end of experiment.

[4]Means within row with different superscripts differ ($P < 0.05$)

[5]Day of experiment

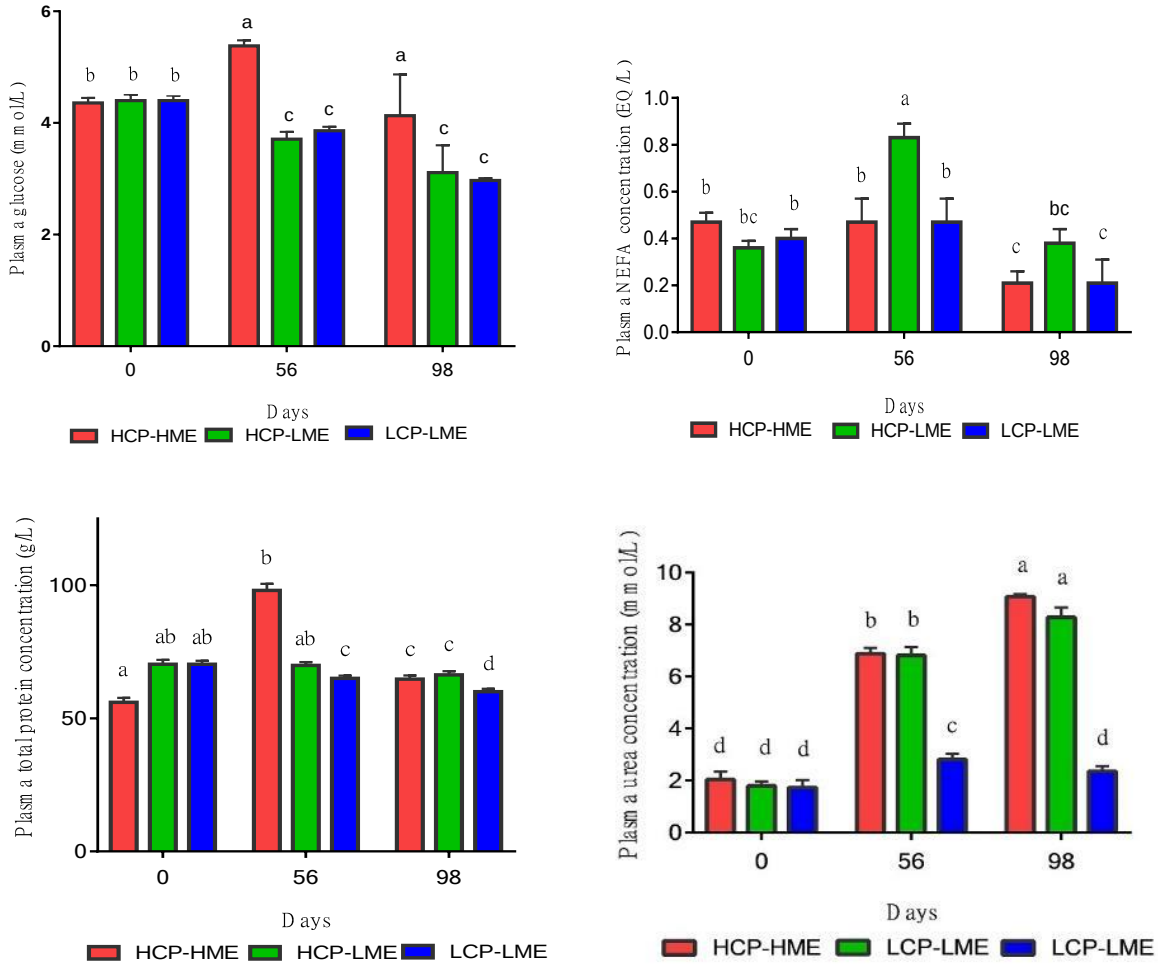


Figure 36. Mean plasma glucose (A), NEFA (B), total protein (C) and urea nitrogen (D) concentrations in steers under different nutritional treatments at the start of the experiment (day 0), at the middle of treatment (day 56) and at the end of the experiment (day 98) and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period. The bars showed standard errors. Means with different letters are significantly different (*P* < 0.05) [1]

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

The plasma concentrations of IGF-I, insulin, total T3, total T4 and leptin in the plasma of steers were affected by the dietary treatments. Steers fed the HCP-HME diet had significantly higher concentrations of these hormones than steers fed HCP-LME and LCP-LME diets at the end of the experiment. There were no significant differences in plasma concentrations of these hormones between steers fed HCP-LME and LCP-LME (Table 9).

The only hormone affected by bST treatment was IGF-1 (Table 9). bST treated steers had higher concentrations of IGF-1 than control animals from 28 days onwards. IGF-1 also significantly changed over time with the main increase occurring between day 0 and day 28 of treatment (Table 10). There was a diet x time interaction as well as time x bST effect for IGF-1 (Table 11). The concentration of IGF-1 was lowest on day 0 and progressively

increased from day 28 onwards. Increased detail on the IGF-1 response to bST is provided in appendix 1.

PTH plasma concentration were variable and there was no diet or bST effect on PTH. However PTH plasma concentrations decreased significantly at each time measured (days 0, 56 and 98) over experimental period (Table 10).

There was a diet x time interaction for total T3 concentration (Table 11). Plasma total T3 did not increase from day 0 to the end of the experiment in steers fed ME restricted diets. Whereas, total T3 concentration in HME-HCP steers increased significantly and reached the highest value at the end of experiment. Prior the experiment, steers in LCP-LME diet had significantly higher plasma total T3, the concentration decreased after dietary treatment. By the end of the experiment, steers in HCP-LME and LCP-LME groups had significantly lower plasma total T3 than those in HCP-HME diet (Table 9 and Figure 37). Similarly, the plasma concentration of total T4 was significantly higher in steers fed the HCP-LME diet than steers in HCP-LME and LCP-LME groups at the end of the experiment (Table 9).

Plasma BAP concentrations at the start of the experiment were the same between the three dietary treatments. There was a strong diet effect with steers offered LCP-LME and HCP-LME having a lower plasma BAP concentration compared to HCP-HME steers at the end of the experiment (Table 9). This indicated that LCP-LME and HCP-LME steers had reduced bone formation compared to HCP-HME counterparts. There was a time effect (Table 10) and an interaction between diet and time (Table 11) with BAP concentrations decreasing further from day 56 to day 98 in low ME treatment steers (Figure 38).

CTX-1 plasma concentrations were more variable.The plasma CTX-1 concentration was significantly ($P<0.05$) increased in steers that were subsequently allocated to the HCP-LME diet (5.1 ng/mL) compared to steers allocated to the HCP-HME (2.0 ng/mL) and LCP-LME (2.3 ng/mL) diets ($P<0.05$). The higher plasma CTX-1 concentration of these steers was due to CTX-1 concentrations in 2 steers of 9.0 and 7.3 ng/mL, prior to the commencement of the experiment. The plasma was re-assayed to confirm these values. The concentration of CTX-1 in this group remained the same throughout the experiment. Analysis of the concentration of CTX-1 on day 98 was performed using the initial CTX-1 concentration as a covariate. No diet effect on plasma CTX-1 concentrations was found (Table 9). There was an interaction between diet and bST (Table 11).

Osteocalcin was only measured at the end of the experiment on day 98. There were no diet or bST effects of osteocalcin with plasma concentrations very similar between groups (Table 7).

Table 9. Plasma hormones and bone markers; diet and treatment effets

Plasma concentrations of insulin-like growth factor (IGF-1; ng/mL), insulin (µIU/mL), parathyroid hormone (PTH; pg/mL), Total T3 (nmol/L), leptin (pg/mL), bone alkaline phosphatase (BAP; U/L), Osteocalcin (OCN; ng/mL) and CTX-1 (ng/mL) from brahman cross steers fed different *diets (D)*[1] and treated with bST[2] or saline control administered subcutaneous every 14 days for a 98 day treatment period [3, 4]. Results are from blood samples collected at the end of treatment phase.

Hormones	Diet (D)					bST treatment (bST)				D x bST
	HCP-HME	HCP-LME	LCP-LME	SE	P	+bST	-bST	SE	P	P
IGF-1	401.0[a]	76.8[b]	55.9[b]	36.9	<.0001	227.6	128.3	30.2	0.02	0.82
Insulin	10.3[a]	6.4[b]	5.1[b]	1.0	<0.01	8.01	6.53	0.79	0.30	0.28
PTH	145.8	149.7	66.2	41.9	0.68	155.9	85.3	34.2	0.28	0.03
Total T3	2.3[a]	1.2[b]	1.3[b]	0.1	<.0001	1.6	1.6	0.1	0.93	0.94
Total T4	69.3[a]	40.6[b]	43.8[b]	5.3	<0.01	53.8	48.64	4.3	0.41	0.46
Leptin	5.9[a]	3.8[b]	4.0[b]	0.5	0.02	4. 7	4.49	0.4	0.82	0.91
Bone biomarkers										
BAP	78.4[a]	30.3[b]	27.9[b]	4.6	<.0001	45.0	46.1	3. 8	0.94	0.78
OCN	117.6	106.5	116.2	11.9	0.59	108.4	118.5	9.7	0.74	0.64
CTX-1	2.3	2.9	2.7	0.3	0.15	2.8	2.4	0.04	0.24	0.24

Table 10. Plasma hormones and bone markers;time effects

Plasma concentrations of insulin-like growth factor (IGF-1; ng/mL), insulin (µIU/mL), parathyroid hormone (PTH; pg/mL), Total T3 (nmol/L), leptin (pg/mL), bone alkaline phosphatase (BAP; U/L), Osteocalcin (OCN; ng/mL) and CTX-1 (ng/mL) from brahman cross steers fed different *diets (D)*[1] and treated with bST[2] or saline control administered subcutaneous every 14 days for a 98 day treatment period [3, 4].

Hormones	Times/days (T)						
	0	28	56	84	98	SE	*P*
IGF-1	59.2[b]	159.5[a]	158.7[a]	171.4[a]	177.9[a]	14.95	<0.0001
Insulin	9.4[a]	N/A	7.4[b]	N/A	7.3[b]	0.52	<0.01
PTH	757.7[a]	N/A	320.2[b]	N/A	120.6[c]	94.5	<0.0001
Total T3	1.6[a]	N/A	1.5[b]	N/A	1.6[a]	0.06	<0.0001
Leptin	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bone biomarkers							
OCN	N/A	N/A	N/A	N/A	113.4	16.8	N/A
BAP	54.5[a]	N/A	51.8[b]	N/A	45.52[c]	3.30	<0.0001
CTX-1	2.7	N/A	2.8	N/A	2.6	0.23	0.91

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Measurements at the end of experiment.

[4]Means within row with different superscripts differ ($P < 0.05$)

[5]Day of experiment

Table 11. Plasma hormones and bone markers; diet, treatment and time interactions

Statistical interactions between diet, bST and time for plasma concentrations of insulin-like growth factor (IGF-1; ng/mL), insulin (µIU/mL), parathyroid hormone (PTH; pg/mL), Total T3 (nmol/L), leptin (pg/mL), bone alkaline phosphatase (BAP; U/L), Osteocalcin (OCN; ng/mL) and CTX-1 (ng/mL) from brahman cross steers fed different diets (D)[1] and treated with bST[2] or saline control administered subcutaneous every 14 days for a 98 day treatment period [3, 4].

Hormones	D*T	D*bST	T*bST	D*bST*T
IGF-1	<0.0001	0.88	<0.02	0.26
Insulin	0.08	0.31	0.72	0.17
PTH	0.56	0.59	0.49	0.03
Total T3	<0.0001	0.79	0.27	0.65
Leptin	N/A	0.82	N/A	N/A
Bone biomarkers				
Osteocalcin	N/A	0.52	N/A	N/A
BAP	<0.0001	0.83	0.31	0.14
CTX-1	0.58	0.04	0.71	0.06

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Measurements at the end of experiment.

[4]Means within row with different superscripts differ ($P < 0.05$)

[5]Day of experiment

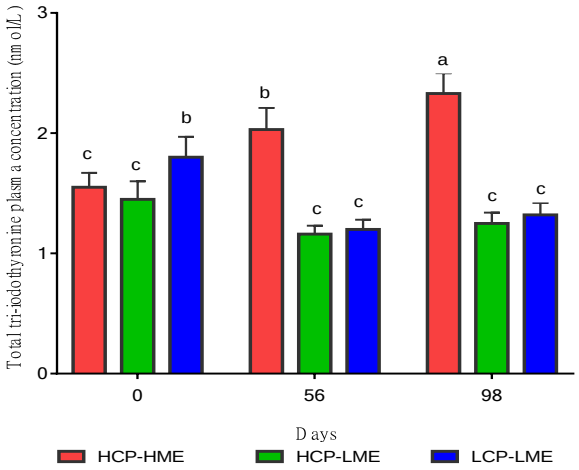


Figure 37. The concentration of total tri-iodothyronine (T3) based on day and diet interaction in the plasma of steers under different nutritional treatments at the start of the experiment (day 0), at the middle of treatment (day 56) and at the end of the experiment (day 98) and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period. The bars showed standard errors. Means with different letters are significantly different (*P* < 0.05) [1]

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

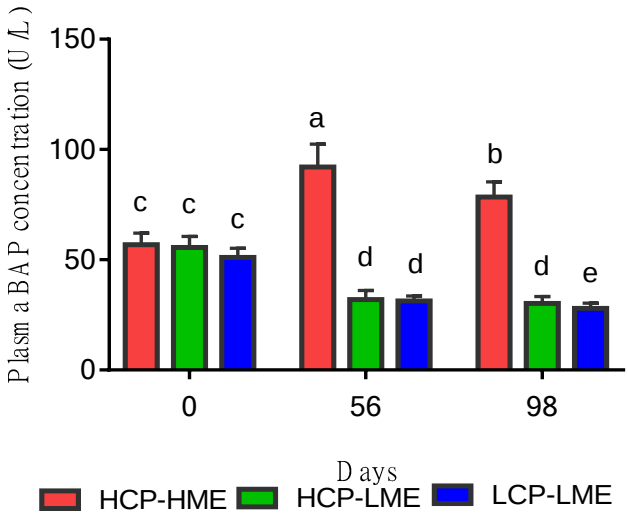


Figure 38. Mean concentration of bone alkaline phosphatase (BAP) in the plasma of steers under different nutritional treatments at the start of the experiment (day 0), at the middle of treatment (day 56) and at the end of the experiment (day 98) and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period. The bars showed standard errors. Means with different letters are significantly different (*P* < 0.05) [1]

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

4.2.3 Growth plate and bone histomorphometry

Histomorphometry was used to measure the thickeness of the different cellular layers of the growth plate in a number of different bones sampled at post mortem (Figure 39). There was a similar pattern of results between the 4 different bones analysed (Table 12). In the distal femur growth plate, the height of the proliferative and hypertrophic zones (PZ and HZ) and the diameter of the terminal hypertrophic chondrocyte (HC) were significantly different between the three diet groups with values for HME-HCP greater than HME-LCP and both these groups greater than LME-LCP.

In the tuber coxae growth plate the PZ, HZ, GP height and HC diameter were all significantly greater in steers offered HME-HCP than the ME restricted groups. In addition, the PZ was greater in LME-HCP than LME-LCP diet groups.

In the distal radius growth plate the HZ, GP height and HC diameter were all significantly greater in steers offered HME-HCP than the ME restricted groups. Steers offered LME-HCP had higher HZ than steers offered LME-LCP.

In the distal tibia growth plate the PZ was significantly greater in steers offered HME-HCP than the ME restricted groups. Steers offered the LME-LCP diet had a shorter PZ than steers offered the HME-HCP and LME-HCP diets.

There was a bST effect on the PZ in the tuber coxae and distal radius with bST treated steers having a significantly greater PZ height than steers receiving saline injections.

There were clear differences in the trabecular bone histomorphometry with steers offered HME-HCP having greater bone volume, trabecular thickness and bone surface than steers in the LME-HCP and LME-LCP groups in every bone measured (Table 13). There were no bST effects for any of the parameters measured in trabecular bone samples.

Measurements of cortical bone thickness in ribs 11 and 12 showed very similar results. The cortical thickess in steers fed HME-HCP was greater than steers in the restriced ME diets. There was no difference between LME-HCP and LME-LCP groups (Figure 43).

Measurement of cortical porosity involves determining the area of a bone section that contains resorption spaces and reflects the degree of cortical bone remodelling/mobilisation. A similar pattern was seen in both the 11^{th} and 12^{th} ribs with porosity greatest in steers fed the LCP-LME diet. The cortical porosity was significantly greater in LME-LCP steers than in the HME-HCP group.

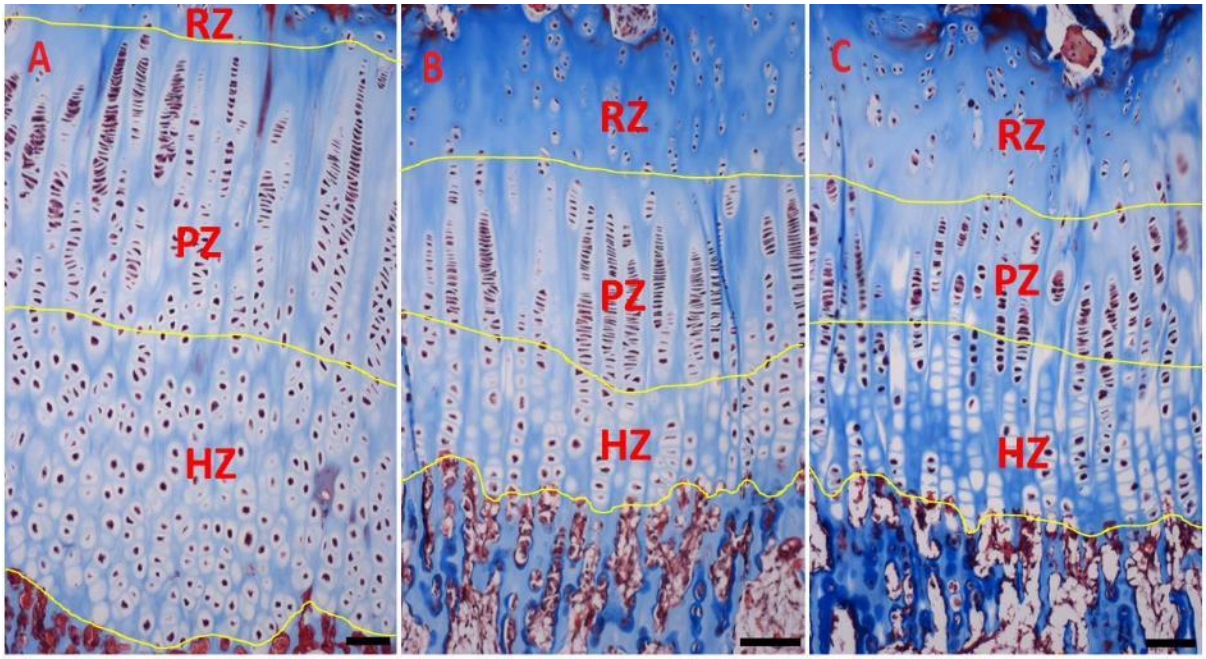


Figure 39. Growth plate histomorphometry. Light micrograph of steers' radius growth plate at 100x magnification, from steers offered HME-HCP diets (A), LME-HCP diets (B) or LME-LCP diets (C). RZ: resting zone, PZ: proliferative zone, HZ: hypertrophy zone. Images from bone samples collected at post mortem at end of treatment phase of steers under different nutritional treatments and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period[1]. The scale bar measure 200 µm in each image.

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

.

Table 12. Growth plate histomorphometry

Height of proliferative (PZ, µm), Hypertrophy zone (HZ, µm), growth plate length (GPL, mm) and diameter terminal HC (µm) of femoral, tuber coxae (TC), radius and tibia bones of steers under different nutritional treatments[1, 3] and receiving either bST[2, 3] or saline administered subcutaneously every 14 days for a 98 day treatment period. Results are from bone samples collected at post mortem at end of treatment phase.

	HCP-HME	HCP-LME	LCP-LME	*P*	SEM	+bST	-bST	*P*	SEM
Femur									
PZ	260^{a}	250^{ab}	210^{b}	0.07	0.02	250	230	0.32	0.01
HZ	140^{a}	120^{ab}	90^{b}	0.01	0.01	130	110	0.32	0.01
GPH?	0.78	0.78	0.70	0.80	0.09	0.76	0.75	0.92	0.08
HC diameter	35^{a}	30^{ab}	30^{b}	0.04	0.002	30	30	0.28	0.001
Tuber coxae									
PZ	280^{a}	240^{ab}	220^{b}	0.05	0.02	250	240	0.05	0.01
HZ	170^{a}	130^{b}	110^{b}	0.01	0.01	140	130	0.73	0.01
GPH	0.46^{a}	0.37^{b}	0.33^{b}	<0.01	0.03	0.40	0.38	0.52	0.02
HC diameter	40^{a}	30^{b}	30^{b}	<0.01	0.002	30	30	0.20	0.001
Radius									
PZ	250	240	220	0.22	0.01	250	220	0.02	0.01
HZ	230^{a}	170^{b}	130^{c}	<0.0001	0.01	180	170	0.65	0.01
GPH	0.60^{a}	0.56^{a}	0.46^{b}	0.02	0.03	0.55	0.53	0.51	0.03
HC diameter	30^{a}	20^{b}	20^{b}	0.05	0.002	20	20	0.36	0.001
Tibia									
PZ	240^{a}	220^{a}	160^{b}	0.05	0.07	220	200	0.58	0.02
HZ	220^{a}	120^{b}	110^{b}	0.05	0.03	170	140	0.35	0.02
GPH	0.54	0.56	0.43	0.05	0.11	0.51	0.51	0.94	0.04
HC diameter	20	20	20	0.55	0.001	20	20	0.37	0.001

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Means within row with different superscripts differ ($P < 0.05$)

Table 13 Trabecular bone histomorphometry

Bone surface (BS, mm^2), average trabecular thickness (Tb.Th, µm) and trabecular volume (BV/TV, %) of distal femur, tuber coxae, distal radius and distal tibial bones in steers under different nutritional treatments[1] and receiving either bST[2,] or saline administered subcutaneously every 14 days for a 98 day treatment period. Results are from bone samples collected at post mortem at end of treatment phase[3].

	HCP-HME	HCP-LME	LCP-LME	*P*	SEM	+bST	-bST	*P*	SEM
Femur									
BS	0.31[a]	0.13[b]	0.10[b]	<0.0001	0.03	0.21	0.16	0.09	0.02
Tb.Th.	290[a]	140[b]	130[b]	<0.0001	0.02	200	170	0.25	0.02
BV/TV	47[a]	17[b]	14[b]	<0.0001	0.04	29	24	0.38	0.03
TC									
BS	0.23[a]	0.12[b]	0.10[b]	<0.01	0.02	0.15	0.14	0.57	0.02
Tb.Th.	190[a]	140[b]	110[b]	<0.01	0.01	150	150	0.84	0.01
BV/TV	36[a]	18[b]	15[b]	<0.01	0.03	24	22	0.55	0.02
Radius									
BS	0.36[a]	0.19[b]	0.17[b]	0.03	<0.01	0.24	0.25	0.26	0.77
Tb.Th.	290[a]	200[b]	150[b]	0.02	<0.0001	210	220	0.73	0.01
BV/TV	58[a]	30[b]	27[b]	0.05	<0.01	37	39	0.77	0.04
Tibia									
BS	0.42[a]	0.22[b]	0.18[b]	<0.01	0.03	0.26	0.28	0.55	0.03
Tb.Th.	350[a]	176[b]	161[b]	<0.01	0.02	209	240	0.21	0.02
BV/TV	69[a]	37[b]	29[b]	<0.01	5.90	42	47	0.54	4.70

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Means within row with different superscripts differ ($P < 0.05$)

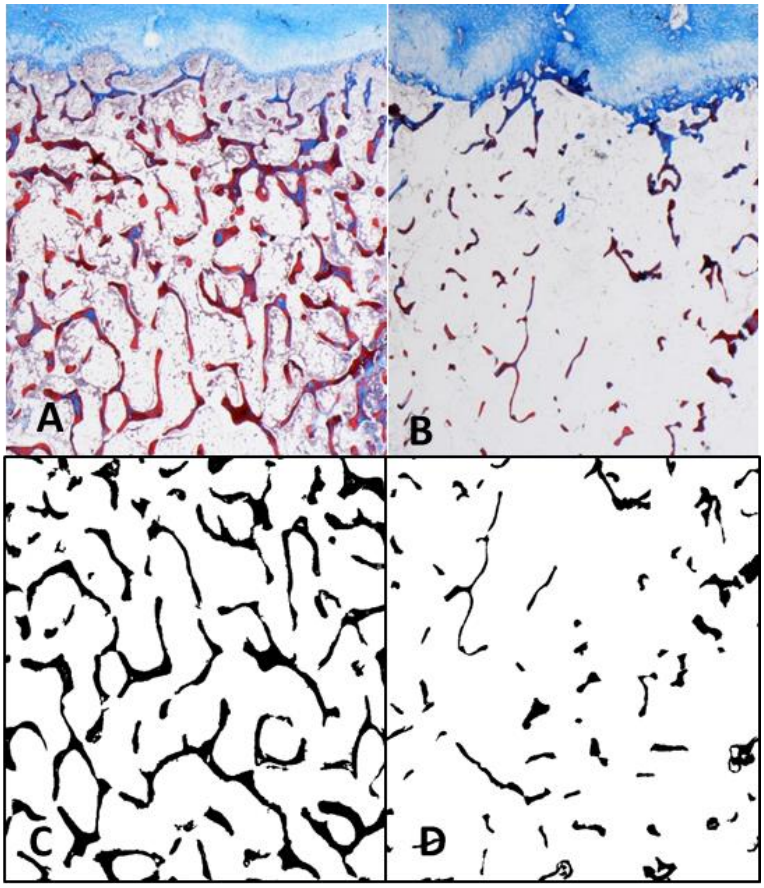


Figure 40 A and B are images of histology sections of tuber coxae bone from steers. In image A the trabecular bone is normal while in image B there is marked loss of bone tissue and thus reduced bone volume (BV). C and D show binary images created in ImageJ that were used to obtain values for bone volume as a percentage of total measured volume (BV/TV, %), trabecular thickness (Tb.Th um) and trabecular separation (Tb.Sp, um) using the ImageJ pluggin BoneJ (Doube et al., 2010).

