The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Increasing lifetime productivity of the heifer:

The role of nutrition during pregnancy upon heifer lifetime productivity within the herd

Dr Viv Perry
Dr Giuliana Miguel-Pacheco
Dr Robert Robinson
Dr Jennifer Edwards
Dr Wendela Wapenaar
Dr Juan Hernandez-Medrano

AHDB Beef & Lamb Funded Project

FINAL REPORT

July 2016

1. EXECUTIVE SUMMARY

Nulliparous maiden heifers (n=188) from 3 farms (A, B, C) were mated to calve at two years of age using fixed timed AI. At farms A (n=44) and B (n=44) heifers were randomly assigned to two diets; high (18%CP) or low (10%CP) 60 days prior to AI. At farm C heifers were randomly assigned to high (14%CP) or low (10%CP) protein diets for 90 days post-conception (dpc) at pasture.

The high protein diet (above NRC recommended intake) tended to decrease ADG of the heifer during the treatment period, however, post calving there was a trend for less weight loss in those heifers receiving high protein treatment either post or pre-conception. In the post-conception group (C) fetal growth was retarded by high protein though only at 36dpc, commensurate with a trend for increase cortisol. As we have recently found in concurrent Australian studies blood flow to the male feto-placental unit was increased compared to female. The combined data from the UK and Australian studies has been published (Hernandez-Medrano et al., 2015). In farm B, calving difficulty increased in those heifers receiving the low protein diet pre-conception followed by increased ADG in late pregnancy and was associated with an increased calf birth weight, length and leg bone size.

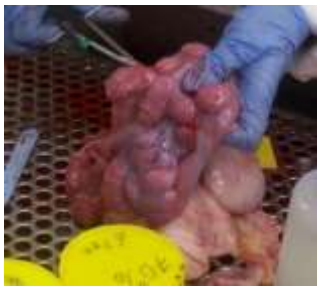
The differing effects in calf size between B and A may have been affected by the different weight gains of the heifers: At site B heifers did not gain weight during the treatment period (2mth prior to AI) whereas site A heifers gained weight at 0.6-1.0kg/d. There were further differences during the pregnancy period. Both groups of heifers were turned out onto pasture after AI. The Farm A group lost weight only during the month of turn out then steadily gained averaging 1kg/d over summer until dropping to approximately 0.7kg at 7mth of pregnancy. In this group there was then effectively weight loss during the final two months of pregnancy at -0.11kg/d for the high group and 0.29 kg/d for the low group as fetal requirements require a gain of 0.4kg/d; (fetal gain requirement being 8MJ/d at 6mth, 11MJ/d at 7mth, 15MJ/d at 8mth and 20MJ/d at 9mths). The poorer straw diet instituted at this site is often used on farm in an attempt to reduce birthweight and dystocia. Some drawbacks may however occur; heifers must rebreed within 85d of calving to maintain 1 calf per 365d. Heifers calving at BCS 2 rather than BCS 3 may cause an increase the days to first cycling from 40 to 90d post calving. Heifers that calve early in the breeding season will continue to do so for the rest of their life e.g. heifers that calve within the first 44d of the calving period have subsequent fertility of 91%. These effects upon conception rates may not be observed in herds that maintain long joining periods and pregnancy test only prior to

autumn housing. It is accepted, however, that BCS below 3 will reduce conception rates (40%) in a subsequent 6-week breeding season (MLA, 2006).

It is interesting to note that the precalving feeding regimen may have influenced the post calving weight as the A heifers were still losing weight at 3mth post partum and did not regain precalving weight until 5mth post calving.

In the B group there was considerable variation in ADG during pregnancy. There was a relative increased in ADG immediately on turnout to 0.5kg/d compared to the housed diet and then an apparent further increase in weight at 2mths in the low group to 1.5kg/d, a lack of scale accuracy until 7mth gestation at this site prevents examination, however, all the metabolites were lower than at Farm A from 3 to 6mths of pregnancy indicating a reduced dietary intake during this period with associated reduction in ADG. At 7mth the low group were gaining at >1.0kg/d whereas the high at 0.6kg/d. We know from previously published work that high rates of gain in the second trimester of pregnancy can increase birthweight (Micke et al., 2010b) as can increased rates of gain in the second and third trimester (Cafe et al., 2006) and this may have affected the increase in birthweight and calf length in this low group rather than the treatment period. Further interpretation of metabolic status will be produced from the blood metabolite analysis of these animals during treatment and pregnancy. During the last two months of pregnancy the ADG in these heifers was 0.5kg/d.

Increasing intake in the midterm of pregnancy sufficient to increase BCS one point subsequent to low intake in early pregnancy however, significantly increased calf birthweight and rates of dystocia.



This resulted from 33% overgrowth of the term placenta in these heifers whereby the placenta grew in early pregnancy enabling sufficient nutrient supply to the fetus but as this period was then followed by increased nutrition in mid pregnancy this large placenta continued to uptake nutrients at the same rate thereby causing an increase in calf birthweight at term. We have previously shown the same effect in a larger group of 2yo calvers where increased protein mid gestation increased birthweight by 8% (3kg).

The C Stabiliser heifers were much smaller at the start of the trial (approximately 280kg) compared to B (300kg) or A (350-400kg) with a lower mature weight (500kg) compared to the South Devon (700kg) at sites A and B. The low protein group tended to have higher weight gain than the high, perhaps due to the mode of delivery of the pellet whereby the

treatment group were keen to chase the daily feed trails, this was further reflected in the trend for higher cortisol levels in this cohort. Progesterone levels were increased however in the high protein group during pellet supplementation. Body weight in these heifers steadily rose throughout pregnancy to reach approximately 550kg at calving.

In Farm A we noted an increase in progesterone associated with the high protein diet. The low level of pregnancy rate however may have masked any effect upon pregnancy rate that we have previously observed (Copping et al., 2016) with increased periconception progesterone levels. To further investigate the physiology behind this effect; in Farm D, we used Angus cross 2yo heifers on 14.6%CP vs 10.5%CP protein diets to examine the effect of dietary protein on follicular dynamics. Increasing protein increased circulating progesterone as well as antral follicle count. High progesterone is directly linked to embryo survival, as it is critical to the production of a satisfactory oviductal and uterine milieu permitting elongation and production of Interferon tau (the pregnancy recognition signal). Studies have shown that low P4 concentrations during early stages of embryo development prior to pregnancy recognition, reduce fertility (pregnancy rate) in cattle, mainly by increasing early embryo mortality.

The associated increase in antral follicle count may increase embryo survival as; prior to 8-cell stage, development is dependent upon the quality of the initial fertilized oocyte, which in turn is dependent upon the proteins and mRNA synthesised during the preovulatory stage of oocyte by ancillary follicles. After the 8-cell stage, the embryo genome is activated and is able to produce its own mRNA and thereby proteins and enzymes enabling regulation of its own survival.

An economically interesting finding from the study in Farm D was that the heifers on the high protein diet also had carcasses of higher quality and reached slaughter weight at a younger age. 35% of the high protein fed heifers made the R grade compared to 19% from the low diet.

Suckling behaviour in the calves was increased in those animals from mothers that received low protein pre-conception diet; supporting previous findings that low protein diet in early pregnancy programs increased neonatal appetite via alteration of hypothalamic expression of orexigenic neuropeptides and anorexigenic precursors. Further, leptin and IGF levels were significantly increased in association with suckling behaviour. As leptin has direct feedback into these brain appetite centres this is an expected corollary. It is of interest that the low protein female progeny gained at the fastest rate in all farms though this was not at a

significant level on two farms, which may have been affected by low numbers in these groups.

There was no effect of diet or farm upon immunoglobulin level in the colostrum in B or A farms completed to date despite supplementary dietary regimens in the months prior to calving aimed at enhancing these levels (A). This effect may have been negated by the feeding of straw and low protein hay at A compared to silage feeding at B as such a regimen may (McGee et al., 2006; Mee et al., 2008) reduce immunoglobulin levels in the colostrum as well as in calf serum.

Milk production from the one farm (B) on which WSW was performed showed that maiden beef breed heifers conservatively produce 15kg of milk per day at one month falling to 10kg by 3mth. This compares to a maximum yield of 10kg per day in *Bos indicus* heifers in Australia and Angus x Fresian in New Zealand previously recorded. No associations were observed between prolactin blood levels with milk production, and no differences were observed between diet groups for prolactin at calving and after calving.

Although progesterone levels were increased in heifers on Farms A, B, C in the high protein versus low protein groups post calving progesterone levels did not rise until 3months post partum in either Farm A or B signalling that these heifers were not cycling. This indicates that nutrition prior to calving was insufficient to allow rebreeding within 80 days of calving as shown in the graphs of their BCS prior to calving being between 2.5 to 2. This late return to cycling is economically important to the farmer as rebreeding later than 80 days is insufficient to allow one calf to be produced per cow per year. Thereby the calving pattern of the herd will fall later and become more disparate each year. BCS did not rise above 2.5 until 4-5mths post partum. The cause of this late return to cyclicity can be observed in the graph of leptin levels. This hormone is produced by fat cells and acts as a signal to the brain that the cow is ready to reproduce. Sufficient levels are essential for the initiation of progesterone production. All the heifers in Farm B and A became pregnant 4 months after calving in a 12 week breeding period, except one heifer from the high protein treatment in Farm A. In the case of site C, 42 heifers became pregnant from a total of 50 heifers that were part of the study. Five of the heifers that did not become pregnant were from the low protein and 3 to the high protein treatment groups. These all became pregnant within 80 days from calving during a 6 week mating period.

Table of Contents

2. INTRODUCTION

There is a well-established relationship between high calf birth weight and an increased risk of dystocia (Cafe et al., 2006; Micke et al., 2010a). Reports upon the effect of maternal nutrient intake during gestation upon calf birthweight and dystocia have however, been inconsistent. These differing results may occur due to the timing of the nutritional intervention. In particular the majority of these studies have investigated maternal nutrient manipulation during either mid- or late-gestation (Bellows et al., 1982; Anthony et al., 1986). None have included the effects of diet prior to pregnancy diagnosis at 39d except ourselves (Micke et al., 2010a). In this study an 8.3% increase in birthweight occurred after protein supplementation in the second trimester alone which may reveal that the 10% increase in birthweight seen when nutrition is increased over both the second and third trimester(Cafe et al., 2006), that the majority of this increase can be achieved by increasing nutrition in the second trimester alone. This is a very different situation to that seen in the sheep perhaps due to the difference in placental development over gestation between the species.

Calf birth weight is directly affected by placental size and functional capacity, both of which are affected by maternal nutrient intake during gestation (Rasby et al., 1990). The maternal pregnancy hormones indicative of placental functional capacity, namely estrone sulphate (ES) (Shah et al., 2007; Sullivan et al., 2009), bovine pregnancy associated glycoprotein (bPAG) (Echternkamp, 1984) and bovine placental lactogen (bPL) (Echternkamp, 1984), are positively correlated with calf birth weight. Successful parturition depends upon adequate pelvic ligament relaxation, cervical ripening and dilation, and sufficient myometrial contractility. These processes are associated with reduced plasma P4 concentration, a direct consequence of increased prostaglandin output and prepartal luteolysis (Kindahl et al., 2002). Given the established relationships between maternal pregnancy hormones and calf birth weight, dystocia and the role of ES and P4 in the parturition process, it may be expected that maternal pregnancy hormones indicative of placental functional capacity could be related to the occurrence of dystocia.

Pelvic area measurement prior to joining has been suggested as a management tool by EBLEX to reduce the incidence of dystocia in heifers calving as 2-year olds within their herd (Johnson et al., 1988). However, this has not been widely taken up in the UK and is not routinely taught in veterinary schools. The value of this measurement is reduced in herds with a large disparity in age of heifer at the point of measurement, which is frequently the case as joining and calving periods extend over 3months.

Our research aims to demonstrate the effects of nutrition *in utero* on lifetime productivity of the heifer in the suckler herd. The current literature on the nutritional requirements of the heifer does not take into account the differing mature size of suckler cows with resulting differing required rates of gain to achieve mating weight. Further, a clear need exists for guidance from farmers and veterinarians on nutritional advice which can reduce dystocia rates in the first calf heifer and maximise subsequent performance within the herd. The heifers are the building blocks of the herd; attention to their nutritional requirement is key to the establishment of a high performing herd. Dystocia is also a welfare concern for the farmer and adds a considerable cost element to a very tight margin.

It has been argued that implementation of dietary regimens for the suckler herd is difficult as the herd calve over an extended 3 month period. This however, does not stop implementation of similar nutritional regimens in the sheep flock. A move to a closer calving period is essential for good management in the beef herd with some farmers clearly already able to do this. Mating well grown heifers for a 6 week period 2 weeks prior to the main cow herd should yield an excellent base from which to implement controlled nutrition. In the main cow herd 65% should calve within the first 3 weeks of mating and 95% in the first 6 weeks. Cows that do not calve in this period will not produce one calf per cow per year, which is the fundamental tenet of profitable beef production as calves born early in season are often 22kg heavier at weaning than those born late. Gradual culling of late calvers as well as the 9 week cow mating period will enable implementation of nutritional regimens. Some of this advice is already available on the EBLEX website but does not appear to be taken up. Our latest research in Australia over the last 3 months has illustrated the importance of both the preconception and immediate post conception nutrition upon the health of the fetus. These effects upon fetal growth are sex dependent. These results suggest that preconception diet influence upon oocyte and embryo development may have long term effects. Assessment of fetal organ and placental development as well as hormone levels will be available at 90dpc and at term. Postnatal development in surviving offspring will also be assessed.

Objectives

- Determination of the effect of nutrient intake during early-gestation on the occurrence of dystocia in nulliparous beef heifer calving at 2 years of age;
- Assessment of the usefulness and ease of pelvic area measurement in these heifers as an aid to minimise the risk of dystocia resulting from the nutritional regimes imposed; and

- Investigation of the association between maternal hormones, indicative of placental functional capacity and metabolites, to be used as indicators of fetal well-being and of dietary supplementation requirements.
- Determination of milk intake of the calf during the first 6 months of life and growth rates to 12 months of age.
- Assessment of effects upon calving to conception interval and effects upon reproductive organ development and development of following offspring.

3. MATERIALS AND METHODS

3.1 Farms

Four farms kindly agreed to take part in this research: A (North Yorkshire), B (Buckinghamshire), C (Northumberland), D (Oxfordshire). Heifers from **A** and **B** were used for the pre-conception diet trial and those from **C,** for the post-conception diet trial. A total of 188 maiden beef heifers were used in this section of the study (A, n=44; B, n=44; and C, n=100) with age ranging from 12 to 15 months of age at joining.

The fourth farm (D) was enrolled to take part in a study investigating the effects of dietary protein content prior to necropsy on the ovary, follicular dynamics and carcass quality *ex vivo*. A total of 320 non-pregnant 14-16mth Angus-Friesian crossed heifers were fed high (14.5%CP) or low (10.4%CP) diets for 60 days prior to slaughter. This period of 60d reflected the period preconception as the ova is developing in the ovary, comparable to the preconception treatment periods in farms A and B.

3.2 Experimental design: Diet management

Heifers on farms A, B and C were allocated to high (H; 18% or 14%CP) or low (L; 10% CP) crude protein diet based on age, weight and genetics at each site. The ration was fed under farm conditions and balanced to be isocaloric and supplemented with a vitamin and mineral commercial preparation. Heifers in the pre-conception groups (in farms A and B) received the diet for 2 months (mo) prior to artificial insemination (AI) while post-conception heifers (C) were fed the diets from AI to 90 days post-conception (dpc). This design resulted in 4 independent groups (Table 1): pre-conception high (preH, n=44; farm A=22 and farm B=22; 375.4±7.7kg BW), pre-conception low (preL, n=44; farm A=22 and farm B=22; 385.8±8.4kg BW), post-conception high (postH, n=51; 392.6±3.8kg BW) and post-conception low (postL, n=49; 393.9±3.6kg BW).

Heifers were group-fed the corresponding diet and managed as individual groups within each farm. The heifers at farm A and farm B were fed either a high protein (18%CP; silage with protein meal supplement) or low protein diet (10% CP; silage alone), both with a mineral and vitamin supplement incorporated into the diet (see Appendix 1 for information on feed nutrient and dried matter analysis).

Table 1. Total number of heifers, initial body weight (average ± standard error of the mean) and initial age at the start of the experimental diets (high, 18%CP and low, 10%CP) in the pre- (farm A and B) and weight at AI in the post-conception groups (farm C; high, 14%CP and low, 10%CP).

Farm	Diet Treatment	Number (n)	Weight (kg) *	Age (mo) *
A	preH	22	384.9 ± 12.6	10.1 ± 0.2
	preL	22	405.9 ± 12.7	10.5 ± 0.3
B	preH	22	365.9 ± 8.8	9.0 ± 0.1
	preL	22	364.8 ± 8.9	8.9 ± 0.1
	Average	*preH (n=44)*	*375.4 ± 7.7*	*9.5 ± 0.1*
		preL (n=44)	*385.8 ± 8.4*	*9.7 ± 0.2*
C	postH	49	393.8 ± 3.8	14.7 ± 3.6
	postL	51	392.6 ± 3.6	14.6 ± 0.1

*Refers to weight and age at the start of the experiment. A and B started experimental diets 2 months before AI, while C started trial after AI, weights are therefore taken 60days later in these animals.

At Farm C, the post-conception treatment compared high protein (14%CP provided by 1kg/day protein pellets plus pasture) and a low protein diets (10% CP un-supplemented pasture) both with access to mineral lick blocks (see Appendix 2 for information on feed nutrient and dried matter analysis). At the end of the dietary intervention, all animals were kept under similar dietary and management practices for the remainder of the pregnancy (see Appendix 3 for information on feed nutrient and dried matter analysis). A table of dietary intake on different farms is available as an appendix.

At farm D, heifers were fed a total mixed ration of either high protein (14.5%CP: pre-slaughter High, preSL-H) or low protein (10.4% CP: pre-slaughter Low, preSL-L) diet with the aim of identifying effects upon oocyte/follicle development. At slaughter, there were a total of 277 heifers necropsied: high protein (n=120 heifers, average carcass cold weight of 305.15±2.63 kg) and low protein (n=157 heifers, average carcass cold weight of 305.00±2.91).

3.3 Animal management and measurements of pre- and post-conception groups

3.3.1 Oestrus Synchronisation

In all farms (A, B and C), synchronisation with fixed timed AI was achieved using a short 5-day protocol as reported by Bridges et al. (2014). Animals were inseminated 24 hours after an injection of GnRH 24 hrs, with semen from a single bull with known estimated breading values for birth weight selected by the farmers. Farm A used a South Devon bull, Farm B a Hereford bull and Farm C a stabiliser.

3.3.2 Pregnancy Diagnosis (PD) and Doppler

Heifers were diagnosed after 36 days post conception/insemination (dpc) and verified again at 60dpc. Heifers that were not pregnant after 60dpc were excluded from the study. In addition to pregnancy diagnosis, fetal crown-rump length (CRL; 36dpc), biparietal distance (BPD; 60dpc) and Doppler indices were recorded monthly up to 7mo of pregnancy in farms A and B only. In C, fetal measurements (CRL and BPD) were carried out on similar days to A and B, but Doppler measurements were only possible at 210dpc. Doppler indices were used to estimate the blood flow volume (BFV) in the uterine artery corresponding to the pregnant uterine horn. Results of these measures have been published(Hernandez-Medrano et al., 2015).

3.3.3 Heifer Measurements and Samples

Body weight, body condition score (BCS), hip height (HH) and blood samples were obtained monthly from all animals starting at 1mo of pregnancy (36dpc) until 3 month postpartum. Blood samples were centrifuged, plasma collected into plastic vials (3 per animal) and stored at -20°C until hormone and metabolite assays were carried out.

3.3.4 Procedures at Calving

Calving occurred during 3 consecutive months as follows: February = farm A, March = farm B and April = farm C. Measurements and samples from heifers and calves were taken as described below for farm A and B (pre-conception groups). Calving at farm C was out at pasture with only sex and calf birth weight being recorded. The following subsections describe the work carried out during calving on farm A and B by a team of 3-4 persons for 2 months on a 24h-continuous monitoring rota.

3.3.5 Calving Process, Samples and Measurements (pre-conception groups)

Animals were continuously monitored over 24hr for signs of parturition. Heifers were allowed to calf in the group pen. After calving the heifer and calf were allowed to bond for

10-20min, before being moved to a separate calving pen where measurements, blood (10ml from each) and colostrum (up to 50ml) samples were collected. Measurements recorded included: weight of heifer and calf (birth weight), BCS, temperature of heifer and calf, cardiac frequency and measurements of calf's size such as crown-rump length (CRL), biparietal distance (BPD), crown-nose length (CNL), cannon size (length and width), coronet (length and width) and withers height (Fig 1).

Once all measurements and samples were collected (15-20min) heifer and calf were placed in individual pens with clean straw bedding. These pens were monitored continuously, and activity recorded (e.g. time to standing, suckling). Heifers were closely observed during the immediate period post-partum for expulsion of the placenta which was collected as soon as expelled, weighed, dissected and samples taken for further histological (4% paraformaldehyde solution) and/or molecular analysis (snap frozen in liquid nitrogen). Heifer and calf were kept for 3-5 days in the individual calving pens and then transferred to a larger post-calving pen. Heifer and calf pairs were visually monitored in the post-calving pen for 7-10 days post-partum. After up to 10 monitoring days post-calving, calves and heifers were managed according the farm procedures.

Figure 1. Calving measurements and samples at farm A and B: (A), (B) crown-rump length (CRL), (C) coronet (width) and (D) colostrum sampling.

3.3.6 Post-Calving Measurements and Samples

3.3.6.1 Postnatal Measurements in Heifer-Calf Pairs

All animals, heifers and calves, were followed during the postpartum period until winter housing. Blood samples, body weight, BCS, HH and milk samples were taken monthly for 3 months from the heifer-calf pairs in farm A and B. In the case of farm C, all the above were taken at ~1 month of age. Blood samples were prepared as described previously for hormone and metabolite measurement. Milk samples were preserved using potassium dichromate tablets and kept in refrigeration (+4-8^{o}C) until assayed for protein and fat content at the National Milk Laboratories (Four Ashes, Wolverhampton, United Kingdom).

3.3.6.2 Weigh-Suck-Weigh Estimation of milk intake

The aim of this section of the study was to monitor calf milk intake and maternal milk production, based on the technique used by Beal et al. (1990): Weigh-Suck-Weigh (WSW). Due to calf health concerns expressed at farm A, however, this farm declined to carry out the WSW, the following data is therefore only from farm B.

Heifers (n=16) and calves (n=16) were kept according to farm management practices and moved into a single shed once every month for sampling and WSW measurements (1 to 3 month post-calving). In order to estimate milk production over a 24h-period, calves were separated from the mothers for a period of 12h (i.e. overnight) allowed visual and olfactory contact (i.e. two contiguous pens). After this period (time 0h), calves were weighed (initial/empty weight), returned to the heifer and allowed to suckle for 15-25min. Calves were then separated from the heifer and weighed again (post-feeding weight) to estimate the amount of milk produced by the heifer. This first measurement was considered as time 0 (T0), the heifer was considered empty at this point. Six hours after the initial WSW period (T6), calves were weighed again and the procedure described above repeated. The WSW procedure was repeated at 12 h (T12) and 24h (T24). After the 3rd suckling period (i.e. T24), calves were returned to the heifers and placed to paddock. This procedure determined the amount of milk produced in a 24h period by each heifer via the intake of the calf.

3.3.6.3 Behavioural Monitoring in the Early Post-Partum (only pre-conception groups)

Prior to calving, individual calving pens were fitted with cameras with infra-red capability to follow individual heifer-calf interactions during the immediate postpartum. Cameras were placed either on the front (farm A) or the back (farm B) of the pen depending on the layout of each pen (Fig 2), with access to full view of the whole pen area. The cameras were connected to a central hard disk drive and set to record continuously for 24h at 32 frames

per second from 6h to 72h post-calving, in order to ensure that good video data was obtained for behavioural analysis.

