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NORTHERN BEEF

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The manipulation of nutrition in pregnancy to increase weaning rates

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Abstract

Varying maternal nutrition during critical periods of foetal development can alter or 'program' body mass and body composition in later life. Protein is the most deficient nutrient in the Australian rangelands and, critically, low protein diets during pregnancy in laboratory animals show significant deleterious effects on post natal development. This experiment aimed to determine if low dietary protein concentration in the first two trimesters of pregnancy alters calf growth and development.

Results show:

- ✓ There was no effect of nutrition during gestation on total calf body weight between 3 and 6 months of age.
- ✓ High nutrition in the second trimester resulted in heavier calf birth weights (2.2kg).
- ✓ Dystocia occurred in 16% of heifers in the trial. This was associated with increased calf birthweight such that a 2.2kg increase in calf birth weight increased risk of dystocia by 3 times.
- ✓ Pelvic area measure taken prior to joining was negatively correlated with dystocia. Low nutrition in the first trimester significantly increased milk intake in the calf.
- ✓ There was a significant effect of nutrition on placental and foetal development *in utero*.
- ✓ There was a significant effect of nutrition during gestation on IGF levels in the heifer
- ✓ There was a significant effect of nutrition in utero on ovarian development in the offspring

Executive Summary

Protein is the most deficient nutrient in the northern Australian rangelands during the pregnancy of the breeder cow. More certainty in the cost benefits from supplementation may be available if the nutritional responses during pregnancy are better understood. The objectives of the experiment were:

1. To examine the effect on weaning weight of targeted protein supplementation at critical times during pregnancy

2. To establish a recommended nutritional regimen for primiparous heifers and a detailed understanding of the effects on the calf, dystocia levels, and weight gains

3. To determine the relationship between dietary protein intake during pregnancy with muscle development, reproductive organ development and weaner liveweight gain

4. To establish the effects of diet during pregnancy on IGF levels in the weaner.

The aim being to increase understanding of breeder nutrition and how this affects weaner performance.

The major results of the experiment include;

I. Protein supplementation in first or second trimester did not affect weaning weight. Catch up growth occurred after 3 months of age.

II. Protein supplementation in the second trimester of pregnancy significantly increased birthweight by approximately 2.2kg (7%) (means ± se of 33.0 ± 0.68 and 30.8 ± 0.62 for high and low respectively) regardless of treatment in the first and third trimester. Dystocia occurred in 16% of heifers in the trial. This was associated with increased calf birthweight such that a 2.2kg increase in calf birth weight increased risk of dystocia by 3 times.

III. There was a significant effect of low protein in 1st trimester on milk production ($p < 0.001$) with higher intake for calves from dams with low protein in the first trimester.

IV. There was a significant effect of nutrition in the first two trimesters on liveweight of the cow until weaning

V. The incidence of dystocia was related to birthweight of the calf and pelvic area of the heifer pre-joining.

Altering protein supplementation in the first 6 months of pregnancy significantly alters birthweight in *Bos indicus* cross heifers. High birth weight is of commercial importance as it is the primary cause of dystocia in the heifer. As well as economic loss to the grazier of the calf and or heifer, there are welfare considerations which need to be addressed in the effective management of the heifer herd. High dietary protein concentration in the second trimester resulted in an 8.3% increase in calf birthweight ($P=0.01$). A 1kg increase in calf birthweight increased the risk of dystocia occurring by 1.44 fold ($P=0.003$).

The effect of nutrition during critical periods of gestation on birth weight and the relationship between birth weight and dystocia is evident.

This study suggests that maternal dietary protein concentration during the second trimester of gestation may be a critical factor in the resultant prevalence of dystocia in 3-year old *Bos indicus* cross heifers. Additionally the study supports the use of pelvic area measurement prior to joining as a useful management tool to aid in decreasing the incidence of of dystocia. The findings from this study may address some of the welfare and economic considerations associated with heifer dystocia in extensively managed beef heifers.

The effects on weaner growth in this study on *Bos indicus* cross heifers calving at three years of age was significantly different to a previous trial on Bos taurus heifers calving at two years of age. In that previous study, nutrition during the second trimester of pregnancy significantly affected weaner weights by 30kg, the growth trajectories being increasingly different over time until six months of age.

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1 Background

Sustainability in rural Australia depends upon supporting existing industries to become more efficient. Australia is the world's second largest beef exporter. Beef cattle production is the main income source for much of rural Australia. Protein is the main limiting nutrient in the Australian rangelands. Protein deficiency during pregnancy, in rats, pigs (Pond et al., 1991) and sheep, results in retarded growth of the offspring in postnatal life, despite adequate post natal nutrition (Rhind, 2001; Roberts et al., 2003).

Understanding the relationship between maternal dietary nutrients, the IGF axis, and foetal growth is at the forefront of international research (Osgerby et al., 2003; Ward et al., 2002; Roberts et al., 2001; Rhind et al., 2001). The species used for this research include laboratory animals, humans, pigs and sheep. The paucity of information on the bovine highlights the need for this study as there is a considerable difference in the development of the foeto-placental unit between these species and the bovine.

In humans, pigs, sheep and rats, the environment in utero influences post natal growth and development (Barker et al., 1993; Ball, 1996). It has been shown that the placenta influences the programming of the foetus, and thus influences postnatal growth (Robinson et al., 1995; Roberts et al., 2001). Nutrient supply or stress during gestation is thought to reprogram the relationship between glucose and insulin, and between growth hormone and the IGF system. There is a substantial body of evidence that suggests that nutritional and hormonal insults experienced in utero can alter gene expression in the foetus with persistent post-natal and life long effects (Godfrey, 1998). This "programming" of the foetal endocrine and cardiovascular systems before birth may also persist over more than one generation (Lumey, 1992; Barker, 1998; Cronje and Adams, 2001). Further, it has recently been shown that diet during pregnancy can effect a phenotypic variation via epigenetic modification of the genome (Waterland and Jirtle, 2003).

Current evidence suggests the IGF (insulin-like growth factor) system determines the relative compartmentalisation of nutrients between the mother, placenta and foetus (Osgerby et al., 2003). It has been shown that protein restriction in cattle during early pregnancy decreases maternal IGF-I, increases placental development (Perry et al., 1999) and significantly decreases IGF-I levels in calves at birth (Perry et al., 2002).

To date, most weaner growth trials have examined the effect of supplementation programs on calves post parturition. Other heifer supplementation trials have concentrated on growth rates from weaning to joining in relation to pregnancy rates (Fordyce et al., 1996) or to dystocia rates (Paterson et al.,1991). Although some interesting work on the effect of diet prior to first mating (Johnston and Obst, 1980) revealed effects on weaner growth this effect was not repeatable with naturally reared calves (Phillips et al., 1982). Decisions on what, when, and how much to spend on supplementation, in a cost recovery budget, are based currently on data from post calving supplementation. In trials at the Goondiwindi Pastoral Veterinary Centre precalving nutrition was studied. This research revealed some direct effects of nutrition at various stages of pregnancy on IGF levels in the heifer and calf at birth (IGF is a growth hormone which influences foetal growth and postnatal growth). (Perry et al 2002) It was shown that this protein supplementation directly affected placental growth (Perry et al 1999).

The unexpected result from this work was a significant increase in weaner weights (30kg at 6 months of age) from the treatment groups which were fed the high levels of protein during the second trimester of pregnancy (V.Perry, unpublished results). This is the first time that this has been shown but the numbers involved in the trial were small.

These experiments have shown that the planned experimental design achieved significant changes in maternal IGF levels, dry cotyledon weight (DCW), foetal trophectoderm development and liveweight gain of progeny. Although numbers of animals were small, significant relationships were found between dry cotyledon weight and protein level. The low protein diets in early pregnancy significantly increased trophectoderm development in the foetal portion of the trophectoderm and decreased IGF-I levels in the maternal circulation.

1.1 Background on specific areas of research in this study

Evidence suggests that under nutrition at different stages of pregnancy leads to phenotypes characterised by various metabolic and physiological abnormalities in rats, pigs, sheep and humans. It is thought that these abnormalities depend on the timing of the under nutrition during pregnancy and its different effects on organs and tissues according to their stage of development. Robinson et al., (1995) suggested that nutrition in the first half of pregnancy may be more important in this regard. It has also been found that nutrition prior to conception affects foetal development as measured by weaner weights (Johnston and Obst, 1980) and further that nutritional status of the mother at the time of conception effects the response to diet during pregnancy (DeBarro, 1992). For this reason diet prior to conception was controlled and all heifers were raised on a uniform nutritional regimen on one property.

Inadequate nutrition during pregnancy has been shown to cause:

- decreased growth rates in progeny in postnatal life despite adequate nutrition;
- decreased numbers of muscle fibres and size (CS) of muscle fibre in the foetus;
- decreased IGF-I levels in offspring and affects on placental function;
- increased age at puberty of offspring;
- decreased brain function in the offspring resulting in significantly longer period to first suckling;
- decreased size of testis and numbers of Sertoli cells in the testis, and significant changes in steroidogenesis in the testis;
- decreased ovarian mass with increased numbers of oocytes;
- increased abnormal gene activation in cattle oocytes and embryos (Rhind et al., 2001; Bielli et al., 2002); and
- Prolonged postpartum anoestrus (Yavas, 2000; Hess, 2005).

Insulin like growth factors (IGFs)

The effect of maternal dietary protein during the first and second trimester of pregnancy on the IGF system and the mechanism by which this system determines the distribution of nutrients between the mother, placenta and foetus is as follows.

Gene ablation studies have shown that the IGFs play a significant role in the control of both foetal and placental growth and maturation (DeChiara et al., 1990; Liu et al., 1993). IGFs may determine the partitioning of nutrients as more receptors are found in tissues which are critical for survival. Both IGF-I and IGF-II increase protein and glycogen synthesis in foetal tissues but IGF-I may have a more prominent role in modulating cell proliferation in specific endocrine and nutritional conditions in utero whereas IGF-II provides a general stimulus for cell growth in utero and may also be responsible for developmental and tissue-specific changes in cell differentiation (Fowden, 1995). Gerard et al. (1994), working with foetuses of double muscled cattle, found that the expression of IGF-II has an explicit role in myogenesis and that a blood-borne factor regulates muscle hypertrophy in foetal cattle.

Treatment of rodents with IGF-I overcomes maternal constraint on foetal growth and alters the usual close correlation between foetal and placental size (Gluckman et al., 1992). This suggests that maternal IGF-I directly or indirectly affects placental function (Gluckman, 1994). Increased levels of IGF binding protein 3 (IGFBP-3) in maternal blood during late pregnancy may enhance the delivery of IGF-I to the placenta. Kind et al. (1995) showed that restriction of placental growth reduces circulating levels of IGF-I in the sheep foetus and reduces the capacity for the production of IGF-I in a number of tissues suggesting that altered production of IGF-I in various tissues may contribute to retarded foetal growth. In pregnant ewes infused with IGF-I, the consequent suppression of maternal insulin led to greater glucose availability and an increase in placental amino acid nitrogen uptake (Harding et al., 1994).

Dwyer (1992) working with guinea pigs found that if the mother is on a restricted diet (40% reduction throughout gestation), levels of IGFs in the foetal blood decrease. Maternal protein deprivation for the last two thirds of gestation reduces concentrations of both IGF-I and II in foetal rat plasma (Pilistine et al., 1984). Long term restriction of nutrient supply is accompanied by sustained reduction in IGF-I and -II concentrations in the rat, whilst administration of IGF-I in late pregnancy promotes the growth of major foetal organs. It has been suggested that altered circulating IGFs in response to altered substrate supply may well contribute to changes in foetal growth (Owens, 1991; Schonecht et al., 1995). In previous trials in the bovine, maternal circulating IGF-I levels decreased significantly in the second trimester in the protein restricted heifers (Perry et al., 2002).

An important role for insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) has been established in sheep and small rodents. However, the impact of varied nutrition on placental growth and function and on foetal growth and the role of the IGF system in this have not been extensively explored in cattle. In a pilot study (Perry et al,. 2002) found that low protein diets in early pregnancy significantly decreased IGF-I levels in the maternal circulation. At birth the calves of heifers that had been on a low protein intake diet in the first trimester had significantly lower IGF levels that those calves born from mothers who had high protein intake in the first trimester.

Placental development

The effects of maternal dietary protein in the first and second trimester in the bovine on the development of the foetus and placenta:

Placental weight is highly correlated with birth weight and therefore survival and growth rate of the newborn mammal (Kelly, 1992). Placental growth in terms of mass and net cellular proliferation is maximal in the first half of gestation (Erhardt and Ball, 1995) suggesting that placental size and even function may be substantially determined at this early stage. The consequences of variable nutrition in the first half of pregnancy for placental growth have yielded conflicting reports. Everitt (1968) found that suboptimal nutrition in the first half of pregnancy in the sheep resulted in reduced foetal growth. In the guinea pig, Dwyer et al. (1992) found that a 40% reduction in maternal feed intake in the first trimester of pregnancy led to foetal and placental weight reductions of 32% and 38% respectively.

Sub-optimal nutrition from day 30 to 100 of gestation induces compensatory growth within the placenta in the ewe (McCrabb et al., 1991; Faichney and White, 1987). The effect of protein restriction in early pregnancy on the development of the bovine placenta is not known and protein deficiency often occurs during the dry season in the bulk of Australian breeding herds. Indeed there is a paucity of information on villus growth in the bovine placentome and a considerable difference exists between the placentome growth curves in the ovine and bovine (Baur, 1977;

Wooding pers comm.1996). The use of the sheep as a model for cattle is therefore questionable, though recent literature reviews can confuse the two (Wu et al., 2006).

Binucleate cells (BNC) play a central role in placental growth and remodelling. They form 15-20% of cells in the foetal trophoblast. They migrate through the foetal-matenal interface to fuse with the maternal epithelium. BNCs produce secretory proteins including placental lactogen (PL) and pregnancy associated glycoproteins (PAGS). These proteins are synthesised within the BNC during maturation and are stored in cytoplasmic granules. Most of these are subsequently transferred to the foetal-maternal syncytium during BNC migration. From there the proteins enter the maternal circulation by exocytosis of the granules at the basal plasmalemma of the foetal-maternal syncytium. Maternal PL is directly related to placental weight in the sheep but little is known about this relationship in the cow. PAGs have been detected in the foetal and maternal circulation in the cow. Little is known about how PAGs or PL enter the foetal circulation but may involve exocytosis of PL granules before migration of BNCs. Hence, BNCs produce tissue for placental growth and biochemical signals to the foetus and from foetus to mother for most of the gestation. It was considered, therefore, that measures of PL and PAG in the maternal circulation would be a valuable indicator of normal placental development.

It was observed in commercial practice that protein restriction in the first half of pregnancy if followed by a good season can enhance birthweight (Norman, 2006). Furthermore, a pilot experiment (Perry et al.,1999) showed that low protein concentration in the first half of pregnancy persistently enhanced trophectoderm growth and that trophectoderm volume was highly correlated with birthweight. From previous studies it has determined that:

Early protein restriction may stimulate trophectoderm growth and function. If the period of low protein in the first trimester is followed by a period of improved nutrition (as often occurs commercially) the increased development of the microvilli will allow the placenta to obtain more nutrition for the foetus from the mother. This may lead to increased birthweight and resultant increases in dystocia.

Post natal growth

Further, Perry et al., 2001 have shown that low protein in early gestation lowers IGF-I levels in the maternal circulation and in the calf at birth (there was no significant effect on birthweight perhaps due to the low numbers in the trial (n=5 per group). In a further study (Perry, unpublished data) where heifers were fed protein supplements in the paddock, despite no effect on birth weight, protein supplementation in the second trimester significantly increased growth rate in calves (30kg difference at 6 months between groups). The nil effect of protein restriction in the first trimester in this trial may be a result of supplementation starting after day 45 of pregnancy; this together with the variation in protein intake and low number (n=5 per group) may account for the nil effect on birthweight. Further, in a paddock trial dietary intake could only be assumed from weight gain

Low IGF levels are associated with lower rates of growth in other livestock species. Other workers have also shown in different species that protein supplementation leads to increased growth rates and muscle development in progeny (Dwyer et al., 1994).
Protein restriction during pregnancy has also been associated with increased levels of disease in progeny (Barker and Clarke, 1997), therefore;

Supplementing protein in early pregnancy may enhance postnatal growth rates in the calf and reduce susceptibility to disease.

In Summary

This is innovative work in an area at the forefront of international science into livestock health and reproduction. The placenta has a critical role in determining growth in utero (Robinson et al.,1995). It directly controls the nutrient supply to the foetus and synthesises hormones, which adapt maternal metabolism and blood flow in favour of foetal growth. Growth in utero determines post-natal viability, disease onset and growth (Barker and Clarke, 1997).

Critically, recent epigenetic studies indicate that the maternal diet can effect a phenotypic variation via epigenetic modification (methylation) of the genome (Waterland and Jirtle, 2003). These epigenetic modifications, which in the past were thought to be cleared and reset on passage through the germline, may be inherited to the next generation (Morgan et al. 1999).

2 Project Objectives

Objectives of the experiment were:

1. To examine the effect on weaning weight of targeted protein supplementation at critical times during pregnancy.

2. To establish a recommended nutritional regimen for primiparous heifers and a detailed understanding of the effects on the calf, dystocia levels, and weight gains.

3. To determine the relationship between dietary protein intake during pregnancy with muscle development, reproductive organ development and weaner liveweight gain.

4. To establish the effects of diet during pregnancy on IGF levels in the weaner.

3 Methodology

3.1 Summary

In summary, 71 live calves were born between 2nd and 22nd August 2005. Two were excluded before weaning (*n*=69 remaining), one due to mis-mothering and another due to death from unidentified causes. One other calf died due to unidentified causes prior to slaughter (*n*= 68 remaining). Bull calves were castrated at 5 months of age and all calves were weaned on 17th February 2006 aged approximately 6 months. On 15th September 2006 aged 13 months they were transferred to “Whylarah” at Surat for back-grounding prior to feedlot entry at “Aronui” Dalby, on 2nd February, 2007 where they were fed for 139 days prior to commercial slaughter on 21st June 2007.

The effects of diet during pregnancy on IGF levels in the dam and calf was established. This was an important addition to the current work being carried out on the importance of IGF levels in relation to feed conversion efficiency.

It had already been established that maternal diet significantly affects IGF levels during pregnancy and in the newborn (Perry *et al.,* 2002). Whether this effect carries through until weaning is yet to be established.

The analysis of gene expression in muscle and fat was undertaken at University of South Australia but does not form part of this report.

Much of the ongoing work was outside of the initial MLA funded project; however, as these calves represented a valuable resource additional funding was sourced from ARC, the WA Cattle Industry Compensation Fund and AACo to continue work during feedlotting and subsequent slaughter so that the effects of early nutrition on development and carcass composition could be assessed

3.2 Methodology

Facilities were established at Goondiwindi in Southern Queensland. These consisted of a two pen feedlot and 60 individual feeding stalls.