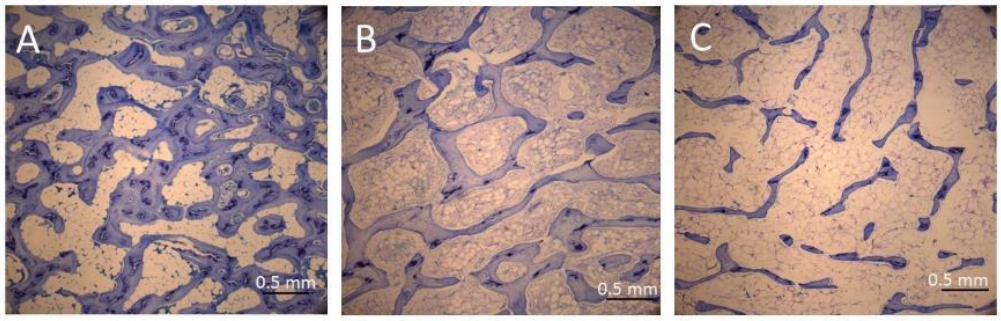


Figure 41. Light micrograph of steers' trabecular bone at 20x magnification, (A) steers offered Lucerne ad libitum (HCP-HME), (B) Lucerne restricted (HCP-LME) and (C) Mitchell grass ad libitum (LCP-LME). Images from bone samples collected at post mortem at end of treatment phase of steers under different nutritional treatments and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

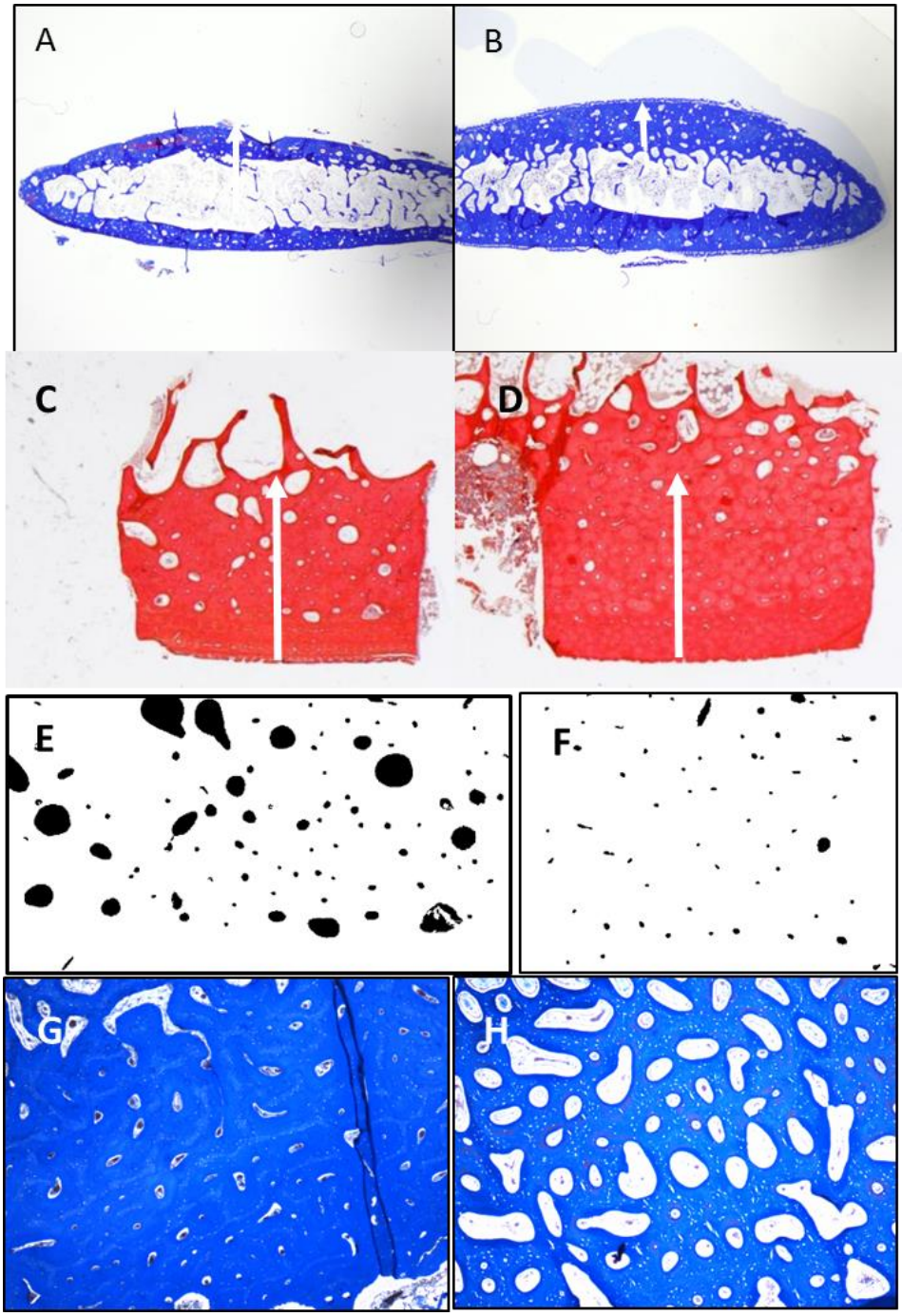


Figure 42. Examples of rib histology to demonstrate aspects of cortical bone volume. A and B are cross sections of rib 12. In A the rib has a thin cortex with a much smaller cross sectional area than seen in B. Typical site of measurement of CBT shown as white arrow. C and D show in higher magnification, typical segments of the outer cortex of a rib obtained by bone biopsy. The rib in C has less CBT than the one in D. E and F are diagrams of the porosity of the rib samples in C and D. The black areas are the "holes" in the bone formed by bone resorption/mobilisation. The white is the remaining cortical bone area that can be used to estimate bone volume in this region. The rib sample in E has greater porosity and thus less bone volume than the rib in F. G and H are greater magnification and again show segments of rib cortical biopsy. The rib in H has extensive bone resorption and thus much greater porosity and reduced bone volume compared to the rib in G.

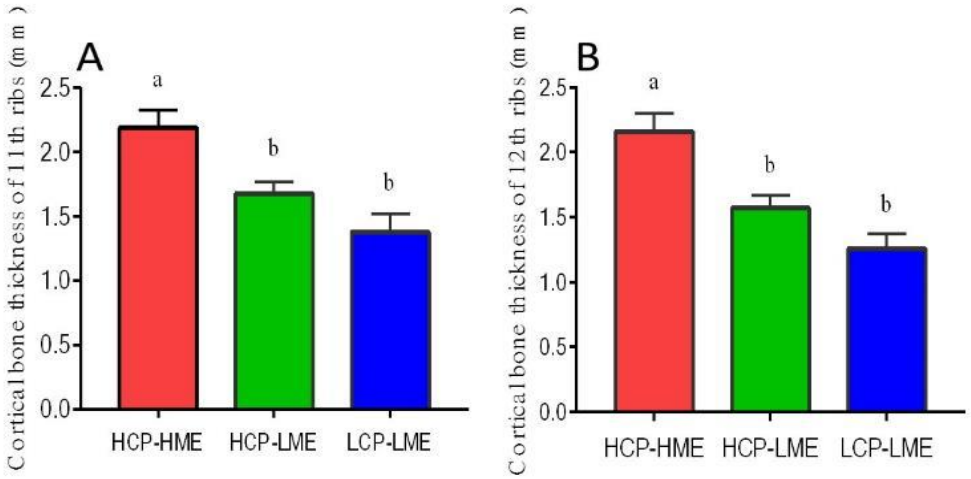


Figure 43. Cortical bone thickness of the 11th (A) and 12th (B) ribs of steers under different nutritional treatments and receiving either bST or saline administered subcutaneously every every 14 days for a 98 day treatment period. Means with different letters are significantly different ($P < 0.05$)[1]. Results are from bone samples collected at post mortem at end of treatment phase.

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

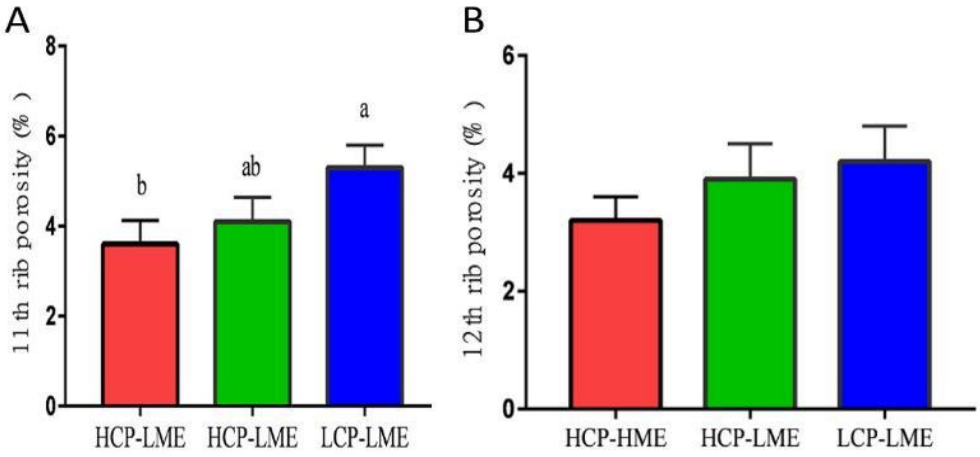


Figure 44. Cortical porosity of 11th rib (A) and 12th rib (B) of steers under different nutritional treatments and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period. Means with different letters are significantly different ($P < 0.05$)[1]. Results are from bone samples collected at post mortem at end of treatment phase.

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

4.2.4 Radiographic measurement of long bones

All bones measured, except for the cannon bone, were longer in steers fed the HCP-LME diet than in steers fed low ME diets (Table 12, Figure 41).

Table 14. Length (mm) measured on digital radiographs of long bones collected at post mortem from steers under different nutritional treatments[1] and receiving either bST[2] or saline administered subcutaneously every 14 days for a 98 day treatment period. Results are from bone samples collected at post mortem at end of treatment phase[3].

	Nutrition			
	HCP-HME	HCP-LME	LCP-LME	*P*
Humerus	283.25[a]	266.6[b]	264.7 [b]	≤0.001
Radius	281.5 [a]	269.7 [b]	266.2 [b]	≤0.001
Cannon	213.9	209.4	212.1	0.42
Tibia	341.0 [a]	324.2 [b]	327.3 [b]	0.009

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

[2]Bovine somatotrophic hormone.

[3]Means within row with different superscripts differ ($P < 0.05$)

A **B**

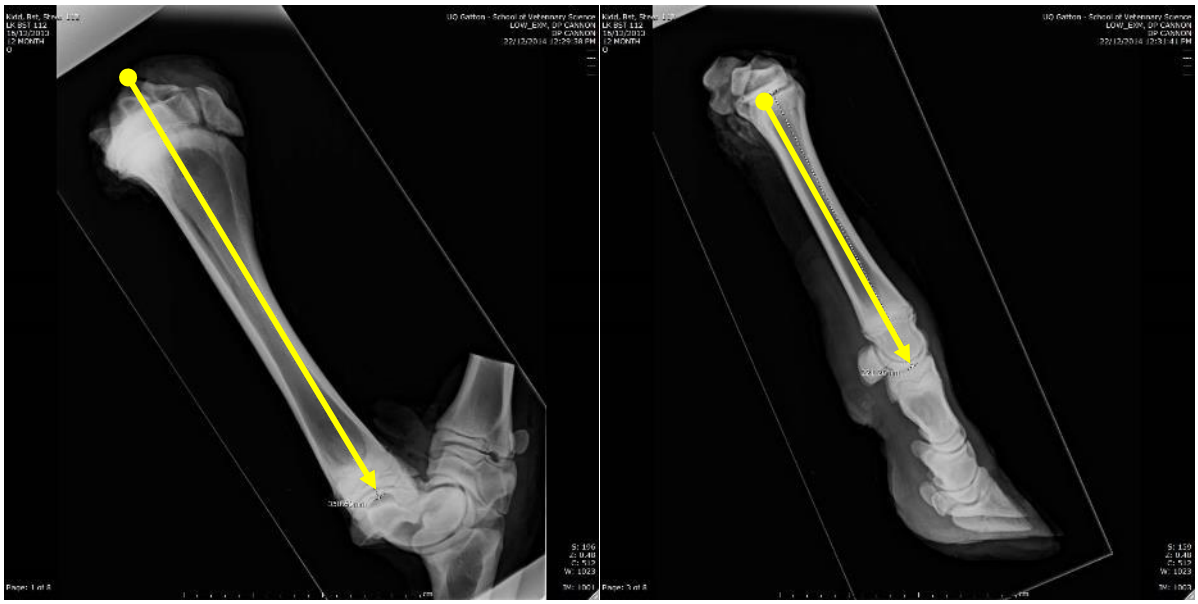


Figure 45. Image of the tibia (A) and cannon bone (B) collected at post mortem from steers. Yellow arrows show the measurement of bone length obtained using a radiographic storage program (Synapse, Fuji). Images from bone samples collected at post mortem at end of treatment phase of steers under different nutritional treatments and receiving either bST or saline administered subcutaneously every 14 days for a 98 day treatment period[1].

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]

4.3 Experiment 3 The effect of changes in feed intake on the molecular regulation of bone elongation in sheep

4.3.1 Dry matter intake, liveweight and liveweight gain

Lambs gained an average of 0.23 kg/day during phase one (AdLib), lost 0.8 kg/day during Phase 2 (Fast) and re-gained .57 kg/day during Phase 3 (ReFeed). By the end of 21 days the 9 animals in the remaining group (ReFeed) had a combined average gain and loss of zero kg LW (Table 13). These treatments were designed to drive rapid skeletal growth, followed by growth stasis and rapid growth again during re-alimentation. Although skeletal growth was not measured over this short time frame it was assumed that these dramatic changes in intake and LW would have the desired effects at the growth plate.

Table 15

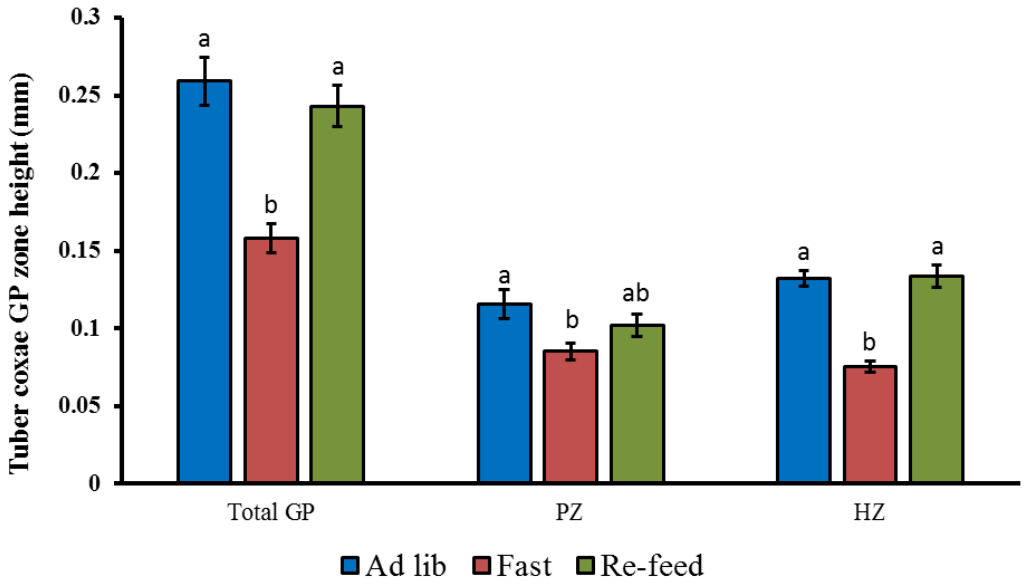
Dry matter (DM) intake (DMI; g/kg LW.day), Liveweight (LW) ADG (kg), of Merino cross male lambs at the end of 3 different phases of a feeding trial (mean ± standard deviation). Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed).

Parameter	AdLib	Fast	ReFeed
Number of animals	27	18	9
LW (kg)	34.1 ± 0.6	28.5 ± 0.8	32.4 ± 1.0
Intake (g DM/kg W.day)	36.0 ± 0.8	1.6 ± 0.2	31.7 ± 1.0
LW gain (7 days)	1.6 ± 0.8	-5.7 ± 1.1	4.0 ± 0.9
LW gain (14 days)		-4.0 ± 1.2	-1.6 ± 1.3
LW gain (21 days)			0.0 ±1.6

4.3.2 Growth plate histomorphometry

There was a significant reduction in total growth plate, PZ and HZ height from the end of the AdLib feeding period to the end of the Fast period in both the tuber coxae and distal radial growth plates (Figure 46 and Figure 48). The results from both growth plates measured were very similar. The total growth plate height in the tuber coxae and distal radius reduced by 46% and 25% respectively. The reduction in height was greater in the hypertrophic than the proliferative zones in both the tuber coxae and radius. This reduction in height of the hypertrophic zones was in part, due to a reduction in the area of the hypertrophic chondrocytes. The area of terminal hypertrophic chondrocyte was approximately 50% less at the end of the Fast feeding period than at the end of the AdLib period (Figure 47 and Figure 49). All of these growth plate measurements were allmost fully restored by the end of the ReFeed period with none of these measurements significanlty different to those from the AdLib group lambs.

A



B

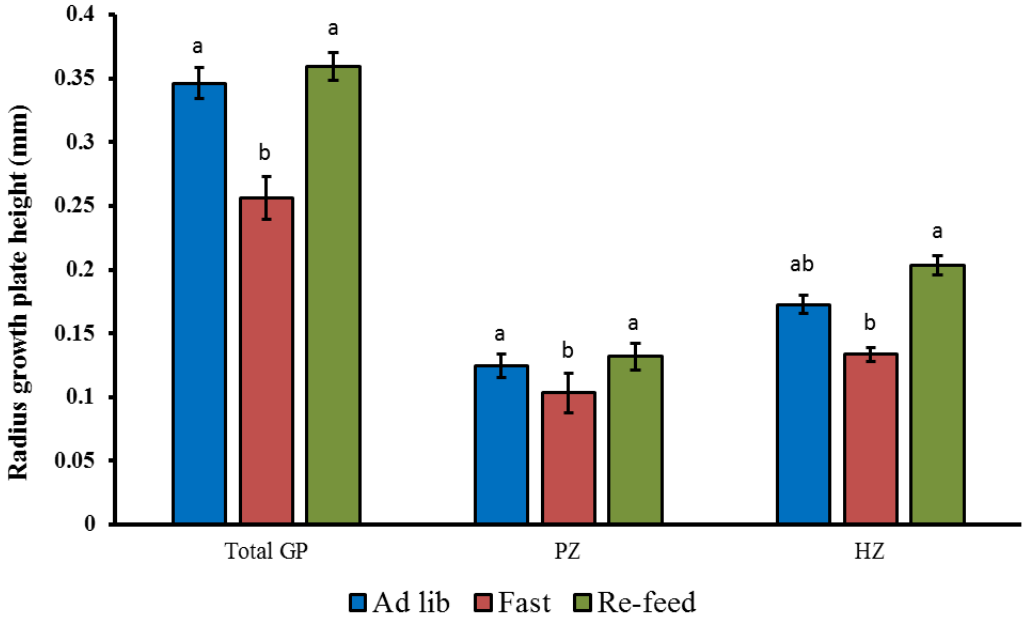
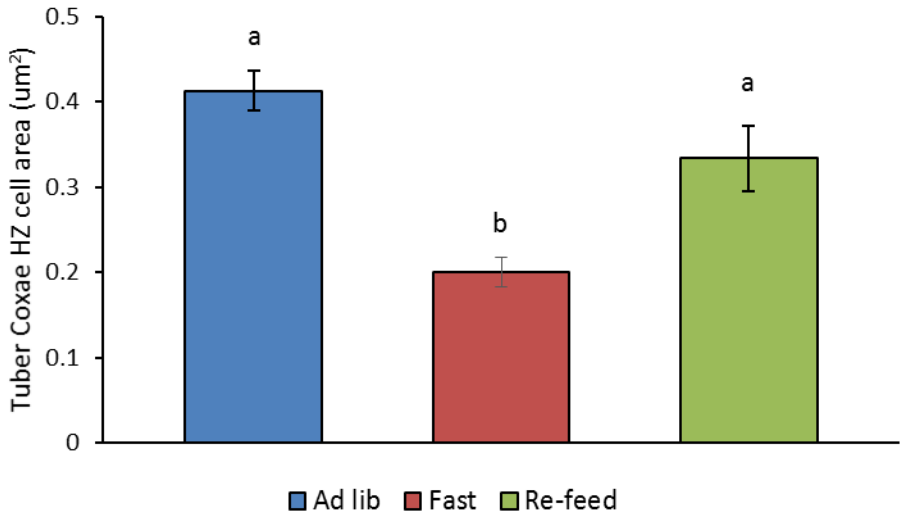


Figure 46. Height (mm) of the total growth plate (GP), the proliferative zone (PZ) or and the hypertrophic zone (HZ) in the growth plate of the tuber coxae (A) and the distal radius (B) of Merino cross weathers lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of ad libitum Lucerne chaff (ReFeed). Means with different letters are significantly different (P ≤ 0.05).

A



B

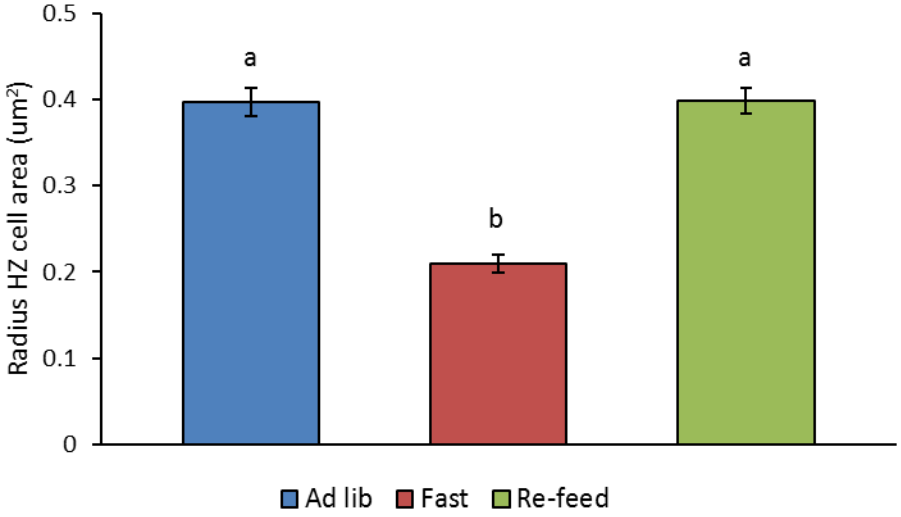


Figure 47. Area (μm^2) of the terminal hypertrophic chondrocytes (HZ) in the growth plate of the tuber coxae (A) and the distal radius (B) of Merino cross weathers lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of ad libitum Lucerne chaff (ReFeed). Means with different letters are significantly different (P ≤ 0.05).

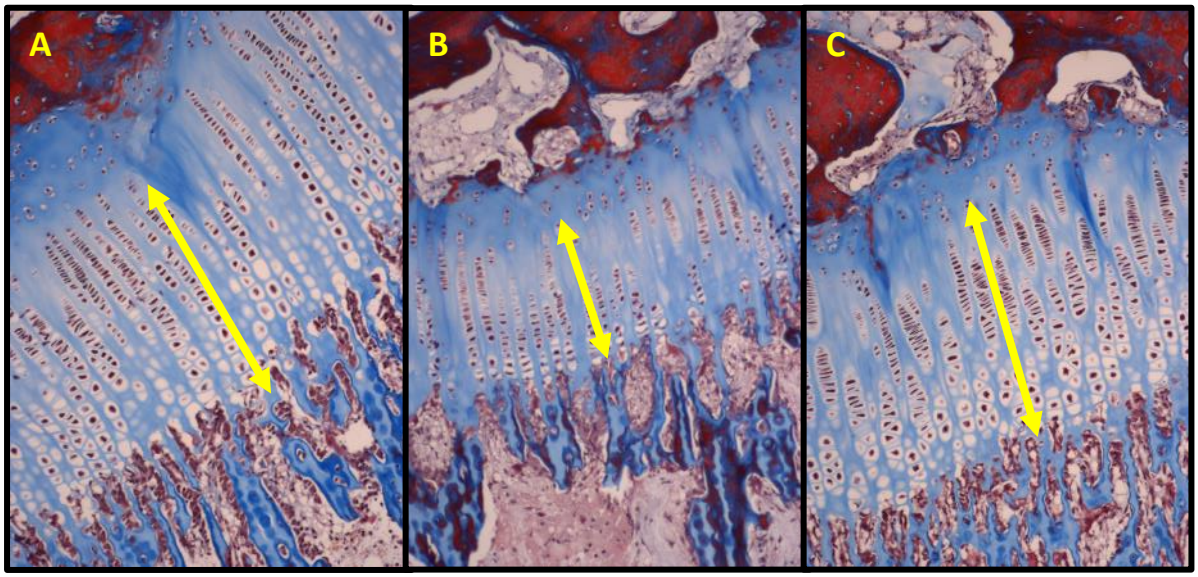


Figure 48. Histological photos showing the growth plate of the distal radius from lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of ad libitum Lucerne chaff (ReFeed). Yellow arrows indicate the height of the combined proliferative (PZ) and hypertrophic (HZ) zones from lambs at the end of Phase 1 (A; AdLib), the end of Phase 2 (B; Fast) and the end of Phase 3 (C; ReFeed). This height is significantly less in Fast than in the AdLib and ReFeed lambs (P ≤ 0.05).