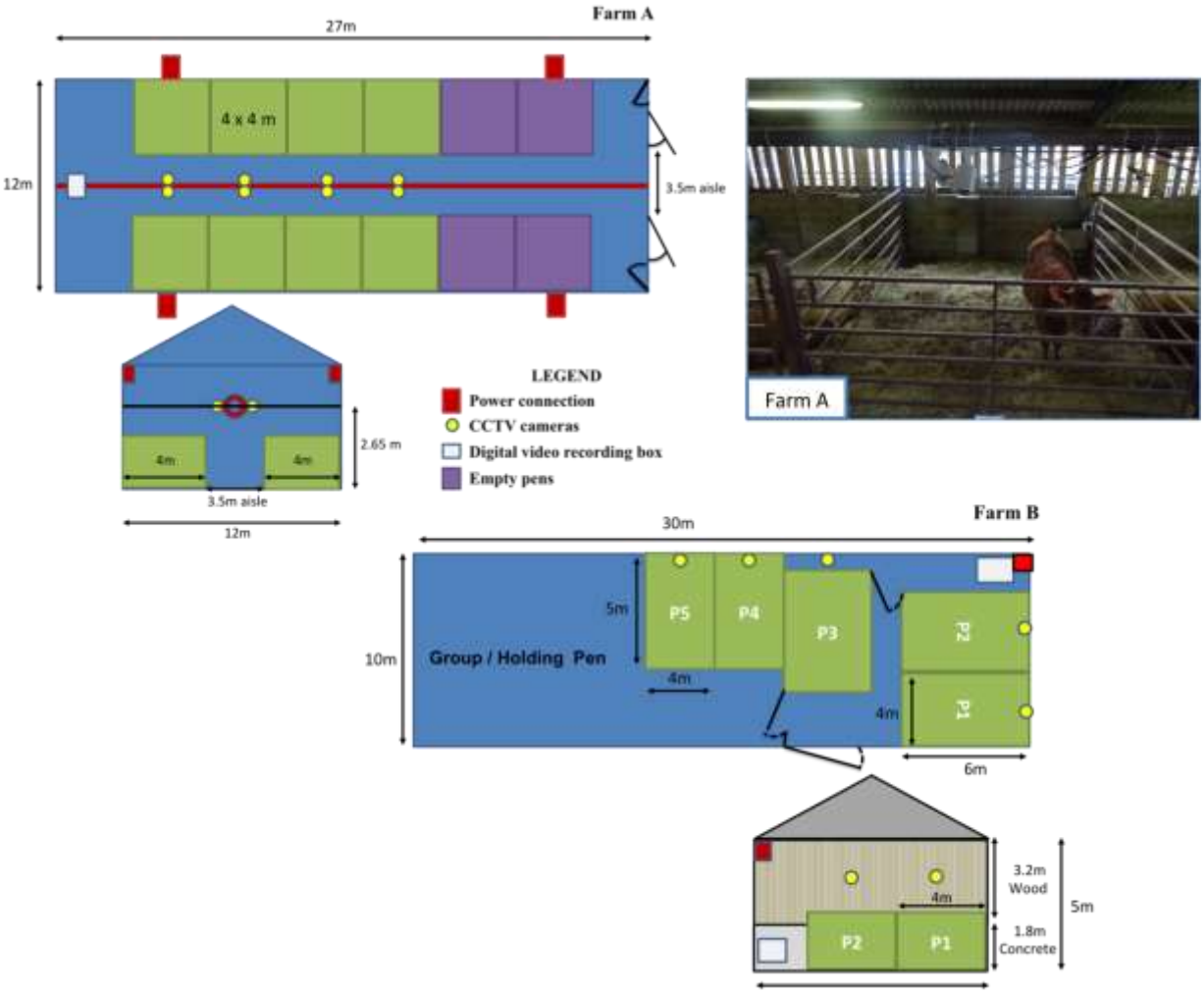


Figure 2. Layout of calving pens and the monitoring system used in farm A (top) and farm B (bottom) to study neonatal/postnatal behaviour in heifer-calf pairs.

Video analysis was carried out using The Observer® XT 11.5 (Noldus, Information Technology bv Wageningen, The Netherlands). A total of 12 hours of video per pair (heifer-calf) was analysed. Video analyses were from 6 to 30 hours after calving. Each 24 hours was divided in blocks of 6 hours and the 3 first hours of each block were continuously analysed. Intra-observer reliability was 99.02% and 99.99% agreement for total duration and frequency respectively for all the behaviours (lying and standing behaviour). Reliability was calculated using The Observer® XT 11.5 reliability analysis. Videos were evaluated using the ethogram described in table 2.

Table 2. Ethogram used to evaluate the behaviour of heifers and calves in the first days after birth.

Subject	Class	Behaviour	Description
Heifer	Posture	Lying	Lying on sternum or side. Head may be rested or raised
		Standing	Body supported by four legs
		Walking	Any movement in pen that is associated with a change in spatial position
	Maintenance	Feeding	Head in feeding trough, or over feeding trough. Includes chewing and sniffing feed.
		Drinking	Muzzle in drinking bowl
		Self-grooming	Licking or sniffing own body. Scratching with their hind feet any part of their body. Rubbing their horns (if present) over fence/walls. Swatting with the tail in an effort to clean all areas of their bodies they can reach.
Heifer-Calf	Social	Touching/sniffing calf	Muzzle in contact with own calf
		Licking calf	Tongue in contact with own calf
Calf	Posture	Lying	Lying on sternum or side. Head may be rested or raised
		Standing	Body supported by four legs
	Play	Locomotor play	Galloping, jumping, bucking or rear kicking
		Social play	Calf pushing forehead against the heifer's forehead, may be accompanied by rotating movements of the head or jumping
	Maintenance	Feeding: Suckling heifer	Head is under the heifer's belly in the udder area (and is actually suckling)

3.4 Animal management and measurements: The effects of dietary protein on follicular dynamics in the beef heifer (Farm D) (*Completed in collaboration with Dr Bob Robinson and Jennifer Edwards*)

Animals were slaughtered between March and June. Whole blood was collected during the bleeding procedure at the abattoir from the jugular vein and prepared for serum analysis. Metabolites; urea, total protein, albumin and globulin were measured using a Randox RX-IMOLA autoanalyser. The globulin was calculated by subtracting albumin concentration from total protein.

Ovaries were collected post-mortem and antral follicle count (AFC) recorded. Follicular fluid and granulosa cells were collected from healthy medium sized follicles (4-9mm). Serum anti-Müllerian hormone concentrations (AMH) were measured by ELISA. RNA was extracted from

granulosa cells (GC). Illumina platform was used to for next-generation sequencing and the differential genes were identified using the *Tuxedo* bioinformatics pipeline.

4. RESULTS

4.1 Pre-Conception Diet Groups

4.1.1 Measurements before and after AI

4.1.1.1 Fertility

Farm A

Reproductive tract examination found one freemartin, which was withdrawn from trial. The number of heifers pregnant to AI was 20 from a total of 43 animals (46.5%; Table 3) with the number increasing to 40/43(93%) after bull removal (87%). From the 20 heifers in the experiment, 2 aborted during mid-pregnancy (between 5-7mo) and were removed. The final number of pregnant heifers that remained in the experiment was: preL n=9 and preH n=9.

Farm B

Palpation of reproductive tract prior to AI resulted in 2 heifers being excluded as freemartin or dysfunctional tract. The remaining 42 heifers underwent the synchronisation and AI protocol. A total of 16 from the 42 animals (38.1%; Table 3) became pregnant. The overall number of pregnant heifers at the time of bull removal was 37/42(88.1%). Of the pregnant heifers 7 were from the preL and 9 from the preH groups.

Table 3. Distribution of animals used in the present study with associated fertility rates (%) for each farm included.

Farm	Diet	Total AI'd	Gx by	Pregnant at			Fertility Rate to AI (%)
				36d	60d	90d	
A	High CP	22	AI	11	11	11	*46.5% (20/43)*
			Bull	0	6	10	
			Empty	11	5	1	
	Low CP	21	AI	10	9	9	
			Bull	1	4	10	
			Empty	10	8	2	
B	High CP	21	AI	10	9	9	*38.1% (16/42)*
			Bull	0	5	11	
			Empty	11	7	1	
	Low CP	21	AI	8	7	7	
			Bull	0	3	10	
			Empty	13	11	4	
C	High CP	49	AI	29	29		*56% (56/100)*
			Bull	1	1		
			Empty	19	19		
	Low CP	51	AI	28	27		
			Bull	3	3		
			Barren	20	21		

AI'd= Artificially inseminated at fixed time, Gx=gestation, CP=crude protein.

4.1.1.2 Heifer Body Weight

Farm A

Body weight (BW) during the pre-conception period was similar between preH and preL (Fig 3-A). Similarly, BW after conception and throughout pregnancy was similar between both groups carrying either male of female fetuses (Fig 3-A). Moreover, there was no difference in the average daily weight gain (ADG) between both groups during the pre-conception period (Fig 4-A), however both groups remained in a similar weight during the 1st month post-conception to then continue gaining on average nearly a kilogram daily (preH = 0.8 ± 0.1 kg/day and preL = 0.9 ± 0.2 kg/day). Conversely as parturition approached both groups lost body weight as reflected in significant drop in ADG during the last trimester (7 to 9mo of pregnancy; Fig 4-A).

Figure 3. Average body weight (±s.e.m.) in heifers in the high (18% CP) or low (10% CP) protein diet groups during the pre-conception and during pregnancy in farm A (A) and B (B).

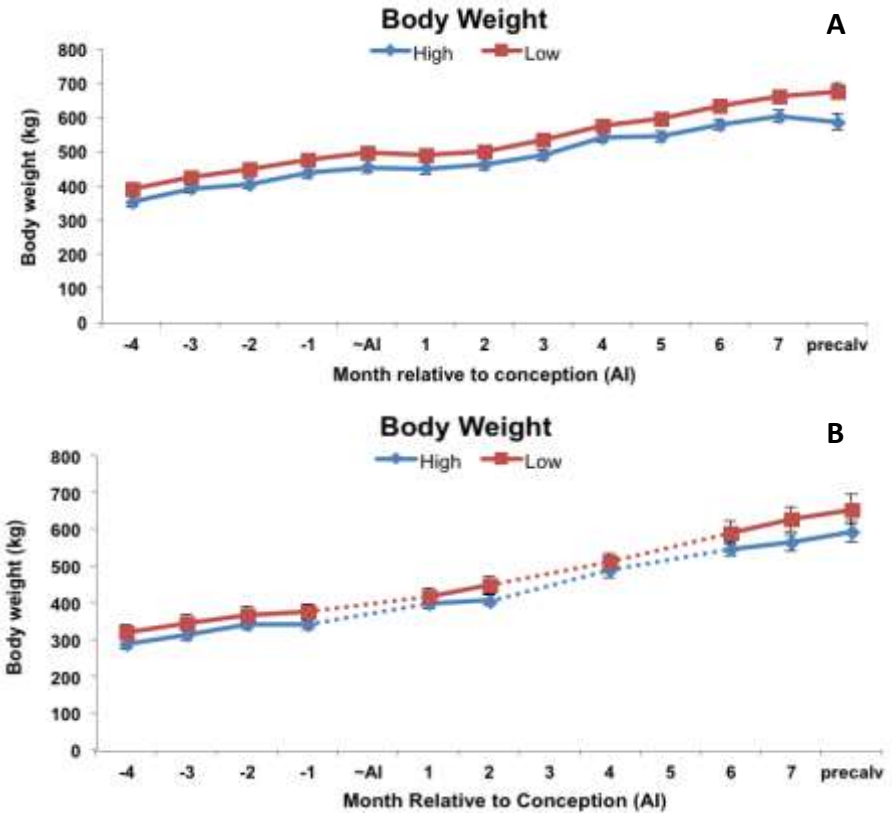
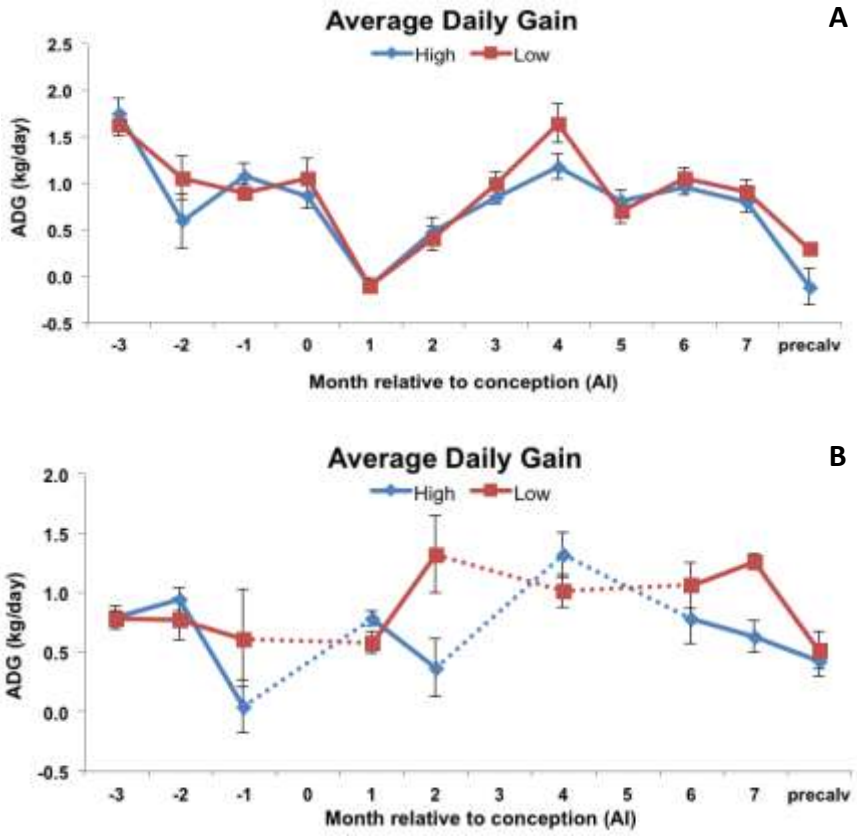


Figure 4. Average daily weight gain (ADG ± s.e.m.) in heifers in the high (18% CP) or low (10% CP) protein diet groups during the pre-conception and during pregnancy in farm A (A) and in B (B).



Farm B

Body weight in heifers was monitored monthly in the pre-conception period and during the post-partum period. However, BW was not recorded at AI or at 3 and 5 months of pregnancy because the scales were not operative. Body weight (Fig 3-B) was not altered during the treatment period during the pre-conception, however there was a large variation in the preH heifers during the period with an ADG for these ranging from -1.4kg to 1.5kg. Moreover, these heifers also showed a lower ADG during the second and seventh month of pregnancy (Fig 4-B). On the other hand, preL heifers showed a more stable ADG of around 1.3kg/day from the 2nd to the 7th month of pregnancy (when scales were functioning again). Both groups showed a decrease in ADG during the last trimester (Fig 4-B) although the decrease in the low group was less.

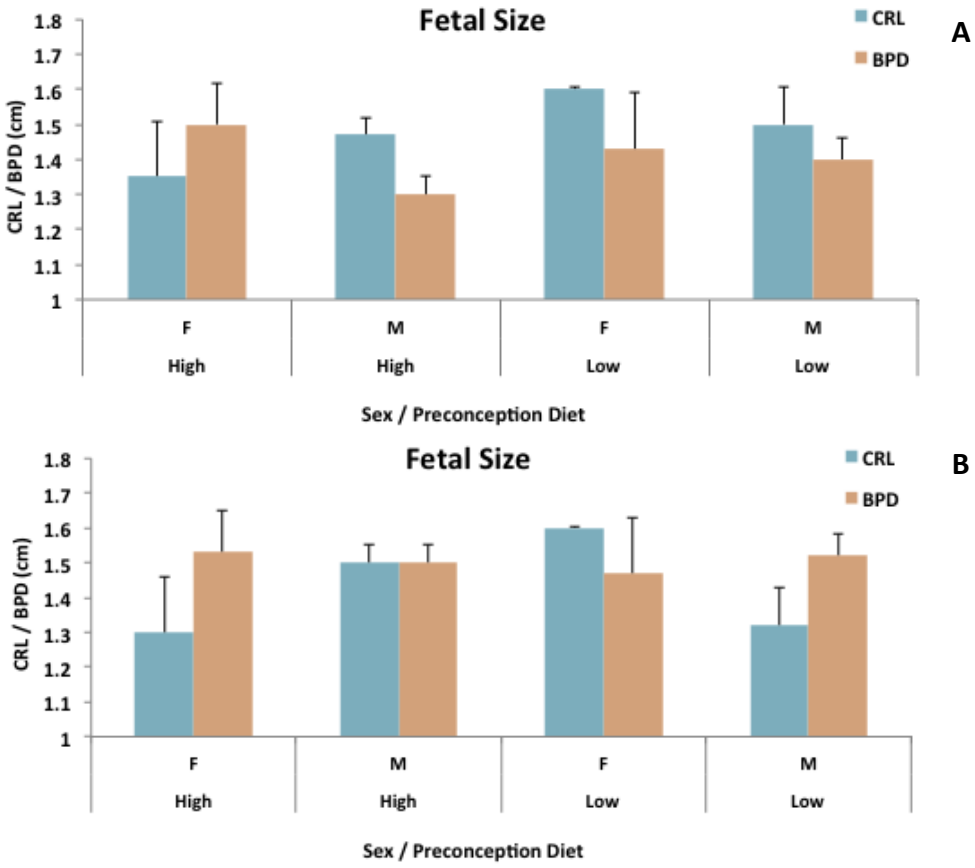
4.1.1.3 Fetal Growth

Farm A

Fetal size was not affected by pre-conception diet, fetuses were of similar size independently of diet treatment: preH, CRL= 1.4±0.09cm and BPD= 1.4±0.07cm; and preL, CRL=1.5±0.08cm and BPD=1.4±0.06cm. Moreover, after analysing the data according to

sex of the fetus it was observed that male and female fetuses were of similar size regardless of dietary treatment during pre-conception (Fig 5-A).

Figure 5. Average (±s.e.m.) crown-rump length (CRL) and biparietal distance (BPD) of male and female fetuses in pregnant heifers that received a high (18% CP) or low (10% CP) protein diet during the pre-conception period in farm A (A) and B (B).



Farm B

Pre-conception diet or sex did not affect size of the fetus during early pregnancy, as CRL (1.4±0.06cm and 1.36±0.05cm for preH and preL, respectively) and BPD (1.52±0.03cm and 1.51±0.05cm for preH and preL, respectively) were similar between preL and preH heifers carrying either male of female fetuses (Fig 5-B).

4.1.1.4 Doppler Measurements

Farm A

Doppler indices were obtained from the first month to the 7th month of pregnancy. Due to technical problems with the ultrasound scanner data for 3rd, 4th and 5th was not used. Blood flow and diameter of the mid-uterine artery (MUA) increased as pregnancy progressed (Fig 6). MUA diameter was not affected by diet treatment during pre-conception. Blood flow in the uterine artery ipsilateral to the pregnant horn was similar between preH and preL heifers during the second trimester of pregnancy (180 and 210

days), but there was a tendency for an increase in the blood flow in preL fetuses during the first trimester, while preH fetuses seemed unaffected during this period. Blood flow and diameter of MUA were similar between male and female fetuses throughout the experimental period.

Figure 6. Mid-uterine Artery (MUA) average (±s.e.m.) blood flow volume and diameter corresponding to the pregnant uterine horn in heifer that received a high (18%) or low (10%) crude protein diet during the pre-conception period in farm A.

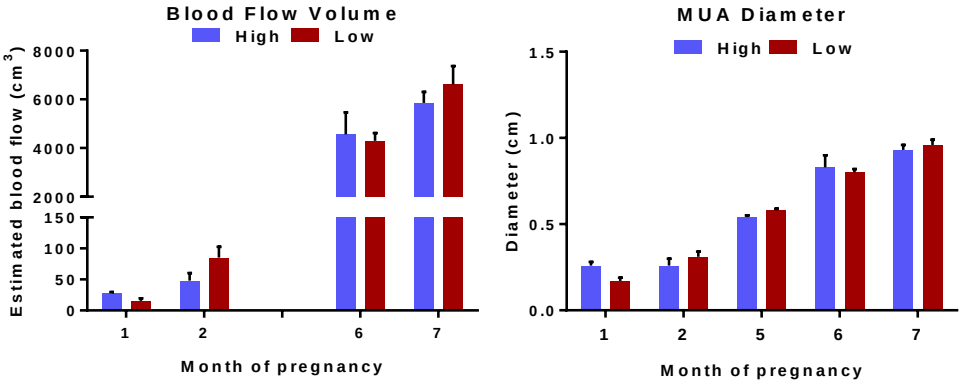
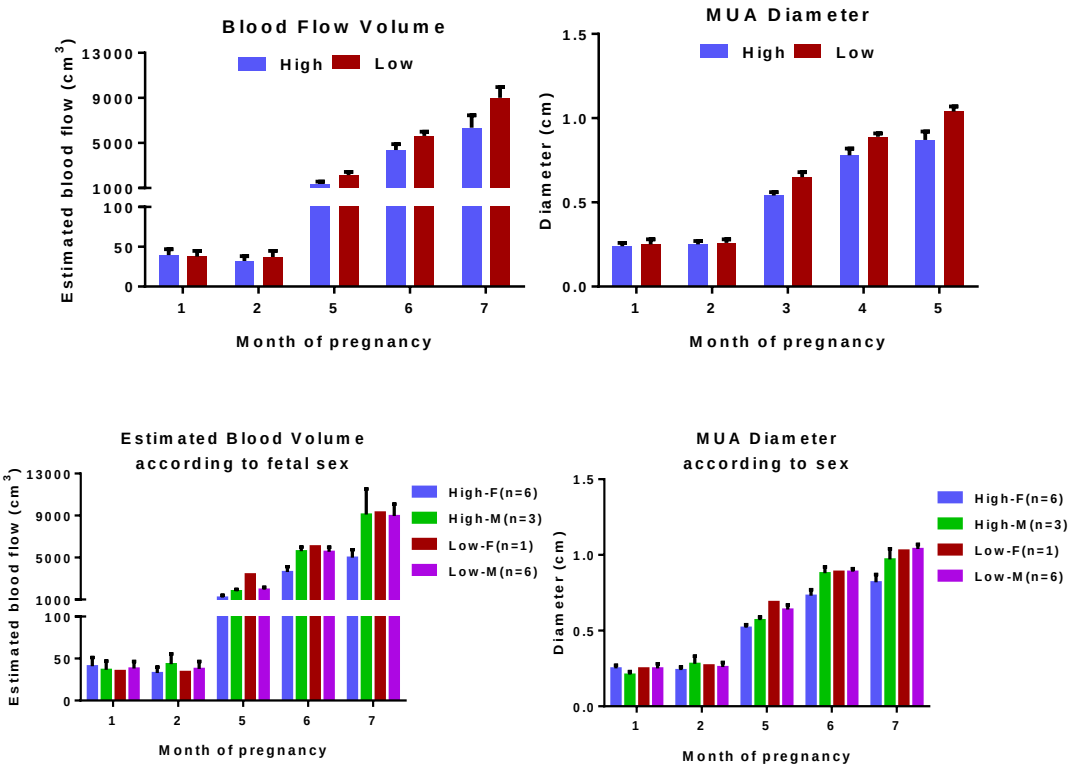


Figure 7. Mid-uterine artery (MUA) average (±s.e.m.) blood flow volume and diameter corresponding to the pregnant uterine horn in heifers that received a high (18%) or low (10%) crude protein diet during the pre-conception period in farm B. Top graph shows the comparison between preH and preL heifers, while bottom graph compares both parameters according to sex of the fetus.