One hundred and twenty two-year-old heifers were obtained from Australian Agricultural Company's herd at Springsure, Queensland (24°12′S, 148°09′E) and relocated to Goondiwindi, Queensland (28°52′S, 150°33′E). Heifers were a *Bos taurus* x *Bos indicus* composite of either ½ Senepol x ¼ Brahman x ¼ Charolais (CBX, *n* = 85) or ½ Senepol x ¼ Brahman x ⅛ Charolais x ⅛ Red Angus (BeefX, *n* = 35). They were selected based on weight, temperament and functional reproductive tracts. All heifers were vaccinated on two occasions four weeks apart against viral and bacterial diseases (Websters Bovine Ephemeral Fever Vaccine (Living)®, Fort Dodge Australia Pty Limited, NSW; Pestigard Vaccine®, Pfizer Animal Health, NSW; Ultravac Botulinum Vaccine®, Pfizer Animal Health, West Ryde, NSW, Ultravac 7 in 1®, Pfizer Animal Health, West Ryde, NSW) and treated for cattle tick (Tixafly®, Coopers Animal Health, Baulkham Hills, NSW) prior to shipment from Springsure.

Heifers were run as one mob during a 45 day acclimatisation period before undergoing a 10-day progesterone-based estrous synchronization program. Intravaginal progesterone releasing devices were inserted on day -12 (progesterone 1.9g, EAZI-BREED™ CIDR® cattle device, Pfizer Animal Health, Australia) and heifers treated intramuscularly with 1mg oestradiol benzoate (Ciderol®, Genetics Australia, Bacchus Marsh, Australia). On day -5 heifers were treated with 25mg dinoprost trometamol intramuscularly (Lutalyse®, Pfizer Animal Health, Australia).

Intravaginal devices were removed on day -2 and heifers were artificially inseminated (AI) with frozen semen from one Senepol bull on day 0 and again on day 1 for any heifers still showing signs of estrus (*n* = 6). Time between the first heifer AI on day 0 until the last heifer AI on day 1 was 24 hours. Two heifers were removed due to temperament-related problems, resulting in 118 heifers at commencement of the study. Pregnancy was positively diagnosed in 77 of the 118 heifers on day 39 via trans-rectal palpation with the aid of a 5MHz linear rectal probe attached to a real time ultrasound scanner (model Aloka-500®, Aloka Inc., Tokyo, Japan). During the study, six spontaneous abortions occurred resulting in a total of 71 heifers that completed the study and gave birth. At the time of AI the heifer age range was 21.6 to 24.6 months.

Experimental design

The study was a two-by-two factorial design. Heifers were divided into treatment groups on the first day of AI (28th October 2004) according to stratification by weight within each composite genotype.

Half of each nutritional treatment group changed to the alternative nutritional treatment at the end of the first trimester of gestation (day 93) giving rise to four treatment groups: high/high (HH; n = 16), high/low (HL; n = 19), low/high (LH; n = 17), low/low (LL; n = 19). At the end of the

second trimester of gestation (day 180) all heifers were run as one treatment group until parturition. All heifers were released into the paddock after successful suckling was observed. One heifer did not continue to suckle the calf and so both the heifer and calf were removed from the trial at 2 weeks post partum. One calf died at 2 months of age leaving 69 heifers and calves (HH = 15; HL = 19; LH = 16; LL = 19).

Nutritional treatments

Diets were formulated using bambatsi hay (*Panicum coloratum*), barley straw (*Hordeum spp*.), cracked sorghum grain (*Sorghum spp*.), cotton seed meal (*Gossypium spp*.), ground limestone and a premix containing vitamins and minerals. The rations fed and their respective energy and protein contents are outlined in Table 1. Values for crude protein and metabolisable energy were measured by both biochemical and near infra red spectroscopy methods (CASCO Agritech, Toowoomba, Qld, Australia). Ground limestone was added to the rations to keep the calcium:phosphorous ratio near 2:1. The premix contained 17g calcium, 9g phosphorous, 2.91g magnesium, 5g sulphur, 27,200 IU vitamin A, 60mg vitamin E, 70mg iron, 150mg zinc, 100mg manganese, 55mg copper, 0.5mg selenium, 3.4mg cobalt and 4.2mg iodine per 100g. Allocation of both the roughage and concentrate components of the rations was calculated on an average intake per head per day basis. The roughage component was fed on a group basis whilst the concentrate portion was fed individually to heifers daily, whilst they were in stanchions.

Table 1. Details of high and low treatment group daily rations fed to dams during each trimester of gestation (as-fed basis)[1]

	Trimester 1 (Day 1 to 93)		Trimester 2 (Day 94 to 180)		Trimester 3 (Day 181 to term)
Item	High	Low	High	Low	All
Sorghum (*Sorghum spp;* kg)	0.65	1.56	1.00	1.20	1.13
Cotton seed meal (*Gossypium spp*; kg)	2.45	0.00	2.50	0.00	1.08
Bambatsi hay (*Panicum coloratum;* kg)	7.88	2.73	5.79	0.00	0.86
Barley straw (*Hordeum spp*; kg)	0.00	5.14	2.21	7.58	7.14
Lime (kg)	0.07	0.02	0.12	0.06	0.08
Premix (kg)	0.07	0.06	0.10	0.10	0.10
DMI (kg)	9.95	8.64	10.51	8.10	9.39
Energy (MJ ME)	76.3	62.5	82.4	63.1	71.4
Energy (% NRC[2])	243	199	229	176	149
CP (kg)	1.37	0.41	1.40	0.38	1.06
CP (% NRC[2])	250	75	228	63	135
DIP balance (g/d NRC[2])	206	-345	214	-464	-11

[1] Data are presented on as fed basis per heifer per d.
[2] Comparison of ration to National Research Council recommended Nutrient Requirements of Beef Cattle (1996) for pregnant Brangus replacement heifers joined at 23 mo with a mature weight of 475 kg and a calf birth weight of 32 kg.
Details of high and low treatment group daily rations fed to dams during each trimester of gestation (as-fed)

Heifer measurements before and during gestation

Heifer liveweight condition score and fat depth at the P8 site were measured at approximately monthly intervals throughout gestation. Blood samples were collected at each of these times.

Pelvic area was measured using a Rice pelvimeter (DLC Australia Pty Ltd, Hoppers Crossing, Vic., Australia) on Day -72 (PA-) and again on Day 117 (PA+).
Pelvic area was calculated from two measures of pelvic diameter. The first was in a horizontal plane as the widest distance between the wings of the ilium and the second in a vertical plane as the shortest distance between the pubis and sacrum. These two measures were then multiplied together to obtain a hypothetical measure of pelvic area.

Fetal calf measurements

Single fetuses (*n* = 71) were measured using trans-rectal ultrasonography on eight separate occasions at 4-weekly intervals between day 39 and 235 of gestation, resulting in a total of 568 measurement events. All images were recorded on VHS-video and digitalised. Measurements of the fetus were taken at the time of ultrasound and from digital images that were later reviewed by a single person. All fetal body measurements were in centimetres.

Crown-rump length (CRL) was measured from a lateral view of the fetus from the tip of the nose to the base of the tail. Biparietal diameter (BPD) was measured from a dorso-ventral view of the cranium. It was measured perpendicular to the sagital crest as the widest span between the most lateral parts of the parietal bone. Crown-nose length (CNL) was measured from a lateral view of the cranium as the distance from the planum nasale to the intercornual protuberance. Eye socket diameter was measured from a lateral view of the cranium in both the vertical (OV) and horizontal planes (OH). Abdominal diameter (AD) was measured from a transverse image at the point of insertion of the umbilical cord. Thoracic diameter (TD) was measured from a lateral image at the level of the twelfth rib. Umbilical cord diameter (UD) was measured at the point of insertion of the umbilical cord to the fetus. Limb cross-sectional diameter was measured on both the fore- and hind-limbs at the levels of the coronet and mid-cannon.
Measurements were across a transverse plane as determined by manual palpation or concurrent visualisation of the hoof. The fetal body measurements were grouped into head (BPD, CNL, OH, OV), trunk (CRL, AD, TD, UD) and limb measurements.

Table 2. Heifer numbers at each ultrasound date during gestation

Date	HH	HL	LL	LH	Total
06-Dec	19	19	19	19	77
04-Jan	17	19	19	19	75
31-Jan	17	19	19	18	73
28-Feb	17	19	19	17	72
27-Mar	17	19	19	17	72
25-Apr	17	19	19	17	72
23-May	16	19	19	17	71
19-Jun	16	19	19	17	71
25-Jul	**16**	**19**	**19**	**17**	**71**

Four heifers aborted after the initial pregnancy diagnosis. Fetal and placental tissues were examined but no causal disease was determined. IBR was suspected on examination of the viral pustular vaginitis but not confirmed.

Newborn calf measurements

At calving, heifers were monitored individually and assistance provided where necessary. Calves were collected for measurement within 15 minutes of birth, prior to standing or suckling.
The whole body and trunk measures that were recorded were calf birth weight (BW), CRL, and abdominal circumference (AC) at the level of the umbilical cord. Cranial measures recorded were BPD and CNL. Limb measures made on both fore- and hind-limbs were metacarpal and metatarsal length and diameter at their mid-points in both the mediolateral and craniocaudal planes, in addition to coronet diameter in both planes.

Limb diameters and BPD were measured using sliding calipers and other measures were obtained using a flexible tape-measure. Trunk and cranial measures were measured to the nearest 0.5cm, limb measures to the nearest 0.1cm and birth weight to the nearest 0.1kg. Gender was also recorded at birth.

Heifer measurements at calving

Heifers were observed 24 hours per day during the calving period. They were brought into calving pens on initiation of labour. Heifers were assisted to calve only after natural progression of calving had ceased and signs of distress were apparent to enable a true indication of natural dystocia levels.

After parturition the heifers were restrained for the collection of blood and colostrum prior to suckling. Time for expulsion of the placenta and level of calving difficulty were also recorded. The placenta was weighed and sampled and then individual cotyledons dissected out.

Heifer measurements post-calving

Milk production was assessed bimonthly using a weigh suck weigh method. The calves were taken off their mothers for 12 hrs overnight and then put with their mothers for 10-15 mins or until suckling had ceased from all calves.
This became the starting point after which the calves were again removed from the mothers for 6 hrs (midday) weighed immediately prior to suckling, allowed to suckle for 10-15mins or until suckling ceased by all calves and then weighed immediately after suckling.
This was repeated 6 hours later (evening). The calves were again removed for 12 hours (overnight) and weigh suck weighed in the morning to give a 24 hr milk intake (6+6+12 =24hr). Once the calves were above 150kg (5months) scales with an accuracy of only 1kg were used. Therefore, weights were only taken at 12 hr intervals (12+12=24 hr).

Progeny measurements post-calving

The calves and heifers were monitored every month for until weaning at 6 months of age. Blood samples were collected and liveweight recorded monthly. Fat depth and eye muscle area was determined using real-time ultrasound at 2, 4 and 6 months. The testis size was recorded in the male calves and follicle number at 5 months was recorded on the female calves. A GnRH challenge for the analysis of follicle stimulating hormone, luteinising hormone and testosterone levels was conducted prior to castration. The males were then castrated and testicular samples collected. Milk production was assessed bimonthly using a weigh suck weigh method. A particularly interesting aspect of previous work is that the heifers and calves maintained a weight difference between groups for 6 months after calving. In the pig and rat foetal nutrition in utero affects later IGF levels and physiological development (Kelley et al., 1995; Langley et al., 1994).

At five months of age, the male calves were weighed and castrated. Testes were removed through 10 cm long cranioventral incisions in the scrotal sac, 2-3 cm either side of the median groove.

The tunica was incised longitudinally to expose the testis and the testis was freed by separation of the tunica from the proper ligament at the tail of the epididymis. The deferent duct, testicular artery and pampiniform plexus were transected 5 cm ventral to the testis and the wound was left open to drain. Left and right testes were used for testis measures. Testis length was measured from the head to the tail of the epididymis using vernier callipers. Testis width was measured horizontally across the widest section of the body of the epididymis. Two width measures were taken at right angles to each other in craniocaudal and mediolateral dimensions. Weight of each testis was recorded. Paired testicular volume was calculated as the sum of volume of the right and the left testicle. Testicles were considered paraboloids, and the equation for calculating the volume was $v = \pi r^2 h$, where r = (width A+ width B)/4 and h= length (Chase, 1997).

Testicles were sectioned in a mid sagital plane and 10mm square sections of testicular parenchyma were taken from the central region of each testicle and fixed in 10 % buffered formalin. Testis samples were embedded in paraffin wax, sectioned (7-µm thick) and stained with hematoxylin-eosin. Average seminiferous tubule diameter was determined from 50 randomly selected tubular cross sections with a visibly round circumferences under 200X magnification using a Nikon Eclipse 80i microscope and a Nikon DS camera and control unit.

Hormone and growth factor assays

IGF-I, *-II and total IGFBP*

Concentrations of IGF-I, -II and total IGFBP were measured in heifer plasma at -14, 28, 82, 179 and 271 d of gestation and in calves at birth, 1 month, 3 months, 6 months, 12 months and 22 months, by RIA after separation of IGF and IGFBP by size-exclusion HPLC under acidic conditions, as detailed by Owens *et al.,* (1990).

Four fractions of eluate (fraction 1, containing IGFBP; fraction 2, inter-peak; fraction 3, containing IGF; and fraction 4, post-peak) were routinely collected for each acidified plasma sample, using collection times based on elution times of ^{125}I-labelled IGF-I and IGF-Immunoreactivity.

Recovery of ^{125}I-IGF-I was 93.3 ± 0.93 ng/mL for ten HPLC runs of heifer plasma and 90.1 ± 0.81 ng/mL for eleven HPLC runs containing plasma from calves. Samples were assayed in triplicate in each assay, and all samples from the same animal were extracted in a single HPLC run and run together in the same assay.

Plasma IGF-I concentrations were measured by analysis of neutralised HPLC fraction 3, in an RIA specific for IGF-I (Francis *et al.,*., 1989), using a rabbit polyclonal antibody to human IGF-I (GroPep, Australia). For heifers, the inter-assay CV for an HPLC eluate fraction 3 pool containing 101.2 ng/mL of IGF-I was 7.1 % (*n* = 15 assays). Covariance for extraction and assay of a pregnant bovine quality control (QC) plasma pool containing 59.8 ng/mL IGF-I was 14.5 %. For progeny, the inter-assay CV was 10.9% (*n* = 18 assays) and the intra-assay covariance for extraction and assay was 22.0% for a calf QC sample containing 31.4 ng/mL of IGF-I.

Total IGFBP concentrations were measured by analysis of neutralised fraction 1 in the same assay. Because IGFBP bind to and sequester ^{125}I-IGF-I in this assay, they can be measured due to their effect of reducing the amount of ^{125}I-IGF-I in the immunoprecipitated pellet, giving an apparent IGF concentration that reflects the total amount and binding affinity of IGFBP present in plasma.

Plasma IGF-II concentrations were measured by analysis of HPLC fraction 3 in a RIA specific for IGF-II (Carr *et al.,* 1995), using a rabbit polyclonal antibody against human IGF-II (GroPep, Australia). For heifers, the inter-assay CV for an HPLC eluate fraction 3 pool containing 339 ng/mL IGF-II was 5.5 (*n* = 9 assays). Covariance for extraction and assay of a pregnant bovine QC plasma pool containing 296.6 ng/mL IGF-II was 25.2 %. For progeny, the inter-assay CV was 9.7% (*n* = 9 assays) and the intra-assay covariance for extraction and assay of 21.6% for a calf QC sample containing 78.0 ng/mL of IGF-II.

Pregnancy hormones

Estrone sulphate concentrations at days 82, 124, 179, 236 and 271 of gestation and at calving were assayed in duplicate by RIA using kits obtained from Diagnostic Serum Laboratories (Webster, Texas, USA). The intra- assay CV was < 10%. The inter-assay CV for low quality control values was 4.35% (mean 0.51 ng/ml) and 10.48% (mean 14.38 ng/ml). The sensitivity of the assays was 0.05 ng/ml.

Progesterone concentrations at days 28, 82, 179, 271 of gestation and at calving were assayed in duplicate by RIA using kits obtained from Diagnostic Serum Laboratories (Webster, Texas, USA). The intra-assay CVs were 5.87% (mean 0.81ng/ml) and 6.30% (mean 8.98 ng/ml), and the inter-assay CVs were 4.30% and 2.30% respectively. The limit of detection was 0.04ng/ml.

Leptin concentrations at days 28, 82, 179, 271 and at calving were measured by RIA developed by Blache *et al.,*. (2000) and subsequently used for cattle samples by Kadokawa *et al.,* (2000). Intra-assay CV were estimated using 3 quality control standards containing 0.54 ng/ ml (4.2%), 0.86 ng/ml (5.2%) and 1.85 ng/ml (4.8%) at a zero binding of 30%. The limit of detection was 0.05 ng/ml.

A monoclonal based ELISA was used to measure bPAG in cow plasma at days 28, 82, 179 and 271 of gestation as described and validated by Green, 2005. 100µl samples were run in duplicate.

Bovine placental lactogen (bPL) concentrations at days 124, 179, 236 and 271 of gestation and at calving were determined by RIA of duplicate samples according to procedures established procedures by Wallace, 1993. The sensitivity of the assay was 0.05 ng/ml.

Progeny GnRH administration and reproductive hormone analysis

Blood collection was by jugular veni-puncture directly into 10ml lithium heparin and 10 ml plain serum vacutainers using 18 G 1 ½ inch at 5 months of age. The vacutainers were gently rotated by hand for 5-10 seconds, labelled and stored on ice for 1- 2 hours prior to centrifuging (5000rpm for10 mins) and plasma recovery. Samples were stored at -22C until analyzed. The calves were then given a dose of synthetic GnRH (0.1mg/ml) (“Fertagyl”- Intervet, Australia Pty Ltd) intramuscularly. The dose rate of 1.5µg/kg (range 1.3- 1.7) was calculated on the average calf weight of 180kg (range 160.3 - 212.2kg) to result in the administration of 270µg / head. In the same order that they were injected, the calves were re-bled 1 hour post GnRH injection.

Plasma concentrations of LH and FSH were determined by double- antibody radioimmunoassay validated by Rawlings and Evans (1995) in pre and post GnRH samples. Plasma concentrations of LH are expressed in terms of NIDDK-bLH-B4 (NHPP National Hormone & Pituitary Program [NHPP], Torrance, CA, USA; 0.125 to 8 ng/ml), using rabbit bovine- LH antiserum (Rawlings *et al.,* 1984) and USDA- bLH-I-1 (NHPP) for tracer labelling.

The sensitivity of the LH assay was 0.06 ng/ml. For the pre-GnRH samples, the intra and inter assay coefficients of variation were 7.2% (assay 1) and 7.1% (assay 2) and 6.6% (mean 0.34 ng/ml) respectively. For the post-GnRH samples, the intra and inter assay CV were 7.1% (assay 1) and 7.5% (assay 2) and 7.5% (mean 0.83 ng/ml) respectively. Plasma concentrations of FSH are expressed in terms of USDA-bFSH-I-1 (NHPP; 0.125 to 16 ng/ml), using rabbit ovine- FSH antiserum (NIDDK- anti-oFSH-1; NHPP) and AFP- 5332B (NHPP) for tracer. The sensitivity of the FSH assay was 0.1ng/ml. For the pre-GnRH samples, the intra-assay CV was 5.3% (mean 0.9ng/ml) and for the post-GnRH samples, the intra-assay CV was 3.7% (mean 3.26 ng/ml).