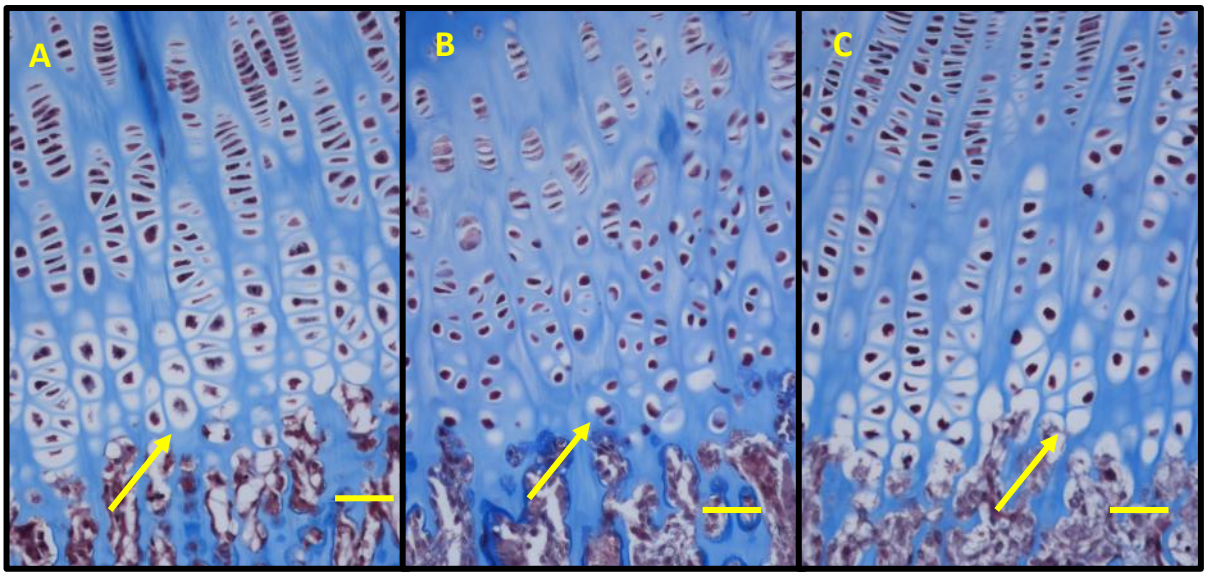


Figure 49. Histological photos showing the growth plate of the distal radius from lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of ad libitum Lucerne chaff (ReFeed). The terminal hypertrophic chondrocytes (yellow arrows) become significantly smaller in area (P ≤ 0.05) after Phase 2 (B, Fast) compared to the Phase before (A, AdLib) and after (C, ReFeed) .

4.3.3 Plasma metabolites, hormones and bone metabolism markers

Plasma concentrations of urea, glucose and calcium were decreased at the end of the Fast feeding period compared to AdLib and ReFeed values and these concentrations had returned to similar values to AdLib by the end of the ReFeed phase of the experiment. Plasma concentrations of total protein and P were increased at the end of Fast compared to the other 2 feeding periods (Figure 50).

The plasma concentrations of insulin followed a similar pattern to glucose and was reduced at the end of Fast compared to AdLib and ReFeed. The plasma concentrations of IGF-1 dropped after Fast and increased after ReFeed but did not return to the level seen at the end of AdLib. IGF-1 concentrations were significantly different at the end of each feeding period (Figure 51). Leptin concentrations did not vary between feeding periods (Figure 52). Total T4 plasma concentration followed a very similar pattern to IGF-1.Total T3 concentrations decreased from the end of AdLib to the end of Fast but had not increased again at the end of ReFeed (Figure 53).

CTX-1, a marker of bone resorption/mobilisation was elevated at the end of Fast and returned to a level similar to AdLib by the end of ReFeed. Osteocalcin and BAB are potential markers of bone formation and both were decreased after the Fast feeding period. While OC increased again after ReFeed, BAP concentrations remained low (Figure 54).

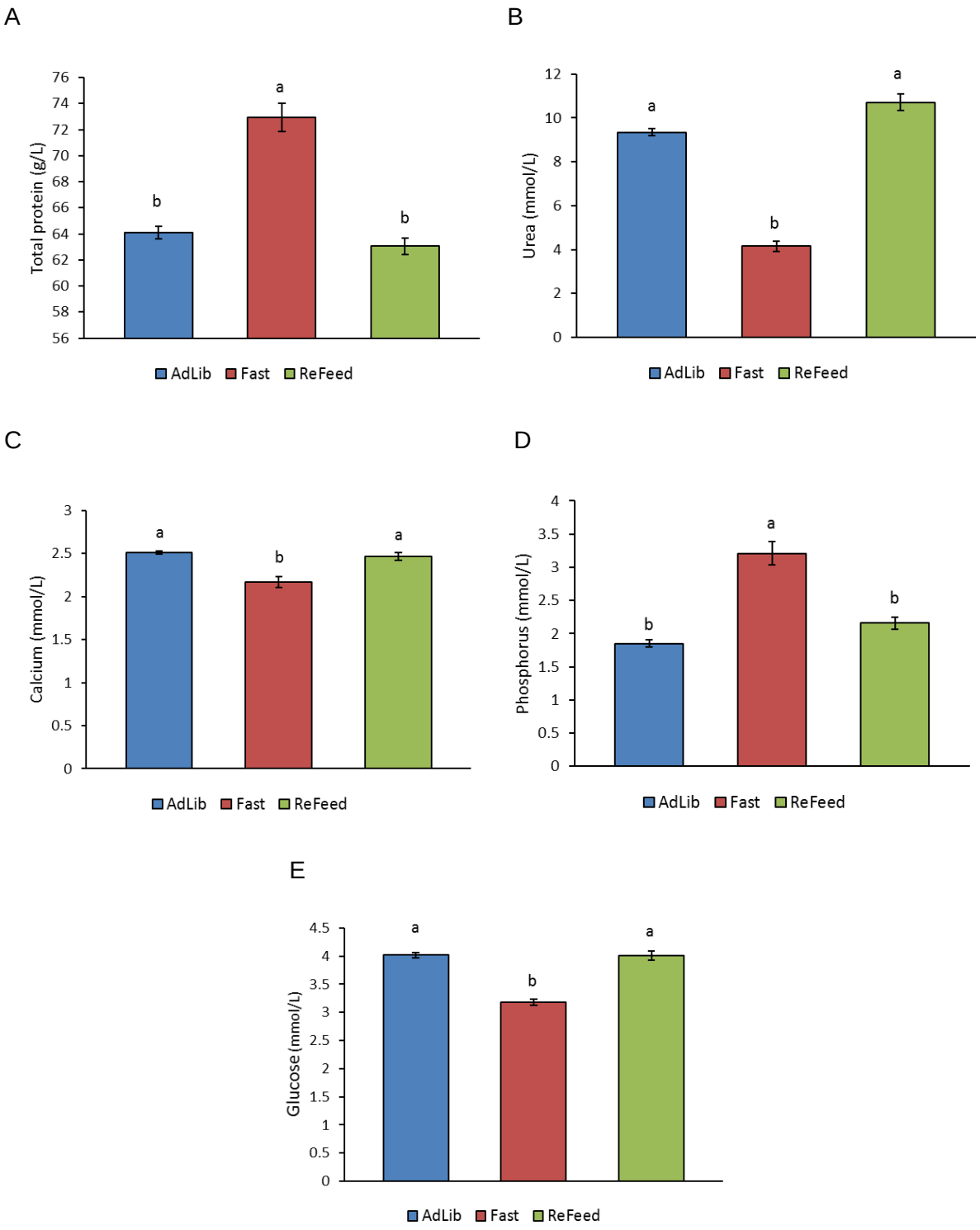


Figure 50

The plasma concentration of (A) total protein (g/L), (B) urea nitrogen (mmol/L), (C) total calcium (Ca; mmol/L), (D) inorganic P (mmol/L) and (E) glucose (mmol/L) in lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed). Means with different letters are significantly different (P≤0.05).

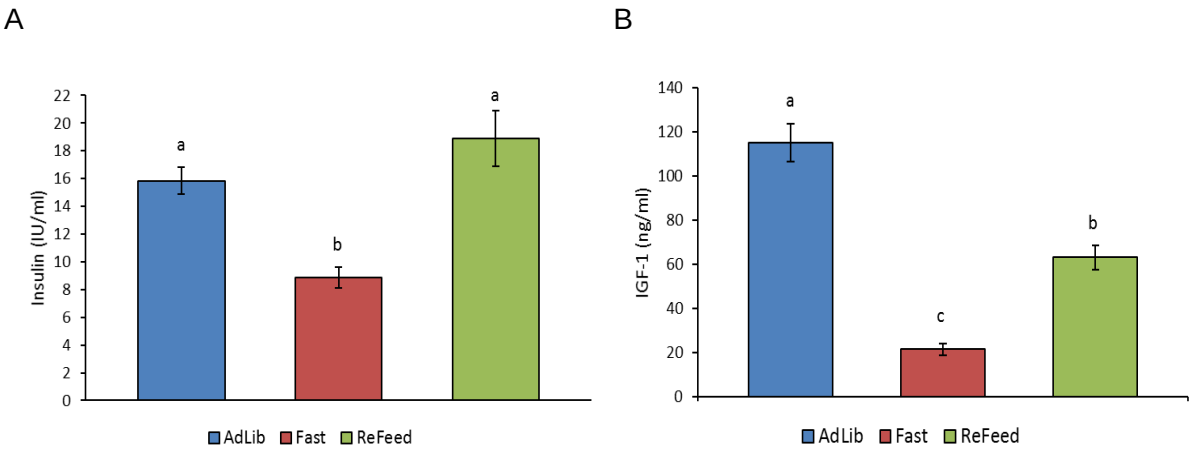


Figure 51

The plasma concentration of (A) insulin (IU/mL; a) and (B) insulin-like growth factor-1 (IGF-1; ng/mL) in lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed). Means with different letters are significantly different (P≤0.05).

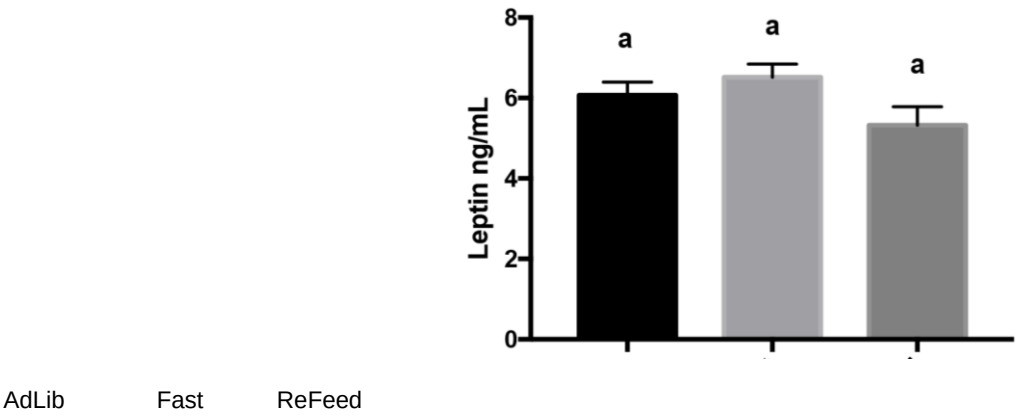


Figure 52

The plasma concentration of leptin (ng/mL) in lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed). Means with different letters are significantly different (P≤0.05).

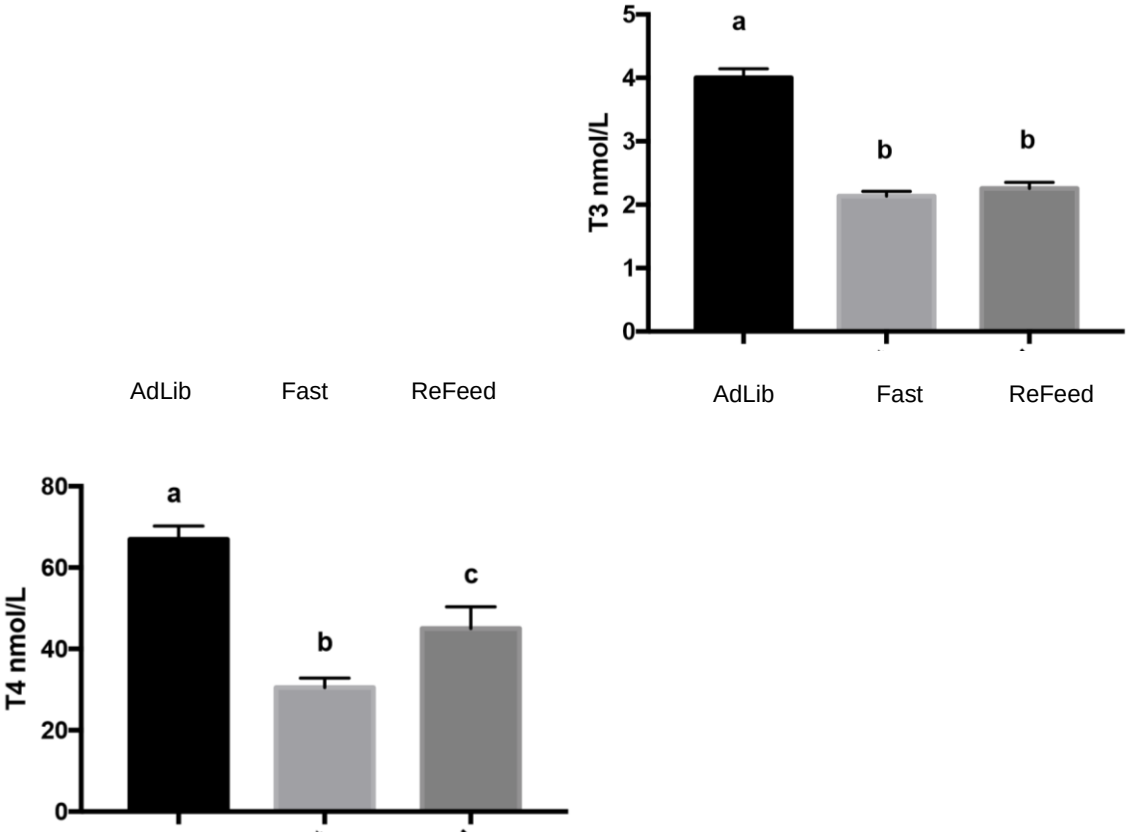


Figure 53

The plasma concentration of (A) triiodothyronine (T3; nmol/L) and (B) thyroxine (T4; nmol/mL) in lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed). Means with different letters are significantly different (P≤0.05).

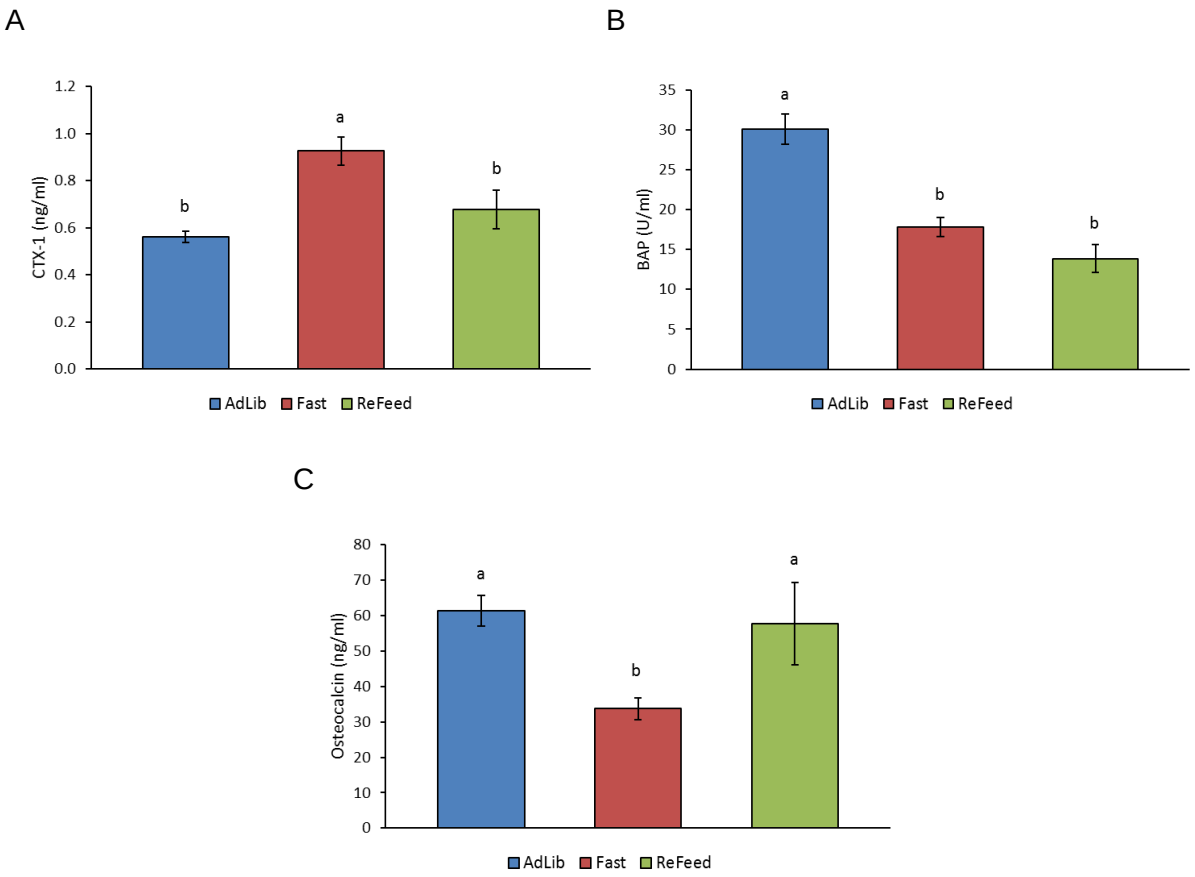


Figure 54

The plasma concentration of (A) CTX-1 (ng/mg), (B) bone alkaline phosphatase (BAP; U/L), (C) osteocalcin (ng/mL) in lambs at the end of 3 different phases of a feeding trial. Lambs were fed Lucerne chaff ad libitum for 7 days (AdLib), followed by 7 days of fasting (Fast) and then a further 7 days of *ad libitum* Lucerne chaff (ReFeed). Means with different letters are significantly different (P≤0.05).

4.4 Gene expression analysis

The total number of genes with higher or lower relative abundance within paired groups comparisons is shown in Table 16. Bioinformatics including cellular pathway analysis revealed similar patterns of response between the 3 experiments. The results of gene expression analysis demonstrated that there is a complex response to reduced nutrition and re-alimentation in the growth plate cartilage and adjacent trabecular bone. Next-generation sequencing of mRNA is able to analyse gene expression across the transcriptome and thus examine for differential expression of thousands of annotated and unknown genes. In all three experiments a large number of genes were differentially expressed and the magnitude of the response was greater in comparisons with a greater effect on growth plate elongation.

In the extreme model of fasting for a week in sheep the number of differentially expressed genes between Fast and either AdLib feeding or ReFeed was in the hundreds. There were very few differentially expressed genes between AdLib and ReFeed and this agreed with the

hormonal and growth plate histology measures where most of the variables measured had returned to AdLib levels by the end of ReFeed.

The greatest number of differentially expressed genes in steers in experiments 1 and 2 were seen between the HCP-HME and the low ME treatments. The main effect on up- or down-regulating gene expression appeared to be ME restriction. Additional CP, bST treatements and genotpe all had minimal effect of changing mRNA expression between treatment groups.

Many of the cellular pathways that were differentially expressed in restricted diets in the cattle and sheep experiments were associated with the immune response, red and white cell production and response to infectious agents. These are likely to represent bone marrow tissue gene expression rather than chondrocyte or bone cell changes. These aspects of gene expression provide very interesting insights into the physiological response of animals to nutritional stressors. Seven days of fasting in lambs or 14 weeks of poor nutrition in steers induced gene expression profiles similar to an animal mounting an immune response to disease processes. These pathways may also provide novel insights into bone and growth plate response to restricted nutrition. There is growing evidence that bone remodelling is influenced by cells in the immune system and some of the differentially expressed genes in this study suggest activation of these emerging mechanisms (Dar et al., 2018). For example, interlukin 17 (IL-17) has only recently been identified as important in activating osteoblasts and chondrocytes to stimulate bone resorption and cartilage matrix breakdown repectively. IL-17 is produced by specific immune cell lineage T “helper” cells (Miossec et al., 2009). T helper cell signalling was identifed as a pathway that was over-represented using the list of genes upregulated in bone/growth plate tissue from steers on low ME diets and fasting lambs.

The list of differentailly expressed genes was analysed to identify genes of known importance in bone and cartilage development and metabolism Table 17 (Mackie et al., 2011, Gat-Yablonski et al., 2013, Murray and Clayton, 2013, Baron et al., 2015). This list was compared to known mediators of proliferation, hypertrophy, extracellular matrix production and organisation and remodelling of the primary spongiosa Table 18. Genes that were significanlty more expressed in steers fed HCP-HME diets compared to low ME diets were visualised using the bioinformatics program String Figure 55 that created a diagram indicating linkages between differentially expressed genes (Szklarczyk et al., 2015). This demonstrated that IGF-1 pathways were central in the relationships between the upregulated genes under high ME and CP nutrition. Upregulation of IGF-1, insulin like growth factor 1 receptor (IGF1R) and insulin like growth factor binding protein 5 (IGFBP5) were shown to be linked with other upregulated genes involved in collagen and cartilage matrix assembly and organisation, chondrocyte proliferation and hypertrophy, angiogenesis and bone formation. These downregulated genes included multiple collagen types, extracellular matrix proteins, growth factors, transcription factors and cell signalling proteins. Alterations in the expression of IGF-1 and related genes demonstrates likely effects on the autocrine and paracrine roles of IGF-1 at the local level of the growth plate.

Table 16. Initial bioinformatics from the Gene Seq analysis created lists of differentially expressed genes (FDR ≥0.05) for each pair-wise camparison undertaken.

Statistical comparison	Number of genes with increased relative expression	Number of genes with decreased relative expression	Total differentially expressed genes due to DIET
Experiment 1 Tuber coxae			
BX HCP-HME vs HCP-LME	80	71	151
BX HCP-HME vs LCP-LME	74	86	160
HF HCP-HME vs HCP-LME	56	39	95
HF HCP-HME vs LCP-LME	25	32	57
HF HCP-LME vs HF LCP-LME	0	0	0
BX HCP-LME vs BX LCP-LME	0	0	0
Experiment 2 Radius -bST			
HCP-HME vs HCP-LME	41	15	54
HCP-HME vs LCP-LME	129	49	178
Experiment 2 Radius +bST			
HCP-HME vs HCP-LME	169	64	233
HCP-HME vs LCP-LME	386	207	593
Experiment 2 Tuber coxae cartilage -bST			
HCP-HME vs HCP-LME	503	449	952
HCP-HME vs LCP-LME	275	342	617
HCP-LME vs LCP-LME	4	1	5
Experiment 2 Tuber coxae bone tissue -bST			
HCP-HME vs HCP-LME	534	363	897
HCP-HME vs LCP-LME	>500	>500	>1000
HCP-LME vs LCP-LME	4	1	5
Experiment 2 Femur +bST			
HCP-HME vs HCP-LME	241	72	313
HCP-HME vs LCP-LME	278	114	393
HCP-LME vs LCP-LME	0	1	1
	Genes with increased relative expression in +bST	Genes with decreased relative expression in +bST	Total differentially expressed genes due to +bST
Experiment 2 Radius			
HCP-HME +bST vs HCP-HME -bST	0	0	0
HCP-LME +bST vs HCP-LME -bST	0	0	0
LCP-LME +bST vs LCP-LME -bST	0	0	0
	Number of genes with increased relative expression in HF	Number of genes with decreased relative expression in HF	Total differentially expressed genes due to genotype
Experiment 1 Tuber coxae			
HF HCP-HME vs BX HCP-HME	0	0	0
HF HCP-LME vs BX HCP-LME	0	0	0
HF LCP-LME vs BX LCP-LME	0	0	0

Table 17. List of genes with known roles in bone and cartilage regulation that had increased relative abundance in HCP-HME diets in experiments 1 and 2

ADAM metallopeptidase domain 12(ADAM12)	hypoxia inducible factor 1 alpha subunit(HIF1A)
ADAM metallopeptidase domain 19(ADAM19)	indian hedgehog(IHH)
ADAM metallopeptidase with thrombospondin type 1 motif 7(ADAMTS7)	insulin degrading enzyme(IDE)
ADAMTS like 1(ADAMTSL1)	insulin like growth factor 1 receptor(IGF1R)
adrenomedullin(ADM)	insulin like growth factor 1(IGF1)
aggrecan(ACAN)	insulin like growth factor binding protein 5(IGFBP5)
alkaline phosphatase, liver/bone/kidney(ALPL)	lactotransferrin(LTF)
bone morphogenetic protein 2(BMP2)	lamin B1(LMNB1)
cartilage intermediate layer protein 2(CILP2)	laminin subunit alpha 4(LAMA4)
cartilage oligomeric matrix protein(COMP)	laminin subunit beta 3(LAMB3)
chondroitin sulfate proteoglycan 4(CSPG4)	matrix metallopeptidase 12(MMP12)
chondroitin sulfate synthase 1(CHSY1)	matrix metallopeptidase 15(MMP15)
chondroitin sulfate synthase 3(CHSY3)	matrix metallopeptidase 19(MMP19)
collagen beta(1-O)galactosyltransferase 1(COLGALT1)	matrix remodeling associated 5(MXRA5)
collagen type IX alpha 1 chain(COL9A1)	parathyroid hormone like hormone(PTHLH)
collagen type VI alpha 3 chain(COL6A3)	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2(PLOD2)
collagen type VIII alpha 1 chain(COL8A1)	procollagen-lysine,2-oxoglutarate 5-dioxygenase 3(PLOD3)
collagen type XII alpha 1 chain(COL12A1)	
collagen type XV alpha 1 chain(COL15A1)	stromal cell derived factor 2 like 1(SDF2L1)
collagen type XXVII alpha 1 chain(COL27A1)	tenascin N(TNN)
C-X-C motif chemokine receptor 2(CXCR2)	TIMP metallopeptidase inhibitor 1(TIMP1)
desmin(DES)	TNF receptor superfamily member 12A(TNFRSF12A)
DGCR8, microprocessor complex subunit(DGCR8)	TNF receptor superfamily member 6b(TNFRSF6B)
elastin(ELN)	transcriptional repressor GATA binding 1(TRPS1)
fibromodulin(FMOD)	tolloid like 1(TLL1)
fibronectin 1(FN1)	transforming growth factor beta receptor 3 like(TGFBR3L)
hedgehog interacting protein(HHIP)	tubulin alpha 4a(TUBA4A)
heparan sulfate proteoglycan 2(HSPG2)	tubulin, alpha 1c(TUBA1C)
hyaluronan and proteoglycan link protein 1(HAPLN1)	Wnt family member 11(WNT11)
transcriptional repressor GATA binding 1(TRPS1)	

Table 18. List of mediators of importance in the the growth plate during skeletal elongation via endochondral ossification identified in recent reviews (Mackie et al., 2011, Baron et al., 2015). and compared to the list of genes differentially expressed in experiments 1 and 2.