Farm B

Doppler indices were obtained from the first month to the 7th month of pregnancy. Blood flow and diameter of the mid-uterine artery (MUA) increased as pregnancy progressed (Fig 7). Despite being similar during the first 2 months post conception, uterine arteries tended to be smaller in preH compared to preL heifers (Fig 7). Moreover, sex of the fetus also had an effect on MUA diameter and blood flow, with female calves having smaller arteries and receiving lower amount of blood to the pregnant horn. The preL group, however, contained only one female fetus (Fig 7).

4.1.1.5 Hormone assessment during pregnancy

Progesterone (P4)

There was a significant difference between farms in the circulating P4 concentrations throughout pregnancy, with heifers in farm A showing higher concentrations than those in farm B (Fig 8-A&B). In terms of the effect of preconceptional diet, overall, there was a tendency for heifers in the preL group having lower P4 concentrations than preH heifers (Fig 8-A&B).

Farm A

A month before AI, circulating P4 concentrations in preL (0.84 ±0.29 ng/ml) group were lower than in preH (6.58 ±1.74) (Fig 8-A). During pregnancy circulating P4 concentrations were no different between groups (Fig 8-A). Circulating P4 concentrations increased after AI until the 3rd month, decrease slowly and then increased again on the 7th month of pregnancy (Fig 8-A).

Farm B

One month before AI, circulating P4 concentrations were not significantly different between heifers in the preL and the preH groups (Fig 8-B). After AI, circulating P4 concentrations were not different between preL and preH heifers throughout pregnancy (Fig 8-B).

Leptin

In the dietary intervention period and at 1 month post AI, there was a significant difference in the circulating leptin concentrations between farms; circulating leptin concentrations were higher in farm A (Fig 8-C) at both time points in comparison with farm B (Fig 8-D). After 4 months of pregnancy, circulating leptin concentrations were higher in heifers in farm B than in heifers in farm A (Fig 8-C&D).

Farm A

At 4 months of pregnancy, circulating leptin concentrations were lower in those heifers with a female fetus than those with a male. Circulating leptin concentrations reduced after one month from AI, after this the circulating leptin concentrations did not change (Fig 8-C).

Farm B

At seven months of pregnancy, there was a significant difference between diets; circulating leptin concentrations were lower in heifers in the preL group than in heifers in the preH group (Fig 8-D). In addition, circulating leptin concentrations were lower in heifers that had a female fetus than in those heifers with a male fetus.

IGF-1

There were significant differences between farms in the circulating IGF-1 concentrations before AI. Circulating IGF-1 concentrations were higher in heifers in farm A (Fig 8-E) than in farm B (Fig 8-F), this was especially evident at 4 and 6 months after AI (Fig 8-E&F).

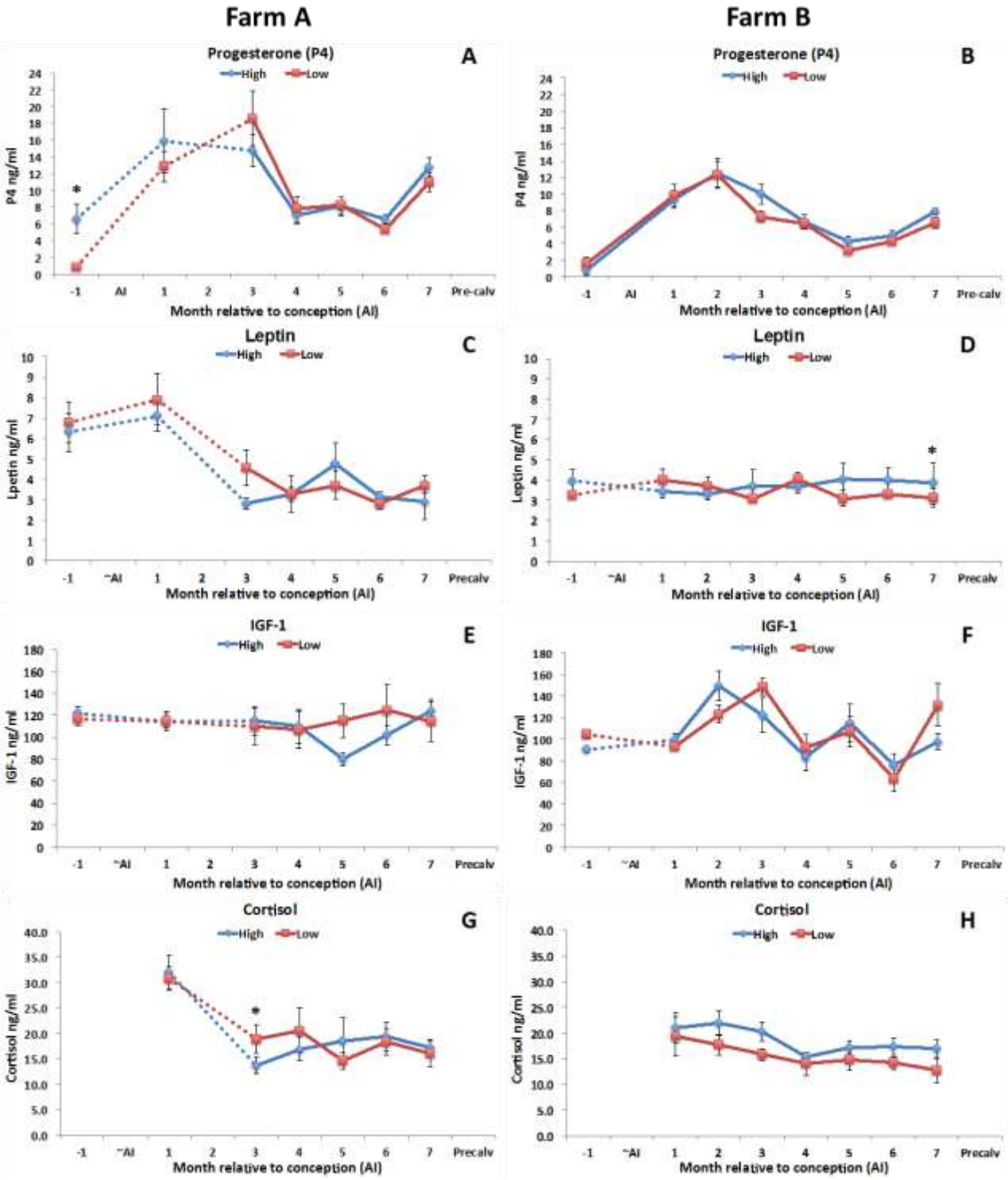
Farm A

No significant differences were observed between preL and preH groups in the circulating lGF-1 concentrations throughout pregnancy (Fig 8-E).

Farm B

No significant differences were observed between preL and preH groups in circulating lGF-1 concentrations (Fig 8-F). Circulating IGF-1 concentrations were different between events; circulating IGF-1 concentrations before and after AI were similar, but they increased and remained elevated until 4 months after AI.

Figure 8. Average (±s.e.m.) of circulating concentrations of P4 (A-B), Leptin (C-D), IGF-1 (E-F) and Cortisol (G-H) before AI and during pregnancy in farm A (right) and farm B (left). This graph shows circulating concentrations of P4, Leptin, IGF-1 and Cortisol one month before and during pregnancy on the heifers that became pregnant after AI. (*) means that there is a significant difference (P<0.05)



Cortisol

There was a significant difference between farms in the circulating cortisol concentrations at 1 month after AI (Fig 8-G&H), and there was also a fetal sex effect at this time point; heifers with a male fetus housed at farm B had lower levels of cortisol than heifers with a male fetus housed in farm A. Three months after AI, preH diet group animals in farm B had higher circulating cortisol concentrations in comparison to preH diet group animals in farm A; but there was no effect of diet within farm.

Farm A

There was a tendency for a difference in the circulating cortisol concentrations between diet groups at 3 months of pregnancy; heifers in the preL group had higher circulating cortisol concentrations than heifers in the preH group. No other significant differences were observed between preL and preH groups in circulating cortisol concentrations (Fig 8-G). Circulating cortisol concentrations decreased two months after AI, but after this they remained constant.

Farm B

There was a fetal sex effect 1 month after AI; heifers with a female fetus had lower circulating cortisol concentrations in comparison with heifers with a male fetus. No other significant differences were observed between preL and preH groups or between events in the circulating cortisol concentrations (Fig 8-H).

Non-Esterified Fatty Acids NEFA)

From 3 to 7 months of pregnancy, there was a significant difference between farms; circulating NEFA concentrations were lower in heifers housed in farm B (Fig 9-B) in comparison with heifers housed in farm A (Fig 9-A). At 5 months of pregnancy, there was a difference between heifers according to fetal sex; circulating NEFA concentrations were lower in heifers with a female fetus than in heifers with a male fetus. At 7 months of pregnancy, circulating NEFA concentrations increased in both farms in comparison with previous months. In farm A (Fig 9-A) and farm B (Fig 9-B) **t**here were no differences between preL and preH diet groups before and after AI in the circulating NEFA concentrations.

Albumin

One month after AI, there was a significant difference between farms; circulating albumin concentrations tended (P=0.07) to be lower in heifers housed in farm B (Fig 9-C) in comparison with heifers housed in farm A (Fig 9-D). From 3 until 7 months after AI, there

was a significant difference between farms; circulating albumin concentrations were lower in heifers housed in farm B (Fig 9-B) in comparison with heifers housed in farm A (Fig 9-A).

Farm A

There was a tendency for a difference between diet groups before AI; circulating albumin concentrations were lower in heifers in the preL diet group than in heifers in the preH diet group (Fig 9-C).

Farm B

At two months of pregnancy there was a significant difference in the circulating albumin concentrations between diets depending on the sex of the fetus; circulating albumin concentrations were higher in heifers with a female fetus in preL group in comparison with heifers with a male fetus in either diet. Furthermore, there was a decrease in both preL and preH in albumin concentrations from the 3rd to the 6th month of pregnancy, to then increase slightly at 7 months.

Total Protein (TP)

At 2 months after AI, there was tendency for a difference in the circulating TP concentrations between diet groups and between fetal sexes; circulating TP concentrations were higher in heifers in the preL group with a female fetus than in heifers in the same diet group but with a male fetus. From 4 until 7 months of pregnancy, there was a significant difference between farms on the circulating TP concentrations; these were lower in heifers housed in farm B (Fig 9-F) than in heifers housed in farm A (Fig 9-E).

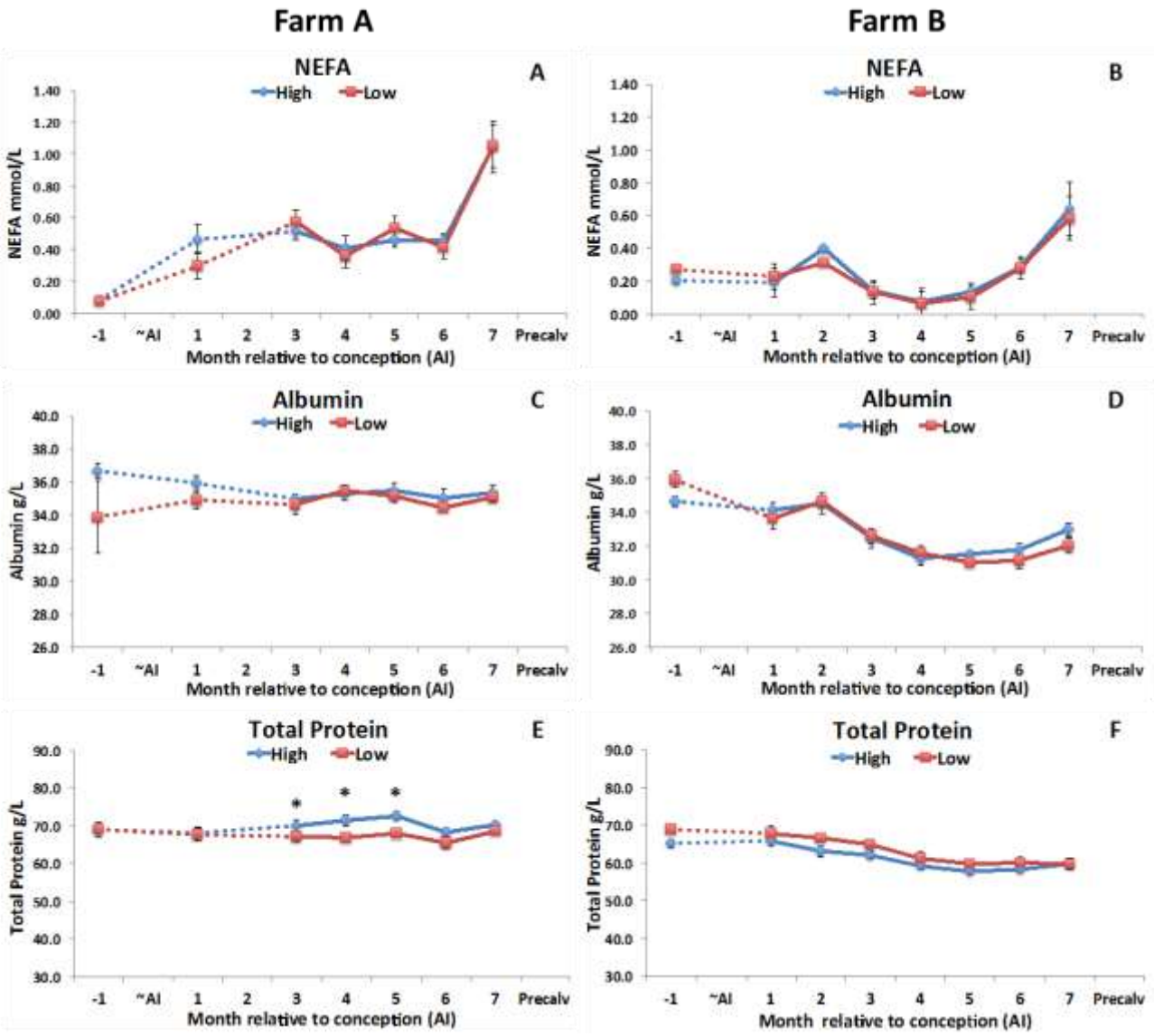
Farm A

There was a significant difference between diet groups at 3 months after AI; circulating TP concentrations were lower in heifers on the preL group than in heifers in the preH group (Fig 9-E). There was a significant difference between diet groups at 4, 5 and 6 months of pregnancy; circulating TP concentrations were lower in heifers in the preL group than in heifers in the preH group (Fig 9-E). No differences were observed between the preL and preH groups after this point (Fig 9-E).

Farm B

There were no significant differences between the preL and preH groups in the circulating TP concentrations (Fig 9-F). But there was a decrease in TP concentrations in both groups from 3 months up to 7 months of pregnancy (Fig 9-F).

Figure 9. Average (±s.e.m.) circulating concentrations of NEFA (A-B), albumin (C-D) and TP (E-F) before AI and during pregnancy in farm A (left) and farm B (right). These graphs show circulating concentrations one month before and during pregnancy on the heifers that became pregnant after AI. (*) means that there is a significant difference (P<0.05)



4.1.2 Measurements at calving

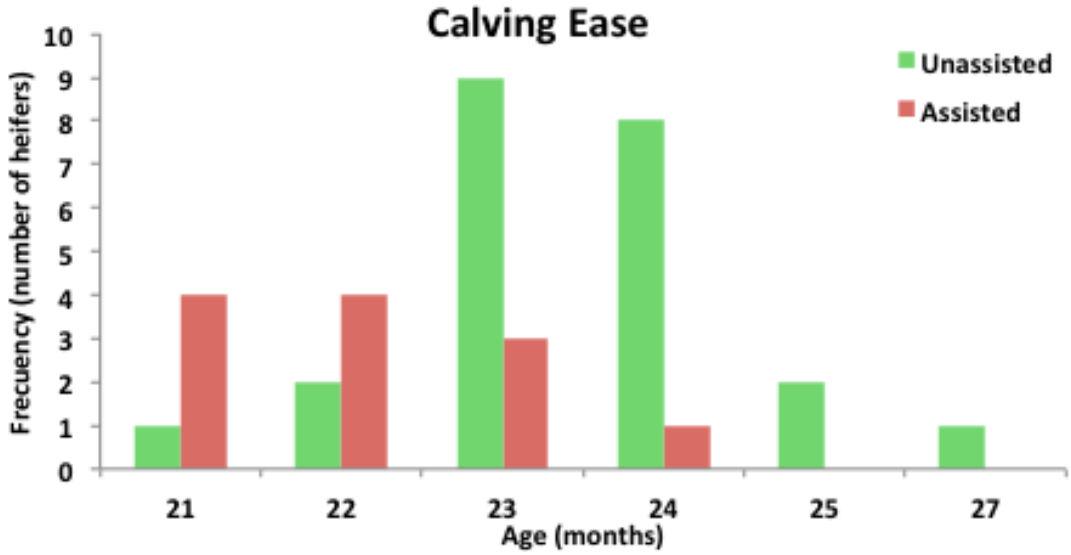
4.1.2.1 Calving

Farm A

Two weeks before the start of the predicted calving, one heifer in the preH group died suddenly. The number of live calves born was: preL female n=2, preL male n=7, preH female n=5, and preH male n=4. Pregnancy length, body condition score and weight at calving were similar between preH and preL heifers (Table 4). There was no difference in these parameters when heifers were carrying a male or a female fetus (Table 4). There was no effect of pre-conception diet on calving ease but the animals younger than 23-24mo at

calving tended to have an increased risk of dystocia (Fig 10). Calf birth weight was not affected by maternal pre-conception diet but calves differed in size depending on diet (Tables 3 and 4). That is calves in the preL diet were longer (CRL, 92.3±1.8cm vs 84.6± 1.5cm) and taller (height, 78.7±1.4cm vs 73.8±1.2cm, for preL and postH respectively) than calves from preH, with no difference between male or female calves.

Figure 10. Distribution of assisted and unassisted calvings in Farm A. This graph is from information received from the farmer and takes into account all heifers in the original group. There was neither effect of diet nor sex of calf on calving ease in experimental heifers.



Farm B

All 16 experimental heifers calved at term, but one calf died due to complications at birth. Results of the measured parameters at calving in heifers and newborn calves are shown in table 3 and 4, respectively. Pregnancy length, body weight at calving, temperature, placenta weight and cotyledon volume and weight were similar between preH and preL heifers at calving (Table 4). However, there was a trend for body condition score at calving to be affected by pre-conception diet: preH heifers tended to be in lower BCS than preL heifers. In the case of farm B, calving difficulty was affected by diet with preL heifers requiring more assistance than preH heifers (70% vs 30%, respectively). Calving ease was not affected by sex of the calf (Table 4).

Contrary to observations in farm A, calf birth weight in Farm B was affected by maternal pre-conception diet and sex (Table 5), with calves from preL heifers being heavier than those from preH, as mentioned above. Moreover, male calves were heavier than female regardless of maternal diet (Table 5). Overall, calf's size (i.e. CRL, BPD or height) was not affected by diet or sex, but the cannon bone and coronet were smaller in female than in male calves. Additional, placental weight and volume were positively correlated with calf's

birth weight in both farms (Fig 11), i.e. heavier placentas corresponded to heavier calves. Placental efficiency (grams of calf body weight per grams of placenta), this was not affected by diet in any of the farms. There was an effect, however, of sex on farm A, male calves had more efficient placentas than female calves.

Table 4. Average (±s.e.m.) parameters at calving for high- (18%) or low-protein (10%) fed heifers during the pre-conception period. Data from farm A and B.

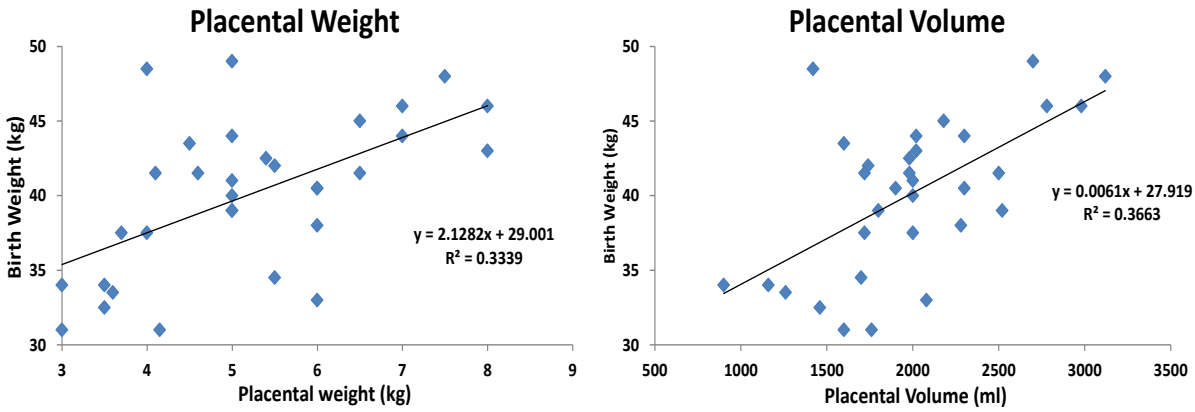
Diet	n	Weight (kg)	BCS	Gx Length (Days)	Heifer Age (months)	Temp (°C)	Placenta		Cotyledon		
							Expulsion Time (min)	Weight (kg)	Number	Weight (kg)	Volume (L)
Farm A											
High CP	9	530.9 ±14.8	2.3 ±0.1	283.9 ±2.5	22.4 ±0.3	38.8 ±0.1	237.6 ±16.1	4.5 ±0.4	102.7 ±6.7	1.9 ±1.5	1.8 ±1.2
Low CP	9	594.2 ±21.1	2.2 ±0.1	286.8 ±2.0	22.9 ±0.5	38.8 ±0.2	242.3 ±13.8	4.6 ±0.2	88.9 ±8.2	1.8 ±1.9	1.8 ±1.5
P value		*0.10*	*0.73*	*0.59*	*0.76*	*0.54*	*0.87*	*0.44*	*0.36*	*0.44*	*0.62*
Farm B											
High CP	9	527.4 ±24.1	2.1 ±0.1	279.7 ±1.5	21.9 ±0.1	38.8 ±0.2	283.8 ±56.6	5.2 ±0.6	86.8 ±8.3	1.9 ±2.3	1.9 ±2.0
Low CP	7	573.7 ±35.6	2.4 ±0.1	282.9 ±0.7	22.0 ±0.1	38.9 ±0.2	262.4 ±38.5	6.7 ±0.4	100.7 ±3.2	2.6 ±1.2	2.5 ±1.5
P value		*0.36*	*0.08*	*0.39*	*0.66*	*0.75*	*0.37*	*0.41*	*0.33*	*0.41*	*0.31*
Overall (both farms)											
P value	*Diet*	*0.08*	*0.31*	*0.27*	*0.39*	*0.88*	*0.41*	*0.33*	*0.95*	*0.20*	*0.28*
	Sex	*0.62*	*0.74*	*0.69*	*0.46*	*0.63*	*0.12*	*0.83*	*0.93*	*0.70*	*0.66*

Table 5. Average (±s.e.m.) measurements at birth from calves from heifers under a high- (18%) or low-protein (10%) diets during the pre-conception period in farm A and farm B. F-B=Front to back measurements; S-S= side to side measurement.