Testosterone concentrations were determined using a total testosterone RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA) but testosterone standards were prepared with purified hormone (Sigma Chemical Co., St. Louis, MO, USA) in charcoal stripped castrate bovine serum as described by Brito *et al.,*. (2007). The sensitivity of the assay was 0.04ng/ml. For the pre-GnRH samples, the inter-assay CV was 7.1% (mean 1.09 ng/ml). The intra-assay CV was 7.6% (mean 1.08 ng/ml) for assay 1 and 6.2% (mean 1.10 ng/ml) for assay 2. For the post-GnRH samples, the inter-assay CV was 4.5% (mean 4.3 ng/ml). The intra-assay CV was 4.7% (mean 4.22 ng/ml) for assay 1 and 2.2% (mean 4.43 ng/ml) for assay 2.

3.2.1 Statistical Analysis

The data has been analysed in distinct sections according to the order in the results section. See Appendix 1 for details of statistical analysis.

4 Results and Discussion

4.1 Summary

A summary of experimental results to date:

- ✓ Protein supplementation in first or second trimester did not affect weaning weight. No significant effect of heifer protein intake on calf weaning weight.
  - o Range 175.8 - 240.8kg
  - o Mean ± SE of 209.6 ± 1.9kg

- ✓ Protein supplementation in the second trimester of pregnancy significantly increased birthweight by approximately 2.2 kgs (means ± se of 33.0 ± 0.68 and 30.8 ± 0.62 for high and low respectively) regardless of treatment in the first and third trimester

- ✓ Dystocia occurred in 16% of the heifers. Two of these calves were dummies and required assistance with suckling for the first 48 hours.

- ✓ Protein supplementation in second trimester significantly affected live weight until two months of age.

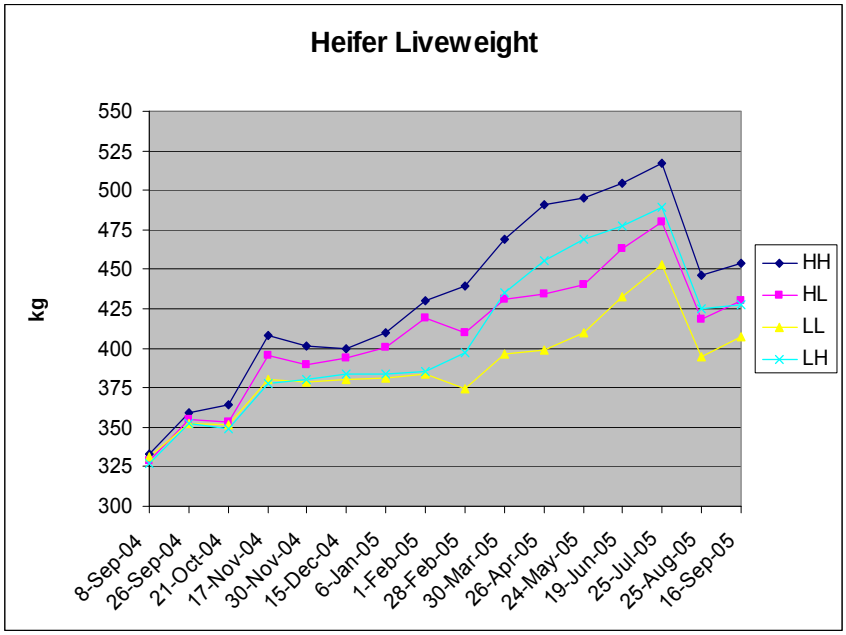
- ✓ Protein supplementation in either the first or second trimester significantly increased foetal growth in utero.
- ✓ Smaller pelvic area at selection, but not during gestation, was associated with an increased risk of dystocia
- ✓ There was a significant effect of protein in 1st trimester on milk production (P <0.001) with higher intake for calves from dams with low nutrition in the first trimester.
- ✓ There was an interaction (P=0.041) between first and second trimester nutrition treatments for milk protein% with LH being significantly lower than LL, HL and HH (3.4 v. 3.6, 3.7 and 3.7 % respectively).
- ✓ There was a significant effect of genotype on milk decline. Milk intake significantly declined over time (P<0.001), with the decline being greater for Beefex (1/2 Senepol,¼ Brahman, 1/8 Charolais, 1/8 Red Angus) than CBX (1/2 Senepol, ¼ Brahman, ¼ Charolais) (P=0.020)
- ✓ There was a significant effect of nutrition in the first two trimesters on liveweight of the cow until weaning
- ✓ Gross Placental Measures; Cotyledon number was significantly higher in heifers receiving high nutrition in the second trimester. Gross placenta weight was significantly higher (P=0.018) in heifers with Beefex dams than CBX dams. There was a significant relationship between calf birthweight and gross cotyledon surface area.

4.2 Results

4.2.1 Heifer liveweight during gestation

Heifers were weighed frequently between allocation to treatments on 8/9/04 and calving (2/8/05 to 22/8/05). Heifer liveweight change from prior to AI until 2 weeks post-calving is shown in Figure1.

Figure 1. Heifer liveweight over pregnancy including one measure 2 weeks post calving.



The following variables were analysed:

- Liveweight at the end of trimester 1 (1/2/05)
- Liveweight at the end of trimester 2 (26/4/05)
- Liveweight at the end of trimester 3 (25/7/05)
- ADG during trimester 1 (21/10/04 to 1/2/05 i.e. 103 days)
- ADG during trimester 2 (1/2/05 to 26/4/05 i.e. 84 days)
- ADG during trimester 3 (26/4/05 to 25/7/05 i.e. 90 days)

For all variables analysed, birthdate was not significant. Significance of dam breed, nutrition treatments and heifer liveweight on 26/9/04 for all variables is shown in Table 3.

Table 3. Probability levels for effects of nutrition, dam breed and liveweight at allocation to treatments

Variable	Dam breed	T1	T2	T1.T2	Liveweight 26/9/04
Liveweight end trimester 1	0.337	<0.001***			<0.001***
Liveweight end trimester 2	0.237	<0.001***	<0.001***	0.412	<0.001***
Liveweight end trimester 3	0.055	<0.001***	<0.001***	0.877	<0.001***
ADG during trimester 1	0.015**	<0.001***			0.755
ADG during trimester 2	0.378	0.023*	<0.001***	0.020*	0.686
ADG during trimester 3	0.090	0.013*	<0.001***	0.567	0.368
Overall ADG	0.003**	<0.001***	<0.001***	0.212	0.944

The effects of trimester treatment group and genotype on heifer weight change over gestation by are outlined in Table 4. Beefex heifers gained more weight than CBX heifers in the first trimester, but not in the remaining trimesters. Over all of gestation average daily gain (ADG) of Beefex heifers was significantly more than CBX heifers despite there being no difference in liveweight between Beefex and CBX heifers at the end of each trimester. During trimester 1, heifers on high nutrition gained more weight and were heavier at the end of trimester 1. During the second trimester, LH heifers gained more weight than HH heifers with HL and LL heifers

gaining the least. At the end of trimester 2, heifers on high nutrition during trimester 1 were heavier than those on low nutrition, with similar results for nutrition in the second trimester. During trimester 3, there was compensatory growth as evidenced by higher ADG for heifers on low nutrition in either trimester 1 or 2. By the end of trimester 3, heifers on high nutrition in trimester 1 or trimester 2 were still heavier than those on low nutrition in either trimester. Heifers on high nutrition in either the first or second trimester gained more weight than those on poor nutrition.

Table 4. Effect of nutrition, dam breed and heifer liveweight at allocation (26/9/04) on heifer liveweight and average daily gain during pregnancy

Dam breed	*n*	Liveweight at end trimester 1	ADG during trimester 1	Liveweight at end trimester 2	ADG during trimester 2	Liveweight at end trimester 3	ADG during trimester 3	Overall ADG
Beefex	20	407±3.4^{a}	0.54±0.028^{a}	458±4.2^{a}	0.51±0.025^{a}	493±4.8^{a}	0.49±0.030^{a}	0.51±0.015^{a}
CBX	53	403±2.1^{a}	0.46±0.017^{b}	452±2.5^{a}	0.48±0.015^{a}	482±2.8^{a}	0.43±0.018^{a}	0.46±0.009^{b}
Trimester 1								
High	36	423±2.7^{a}	0.66±0.022^{a}	467±3.3^{a}	0.46±0.020^{a}	500±3.7^{a}	0.43±0.024^{a}	0.52±0.012^{a}
Low	37	387±2.6^{b}	0.35±0.021^{b}	443±3.2^{b}	0.52±0.019^{b}	476±3.6^{b}	0.50±0.023^{b}	0.45±0.011^{b}
Trimester 2								
High	35			483±3.4^{a}	0.80±0.020^{a}	505±3.8^{a}	0.36±0.024^{a}	0.54±0.012^{a}
Low	38			427±3.1^{b}	0.19±0.019^{b}	470±3.5^{b}	0.57±0.022^{b}	0.43±0.011^{b}
T1.T2								
High high	17				0.74±0.027^{b}			
High low	18				0.19±0.026^{c}			
Low high	19				0.86±0.027^{a}			
Low low	19				0.19±0.026^{c}			

4.2.2 Gross placental measures and pregnancy hormones

Eight placentas were partially eaten or fetal membranes were retained, the data were regarded as missing for all variables except gestation length. The relationship between calf birth weight and both average and total cotyledon surface area was investigated. Initially influence of calf sex on the relationship was tested as this being absent simple correlations were completed. For all variables analysed, calf sex and its interactions with nutrition treatments, birthdate and heifer weight on 26/9/04 were not significant. Significance of dam breed and nutrition treatments for all variables is shown in Table 5. Table 6 presents predicted means ± SE for dam breed and nutrition treatments.

Gross placenta weight was significantly higher in heifers with Beefex dams than CBX dams (P =0.018). Cotyledon number, excluding accessory cotyledons, was higher in the HH treatment than HL, LH and LL treatments. However, when the accessory cotyledons were included the interaction was no longer significant, but cotyledon number was significantly higher in heifers receiving high nutrition in the second trimester (Table 5.)

Table 5. Probability levels for effects of nutrition and dam breed

Variable	Dam breed	T1	T2	T1.T2
Gestation length	0.939	0.573	0.617	0.157
Placental expulsion time	0.543	0.255	0.607	0.531
Gross placental wt	0.018*	0.861	0.896	0.289
Cotyledon no excluding accessory cotyledons	0.054	0.100	0.045*	0.014*
Cotyledon no including accessory cotyledons	0.121	0.423	0.026*	0.227
Wet cotyledon wt	0.209	0.830	0.654	0.401
Dry cotyledon wt	0.910	0.112	0.872	0.497
Total cotyledon surface area	0.539	0.665	0.380	0.136
Average cotyledon surface area	0.231	0.101	0.070	0.469

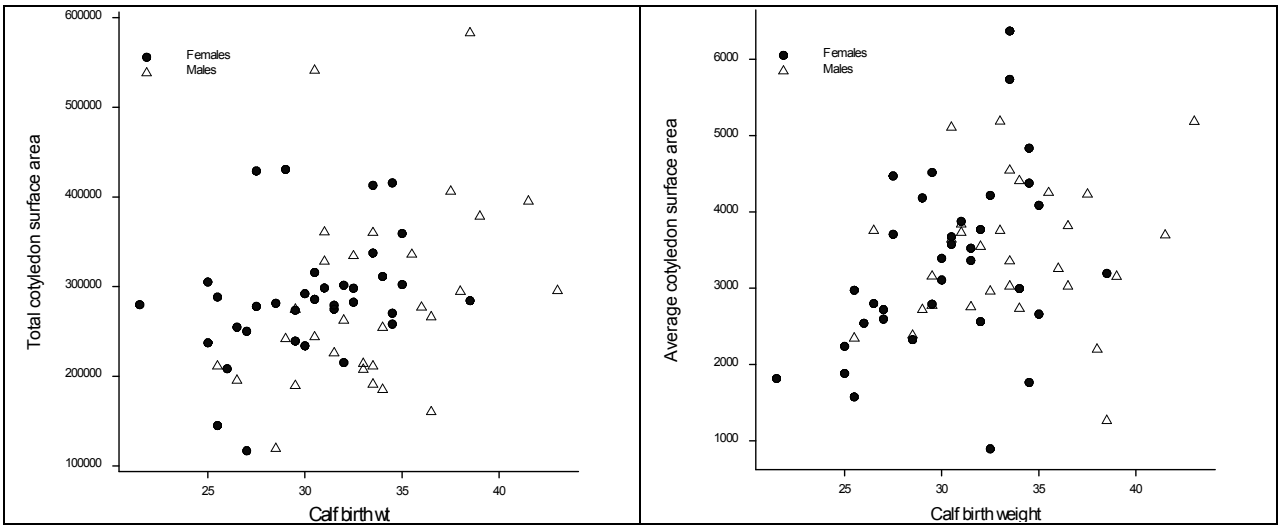
Table 6. Effect of nutrition and dam breed on log transformed placental traits

	n	Cotyledon no excluding accessory cotyledons		Cotyledon no including accessory cotyledons		Total cotyledon surface area (includes access cots)	
Dam breed		*Mean±SE	Back-transformed mean	*Mean±SE	Back-transformed mean	*Mean±SE	Back-transformed mean
Beefex	17	4.59±0.08[a]	98	4.62±0.10[a]	102	12.6±0.06[a]	294341
CBX	46	4.42±0.05[a]	83	4.45±0.06[a]	85	12.6±0.04[a]	281105
Trimester 1							
High	30	4.59±0.06[a]	98	4.58±0.08[a]	97	12.6±0.05[a]	283521
Low	33	4.42±0.06[a]	83	4.49±0.07[a]	90	12.6±0.05[a]	291833
Trimester 2							
High	28	4.59±0.06[a]	98	4.65±0.08[a]	104	12.6±0.05[a]	296214
Low	35	4.42±0.06[a]	83	4.42±0.07[b]	83	12.5±0.05[a]	279328
T1.T2							
High high	12	4.77±0.09[a]	118				
High low	18	4.41±0.08[b]	82				
Low high	16	4.41±0.08[b]	82				
Low low	17	4.44±0.08[b]	84				

*Log transformation applied

Figure 2 shows the relationship between calf birth weight and both average and total cotyledon surface area. Correlations were r = 0.300; P =0.015 and r = 0.332; P =0.007 for calf birth weight v. average cotyledon surface area and total cotyledon surface area, respectively.

Figure 2.Relationships between cotyledon surface area and calf birth weight



Nutrition during pregnancy affected cotyledon number but not any of the other placental variables. There was a significant relationship between calf birth weight and both average and total cotyledon surface area.

Placental Hormones

Progesterone levels were significantly increased by increased nutrition in both the first and second trimesters ($p < 0.05$).

Increased circulating bovine placental lactogen (bPL) occurred in first trimester with increased protein whereas in second trimester the opposite occurred ($p = 0.02$). At day 271 bPL was positively associated with calf birth weight ($p = 0.02$)

Circulating concentrations of estrone sulphate (ES) were positively associated with calf birth weight from day 231 to term ($p < 0.05$). Concentrations of ES were differentially affected by protein with high protein in the first trimester reducing ES with the opposite effect occurring in the second trimester.

There was no significant effect of treatment on bovine pregnancy associated glycoprotein (bPAG) concentrations except during the first trimester were increased protein lowered levels of bPAG.

Placental measures at term and their relationship with placental hormones.

Gross placental weight was significantly negatively correlated with heifer age (P =0.005, 0.03, 0.04) and leptin at day 28 (P =0.008) and at calving (P =0.02). Placental weight positively correlated with calf birth weight (P =0.003, 0.001, 0.005, <0.001, 0.001), bPAG at day 271 (P =0.01) and P4 at calving (P =0.02). BeefX heifer had significantly (P =0.03) heavier placental weights than CBX.

Cotyledonary weights

Dry cotyledonary weight was significantly negatively correlated with bPAG at day 28 (P =0.02), IGF-I at day 82 (P =0.01) and heifer weight at day 179(P =0.045). Dry cotyledonary weight was significantly positively associated with calf birthweight (P =0.02) and ES at calving (P =0.006). Heifers on high protein in first trimester had significantly (P =0.03) lighter dry cotyledonary weights than those on high protein.

Wet cotyledonary weight was significantly positively correlated with calf birthweight (P =0.02, 0.007, 0.03), ES at days 124 (P < 0.001), 236 (P =0.002) and 271 (P =0.001) and P4 at calving (P =0.003).

Cotyledon surface area

Cotyledon surface area was significantly negatively correlated with IGFBPs at day 28 (P =0.03) and gestation length (P =0.03). Cotyledon surface area was significantly positively correlated with calf birthweight (P =0.007), ES at days 82 (P =0.02), 124 (P < 0.001), 236 (P =0.005) and 271 (P < 0.001) and leptin at day 82 (P =0.04). Placentas from male calves had significantly larger cotyledonary surface areas than female calves (P =0.04, 179). Heifers on high protein in second trimester had significantly (P =0.002) larger cotyledonary surface areas than heifers on low protein, and heifers on high protein in both trimesters had the highest surface area (P =0.003).

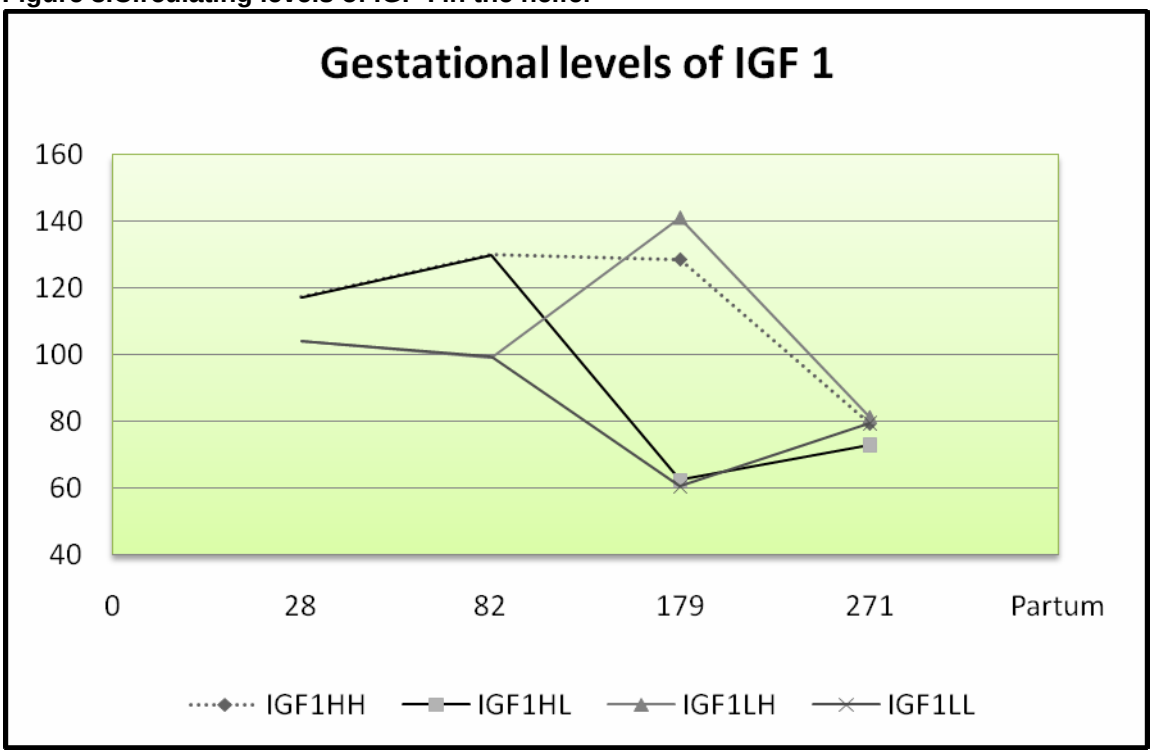
Calf measures

Calf birth weight was significantly positively correlated with placental weight (P =0.008), the total cotyledonary surface area of the expelled placenta (P =0.03), age of the dam (P =0.03) and gestational length (P =0.002). Male calves were significantly (P =0.007) heavier than female calves.