	Well described mediators of endochondral ossification	Effect of low ME diet in steers in Experiments 1 and 2
Stimulation of chondrocyte proliferation in the proliferative zone (PZ)	Hormonal control • Growth hormone • IGF-1 Local control and expression • IGF-1, IGF-1 receptors • IHH • WNTs • BMPs • BMP receptors • Perlecan • TRPS1 Suppression of proliferation • FGFs, FGF receptors	Reduced plasma IGF-1 Reduced expression of: • IGF-1, IGF1R1, IGFBP5 • IHH • WNT 11 • BMP-2 • Perlecan • TRPS1 Increased expression of • FGFR2
Extracellular matrix formation, organisation and signalling	Local factors • IGF-1 • BMPs Local control and expression • Hyaluronin • Aggrecan • Collage type 2 • Collagen types 6, 9, 11, 12, 14, • Decorin • Biglycan • Fibromodulin • Thrombospondin • COMP • SOX9	Reduced expression of: • IGF-1, IGF1R1, IGFBP5 • BMP-2 • Aggrecan • Collagen types 6, 9, 12, 15, 27 • Fibromodulin • Thrombospondin • COMP
Stimulation of chondrocyte hypertrophy in the hypertrophic zone (HZ)	Hormonal control • IGF-1 • Thyroid hormones Local control and expression • IHH • PTHrP • Collagen type 10 • MMP 13 • ADAMTS • WNTs • B-catenin • RUNX2 • Zfp521 • TRPS1	Reduced plasma: • IGF-1 • T3, T4 Reduced expression of: • IHH • PTHrP • Collagen type 8,10 • MMP 12, 15, 19 • ADAMTS7 • WNT 11 • B-catenin • TRPS1
Degradation and invasion of growth plate cartilage	Local control and expression • Annexins • Alkaline phosphatase • PHOSPHO1 • Carminerin • MMP 9, MMP13 • Cathepsin C, Cathepsin K • VEGF • HMGB1 • RANKL • BMPs, WNTs	Reduced expression of: • Alkaline phosphatase • MMP 12, 15, 19 • Cathepsin C, • VEGF • HMGB1 • BMP-2 • WNT 11

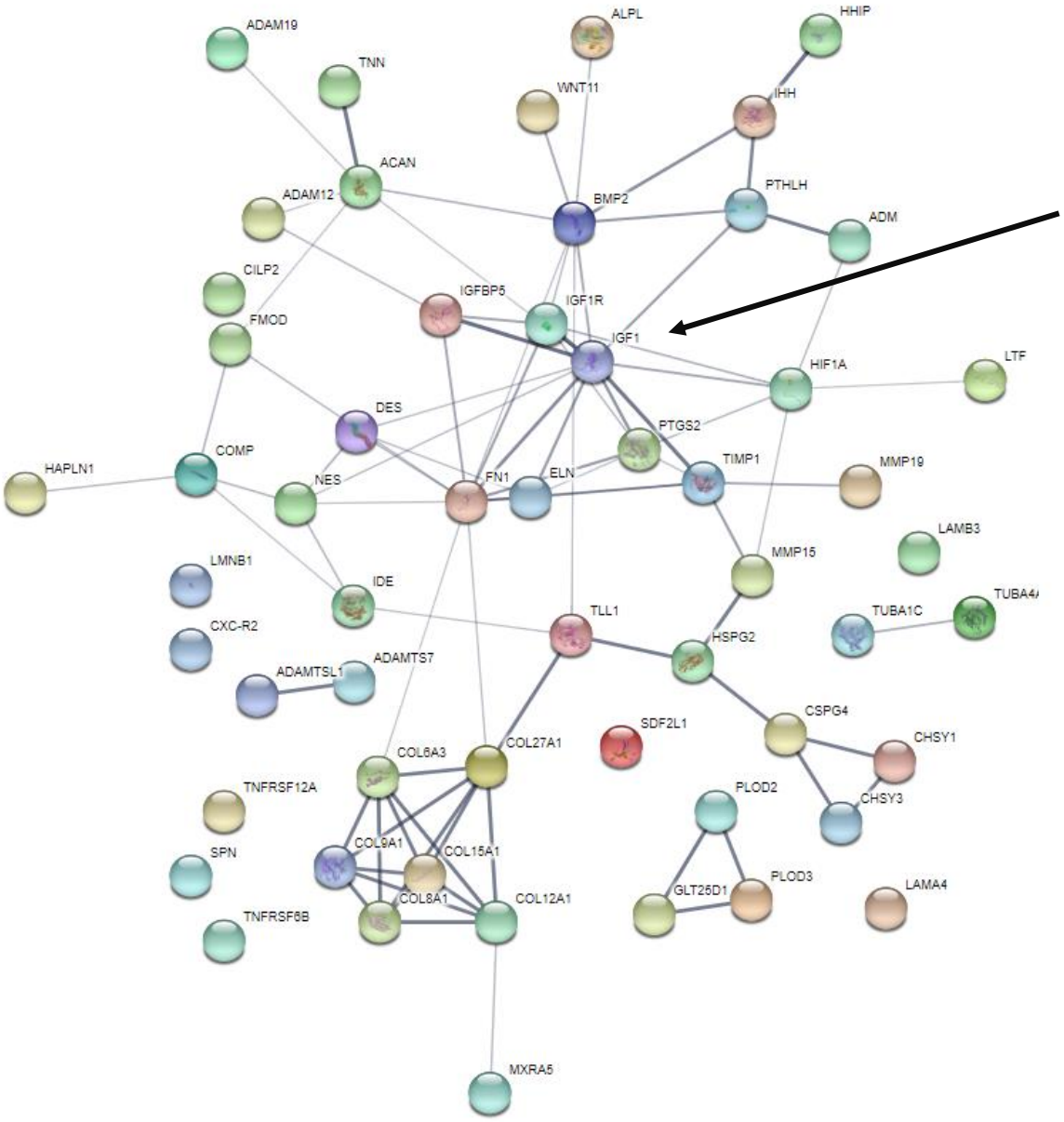


Figure 55. Pathway linkage illustration created using the list of genes created in table 17 and analysed in the protein network program String. The black arrow indicates the position if IGF-1 in the centre of the network analysis with multiple known functional connections the the other proteins identifies in gene expression analysis.

5 Discussion

This project investigated the interaction between nutrition, liveweight gain and skeletal elongation in cattle and sheep undergoing periods of nutritional restriction and re-alimentation. Although cattle appear to have an allometric relationship between HH and LW gain during growth, periods of poor nutrition can alter this relationship. One of the major aims of this project was to determine if it is possible to “un-couple” growth in skeletal height with LW gain in growing steers and drive additional frame size in cattle in the dry season to enhance compensatory LW gain in the wet season. Skeletal growth was examined using a

number of different strategies. Experiment 1 examined the effect of dietary crude protein (CP) content or metabolisable energy (ME) intake on skeletal growth, subsequent compensatory growth and bone gene expression in different phenotype steers; bos indicus cross (BX) and Holstein/Fresion (HF). A second experiment (Experiment 2) employed similar nutritional interventions to Experiment 1 but used a single strain of *bos indicus* cross steers and imposed an additional treatment of growth hormone/bovine somatatropin (bST). Fasting and re-feeding in lambs was used as nutritional model of dramatic, acute changes of bone growth (Experiment 3). This experimental strategy was used to determine the maximal growth possible at each nutritional level and what the cellular response to nutrition and exogenous bST revealed about drivers of skeletal elongation.

The project objectives were to have:

1. Identified the important growth factors and cellular signalling mediators within the growth plate.
2. Determined the effect of CP content or level of ME intake on skeletal growth, subsequent compensatory growth and growth plate gene expression in Bos indicus crossbred and Holstein genotype steers.
3. Examined the effect of bovine somatotrophic hormone (BST) on skeletal growth and growth plate gene expression in Bos indicus cross steers.
4. Identified possible strategies and options to increase dry season skeletal elongation so as to maximise compensatory growth in the wet season and potentially reduce or change dry season supplementation.
5. Developed recommendations for subsequent research to capture the benefits of this approach under commercial conditions.

The effect of CP content or level of ME intake on skeletal growth

Both experiment 1 (Exp 1) and Experiment 2 (Exp 2) examined the effect of CP and low or high ME diets on skeletal growth. Experiment 1 was designed using BX steers typical of northern Australia as well as a tall, rapidly growing HF steers in pen experiments. Experiment 2 used a single strain of BX steers. The experimental design was successful in creating 2 diets with matched low ME and differing CP content but the same LWG in both cattle. While steers fed HCP-HME diets achieved rapid LWG of approximately 1.2 kg/d while both the ME restricted diets produced steers with static or slow LWG of between -0.2 and 0.25 kg/day.

Contrary to our original hypothesis, adding CP to a low ME diet was not able to drive useful increases in skeletal elongation. While there were some significant increases in HH gain in the overall the effects on skeletal elongation of additional CP to ME restricted diets were modest. During the dietary treatment phases steers eating HCP-HME gained HH rapidly with a rate of 105 mm/100days (Exp 1) and 99 mm/100d (Exp 2) which was significantly faster than in the restricted ME groups in each experiment. In Exp 1 steers fed HCP-LME and LCP-LME diets both grew in height more slowly at 41.8 mm/100 days for the LME-HCP group which was significantly greater than 30.3 mm/100 days for the LCP-LME group. This suggested that there was an effect of CP driving some additional gain in HH. The LW:HH was significantly less in the HCP-LME than the LCP-LME steers also indicating skeletal growth was increased over and above LW gain. There was an interaction between diet and genotype but statistical differences were not robust enough to extend to differences between

individual genotype/diet groups of steers. The changes in HH in steers in experiment 2 were of a similar order of magnitude (39mm/100 days in the HCP-LME and 31 mm/100d in LCP-LME) but there were no statistical differences in HH between these two low ME groups. As expected, HF steers had greater ME intake, LWG and HH gain than BX steers when CP and ME were not limiting. There was a strong similarity in IGF-I concentration between steers fed the HCP-LME and LCP-LME diets indicating that CP intake had little influence on plasma IGF-I concentration. The effect of ME and CP have been investigated previously in *bos taurus* steers. In one study, the plasma concentration of IGF-I increased linearly in beef steers fed a high energy diet with an increasing CP content (80 to 140 g CP/kg DM) but was unchanged in response to diet CP content when the diet had a lower energy content (Elsasser et al., 1989). It appeared that the ME of the diet accounted for most of the differences in skeletal growth in steers in this project.

The different dietary treatments and re-alimentation created a larger effect on LWG than HH gain or other body dimension measurments. During nutritional restriction in Exp 1, HHG was proportionally less affected (reduced by 66% - average of HCP-LME and LCP-LME) than LWG (reduced by 89% - average of HCP-LME and LCP-LME) resulting in a disconnection between hip height and LW. Over the re-alimentation period (i.e. Phase 2), LWG (increased by 50% - average of HCP-LME and LCP-LME) was proportionally greater than the changes observed in HHG (increased by 18% - average of HCP-LME and LCP-LME) when compared to control group (i.e. HCP-HME). Similar findings were seen in previous studies, which examined the effect of feed restriction and re-alimentation on LW and body dimensions in sheep and *bos taurus* cattle (Pálsson and Vergés, 1952, Lawrence and Pearce, 1964, Keogh et al., 2015). The magnitude skeletal elongation varied between the body dimension but most followed a similar pattern to HH with slower elongation in ME restricted groups and faster elongation during re-alimentation. Measuring body dimensions in experiment 1 relied on identifying external bony landmarks that were variably covered in soft tissue. Although this may lead to increased error in measuring these distances, repeated measurements followed a biologically plausible and smooth growth curve. Measuring the width of the pelvis from one tuber coxae to another is measuring the combined effect of the growth plate in each tuber coxae, the site examined histologically in all 3 experiments. In experiment 2 individual bones were removed and measured using radiographs. Greater differences were seen between dietary treatments in the longer bones (radius, femur) than in the short cannon bone of the lower limb. The growth plates of the lower limb bones close earlier and there is less contribution of these bones to the overall length of the limb than the long bones in the upper limb (Lawrence and Pearce, 1964).

The response of the growth plate to longer term nutritional restriction, especially in large animals has not been well examined. Analysis of plasma hormones, growth plate morphology, gene expression and patterns of bone elongation after a period of growth restriction has been undertaken in a number of studies in rabbits (Baron et al., 1994; Gafni et al., 2001b) and rats (Farnum et al., 2003, Marino et al., 2008). There are, however, no previous reports of using biopsies or post mortem material to examine growth plate dynamics, gene expression or bone histomorphometry to investigate the response of these tissues to nutritional restriction and re-alimentation in sheep or cattle.

In this project steers offered the low ME diets had shorter proliferative and hypertrophic zones (PZ and HZ), number of HZ chondrocytes and smaller terminal hypertrophic chondrocyte than in the steers fed HCP-HME in all the bones examined in experiments 1

and 2. Although the PZ is about 30-40% longer than the HZ in most bones studied, the reduction in height was of a similar absolute magnitude in both the PZ and HZ with HZ losing proportionately more height in some bones. This was associated with a greater reduction in size of the HZ chondrocytes. All of these differences were lost during re-alimentation in Exp 1 and many of the measurements returned to pre-restriction sizes. These growth plate measures of skeletal elongation (SER) rate corresponded well to the SER observed with HH change indicating that histological and gene expression results from biopsies would be reflecting the true biological response.

In experiment 3 which was a more extreme but short duration model of nutritionally induced growth plate stasis, there were dramatic reductions in PZ, HZ and terminal chondrocyte area in the growth plate. These changes were all fully reversed after 7 days of re-alimentation. These finding were very similar to those seen in numerous rodent studies demonstrating reduction in growth plate zone height with fasting followed by compete restoration of growth plate height when feeding resumes (reviewed by (Gat-Yablonski et al., 2013). This suggests that the response of the growth plate to nutrition is similar between small animal models and our larger ruminants of interest to livestock production. Both proliferative and hypertrophic zones were involved in the shortening of the growth plate with a reduction in size and number of cells in each column within the zone. The ability of the growth plate to rapidly resume normal size and growth after fasting demonstrates that the growth plate is very responsive to nutritional stimuli and that short term sessation of growth is fully reversible in animals this age.

In this sheep experiment the growth plate histomorphology changes were almost identical between the distal radius and the tuber coxae. This was reassuring and showed that the model has low variability and that changes seen in the tuber coxae are reflective of changes seen in the growth plates of rapidly elongating, load bearing limb bones. Obtaining samples from these load bearing bones can only be undertaken at post mortem so obtaining biopsies of the tuber coxae allows the many advantages of repeated measurements from live animals.

Low ME intake had widespread effects on plasma metabolite, biomarker and hormone measurements but these were independent of diet CP in all cases except the thyroid hormones. In Exp1, steers with the higher CP intake during ME restriction had higher concentration of triiodothyronine (T3) in the plasma and this was correlated with larger terminal hypertrophic chondrocytes at the tuber coxae growth plate as well as increased hip height gain. This was not the case in Exp2 where thyroid hormones were only elevated in steers offered HCP-HME diet. Steers fed low ME diets had markedly reduced plasma glucose, insulin and insulin-like growth factor 1 concentration in both experiments. Plasma urea nitrogen was markedly reduced in steers fed LCP-LME diets, reflecting the very low CP intake in these groups. All of these plasma metabolites and hormones had returned partially or completely to control levels during re-alimentation following nutritional restriction in steers in Exp1.

Adiponectin was unaffected by diet in Exp1. Leptin was reduced in steers eating low ME diets but this was only a significant change in Exp2. BX steers had higher leptin than HF steers at the start of Exp1 so there may be genotype differences in fat tissue or leptin response to diet. Leptin did not decrease during fasting in lambs in Exp3. As leptin is produced in adipose tissue there was unlikely to be sufficient time for major changes in

adiposity in these lambs. Leptin has previously been identified as an important mediator of skeletal catch-up growth in rats (Gat-Yablonski et al., 2004) but it does not appear that circulating leptin changes, at least, are critical to the increased skeletal elongation rate seen following nutritional restriction in ruminants in this project.

One week of fasting in lambs led to an increase plasma protein which was likely to be due to dehydration. This was associated with an increased plasma P, a common sequelae of haemoconcentration and lack of saliva production in ruminants. Plasma insulin, glucose, calcium and urea nitrogen all decreased during fasting and returned to previous levels following 1 week re-feeding. Plasma concentrations of IGF-1 and T4 were reduced during fasting and had partly returned to previous levels following re-feeding while T3 was reduced during fasting in lambs and had not increased again after re-feeding. The growth plate and separate zones within it had completely returned to the same height following re-feeding as during the initial ad lib feeding. This corresponded well with the gene expression profile. During fasting there were hundreds of differentially expressed genes compared to the growth plate of AdLib or ReFeed lambs but there were minimal numbers of differentially expressed genes between the AdLib and ReFeed groups. This indicated that gene expression had more completely returned to previous levels than the circulating concentrations of IGF-1 or thyroid hormones. Local autocrine and paracrine signalling at the level of the growth plate were likely to be contributing to the rapid return to growth plate elongation.

Catch-up growth in skeletal height

Rapid gains in LW following a period of nutritional restriction is commonly described in livestock as compensatory LW gain. Descriptions of skeletal growth and changes in height are not as well developed for livestock. In the medical literature, increased rate of height gain seen after a period of reduced growth is moslty described as catch-up growth (Pando et al., 2010, Finkielstain et al., 2013). As maturity is reached there is a progressive reduction in the rate of growth of organs including the skeleton. Growth velocity therefore slows with increasing age. The growth trajectory and eventual size of the skeleton is largely genetically predetermined but periods of reduced growth velocity appear slow maturation leaving an animal with a younger “bone age” (Rosello-Diez and Joyner, 2015). The mechanism by which catch-up growth in height occurs is still not well understood but has been studied in rodent models because of its importance in human health. Stunting of growth is a major problem in children throughout the world. Malnutrition, chronic disease, hormonal disorders and anorexia nervosa can all lead to reduced growth in height (stunting) and weight (wasting) but catch up growth in response to improved nutrition is well recognised (Angood et al., 2016, de Onis and Branca, 2016, Millward, 2017).

The morphological changes observed at the growth plate level in steers and lambs in this project agree with previous work in rodents showing that growth plates are able to slow and the accelerate elongation in response to nutrition and that growth acceleration is not determined by chronological age alone. Many authors agree that this is likely to be due to a delay in growth plate senescence (Lui et al., 2011, Baron et al., 1994; Gafni et al., 2001a; Nilsson and Baron, 2004; Gat-Yablonski et al., 2008; Marino et al., 2008; Jobling, 2010). The elongation rate of long bones is rapid in prenatal and early postnatal life and it decreases naturally as animals grows older. The reason for the decrease in growth rate in rats is due to a reduction in chondrocytes proliferation and size of terminal hypertrophic chondrocytes. During a period of growth inhibition, such as nutritional restriction, it is proposed that the

pace of the growth plate senescence is reduced as well as the growth rate of bones. Once the growth limiting conditions are resolved the growth plate of restricted animals are more "physiologically immature" than unrestricted cohorts of the same age. This effect in turn leads to an increased rate of bone elongation in these previously restricted animals (Forcinito et al., 2011, Rosello-Diez and Joyner, 2015). In Exp 1, the limited ME intake during Phase 1 is assumed to have reduced the pace of growth plate senescence as well as it reduced the heights of the proliferative, hypertrophic zones and terminal hypertrophic cell size. During the re-alimentation phase the restricted animals had a more "immature" growth plate so they were able to show greater bone elongation rate when compared to the un-restricted cohorts.

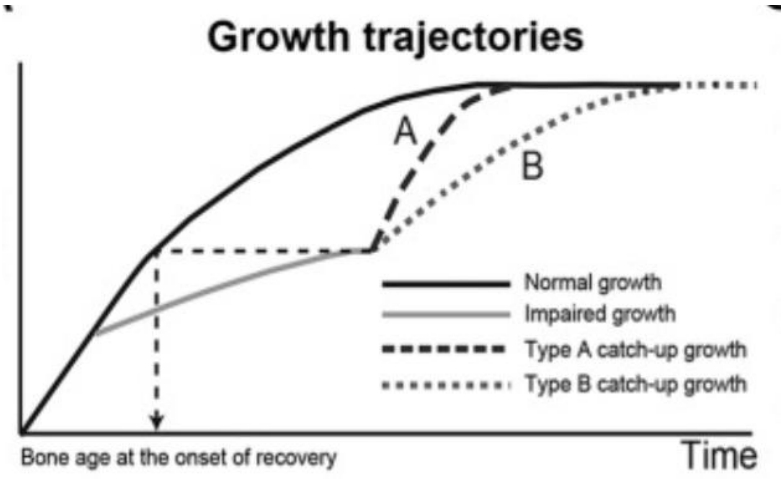


Figure 56. Types of skeletal catch-up growth. Idealized growth trajectories for height showing progressive loss of growth potential and 2 potential types of catch-up growth following nutritional restriction. In type A catch-up growth (A) the rate of gain in height is faster than a younger, unrestricted animal at the same height or "bone age". In type B catch up growth the rate of gain is faster than unrestricted animals at the same chronological age but not faster than younger animals at the same height or "bone age". The growth curve in Type B catch-up growth essentially returns to a normal trajectory but delayed in time (Rosello-Diez and Joyner, 2015).

In this project the improvement in bone elongation during re-alimentation with previously nutritionally restricted steers growing faster than their un-restricted counterparts is greater than seen in other previous studies in *bos indicus* steers (Quigley, 2013, Mclennan, 2014). There was a genotype interaction with diet suggesting that this effect may have been at least partly due to the more rapidly growing phenotype of the Holstein steers. The very high increases in HH seen in steers eating lucerne chaff *ad libitum* during phase 1 of Exp 1 and in Exp 2 may have contributed to these steers reaching a level of maturity at which they had already started to slow in HH change by the start of Phase 2. The previously ME restricted animals were still sufficiently early in maturity to allow a relatively rapid increase in height during re-alimentation in a very high quality diet.

Different types of catch-up growth in height have been described as types A and B (Rosello-Diez and Joyner, 2015). In type A catch-up growth the rate of gain in height is faster than a younger, unrestricted animal at the same height or "bone age". In type B catch up growth the rate of gain is faster than unrestricted animals at the same chronological age but not faster than younger animals at the same height or "bone age". The growth curve in Type B catch-

up growth essentially returns to a normal trajectory but delayed in time. Type A catch-up growth allows rapid return to pre-restriction height. In type B faster growth needs to continue for a longer time and past the age that a control animal would have stopped gaining height (Figure 56).

The increased rate of growth in height seen in steers in the current study follows a Type B pattern. This is similar to findings from other studies in beef steers (Quigley, 2013, Mclennan, 2014) and further supports previous work suggesting that rate of change in HH in growing steers cannot be increased beyond that seen on a normal growth curve.

Bone quality

A very interesting finding in this project is the degree to which overall bone quality and structure was affected by nutrition. The role of mineral deficiencies is well recognised, but the effect of reduced energy and protein nutrition on bone health has not been widely described in cattle. Bone histomorphometry analysis of trabecular bone volume (BV/TV), trabecular thickness (Tb.Th) and separation (Tb.Sp) and cortical bone thickness and porosity are all measures of the amount and structure of bone tissues in a given volume. These are established methods of examining changes in bone volume and structure and have standardised units of measure (Dempster et al., 2013). While bone volume does not measure actual bone mineral density, the degree of mineralisation within bone tissue will not vary widely unless there is a severe mineral deficincy. Bone strength is strongly related to the bone volume, size, structure and geometry of a given bone as well as the mineral density.