Diet	Sex	n	Calf wt (kg)	CRL (cm)	BPD (cm)	Height (cm)	HR (bt/min)	Temp (°C)	Cannon bone			Coronet	
									Length (cm)	F-B (mm)	S-S (mm)	F-B (mm)	S-S (mm)
Farm A													
High	Female	5	38.7 ±1.7	85.2 ±2.6	13.0 ±0.6	74.6 ±1.6	200.0 ±6.9	39.1 ±0.2	16.7 ±0.41	34.6 ±1.2	31.2 ±0.97	45.4 ±1.4	56.4 ±1.5
	Male	4	39.4 ±2.5	83.9 ±1.7	12.3 ±0.5	72.9 ±1.9	174.7 ±13.5	38.6 ±0.1	16.9 ±0.9	39.3 ±3.3	31.0 ±1.7	43.0 ±1.5	59.7 ±2.3
Low	Female	2	39.5 ±0.5	91.3 ±6.8	13.0 ±1.0	78.5 ±2.5	158 ±10.0	39.3 ±0.6	15.5 ±0.5	35.0 ±1.0	33.5 ±3.5	45.0 ±0.0	58.0 ±1.9
	Male	7	42.1 ±1.6	92.6 ±1.9	13.4 ±0.4	78.8 ±1.7	169.1 ±12.8	39.1 ±0.2	18.4 ±0.4	37.4 ±1.2	33.3 ±0.8	47.2 ±1.9	64.2 ±0.9
P value	*Diet*		*0.44*	*0.02*	*0.38*	*0.04*	*0.12*	*0.25*	*0.77*	*0.70*	*0.13*	*0.38*	*0.09*
	Sex		*0.47*	*0.99*	*0.81*	*0.74*	*0.62*	*0.25*	*0.02*	*0.08*	*0.90*	*0.96*	*0.02*
Farm B													
High	Female	6	34.2 ±1.6	88.2 ±2.9	12.7 ±0.5	75.9 ±0.7	192.0 ±8.5	39.2 ±0.2	18.1 ±0.4	32.2 ±0.9	29.2 ±0.6	44.0 ±0.9	56.2 ±0.7
	Male	3	39.3 ±3.8	88.2 ±1.2	13.0 ±0.6	77.8 ±2.9	194.0 ±5.9	39.1 ±0.0	17.3 ±0.6	37.3 ±3.5	34.7 ±2.2	47.7 ±2.0	62.0 ±1.5
Low	Female	1	38.0	93.0	12.0	80.0	200.0	39.5	17.5	32.0	28.0	46.0	58.0

	Male	6	45.8 ±0.9	93.2 ±1.8	13.5 ±0.2	79.4 ±1.2	190.3 ±9.5	39.2 ±0.3	18.1 ±0.7	38.3 ±1.4	35.4 ±1.1	51.5 ±1.1	66.5 ±0.9
P-value	*Diet*		*0.07*	*0.19*	*0.90*	*0.21*	*0.88*	*0.62*	*0.93*	*0.86*	*0.90*	*0.11*	*0.04*
	Sex		*0.03*	*0.98*	*0.19*	*0.76*	*0.79*	*0.67*	*0.93*	*0.03*	*0.002*	*0.02*	*0.002*
Overall (both farms)													
P-value	*Diet*		*0.04*	*0.008*	*0.40*	*0.02*	*0.20*	*0.23*	*0.93*	*0.92*	*0.19*	*0.12*	*0.007*
	Sex		*0.02*	*0.98*	*0.53*	*0.92*	*0.76*	*0.25*	*0.11*	*0.004*	*0.03*	*0.12*	*<0.001*

Figure 11. Placental weight and volume correlated positively with calf's birth weight (r=0.58 and r=0.61 for weight and volume, respectively*).* This graph shows data from farm A and B.



4.1.2.2 Behavioural assessment

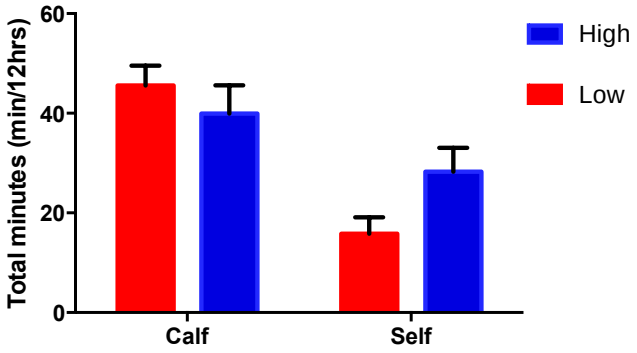
A total of 26 heifer-calf pairs were observed, n=14 in Farm A and n=12 in Farm B. From these, 14 were preL (3 heifer- and 11 bull-calves) and 12 were preH (8 heifer- and 4 bull-calves).

Heifer behaviour

Licking calf

Heifer dietary treatments did not have any effect on the duration of heifers licking their calf. Heifers in the preL group licked their calves on average 45.6 ±4.0 minutes/12hrs while heifers in the preH group licked their claves on average 39.9±5.7 minutes/12hrs (Fig 12). Similarly, farm and calf's sex did not have any effect in the duration of heifers licking their calf. There was no significant correlation between this heifer behaviour and any of the hormones studied.

Figure 12. Average (±s.e.m.) time duration of heifers licking their calf and licking themselves by diet treatment (preH and preL) in both farms (farm A and farm B).



Licking self

Overall, self-grooming duration was similar in both dietary treatments (Fig 12), regardless of the sex of the calf. However, farm had a significant effect on heifer self-grooming duration; heifers in farm B spent 17.4 minutes more on this behaviour than heifers in farm A. Looking at this behaviour by farm, in farm A self-grooming behaviour duration was lower in preL heifers with bull calf progeny than in preH heifers with a bull calf. In farm B, self-grooming behaviour duration was lower in preL heifers with heifer calf progeny than in preL heifers with bull progeny. There was no observed correlation between this behaviour and any of the hormones studied.

Standing behaviour

Overall, heifer dietary treatment had a significant effect on the total standing time; heifers in the preL group stood for 135 minutes longer than heifers in the preH group (Fig 13-A). Average standing bout duration was not significantly different between diet treatments; and similarly, frequency of standing bouts was not significantly different between diet treatments (Fig 13-B and C). However when separated by farm, preL heifers in farm B with heifer progeny spent more time standing than those with bull calf progeny or those in the preH treatment regardless of sex of the progeny; and heifers from the low diet in farm B had less standing bouts that heifers in the high diet group.

Heifer circulating IGF-1 concentration was positively associated with their total standing time and the standing bout duration (Fig 14). Heifers that spent more time standing and with longer standing bout duration had higher circulating IGF-1 concentrations.

Figure 13. Average (±s.e.m.) heifer standing behaviour for the variables: A= total standing time, B= number of bouts, and C= bout duration by diet treatment (preH and preL) in both farms (farm A and farm B), * denotes P≤0.05

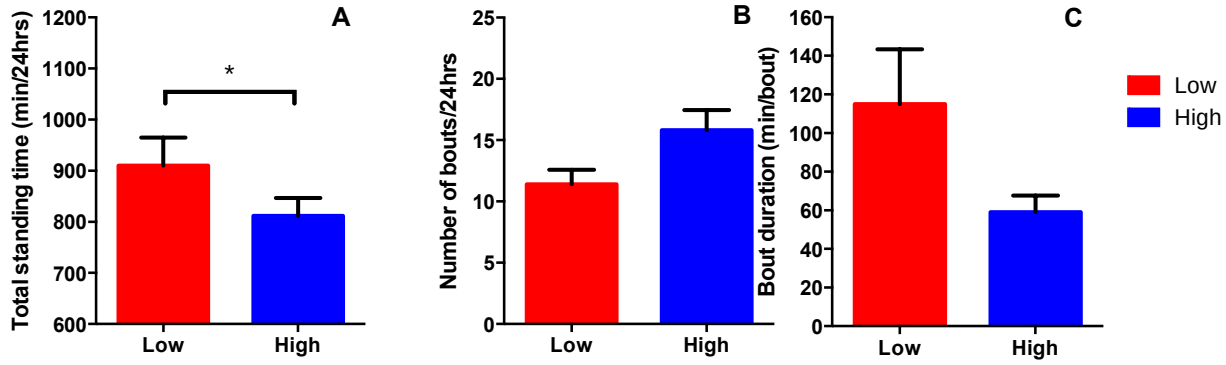
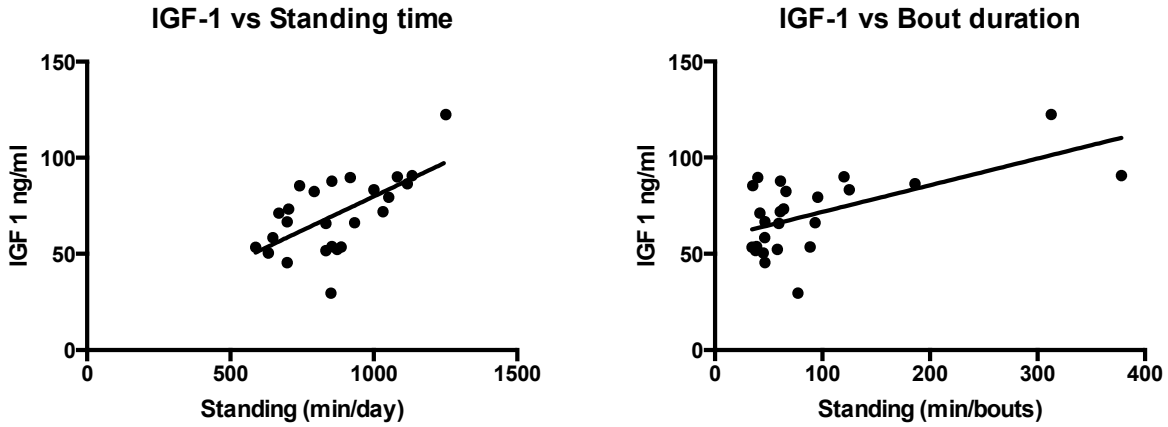


Figure 14. Association between heifer circulating IGF-1 concentrations with standing duration (left) and number of standing bouts (right).

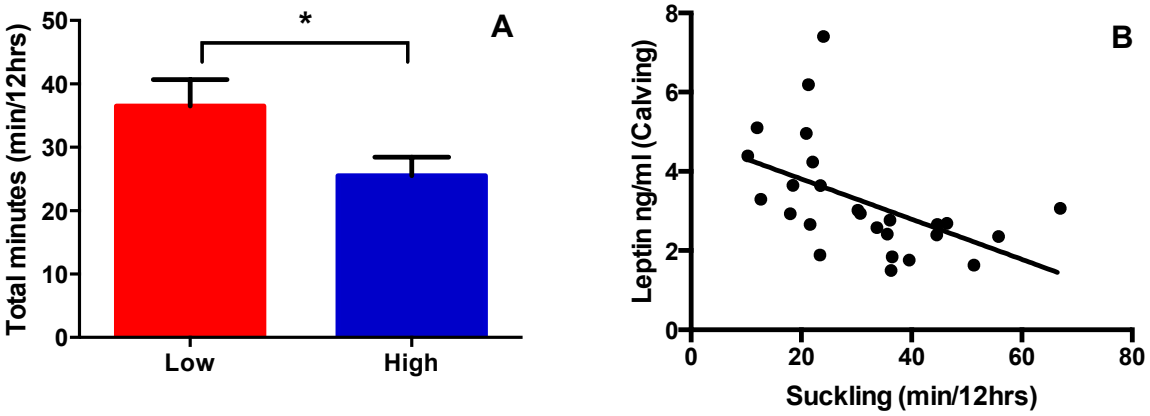


Calf behaviour

Suckling

Suckling duration was different between treatments, preL calves suckled for a longer period than preH calves (Fig 15). Suckling duration was negatively correlated with circulating calf leptin concentrations (r=-0.53, P=0.005; Fig 15) and positively correlated with the time mothers spent licking their calves (r=0.44, P= 0.02), calves that suckled for longer had lower circulating leptin concentrations and were licked by their mothers for longer. No other significant effect or hormone correlation was observed.

Figure 15. Graph A: Average (±s.e.m.) calf suckling duration according to dam's pre-conception diet treatment; * denotes P≤0.05. Graph B: Association between circulating calf leptin concentrations and suckling duration on the first 12 hours after calving.



Locomotor play

Heifer dietary treatment did not have any effect on the amount of locomotor play in their calves. PreL calves ran for 1.3±0.2 minutes and preH calves run for 1.7±0.6 minutes in the 12hrs observation period. However, there was a sex effect on the amount of locomotor play, bull calves ran (1.1 min/12hrs) less than heifer calves (2 min/12hrs). No other significant effect was observed for this behaviour, and no significant correlation was observed between locomotor play and any measured hormones levels (leptin, cortisol or IGF-1).

Standing behaviour

Heifer dietary treatment did not have any effect on calves total standing time, bout frequency and bout duration. Similar findings were observed between the standing variables and calf's sex and farm. Circulating IGF-1 concentrations in the calf was negative associated with the number of standing bouts (P=0.05). Calves that had less standing bouts had higher circulating IGF-1 concentrations. No other associations were observed between other circulating hormones (cortisol and leptin) concentrations and standing behaviour.

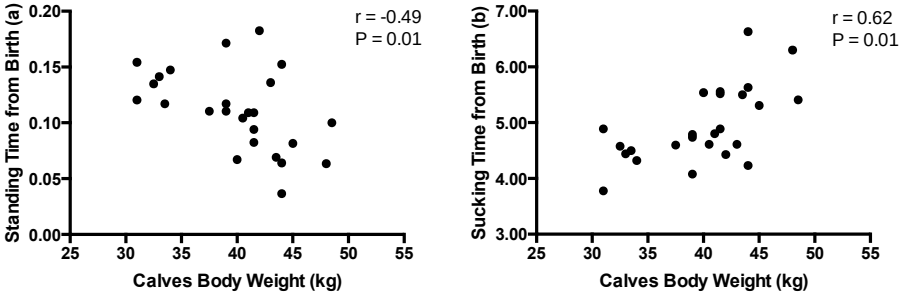
Time from calving to standing

Heifer dietary treatment did not have any effect on the time between calving to standing. However, there was a significant negative correlation between this variable and calf body weight; lighter calves took longer to stand after calving (Fig 16).

Time from calving to suckling

Heifer dietary treatment did not have any effect on the time between calving to suckling. However, there was a significant positive correlation between this variable and calf body weight; heavier calves took longer to suckle after calving (Fig 16).

Figure 16. Pearson correlation between Standing Time from Birth (a=1/Square) and Suckling Time from Birth (b=Log) with Calves Body Weight.



4.1.3 Postnatal Measurements in Heifers

4.1.3.1 Body weight

Farm A

Relative to pre-calving weight, heifers in both experimental groups lost weight after calving with heifers in the preL diet losing significantly more weight at calving than preH heifers (-84.3kg and -66.4kg, respectively). After birth, heifers in the preH diet had a decreased body weight gain from calving to 21mo postpartum (Fig 17-A) compared to heifers in the preL diet. This effect was no longer observed when weight was expressed as a ratio of change relative to the initial weight of the heifers before the start of the experiment (Fig 18-A).

Farm B

Relative to pre-calving weight, heifers in both experimental groups lost a similar amount of weight after calving and remained on a similar weight for the following 3 months (Fig 17-B). Moreover, when weight was expressed as a ratio of change relative to the initial weight of the heifers before the start of the experiment (February 2013) it was observed that both groups (preL and preH) followed a similar pattern throughout pregnancy and before and after parturition (Fig 18-B).

Figure 17. Average body weight post-calving in heifers/heifers that received either high (18% CP) or low (10% CP) protein diet during the pre-conception stage (-60 days to conception, AI; Farm A (A) and Farm B (B)) (pp= months postpartum).

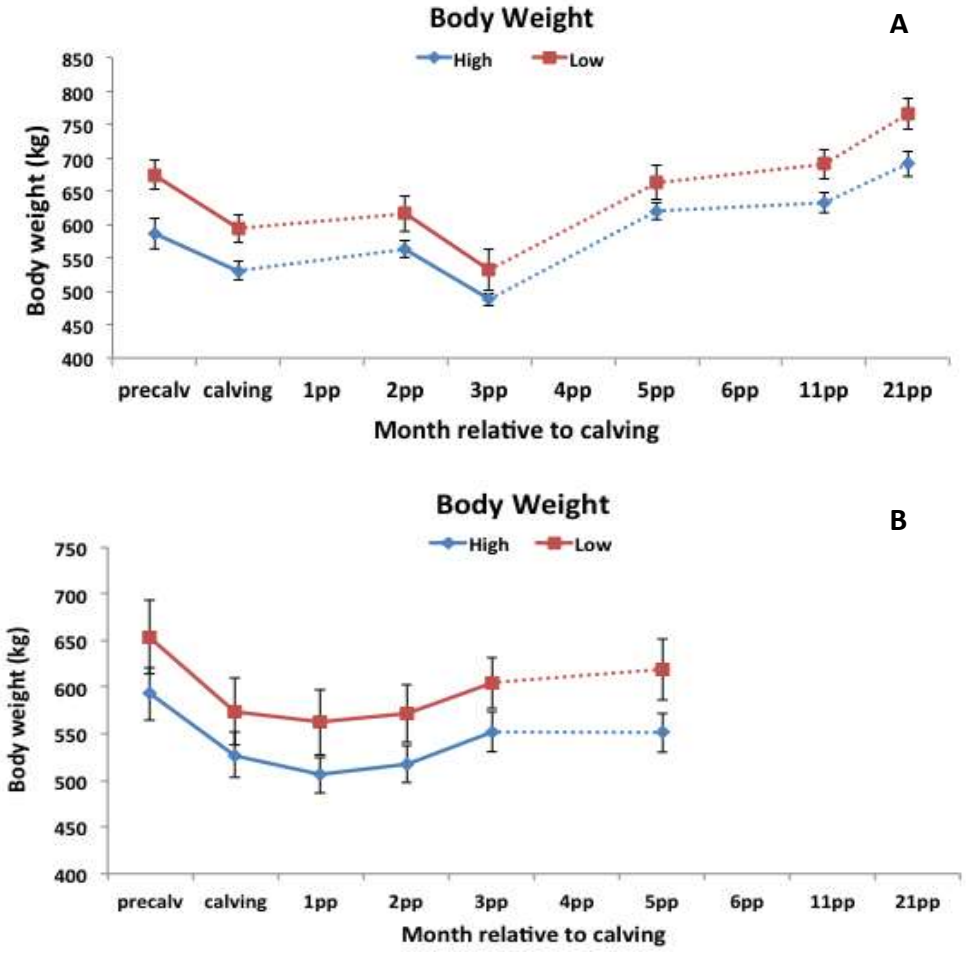
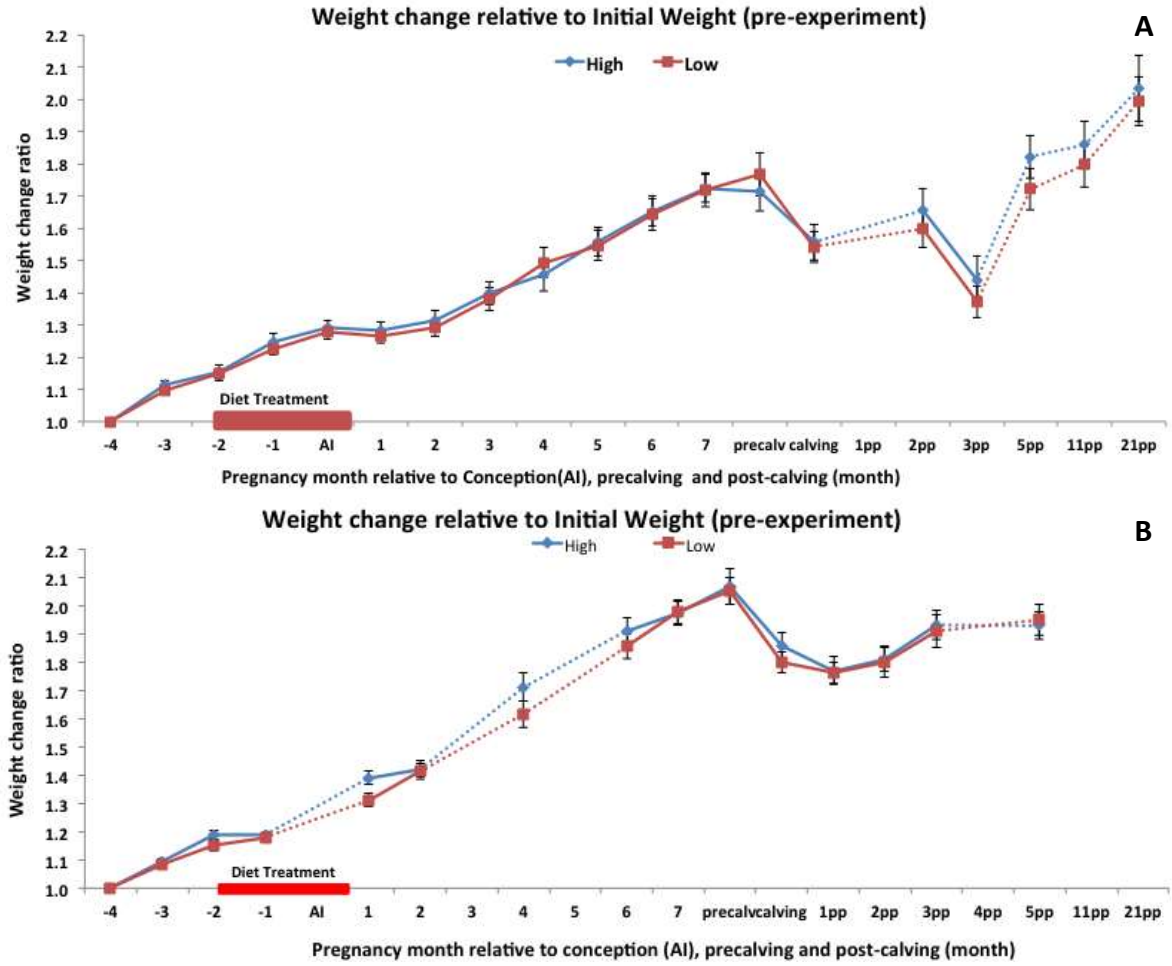


Figure 18. Change in body weight relative to the initial weight (i.e. pre-experimental; Farm A: 13-January-2013, Farm B: 6-February-2013) in experimental heifers at Farm A (A) and at Farm B (B). Heifers received a high (18% CP) or low (10% CP) protein diet during the pre-conception period (red bar; -60day to conception, AI) with weight taken every month.



4.1.3.2 Milk composition

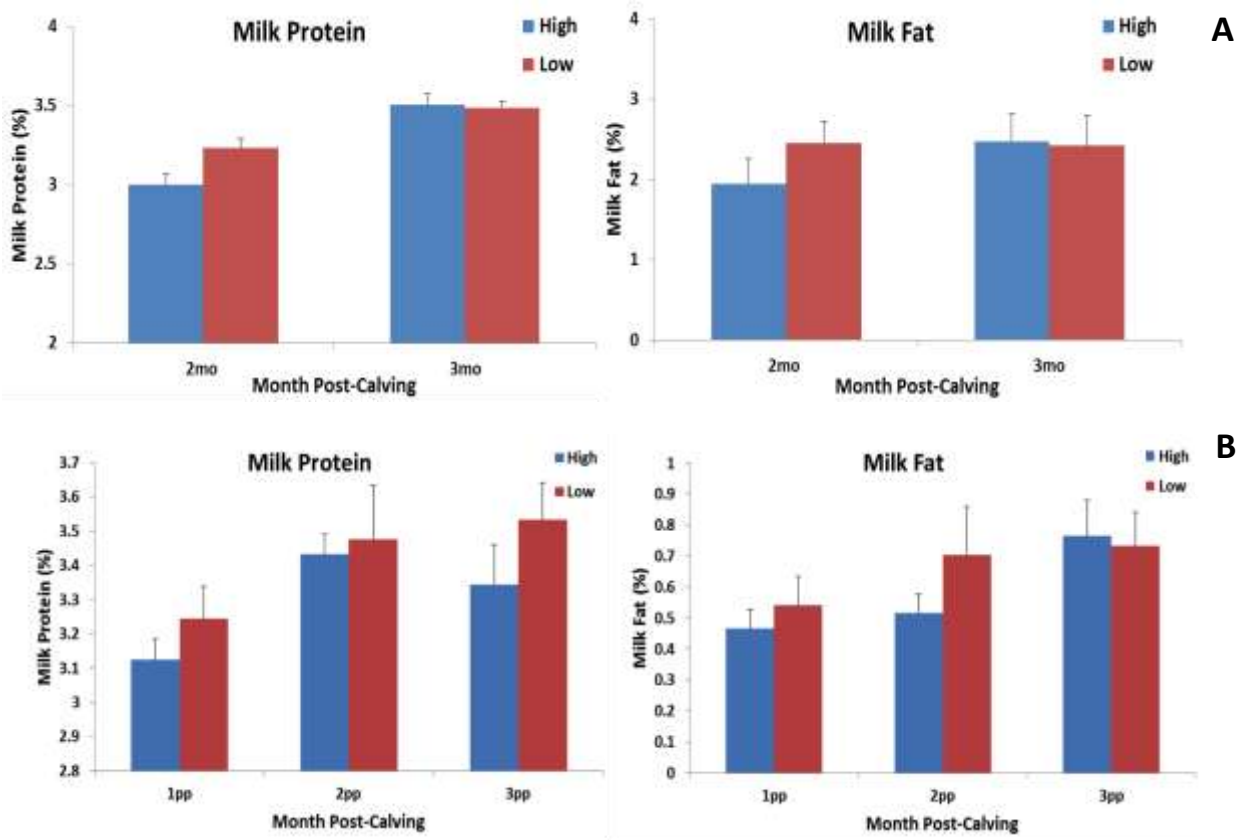
Farm A

Milk composition was not affected by diet treatment during the first 3 months post-partum as observed in Fig 19-A.