Circulating concentrations of IGF during gestation

Circulating levels of IGF-I in the heifer is shown in figure 3. Protein supplementation had a rapid (<28 day) effect on increasing IGF-I levels such that by d 28 the high protein diet heifers significantly increased in IGF-I (p =0.04) by the end of the first trimester (day 89) this effect was enhanced (p =0.001). Lack of protein supplementation in the second trimester led to immediate drops in IGF-I levels in the low protein treatment groups regardless of treatment in the first trimester. No effect of treatment group remained at day 271.

Figure 3.Circulating levels of IGF-I in the heifer

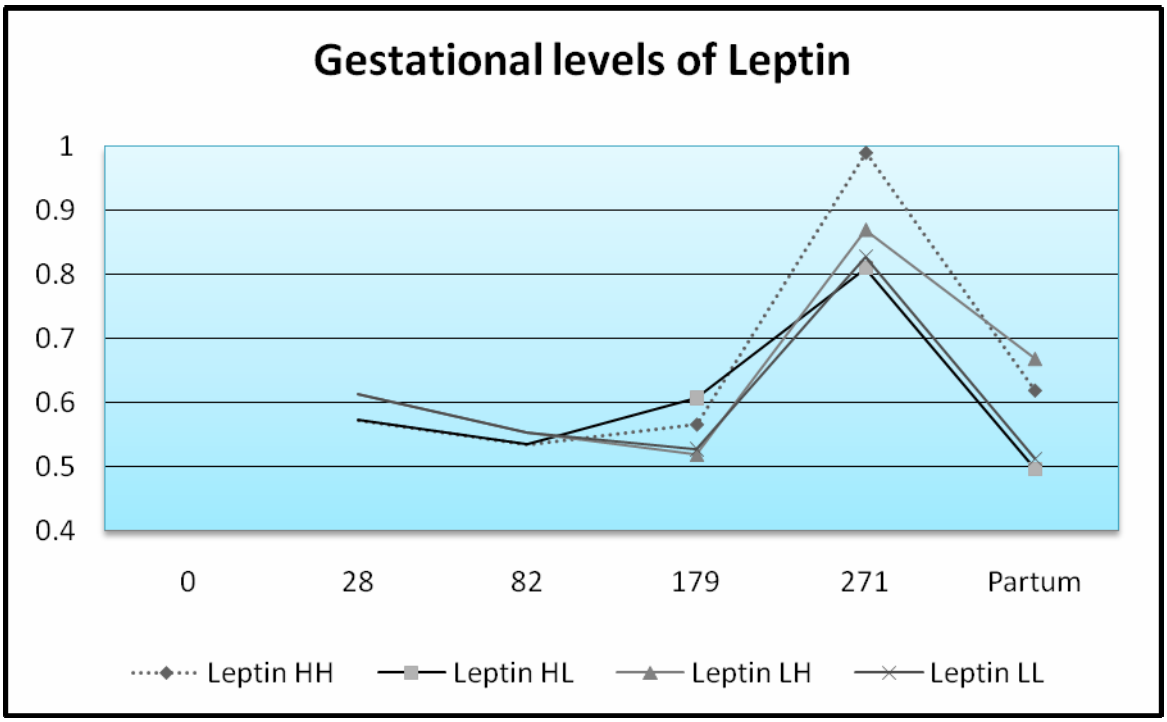


Increased dietary protein increased maternal plasma IGF-II (*P* = 0.01 on day 82; *P* = 0.04 on day 271). There was a significant effect of genotype throughout pregnancy with Beefex heifers having significantly higher levels of IGF-II until 271 days. Similarly IGFBPs were increased by increased dietary protein (*P* = 0.002 on d 82; *P* = 0.005 on d 179; *P* = 0.03 on d 271). IGFBPs were also significantly affected by genotype throughout pregnancy with CBX heifers having higher levels than Beefex

Leptin

Circulating leptin during gestation in the heifer are shown in figure 4. There was no effect of treatment upon levels of circulating leptin except at calving when high protein in the second trimester significantly increased leptin levels (p =0.002).

Figure 4. Circulating leptin during gestation in the heifer

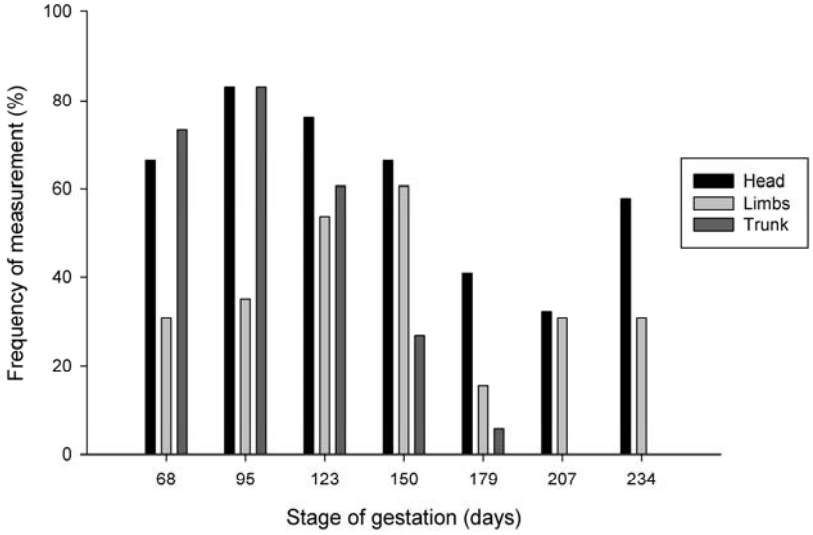


4.2.3 Fetal development and the calf at birth

Fetal measurement ability

The ability to measure the fetus declined by day 179 to approximately half that of days 68 to 150 due to the position of the fetus within the abdominal cavity. By day 234, the frequency of successful fetal measurement increased (see Figure 5). Head and limb measures were obtained throughout gestation; however, trunk measures were not obtained beyond day 179. Fetal head measures were the most frequently obtained with at least one type of head measurement made on 60.4% (300/497) of all scanning events. Trunk and limb measures were similar in their frequency of success with at least one type of trunk or limb measure being obtained at 35.6% (177/497) and 36.8% (183/497) of all scanning events respectively.

Figure 5. Frequency of fetal body part measurement at each scanning event during gestation across all treatment groups



First trimester fetal growth

Between days 39 and 95 of gestation sufficient data for statistical analysis was available on five head (OH, OV, CNL, CRL, and BPD), three trunk (AD, TD and UD) and two limb (forelimb mid-cannon and coronet diameter) variables. At day 39 of gestation, L fetuses had a significantly shorter CRL than H fetuses (P <0.01, Table 7). At day 68 of gestation CNL of L fetuses had a tendency to be significantly longer that H fetuses (P <0.10). At day 95 of gestation L fetuses had a significantly wider TD than H fetuses (P <0.01) but had significantly smaller UD than H fetuses (P <0.05). There were no other statistically significant treatment group differences for fetal measurements obtained during the first trimester of gestation.

Table 7. Fetal body measurements that were affected by maternal nutrition during the first trimester of gestation

Stage of gestation	Maternal nutritional group				*P*-value
	n	High	*n*	Low	
Day 39					
CRL (cm)	33	1.79 ± 0.05	33	1.55 ± 0.05	< 0.01
Day 68					
CNL (cm)	23	3.06 ± 0.06	24	3.23 ± 0.06	< 0.10
Day 95					
UD (cm)	20	1.19 ± 0.03	26	1.11 ± 0.02	< 0.05
TD (cm)	12	4.04 ± 0.08	24	4.35 ± 0.06	< 0.01

Values are predicted means ± S.E.M. from models described in the 'Statistical analyses' section of Materials and Methods. CRL = crown-rump length; CNL = crown-nose length; UD = umbilical cord diameter; TD = thoracic diameter.

Second and third trimester fetal growth

Between day 123 and 234 of gestation sufficient data for statistical analysis was available for OH, OV, BPD and UD. At day 123 of gestation HL and LL fetuses had a significantly greater UD than HH and LH fetuses ($P < 0.05$, Table 8). There was no interaction between first and second trimester treatment group effects. At days 123 ($P < 0.01$) and 235 ($P < 0.10$) female fetuses had smaller OH than males (Table 9). There were no other statistically significant treatment group differences for fetal measurements obtained during the second and third trimesters of gestation.

Table 8. **Fetal body measurements that were affected by maternal nutrition during the second trimester of gestation**

Stage of gestation	Maternal nutritional group								*P* - value
Day 123	*n*	HH	*n*	HL	*n*	LH	*n*	LL	
UD (cm)	6	1.72 ± 0.07	10	1.93 ± 0.5	10	1.78 ± 0.05	10	1.86 ± 0.05	< 0.05[a]

Values are predicted means ± S.E.M. from models described in the 'Statistical analyses' section of Materials and Methods. HH = high/high; HL = high/low; LH = low/high; LL = low/low; UD = umbilical cord diameter; a = -/H vs -/L

Table 9. Means of fetal and calf body measurements during gestation and at birth that were affected by gender

	n	Male	*n*	Female	*P*-value
OH at day 123 (cm)	25	1.64 ± 0.02	27	1.53 ± 0.02	< 0.01
OH at day 235 (cm)	22	2.14 ± 0.04	19	2.04 ± 0.04	< 0.10
Birthweight (kg)	32	33.37 ± 0.64	38	30.43 ± 0.59	< 0.01
CNL at birth (cm)	33	20.48 ± 0.26	38	19.84 ± 0.24	< 0.10
CRL:BW	32	2.55 ± 0.04	38	2.71 ± 0.04	< 0.01

Values are predicted means ± S.E.M. from models described in the 'Statistical analyses' section of Materials and Methods.OH = horizontal eye socket diameter; CNL = crown–nose length; CRL:BW = crown-rump length:birthweight

Fetal growth over time

Eye socket diameter in the horizontal plane was the only variable that had a sufficient number of consecutive measurements between days 68 and 234 of gestation to allow assessment of the effect of dam treatment group on head growth over time. There was no significant effect of first or second trimester treatment group, their interaction term or gestation length on this measure, however female fetuses had a significantly lower OH than males (Coef.: -0.063; 95% CI -0.127 -0.001; $P < 0.05$).

Foreleg mid-cannon bone diameter and crown-nose length had sufficient numbers of consecutive measurements between days 68 and birth to allow the assessment of treatment group on fetal skeletal and head development over time. Univariate analysis indicated that progeny in the low protein treatment group in the second trimester of gestation had smaller foreleg cannon bone diameters from day 68 to birth than their high protein treatment group counterparts (Coef.: -0.584; 95% CI -0.858 -0.310; $P < 0.01$). This effect was not significant in a multivariate model that also contained fetal gender. There was no significant effect of gestation length on foreleg mid-cannon bone diameter over time. There were no significant effects of either first or second trimester treatment group, their interaction term or gestation length on measures of crown-nose length over this time period.

Calf biometry at birth

Low dam nutrition in the second trimester decreased BW ($P < 0.05$, Table 10). There was no interaction between first and second trimester treatment group effects. Female calves had significantly lower BW than male calves ($P < 0.01$, Table 9). Increased gestation length tended to be associated with an increased BW (Coef.: 0.234; 95% CI 0.019 0.448; $P = 0.053$).

Table 10. Calf body measurements at birth that were affected by maternal nutrition during gestation

	Maternal nutritional group								*P*-value
	n	HH	*n*	HL	*n*	LH	*n*	LL	
BW (kg)	16	32.9 ± 0.9	18	31.1 ± 0.9	17	33.4 ± 0.9	19	29.9 ± 0.8	< 0.05[a,b,c]
AC (cm)	16	74.4 ± 0.9	19	75.8 ± 0.8	17	77.4 ± 0.9	19	74.4 ± 0.8	< 0.10[d]

Values are predicted means ± S.E.M. from models described in the 'Statistical analyses' section of Materials and Methods. HH = high/high; HL = high/low; LH = low/high; LL = low/low; BW = birth weight; AC = abdominal circumference; a = -/H vs -/L; b = HH vs LL; c = LH vs LL; d = LH v HH

Abdominal circumference tended to be greater in LH ($P < 0.10$, Table 10) than in HH calves. There was no interaction between first and second trimester treatment group effects. Both AC (Coef.: 0.24; 95% CI 0.0025 0.487; $P < 0.05$) and CRL (Coef: 0.73; 95% CI 0.41 1.05; $P < 0.01$) at birth were associated positively with length of gestation. There was a tendency for CNL to be shorter in female than male calves ($P < 0.10$, Table 9). There were no other significant effects on the other calf physical body variables at birth.

Indices of disproportionate growth

The ratio between the BPD and the AD (BPD:AD) was used on days 68 and 95 of gestation to investigate the effect of maternal diet in the first trimester on proportional fetal growth. At days 68 and 95 of gestation there were no significant differences in BPD:AD due to maternal nutrition or fetal gender.

At birth, there were no effects of first or second trimester treatment group, nor their interaction term, on either CRL:BW or CNL:CRL. Female calves had significantly higher CRL:BW ratios than male calves ($P < 0.01$, Table 9) and calves of longer gestation lengths had increased CRL:CNL (Coef.: 0.03; 95% CI 0.001 0.052; $P < 0.01$).

Relationships between fetal measures during early and late gestation and calf birth weight

Univariate screening of the relationships between fetal measures of optic diameter and foreleg mid-cannon diameter obtained on days 68 and 95 with those obtained on days 207 and 234, revealed that foreleg mid-cannon diameter on day 95 was closely associated with its subsequent diameter on day 207 (Coef.: 0.13; 95% CI 0.09 0.18; $P < 0.01$). There were no other significant associations between variable measurements obtained during early and late gestation.

Univariate screening of fetal measurements obtained during the first and second trimesters and calf birth weight revealed that increased calf birth weight was associated with increased optic diameter in the vertical plane on days 95 (Coef.: 0.009; 95% CI: 0.001 .02; $P < 0.05$) and 123, (Coef.: 0.02; 95% CI 0.008 0.02; $P < 0.01$). Calf birth weight tended to be increased in association with increased abdominal cross section on day 65 (Coef.: 0.01; 95% CI -0.001 0.02; $P < 0.10$).

Similarly on day 123, increased biparietal diameter (Coef.: 0.03; 95% CI -0.003 0.06; P < 0.10) and hind limb mid-cannon diameter (Coef.: 0.03; 95% CI -0.004 0.06, P < 0.10) tended to be associated with increased calf birth weight.

There was no significant association between fetal-crown rump length at day 39 and calf birth weight, nor any of the other fetal variable measurements obtained during early- and mid-gestation and calf birth weight.

4.2.4 Dystocia

A total of 71 heifers calved (LL = 19; LH = 17; HL = 18; HH = 17) over a 20 day period from 2/8/05 to 22/8/05. Calves were weighed within 15 minutes of birth prior to suckling and each delivery catergorised as eutocic (*n* = 61) or dystocic (*n* = 10) with dystocic calvings including those due to both malpresentation (*n* = 4) and fetopelvic disproportion (*n* = 6). The distribution of calves by delivery type and gender across treatment groups is shown in Table 11. A 1kg increase in calf birthweight increased the risk of dystocia occurring by 1.44 fold (P =0.003). Seven of the assisted deliveries were male calves.

Table 11. The number and birth weight of assisted and unassisted calf deliveries by treatment group. Values are means ± SD.

	HH				HL				LH				LL			
	n	Male	*n*	Female	*n*	Male	*n*	Female	*n*	Male	*n*	Female	*n*	Male	*n*	Female
Unassisted	4	31.4 ± 4.44	8	30.8 ± 4.54	8	32.8 ± 2.31	9	29.9 ± 3.43	8	33.6 ± 3.17	7	31.9 ± 2.21	6	31.6 ± 4.90	11	28.6 ± 3.40
Assisted	3	38.8 ± 2.75	1	38.5	1	33.0	1	32.5	1	43	1	32.5	2	32.0 ± 2.12	-	-

HH = high/high; HL = high/low; LH = low/high; LL = low/low; EUT = eutocic deliveries; FPD = fetopelvic disproportion deliveries; MP =malpresentation deliveries

Pelvic area by delivery type is shown in Table 12. Pelvic area prior to joining tended to be less in heifers that experienced dystocia compared with those that had unassisted deliveries. A 1cm^2 increase in pelvic area tended to reduce the risk of dystocia by 0.96 (P =0.07). Pelvic area measurement on day 117 of gestation was not associated with delivery type.

Table 12. Mean (± SEM) pelvic area on Day -72 and Day 117 for unassisted and assisted calf deliveries

	EUT	FPD+MP	*P*-value
- 72 days (cm^2)	226.1 ± 2.6	214.2 ± 6.2	0.08
+ 117 days (cm^2)	273.0 ± 3.2	259.9 ± 7.8	ns

PA- = pelvic area on Day -72, PA+ = pelvic area on Day 117, EUT = eutocic deliveries, FPD+MP =fetopelvic and malpresentation deliveries; ns = not significantly different

4.2.5 Heifer liveweight post-calving

Heifers were weighed and condition scored 7 times between 25/8/05 (immediately after the calving period finished) and 18/2/06. Figure 6 shows the liveweight growth paths for the individual heifers for each nutrition treatment. Figure 7 shows the mean liveweight growth paths for the 4 nutrition treatments. The general trend was for the liveweight differences between treatments that existed after calving to be maintained over time.

Figure 6. Liveweight growth paths post calving for individual heifers by nutrition treatment.