The reduced cortical and trabecular bone volume seen in the steers fed low ME diets in this project appeared to be independent of the reduced growth seen at the growth plate. While the nutritional and other factors that determine the rate of bone elongation are likely to be similar to those affecting bone formation it is possible for total bone mass and bone elongation to be affected differently (Wongdee et al., 2012). Reduced rib cortical thickness and increased cortical porosity (area of removed bone) seen in experiment 2 demonstrated bone resorption and reduced bone formation at a site unaffected by bone elongation. Trabecular bone volume was measured adjacent to the growth plate in steers from experiments 1 and 2 so may have also been affected by the activity seen at the growth plate. However, plasma concentrations of markers of bone formation (BAP) and resorption (PYD), appeard to correlate well with bone structural changes so it is likely that the effect was generalised rather than local to regions adjacent to growth plates.

Bone from multiple sites showed consistent, marked effect of reduced nutrition. This was seen as reduced trabecular bone volume and thinning of rib cortical bone. Trabecular bone volume, trabecular thickness and bone surface were all lower in steers fed low ME diets in every bone measured in experiments 1 and 2. Plasma BAP, a marker of bone formation, was markedly reduced in steers fed low ME diets in both experiments while markers of bone resorption were only mildly affected by diet and were much more variable. This suggests that changes in bone volume and cortical thickness are likely to be due to an overall reduction in bone formation rather than markedly increased bone mobilisation/resorption. Cortical bone constitutes 80% of bone volume and provides most of the strength and structural integrity of long bones. At this stage of an animal’s life, changes in cortical bone are independent of growth plate changes. Changes in cortical thickness were more prominent than increases in

porosity. This once again suggests reduced trabecular bone formation and reduced growth in cortical thickess is more important than bone mobilisation but there is likely to be a combination of bone loss and reduced bone gain contributing to the overall reduction in bone tissue at all sites.

Structural changes in bone volume were not measured in Exp3 as there were unlikley to be significant chages after only one week. However theew was a significant increase in CTX-1 and a decrease in osteocalcin and BAP during fasting. These plasma concentrations returned to previous levels for OC and CTX-1 but not BAP. Thus changes in bone turnover with increased bone formation and increased bone resorption are initiated rapidly with severe restrictions in ME and CP.

Severe nutrient restriction leads to the well-recognised reductions in bone volume, density and quality in adolescents with anorexia nervosa (El Ghoch et al., 2016). This loss of bone mass is associated with reduced IGF-1 and leptin as well as other hormonal changes including elevated cortisol and adiponectin and reduced thyroid hormone, insulin, oxytocin and oestrogen. All of these changes can contribute to reduced bone mass and slowing of bone elongation. Similar endocrine and bone changes have been reported in experimental studies on malnutrition in rats (Bourrin et al., 2000, Hamrick et al., 2008). Steers in this project demonstrated some of the hormonal changes associated with bone loss seen in humans and rats with nutrition-induced bone loss. Plasma IGF-1, insulin, total T3 and T4 were reduced in steers fed low ME diets in both experiments. Plasma leptin was higher in steers fed HME-HCP than in steers fed low ME diets in experiment 2. Malnutrition is recognised as a cause of bone loss in cattle (Dittmer et al., 2016) but it has not previously been documented with detailed histomorphometry in controlled pen trials. In Exp 2 all of the differences between groups were reversed at the end of the re-alimentation period indicating that bone volume was rapidly improving during re-alimentation on a high quality diet.

It is unclear what effect this reduced bone mass might have on production in growing beef steers. In northern Australia pasture P deficiency is a common problem and at its worst can lead to cattle incurring fractures due to severe loss in bone mass and mineralisation (Dixon et al., 2017). Rib cortical thickness has been widely used to estimate P status in cattle (Coates et al., 2016) but rib CBT is clealry affected by a low ME diet with adequate minerals. This will be important to consider when rib CBT is being used to investigate P deficincies. During the dry season P is not the limiting nutrient but the results of this project suggest that growing animals may have poor bone quality going into the wet season when P may be inadequate. It is possible that some of the worst manifestations of bone loss seen in P deficiency regions are a result of poor nutrition in the dry season combined with P deficiency in the wet season if animals are not supplemented. Whether or not there is P deficiency, the rapid gain in bone volume seen with re-alimentation in this project indicate that it is important to ensure that growing animals have adequate minerals at the start of the wet season when the skeleton will be attempting to quickly increase bone mass as well bone elongation and LW.

The effect of CP content or level of ME intake on subsequent compensatory LW growth in Bos indicus crossbred and Holstein genotype steers.

Hooper (1978) reporting on bone length in mice suggested that bone elongation might be the “pacemaker” for muscle growth (Hooper, 1978). The effect of bone elongation on muscle

length is mainly derived from a stretch stimulus, since they are attached, and this concept was further supported in studies involving chicken and pigs (Swatland, 1982, Holly et al., 1990). This concept led McLennan and Poppi (2011) to suggest a new approach to exploit compensatory growth in cattle by stimulating bone elongation during periods of under-nutrition (e.g. in the dry season in northern Australia) potentially resulting in a larger frame size for the muscle to rapidly realiment towards normal allometry when the quality and quantity of nutrients is increased (e.g. in the wet season in northern Australia). In the current project CP was chosen as the nutritional approach most likely to drive increased height gain under conditions of restricted dietary ME. Steers fed low ME diets in Exp 1 and 2 had minimal LWG over a 14 week period. In Exp 2, realimentation led to compensatory LWG, i.e. an increase in LWG at a rate that was greater than the control animals at the same time as well as being greater than the rapid LWG the control animals achieved during Phase 1. While small increases in HH gain were achieved by feeding additional CP to steers on a low ME diet in Exp 1, the increased HH seen in steers eating HCP-LME over those eating LCP-LME was not, able to create greater compensatory gain during the re-alimentation period.

The effect of bovine somatotrophic hormone (BST) on skeletal growth and bone gene expression in Bos indicus cross steers.

Treatment with bST was an attempt to exaggerate the growth response of the skeleton to nutritional interventions. The primary effect seen with bST treatment was to increase LW gain in steers on the HCP-HME diet. This LW response to bSTwas not seen in steers offered the HCP-LME or LCP-LME diets. There was no effect of bST on any animal-scale measures of skeletal elongation. There were significant increases in circulating IGF-1 induced by the bST injection and this is likely to have contributed to the increase in LW gain in steers fed the HCP-HME diet. This increased LWG was not, however, associated with similar bST effects on skeletal elongation as measured by HH change, hip width or bone length measured on X rays. The similarity in IGF-1 concentrations between the HCP-LME + bST and LCP-LME + bST treatment groups also suggests that the response of IGF-I concentration to bST administration was not affected by CP intake at low ME intakes. There are conflicting reports on the effect of bST on growing cattle. In one study, bST-treated gowing *bos taurus* beef heifers had earlier puberty with no change in LWG despite elevated circulating IGF-1 concentrations over a 210 day treatment period (Cooke et al., 2013). In contrast to this, treatment with bST led to faster HH gain and increased HH along with increased LW and LWG in growing Holstein dairy heifers (Moallem et al., 2004, Radcliff et al., 1997) and growing Holstein steers (Schlegel et al., 2006) when animls were fed high quality diets. There may, therefore be some genotype differences in the response to bST. But steers in this study had increased plasma IGF-1 concentrations and LWG without HH gain. It is possible that the very high quality of the HCP-HME lucerne diet was driving almost maximal growth in HH such that no further gains were possible.

Project objective: Identify the important growth factors and cellular signalling mediators within the epiphyseal growth plate (EGP).

Bone elongation during growth occurs at the growth plate by the process of endochondral ossification. Chondrocytes within a cartilage template form columns of cells that progress from resting to proliferation, hypertrophy and eventual cell death. The cartilage canals left empty by chondrocyte death are invaded by vessels and progressive mineralisation and creation of the primary spongiosa (bone). Invasion of bone cells and ongoing bone

remodelling results in trabecular bone formation in the metaphysis. Ongoing proliferation and matrix production is required to ensure ongoing elongation of the bone (Byers et al., 2000, Rauch, 2012).

This study sought to understand the mechanisms behind changes in dimensional characteristics of bone in response to levels of nutrition, in terms of both ME intake and CP intake. Histomorphometry of the growth plates revealed changes in bone length were in response to cellular dimensional changes at the growth plate.

Nutrition has its effect on specific supply of nutrients (type and quantity) at a tissue and cellular level and its effect is largely mass action on quantitative metabolism (anabolism and catabolism). However nutrient supply also mediates its effect by the nutrient-endocrine axis whereby total nutrient supply (usually measured as ME intake or metabolisable nutrient intake) or specific nutrient supply (eg amino acids, specific amino acids, glucose etc) elicit an endocrine response from a tissue or organ (eg liver) for systemic circulation or elicits a localised target tissue endocrine response. The Growth hormone-IGF-1 Axis is important in bone dimensional growth (Yakar and Isaksson, 2016).

Endochondral ossification at the growth plate is tightly regulated by a number of hormones, growth factors, extracellular matrix components and transcription factors. Each phase of the process is regulated by different factors. Hormones known to have effects on bone elongation include GH, IGF-1, thyroid hormones, sex steroids, leptin, insulin, PTH and PTHrP. Of these the GH/IGF-1 axis and thyroid hormones are possibly most important and most likely to respond to nutritional influences (Mackie et al., 2011, Gat-Yablonski et al., 2013, Murray and Clayton, 2013).

Hence the resultant endocrine changes were monitored in response to the three dietary treatments. Because of the known role of amino acid supply to the liver and the IGF-1 response to this, it was hypothesized that increasing CP supply in the diet would elicit a higher IGF-1 response. Hence an overlay of +/- bST should further elicit the responsiveness of skeletal elongatation rate and bone cellular parameters to changes in IGF-1 either through dietary or exogenous bST treatment.

Gene expression

The gene expression results correlated well with the animal measurement outcomes in growth and plasma concentrations of hormones and metabolites which were mosly affected by dietary ME. In all the experiments the agreement between expression results and other biological data suggests that these were biologically relevent changes in gene expression reflected processes that were important responses to nutrient restriction.

The hormonal and gene expression results from the 3 experiments were all consistent with Insulin-like growth factor -1 (IGF-1) being central to the growth plate response to nutrition and the control of skeletal elongation. Rapid growth in HH along with histological and X ray evidence of rapid bone elongatation occurred when IGF-1 levels were high. IGF-1 plays a leading role in controlling bone elongation and IGF-1 is very sensitive to nutritional status (Gat-Yablonski et al., 2013). Feed restriction imposed by controlling intake of balanced diets consistently decreases the concentration of circulating IGF-1 in cattle (Hornick et al. 2000). High protein diets maintained serum IGF-1 levels of caloric restricted humans (6-8% body weight loss in 14 days) to the same level prior to restriction (Musey et al. 1993). However the

effect of a high CP content diet during energy restriction has not previously been described for cattle. If increased CP in an energy restricted diet was going to be able to increase skeletal elongation it would likely need to act through the IGF-1/GH axis. However in both experiments plasma IGF-1 concentrations appeared to be predominantly responsive to ME as there were was no differences between the HCP- LME and LCP-LME diets but a significant increase in high ME groups. The ME in restricted diets was sufficiently low to prevent any LWG and thus may have been the limiting factor to IGF- production in the liver.

Nutrient restriction induced upregulation of genes in pathways associated with negative regulation of endochondral ossification. Some mediators of endochondral ossification have varied roles in the different cell types of the growth plate. For example fibroblastic growth factors (FGFs) will reduce chondrocyte proliferation and production of extracellular matrix while stimulating hypertrophic differentiation but decreasing the size of the hypertrophic chondrocytes (Baron et al., 2015). In the current study FGF2 and FGF-related receptors and processors were upregulated in in the cartilage of energy restricted steers. This pathway may be associated with the reduced proliferative zone and smaller hypertrophic chondrocytes seen in the growth plate of energy restricted steers.

Many of the differentially expressed genes were known to be involved in endochondral ossification or bone remodelling. There were however a number of genes that were differentially expressed across a number of different treatment comparisons suggesting they may be key regulators of changes in cell activity in the growth plate. MicroRNAs (miRs) negatively regulate post transcriptional gene expression by inhibiting protein translation. The formation of precursor miRs requires DiGeorge syndrome critical region gene 8 (DGCR8). In this project, marked increases in expression of the DGCR8 gene was a associated with low ME diests or fasting across the 3 experiments. The roles of miRNAs in bone biology have not been widely examined but there is emerging evidence that DGCR8 may be an important inhibitor of bone formation (Choi et al., 2017). This is consistent with upregulation of DGCR8 in animals demonstrating reduced bone volume and decreased plasma concentrations of biomarkers of bone formation.

Project objective: Identified possible strategies and options to increase dry season skeletal elongation so as to maximise compensatory growth in the wet season and potentially reduce or change dry season supplementation.

Developed recommendations for subsequent research to capture the benefits of this approach under commercial conditions.

Compensatory growth in weight appears to be modulated by the process where a previously restricted animal increases growth rate and energy intake until reaching its LW-for-HH. Using LW and HH data from steers in this project and from a range of other projects undertaken by this group, Silva (2017) developed a curve for normal LW:HH over time during growth. The model was then used to compare the effect of the difference between the liveweight predicted by the model to the observed liveweight during the compensatory growth phase of steers when the level of nutrition was increased after a period of restriction. The results of the study suggest that the potential for compensatory growth may be defined by the difference between the measured LW and the expected LW based on the animals LW-for-HH at the end of a period of nutritional restriction. The results for steers in Exp 1 in this project were consistent with this theory. LW:HH values increased over time in HCP-HME

groups steers while LW:HH values steers fed HCP-LME and LCP-LME diets remained lower and progressively diverged further from the normal curve during Phase 1. During realimentation previously restricted steers had rapid LWG and the LW:HH relationship increased over time to be closer to that of the HCP-HME steers by the end of Phase 2 (Figure 57) .

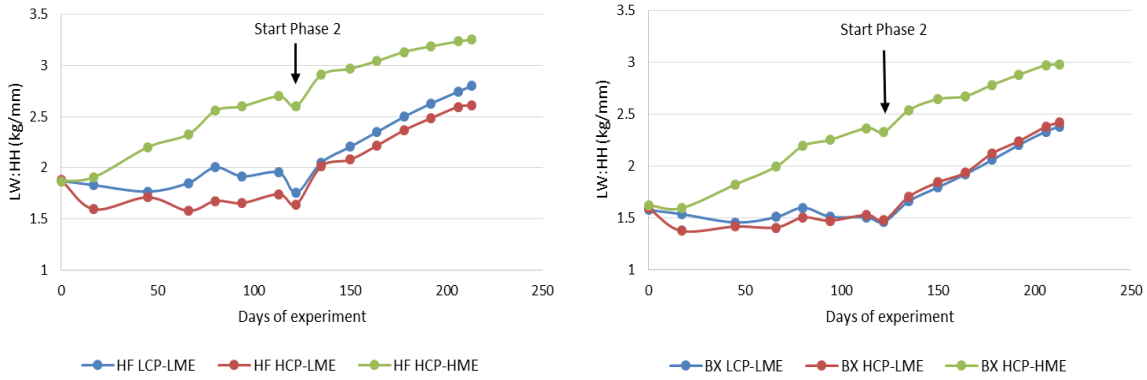


Figure 57. Liveweight:hip height (kg/mm) of *Bos taurus* (HF) and *Bos indicus* (BX) steers fed different nutritional treatments[1] (Phase 1) and undergoing re-alimentation (Phase2).

[1]See materials and methods for description of nutritional treatments [high crude protein intake and high MEI (HCP-HME), high crude protein intake and low MEI (HCP-LME) and low crude protein intake and low MEI (LCP-LME)]. Each point is the arithmetic mean of 5 steers with standard error of the mean.

The liveweight-to-hip height relationship is therefore central to compensatory growth and developing strategies to manipulate this relationship is still an attractive prospect. This project demonstrated that adding CP to a low ME diet was not able to drive meaningful increases in HH. This diet was, however, sufficiently low in ME to inhibit weight gain in growing steers. Under these circumstances, HH gain, growth plate zone thickness, individual body dimensions, bone length, plasma IGF-1 and changes in growth plate gene expression were all primarily responsive to the level of ME in the diet rather than CP. CP is able to increase IGF-1 and bone elongation when the diet contains a higher level of ME (Moallem et al., 2004). It is therefore possible that there are nutritional strategies where CP added to a diet slightly higher in ME may contribute to intermediate growth pathways where LW:HH still deviates from the normal relationship but with some gain in LW and relatively more gain in HH. Small amounts of ME and CP supplementation under these circumstances may produce cost effective increases in compensatory LWG if divergence from the LW:HH can still be generated alongside some LWG. The greater the deviation of the LW-for-HH, the greater the potential increase in LWG during the compensatory growth period. The absolute valuses for LW and HH are likely to be less important but this would need to be tested. This project demonstrated that skeletal growth and the endocrine and local regulators of bone elongation at the growth plate are sensitive to dietary ME. Investigating varying ME intakes to create the optimal relationship between LWG and HH gain to create maximum compensatory LWG is worth pursuing.

6 Conclusions

- In growing steers, skeletal elongation and thus gain in HH progresses in the face of low ME diets and minimal LWG. This leads to a divergence of the normal LW:HH relationship during growth and this difference between expected LW and actual LW contributes to the potential for compensatory LWG.
- When growing steers were fed diets resulting in minimal LWG, ME was the primary nutritional component that controlled growth, skeletal elongation, bone formation and hormonal and growth plate gene expression response to the diet. Under these low ME nutritional conditions it may not be possible to drive additional HH gain or divergence from the LW:HH relationship to achieve additional compensatory LWG.
- Skeletal elongation at the growth plate is very responsive to additional diet ME and can slow down and rapidly speed up in response to nutrition. Growth in HH was able to return to a faster velocity than unrestricted age-matched animals of the same size suggesting restricted animals have a reduced “bone age” following restriction that explains observed “catch-up” growth in HH.
- While HH gain more rapid than age-matched control animals is possible following restriction (Type B catch-up graowth), HH growth verolity greater than the growth curve of unrestricted animals of the same age does not appear to be possible in growing steers.
- Gene expresison of growth plate tissue and plasma hormone analysis indicates that IGF-1 is central to the cellular pathways responsibe for controlling changes in skeletal elongation in response to nutrition.
- The GH/IGF-1 axis is complex and difficult to manipulate when there are extremes of growth. Steer on low ME diets were not able to respond to exogenous bST treatment which is likely to be due to GH resistance. Steer on high ME and CP diets responded to exogenous bST with increased LWG but not skeletal elongation. This is likely to be due to the differences in maximal LWG and HH gain that are possible.
- Growing steers on low ME diets and minimal LWG have reduced bone formation leading to cortical thinning and reduced bone volume. This could contribute to poor bone quality when superimposed on P deficiency.

Recommendations for future research

- This project examined sleletal elongation under the relative extremes of rapid LWG and minimal LWG. Investigating varying ME and CP intakes to create the optimal relationship between LWG and HH gain may determine the most cost efficient supplemetation strategy for driving divergence of LW:HH relationship to create maximum compensatory LWG.
- As compensatory LWG is an important tool in northern Australian cattle production, selecting cattle with the ability to maximise skeletal growth during poor nutrition may improve compensatory LWG potential. Investigating the LWG responses of animals with different skeletal phenotypes may lead to markers of useful improvements in skeletal elongation rate.
- As considerable bone volume is due to low ME/CP independent of mineral deficiencies, it would be very useful to investigate the importance of this cause of

bone loss superimposed on P deficicny that is a common feature of northern Australian production systems.

- The gene expression results from the bone marrow adjacent to the growth plate suggested a considerable immune, inflammaory, host-defence and haemopoetic cell response. Acute or chronic under-nutrition may provide an excellent model of stress on livestock that is not associated with pain or procedures. Further hormonal (ie cortisol) and inflammatory mediator analysis, possibly using plasma proteomics, potentially using samples remaining from this project could further our understanding of the physiological response to poor nutrition. It could also help to differentiate biomarkers related to stress, pain or inlfammation.

7 Key messages

- These experiments were conducted under situations where ME intake was so low that LW gain was static. Under these circumstances even exogenous bST was not able to induce a higher plasma concentration of IGF-1 in response to CP addition to a low ME diet.
- This project has demonstrated that it is unlikely that we will be able to sufficiently un-couple skeletal height from LW to yield meaningful increases in compensatory growth under this level of energy restriction.
- The period of stasis in LW gain is also associated with considerable reductions in skeletal mass as well as slowing of skeletal elongation.
- Supplementation strategies aimed at providing slightly higher ME diets that are sufficient to be able to exploit benefits of additional protein might be more successful in generating cost effective improvements in growth rate.

8 Bibliography

ANGOOD, C., KHARA, T., DOLAN, C. & BERKLEY, J. A. 2016. Research Priorities on the Relationship between Wasting and Stunting. *PLoS One,* 11**,** e0153221.

BALCELLS, J., GUADA, J. A., PEIRÓ, J. M. & PARKER, D. S. 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications,* 575**,** 153-157.

BARON, J., SAVENDAHL, L., DE LUCA, F., DAUBER, A., PHILLIP, M., WIT, J. M. & NILSSON, O. 2015. Short and tall stature: a new paradigm emerges. *Nat Rev Endocrinol,* 11**,** 735-46.

BONJOUR, J. P. 2016. The dietary protein, IGF-I, skeletal health axis. *Horm Mol Biol Clin Investig,* 28**,** 39-53.

BOURRIN, S., AMMANN, P., BONJOUR, J. & RIZZOLI, R. 2000. Dietary Protein Restriction Lowers Plasma Insulin-Like Growth Factor I (IGF-I), Impairs Cortical Bone Formation, and Induces Osteoblastic Resistance to IGF-I in Adult Female Rats 1. *Endocrinology,* 141**,** 3149-3155.

BOWEN, M. K., POPPI, D. P., MCLENNAN, S. R. & DOOGAN, V. J. 2006. A comparison of the excretion rate of endogenous purine derivatives in the urine of Bos indicus and Bos taurus steers. *Australian Journal of Agricultural Research,* 57**,** 173-177.

BYERS, S., MOORE, A. J., BYARD, R. W. & FAZZALARI, N. L. 2000. Quantitative histomorphometric analysis of the human growth plate from birth to adolescence. *Bone,* 27**,** 495-501.

CAKE, M. A., GARDNER, G. E., HEGARTY, R. S., M.D.BOYCE & PETHICK, D. W. 2006,. Effect of nutritional restriction and sire genotype on forelimb bone growth and carcass composition in crossbred lambs *Australian Journal of Agricultural Research* 57**,** 605–616.

CHEN, X. B. & GOMES, M. 1995. *Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details*, International Feed Resources Unit.

CHOI, Y. J., JEONG, S., YOON, K. A., SUNG, H. J., CHO, H. S., KIM, D. W. & CHO, J. Y. 2017. Deficiency of DGCR8 increases bone formation through downregulation of miR-22 expression. *Bone,* 103**,** 287-294.

COATES, D. B., DIXON, R. M., MAYER, R. J. & MURRAY, R. M. 2016. Validation of single photon absorptiometry for on-farm measurement of density and mineral content of tail bone in cattle. *Animal Production Science,* 56**,** 2054-2059.

COOKE, R. F., BOHNERT, D. W., FRANCISCO, C. L., MARQUES, R. S., MUELLER, C. J. & KEISLER, D. H. 2013. Effects of bovine somatotropin administration on growth, physiological, and reproductive responses of replacement beef heifers. *J Anim Sci,* 91**,** 2894-901.

CSIRO 2007. *Nutrient requirements of domesticated ruminants*, CSIRO PUBLISHING.

DAR, H. Y., AZAM, Z., ANUPAM, R., MONDAL, R. K. & SRIVASTAVA, R. K. 2018. Osteoimmunology: The Nexus between bone and immune system. *Front Biosci (Landmark Ed),* 23**,** 464-492.

DE ONIS, M. & BRANCA, F. 2016. Childhood stunting: a global perspective. *Matern Child Nutr,* 12 Suppl 1**,** 12-26.

DEMPSTER, D. W., COMPSTON, J. E., DREZNER, M. K., GLORIEUX, F. H., KANIS, J. A., MALLUCHE, H., MEUNIER, P. J., OTT, S. M., RECKER, R. R. & PARFITT, A. M. 2013. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res,* 28**,** 2-17.

DITTMER, K. E., HITCHCOCK, B., MCDOUGALL, S. & HUNNAM, J. C. 2016. Pathophysiology of humeral fractures in a sample of dairy heifers. *N Z Vet J,* 64**,** 230-7.

DIXON, R. M., KIDD, L. J., COATES, D. B., ANDERSON, S. T., BENVENUTTI, M. A., FLETCHER, M. T. & MCNEILL, D. M. 2017. Utilising mobilisation of body reserves to improve the management of phosphorus nutrition of breeder cows. *Animal Production Science,* 57**,** 2280-2290.

DOUBE, M., KŁOSOWSKI, M. M., ARGANDA-CARRERAS, I., CORDELIÈRES, F. P., DOUGHERTY, R. P., JACKSON, J. S., SCHMID, B., HUTCHINSON, J. R. & SHEFELBINE, S. J. 2010. BoneJ: Free and extensible bone image analysis in ImageJ. *Bone,* 47**,** 1076-1079.

EL GHOCH, M., GATTI, D., CALUGI, S., VIAPIANA, O., BAZZANI, P. V. & DALLE GRAVE, R. 2016. The Association between Weight Gain/Restoration and Bone Mineral Density in Adolescents with Anorexia Nervosa: A Systematic Review. *Nutrients,* 8.

ELSASSER, T. H., RUMSEY, T. S. & HAMMOND, A. C. 1989. Influence of Diet on Basal and Growth Hormone-Stimulated Plasma Concentrations of IGF-I in Beef Cattle1. *Journal of Animal Science,* 67**,** 128-141.

FARNUM, C. E., LEE, A. O., O'HARA, K. & WILSMAN, N. J. 2003. Effect of short-term fasting on bone elongation rates: an analysis of catch-up growth in young male rats. *Pediatr Res,* 53**,** 33-41.