Farm B

Milk composition was not affected by dietary treatment during the first 3 months post-partum as observed in Fig 19-B. However, in comparison to Farm A, heifers in farm B produced milk with significantly lower fat content (Fig 19-B). This may be linked to their current diet or to a dilution effect as samples from these animals were collected 12 hours after separation from the calf, i.e. weigh-suck-weigh point time 0. It should also be noted that based on the calf weight gain in farm A compared to farm B, milk production may have been higher in the farm B heifers thereby diluting the fat content.

Figure 19. Average (±s.e.m.) protein (%) and fat (%) content in milk from heifers that received a high (18% CP) or low (10% CP) protein diet during the pre-conception period in Farm A (A) and B (B).



4.1.3.3 Hormonal assessment at calving and during lactation.

Progesterone (P4)

There was a significant difference between farms in the circulating P4 concentrations at calving; circulating P4 concentrations were higher in heifers housed in farm B than in heifers housed in farm A. However, at 2 months after calving, none of the cows in either farm were cycling as animals had circulating P4 concentrations lower than 1ng/ml. No differences were observed in the circulating P4 concentrations between preL and preH heifers after calving at farm A (Fig 20-A), or at farm B (Fig 20-B).

Leptin

There was a significant difference between farms at calving in circulating leptin concentrations: heifers in farm B had higher circulating leptin concentrations than heifers in farm A. At three months after calving, there was a significant difference between diet groups; circulating leptin concentrations were lower in heifers in the preL diet than in heifers in the preH diet. There was a tendency for a difference between farms at 3 months after calving with circulating leptin concentrations being lower in heifers at farm B than at farm A.

Farm A

There was no significant difference in the circulating leptin concentrations between preH and preL after calving (Fig 20-C).

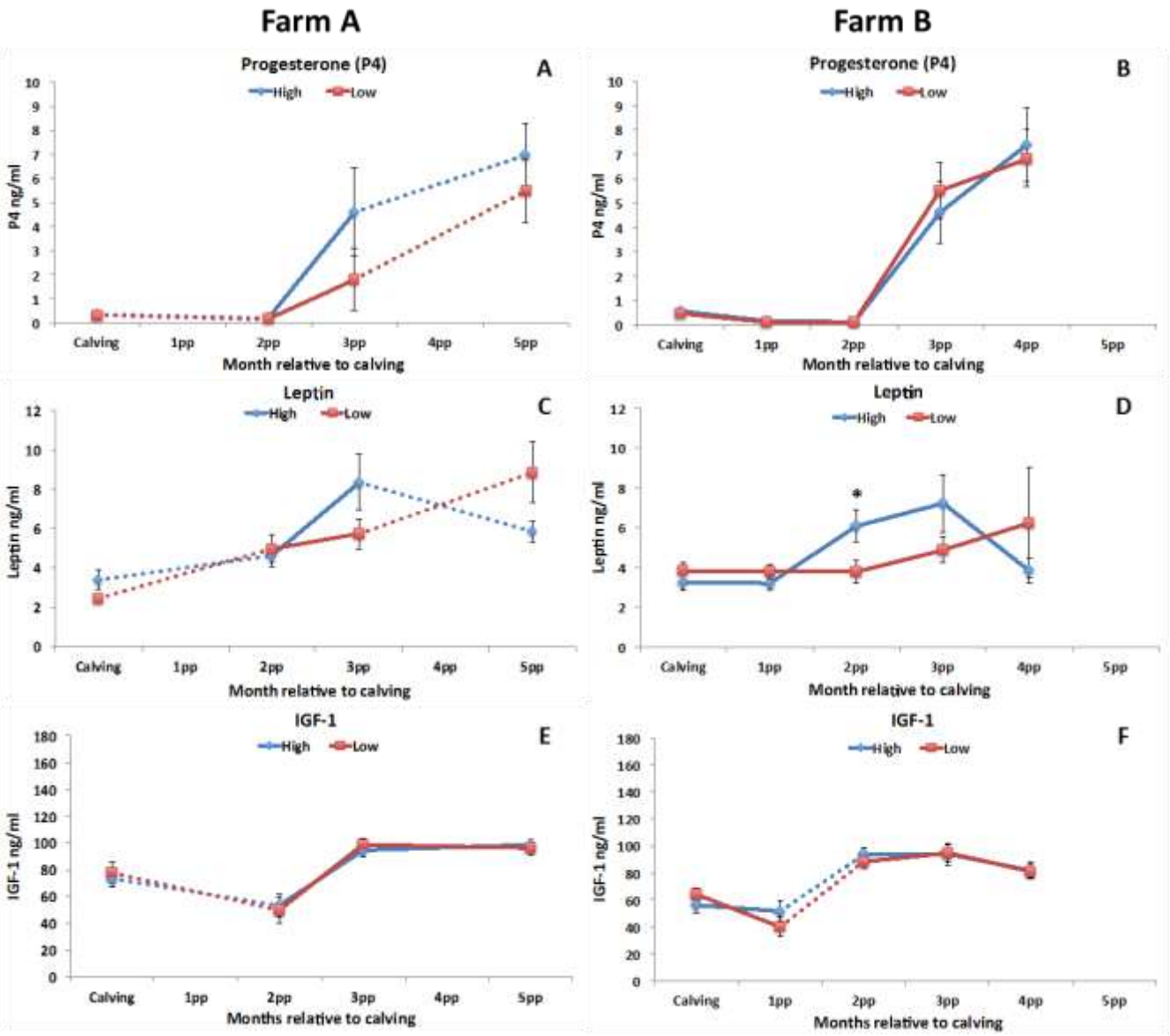
Farm B

There was a significant difference at two months after calving; circulating leptin concentrations were lower in heifers in the preL diet than in heifers in the preH diet (Fig 20-D).

IGF-1

There was a significant difference between farms at 2 months after calving, circulating IGF-1 concentrations were higher in heifers housed at farm B than in heifers housed at farm A. There was no significant difference between preH and preL in the circulating IGF-1 concentrations after calving at farm A (Fig 20-E), or at farm B (Fig 20-F).

Figure 20. Average (±s.e.m.) circulating P4 (A-B), Leptin (C-D) and IGF-1 (E-F) concentrations after calving in farm A (left) and B (right). (*) means that there is a significant difference (P<0.05)



Prolactin

At calving, there was a significant difference between farms; circulating prolactin concentrations were higher in heifers housed at farm B than in heifers housed at farm A.

Farm A

There was no significant difference between preH and preL heifers in the circulating prolactin concentrations after calving (Fig 21-A).

Farm B

There was a tendency for a difference between diet groups at calving; circulating prolactin concentrations were higher in heifers in the preL diet than in heifers in the preH diet. There

was no other significant difference between preH and preL heifers in the circulating prolactin concentrations after calving (Fig 21-B).

Oxytocin

There was a significant difference between farms at 2 months after calving; circulating oxytocin concentrations were lower in heifers housed at farm B than in heifers housed at farm A.

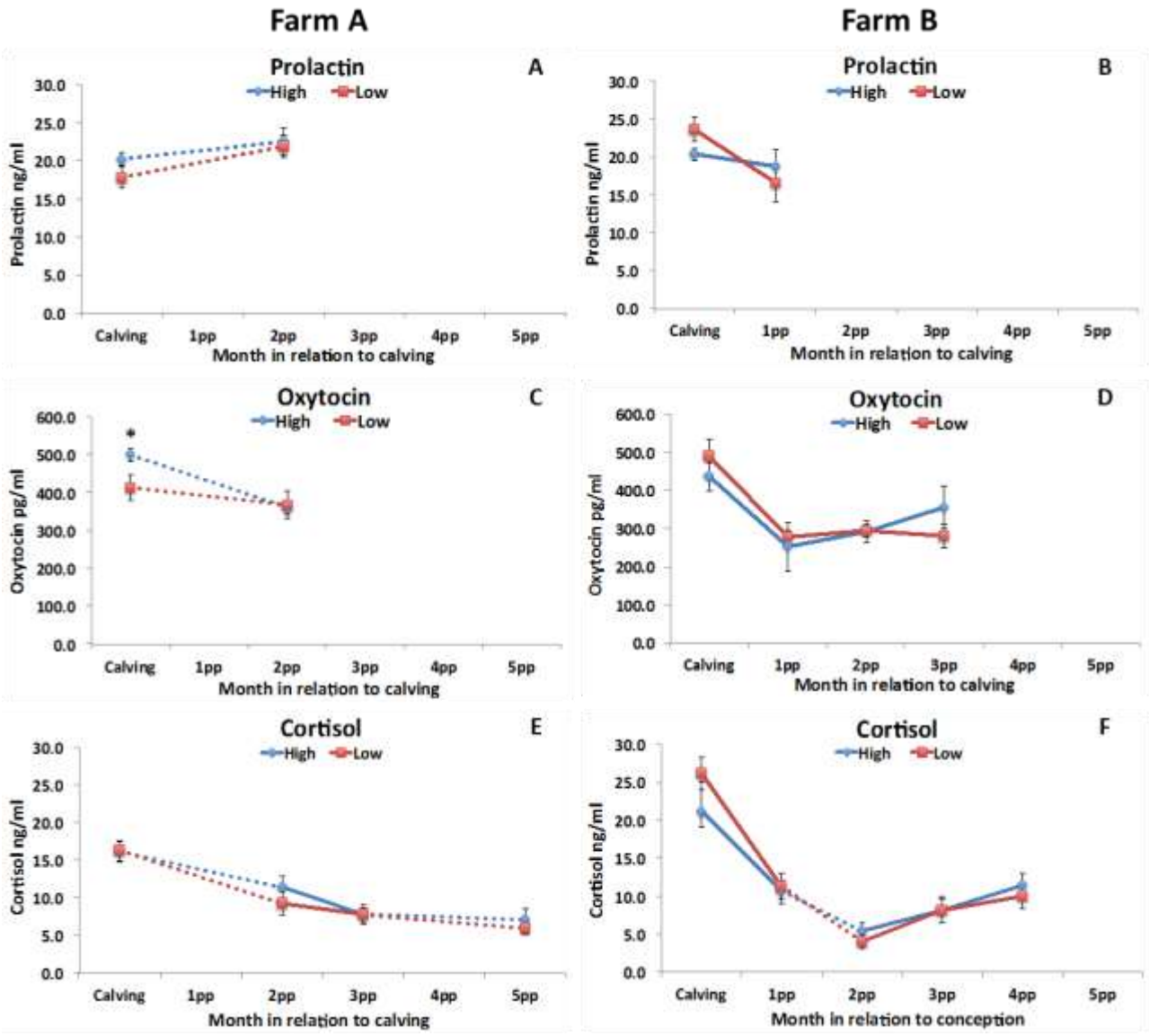
Farm A

There was a significant difference in circulating oxytocin concentrations at calving; circulating oxytocin concentrations were lower in heifers in the preL diet than in heifers in the preH diet. No other significant difference was observed between preH and preL in the circulating oxytocin concentrations after calving (Fig 21-C).

Farm B

There was no significant difference between preH heifers and preL heifers in circulating oxytocin concentrations after calving (Fig 21-D). But oxytocin concentrations were lower in the postpartum than at calving in both groups.

Figure 21. Average (±s.e.m.) of the circulating Prolactin (A-B), Oxytocin (C-D) and Cortisol (E-F) concentrations after calving in farm A (A) and farm B (B). (*) means that there is a significant difference (P<0.05)



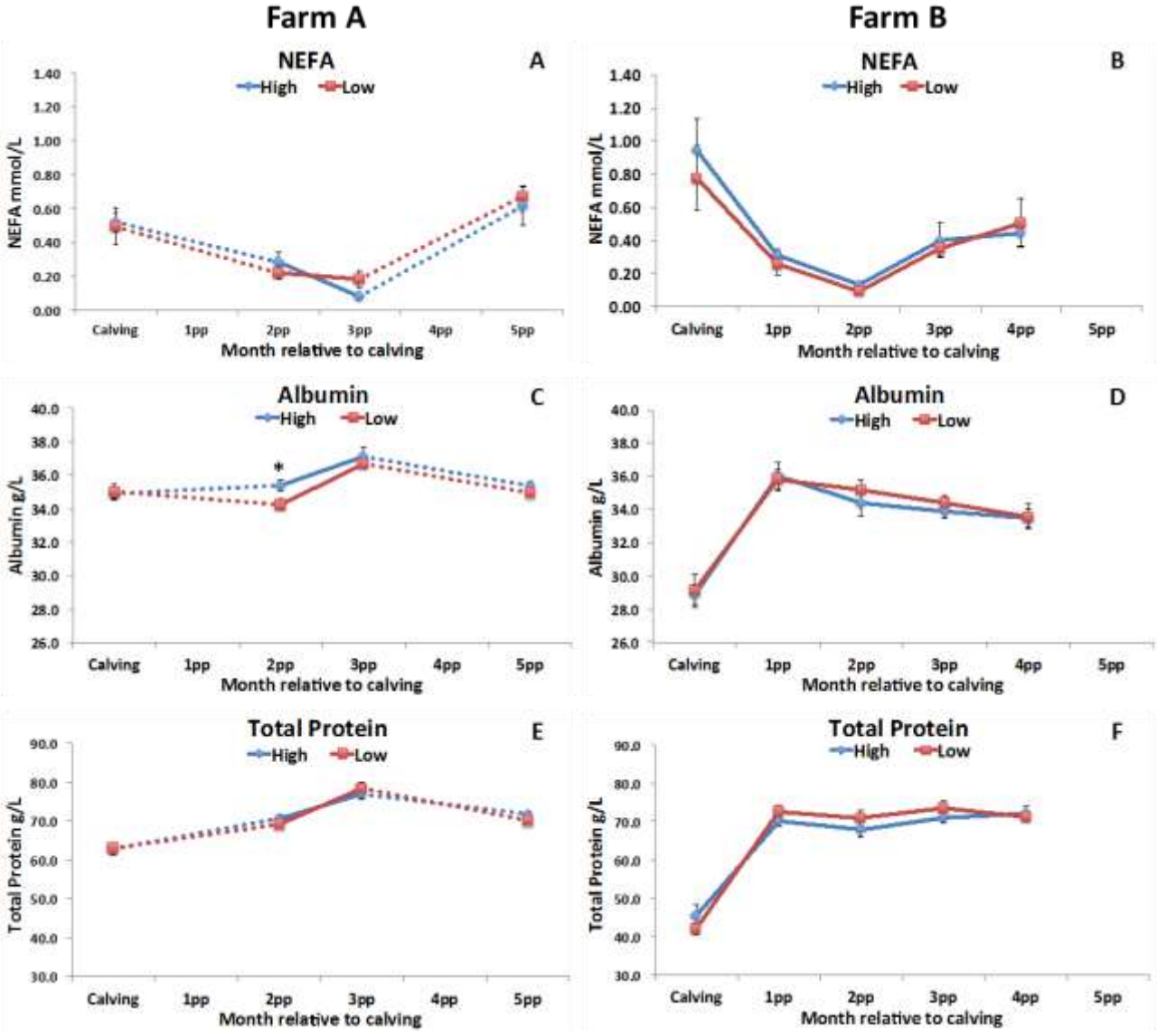
Cortisol

There was a significant difference between farms at calving in circulating cortisol concentrations; heifers at farm B had higher circulating cortisol concentrations than heifers at farm A. At 2 months after calving, there was a significant difference between farms; heifers at farm B had lower circulating cortisol concentrations than heifers at farm A. There was no significant difference between preH diet and preL diet in the circulating cortisol concentrations levels after calving at farm A (Fig 19-E), or at farm B (Fig 19-F).

Non-Esterified Fatty Acids (NEFA)

There was a significant difference at calving in circulating NEFA concentrations; heifers at farm B had higher circulating NEFA concentrations than heifers at farm A. There was a significant difference between farms at 2 months after calving; circulating NEFA concentrations were lower in heifers at farm B than in heifers at farm A. At 3 months after calving, circulating NEFA concentrations were higher in heifers at farm B than in heifers at farm A. There was no significant difference between preH diet and preL diet in the circulating NEFA concentrations after calving at farm A (Fig 22-A), or at farm B (Fig 22-B).

Figure 22. Average (±s.e.m.) circulating NEFA (A-B), albumin (C-D), and TP (E-F) concentrations after calving in farm A (left) and farm B (right) farms. (*) means that there is a significant difference (P<0.05)



Albumin

There was a significant difference in the circulating albumin concentrations between farms at calving; circulating albumin concentrations were lower in heifers at farm B than in heifers at farm A. At 3 months after calving, a similar difference was observed; circulating albumin concentrations were lower in heifers at farm B than in heifers at farm A.

Farm A

At 2 months after calving, there was a significant difference in circulating albumin concentrations between diet groups; circulating albumin concentrations were lower in heifers in the preL diet than in heifers in the preH diet. There was no other significant difference between preH and preL in the circulating albumin concentrations after calving (Fig 22-C).

Farm B

There was no significant difference between preH heifers and preL heifers in the circulating albumin concentrations after calving (Fig 22-D).

Total Protein (TP)

There was a significant difference in the circulating TP concentrations between farms at calving; circulating TP concentrations were lower in heifers at farm B than in heifers at farm A. A similar difference was observed at 3 months after calving; circulating TP concentrations were lower in heifers at farm B than in heifers at farm A.

Farm A

There was a tendency for a difference in the circulating TP concentrations between diet groups at 5 months after calving; circulating TP concentrations were lower in heifers in the preL group than in heifers in the preH group. There was no significant difference between preH and preL in the circulating TP concentrations after calving (Fig 22-E).

Farm B

There was no significant difference between preH heifers and preL heifers in the circulating TP concentration after calving (Fig 22-F).

4.1.3.4 Pregnancy diagnosis after 1st calving

In Farm A, from the 18 heifers within the study, 17 heifers were pregnant at the end of the 12wk breeding season: ten heifers were in the preL diet and seven heifers in the preH diet. Heifers in the preH diet had an average of 18 weeks from calving to conception, the range being between15 to 23 weeks; similarly, heifers in the preL diet had an average of 19 weeks from calving to conception, with a range of 15 to 24 weeks. Only one heifer in the preL diet at her second calving had an assisted calving.

In Farm B, all the heifers within the experiment became pregnant by the end of the 12 wk breeding season. Heifers in the preH diet conceived within 17 weeks from calving, with a range of 14 to 20 weeks; similarly, heifers in the preL diet had an average of 16 weeks from calving to conception, with a range of 15 to 19 weeks.

4.1.3.5 Heifers ongoing reproduction and calving success

Farm A

There was no significant effect of heifer diet at the following calving events. Days from calving to calving was not significant different between diet groups (preL 390.2 ±8.7 days; preH 390.1 ±8.9 days); the first calving interval between 2yo and 3yo calving (preL 416.8 ±7.5; preH 418.9 ±8.5) was longer than the interval between 3yo and 4yo calving (preL 363.6 ±6.1; preH 361.2 ±7.8).

The calves' birth weight increased between calvings with cow age but it was not affected by heifer diet (Fig 23). In the second calving, only 15 heifers calved (preL =8 and preH =7) mainly bull calves (n=13). The weight progress of these 15 calves by diet can be seen in Fig 24.

Figure 23. Average birth weight (±s.e.m.) of the heifers' progeny at calving in farm A grouped by heifers study diet (during the first calving)

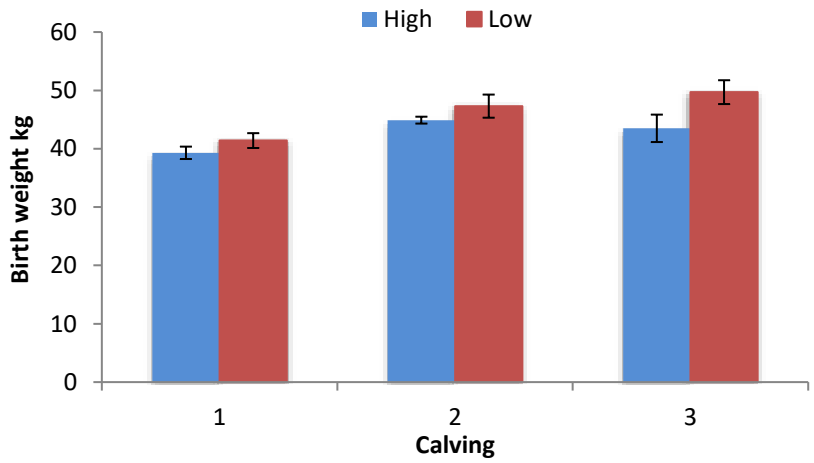
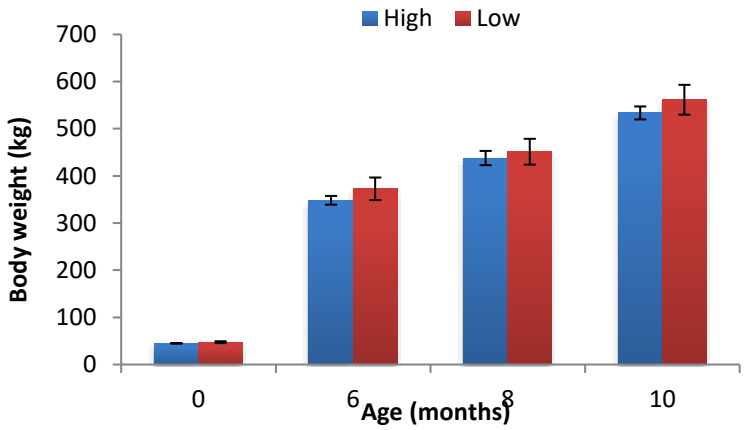


Figure 24. Average weight (±s.e.m.) of the calves from 2nd calving grouped by heifers experimental diet (during first calving) in farm A



4.1.4 Postnatal measurement in calves

Measurements taken in calves during the postpartum period focused on body weight, height and hormonal assessment in both farms. Measurement of milk intake using the weight-suck-weight technique was used only in farm B as it was not possible to measure this in farm A.

4.1.4.1 Body weight and height

Farm A

Birth weight in preL and preH calves was similar between male and female calves. However, female calves from preH heifers seemed to be lighter than calves from preL heifers (male and female) or males from preH heifers at 3 and 5 months of age (Fig 25-A). From 11 months of age, preL calves were heavier than preH calves; female calves from preH heifers seemed lighter than preH and preL males. PreH female calves showed lower height at birth compared to other groups and continued to be so up to 5 months of age (Fig 26-A).

Farm B

As mentioned in table 4, birth weight was affected by diet and sex, but this effect was lost in the months following birth (Fig 25-B). Calf height was not different in calves born to preL or preH heifers in the months following calving (Fig 26-B).

Figure 25. Average (±s.e.m.) calf weight during the post-partum period in calves from heifers receiving a low (10% CP) or high (18% CP) diet during the pre-conception period (-60d to conception, (AI) in farm A (A) and in farm B (B).