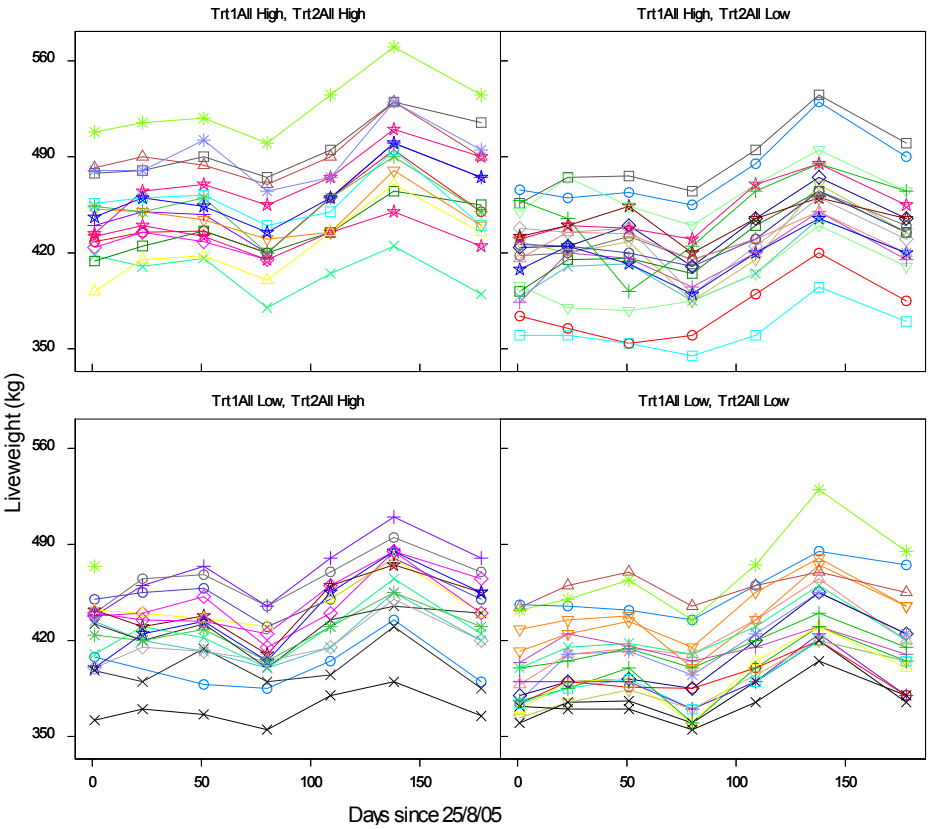
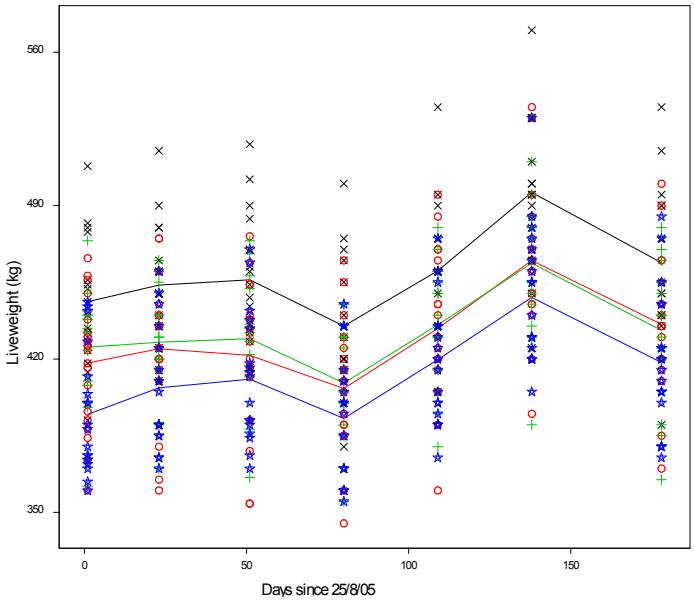


Figure 6 illustrates the variation between individuals within the same treatment group in total liveweight. For example the lowest weight animal in the high high group weighed a maximum of 420 kg during lactation whereas the highest weight animal had a maximal weight of 560kg.

Figure 7. Mean liveweight growth paths of heifers post calving by nutrition treatment (green=LH, red=HL, blue=LL, black=HH).



Heifers lost weight in the last month of the trial due to seasonal conditions and lack of feed availability despite being supplemented with whole cottonseed.

Predicted mean liveweight and body condition scores for the fixed effects are presented in Table 13. High nutrition in both trimesters continued to have a positive effect on heifer liveweight post-calving.

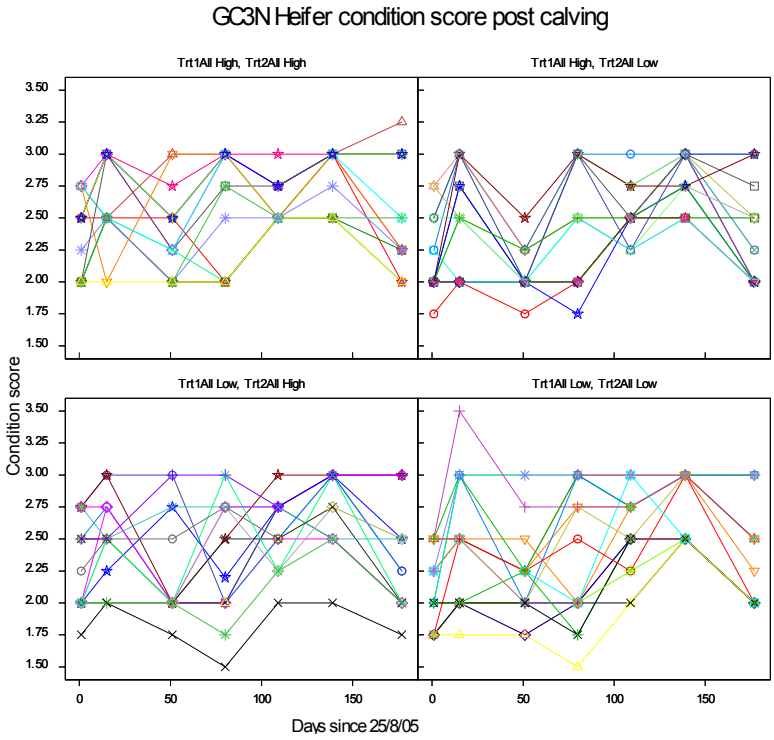
Table 13. Effect of dam breed and nutrition treatments on heifer liveweight and body condition score post-calving.

Dam breed	No.	Liveweight	Condition score
Beefex	126	435±6.5 a	2.4±0.06
CBX	361	433±3.9 a	2.5±0.04
Trimester 1			
High	242	447±5.2 a	2.4±0.04
Low	245	425±5.0 b	2.5±0.04
Trimester 2			
High	221	447±5.3 a	2.5±0.05
Low	266	421±4.9 b	2.4±0.04
Time			
25/8/05	71	422±3.9 b	2.2±0.05
16/9/05	69	429±3.9 c	2.5±0.05
14/10/05	70	431±4.1 c	2.2±0.05
12/11/05	70	412±3.9 a	2.4±0.07
11/12/05	69	439±4.1 d	2.6±0.03
9/1/06	69	469±4.3 e	2.8±0.04
18/2/06	69	439±4.4 d	2.4±0.06

Body condition score

Figures 8 and 9 show the trends for body condition score, which tended to fluctuate over time. A significant part of this fluctuation was attributed to a lack of operator experience. Predicted means for the fixed effects are presented in Table 13. Nutrition during pregnancy did not influence body condition score post-calving, but the effect of dam breed varied over time (Figure 10). Condition scores of the dam breeds were similar at all times except 12/11/05 and 11/12/05.

Figure 8. Body condition score for individual heifers by nutrition treatment.



This figure illustrates the variation in Body condition score for the individual. It reflects the inexperience of the operator. The fat scan analysis provides a more accurate picture of body condition.

Figure 9. Mean body condition score of heifers response by nutrition treatment (green=LH, red=HL, blue=LL, black=HH) post calving.

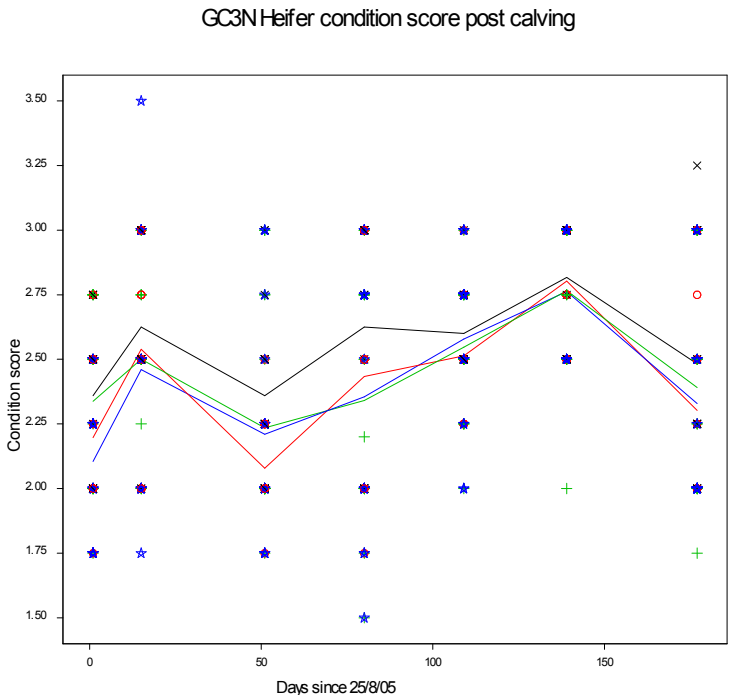
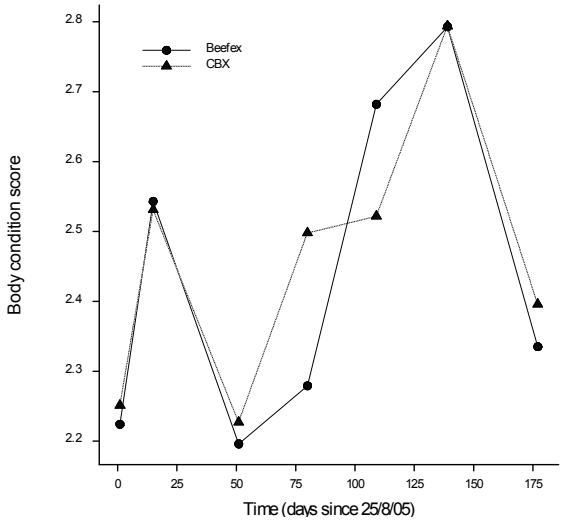


Figure 10. Post calving body condition score for dam breeds over time.



4.2.6 Milk production

Milk intake of the 69 calves was assessed five times between birth and weaning using a weigh, suck, weigh process over a 24 hour period. Figure 11 indicates the overall pattern of milk intake for the 4 nutrition treatments. There was a decline in milk intake over time with considerable variation from calf to calf within treatments. Results are presented in Table 14 as predicted means ± SE.

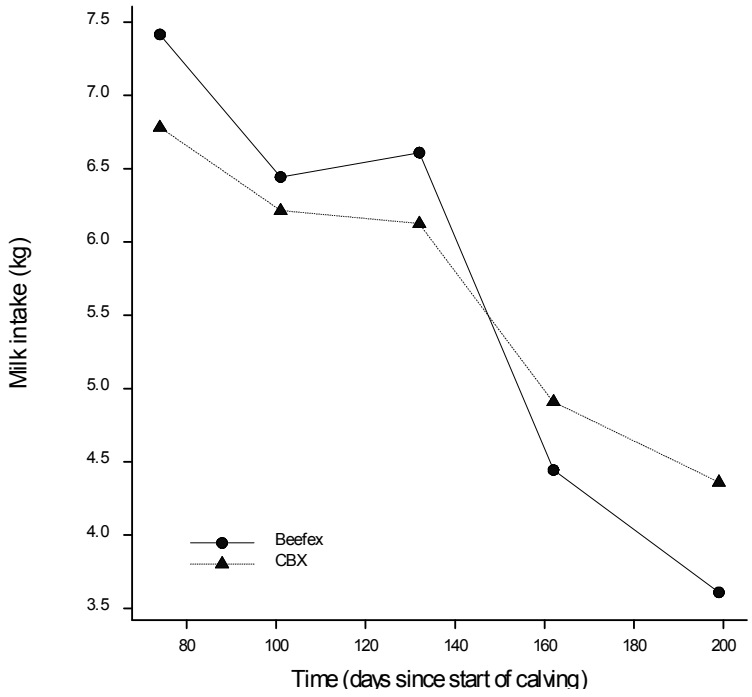
Table 14. Predicted mean milk intake by calves

Term	Levels	N	Mean ± SE
T1	High	170	5.3 ± 0.18 a
	Low	175	6.1 ± 0.17 b
T2	High	155	5.6 ± 0.18 a
	Low	190	5.8 ± 0.17 a
Sex	Female	180	5.6 ± 0.18 a
	Male	165	5.7 ± 0.17 a
Dam breed	Beefex	90	5.7 ± 0.24 a
	CBX	255	5.7 ± 0.14 a
Time	74	69	7.1 ± 0.18 a
	101	69	6.3 ± 0.20 b
	132	69	6.4 ± 0.25 b
	162	69	4.7 ± 0.21 c
	199	69	4.0 ± 0.19 d

Within terms, means not followed by a common letter are significantly different (P <0.05).

It can be seen that nutrition in the first trimester influenced milk intake (P <0.001) with higher intake for calves from dams with low nutrition in the first trimester, but there was no effect of second trimester nutrition (P =0.560) or calf sex (P =0.605). Milk intake significantly declined over time (P <0.001), with the decline being greater for Beefex than CBX (P =0.020; Figure 11).

Figure 11. Predicted milk intake over time for Beefex and CBX dam breeds.



Milk Protein

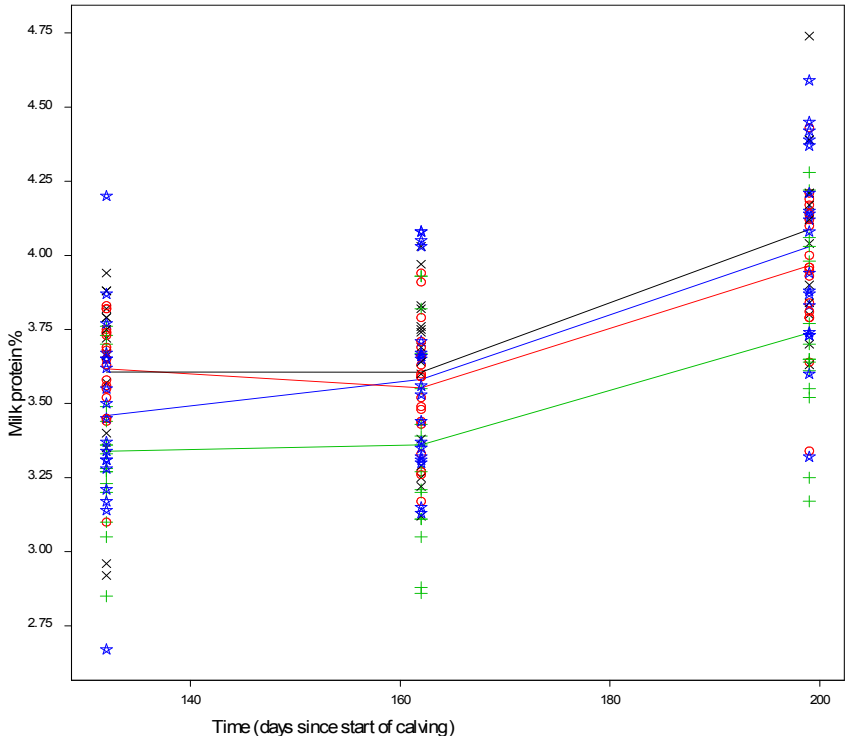
Milk protein percentage was assessed 3 times between birth and weaning. Figure 12 indicates the overall pattern of milk protein percentage for the 4 nutrition treatments. Predicted mean milk protein percentage by fixed effects is shown in Table 15. There was an interaction (P =0.041) between first and second trimester nutrition treatments for milk protein% with LH being significantly lower than LL, HL and HH (3.4 v. 3.6, 3.7 and 3.7 % respectively). There was no effect of calf sex (P =0.446) on milk protein%. Heifers from CBX dams had significantly (P =0.016) higher milk protein% than Beefex dams. Milk protein% increased significantly (P <0.001) at weaning, the last time of measurement.

Table 15. Predicted mean milk protein percentage

Term	Levels	*n*	Mean ± SE
T1	High	99	3.7 ± 0.05[a]
	Low	104	3.5 ± 0.04[b]
T2	High	90	3.6 ± 0.05[a]
	Low	113	3.7 ± 0.04[a]
Sex	Female	106	3.6 ± 0.04[a]
	Male	97	3.7 ± 0.04[a]
Dam breed	Beefex	54	3.5 ± 0.06[a]
	CBX	149	3.7 ± 0.03[b]
Time	132	69	3.5 ± 0.04[a]
	162	69	3.5 ± 0.04[a]
	199	65	3.9 ± 0.04[b]

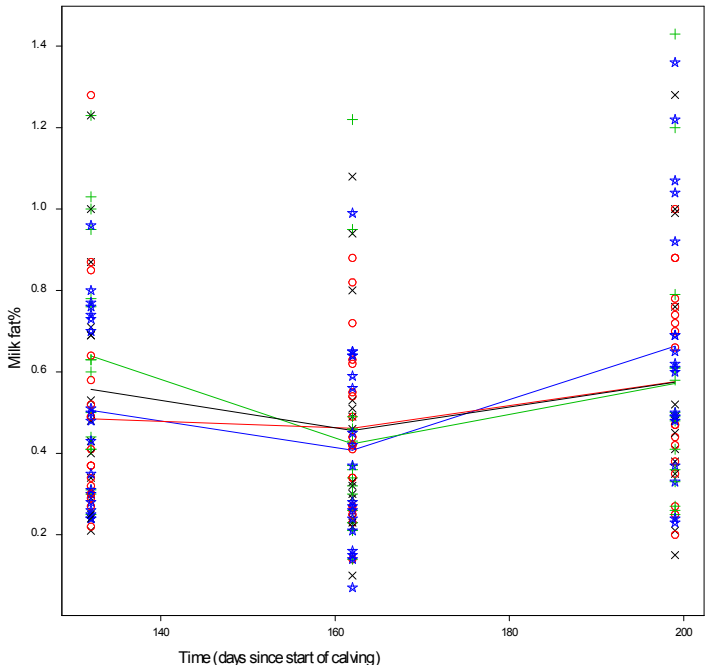
Within terms, means not followed by a common letter are significantly different (P <0.05).

Figure 12. Milk protein percentage means for each nutrition treatment (HH – black, HL – red, LH – green and LL – blue).



Milk fat percentage was assessed at the same times as protein. Figure 13 below indicates the overall pattern of milk fat percentage for the 4 nutrition treatments and the patterns for individual calves.

Figure 13. Milk fat percentage over lactation for each nutrition treatment (HH – black, HL – red, LH – green and LL – blue).



Statistical analysis was identical to that for milk protein percentage. For milk fat percentage, there was no correlation between times for individual calves. Hence the analysis was simply a split-plot in time.