FINKIELSTAIN, G. P., LUI, J. C. & BARON, J. 2013. Catch-up growth: cellular and molecular mechanisms. *World Rev Nutr Diet,* 106**,** 100-4.

FORCINITO, P., ANDRADE, A. C., FINKIELSTAIN, G. P., BARON, J., NILSSON, O. & LUI, J. C. 2011. Growth-inhibiting conditions slow growth plate senescence. *J Endocrinol,* 208**,** 59-67.

GAT-YABLONSKI, G., BEN-ARI, T., SHTAIF, B., POTIEVSKY, O., MORAN, O., ESHET, R., MAOR, G., SEGEV, Y. & PHILLIP, M. 2004. Leptin reverses the inhibitory effect of caloric restriction on longitudinal growth. *Endocrinology,* 145**,** 343-50.

GAT-YABLONSKI, G., PANDO, R. & PHILLIP, M. 2013. Nutritional catch-up growth. *World Rev Nutr Diet,* 106**,** 83-9.

HAMRICK, M. W., DING, K. H., PONNALA, S., FERRARI, S. L. & ISALES, C. M. 2008. Caloric restriction decreases cortical bone mass but spares trabecular bone in the mouse skeleton: implications for the regulation of bone mass by body weight. *Journal of Bone and Mineral Research,* 23**,** 870-878.

HOLLY, R. G., BARNETT, J. G., ASHMORE, C. R., TAYLOR, R. G. & MOLE, P. A. 1990. Stretch-induced growth in chicken wing muscles: a new model of stretch hypertrophy. *The American Physiological Society***,** 62-71.

HOOPER, A. C. 1978. Muscles and bones of large and small mice compared at equal body weights. *J Anat,* 127**,** 117-23.

KEOGH, K., WATERS, S., KELLY, A. & KENNY, D. 2015. Feed restriction and subsequent realimentation in Holstein Friesian bulls: I. Effect on animal performance; muscle, fat, and linear body measurements; and slaughter characteristics. *Journal of animal science,* 93**,** 3578-3589.

KIDD, J. & MCLENNAN, S. 1998. Relationship between liveweight change of cattle in the dry season in northern Australia and growth rate in the following wet season. *Animal Production in Australia,* 22**,** 363-363.

LAWRENCE, T. L. J. & PEARCE, J. 1964. Some effects of wintering yearling beef cattle on different planes of nutrition: I. Live-weight gain, food consumption and body measurement changes during the winter period and the subsequent grazing period. *The Journal of Agricultural Science,* 63**,** 5-21.

MACKIE, E. J., TATARCZUCH, L. & MIRAMS, M. 2011. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. *J Endocrinol,* 211**,** 109-21.

MARINO, R., HEGDE, A., BARNES, K. M., SCHRIER, L., EMONS, J. A., NILSSON, O. & BARON, J. 2008. Catch-up growth after hypothyroidism is caused by delayed growth plate senescence. *Endocrinology,* 149**,** 1820-8.

MCLENNAN, S. 2014. Optimising growth paths of beef cattle in northern Australia for increased profit. *Meat & Livestock Australia Limited,* B.NBP.0391.

MCLENNAN, S. R. & POPPI, D. P. Recent advance in nutrition for improving liveweight gain. Update Conference, 3 & 4 August 2011 2011 Darwin. Australia: North Australia Beef Research Council, 94-101.

MILLWARD, D. J. 2017. Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev,* 30**,** 50-72.

MIOSSEC, P., KORN, T. & KUCHROO, V. K. 2009. Interleukin-17 and type 17 helper T cells. *N Engl J Med,* 361**,** 888-98.

MOALLEM, U., DAHL, G. E., DUFFEY, E. K., CAPUCO, A. V. & ERDMAN, R. A. 2004. Bovine somatotropin and rumen-undegradable protein effects on skeletal growth in prepubertal dairy heifers. *J Dairy Sci,* 87**,** 3881-8.

MODAN-MOSES, D., YAROSLAVSKY, A., KOCHAVI, B., TOLEDANO, A., SEGEV, S., BALAWI, F., MITRANY, E. & STEIN, D. 2012. Linear growth and final height characteristics in adolescent females with anorexia nervosa. *PLoS One,* 7**,** e45504.

MURRAY, P. G. & CLAYTON, P. E. 2013. Endocrine control of growth. *Am J Med Genet C Semin Med Genet,* 163c**,** 76-85.

OSBORNE, J. W. 2010. Improving your data transformations: Applying the Box-Cox transformation. *Practical Assessment, Research & Evaluation,* 15**,** 1-9.

PÁLSSON, H. & VERGÉS, J. B. 1952. Effects of the plane of nutrition on growth and the development of carcass quality in lambs Part I. The effects of High and Low planes of nutrition at different ages. *The Journal of Agricultural Science,* 42**,** 1-92.

PANDO, R., GAT-YABLONSKI, G. & PHILLIP, M. 2010. Nutrition and catch-up growth. *J Pediatr Gastroenterol Nutr,* 51 Suppl 3**,** S129-30.

QUIGLEY, S. P., D AND SCHATZ, T 2013. Validation and demonstration of a diagnostic tool for phosphorus status of beef cattle. Final report presented to MLA for project B.NBP.0537

RADCLIFF, R. P., VANDEHAAR, M. J., SKIDMORE, A. L., CHAPIN, L. T., RADKE, B. R., LLOYD, J. W., STANISIEWSKI, E. P. & TUCKER, H. A. 1997. Effects of diet and bovine somatotropin on heifer growth and mammary development. *J Dairy Sci,* 80**,** 1996-2003.

RAUCH, F. 2012. Chapter 16 - Pediatric Bone Histomorphometry. *In:* JÜPPNER, F. H. G. M. P. (ed.) *Pediatric Bone (Second Edition).* San Diego: Academic Press.

ROBINSON, L., ALDRIDGE, V., CLARK, E. M., MISRA, M. & MICALI, N. 2016. A systematic review and meta-analysis of the association between eating disorders and bone density. *Osteoporos Int,* 27**,** 1953-66.

ROSELLO-DIEZ, A. & JOYNER, A. L. 2015. Regulation of Long Bone Growth in Vertebrates; It Is Time to Catch Up. *Endocr Rev,* 36**,** 646-80.

SCHLEGEL, M. L., BERGEN, W. G., SCHROEDER, A. L., VANDEHAAR, M. J. & RUST, S. R. 2006. Use of bovine somatotropin for increased skeletal and lean tissue growth of Holstein steers. *J Anim Sci,* 84**,** 1176-87.

SCHORR, M. & MILLER, K. K. 2016. The endocrine manifestations of anorexia nervosa: mechanisms and management. *Nat Rev Endocrinol*.

SWATLAND, H. J. 1982. Histology of allometric growth in hindlimb muscles of pigs. *The Journal of Agricultural Science,* 98**,** 629-632.

SZKLARCZYK, D., FRANCESCHINI, A., WYDER, S., FORSLUND, K., HELLER, D., HUERTA-CEPAS, J., SIMONOVIC, M., ROTH, A., SANTOS, A., TSAFOU, K. P.,

KUHN, M., BORK, P., JENSEN, L. J. & VON MERING, C. 2015. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res,* 43**,** D447-52.

VAN SOEST, P. J., ROBERTSON, J. B. & LEWIS, B. A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science,* 74**,** 3583-3597.

VELAYUDHAN, B. T., GOVONI, K. E., HOAGLAND, T. A. & ZINN, S. A. 2007. Growth rate and changes of the somatotropic axis in beef cattle administered exogenous bovine somatotropin beginning at two hundred, two hundred fifty, and three hundred days of age. *J Anim Sci,* 85**,** 2866-72.

WANG, Z. & GOONEWARDENE, L. 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. *Canadian Journal of Animal Science,* 84**,** 1-12.

WONGDEE, K., KRISHNAMRA, N. & CHAROENPHANDHU, N. 2012. Endochondral bone growth, bone calcium accretion, and bone mineral density: how are they related? *J Physiol Sci,* 62**,** 299-307.

YAKAR, S. & ISAKSSON, O. 2016. Regulation of skeletal growth and mineral acquisition by the GH/IGF-1 axis: Lessons from mouse models. *Growth Horm IGF Res,* 28**,** 26-42.

YOUNG, M. J. 1988. *Bone and muscle growth in sheep: with particular reference to the effects of nutrition and intestinal parasitism.* Lincoln College, University of Canterbury.

9 Appendix

Appendix 1

Draft manuscript (not yet submitted for publication)

Differential abundance of mRNA of genes involved in the activity of muscle cells and muscle growth in *Bos taurus* and *Bos indicus* cattle undergoing growth restriction followed by re-alimentation.

Introduction

Within the context of northern Australian beef production systems compensatory growth has the potential to occur during the wet season when the quality of available feed (100 g CP and 10 MJ ME/kg DM) provides sufficient nourishment for growing cattle to rapidly gain LW after a period of growth restriction during the previous dry season when the quality of available feed was low (e.g. 35 g CP and 7 MJ ME/kg DM). Cattle undergoing compensatory growth display a more rapid rate of LWG than normally grown counterparts at an equivalent LW, and this is often achieved through greater feed efficiency. If cattle undergoing compensatory growth attain the same final LW as normally grown counterparts, then significant cost savings in dry season supplementation may be made by industry (McLennan, 2014). The rate of compensation and the duration of the compensation period are critical factors in determining the likely impact on annual LW production by growing cattle and these are likely to be affected by both the timing (pre- or post-weaning), severity and duration of the restriction period and the quality and quantity of feed available during the re-alimentation period.

Skeletal muscle accounts for approximately 50% of the body mass of young growing cattle and as such is one tissue which is likely to undergo compensatory growth in cattle. Skeletal muscle growth (and hence LWG) is ultimately driven by the accretion of proteins in myofibres within the skeletal muscle which is regulated at the molecular level by a large number of gene's and gene pathways in response to genotypic and environmental signals, including nutrition. The insulin-like growth factor and transforming growth factor-β (TGFβ) gene families are expressed locally in muscle and act in an autocrine/paracrine manner to regulate cellular proliferation, differentiation and protein synthesis in muscle by mediating the effects of nutrition, genotype, physiology, steroids and growth hormone on skeletal growth and as such were identified as candidate genes potentially involved in the regulation of compensatory growth of skeletal muscle in cattle. The objective of this activity within experiment 1 of this project was to determine the effect of nutrient restriction and re-alimentation on the abundance of mRNA's from candidate within the IGF and TGFβ families and other individual genes thought to have roles in skeletal muscle growth.

While the current experiment used a candidate gene approach to focus on differential expression of key genes known to be involved in muscle cell proliferation, differentiation and protein accretion others have used high throughput genome wide sequencing (Keogh et al. 2016) or micro-array technologies (Lehnert et al. 2006). Keogh et al. (2016) reported differential expression of genes involved in lipid metabolism and energy production in response to nutrient restriction and genes involved in cellular function and organisation during compensatory growth of Holstein bulls. Lehnert et al. (2006) reported down-regulation

of genes involved in muscle structure and muscle metabolism, particularly those associated with muscle glycolytic activity, in cattle under-fed for 114 days with the majority of these genes returning to normal levels after 84 days of re-alimentation. This whole-genome approach has clear advantages in identifying key pathways and gene's through co-expression network analysis which may not have been previously implicated in muscle growth and metabolism. Nevertheless, a candidate gene approach was undertaken in this experiment to determine the response and roles of the IGF and TGFβ family of genes in skeletal muscle to nutrient restriction and re-alimentation in growing cattle of diverse genotypes.

Materials and methods

Muscle biopsies were collected and gene expression studies conducted on the steers in experiment 1. The animals, diets, measurements conducted and the phenotypic results are described in detail elsewhere in this report.

Muscle biopsy

On days 104 (end of restriction), 140 and 207 (early and end of Phase 2) of the experiment biopsies were collected from the *semitendionosus* muscle of all steers. The biopsies were collected from the left (day 140) and right (days 104 and 207) sides of the steer, with the second biopsy on the right side collected ventral to the first biopsy. The biopsy site was clipped and cleaned with Chlorhexadine surgical scrub and infiltrated with Lignocaine (20 mg lignocaine hydrochloride/mL). A 30 mm incision was made through the skin and an 8 mm diameter muscle core was collected using a disposable biopsy punch (Kai Medical; Tokyo, Japan). The biopsy was chopped into smaller 8 x 4 mm cubes, placed in a cryovial and snap frozen in liquid N before storage at -80^{o}C. The incision site was closed with absorbable sutures and skin staples which were removed approximately 7 days after biopsy.

Laboratory

RNA was extracted from 100 mg of tissue in 1 mL of TRIzol (Invitrogen; Thermo Fisher Scientific, Scoresby, VIC, Australia) and purified through a RNeasy mini-column (QIAGEN, Chadstone, VIC, Australia) with an on-column DNAse treatment (QIAGEN) according to the manufacturer's instructions. The quality and quantity of RNA was confirmed by electrophoresis through a 1% agarose gel and reading absorbance at 260 and 280 nm on a Nanodrop (Thermo Fisher Scientific). RNA (1000 ng) was reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific) with OligoDT and random hexamer primers. cDNA was then diluted 1:20 in 10 mM Tris pH 8.0 for quantitative PCR (qPCR). Quantitative PCR were conducted with 1:20 cDNA, Power Sybr PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific) and 300 nM forward and reverse primers (Sigma Aldrich, Castle Hill, NSW, Australia) (Table 1) with relative abundance determined from a 6 point 2-fold serial dilution standard curve of a pooled 1:8 diluted cDNA representative of all samples including in the experiment. Quantitative PCR was performed on a 384 well real-time PCR machine (7900; Applied Biosystems) for 1 cycle at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min with data acquisition occurring at the 60°C step in each cycle. Amplification of specific PCR products of expected size was confirmed by melt-curve analysis and agarose gel electrophoresis.

Data normalisation and statistical analysis

The geometric average of the two most stably expressed genes across the entire data set (RPLPO & TGFBR2) was used to normalise qPCR data. Statistical analysis of the data was performed using the GLM procedure in SAS (SAS Institute); significant differences between least-square means were determined using the Tukey procedure. The overall statistical model included genotype, nutritional treatment, stage of experiment and all two-way interactions; interactions were removed from the model if not significant (P>0.05). Data were further analysed to examine genotype and nutritional treatment affects and their interaction within each stage of the experiment. Data are expressed as treatment least-square means and standard errors relative to one of the treatment means in the comparison (set at the arbitrary value of 1). Pearson correlation coefficients between candidate genes and intake and LWG of cattle were determined at the end of the restriction period and early and late stages of the re-alimentation period using the CORR procedure in SAS.

Table 1. Sequence of forward and reverse primers used in candidate gene assays.

Name	Code	Forward (5' – 3')	Reverse (5' – 3')
Insulin-like growth factor-1	IGF1_501_617	gcggagacacaggggcttttat	gcctcctcagatcacagctc
Insulin-like growth factor-2	IGF2_690_794	gagacttactgtgccacccc	ttggaagaacttgcccacgg
Insulin-like growth factor type-1 receptor	IGF1R_22_77	cctctgtgaacccggagtat	ggctcatggtgatcttcttcc
Insulin-like growth factor binding protein 3	IGFBP3_513_596	gcccttacctgctaccgtc	cagcctggttctctgtgctc
Insulin-like growth factor binding protein 5	IGFBP5_668_782	tgtacctgcccaactgtgac	ggcagcttcatcccgtactt
Myosin Heavy Chain 1	SMYH7(1)		
Myosin Heavy Chain 2a	MYH1 (2a)		
Myosin Heavy Chain 2x	MYH2 (2x)	actgaggaggaccgaccgcaaga	tgcttgcagtttatccacca
Transforming growth factor β-1	TGFβ-1_687_798	ctggagttgtgcggcagt	ccgttaatgtccacttgaagcg
Transforming growth factor β-2	TGFβ-2_1528_1640	gctcagacactcagcacagt	gtagagaatggtgagcggct
Transforming growth factor β-1 receptor	TGFβ1R_1556_1664	tctgggaagtagctcgacga	tgatggatcggaaggtacaagat
Transforming growth factor β-2 receptor	TGFβ2R_1528_1614	gacttcgggctctcactgc	catgtatctcgccgccgttccca
Myostatin	GDF8	tcttgctgtaaccttcccaga	tggtgtgtctgttaccttgacttc
Follistatin	FSTN_69_101	cgtgctgctggaagtgaag	aggaaagctgtagtcctggtctt
Kinesin Family Member 21A	KIF21A_303_388	agcctctggaaatgctgtca	acataacagggcctagatgtcc
RAC-alpha serine/threonine-protein kinase	Akt1544/633	aagaaggaggtcatcgtggc	gtcgtgtgtctggaaggagt
Large Ribosomal Protein	RPLPO	caaccctgaagtgcttgacat	gcaagtgggaaggtgtaatca
Ubiquitin carboxyl-terminal esterase 15	UHCL15_122	agcagagttacaaagacaacttgc	gcatctgatttttggcaacc
Elongation Factor 1A2	EEFIA2_59	ggaacttgatcgtatcattgcat	ggatcccctgtctccactcg

Results and discussion

Overall effects

The initial overall statistical model demonstrated significant stage x diet interactions for the abundance of IGF1, IGF1R and IGFBP3 mRNA in the *semitendinosus* muscle of steers undergoing different growth trajectories in response to dietary treatment (Table 2). The significant interaction affects are largely attributed to differences in mRNA abundance at the end of the restriction period.

The relative abundance of IGF1 mRNA was higher in the *semitendinosus* muscle of rapidly growing steers with the HCP-HME intake at the end of the restriction period (Figure 1). Insulin-like growth factor-1 stimulates proliferation, differentiation and protein synthesis and inhibits protein degradation of muscle cells. (Dayton and White, 2008). While the concentration of IGF-1 in the circulation of growing cattle is responsive to compensatory LWG after a restriction in energy intake (Hayden et al. 1993), feed deprivation (Wu et al. 2008), dietary protein supply (Liu et al. 1997), age and gender (Plouzek and Trenkle, 1991; Dahlanuddin et al. 2014), the administration of hormonal growth promotants (Pampusch et al., 2003) and bST (Lemal et al., 1989), with which the response of circulating IGF-1 is itself dependent on the nutritional status of the animal (Elsasser et al. 1989; Rausch et al. 2002), the effects on skeletal muscle IGF1 mRNA are less consistent. Skeletal muscle IGF1 mRNA has been shown to be responsive to sex-steroid based hormone growth promotants (Pampusch et al. 2003) and nutrient status but is not associated with bST administration (Ge et al. 2012) or residual feed intake (Kelly et al. 2013) in cattle. Treatment of bovine satellite cells with IGF1 in culture increased differentiation while administration of IGF1 to cattle nutrient restricted growing heifers was able to conserve skeletal muscle proteins (Hill et al. 1999) probably by decreasing protein degradation (Oddy and Owens, 1996). Previous studies have demonstrated direct effects of insulin, glucose and amino acids on IGF1 mRNA in various species and *in vitro*, so the diet effects reported in the current experiment were expected. Overall the literature suggests that IGF1 expression in skeletal muscle is associated with more efficient production of muscle in cattle, for example increased IGF1 mRNA in the skeletal muscle was associated with increased heavier carcasses and increased eye muscle area in cattle selected for high or low carcass weight (Keady et al. 2011). Interestingly, the abundance of IGF1 mRNA in the *semitendinosus* muscle of steers with the HCP-HME intake during the restriction period declined during the re-alimentation period in the current experiment, while the abundance of IGF1 mRNA in steers with the LCP-LME intake during the restriction period increased during the re-alimentation period. These differences during the re-alimentation period may reflect the relative maturity of the steers at this time when HCP-HME steers would be undergoing decreasing rates of myoblast activity and protein accretion as they approach maturity relative to LME steers.

In contrast to the mRNA profile of IGF1, IGF1R and IGFBP3 mRNA were higher in the *semitendinosus* muscle of the slow growing steers fed the LME treatments respectively at the end of the restriction period. There were no differences in the abundance of IGF1R and IGFBP3 mRNA in the *semitendinosus* muscle of steers that consumed different diets during the restriction period early or late in the re-alimentation period of the experiment, suggesting that these mRNA were responding to, or associated with, nutrition and/or growth rate at the time at which the sample was collected rather than any carry-over effects of the dietary/growth restriction *per se*. The lower IGF1R mRNA levels detected for HCP-HME

steers in the current experiment is contrary to expected findings where a higher receptor expression would be expected to facilitate the anabolic actions of IGF1, which has a greater affinity for this receptor than other IGF receptors. For example, lower IGF1R mRNA abundance in skeletal muscle was reported in calves with dwarfism (Blum et al. 2007) and in cattle with higher RFI (low feed conversion efficiency) (Kelly et al. 2013). However, our findings are in support of those of Busato et al. (2016) who reported upregulation of the IGF1R mRNA in steers that were offered a restricted intake (55% of *ad libitum* fed counterparts) across both *Bos indicus* (Nellore) and *Bos taurus* (Angus) steers.

The results suggest IGFBP3 mRNA is upregulated in the skeletal muscle of steers specifically in response to CP intake; this appears to be a novel finding not previously reported for cattle. Previous studies have demonstrated upregulation of IGFBP3 in the skeletal muscle of less muscled Angus steers compared with extreme muscled Belgian Blue steers and a negative association between IGFBP3 and muscle area (Keady et al. 2011) or no differences due to genotype (Nellore v Angus) or level of feeding (Busato et al. 2016) but evidence of a nutrient specific effect on IGFBP3 mRNA in the literature do not exist.

The TGFβ family of proteins are known to be potent inhibitors of proliferation and differentiation in muscle cells (Greene and Allen, 1991). The most commonly known of these is myostatin (GDF-8) which is a potent inhibitor of myogenesis; different mutations in the GDF-8 sequence result in a non-functional copy of the gene resulting in extreme musculature in cattle (e.g. Belgain Blue, Piedmontese breeds) (McPherron and Lee, 1997). Myostatin mRNA is upregulated in animals with a mutation suggesting a breakdown in the negatve feedback loop (Oldham et al. 2001). Despite the profound effects of mutations in GDF-8 on muscle growth in cattle, the mRNA levels of the gene itself under normal conditions are variable in response to nutrition, stage of development in post-natal muscles and exogenous treatments (e.g. steroids). No differential myostatin mRNA expression occurred in the current experiment where the gene was stably expressed regardless of dietary treatment, genotype and timing of sample collection. The mRNA abundance of TGFβ1 and TGFβ2 were higher in steers fed HCP-HME treatments, BX compared with HF and at the end of the re-alimentation period compared to the end of the restriction and early re-alimentation period. The TGFβ1 mRNA was upregulated in steers that received the HCP-HME treatment compared to steers that received the HCP-LME treatment but was stable between genotypes and across the experiment. The upregulation of TGFβ in the current experiment probably reflects the normal modulation of allometric muscle growth that is required by growing animals. An upregulation of TGFβ's as animals approach maturity might be expected to inhibit the continued proliferation and differentiation of satellite cells, leading to a slowing and eventual cessation of muscle cell activity, except in response to injury.

KIF21A is a transport carrier protein that is best recognised for its role in the development of ocular muscles; an inherited missense mutation in KIF21A results in congenital fibrosis of the extraocular muscles (CFEOM) which suppresses eye movement and positioning (Yamada et al. 2003). It is widely expressed in developing human and mice

central and peripheral nervous systems, brain ocular and skeletal muscles (Desai et al. 2012). Whilst there is limited published information regarding the expression of KIF21A in the skeletal muscle of cattle, unpublished work (Quigley et al. unpublished, demonstrated marked up-regulation of this mRNA in steers and cows undergoing re-alimentation under scenario's common to northern Australia cattle production systems). In the current

experiment KIF21A was upregulated in responses to the HCP-HME treatment and growth with this effect persisting during the early part of re-alimentation, with abundance also varying in response to genotype and stage of maturity across the experiment. While an explanation for the association of this gene in skeletal muscle growth in cattle remains unknown it is something that warrants further investigation.

Overall, IGF1R and IGFBP3 mRNA were more abundant at the end of the restriction period, when steers were fed different diets resulting in divergent growth (Table 2). In contrast, the abundance of IGFBP5, KIF21A and TGFβ2 mRNA were all more abundant in the *semitendinosus* muscle of steers at the end of the re-alimentation phase, as the steers were at a greater stage of maturity. Again these findings would suggest increased sensitivity of IGF1R and IGFBP3 to immediate nutrition and growth at the time of sampling, whereas IGFBP5, KIF21A and TGFβ2 were all elevated at a time when the total rate of muscle accretion across all steers was at its greatest (albeit probably declining in the physiologically more mature steers that consumed the HCP-HME diet during the restriction period).

Differences in mRNA abundance of candidate genes between HF and BX steers in the current study were limited and variable between the different periods of the experiment. Overall BX steers had a higher abundance of MYH1, MYH2, TGFβ1 and TGFβ2 and KIF21A mRNA. The increased relative abundance of MYH1 and MYH2 (expressed in Type IIA and IIB myofibres respectively) may indicate a genotypic difference in the metabolic and contractile properties of the *semitendinosus* muscle in the steers. Surprisingly reports of comparison of myofibre type composition between *Bos indicus* and *Bos taurus* cattle are rare. Those papers that compare myofibre type comparisons between these cattle species tend to report no differences in fibre type composition (at an equivalent age/stage of maturity) (Solomon et al. 1986; Seidaman, 1985). While the findings of the current study may therefore be in contrast to the literature it may be significant that the *Bos taurus* breed used in the current study was one not selected for meat production. In support of this potential (or perhaps greater) difference in myofibre type composition between *Bos taurus* beef v milk genotypes and *Bos taurus* and *Bos indicus* beef genotypes, Wegner et al. (2006) report that Belgian Blue (extreme muscling *Bos taurus* cattle) have an increased frequency and area of fast myofibre types compared with HF cattle. The differences in mRNA abundance between genotypes within each stage of this experiment (Tables 3, 4 and 5) were minor and unlikely to be of biological significance. The lack of difference between the genotypes used in this study demonstrates that diet had a greater influence over mRNA abundance of candidate genes than genotype.