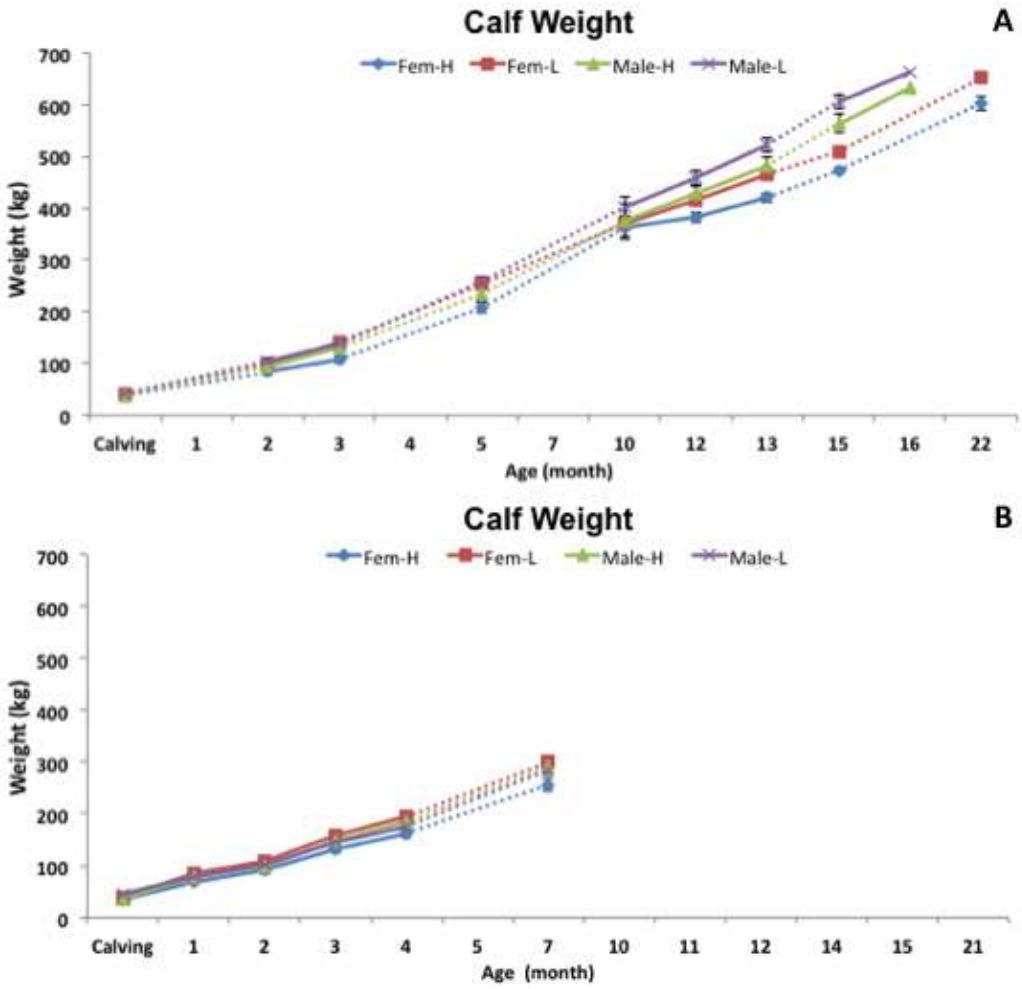
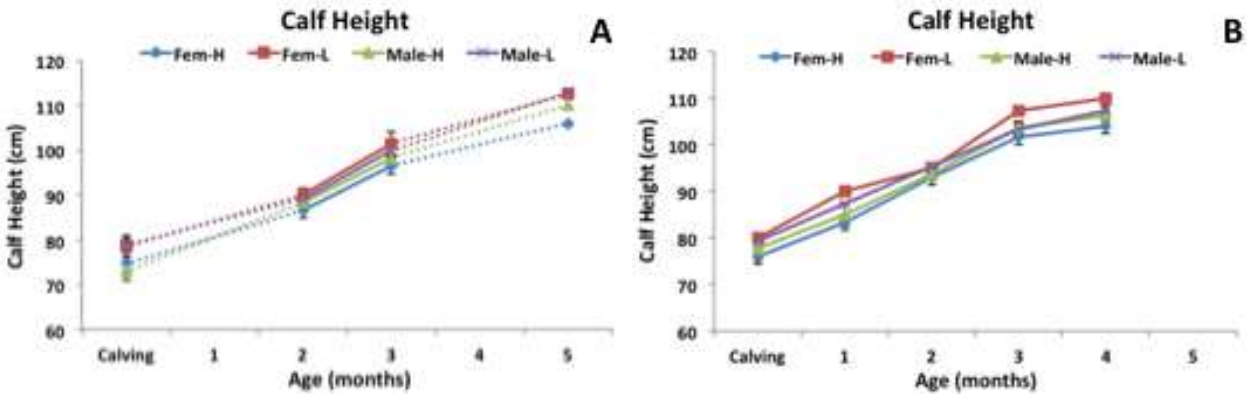


Figure 26. Average (±s.e.m.) calf height during the post-partum period in calves from heifers receiving a low (10% CP) or high (18% CP) diet during the pre-conception period (-60d to conception, AI) in farm A (A) and in farm B (B).

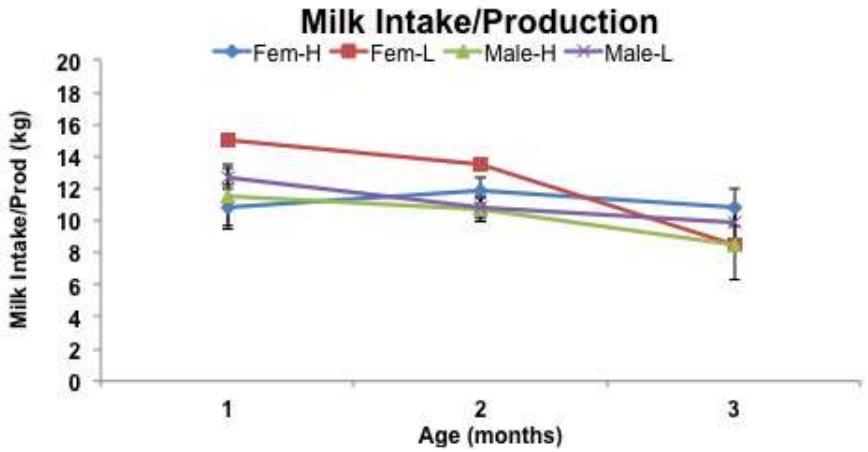


4.1.4.2 Milk intake/Milk Production (Weigh-Suck-Weigh)

Farm B

Weigh-suck-weigh was carried out in farm B to evaluate milk production/calf intake in a 24h period. There was a decrease in milk production/intake with age from 1st to 3rd month of age (Fig 27). The amount of milk produced/consumed by preL was similar to preH heifers. Interestingly, there was an association between milk production and circulating oxytocin concentrations in the heifer.

Figure 27. Average (s.e.m) milk intake/production in a 24h period as estimated by the Weigh-Suck-Weigh technique (WSW) in calves/heifers from 1 to 3 months post-partum in farm B.



4.1.4.3 Calf hormonal assessment

Cortisol

At calving there was a tendency for a difference in the circulating cortisol concentrations between male and female calves by farm; male calves at farm B had lower circulating cortisol concentrations than male and female calves at farm A. Cortisol concentrations decreased following parturition in all calves in both farms.

Farm A

The circulating cortisol concentrations were not significantly different in preL and preH calves in the months following calving (Fig 28-A).

Farm B

From calving and up to 4 months post-calving, concentrations of cortisol tended to be higher in female calves than in male calves (Fig 28-B).

Leptin

Circulating leptin concentrations were different between farms; circulating leptin concentrations were lower in calves at farm B than in calves at farm A. Similarly, at 3 months after calving, circulating leptin concentrations were lower in calves at farm B than

in calves at farm A. Circulating leptin concentrations were not different between preL and preH calves in the months following calving at farm A (Fig 28-C) and at farm B (Fig 28-D).

IGF-1

At 3 months after calving, there was a significant difference in the circulating IGF-1 concentrations according to diet and calf sex; circulating IGF-1 concentrations were lower in preL calves than in preH calves and lower in female calves than in male calves. Overall, IGF-1 concentrations increased after calving in all calves in both farms, regardless of diet.

Farm A

At calving, circulating IGF-1 concentrations were lower in preH female calves than in preH male calves; and circulating IGF-1 concentrations were higher in preL female calves than in preL male calves (Fig 28-E).

Farm B

At 5 months after calving, circulating IGF-1 concentrations were lower in preL calves than in preH calves (Fig 28-F).

Non-Esterified Fatty Acids (cNEFA)

At two and three months after calving, circulating cNEFA concentrations were higher in calves at farm B than in calves at farm A. The circulating cNEFA concentrations were not different between preL and preH calves at farm A (Fig 29-A) or at farm B (Fig 29-B). But concentrations seemed to decrease after calving in both farms.

Albumin

At calving there was a significant difference between farms in circulating albumin concentrations; albumin concentrations were higher in calves housed at farm B than those in farm A. One month after calving, female calves tended to have higher albumin circulating concentrations than male calves in farm B (Fig 29-D). Two months after calving, circulating albumin concentrations were lower in calves housed at farm B than in calves housed at farm A. The circulating albumin concentrations were not different between preL and preH calves in the months following calving at farm A (Fig 29-C) or at farm B (Fig 29-D).

Figure 28. Average (±s.e.m.) circulating cortisol (A-B), leptin (C-D) and IGF-1 (E-F) concentrations during the post-partum period in calves from heifers receiving a low (10% CP) or high (18% CP) diet during the pre-conception period (-60d to conception, AI) in farm A (Left) and in farm B (Right).

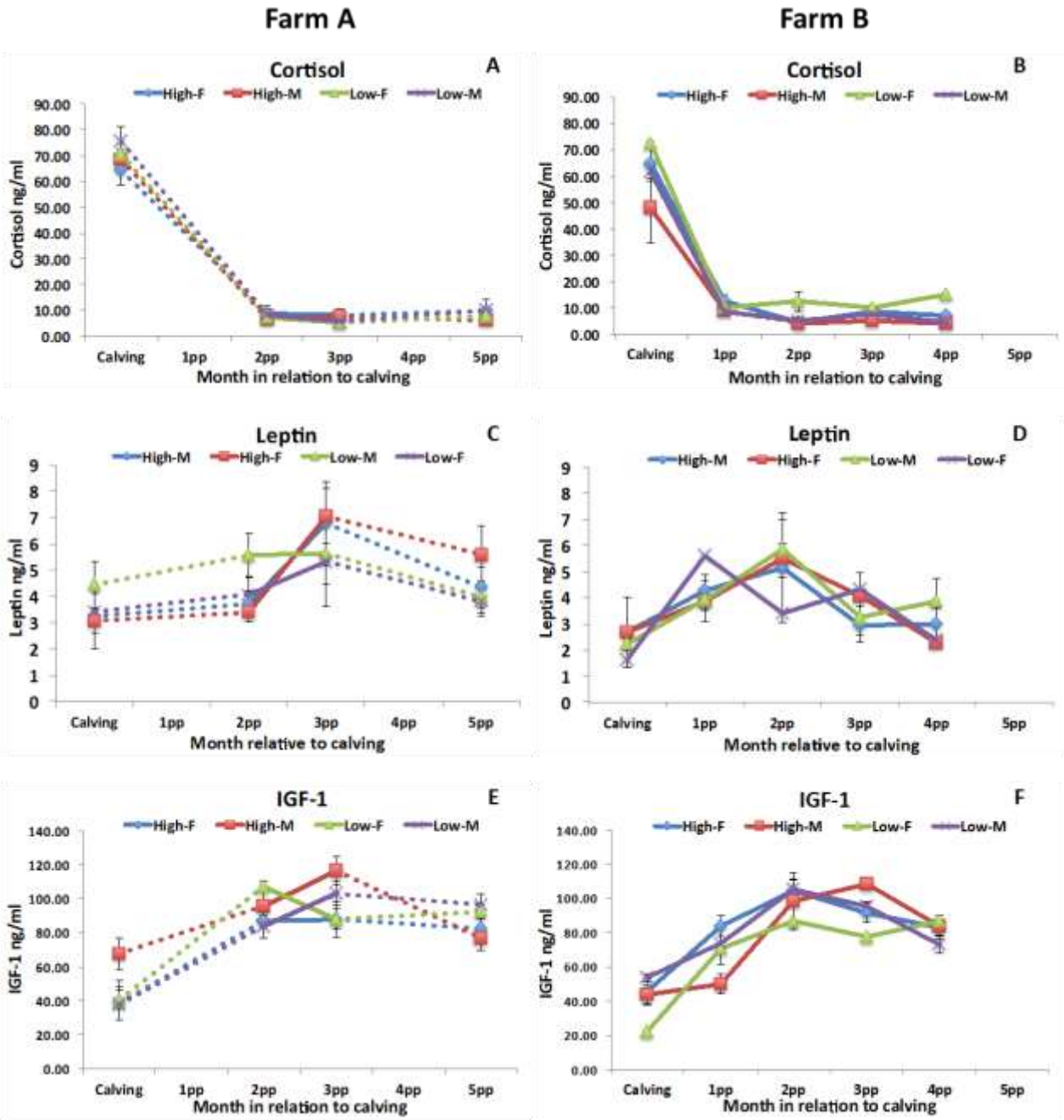
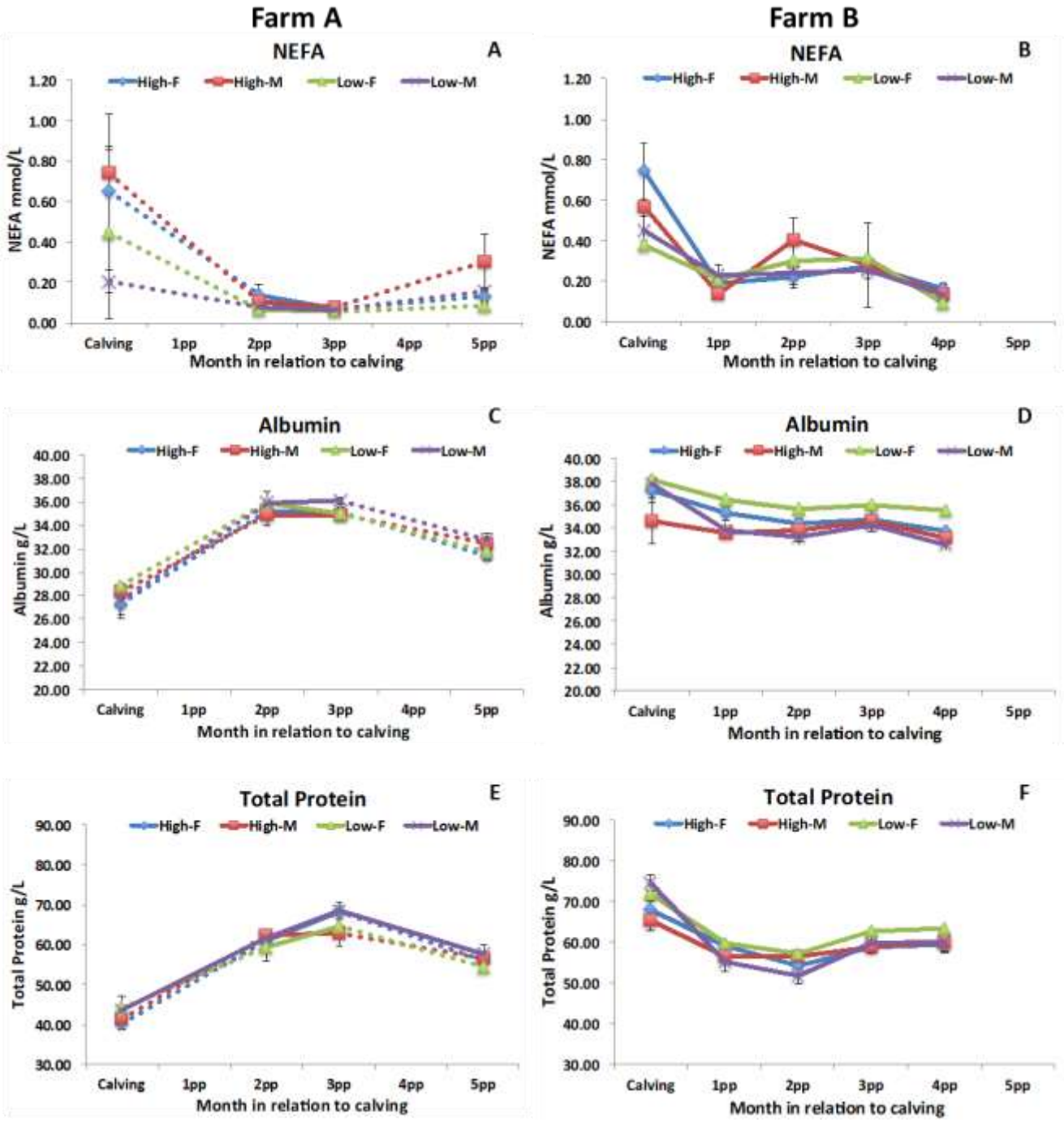


Figure 29. Average (±s.e.m.) circulating NEFA (A-B), albumin (C-D) and total protein (E-F) concentrations during the post-partum period in calves from heifers receiving a low (10% CP) or high (18% CP) diet during the pre-conception period (-60d to conception, AI) in farm A (Left) and in farm B (Right).



Total Protein (TP)

At calving circulating TP concentrations were higher in calves housed in farm B than those in farm A, while at two months after birth the opposite was observed. Moreover, circulating TP concentrations were higher in preL calves than in preH calves.

Farm A

Throughout the study period, TP concentrations were similar in calves form heifers in both diet groups, but at two months circulating TP concentrations were higher in preL female calves than in preL male calves (Fig 29-E).

Farm B

At calving, circulating TP concentrations were higher in preL calves than in preH calves (Fig 29-F). Following birh, TP concentrations were similar between both groups of calves.

4.1.4.4 Carcass weights

Farm A

At 16 months of age, 6 bulls from the 18 calves were slaughtered, 5 of these were preL calves and had an average carcass net weight of 367 kg. These carcasses were classified as R3 (3), R2 (1) and U-3 (1). The remaining bull was a preH treatment calf. It had a carcass net weight of 356 kg and was classified as U+3.

4.1.4.5 Heifers progeny

Farm A

From the seven heifer calf progeny (preL= 2 calves and preH = 5 calves), two did not calve (one from preL group and one from preH group); the remaining calves had an easy calving except one heifer calf from the preH that needed to be pulled, and the average progeny weight was 40.8 (±1.3) Kg.

4.2 Post-Conception Diet Treatment

4.2.1 Measurements before calving

4.2.1.1 Fertility

Farm C heifers (n=100) were synchronised and fixed-timed inseminated using the same protocol as at farm A and B and subjected to high and low protein diets from insemination/conception to 90dpc (postH, n=49 and postL, n=51). Pelvic measurement using a Rice pelvimeter was performed on the heifers one month prior to initiation of the trial. Fertility rate at 36dpc was 57% (postH, n=29 and postL, n=28) based on the ultrasound scans and the size of the fetus (Table 3). At 60dpc, second US scan, one heifer in the low protein post-conception diet had lost the pregnancy, the resulting experimental group was therefore: postH, n=29 and postL, n=27.

4.2.1.2 Heifer Body Weight

Observed body weight in the experimental heifers in Farm C was similar from one month prior to the start of the experimental dietary treatment post-conception (0-90dpc) (Table 1) and at the 1st, 2nd and 7th month of pregnancy (Fig 30-A). Moreover, average daily

weight gain followed a similar pattern between postH and postL groups and was not different at 1 and 7 months of pregnancy; Fig 30-B). However, there was a significant difference in the average daily weight gain at 2 months post AI (during the experimental treatment), whereby the postH heifers gained less weight than postL heifers. It was apparent that ADG increased post-conception (1st month) to then decrease between the 1st and 2nd month of pregnancy, with a more pronounced drop in ADG in the postH heifers receiving the post-conception high diet (postH; 14%CP). After the dietary treatment had finished postH and postL heifers showed comparable ADGs (Fig 30-B).

4.2.1.3 Fetal Growth

Fetal size, as indicated by crown-rump length (CRL at 36dpc) and biparietal distance (BPD at 60dpc), was affected by post-conception diet (Fig 31). That is, fetuses from postH heifers were smaller at 36dpc (CRL) than postL fetuses. However, this effect was not apparent at 60dpc (BPD) when fetuses from both groups were of similar size (Fig 31).

4.2.1.4 Doppler Measurements

Doppler indices were obtained at 7th month of pregnancy. Blood flow volume and mid-uterine artery diameter corresponding to the pregnant horn were not affected by the post-conception diet, (Fig 32) nor by fetal sex.

Figure 30. Average (±s.e.m.) body weight (A) and daily gain (ADG; B) in heifers that received a high (14% CP) or low (10% CP) protein diet during the post-conception period at farm C. **Initial ADG was taken from the original files and was calculated by the farmer.*

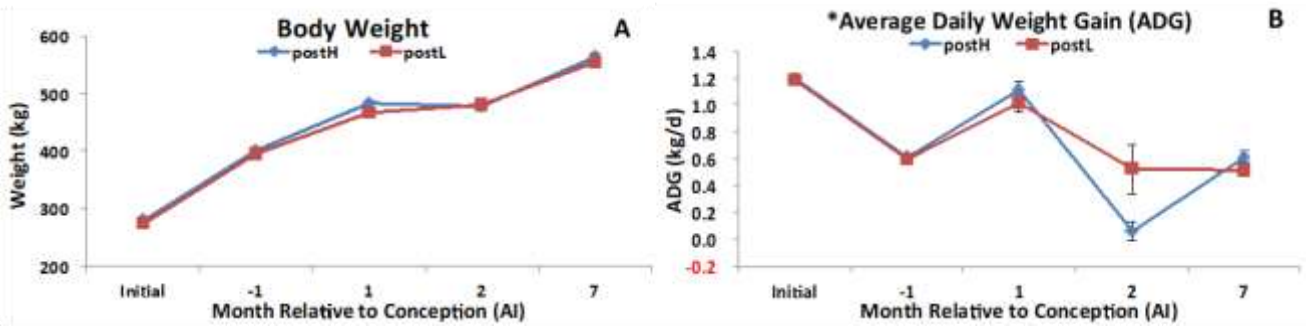


Figure 31. Average (±s.e.m.) crown-rump length (CRL) at 36dpc and biparietal distance (BPD) at 60 in pregnant heifers that received a high (14% CP; postH) or low (10% CP; postL) protein diet during the post-conception period at farm C. ** Indicates a significant difference when comparing postH vs postL.*

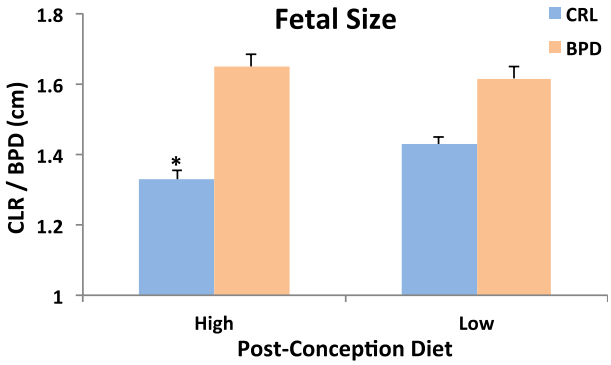
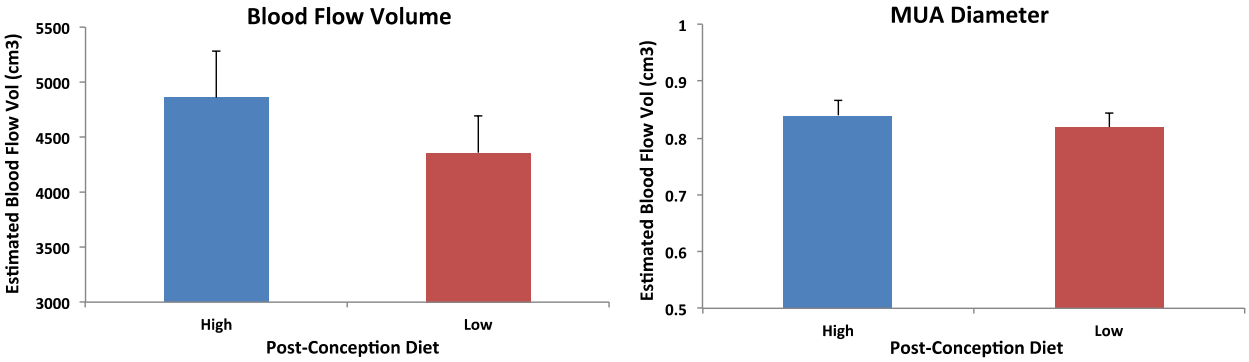


Figure 32. Mid-uterine artery (MUA) average (±s.e.m.) blood flow volume and diameter corresponding to the pregnant uterine horn in heifers that received a high (14%) or low (10%) crude protein diet during the post-conception period.