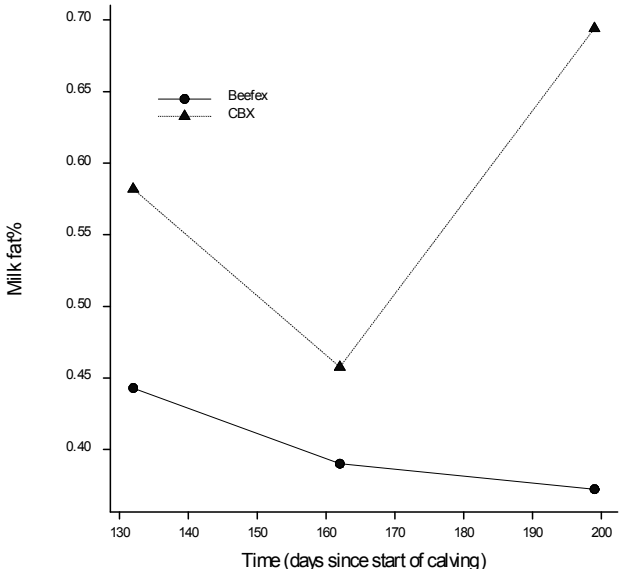
There was no effect of first (P =0.640) or second (P =0.733) trimester nutrition on milk fat percentage. Calf sex (P =0.003) influenced milk fat percentage, with higher fat percentage for male calves (Table 16). Heifers from CBX dams had significantly (P <0.001) higher milk fat percentage than Beefex dams overall, but the size of the difference varied over time (Figure 14). Milk fat percentage was lower at the second time of measurement that at other times (P =0.013).

Table 16. Predicted mean milk fat percentage

Term	Levels	*n*	Mean ± SE
T1	High	99	0.48 ± 0.034 a
	Low	104	0.50 ± 0.032 a
T2	High	90	0.50 ± 0.035 a
	Low	113	0.48 ± 0.031 a
Sex	Female	106	0.42 ± 0.033 a
	Male	97	0.56 ± 0.033 b
Dam breed	Beefex	54	0.40 ± 0.042 a
	CBX	149	0.58 ± 0.026 b
Time	132	69	0.51 ± 0.036 a
	162	69	0.42 ± 0.036 b
	199	65	0.53 ± 0.036 a

Within terms, means not followed by a common letter are significantly different (P <0.05).

Figure 14. Predicted milk fat percentage over time by dam genotype



Milk intake, fat and protein percentage were measured simultaneously at 3 times, 11/12/05, 10/1/06 and 17/2/06. Pearson correlations were calculated between these traits at each time and are presented in Table 17. There were no consistent correlations between milk parameters.

Table 17. Correlations between milk parameters at 3 times of measurement

	11/12/05	10/1/06	17/2/06
Intake v Fat%	0.031	0.362**	0.029
Intake v Protein%	-0.012	-0.020	-0.080
Fat% v Protein%	0.106	0.232	0.342**

*** P <0.001; ** P <0.01; * P <0.05

4.2.7 Calf growth pre-weaning

Mean calf liveweight by treatment group and gender are presented in Table 18. Average daily gain ranged from 0.78 to 1.10 kg/day with mean ± se of 0.93 ± 0.01 kg/day. There was no effect of nutrition or dam breed on ADG. Calf sex was highly significant (P <0.001) with male calves (0.97 ± 0.011 kg/day) gaining more weight per day than female calves (0.90 ± 0.011 kg/day).

Table 18. Calf weights from birth to weaning (mean ± SE)

	FEMALES				MALES			
	HH	**HL**	**LH**	**LL**	**HH**	**HL**	**LH**	**LL**
Liveweight (kg)	(*n* = 8)	(*n* = 10)	(*n* = 7)	(*n* = 11)	(*n* = 7)	(*n* = 9)	(*n* = 9)	(*n* = 8)
Birth	31.1 ± 1.8	30.2± 0.8	31.7 ± 1.1	28.6 ± 1.0	34.6 ± 2.0	32.8 ± 1.4	34.6 ± 0.8	31.7 ± 1.5
25/08/2005	47.3 ± 2.4	44.8 ± 2.4	46.6 ± 1.0	45.7 ± 1.8	47.1 ± 2.2	45.9 ± 2.5	48.9 ± 1.6	46.6 ± 3.3
8/09/2005	63.6 ± 2.7	61.9 ± 2.2	64.3 ± 1.1	60.7 ± 1.8	63.9 ± 2.6	63.5 ± 2.9	68.1 ± 1.6	65.1 ± 3.3
14/10/2005	92.8 ± 3.8	90.8 ± 1.7	93.5 ± 1.5	88.6 ± 2.4	94.4 ± 4.2	97.0 ± 3.7	100.0 ± 2.2	99.5 ± 3.5
12/11/2005	115.6 ± 4.4	114.2 ± 2.1	117.4 ± 2.1	111.5 ± 2.9	118.8 ± 5.0	122.7 ± 4.0	126.2 ± 2.3	123.6 ± 3.5
11/12/2005	144.4 ± 4.4	143.3 ± 2.0	145.8 ± 2.6	139.1 ± 3.3	149.4 ± 6.6	155.3 ± 5.0	159.6 ± 2.4	158.0 ± 4.4
10/01/2006	175.9 ± 4.9	174.6 ± 2.3	175.6 ± 3.1	169.6 ± 3.3	182.6 ± 7.5	188.6 ± 6.4	195.1 ± 2.8	189.6 ± 4.7
17/02/2006 (weaning)	204.8 ± 5.6	205.1 ± 2.3	205.1 ± 3.4	197.7 ± 3.9	209.7 ± 7.5	215.5 ± 6.6	222.6 ± 2.5	218.8 ± 4.8

At the weighing on 25/8/05, significant effects on calf weight were calf age (P <0.001), calf sex (P =0.041) and treatment in the second trimester (P =0.036). Means are presented in Table 19. Therefore, on 25/8/05, older calves were heavier, male calves were heavier and calves whose dams had high nutrition in the second trimester were heavier.

At the weighing on 8/9/05, significant effects on weight were calf age (P <0.001), calf sex (P =0.005) and treatment in the second trimester (P =0.034). Means are presented in Table 19. Results were similar to those for calf weight on 25/8/05.

*At the weighing on 14/10/05***,** significant effects were calf age (P <0.001) and calf sex (P <0.001). Nutritional regimen in first (P =0.766), or second trimester (P =0.277), dam breed (P =0.254) and interaction (P =0.147) were not significant. Means are presented in Table 19. This shows that there is no longer an effect of nutrition in the second trimester on calf weight by 14/10/05 (average age of calves of 65 days), but there is still an effect of calf sex and calf age.

Table 19. Predicted mean (± se) calf weight for factors and coefficient for the covariate calf age on 25/8/05, 8/9/05 and 14/10/05.

Term	Levels	*n*	25/8/05	8/9/05	14/10/05
T1	High	34	47.2 ± 0.88[a]	64.2 ± 1.05[a]	94.9 ± 1.47[a]
(trimester 1)	Low	35	47.2 ± 0.84[a]	64.7 ± 1.01[a]	95.5 ± 1.41[a]
T2	High	31	48.4 ± 0.90[a]	65.9 ± 1.08[a]	96.2 ± 1.51[a]
(trimester 2)	Low	38	46.0 ± 0.82[b]	63.0 ± 0.98[b]	94.1 ± 1.38[a]
Sex	Female	36	46.0 ± 0.87[a]	62.5 ± 1.04[a]	91.3 ± 1.47[a]
	Male	33	48.4 ± 0.87[b]	66.4 ± 1.03[b]	99.1 ± 1.45[b]
Dam breed	Beefex	18	48.2 ± 1.11[a]	65.5 ± 1.33[a]	96.4 ± 1.87[a]
	CBX	51	46.2 ± 0.66[a]	63.4 ± 0.79[a]	93.9 ± 1.11[a]
Calf age		69	0.93 ± 0.14	0.84 ± 0.17	0.83 ± 0.23

Within terms, means not followed by a common letter are significantly different (P <0.05).

Calf weaning weight (17/2/06)

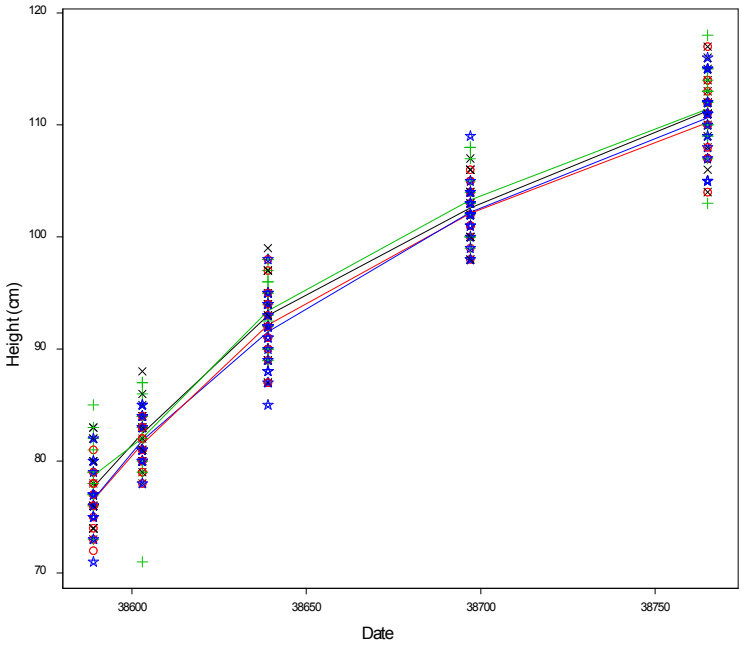
Calf weaning weight ranged from 175.8 to 240.8 kg with mean ± se of 209.6 ± 1.9. Calves were weaned on 17th February 2006. There was no effect of treatment on body weight at weaning the only significant effect on weaning weight was sex (217.0 ± 2.4 and 202.8 ± 2.3 for male and female calves respectively).

Therefore, by three months of age those calves that were born to heifers unsupplemented in the second trimester had caught up to the other calves such that there was no difference between the four treatment groups at weaning.

Calf hip height from birth to weaning

Hip height (cm) of the 69 calves was assessed 5 times between birth and weaning. Figure 15 indicates the overall pattern of calf height for the 4 nutrition treatments and the patterns for individual calves. Hip height increased over time in a curvilinear trend.

Figure 15. Hip height of calves means (cms) for each nutrition treatment group (HH – black, HL – red, LH – green and LL – blue).



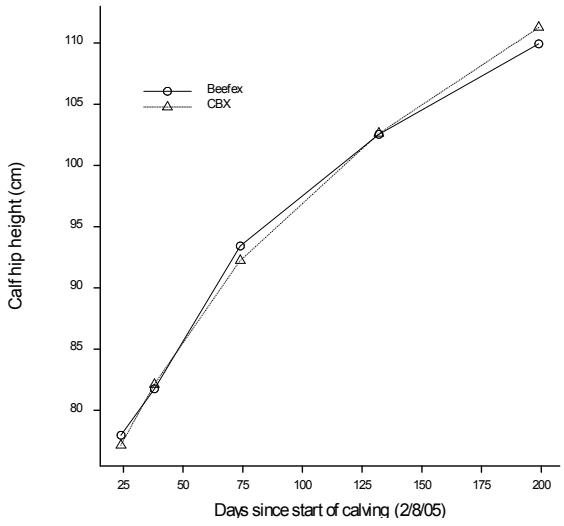
Results are presented in Table 20 as predicted means ± SE. Calf date of birth (P =0.521) and nutrition in either trimester did not influence calf hip height (P =0.734 and P =0.132 for T1 and T2). Male calves were significantly taller than female calves (P <0.001). Hip height significantly increased over time (P <0.001), but the pattern varied with dam breed (P =0.012; Figure 16).

Table 20. Predicted mean hip height of calves

Term	Levels	*n*	Mean ± SE
T1	High	170	93.0 ± 0.42[a]
	Low	175	93.2 ± 0.41[a]
T2	High	155	93.5 ± 0.43[a]
	Low	190	92.7 ± 0.40[a]
Sex	Female	180	92.1 ± 0.42[a]
	Male	165	94.1 ± 0.42[b]
Dam breed	Beefex	90	93.1 ± 0.55[a]
	CBX	255	93.1 ± 0.32[a]
Time	74	69	77.6 ± 0.38[a]
	101	69	81.9 ± 0.36[b]
	132	69	92.8 ± 0.43[c]
	162	69	102.6 ± 0.33[d]
	199	69	110.6 ± 0.44[e]

Within terms, means not followed by a common letter are significantly different (P <0.05).

Figure 16. Predicted calf hip height over time for Beefex and CBX dam breeds.



4.2.8 Calf insulin-like growth factors

There was no effect of treatment during gestation on circulating IGF concentrations in the calf at birth. Plasma concentration of IGF-I by treatment group is shown in Figure 17, IGF-I*I* in Figure 18 and total IGFBPs in Figure 19. IGF-I concentrations were significantly positively related to growth rates ($p < 0.001$) and IGF-I*I* levels significantly negatively related to growth rates in the calves ($p = 0.002$)

Figure 17.Circulating IGF-I concentration in the calf

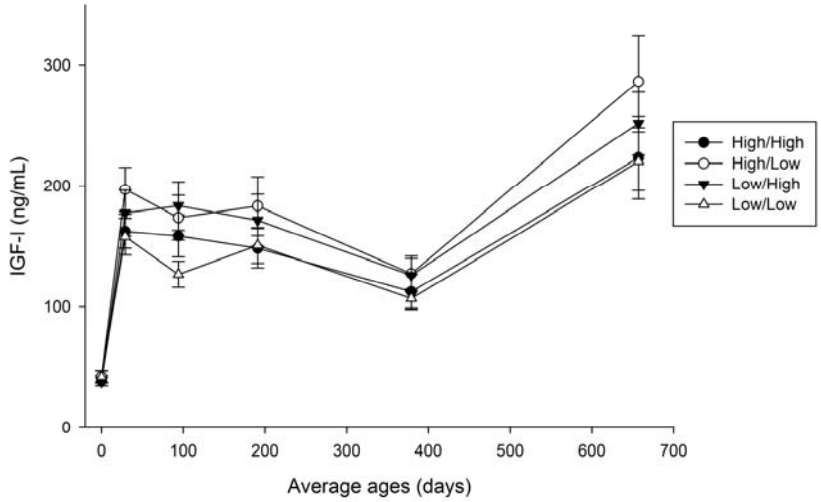
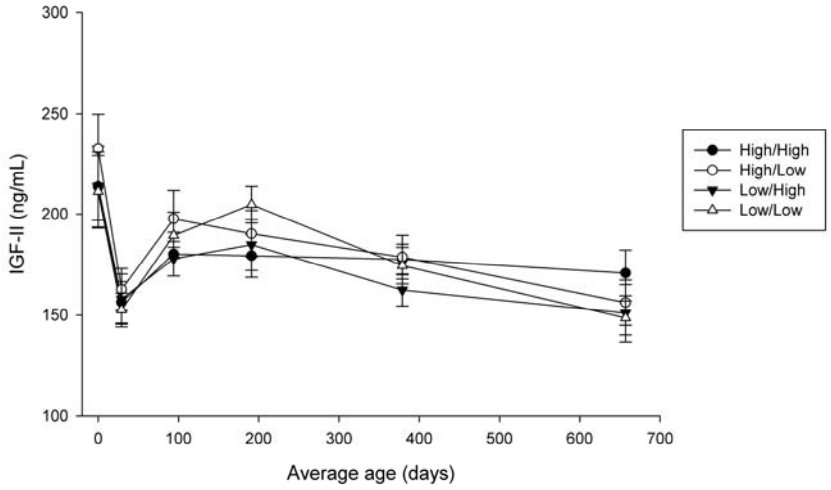


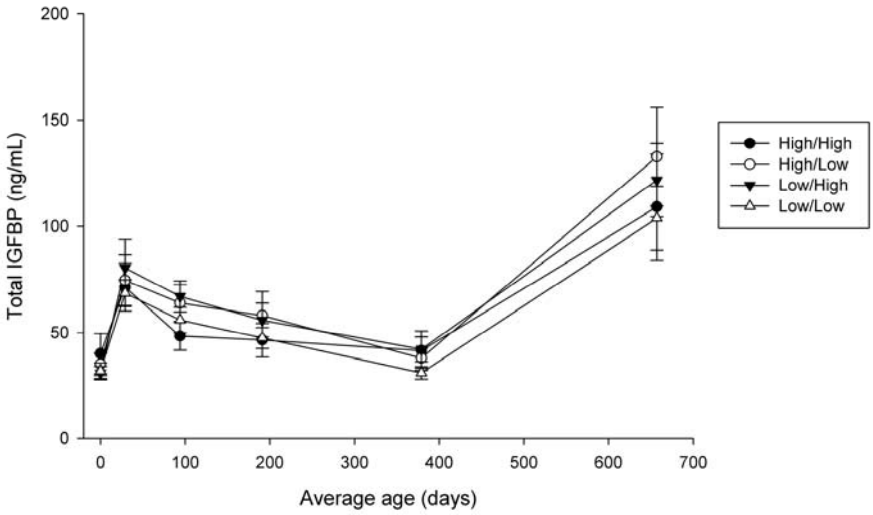
Figure 18. Circulating IGF-I*I* in the calf



IGFBPs

There was an effect of genotype on concentrations of total IGFBPs in the calf at birth with the CBX being higher ($p = 0.01$). IGFBPs were also positively related to calf growth ($p < 0.001$). Blood glucose and BUN were also positively associated ($p < 0.001$) with calf growth rates.

Figure 19. Circulating IGFBPs in the calf



4.2.9 Calf reproductive hormones and organs

Bull Testicular Measures
Low maternal dietary protein levels during gestation were associated with increased prepubertal FSH concentrations (P = 0.03) and paired testicular volume (P = 0.04) in male offspring and tended to be associated increased with seminiferous tubule diameter (P = 0.07) and LH concentrations (P = 0.09).

Serum LH (P < 0.001) and FSH concentrations (P = 0.04) were correlated with seminiferous tubule diameter. Testosterone concentrations were positively correlated with testis measures: paired testicular weight (P < 0.001), volume (P = 0.03) and seminiferous tubule diameter (P < 0.001). Although leptin concentrations were associated with prepubertal age (P = 0.04) and body weight (P = 0.006), they not associated with any of the measures of reproductive development, but insulin-like growth factor-I was associated with prepubertal FSH (P = 0.005). In conclusion, prepubertal reproductive development of bulls may be affected by prenatal nutrition during early and mid gestation.

Heifer Ovarian Measures
Ovarian measures in the female progeny were performed at slaughter (23 months of age). (Appendix 2) The results showed that a low protein diet in both the first and second trimesters resulted in the highest percentage and density of healthy follicles and the lowest percentage of atretic follicles. A high protein diet in both the first and second trimesters HH, however, results, in the lowest percentage of healthy follicles and the highest percentage of atretic follicles.

The HL group whose mothers were fed a high protein then low protein diet, had the second highest percentage of healthy follicles and the second lowest percentage of atretic follicles. The cows in this group had significantly higher ovarian weights compared to other Groups. However, after taking this difference into account with follicle density, the HL still had the second highest healthy follicle density.

5 Discussion

Optimal growth of the fetus is dependent upon the supply of substrates such as glucose, amino acids and lactate from the mother. Foetal growth is limited by the mother's ability to supply these nutrients. The partitioning of nutrients between mother, placenta and fetus is facilitated by hormones such as insulin, growth hormone (GH), insulin like growth factors (IGFs), thyroid hormone, leptin, placental lactogen, progesterone, and ghrelin.