End of restriction period

At this stage of the experiment any differences in mRNA abundance are likely due to either genotype or the treatment diet (or growth rate) of the steers at that point in time. At the end of the restriction period steers that were offered the HCP-HME treatment diet had significantly higher abundance of IGF1, IGFBP5, KIF21A, TGFβ1, TGFβ2 and TGFβ1R (Table 3) mRNA in the *semitendinosus* muscle compared to steers that consumed ME restricted diets. In contrast, the abundance of IGF1R and IGFBP3 mRNA were significantly lower in the *semitendinosus* muscle of HCP-HME steers compared to steers that consumed ME restricted diets.

Consistent with other findings in this experiment, the consumption of additional CP at an equivalent ME intake had little effect on the abundance of mRNA in skeletal muscle of steers at the end of the restriction period and during re-alimentation. The exception to this was the down-regulation of SMYH7 mRNA in steers that consumed the HCP-LME treatment diet compared with steers that consumed the LCP-LME diet, suggesting a possible CP effect on myofibre type independent of ME intake. The SMYH7 gene encodes Myosin Type I and as such may suggest the potential for a more slow-twitch/oxidative muscle. A more oxidative myofibre composition in the *semitendinosus* muscle has been reported previously in both cattle (Brandstetter et al. 1998) and sheep (Greenwood et al. 2006). However, a specific down-regulation of SMYH7 in response to a higher CP intake is somewhat in contrast to the mechanism proposed by Lehnert et al. (2006) and Greenwood et al. (2009) where the net effect of an increased oxidative myofibre area in the muscle was due to a decrease in the size of glycolytic fibres rather than any change in the size of oxidative myofibres. Regardless of the exact mechanism, in the current experiment that nutrient restriction resulted in a higher abundance of mRNA that encode proteins associated with more oxidative muscle metabolism.

Early restriction period

At this stage of the experiment any differences in mRNA abundance are likely due to either genotype, or growth rate of the steers at that point in time rather than specific diet effects. IGF2 mRNA was down-regulated in steers previously fed the HCP-HME treatment compared to steers fed the LCP-LME treatment during the restriction period of the experiment (Table 4). KIF21A and TGFβ1R mRNA were both more abundant in the skeletal muscle of steers previously fed the HCP-HME treatment compared to steers fed the LME treatments during the restriction period of the experiment. ATK1, IGFBP3, and TGFβ2 mRNA more abundant in the skeletal muscle of steers previously fed the HCP-HME treatment compared to steers fed the HCP-LME treatment during the restriction period of the experiment, with no difference between HCP-LME and LCP-LME steers for these genes at this time. Overall these results support accelerated muscle cellular activity in the skeletal muscle of steers previously fed the LME treatments. For example, downregulation of TGFβ1R in steers undergoing compensatory gain may suppress the potent inhibitory effects of the TGFβs allowing more accelerated muscle growth at this time.

Late restriction period

At this stage of the experiment any differences in mRNA abundance are likely due to either genotype, maturity or growth rate of the steers at that point in time rather than specific diet effects. At the end of the re-alimentation period (100 days in duration) there were no significant differences in mRNA abundance in the *semitendinosus* muscles of steers that were offered different treatment diets during the restriction period (Table 5). This indicates a normalisation of muscle and myofibre growth regulation when cattle have unlimited access to a high quality diet under which growth is unlikely to be restricted, and is beyond the time at which maximum responses in compensatory gain are likely to occur (approximately 8 weeks after re-alimentation commences). These findings are consistent with the phenotypic, metabolic and histomorphometry data collected during the experiment which all demonstrated little difference between steers undergoing divergent growth during a restriction period after approximately 100 days of re-alimentation.

Gene association with liveweight gain

Correlation coefficients between genes and between genes and ME intake and LWG are presented in Tables 6. At the end of the restriction period, IGF1 and IGFBP5 mRNA were positively associated with LWG (kg/day) in the steers, while IGF1R and IGFBP3 were negatively associated with LWG. KIF21A, TGFβ1, TGFβ2 and TGFβ1R were also positively associated with LWG at the end of the restriction period. Consistent with the end of the restriction period, IGF1R and TGFβ1R mRNA abundance in skeletal muscle of steers were negatively and positively associated respectively with LWG after 37 days of re-alimentation. There were no significant associations between gene abundance and LWG of steers at the end of the re-alimentation; this was largely expected as there were no significant treatment differences on LWG and gene expression at this time. These results do suggest a greater association of growth factor receptors in skeletal muscle of growing steers with LWG rather than the growth factors themselves, and demonstrate the importance of the receptor's in mediating the effects of the various growth factors.

Table 2. Relative mRNA abundance of candidate genes in the *semitendinosus* muscle of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers consuming diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) for 104 days (restriction period) followed by a re-alimentation period (Phase 2)[1,2]

	Nutrition (N) treatment in Stage 1					Genotype (G)				Stage (S) sample collected[3]					Interaction (P value)		
Gene[4]	**HCP-HME**	**HCP-LME**	**LCP-LME**	SEM	P	**BX**	**HF**	SEM	P	**1**	**2**	**3**	SEM	P	NxG	NxS	GxS
AKT1	***1.21b***	***0.93a***	***1.00a***	0.05	0.01	***1.00***	***0.78***	0.03	0.001	1.00	1.01	0.98	0.05	0.86	0.99	0.81	0.70
EEFIIA2	***1.24b***	***0.88a***	***1.00a***	0.06	0.001	1.00	1.06	0.05	0.39	***1.00a***	***1.29b***	***1.47b***	0.07	0.001	0.06	0.08	0.55
FSTN	0.66	0.69	1.00	0.13	0.32	1.00	0.82	0.13	0.32	1.00	0.84	1.17	0.17	0.4	0.81	0.91	0.53
GDF8	0.99	0.99	1.00	0.10	0.99	1.00	1.16	0.09	0.16	1.00	0.75	0.94	0.09	0.12	0.37	0.50	0.27
IGF1	1.17	1.00	1.00	0.06	0.63	1.00	1.09	0.05	0.53	1.00ab	0.95a	1.06b	0.06	0.45	0.06	**0.01**	0.86
IGF1R	***0.74a***	***0.84a***	***1.00b***	0.07	0.03	1.00	0.88	0.06	0.17	***1.00b***	***0.49a***	***0.55ab***	0.05	0.001	0.88	**0.00**	0.98
IGF2	***0.67a***	***0.78ab***	***1.00b***	0.06	0.002	1.00	1.13	0.07	0.20	1.00	1.11	1.12	0.09	0.54	0.72	0.74	0.31
IGFBP3	***0.77a***	***0.69a***	***1.00b***	0.06	0.002	***1.00***	***1.29***	0.07	0.01	***1.00b***	***0.74a***	***0.87ab***	0.07	0.03	0.16	**0.00**	0.40
IGFBP5	***1.40b***	***0.90a***	***1.00a***	0.08	0.001	***1.00***	***1.05***	0.06	0.001	***1.00a***	***1.10a***	***1.47b***	0.09	0.56	0.13	0.47	0.12
KIF21A	***1.41b***	***0.96a***	***1.00a***	0.08	0.004	***1.00***	***0.76***	0.05	0.002	***1.00a***	***0.95a***	***1.64b***	0.09	0.001	0.18	0.31	0.96
MYH1	***1.10b***	***0.83a***	***1.00ab***	0.07	0.04	***1.00***	***0.78***	0.06	0.01	1.00	0.79	0.80	0.06	0.04	0.80	0.84	0.30
MYH2	0.95	0.84	1.00	0.11	0.56	***1.00***	***0.74***	0.08	0.03	***1.00ab***	***0.62a***	***1.43b***	0.12	0.001	0.24	0.18	**0.03**
SMYH7	1.05	0.70	1.00	0.13	0.11	1.00	0.80	0.10	0.17	***1.00b***	***0.44a***	***1.35b***	0.13	0.001	0.31	0.13	0.49
TGFβ1	***1.17b***	***0.89a***	***1.00ab***	0.07	0.01	***1.00***	***0.77***	0.05	0.001	***1.00a***	***1.17a***	***1.73b***	0.09	0.001	0.16	0.67	0.90
TGFβ1R	***1.29b***	***0.95a***	***1.00ab***	0.05	0.001	1.00	0.91	0.04	0.11	1.00	0.94	1.05	0.05	0.25	0.19	0.12	0.81
TGFβ2	***1.44b***	***0.80a***	***1.00a***	0.09	0.001	***1.00***	***0.74***	0.06	0.002	***1.00a***	***1.06a***	***1.74b***	0.10	0.001	0.40	0.78	0.20
UCHL15	1.11	0.87	1.00	0.09	0.18	1.00	0.93	0.07	0.53	***1.00ab***	***0.69a***	***1.28b***	0.09	0.001	0.19	0.87	0.98

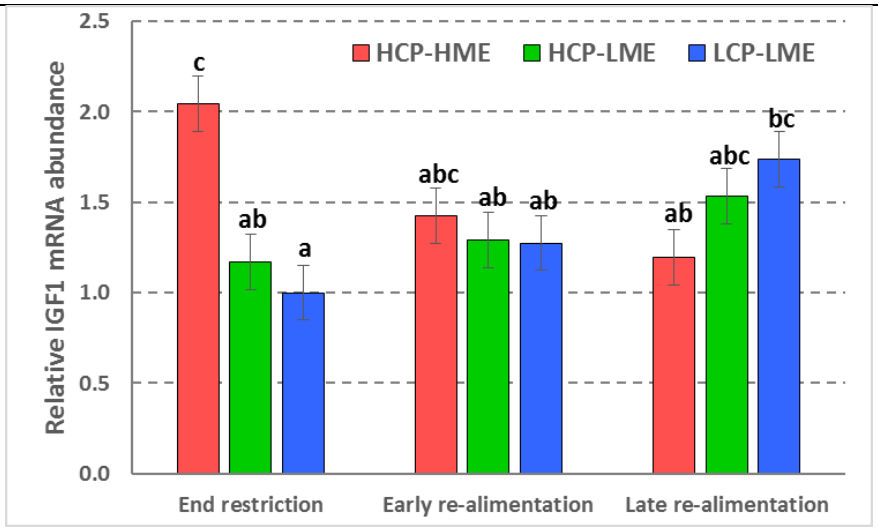
[1]values are least-square means with standard error of the mean (SEM), different alphabetical letters across a row within a main effect (N and S) indicate treatment means are significantly different (P<0.05)

[2]mRNA abundance is expressed relative to LCP-LME (N), BX (G) and stage 1 (S) for each of the main effects included in the statistical model

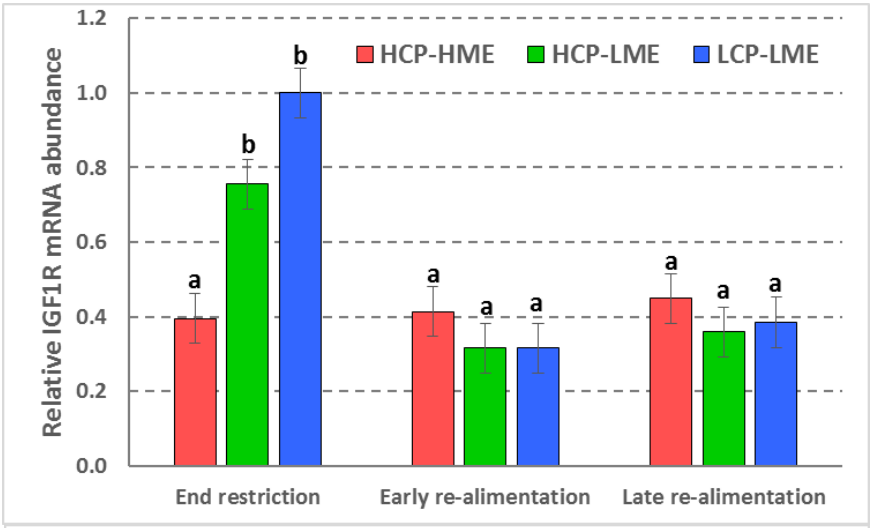
[3]steers were fed three different diets during the restriction phase (Phase 1) and a single diet during the compensation phase (Phase 2) with samples collected at the end of Phase 1 (stage 1) and 37 (stage 2) and 100 (stage 3) days after the commencement of Phase 2

[4]AKT1, RAC-alpha serine/threonine-protein kinase; EEF1A2, Elongation factor 1A2; FSTN, Follistatin; GDF-8, Myostatin; IGF1, Insulin-like growth factor-1; IGF1R, Insulin-like growth factor receptor 1; IGF2, Insulin-like growth factor 2; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP5, Insulin-like growth factor binding protein 5; KIF21A, Kinesin Family Member 21A; MYH1, Myosin heavy chain 1; MYH2, Myosin heavy chain 2; SMYH7, Slow myosin; TGFβ1, Transforming growth factor beta 1; TGFβ1R, Transforming growth factor beta 1 receptor; TGFβ2, Transforming growth factor beta 2; UCHL15, ubiquitin carboxyl-terminal esterase 15

a.



b.



c.

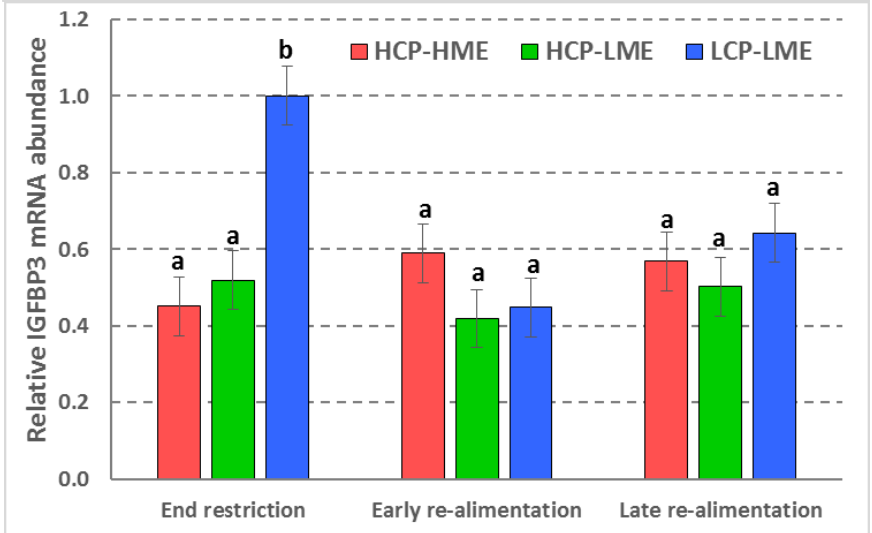


Figure 1. Relative abundance of insulin-like growth factor-1 (IGF1) (a.), IGF type 1 receptor (IGF1R) (b.) and IGF binding protein 3 (IGFBP3) (c.) in the *semitendinosus* muscle of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers consuming diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) (restriction period) followed by a re-alimentation period. Samples were analysed at the end of the restriction period and on days 37 (early) and 100 (late) of the re-alimentation period. mRNA abundance is expressed relative to steers fed the LCP-LME diet at the end of the restriction period.

Table 3. Relative mRNA abundance of candidate genes in the *semitendinosus* muscle of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers consuming diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) (end of restriction period) [1,2]

	Nutrition (N) treatment during Phase 1					Genotype (G)				Interaction (P value)
Gene[3]	**HCP-HME**	**HCP-LME**	**LCP-LME**	SEM	P	**BX**	**HF**	SEM	P	NxG
AKT1	1.16	1.01	1.00	0.08	0.280	1.00	1.19	0.07	0.06	0.74
EEFIIA2	***1.68b***	***0.85a***	***1.00a***	0.07	0.001	1.00	1.01	0.05	0.94	0.15
FSTN-101	0.76	0.75	1.00	0.14	0.390	1.00	0.93	0.14	0.72	0.22
GDF8	1.00	0.94	1.00	0.15	0.930	1.00	1.01	0.12	0.93	0.68
IGF1	***2.07b***	***1.17a***	***1.00a***	0.13	0.001	1.00	0.94	0.07	0.9	0.73
IGF1R	***0.39a***	***0.76ab***	***1.00b***	0.11	0.001	1.00	1.08	0.12	0.64	0.97
IGF2	0.66	0.79	1.00	0.10	0.535	***1.00***	***0.72***	0.08	0.023	0.4
IGFBP3	***0.45a***	***0.52a***	***1.00b***	0.06	0.000	***1.00***	***0.70***	0.06	0.002	***0.003***
IGFBP5(40)	***1.77b***	***0.85a***	***1.00a***	0.21	0.009	1.00	1.22	0.16	0.33	0.41
KIF21A	***1.79b***	***1.03a***	***1.00a***	0.16	0.002	1.00	1.35	0.12	0.052	0.74
MYH1	0.98	0.77	1.00	0.14	0.450	***1.00***	***1.44***	0.15	0.0495	0.89
MYH2	0.57	0.61	1.00	0.14	0.079	1.00	1.01	0.16	0.95	***0.045***
SMYH7	***0.60ab***	***0.50a***	***1.00b***	0.14	0.036	1.00	1.04	0.16	0.85	0.32
TGFβ1	***1.42b***	***0.80a***	***1.00a***	0.08	0.000	***1.00***	***1.31***	0.07	0.0031	*0.03*
TGFβ1R	***1.40b***	***1.09a***	***1.00a***	0.09	0.008	1.00	1.11	0.07	0.22	0.89
TGFβ2	***1.72b***	***0.73a***	***1.00a***	0.08	0.000	***1.00***	***1.20***	0.06	0.036	0.99
UCHL15	1.06	0.77	1.00	0.16	0.400	1.00	1.04	0.14	0.84	0.54

[1]values are least-square means with standard error of the mean (SEM), different alphabetical letters across a row within a main effect (N) indicate treatment means are significantly different (P<0.05)

[2]mRNA abundance is expressed relative to LCP-LME (N) and BX (G) for each of the main effects included in the statistical model

[3]AKT1, RAC-alpha serine/threonine-protein kinase; EEF1A2, Elongation factor 1A2; FSTN, Follistatin; GDF-8, Myostatin; IGF1, Insulin-like growth factor-1; IGF1R, Insulin-like growth factor receptor 1; IGF2, Insulin-like growth factor 2; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP5, Insulin-like growth factor binding protein 5; KIF21A, Kinesin Family Member 21A; MYH1, Myosin heavy chain 1; MYH2, Myosin heavy chain 2; SMYH7, Slow myosin; TGFβ1, Transforming growth factor beta 1; TGFβ1R, Transforming growth factor beta 1 receptor; TGFβ2, Transforming growth factor beta 2; UCHL15, ubiquitin carboxyl-terminal esterase 15

Table 4. Relative mRNA abundance of candidate genes in the *semitendinosus* muscle of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers after 37 days of re-alimentation after a period of growth restriction during which the steers consumed diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) [1,2]

	Nutrition (N) treatment during Phase 1					Genotype (G)				Interaction (P value)
Gene[3]	**HCP-HME**	**HCP-LME**	**LCP-LME**	SEM	P	**BX**	**HF**	SEM	P	NxG
AKT1	***1.26b***	***0.91a***	***1.00ab***	0.08	0.02	***1.00***	***1.29***	0.07	0.01	0.81
EEFIIA2	***1.23b***	***0.92a***	***1.00ab***	0.09	0.05	1.00	1.00	0.07	0.16	0.24
FSTN-101	0.73	0.79	1.00	0.23	0.69	1.00	1.24	0.26	0.52	0.54
GDF8	1.41	1.26	1.00	0.20	0.35	1.00	0.91	0.13	0.62	0.77
IGF1	1.12	1.01	1.00	0.10	0.85	1.00	0.88	0.07	0.83	0.46
IGF1R	1.31	1.00	1.00	0.11	0.08	1.00	1.16	0.09	0.19	0.86
IGF2	***0.58a***	***0.83ab***	***1.00b***	0.11	0.03	1.00	0.92	0.10	0.58	0.81
IGFBP3	***1.31b***	***0.93a***	***1.00ab***	0.10	0.03	1.00	0.86	0.07	0.18	0.52
IGFBP5(40)	***1.55b***	***0.98a***	***1.00a***	0.10	0.000	***1.00***	***0.75***	0.06	0.001	0.37
KIF21A	***1.84b***	***1.00a***	***1.00a***	0.15	0.000	*1.00*	*1.41*	0.11	0.02	0.79
MYH1	1.29	0.86	1.00	0.13	0.06	1.00	1.34	0.12	0.04	0.87
MYH2	1.05	0.98	1.00	0.13	0.93	1.00	1.11	0.12	0.49	***0.04***
SMYH7	1.52	0.84	1.00	0.23	0.11	1.00	1.31	0.19	0.27	0.09
TGFβ1	1.08	0.96	1.00	0.08	0.56	***1.00***	***1.34***	0.07	0.004	0.65
TGFβ1R	***1.51b***	***0.92a***	***1.00a***	0.08	0.000	1.00	1.04	0.06	0.59	0.77
TGFβ2	***1.47b***	***0.93a***	***1.00ab***	0.15	0.03	1.00	1.29	0.12	0.10	0.49
UCHL15	***1.43b***	***0.88a***	***1.00a***	0.11	0.01	1.00	1.13	0.09	0.34	0.53

[1]values are least-square means with standard error of the mean (SEM), different alphabetical letters across a row within a main effect (N) indicate treatment means are significantly different (P<0.05)

[2]mRNA abundance is expressed relative to LCP-LME (N) and BX (G) for each of the main effects included in the statistical model

[3]AKT1, RAC-alpha serine/threonine-protein kinase; EEF1A2, Elongation factor 1A2; FSTN, Follistatin; GDF-8, Myostatin; IGF1, Insulin-like growth factor-1; IGF1R, Insulin-like growth factor receptor 1; IGF2, Insulin-like growth factor 2; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP5, Insulin-like growth factor binding protein 5; KIF21A, Kinesin Family Member 21A; MYH1, Myosin heavy chain 1; MYH2, Myosin heavy chain 2; SMYH7, Slow myosin; TGFβ1, Transforming growth factor beta 1; TGFβ1R, Transforming growth factor beta 1 receptor; TGFβ2, Transforming growth factor beta 2; UCHL15, ubiquitin carboxyl-terminal esterase 15

Table 5. Relative mRNA abundance of candidate genes in the *semitendinosus* muscle of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers after 100 days of re-alimentation after a period of growth restriction during which the steers consumed diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) [1,2]

	Nutrition (N) treatment during Phase 1					Genotype (G)				Interaction (P value)
Gene[3]	**HCP-HME**	**HCP-LME**	**LCP-LME**	SEM	P	**BX**	**HF**	SEM	P	NxG
AKT1	1.19	0.87	1.00	0.10	0.09	***1.00***	***1.35***	0.10	0.02	0.50
EEFIIA2	0.99	0.88	1.00	0.10	0.65	1.00	0.98	0.09	0.88	0.50
FSTN-101	0.54	0.59	1.00	0.27	0.42	1.00	1.54	0.40	0.34	0.53
GDF8	0.73	0.87	1.00	0.16	0.50	1.00	0.67	0.13	0.08	0.19
IGF1	0.69	0.88	1.00	0.11	0.32	1.00	0.93	0.10	0.53	0.14
IGF1R	1.16	0.93	1.00	0.10	0.28	1.00	1.20	0.09	0.12	0.20
IGF2	0.76	0.72	1.00	0.13	0.27	1.00	1.04	0.123	0.83	0.82
IGFBP3	0.88	0.78	1.00	0.16	0.61	1.00	0.81	0.13	0.32	0.86
IGFBP5(40)	1.10	0.88	1.00	0.12	0.41	1.00	0.96	0.09	0.77	0.58
KIF21A	1.03	0.91	1.00	0.13	0.78	1.00	1.25	0.12	0.14	0.06
MYH1	1.10	0.89	1.00	0.11	0.42	1.00	1.08	0.10	0.54	0.73
MYH2	1.30	1.01	1.00	0.20	0.51	***1.00***	***1.83***	0.20	0.01	0.68
SMYH7	1.43	0.89	1.00	0.26	0.30	1.00	1.40	0.23	0.23	0.15
TGFβ1	1.10	0.88	1.00	0.14	0.51	1.00	1.24	0.12	0.17	0.49
TGFβ1R	1.24	1.04	1.00	0.11	0.42	1.00	1.12	0.08	0.32	0.17
TGFβ2	1.28	0.76	1.00	0.16	0.08	***1.00***	***1.48***	0.15	0.04	0.63
UCHL15	1.00	0.94	1.00	0.16	0.95	1.00	1.07	0.13	0.72	***0.04***

[1]values are least-square means with standard error of the mean (SEM), different alphabetical letters across a row within a main effect (N) indicate treatment means are significantly different (P<0.05)

[2]mRNA abundance is expressed relative to LCP-LME (N) and BX (G) for each of the main effects included in the statistical model

[3]AKT1, RAC-alpha serine/threonine-protein kinase; EEF1A2, Elongation factor 1A2; FSTN, Follistatin; GDF-8, Myostatin; IGF1, Insulin-like growth factor-1; IGF1R, Insulin-like growth factor receptor 1; IGF2, Insulin-like growth factor 2; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP5, Insulin-like growth factor binding protein 5; KIF21A, Kinesin Family Member 21A; MYH1, Myosin heavy chain 1; MYH2, Myosin heavy chain 2; SMYH7, Slow myosin; TGFβ1, Transforming growth factor beta 1; TGFβ1R, Transforming growth factor beta 1 receptor; TGFβ2, Transforming growth factor beta 2; UCHL15, ubiquitin carboxyl-terminal esterase 15