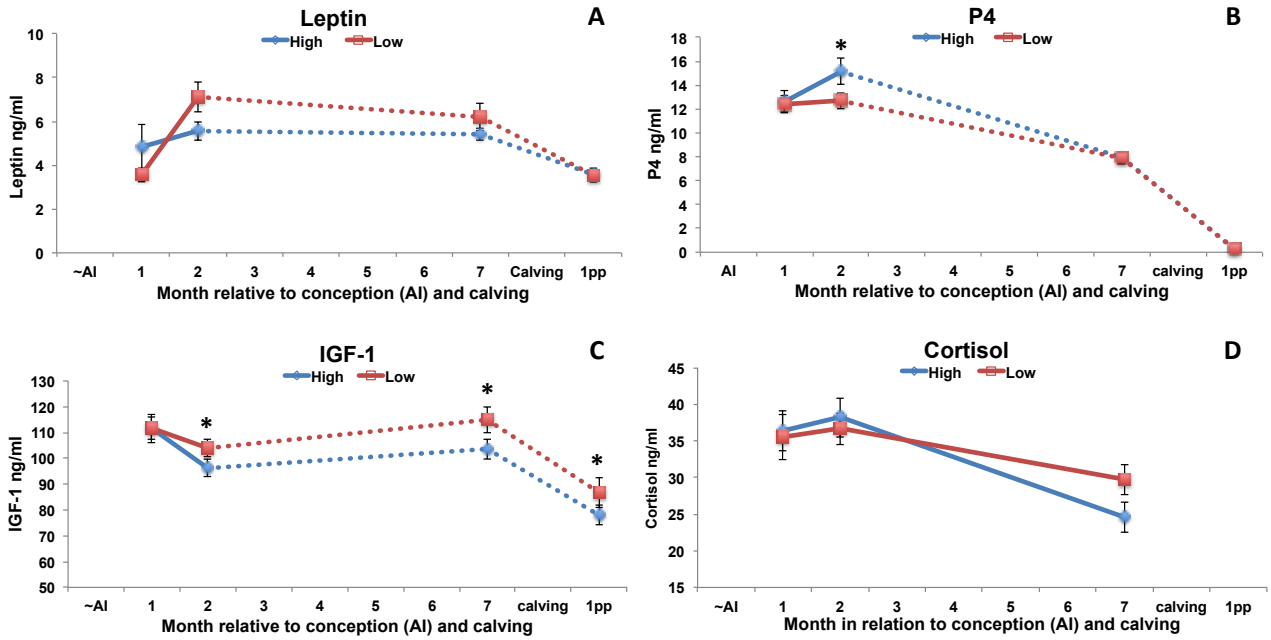


4.2.1.5 Heifer hormonal assessment before and after calving

Leptin

Concentrations of leptin in the postL group increased from the first to the 2nd month of pregnancy, while concentrations in the post H remained similar throughout pregnancy. One month after calving, concentrations of leptin return to levels comparable to those at 1 month of pregnancy in both groups (Fig 33-A).

Figure 33. Average (±s.e.m.) circulating leptin, P4, IGF-1 and cortisol concentrations during pregnancy, calving and in the immediate post-calving period, in heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period. ** Indicates a significant difference when comparing postH vs postL.*



Progesterone (P4)

Concentrations of progesterone were affected by month of pregnancy, with levels being lower during the 7th compared to 1st or 2nd months of pregnancy (Fig 33-b). Moreover, progesterone concentrations were higher in postH compared to postL heifers during the 2nd month of pregnancy. At seven months after AI, there was a significant difference in the circulating P4 concentrations between heifers depending on the sex of the fetus; circulating P4 concentrations were lower in heifers with a female fetus than in heifers with a male fetus. No other differences were observed for the P4 blood levels.

IGF-1

IGF-1 concentrations remained relatively constant during pregnancy at 1,2 and 7mths, after calving however, there was a significant decrease in circulating IGF-1 concentrations (Fig 33). At two, 7 months post conception (AI) and at the 1st month post-calving, circulating IGF-1 concentrations were higher in postL compared to postH heifers (Fig 33-C).

Cortisol

Circulating cortisol concentrations increased at 2 months after conception (AI), to then decrease towards the end of pregnancy (7 months post-conception). There was a tendency for a difference in the circulating cortisol concentrations between postH and postL heifers

at the 7th month of pregnancy (Fig 33-D), with higher circulating cortisol concentrations in postL heifers.

Non-Esterified Fatty Acids (NEFA)

Circulating NEFA concentrations increased from the 2nd to the 7th month of pregnancy and 1st month post-partum in all heifers. At one month after AI, circulating NEFA concentrations tended to be higher in postL heifers than in postH heifers (Fig 34-A). This trend was the opposite at two months after AI with postL heifers showing lower NEFA concentrations than the postH heifers (Fig 34-A). At 7 months after AI, circulating NEFA concentrations were lower in postH heifers carrying a female fetus than in heifers in the same diet group but with a male fetus. In addition, circulating NEFA concentrations were higher in heifers with a female fetus if they were in the postL group than in the postH group.

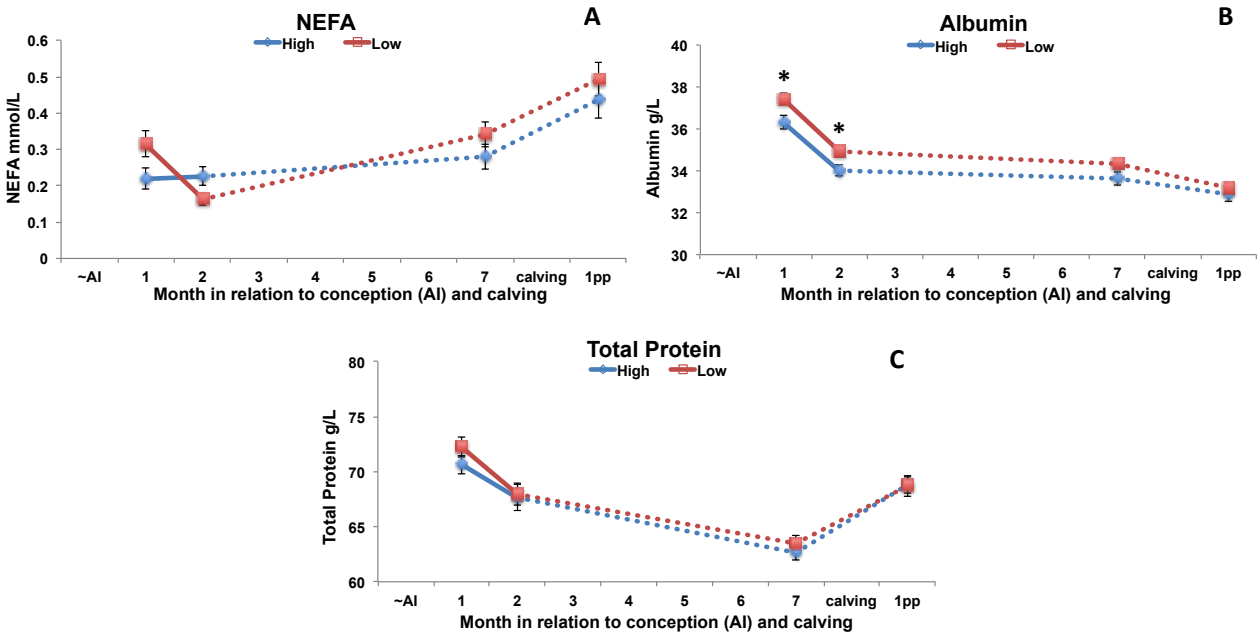
Albumin

Circulating albumin concentrations were higher in postL than postH heifers in the first and second month of pregnancy (Fig 34-A). Overall, circulating albumin concentrations were higher in the 1st month of pregnancy to then decrease at 2 months, remaining at constant level until after parturition.

Total Protein (TP)

No differences were observed in the circulating total protein concentrations between postH and postL heifers (Fig 34-C). However, TP concentrations were higher at 1month, decreased during pregnancy, reaching their lowest level at 7 months of pregnancy, to then increase again at 1-month post-calving.

Fig 34. Average (±s.e.m.) cNEFA, cAlbumin and cTP blood levels during the pregnancy, calving and in the inmediate post-calving period in heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period. ** Indicates a significant difference when comparing postH vs postL.*



4.2.2 Measurements at Calving

At Farm C, calving occurred out at pasture with the calf body weight taken as soon as practically possible within 12hrs of calving. From the 56 heifers pregnant to AI, 55 calved at term. However, 5 calves died during or soon after calving and 2 had a twin pregnancy, therefore these animals were removed from analysis. The total number of animals with their average weight at calving is shown in table 5. No significant correlation occurred between pelvic area and calf birthweight or between pelvic area and dystocia. (The low numbers experiencing dystocia may have influenced this latter effect).

Age at calving was similar between both postH and postL heifers. However, gestation length tended to be affected by calf sex, with pregnancies carrying females being slightly shorter than those with males (Table 6). Only 2 experimental heifers required assistance therefore diet or sex did not affect calving difficulty. All animals were approximately 24 months at calving.

At birth, postL calves were heavier than postH calves and male calves were heavier than female calves (Table 6). PostL males were 3kg heavier than the preL females. After birth, female calves gained more weight tending to be heavier than males at 2 months of age (Fig 35). This was reflected in their ADG, with heifer calves showing higher gains over the 2-month period after birth (Fig 36). Notably as in the other farms (A, B) the postL females

gained weight at the fastest rate. At 7 months of age, no difference in calf weight between the diet groups was observed (Fig 36). At 13months of age only female calf data was available, with male data available between 15-18 months dependent upon sale date.

Table 6. Average (±s.e.m.) age at calving, gestation length and birth weight in heifer and calves corresponding to either high (14% CP) or low (10% CP) protein diet during the post-conception period.

Group	Sex	n	Age @ Calving (months)	Gestation length (days)	Calf Birth Weight (kg)
postH	M	10	23.9 ± 0.1	282.5 ± 1.4	34.4 ± 1.7
	F	15	24.1 ± 0.1	281.7 ± 1.5	34.4 ± 0.7
postL	M	17	24.0 ± 0.1	282.6 ± 0.7	37.25 ± 0.9
	F	7	23.6 ± 0.2	278.4 ± 0.4	34.3 ± 1.6
P-value		*Diet*	*0.39*	*0.62*	*0.05*
		Sex	*0.36*	*0.07*	*0.04*

Figure 35. Average (±s.e.m.) body weight during the post-calving period in male and female calves from heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period.

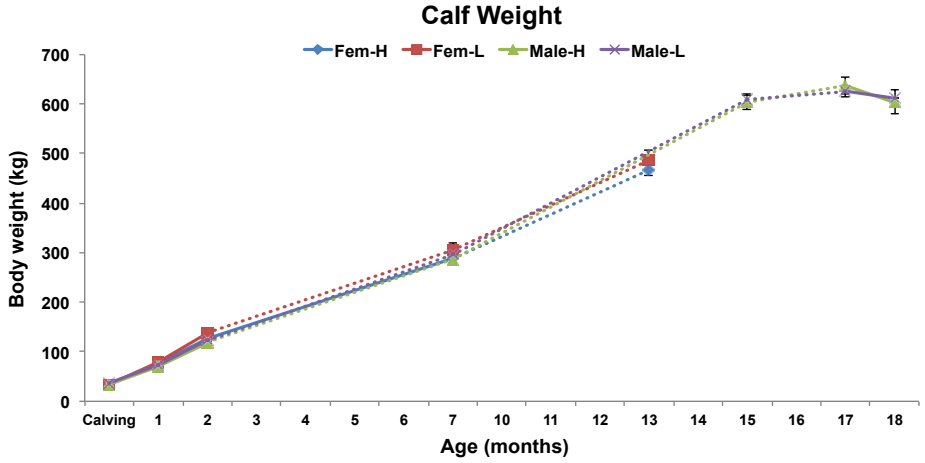
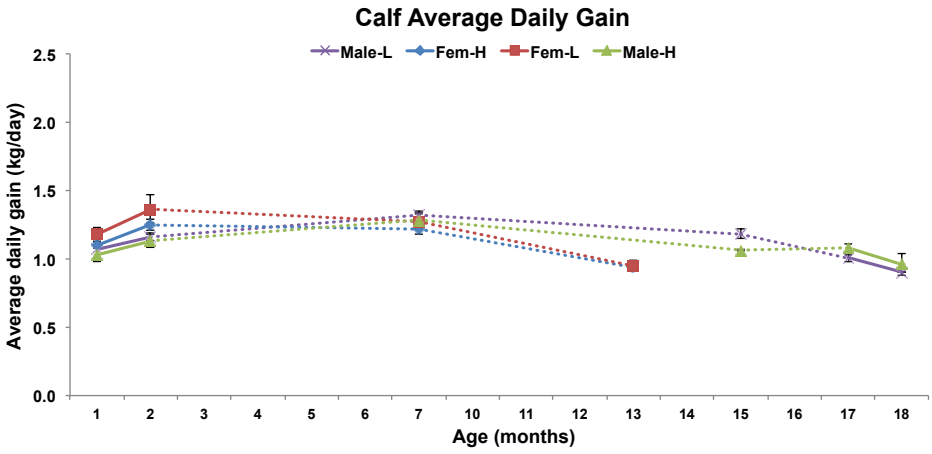


Figure 36. Average (±s.e.m.) daily weight gain during the post-calving period in male and female calves from heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period.



4.2.3 Heifers Post-Partum Measurements

4.2.3.1 Body weight

Body weight in heifers was not recorded at calving at Farm C, but there was a loss of weight between 7th months of pregnancy and the 1st postpartum month (also observed in ADG), a loss that was similar between postH and postL (Fig 37). Following parturition, heifers in both experimental groups had similar body weights at 1 and 2 months post-calving with weight 2 months after parturition increased in both groups. At 6 months post-calving both groups had increased their body weight with a tendency for the postH group (P=0.06) to have increased more than the postL. Both groups, however, showed a reduction in ADG at this time in comparison to the ADG at 2 months post-calving.

When heifer weight was expressed as a ratio to the pre-experimental weight one month prior to insemination (20 –May –2013) it was observed that postH and postL heifer growth followed a similar pattern throughout the experiment (Fig 38).

Figure 37. Average (±s.e.m) body weight (A) and daily weight gain (ADG; B) in experimental heifers that received either a high (14%) or low (10%) protein diet during the post-conception period.

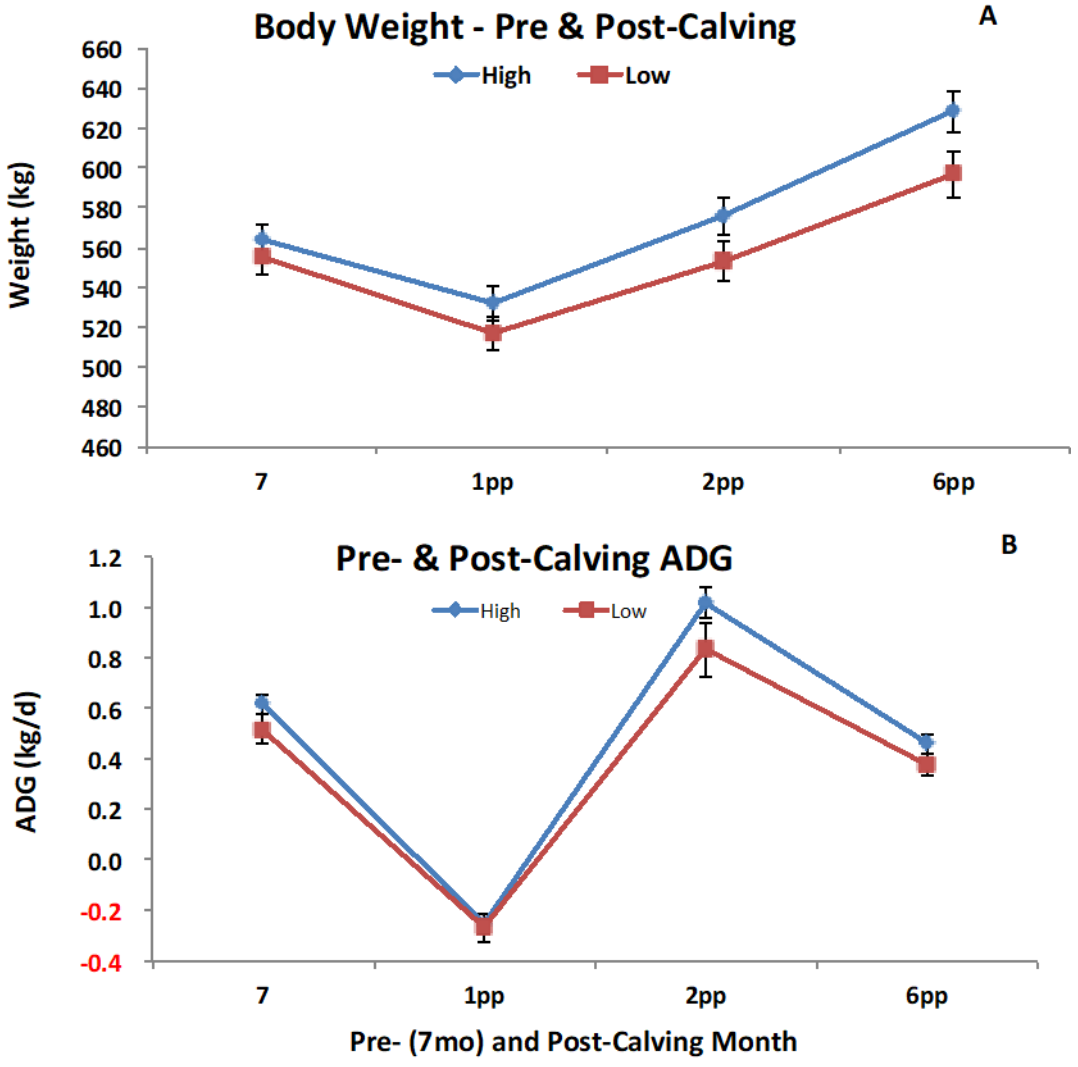
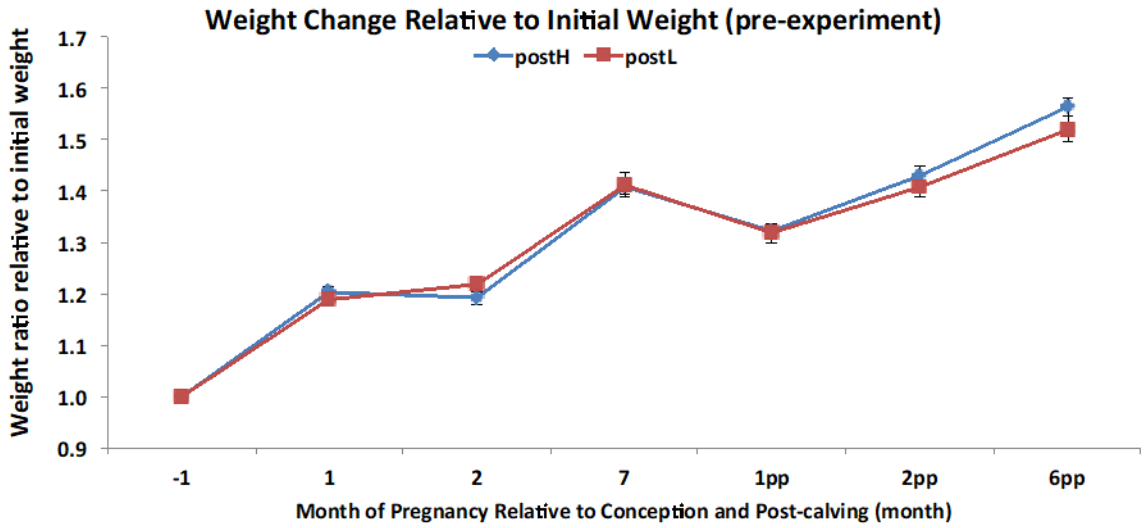


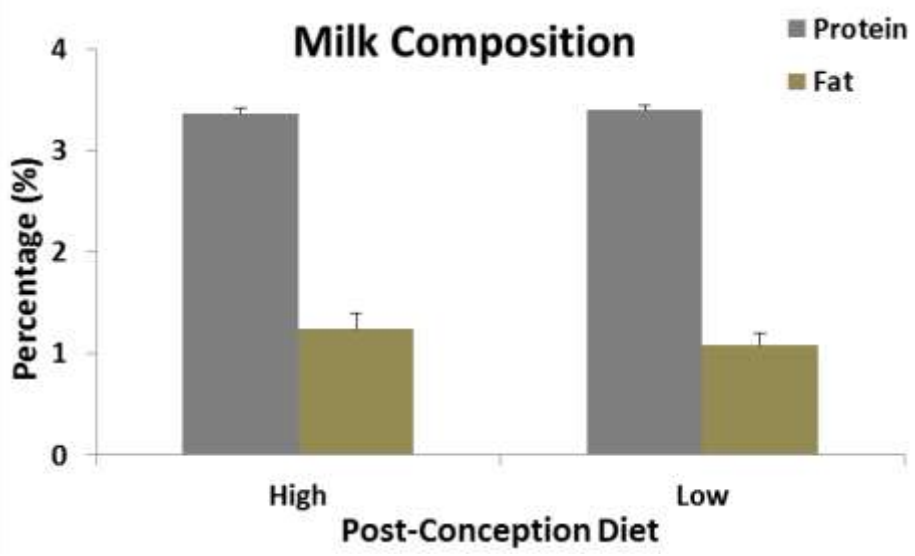
Fig 38. Change in body weight relative to the initial weight (i.e. pre-experimental; 20-May-2013) in experimental heifers that received a high (14% CP) or low (10% CP) protein diet during the post-conception period.



4.2.3.2 Milk composition

Milk composition was not affected by diet treatment or sex of the calf in samples taken at 1-month post-calving (Fig 39). Milk protein concentrations were comparable to those observed in the pre-conception diet farms (A and B) with milk fat intermediate between those farms. As with the farm B milk fat, this may have been influenced by the different diets at the time of milk sample collection and also by dilution. The rate of growth of the farm C calves was higher than the farm B group that as we have noted was higher than farm A. This rate of gain indicates a probable higher milk output from these heifers, which we were unable to ascertain as no WSW was carried out.

Figure 39. Average (±s.e.m.) milk protein and fat content in samples taken at 1-month post-partum in heifers that received a high (14% CP) or low (10% CP) protein diet during the post-conception period at farm C.



4.2.3.3 Pregnancy diagnosis after 1st calving

From the 50 heifers within the study, 42 became pregnant in the subsequent mating period. Seven heifers were categorised by the farm as late pregnant at 6 months after calving. From these late-pregnant 5 belonged to the postH group and 2 to the postL group. Five heifers did not become pregnant six months after calving, 2 of these belonged to the postH group and 3 to the postL group.