It is clear from the results of liveweight in the heifers that we achieved a significant difference in weight gain during gestation, and it is evident from the effect on birthweight and foetal growth that we were able to effect partitioning of nutrients to the fetus. This is the first study in bovines in which nutrition from conception has been manipulated and measured. Most other studies have waited until after pregnancy is confirmed before starting the dietary regimens (Perry et al., 2001; Café et al 2006, Greenwood et al., 2006). Diet during the first trimester has been little considered perhaps because of the length of time prior to calving and the references to the last trimester as being the most significant in determining calf birthweight (Ferrell et al., 1976; Tudor 1972). However, more detailed analysis of the effects of nutrition during gestation on foetal development in small ruminants has clearly shown that each stage of gestation is a window within which alteration of substrate supply can lead to quite different birth phenotypes with different post natal development, interestingly these effects are not always revealed in the gross measure of birth weight (Harding, 2001).

This study highlights the importance of nutrition in the first two trimesters of pregnancy in the development of the feto-maternal unit. Calves from heifers on high protein diet in the second trimester were 8.2% heavier than those who received low protein during this three month period. It is interesting to note that this is a similar difference in birth weight to that achieved by Café et al (2006) when cows were fed on a high nutrition diet for both the second and third trimesters. High birth weight calves are the primary cause of dystocia in heifers (Arthur et al., 2000).

While the mortality of large calves due to dystocia and its associated susceptibility to disease is well recognised (Basarab et al., 1993) the death of low birth weight calves is often overlooked. Mortality rates for low birth weight calves are reported to be similar to those of high birth weight (Holland and Odde, 1992) so the effects seen in the LL group should not be overlooked. However, with increasing selection of EBVs for growth, calf birth weight is increasing annually as the EBVs for yearling weight and calf birth weight are directly correlated. Estimates of the genetic correlation between direct effects on birth weight and yearling weight are 0.5 across breeds (Koots and Gibson, 1996). This study shows that this trend could be exacerbated by diet during gestation. The measurement of in utero foetal development was able to show that by day 39 low protein diets had effected foetal growth ($P=0.0001$) as measured by crown rump length. By mid gestation (day 123) nutrition in second trimester influenced calf size (cranial measures) regardless of first trimester protein levels. It is apparent that foetal growth responds rapidly to variations in maternal diet.

The effect of nutrition during the first six months of pregnancy on heifer liveweight persisted until weaning; however, by three months of age the liveweight of the calves did not differ between treatment groups. There was no relationship between the milk intake and calf growth. Obviously the effect of the low protein in the first trimester enhancing milk yield may have masked some of the effects in the lower birth weight calves enabling them to increase weight.

In a recent study Café et al (2006), calves from cows on high nutrition in the second and third trimester grew 16.7% faster than the low nutrition counterparts between birth and weaning.

However, there are considerable differences between the two trials apart from the different periods of gestation chosen the Café study used mature cows whereas heifers were used in this study. In the sheep there is considerable difference between the results of nutritional stress to adult ewes compared to adolescent ewes (Wallace et al., 2005). Also, the heifers in this trial did not lose weight during gestation- the low group were only gaining at 0.2kg a day often but this differs to the lows in the Café trial that lost approximately 40 kg over the last two trimesters.

Significantly there was only a 52kg difference between the extreme treatment groups (HH vs LL) at parturition and a 10kg difference between cross over treatments (LH vs HL). This compares to previous trials where differences in body weight at parturition were approximately 100kg (Café et al., 2006) or 40kg (Freetly et al., 2000) after high or low feed regimens in the second and third trimester. Tudor (1971) in a classic experiment where cows of various parity were fed a high ration to gain 64kg during the last trimester or to lose 37kg achieved a difference in body weight at parturition of 125kg. The difference in birthweight in the Tudor trial was 6kg or 22%. There was no significant difference between treatment groups in this study in BCS at parturition, although a significant difference existed in body weight between the two extreme treatments of HH and LL.

Winks et al., (1978) achieved a significant difference between high and low birth weight calves in weaning weight. This suggested that high birth weight calves were able to extract more milk and utilize it more efficiently than low birth weight calves. Other studies also reported that the weight of the calf in early lactation positively influenced the dam's subsequent yield (Byatt, 1994; Saner, 1988; Rutledge, 1971; Fiss, 1993; Minnick 2001). It has been suggested that the positive influence of calf's birth weight may arise from an increased placental lactogen secretion from heavier foetuses that in turn stimulates increased milk yield in the subsequent lactation (Saner, 1988; Mallinckrodt, 1993). In this trial however, there was no correlation between milk intake and birth weight despite placental lactogen being correlated with birth weight. Similarly Tudor (1972) found no correlation between birthweight and weaning weight, these latter results were from hand reared calves so post natal effects of a larger calf perhaps being able to suckle more were unable to be measured.

Boggs (1980) and Mieckle et al., 2004 found that cows in less than BCS 3 produced less milk than their counterparts but Savage (2005) considers that higher (>3) body condition score cows produced less milk as measured by weaning weight of their calves). In beef cows weaning weight and milk production are highly correlated (Freking, 1992; Miller, 1999), with milk quantity rather than quality being more important in its influence upon weaning weight (Rutledge, 1971). There is also evidence from the Freetly et al., (2002) trial that showed that low body weight heifers that had been restricted for the second and third trimesters produced similar quantity of milk to high body weight cows even whilst on a more restricted diet but once on an increased diet from 28-58days post partum the growth rate of their calves was 15% higher than in high body weight cows on the same ration suggesting that low body weight enabled diversion of nutrients to milk production over body maintenance requirements.

Notwithstanding, in this trial it is clear that nutrition during the first trimester affected milk intake with lower nutrition enhancing either milk production or the calves ability to extract more nutrients from the dam after birth. The difference in body condition between LL cattle in this trial was significant but despite no difference in BCS or liveweight between the LH and HL groups there was still a difference in milk intake.

The effect of nutrition in the first trimester on the development of mammary glands is still unclear. In cattle, mammary development is essentially complete at calving (Svenersten, 2005).

Mammogenesis (the proliferation of the mammary epithelium) is dependent upon oestrogen and progesterone.

Low protein in the first trimester reduced progesterone but did increase estrone sulphate levels and this may have been sufficient to induce greater ductal growth of mammary gland.

In the standard lactation curve in mammals the amount of milk increases with the increasing energy requirements of the offspring, peaks when the mother can no longer supply all the energy required by the calf and then slowly declines as milk is substituted by other food (Landete, 2000). Peak milk production has been reported at 45 days (Shell, 1995) and 60 days post partum (Williams, 1979). In this trial the initial estimate of milk production via weigh suck weigh was completed on 14/10/05 when the calves were approximately 74 days of age milk intake. Their peak production may have been prior to this as milk intake declined after this time.

In this study there was no effect of sex on milk output: other studies show an inconsistent effect. Dams nursing female calves produced significantly more milk than those nursing male calves (Ruttledge, 1971) opposite to later studies found that sex of calf had significant effect on milk yields at 3 to 4 months when milk production was significantly higher for male calves than female calves (McCarter, 1991, Saner, 1988).

Differences existed between genotypes in 'milk intake decline' in this study. Despite only an 1/8 (12%) infusion of Red Angus being the only difference between the heifers there was a significantly faster decline in milk intake in the calves from the Beefex cross heifers than the CBX cross heifers. Beef cattle breed has been shown to significantly effect milk yields (Gregory, 1992, Masilo, 1992, Freking, 1992).

Prior to calving, the calving pens were gravelled due to rain. The gravel unfortunately adhered to the placental tissue on expulsion such that is could not be removed even after cotyledon dissection. This differentially affected the weights of the placentae however a correlation between calf weight and placental weight was found (Anthony *et al*. 1986b; Echternkamp 1993; Zhang *et al*. 1999, Perry et al., 1999). In the cow, placental implantation occurs between days 20 and 28 (Wathes and Wooding, 1980) and definite adhesions between the foetal trophectoderm and uterine epithelia are present by day 20. Microvilli are forming and many giant cells are present in the uterine epithelia by day 24. By day 28, interdigitation between trophetoderm and maternial microvilli is complete. However, there is a paucity of literature on villus growth in the cow. Baur (1977) showed that a considerable difference in growth curve occurs between the sheep and the cow in that in the latter placentoma weight and surface area increases continually, whereas, in the sheep, the placenta attains most of its mass of dry tissue, protein, and DNA by mid-gestation (Ehrhardt and Bell, 1995). Perry et al., (1999) found a significant effect on placental development and increase in weight at term when nutrient restriction occurred in the first trimester in primiparous heifers. Rasby et al. found that placental weight was significantly greater in multiparous cows fed sub-optimal diets during mid to late gestation.

The previous studies show that low protein in the first trimester (diet regimen introduced at 36d of pregnancy) should significantly enhance placental growth and in this study low protein in the first trimester did enhance placentally produced bovine pregnancy associated glycoproteins (bPAG) but in the term placenta there was no effect of low nutrition on increased placental size. This may be because a) we could only collect the foetal portion of the expelled cotyledon and b) that the foetal portion of the placenta increases between days 231-271 (Ferrell, 1991). This growth is under foetal influence and may be a compensatory mechanism.

The Bos indicus cross (25% Brahman) genotype used in this study may also have effected the growth of the fetus and placenta, in comparison to previous studies which have only used Bos taurus animals. It has been shown in the classic experiment of Comerford et al., (1987) that in a diallel mating plan involving Simmental , Limousin, Poll Hereford and Brahman breeds, a large negative maternal additive effect for birth weight exists in the purebred Brahman female.

A further experiment (Morrison et al., 1989) using Brahman, Chianina, Maine Anjou and Simmental sires mated to Hereford/Angus females showed that the 50% Brahman cross cows were able to suppress the birth weight of their calves. Comerford et al. (1987) suggest that the Brahman female has some physiological mechanism in the maternal environment for reducing foetal growth rate regardless of paternal geno type and further that this was unique to the Brahman female in their study. Further, Morrison et al. state the ratio of calf birth weight to pre-calving cow weight and the ratio of calf birth weight to pelvic area was significantly smaller in the 50% Brahman females. Whether such physiological mechanisms occur in 25% Brahman females is unclear. Despite the strong effect on IGF-I levels we achieved in the heifer dams we did not achieve the difference in calf IGF-I that we achieved in our previous study. The strong correlation between IGF-I and growth rates found in this study may partly explain why the weaning weights were not different in this breed type.

5.1 In Summary

Significantly, this trial differs from the previous experiments, in that heifers that were at 66% (330kg) of mature body weight, rather than mature cows, were used. It is increasingly common in Northern Australia to aim to mate heifers at 14-15 months of age at 65% of their mature body weight to enable calving at two years of age. The birth weights of first parity progeny in calves and lambs are 10-15% lower compared with offspring from mature dams (Bellows and Short, 1978, Wu et al, 2006). This can be explained by the fact that mother and fetus grow substantially and compete for nutrients during pregnancy (Redmer et al, 2004).

It has been proposed that nutrient restriction in early pregnancy would not affect foetal development in the cow as placental growth continues throughout pregnancy, unlike the sheep, and further that maximal weight increase occurs in the last trimester. However, this study shows that, in the growing heifer, nutritional demands of the fetus must compete with the requirements of the heifer such that the placenta is unable to compensate for early nutritional stress, resulting in phenotypic changes in the fetus.

In a previous trial where a small number of Hereford heifers were mated at 270kg (58% of mature body weight) at 15 months of age the effects of nutritional restriction in early pregnancy were reflected in significantly lower weaning weights and carcass muscle to fat ratios. In this study the differences in weight at birth were not maintained to weaning either due to increased milk intake or some physiological mechanism that allowed the low birthweight calves to catch up.

These studies in combination infer that a) maturity of the dam could affect the level of intrauterine growth restriction that occurs (ie. there would be more competition for nutrients in the 14-15month heifers than in 26-27month old heifers and subsequently greater phenotypic effect on the calf of any intrauterine growth restriction) and b) that the stage of gestation at which nutritional restriction occurred could affect foetal development differently, that is, a different phenotype was produced. Clearly, in the sheep this is known to be the case. There are discrete windows during gestation at which nutritional intervention causes distinct developmental changes resulting in characteristics phenotypes at birth (McMillen and Robertson, 2005).

The nutrient intake, in particular protein, of unsupplemented grazing heifers during the winter months in Northern Australia is often inadequate to support optimal reproductive performance, such that foetal growth and lactational performance are seriously compromised (Patterson et al, 2003). Further, in tropical and subtropical Australia high environmental temperatures reduce feed intake by pregnant dams grazing extensive pasture. This thermal stress is sufficient to cause intra uterine growth retardation (Reynolds et al., 1985).

We have clearly shown that mild protein restriction for short periods during gestation can alter birth weight. Birth weight is significant because of its relationship post natal growth and dystocia.

a) Post natal growth has been shown to be affected by birth weight in cattle. Guerra-Martinex et al (1990) showed that the efficiency of feed utilization is lower for twins than singletons, and Cundiff et al, (1986) and Café et al., (2006) found that low birth weight calves grew more slowly than high birth weight calves.

b) High birth weight of the calf is the major cause of dystocia in extensively managed beef herds (Arthurs et al 2000)

6 Success in achieving objectives

Objectives of the experiment were:

1. To examine the effect on weaning weight of targeted protein supplementation at critical times during pregnancy

Pre calving nutritional treatments had no effect on final weaning weight but treatments did affect weaner development, and lactation of the dam differentially

2. To establish a recommended nutritional regimen for primiparous heifers and a detailed understanding of the effects on the calf, dystocia levels, and weight gains.

Nutritional regimen in the second trimester significantly affected calf birthweight and thereby associated dystocia levels. It is clear that the nutritional regimens influenced placental development, calf growth in utero, lactation and initial weight gain pre-weaning, although not at weaning. Based on our findings, high protein maternal diets should be avoided during both the first and second trimester of gestation. This recommendation is aimed at firstly minimising the risk of delivering a high birth weight calf to minimise the risk of dystocia and secondly, to maximise milk yield to promote pre-weaning growth. Whilst it is possible to directly manipulate pasture protein content using pasture composition and nitrogenous fertiliser compounds, it would be difficult to directly manipulate protein content in a downwards direction in a grazing situation. Practical application of these recommendations would be:

- *preferential allocation of paddocks with known lower legume content to pregnant heifers;*
- *intensify the grazing system to limit total dry matter and thus protein intake per day;*
- *in the event of unseasonal winter rainfall in a mixed farming system, allocate any crop paddocks with standing stubble that have been left out of cropping rotations to pregnant heifers;*
- *in a mixed farming system with sheep, graze heifers behind sheep so that sheep preferentially graze legumes first;*

- *feed cereal straw, although this is unlikely to be cost effective when labour is taken into account; and*
- *avoid supplementation of pregnant heifers above 14% CP.*

3. To determine the relationship between dietary protein intake during pregnancy with muscle development, reproductive organ development and weaner liveweight gain.

Dietary protein in utero affected reproductive organ development in the female offspring.

4. To establish the effects of diet during pregnancy on IGF levels in the weaner.

IGF concentrations were significantly increased by increased protein treatments in these heifers, however the nutritional treatments in utero did not affect IGF -I levels in the calf. The IGF -I levels in the calves were significantly positively associated with growth rate ($p<0.001$).

6.1 Impact on the meat & livestock industry – now & in five years time

It is estimated that the cost of dystocia to the Australian national herd is, $48 million annually (Howard et al.,1993). Dystocia is often thought to be a problem of British breed heifers but this study shows that significant losses could also be occurring in Northern Australian composite herds. Indeed, losses between pregnancy diagnosis and weaning in North Queensland heifer herds have been recorded at 20% (Fordyce and Burns, 2007). A study based on cattle prices in 1995 found that in southern Queensland, for each percentage decrease in the dystocia incidence, the increase in gross margin was $0.13 per hectare. For the average sized beef cattle property in the study (2090 hectares), this represented an increase in the annual total gross margin of approximately $272 for each percent decrease in the heifer dystocia rate. These figures may assist decisions on how much should be spent on dystocia control methods. For example, reducing the dystocia incidence by 10% on an average sized southern Queensland property described above would provide approximately $2,720 extra annual income (Norman, 2006). The number of graziers in Northern Australia reducing age at calving to two year of age is increasing (Bortolussi et al, 2005). The difference between management regimens between three year old and two year olds in the effects of nutrition during pregnancy should be further examined and appreciated by industry advisors in Northern pastoral areas.

This study shows that protein supplementation during early pregnancy in the heifer significantly affects foetal growth in terms of calf birth weight and foetal development in utero. The nutritional regimen we implemented could increase dystocia in *Bos indicus* cross heifers calving at three years by 3 times. We have also shown that pelvic area measurement pre joining could be a useful tool in the reduction of levels of dystocia.

It is apparent from comparisons between this study and others in the published literature that:

a) the greater the immaturity of the heifer in comparison to the final body weight of the cow eg 58% compared to 65% -the greater will be the effect of nutritional restriction during pregnancy on calf growth and development;
b) the timing of the nutritional restriction during pregnancy may affect the phenotype of the calf produced- eg lower diet in the last trimester may reduce birth weight more significantly but not weaner growth rates whereas restriction in the second trimester may reduce birth weight and postnatal growth eg reproductive organ development in the heifer;
c) nutrition during early pregnancy can significantly effect lactation in the heifer;

d) genotype of the heifer- particularly the inclusion of *Bos indicus* genetics may alter the effects of foetal programming.

Although this trial was unable to show an effect of nutrition in second trimester on growth rate to weaning previous studies by Perry (unpublished data) and by Café (2006) showed a response. The effects of in utero nutrition on carcass characteristics will be available on completion of the work on gene expression in marbling and muscle factors.

7 Conclusions and Recommendations

Conclusions

- ✓ Nutrition in the first and second trimester significantly effects foetal growth in the pregnant 2 year old heifer and calf birth weight but did not have any significant effect on weaning weight.

- ✓ The level of maturity or body weight, as a function of mature body weight at mating, influences the effect of nutritional insult i.e. the lower the body weight of heifer at mating compared to mature cow weight the greater the effect of nutritional stress. It is thought that this phenomenon occurs due to the ability of the mature cow to compensate in late pregnancy by increasing placental growth whereas the increased competition for nutrients in the growing heifer means the placenta receives insufficient nutrients for compensatory growth to occur.

- ✓ Nutrition in early pregnancy significantly effected milk intake in the calf.

- ✓ The effects of nutritional stress on postnatal growth are influenced by timing of nutritional insult and parity of the dam.

- ✓ Dystocia in an industry wide economic problem affecting both *Bos taurus* heifers calving at two years of age and *Bos indicus* cross heifers calving at three years of age.

Recommendations

1) Research into the effect of nutritional stress during pregnancy on *Bos indicus* cross heifers calving at two years of age. In reporting the results of the study to RBRCs it is apparent that more producers in Northern Australia are moving to calving at two years of age.

2) Survey of producers in Northern Australia on exact year of age at calving of heifers as the current surveys do not detail this.

3) Further study into the effects of nutritional stress on distinct stages of gestation such that the effect of one stage only is seen in the postnatal growth of the calf. In the light of recent study in the sheep, nutrition immediately prior to conception should be included in this.