Table 6. Correlation coefficients for candidate gene abundance in the *semitendinosus* muscle, metabolisable energy intake (MEI; MJ ME/kg LW.day) and liveweight gain (LWG, kg/day) of *Bos indicus* crossbred (BX) and *Bos taurus* (HF) steers after a period of growth restriction during which the steers consumed diets that provided high crude protein (CP) and high metabolisable energy (ME) intake (HCP-HME), high CP and low ME intake (LCP-LME) and low CP and low ME intake (LCP-LME) and after 37 and 100 days of re-alimentation with high CP and high ME intake[1,2, 3]

	FSTN	GDF8	IGF1R	IGF1	IGF2	IGFBP3	IGFBP5	KIF21A	MYH1	MYH2	SMY7	TGFβ1	TGFβ1R	TGFβ2	MEI	ADG
							End of restriction period									
AKT1	**-0.59**	-0.33	0.16	**0.40**	-0.03	**-0.36**	0.32	-0.05	0.14	-0.32	-0.18	**0.41**	**0.66**	0.31	**0.36**	0.29
FSTN	1	0.24	-0.10	-0.15	0.09	**0.63**	-0.30	0.25	0.08	**0.81**	**0.42**	-0.04	**-0.51**	-0.01	-0.17	-0.19
GDF8		1	-0.16	-0.09	**-0.41**	-0.01	0.20	**0.40**	**0.35**	0.12	-0.09	-0.11	0.07	0.05	0.00	0.06
IGF1R			1	**-0.40**	**0.37**	0.32	-0.22	-0.35	0.29	0.05	0.19	-0.28	-0.19	-0.23	**-0.57**	**-0.61**
IGF1				1	-0.26	-0.29	0.36	0.19	-0.14	-0.28	-0.27	**0.45**	**0.63**	**0.69**	**0.74**	**0.70**
IGF2					1	**0.42**	**-0.39**	**-0.48**	-0.29	0.27	**0.37**	-0.27	-0.34	-0.32	-0.34	**-0.36**
IGFBP3						1	**-0.45**	-0.25	0.00	**0.63**	**0.51**	-0.17	**-0.52**	-0.20	**-0.48**	**-0.51**
IGFBP5							1	**0.38**	0.12	-0.36	-0.14	**0.46**	**0.50**	**0.38**	**0.53**	**0.59**
KIF21A								1	**0.55**	0.18	0.11	**0.51**	0.17	**0.54**	**0.54**	**0.61**
MYH1									1	0.13	0.15	0.25	0.04	**0.37**	0.09	0.11
MYH2										1	**0.61**	0.05	**-0.43**	-0.13	-0.25	-0.25
SMYH7											1	0.06	**-0.42**	-0.02	-0.17	-0.12
TGFβ1												1	**0.40**	**0.58**	**0.67**	**0.65**
TGFβ1R													1	**0.49**	**0.56**	**0.55**
TGFβ2														1	0.81	0.79
MEI															1	0.97
							Early re-alimentation period									
AKT1	-0.22	-0.16	**0.48**	0.07	-0.27	-0.22	0.11	0.18	0.12	0.08	**0.65**	0.18	**0.40**	0.06	0.18	-0.35
FSTN	1	**0.43**	-0.06	0.18	**0.47**	0.15	-0.07	0.29	**0.50**	**0.54**	-0.05	0.17	-0.05	**0.43**	0.07	0.13
GDF8		1	0.01	-0.01	0.13	**0.38**	**0.40**	**0.43**	**0.51**	0.20	0.04	-0.23	**0.39**	**0.36**	-0.28	-0.26
IGF1R			1	0.28	-0.34	0.02	**0.39**	0.25	0.21	0.00	**0.50**	0.22	**0.48**	0.29	-0.14	**-0.38**
IGF1				1	0.13	0.29	0.30	-0.15	-0.14	0.01	0.16	-0.03	0.07	-0.03	-0.25	0.10
IGF2					1	0.03	**-0.46**	-0.16	-0.17	0.32	-0.06	0.02	-0.32	-0.06	0.21	0.14
IGFBP3						1	**0.38**	**0.49**	0.34	0.12	-0.04	0.02	**0.52**	**0.48**	**-0.59**	-0.17
IGFBP5							1	0.33	0.25	-0.12	0.19	-0.17	**0.60**	0.30	**-0.67**	-0.26
KIF21A								1	**0.72**	0.21	0.23	**0.42**	**0.64**	**0.84**	-0.10	-0.33
MYH1									1	0.23	0.07	0.17	**0.58**	**0.65**	-0.19	-0.19
MYH2										1	**0.46**	0.30	0.06	0.20	0.00	-0.15
SMYH7											1	0.25	**0.40**	0.08	-0.03	-0.31

TGFβ1												1	0.00	0.34	**0.45**	-0.03
TGFβ1R													1	**0.50**	**-0.48**	**-0.45**
TGFβ2														1	-0.09	-0.18
MEI															1	0.23
							Late re-alimentation period									
AKT1	-0.34	**-0.59**	**0.47**	-0.26	0.00	**-0.42**	0.33	-0.06	0.13	-0.06	**0.37**	0.19	0.47	-0.02	0.02	-0.29
FSTN	1	0.08	-0.28	0.08	**0.61**	0.09	-0.34	0.03	0.00	**0.42**	-0.08	0.19	-0.28	**0.57**	0.17	0.23
GDF8		1	**-0.45**	0.04	-0.35	0.18	-0.12	**0.36**	0.37	-0.05	-0.03	-0.28	-0.12	-0.31	-0.08	0.23
IGF1R			1	-0.07	0.00	0.06	**0.41**	-0.13	0.02	-0.08	-0.12	-0.03	0.23	0.15	-0.01	-0.19
IGF1				1	0.15	**0.61**	0.16	**-0.37**	-0.32	-0.13	-0.29	0.35	-0.22	0.02	0.02	0.24
IGF2					1	0.13	-0.17	-0.27	-0.25	0.09	-0.07	0.24	-0.29	**0.40**	0.13	0.08
IGFBP3						1	0.33	-0.07	-0.06	0.12	-0.29	0.25	-0.17	0.35	-0.29	0.13
IGFBP5							1	0.04	0.18	-0.12	0.16	0.22	0.28	-0.03	-0.22	-0.02
KIF21A								1	**0.55**	**0.60**	**0.54**	0.19	-0.13	0.10	0.06	0.18
MYH1									1	0.16	0.22	-0.06	0.21	-0.09	-0.14	-0.02
MYH2										1	**0.50**	**0.60**	-0.24	**0.60**	-0.05	-0.02
SMYH7											1	**0.55**	-0.01	0.05	-0.14	-0.21
TGFβ1												1	-0.31	**0.44**	-0.23	-0.08
TGFβ1R													1	-0.11	0.08	-0.07
TGFβ2														1	-0.17	-0.10
MEI															1	0.73

[1]correlation coefficients in bold are significant (P<0.05)

[2]data was pooled for HF and BX steers at each stage of the experiment

[3]AKT1, RAC-alpha serine/threonine-protein kinase; FSTN, Follistatin; GDF-8, Myostatin; IGF1, Insulin-like growth factor-1; IGF1R, Insulin-like growth factor receptor 1; IGF2, Insulin-like growth factor 2; IGFBP3, Insulin-like growth factor binding protein 3; IGFBP5, Insulin-like growth factor binding protein 5; KIF21A, Kinesin Family Member 21A; MYH1, Myosin heavy chain 1; MYH2, Myosin heavy chain 2; SMYH7, Slow myosin; TGFβ1, Transforming growth factor beta 1; TGFβ1R, Transforming growth factor beta 1 receptor; TGFβ2, Transforming growth factor beta 2

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References

Blum JW, Elasser TH, Greger DL, Wittenberg S, de Vries F and Distl O. 2007. Insulin-like growth factor type-1 receptor down-regulation associated with dwarfism in Holstein calves. *Domestic Animal Endocrinology*. **33**, 245-268.

Brandstetter AM, Picard B and Geay Y. 1998. Muscle fibre characteristics in four muscles of growing male cattle II. Effect of castration and feeding level. *Livestock Production Science*. **53**, 25-36.

Busato KC, Gomes RA, Ladeira MM, Duarte MS, Freitas NC, Rodrigues AC, Chalfun-Junior A, Paiva LV and Chizzotti ML. 2016. Expression of genes related to regulation of muscle protein turnover in Angus and Nellore bulls. *Journal of Animal Science*. **94**, 1472-1481.

Dahlanuddin, Ningsih BS, Poppi DP, Anderson ST and Quigley SP. 2014. Long-term growth of male and female Bali cattle fed Sesbania grandiflora. *Animal Production Science*. **54**, 1615-1619.

Dayton WR. And White ME. 2008. Cellular and molecular regulation of muscle growth and development in meat animals. *Journal of Animal Science*. **86** (Sup.), E217-E255.

Desai J, Velo MPR, Yamada K, Overman LM and Engle EC. 2012. Spatiotemproal expression pattern of KIF21A during normal embryonic development and in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). *Gene Expression Patterns*. **12**, 180-188.

Ge X, Yu J and Jiang H. 2012. Growth hormone stimulates protein synthesis in bovine skeletal muscle cells without altering insulin-like growth factor-1 mRNA expression. *Journal of Animal Science*. **90**, 1126-1133.

Greene EA and Allen RE. 1991. Growth factor regulation of bovine satellite cell growth *in vitro*. *Journal of Animal Science*. **69**, 146-152.

Greenwood PL, Gardner GE and Hegarty RS. 2006. Lamb myofibre characteristics are influenced by sire estimated breeding values and pastoral nutritional system. *Australian Journal of Agricultural Research*. **57**, 627-639.

Greenwood PL, Tomkins NW, Hunter RA, Allignham PG, Harden S and Harper GS. 2009. Bovine myofibre characteristics are influenced by postweaning nutrition. *Journal of Animal Science*. **87**, X-X.

Hill RA, Hunter RA, Lindsay DB and Owens PC. 1999. Action of long(R^3)-insulin-like growth factor-1 on protein metabolism in beef heifers. *Domestic Animal Endocrinology*. **16**, 219-229.

Houseknecht KL, Boggs DL, Campion DR, Sartin JL, Kiser TE, Rampacek GB and Amos HE. 1988. Effect of dietary energy source and level on serum growth hormone, insulin-like growth factor-1, growth and body composition in beef heifers. *Journal of Animal Science* **66**, 2916-2923.

Keady SM, Kenny DA, Keane MG and Waters SM. 2011. Effect of sire breed and genetic merit for carcass weight on the transcriptional regulation of the somatotrophic axis in longissimus dorsi of crossbred steers. *Journal of Animal Science*. **89**, 4007-4016.

Kelly AK, Waters SM, McGee M, Browne JA, Magee DA and Kenny DA. 2013. Expression of key genes of the somatotrophic axis in *longissimus dorsi* muscle of beef heifers phenotypically divergent for residual feed intake. *Journal of Animal Science*. **91**, 159-167.

Keogh K, Kenny DA, Cormican P, McCabe MS, Kelly AK and Waters SM. 2016. Effect of dietary restriction and subsequent re-alimnetation on the transcriptional profile of bovine skeletal muscle. PLoS ONE. 11, e0149373. Doi: 10.1371/journal.pone.0149373

Lehnert SA, Byrne KA, Reverter A, Nattrass GS, Greenwood PL, Wang YH, Hudson NJ and Harper GS. 2006. Gene expression profiling of bovine skeletal muscle in response to and during recovery from chronic and severe undernutrition. *Journal of Animal Science*. **84**, 3239-3250.

Lemal D, Renaville V, Claes V, Ruelle L, Fabry J, Burny A, Underwood LE and Ketelslegers J-M. 1989. Effect of pituitary somatotropin injections on plasma insulin-like growth factor-1 and somatotropin profiles in growing heifers. *Journal of Animal Science* **67**, 2715-2723.

Liu SM, MacLeod NA, Luo QJ, Chen XB, Kyle DJ, Nicol P, Harbron CG and Orskov ER. 1997. The effects of acute and chronic protein depletion and accretion on plasma concentrations of insulin-like growth factor-1, fibronectin and total protein for ruminants nourished by intragastric infusion of nutrients. *British Journal of Nutrition* **78**, 411-426.

McGuire MA, Vicini JL, Bauman DE and Veenhuizen JJ. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *Journal of Animal Science*. **70**, 2901-2910.

McLennan SR. 2014. *Optimising growth paths of beef cattle in northern Australia for increased profitability*. (MLA Project, NBP.391). Meat & Livestock Australia Limited, North Sydney, Australia.

McPherron AC and Lee S-J. 1997. Double muscling in cattle due to mutations in the myostatin gene. *Proc. Natl. Acad. Sci., USA*. **94**, 12457-12461.

Oddy H and Owens PC. 1997. Insulin-like growth factor I inhibits degradation and improves retention of protein in hindlimb muscle of lambs. *The American Journal of Physiology* **271**, E973-82

Oldham JM, Martyn JAK, Sharma M, Jeanplong F, Kambadur R and Bass JJ. 2001. Molecular expression of myostatin and myoD is greater in double-muscled than

normal-muscled cattle foetuses. *American Journal of Physiology : Regulatory Integrative Comparative Physiology*. **280.** R1488-R1493

Pampusch MS, Johnson BJ, White ME, Hathaway MR, Dun JD, Waylan AT and Dayton WR. 2003. Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and non-implanted steers. *Journal of Animal Science*. **81**, 2733-2740

Plouzek CA and Trenkle A. 1991. Insulin-like growth factor-1 concentration in plasma of intact and castrated male and female cattle at four ages. *Domestic Animal Endocrinology*. **8**, 73-79.

Seideman SC. 1985. Muscle fibre studies comparing *Bos indicus* and *Bos taurus* cattle. *Roman L Hruska US Meat Animal Research Centre*. Paper **60**.

Solomon MB, West RL and Hentges JF. 1986. Growth and muscle development characteristics of purebred Angus and Brahman bulls. *Growth*. **50**, 51-67

Wegner J, Albrecht E, Fiedler I, Teuscher F, Papstein HJ and Ender K. 2000. Growth- and breed-related changes of muscle fibre characteristics in cattle. *Journal of Animal Science*. **78**, 1485-1496.

Wu MZ, Wang AH, Bernard GC, Hall JB, Beal WE, Akers RM, Boisclair YR and Jiang HL. 2008. Increased degradation of insulin-like growth factor-1 in serum from feed-deprived steers. *Domestic Animal Endocrinology* **35**, 343-351.

Yamada K, et al. 2003. Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscle type 1 (CFEOM1). *Nature Genetics*. **35**, 318-321.

Appendix 2

The following information is modified from a poster and one-page paper presented at the Northern Beef Research Update Conference (2016) and was the basis of an Honours thesis submitted by Mr David Innes in the School of Agriculture and Food Sciences, The University of Queensland (2015).

Introduction

The relationship between liveweight (LW) gain and skeletal growth in cattle is well established. Experiment 2 in this project established an *in vivo* model using bovine somatotropin (bST) and different dietary regimes to manipulate and better understand the mechanisms that regulate skeletal growth in cattle. It is recommended that bST is administered to dairy cattle every 14 days in the USA to increase milk production. Many of the responses of cattle to bST are attributable to an increase in the concentration of Insulin-like Growth Factor-1 (IGF-1) in the circulation most of which is hepatic in origin (rather than local). The current experiment examined the validity of those dosage recommendations for young *Bos indicus* steers fed high (*Medicago sativa)* and low (*Astrebla* spp.) quality roughage diets. The concentration of IGF-1 in plasma was measured as an indicator of the response of growing steers fed different diets to bST within a 14 day dosage period.

Materials and methods

Bos indicus steers (n=30; 194 ± 10 kg LW, mean ± S.D.) were allocated to one of three dietary treatments [high crude protein (CP)-high metabolisable energy (ME) intake (HCP-HME), high CP-low ME intake (HCP-LME), low CP-low ME intake (LCPLME; n=10/treatment]. Within each dietary treatment, steers were administered bST (500 mg Sometribove zinc suspension, Elanco Animal Health; n=5) or saline (n=5) subcutaneously every 14 days for 98 days. Within one 14-day dose interval, blood samples were collected from the jugular vein of all steers prior to feeding on day 0 (pre-dose) and 1, 2, 5, 8, 12 and 14 days after bST or saline administration and centrifuged at 2250 *g* at 4ºC for 10 min. The concentration of IGF-1 in plasma was measured using an immunoradiometric kit (Beckman Coulter). Data were $\log_{10}$ transformed prior to analysis using a Mixed model in SAS which included Hormone, Diet, Day and interactions.

Results and discussion

The concentration of IGF-1 in the plasma of steers was 59 ± 6 ng/mL prior to commencement of treatment diets and bST administration (day 0). During the 14 day dosage period, the concentration of IGF-1 in the plasma of steers administered bST was significantly higher 24 hours after bST administration compared to pre-dosage concentrations regardless of treatment diet. The mean plasma IGF-1 concentration was higher ($P<0.001$) in steers injected with bST than steers injected with saline (275 and 107 ng/mL), and in steers fed the HCP-HME treatment (372 ng/mL) compared to steers fed HCP-LME and LCP-LME (80 and 63 ng/mL) treatments over the 14 day period (Figure 1). Plasma IGF-1 concentration was higher on 1 day after bST injection and peaked 2 (LCP-LME) or 8 (HCP-HME, HCP-LME) days after bST injection, returning to pre-injection concentration 12 days post-injection, regardless of diet. The proportional increase in plasma IGF-1 in response to bST was similar between diets (1.8-fold above baseline; Figure 2), albeit the absolute increase in concentration was higher in steers fed the HCP-HME

treatment (387 ng/mL) compared to steers fed the HCP-LME and LCP-LME (82 and 51 ng/mL) treatments. Plasma IGF-1 concentration did not change within the 14 day period for steers injected with saline.

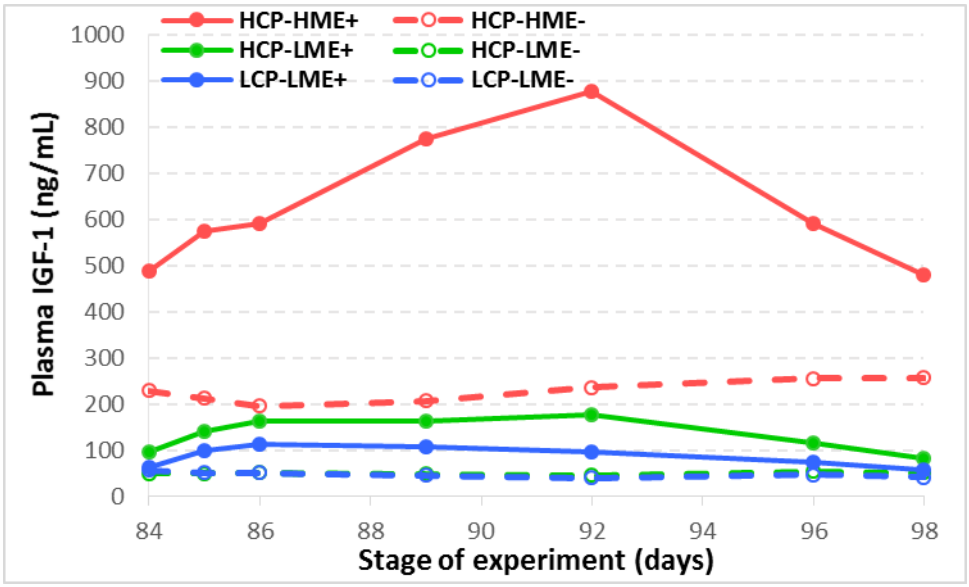
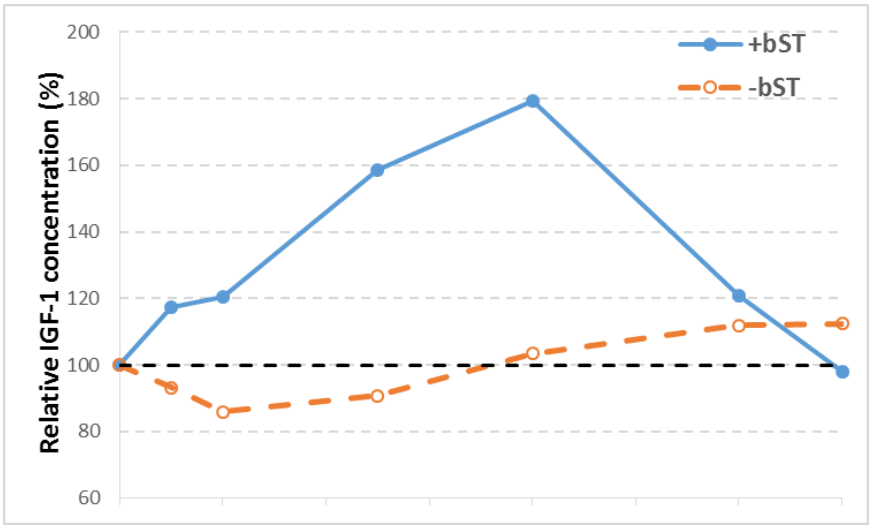


Figure 1. Concentration of insulin-like growth factor-1 (IGF-1) in the plasma of steers with high crude protein (CP) and high estimated metabolisable energy (ME) (HCP-HME), high CP and low ME (HCP-LME) and low CP and low ME (LCP-LME) intakes administered bovine somatotropin (+) or saline (-) on day 84 of the experiment.

a.



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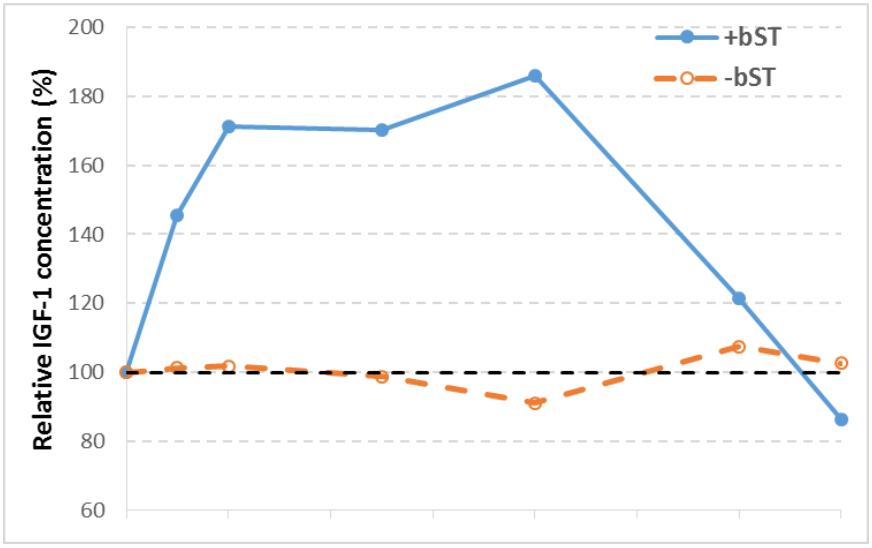

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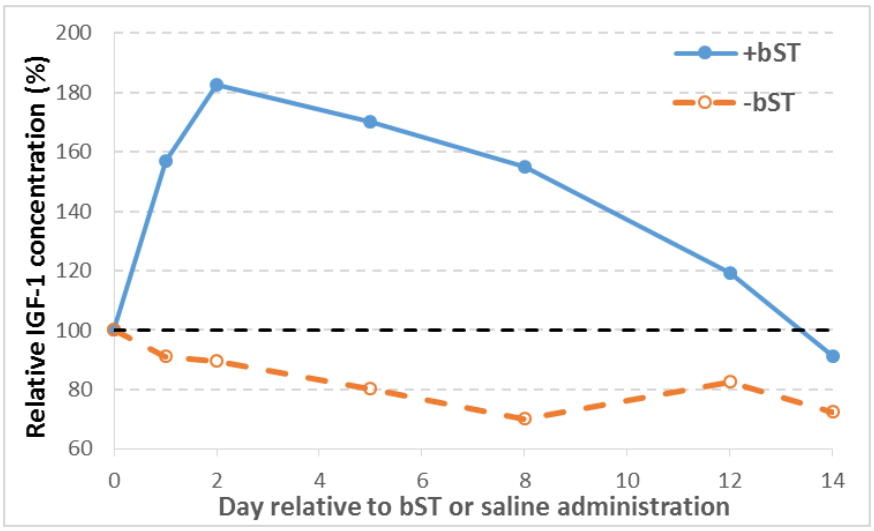

Figure 2. Concentration of insulin-like growth factor-1 (IGF-1) in the plasma of steers with high crude protein (CP) and high estimated metabolisable energy (ME) (a. HCP-HME), high CP and low ME (b. HCP-LME) and low CP and low ME (c. LCP-LME) intakes administered

bovine somatotropin (+bST) or saline (-bST) on day 84 of the experiment. Values are expressed relative to the concentration of IGF-1 in plasma prior to the administration of bST or saline on day 0 (day 84 of overall experiment).

Conclusions

A 14 day dose interval is appropriate to induce an increase in plasma IGF-1 of *Bos indicus* steers at the dose rates used in this experiment. The relative increase in plasma IGF-1 concentration to bST administration in steers was similar regardless of diet (1.8-fold above pre-dose) however a high energy intake with bST administration is required to achieve the maximum and a biologically significant increase in the concentration of IGF-1 in the plasma of growing steers (880 ng/mL).

References

Innes D. 2015. *A high protein and high energy diet and sustained release recombinant bovine somatotropin increase the concentration of insulin-like growth factor-1 in the plasma of growing Bos indicus steers*. Honours Thesis, The University of Queensland. pp 52.

Innes D, Anderson ST, Lisle A, Kidd LJ, Antari R, Poppi DP and Quigley SP. 2016. Exogenous bovine somatotropin increases the concentration of Insulin-like Growth Factor-1 in plasma of *Bos indicus* steers fed low and high quality diets. *Proceedings of the Northern Beef Research Update Conference, Rockhampton*, *August 2016.* 91