4) Study into the effects of nutrition during early gestation on milk yield as well a weaner growth should be completed in two year old and three year old beef heifers. The information from this study on the effects of nutrition during gestation on milk production in beef cattle is unique and justifies further study.

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9 Appendices

9.1 Appendix 1 Statistical Method

Heifer liveweight during gestation

A linear mixed model was used to analyse liveweight variables with estimation using the method of residual maximum likelihood (REML) in GenStat. The random model was simply the residual variation between heifers. The full model for the fixed effects for liveweight at the end of the first trimester and ADG during the first trimester was:

T1 + dam breed + birthdate + dam wt

where T1 is a factor with 2 levels for nutrition treatment in the first trimester (high, low)

Dam breed is a factor with 2 levels for the breed of the dam of the heifer ;Beefex (Senepol sire with dam of half Brahman, ¼ Charolais, ¼ Red Angus), CBX (Senepol sire with dam of half Brahman half Charolais)-

Birthdate is a covariate - the date of birth of the heifer (reflects age of the heifer)
Dam wt is a covariate - the weight of the heifer on 26/9/04

The full model for the fixed effects for liveweight at the end of the second and third trimesters and ADG during the these trimesters was;T1 * T2 + dam breed + birthdate + dam wt where T2 is a factor with 2 levels for nutrition treatment in the second trimester (high, low)

T1 * T2 represents the main effects of T1 and T2 and their interaction

Non-significant (P>0.05) fixed effects were removed from the model while observing the principle of marginality (ie if an interaction is significant, the main effects for that interaction remain in the model) to arrive at the final model. Results are presented as means ± SE for significant effects. Means for T1, T2 and dam breed are always presented. Additional effects are presented if they were significant.

For all variables analysed, birthdate was not significant. Significance of dam breed, nutrition treatments and heifer liveweight on 26/9/04 for all variables is shown in Table 1. Table 2 presents predicted means ± SE for dam breed and nutrition treatments and the regression coefficient for the covariate of heifer weight on 26/9/04 (when significant).

Heifer liveweight after calving until weaning

Heifers were weighed and condition scored 7 times between 25/8/05 (immediately after the calving period finished) and 18/2/06. A repeated measures linear mixed model was used to analyse heifer weight and body condition score post-calving, with estimates using the method of residual maximum likelihood (REML) in GenStat. Since the time points of the repeated measures were not equally spaced, the covariance models investigated for modelling the residual variance (heifer.time) were antedependence orders 1 and 2, power and unstructured models. The full model for the fixed effects is shown below.

(T1 + T2 + T1.T2 + dam breed) + time + T1.time + T2.time + T1.T2.time + dam breed.time

where T1 is a factor with 2 levels for nutrition treatment in the first trimester (high, low)

T2 is a factor with 2 levels for nutrition treatment in the second trimester (high, low)

T1.T2 is the interaction between T1 and T2

Dam breed is a factor with 2 levels for the breed of the dam of the heifer (Beefex, CBX)

Time is a factor representing days since 25/8/05 with 7 levels (1, 23, 51, 80, 109, 138, 178)

Likelihood ratio tests were used to determine which model provided the best fit to the residual variance structure. Once the best model was determined, the fixed effects were assessed using Wald tests. Non-significant (P>0.05) fixed effects were removed from the model while observing the principle of marginality (ie. if an interaction is significant, the main effects for that interaction remain in the model) to arrive at the final model. Results are presented as means ± SE for significant effects, except that the main effects of dam breed, T1 and T2 were always retained in the model.

The best model for describing the correlation between times was with an antedependence order 2 structure ie for each heifer the liveweight at any time was related to the liveweight from the previous 2 times. The final fixed effects model was dam breed (P=0.773) + T1 (P=0.003) + T2 (P<0.001) + Time (P<0.001).

Body condition score

The best model for describing the correlation within heifers across times was an unstructured model ie this allows for changes in variability over times and varying correlation between times. The final fixed effects model was dam breed (P=0.010) + T1 (P=0.898) + T2 (P=0.431) + time (P<0.001) + dam breed.time (P=0.017).

Calf liveweight until weaning

A linear mixed model was used to analyse calf birth weight, calf weaning weight and average daily gain (ADG kg/day) between birth and weaning with estimation using the method of residual maximum likelihood (REML) in GenStat.

Calf IGFs

All statistical analyses were carried out using the statistical software, Stata SE Version 9.2 (Stata Corporation, College Station, TX). The variables IGF-I and total IGFBPs were transformed to their natural logarithm based on visual inspection of the density distribution plots of the values of the continuous outcome. Univariable linear models were used as screening tests to determine the relationship between nutritional treatment (nutritional treatment in the first and second trimesters and their interaction term), calf gender and gestation length (to account for the 3 week age distribution of calves) and offspring plasma concentrations of IGF-I, -II, total IGFBPs using animal ID as a random effect to account for the repeated measurement design of the study. General linear models (GLMs) were then developed using these same effects and outcomes, again using animal ID as a random effect to account for the repeated measurement design of the study. Biologically meaningful first-order interactions were assessed for statistical significance. Associations of progeny liveweight and average daily gain (AGD) at each sampling event with the respective plasma concentrations of IGF-I, -II, total IGFBPs were assessed using Pearson and Spearman correlation analyses.

Associations between birth weight and the above named factors within each treatment group were also assessed. Significance was accepted at $P < 0.05$.

Milk Intake

Milk intake of calves over time was subjected to a repeated measures analysis using the method of residual maximum likelihood (REML) in GenStat. A range of covariance structures for the residual variance calf.time, where time is a factor with 5 levels, were tested to model the correlation between times for individual calves. These were compared using likelihood ratio tests to determine the best fit, with an antedependence model of order 2 being the preferred covariance structure. The full model tested for the fixed effects was

(T1 * T2 * calf sex + dam breed) * Time + calf DOB + calf birth wt

where T1 is a factor with 2 levels for nutrition treatment in the first trimester (high, low)
T2 is a factor with 2 levels for nutrition treatment in the second trimester (high, low)
Calf sex is a factor with 2 levels (female, male)
Dam breed is a factor with 2 levels for the breed of the dam of the heifer (Beefex, CBX)
Time is a factor with 5 levels (74, 101, 132, 162 and 199 days since the start of calving)
CalfDOB is a covariate, the date of birth of the calf (reflecting calf age at the start of suckling) calf birth wt is a covariate

Non-significant (P>0.05) fixed effects interactions and covariates were removed from the model while observing the principle of marginality to arrive at the final model.

Milk Protein and Fat

Statistical analysis was identical to that for milk intake except there were only 3 measurements (132, 162 and 199 days since the start of calving). For milk protein and fat%, the best covariance structure was an antedependence model of order 1.

Placental measures

A linear mixed model was used to analyse placenta variables with estimation using the method of residual maximum likelihood (REML) in GenStat. The random model was simply the residual variation between heifers.

The full model for the fixed effects for all variables was;

T1 * T2 * calf sex + dam breed + birthdate + dam wt

where: T1 is a factor with 2 levels for nutrition treatment in the first trimester (high, low)
T2 is a factor with 2 levels for nutrition treatment in the second trimester (high, low)
Calf sex is a factor with 2 levels (female, male)
Dam breed is a factor with 2 levels for the breed of the dam of the heifer (Beefex, CBX)
Birthdate is a covariate - the date of birth of the heifer (reflects age of the heifer)
Dam wt is a covariate - the weight of the heifer on 26/9/04
T1*T2*calf sex specifies all interactions between these 3 factors

Non-significant (P>0.05) fixed effects were removed from the model while observing the principle of marginality (ie if an interaction is significant, the main effects for that interaction remain in the model) to arrive at the final model. Results are presented as means ± SE for significant effects. Means for T1, T2 and dam breed are always presented. Additional effects

are presented if they were significant. A log transformation was applied to cotyledon number and total cotyledon surface area prior to analysis to stabilise the variance. Some placentas were partially eaten or foetal membranes were retained. In these cases (n=8), the data were regarded as missing for all variables except gestation length. The relationship between calf birth weight and both average and total cotyledon surface area was investigated. Initially influence of calf sex on the relationship was tested and as this was absent, simple correlations were completed.

Calf hip height from birth to weaning

Hip height of calves over time was subjected to a repeated measures analysis using the method of residual maximum likelihood (REML) in GenStat. A range of covariance structures for the residual variance calf.time, where time is a factor with 5 levels, were tested to model the correlation between times for individual calves. These were compared using likelihood ratio tests to determine the best fit, with an antedependence model of order 2 being the preferred covariance structure. The full model tested for the fixed effects was:

(T1 * T2 * calf sex + dam breed) * Time + calf DOB

where T1 is a factor with 2 levels for nutrition treatment in the first trimester (high, low).T2 is a factor with 2 levels for nutrition treatment in the second trimester (high, low). Calf sex is a factor with 2 levels (female, male).Dam breed is a factor with 2 levels for the breed of the dam of the heifer (Beefex, CBX).Time is a factor with 5 levels (24, 38, 74, 132 and 199 days since the start of calving)

CalfDOB is a covariate for date of birth of the calfNon-significant (P>0.05) fixed effects interactions were removed from the model while observing the principle of marginality to arrive at the final model.

Statistical analyses of hormone levels during gestation

Data were analysed using Intercooled Stata 9.0 (StataCorp, College Station TX 77845, Texas, USA) software. General linear models (GLMs) were developed to test the effect of maternal genotype, calf gender, calf birth weight, calf crown rump length at birth, heifer age, weight at corresponding stage of gestation, nutritional treatment, IGFs and leptin on progesterone, ES, bPAG and bPL concentrations.

In addition, GLMs were then developed to test the effect of maternal genotype, calf gender, gestation length, nutritional treatment, heifer age, progesterone, ES, bPL and bPAG on two calf outcomes measured at birth: calf weight and crown rump length.
Non-significant effects were removed from the models in a backward stepwise manner until all remaining effects were significant (P<0.05). Model checking included inspection of residuals and leverage, Cook's distance and difference of fits (DFITs) statistics (Doohoo et al., 2003). Any outliers or influential covariate patterns identified were removed from the data set and analyses repeated to check for the effect of these data points. Follow-up tests were performed to investigate significant effects and to investigate *a priori* defined comparisons involving treatment group means.

Comparisons were made between a single treatment group and all other treatment groups, and between combinations of treatment groups with commonality in first and/or second trimester treatment groups for measures on days 179 and 271. Comparisons were made between first trimester treatment groups only for days 28 and 82.

Outcome variables were transformed using natural logarithms to normalise a right-skewed distribution. In these situations marginal means derived from statistical models are presented as geometric means and confidence intervals in order to present results in the original scale. All other results are reported as the mean +/- standard error (SE) of the mean. Statistical significance is reported at $P<0.05$.

Statistical analyses of male reproductive organs and hormones

Data were analysed using Intercooled Stata 9.0 (StataCorp, College Station TX 77845, Texas, USA) software. Paired ttests indicated no difference between left and right testicle lengths and widths, so averages were used. General linear models (GLMs) were developed to test the effect of maternal genotype, calf gender, calf birth weight, calf age, heifer age, nutritional treatment, IGF-I and leptin on FSH, LH and testosterone pre- and post- GnRH. In addition, GLMs were then developed to test the effect of maternal genotype, calf gender, calf birth weight, calf age, nutritional treatment, calf weight at 5 months of age, IGF-I, leptin, FSH, LH and testosterone concentrations on testicle measures (average tubule diameter, testicle length, width, length:width, and total testicular weight and volume). Non-significant effects were removed from the models in a backward stepwise manner until all remaining effects were significant ($P<0.05$). Model checking included inspection of residuals and leverage, Cook's distance and difference of fits (DFITs) statistics (Doohoo et al., 2003). Any outliers or influential covariate patterns identified were removed from the data set and analyses repeated to check for the effect of these data points. Follow-up tests were performed to investigate significant effects and to investigate *a priori* defined comparisons involving treatment group means. Comparisons were made between a single treatment group and all other treatment groups, and between combinations of treatment groups with commonality in first and/or second trimester treatment groups.

Outcome variables were transformed using natural logarithms to normalise a right-skewed distribution. In these situations marginal means derived from statistical models are presented as geometric means and confidence intervals in order to present results in the original scale. All other results are reported as the mean +/- standard error (SE) of the mean. Statistical significance is reported at $P<0.05$ and tendency at $P<0.10$.

Statistical analyses of Dystocia

Normality of data was examined by inspection of distribution plots and associations between maternal factors screened using Pearson correlation analyses. Two-way ANOVA were used to explore differences between trimester treatment groups and their interaction term for maternal factors potentially associated with the occurrence of dystocia. Odds ratios (OR) to estimate the association between dystocia and maternal and calf factors were derived using multivariable logistic regression models. Statistical significance was set at $p < 0.05$, and data are expressed as means ± SEM unless otherwise stated. Data was analysed using Intercooled Stata 9.0 (StataCorp, College Station TX 77845, Texas, USA) software.

9.2 Appendix 2 Results of ovarian work on female progeny

At slaughter (23 months of age) both heifer ovaries were collected, measured and weighed. For each heifer, and for each ovary, there were in most cases, multiple slides. These were scanned using the NanoZoomer Digital Pathology Scanner (Hamamatsu Photonics KK).

Antral follicle counts The total area of each section from which counts were made was measured using the NanoZoomer software. Maximum and minimum diameters of the antral follicles were measured using the software, and these values were averaged to give an average

size of the antral follicles. These follicles were then classified as either healthy or atretic, based on the shape of the granulosa cells and the presence of pyknotic nuclei.

The total number of follicles counted per animal was also recorded, and percentages of healthy and atretic follicles over total follicles were calculated per cow.

Table 19. Ovarian Follicle Counts

Group	Animals	Total ovary weight (g)	Area mm^2 (mean ± SEM)	Total follicles (n)	Healthy follicles (n)	Healthy follicles (%)	Atretic follicles (n)	Atretic follicles (%)
HH	8	18.3 ± 3.3 [a]	385 ± 65	15 ± 4	4.9 ± 2.4	27.4 ± 4.4 [a]	10.3 ± 2.4	72.8 ± 4.4 [a]
HL	9	27.5 ± 2.8 [b]	458 ± 77	18 ± 6	7.9 ± 2.7	46.7 ± 7.1 [b]	9.7 ± 4.0	53.1 ± 7.1 [b]
LH	7	19.1 ± 3.6 [a]	530 ± 82	14 ± 3	5.0 ± 1.5	38.1 ± 8.3 [a,b]	8.7 ± 1.9	61.9 + 8.3 [a,b]
LL	11	19.3 ± 2.1 [a]	438 ± 57	17 ± 3	7.6 ± 3.3	52.8 ± 6.3 [b]	9.1 ± 2.1	47.3 ± 8.1 [b]

Group	Healthy follicle density ($n/100mm^2$)	Atretic follicle density ($n/100mm^2$)
HH	1.0 ± 0.3 [a]	2.5 ± 0.6
HL	1.5 ± 0.3 [a,b]	2.0 ± 0.5
LH	0.9 ± 0.2 [a]	1.6 ± 0.3
LL	1.7 ± 0.1 [b]	2.0 ± 0.3

Values with different superscripts differ at $P<0.05$
Area = area of whole section

The average diameters of the antral follicles were distributed comparatively across all four groups. Whilst the total number of follicles sampled from each group differed, the percentages of each approximate size were similar. For instance, the percentage of antral follicles with average diameter of less than or equal to 1mm ranged from 8 to 13 percent across all groups. Between 20 and 30 percent of antral follicles sampled in all four groups had an average diameter of between 1 and 2 mm. Between 30 and 40 percent had a diameter of between 2 and 3 mm, 10 to 15 percent were between 3 and 4 mm, and 3 to 6 percent were between 4 and 5mm. 9 to 12 percent of the antral follicles had an average diameter of greater than 5 mm. The Duncan test on the total ovarian weight per cow showed that HL differed significantly to the other groups, with the highest weights.

The final collation of data included values per heifer for total slide area, total follicles counted, number, percentage and density of healthy follicles, number, percentage and density of atretic follicles and total ovarian weight. Data was analysed using SPSS Data Editor Software (version 15) and ANOVA and Duncan post hoc test. At a significant level of 0.05, it was found that the total section area, total follicles, number of healthy follicles and number and density of atretic follicles per heifer were not significantly different across the groups. However, it was found that the percentage of healthy follicles in the HH group (27%) was significantly lower than that of the HL group (47%) and LL (53%). The heifers in the LL group had the highest healthy follicle density, and this was significantly greater than HH or LH.

9.3 Appendix 3 Media Releases

ABC Regional Radio Longreach July 2004 Manipulation of protein during gestation and weaner weights

Dalby Herald 4/2/05 Study looks at increasing weaning weights

Queensland Country Life 20/1/05 Test of protein power

Rural Weekly 14/1/05 Research to produce beefier cows

Mackay Bush Telegraph 1/2/2005 Beefing up those kilos

UQ News February 2005 Increasing profits for beef Farmers

9 Win TV News January 2005 Protein in pregnant beef cows.

9.4 Appendix 4

Conference Proceedings

NGED ARCnetwork Congress 2005 Adelaide
NGED ARCnetwork Congress 2006 Cairns
Society for Reproductive Biology Conference, Gold Coast, August 2006.
AVA conference 2008 Perth Australia

9.5 Appendix 5 published papers

"Dietary protein during gestation affects hormonal indicators of placental function and fetal development in heifers" T.M. Sullivan, G.C. Micke, R.S. Magalhaes, G.B. Martin, C.R. Wallace, J.A. Green, **V.E.A. Perry** (2009) Placenta; in press

"Dietary protein and energy during gestation affects maternal IGF, IGFBP, leptin and fetal growth in heifers" T.M. Sullivan, G.C. Micke, N. Perkins, G.B. Martin, C.R. Wallace, K.L. Gatford, J.A. Owens, **V.E.A. Perry** (2009) J.Anim.Sci (prov.accepted).

"Heifer nutrition during early- and mid-pregnancy alters fetal growth trajectory and birth weight" G.M. Micke, T.M. Sullivan, S. Norman S, P. Rolls, **Perry VEA** Anim.Reprod.Sci (2009) **;**in press

"**Dietary protein during gestation affects placental development in heifers**". Theriogenology Sullivan TM, Micke GC, Magalhaes RS, Phillips NJ, Perry VEA 2009;in press.

"**Dietary manipulation of *Bos indicus X* heifers during gestation affects the reproductive development of their heifer calves**." Sullivan TM, Micke GC, Greer RM, Irving-Rodgers H, Perry VEA Reproduction, Fertility and Development 2009;in press.

Dietary manipulation of *Bos indicus X* heifers during gestation affects the prepubertal reproductive development of their bull calves Sullivan TM, Micke GC, Greer RM, Perry VEA.. Anim Reprod Sci 2009; submitted.

Fibrillins and latent TGF_ binding proteins in bovine ovaries of offspring following high or low protein diets during pregnancy of dams Mark J. Prodoehla, Helen F. Irving-Rodgers,Wendy M. Bonner, Tracy M. Sullivan, Gina C. Micke, Mark A. Gibson, Vivienne E. Perry, Raymond J. Rodgers Mol Cell Endocrinol 2009; in press.