4.2.4 Calf Post-Partum measurements

4.2.4.1 Hormonal assessment

Leptin

The circulating leptin concentrations were lower in postL female calves in comparison with postH female calves and postL male calves (Fig 40).

IGF-1

No differences were observed in the circulating IGF-1 concentrations between postH and postL calves (Fig 40).

Non-Esterified Fatty Acids (NEFA)

There was a tendency for a difference between diet group and calves sex; circulating NEFA concentrations tended to be higher in postL female calves than in postH female calves, and in female calves than in male calves born from heifers in the postL group (Fig 41).

Figure 40. Average (±s.e.m.) circulating leptin and IGF-1 concentrations one month after calving in male and female calves from heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period (0-90dpc).

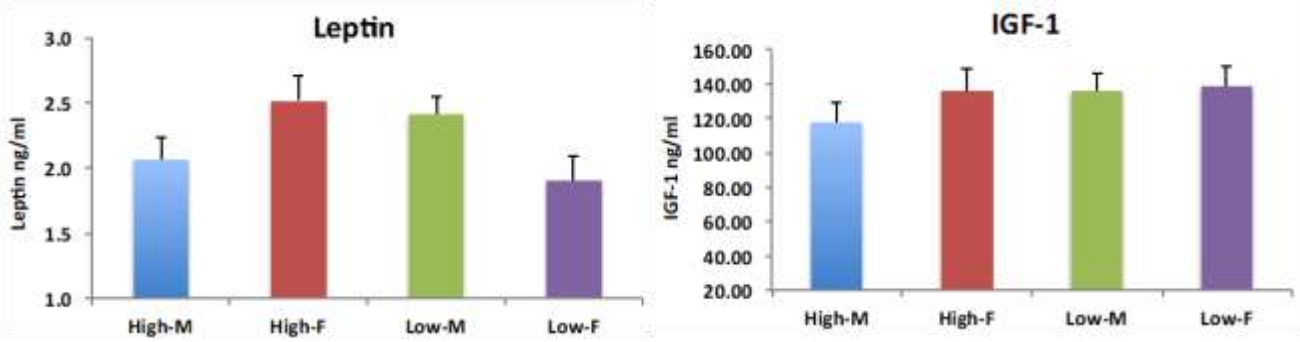
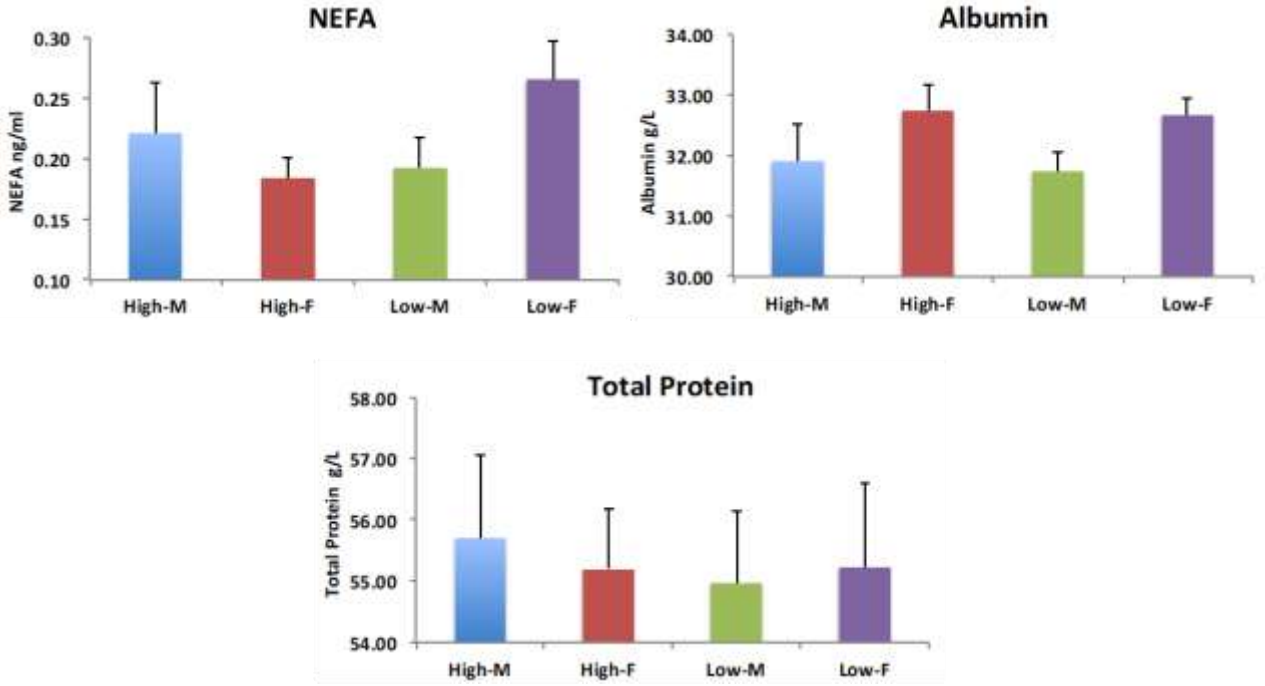


Figure 41. Average (±s.e.m.) circulating NEFA, albumin and TP concentrations one month after calving in male and female calves from heifers that received a high (14% CP) or low (10%CP) protein diet during the post-conception period.



Albumin

There was a significant difference between male and female calves; circulating albumin concentrations were higher in female calves than in male calves. However, heifer diet did not affect circulating albumin concentrations (Fig 41).

Total Protein (TP)

Heifer diet or calf did not affect TP circulating concentrations in this study (Fig 41).

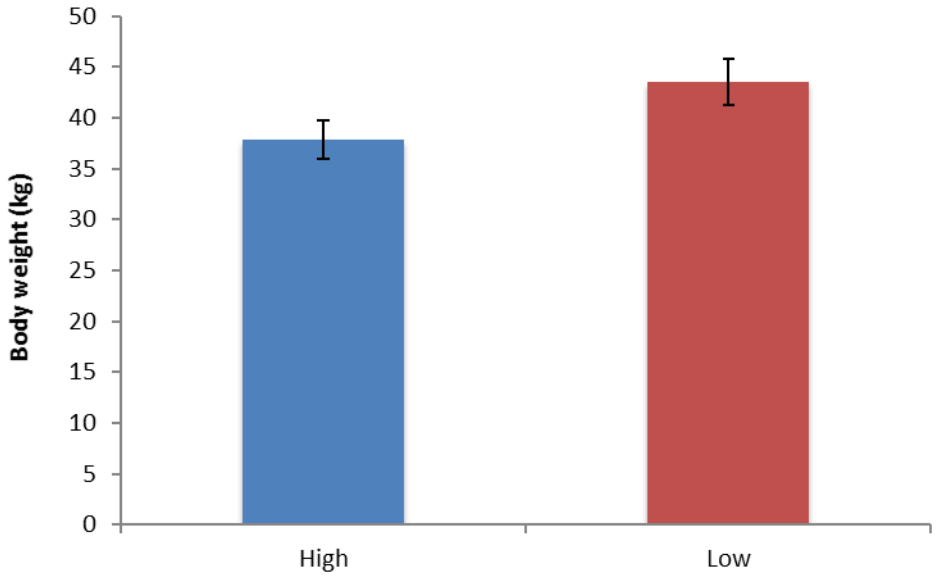
4.2.4.2 Pelvic measurement of heifer progeny

The daughters of experimental heifers had internal pelvimetry measured at 13 months of age. Heifers from postH mothers (n=14) had an average measurement of 174.14 ±4.10 cm (mean ±s.e.m) while those from postL mother (n=7) had 182.14 ±7.37 cm. None of these measures were related to their mother's pelvic measurement at the beginning of the trial (Heifers on High diet: 155.24 ±2.30; heifers on Low diet: 153.12 ±2.12).

4.2.4.3 Calving success of heifer progeny

Only 10 heifers (67%) from postH mothers calved; 3 heifer calves and 7 bull calves. Five (5) heifers (72%) from postL mothers calved; 4 bull calves and 1 heifer calf. In both diet groups 1 calf died at birth. There was no significant effect of diet on their calve's birth weight (Fig 42).

Figure 42. Calves average (±s.e.m.) body weight at calving from heifers progeny (F1) that received a high (14% CP) or low (10%CP) protein diet during the post-conception period.



4.3 Effects of dietary protein upon follicular dynamics in the beef heifer (*Completed in collaboration with Dr Bob Robinson and Jennifer Edwards*)

4.3.1 Carcass quality

In Farm D heifers were fed either a high protein (14.5%CP) or low protein (10.4%CP) diet for 2 months (60d) prior to slaughter, a total of 276 heifers were necropsied. One hundred and twenty carcasses from the high protein group graded as follows: 81 heifers were graded O (O= 20, O-= 1 and O+ = 60) and 39 heifers were graded R (R= 4, R- = 33 and R+ = 2) (Table 7). The remaining 157 carcasses from the low protein group graded as follows: 127 heifers were graded O (O= 57 and O+ =70), 29 heifers were graded R (R= 10 and R- = 19) and 1 heifer was graded as U- (Table 7). A higher percentage of carcasses from the high protein diet group were of good carcass quality (Conformation class R) than carcasses from the low protein diet group. In addition, a higher percentage of carcasses from the low protein group were of fair quality (Conformation class O) than carcasses from the high protein group.

Seven high protein carcasses were graded as fat level ≤ 3, 103 carcasses were graded as fat level 4 and 10 carcasses were graded as fat level 5. Similarly, seven low protein carcasses were graded as fat level ≤ 3, 135 carcasses were graded as fat level 4 and 14 carcasses were graded as fat level 5. There was no effect of protein diet on the fat level grading (Table 7).

Average carcass cold weight from the heifers from the high diet group was 305.15±2.63 kg and from the low protein group 306.74±2.35 kg. No effect of diet was observed on the carcass cold weight.

Table 7. Total number and percentage of heifers carcasses according to either high (14% CP) or low (10% CP) protein diet group fed during 60 days prior slaughter categorised by carcasses conformation class and fat level. ** Indicates a significant difference when comparing High vs Low diet.*

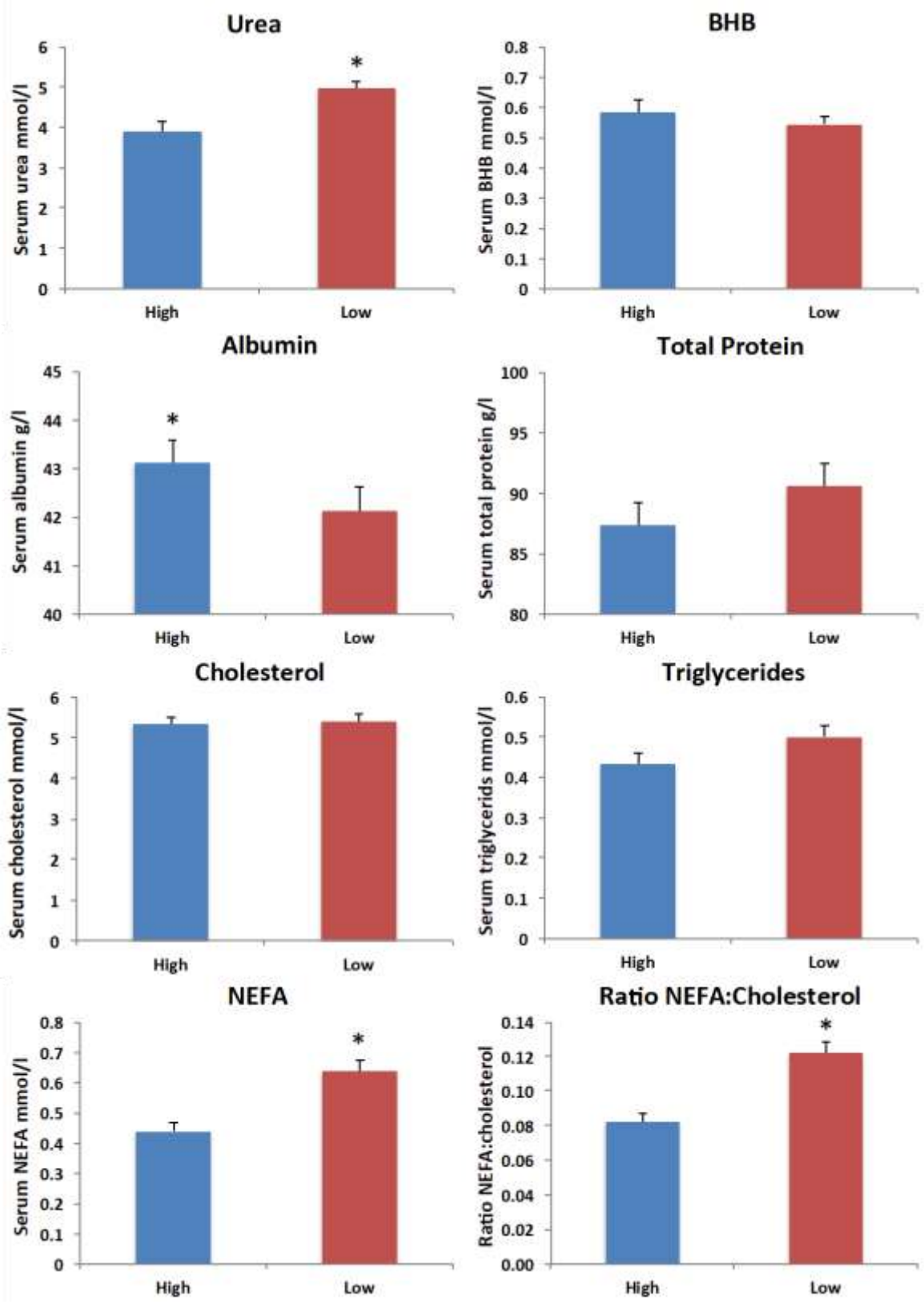
Carcass	High (n=120)	Low (n=156)
Conformation class		
O	81*	126*
	67.50%	80.90%
R	39*	29*
	32.50%	18.47%
U		1
		0.64%
Fat Level		
2	1	
	0.83%	
3	6	7
	5.00%	4.46%
4	103	136
	85.83%	86.62%
5	10	14
	8.33%	8.92%

4.3.2 Serum metabolite results

Serum metabolites were analysed in the blood of 91 heifers, which were slaughtered after overnight lairage to avoid bias on the results due to period off feed prior to slaughter.

Pre-slaughter diet affected circulating urea, albumin and NEFA concentrations. Circulating urea and NEFA concentrations were higher in heifers on the low diet group than in heifers on the high diet group (Fig 43); similarly, the ratio NEFA:cholesterol was also higher in heifers on the low diet group than in heifers on the high diet group. While, circulating albumin concentrations were lower in heifers on the low diet group (Fig 43). No other serum metabolite was affected by diet.

Figure 43. Average (±s.e.m.) circulating urea, BHB, albumin, total protein, cholesterol, triglycerides and NEFA concentrations, and ratio NEFA: cholesterol at slaughter from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low group.*



Heifers were not synchronised prior to slaughter. Phase of oestrous cycle therefore is important to consider when analysing progesterone. Diet had no effect on the overall circulating progesterone concentrations (Fig 44-A), however, an effect during the luteal phase was observed whereby circulating progesterone concentrations were higher in heifers from the high protein diet group (Fig 44-B). The circulating progesterone concentrations were also affected by diet depending on the day of the oestrous cycle, circulating progesterone concentrations were higher in heifers on the high diet group between the 1st and 2nd day of the oestrous cycle (Fig 45).

Figure 43. Average (±s.e.m.) circulating progesterone (P4) at slaughter (A) and categorised by oestrous cycle stage (B) from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low.*

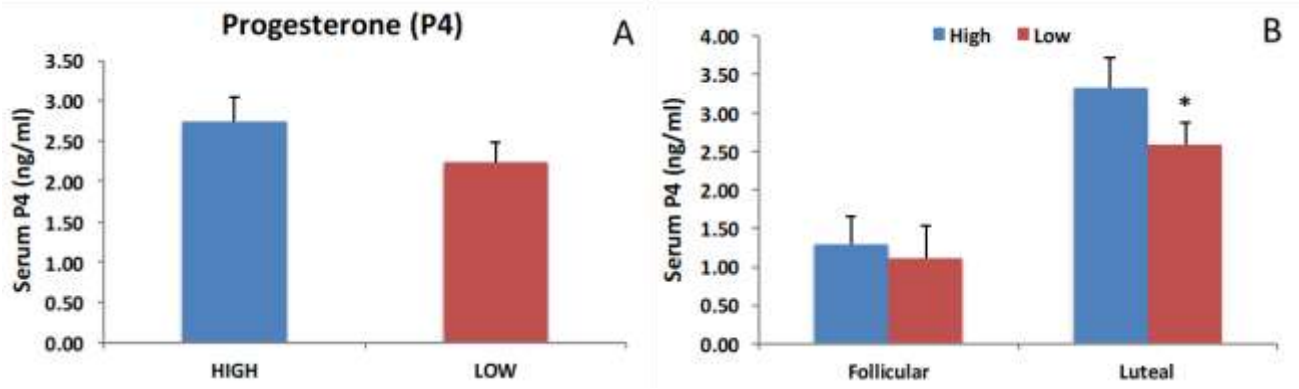
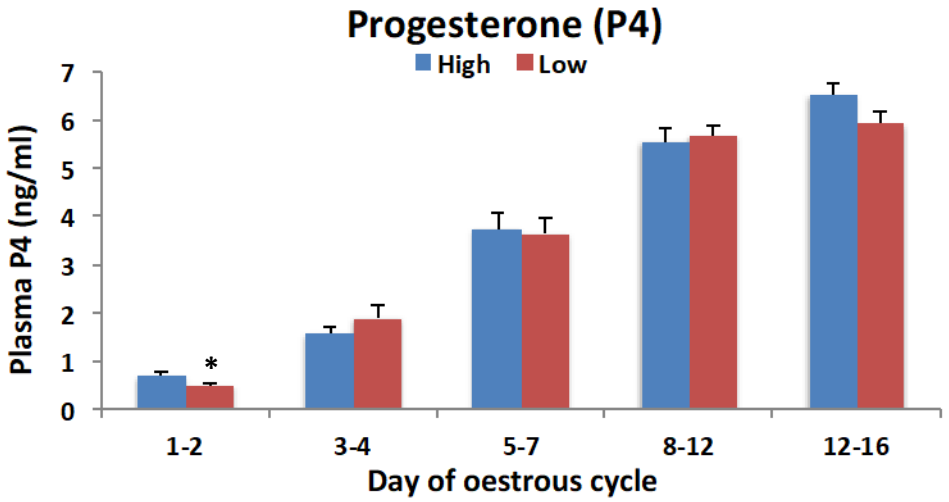


Figure 45. Average (±s.e.m.) circulating progesterone (P4) at slaughter categorised by day of the oestrous cycle stage from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low.*



4.3.3 Ovarian characteristics

Ovary weight was not affected by diet treatment (Table 8). However, diet had a significant effect on antral follicle count and dominant follicle diameter (DF), and tended to affect

corpus luteum (CL) weight. Antral follicle count was higher in heifers from the high protein diet group than from heifers on the low diet. CL weight, on day 12-16 of the oestrus cycle, also tended to be higher in heifers from the high diet group than in heifers from the low diet group. The DF in the luteal phase was larger in heifers on the high diet group than in heifers from the low diet group (Table 8). Diet had no effect on oocyte yield (Low diet: 4.50 ±0.58 oocytes per heifer; High diet: 4.72 ±0.56 oocytes per heifer), double ovulation rate (Low diet: 2.0%; High diet: 4.1%), ovarian cysts (Low diet: 2.6%; High diet: 4.1%) or on the degree of vascularisation in the mature CL.

Diet had an effect on antral follicle count depending on the size of follicle (Fig 46); heifers on the high diet had higher antral follicles counts smaller than 4mm and also bigger than 9mm. This effect was particularly significant during the luteal phase (Fig 46).

Table 8. Ovarian characteristics (average ±s.e.m.) from ovaries collected at slaughter from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter.

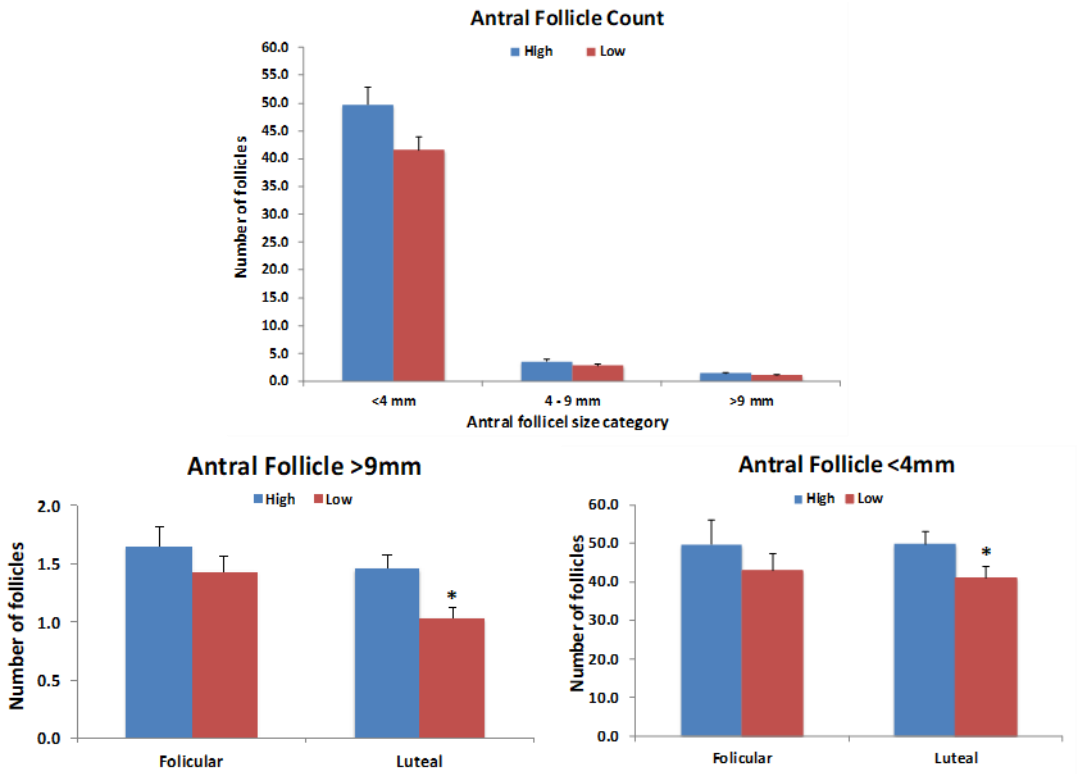
		LOW	HIGH	P value
Cold weight (kg)		306.7 ±2.3	305.1 ±2.6	ns
Age (days)		814.0 ± 6.2	780.8 ± 6.9	P<0.001
Ovary weight (g)	Overall	17.1± 0.6	16.2 ± 0.5	ns
	[1]Follicular phase	19.5 ± 1.2	18.3 ± 0.9	ns
	[1]Luteal phase	15.9 ± 0.6	15.4 ± 0.6	ns
Antral follicle count	Overall	45.5 ± 2.4	54.8 ± 3.2	P=0.006
	Follicular phase	46.5 ± 4.3	54.2 ± 6.4	ns
	Luteal phase	45.2 ± 2.8	55.0 ± 3.6	P=0.03
CL weight (g)	Mature	5.3 ± 0.2	5.7 ± 0.2	ns
	[2]Day 8-12	5.7 ± 0.2	5.6 ± 0.3	ns
	[2]Day 12-16	5.9 ± 0.2	6.5 ± 0.2	P=0.07
DF (mm)	[3]Follicular	16.2 ± 0.6	15.4 ± 0.7	ns
	[3]Luteal	11.6 ± 0.7	14.4 ± 0.6	P=0.003
	Interaction			P=0.01

[1]significant effect of stage of oestrous cycle P<0.001

[2]significant effect of estimated day of oestrous cycle P<0.001

[3]significant effect of stage of oestrous cycle P<0.001

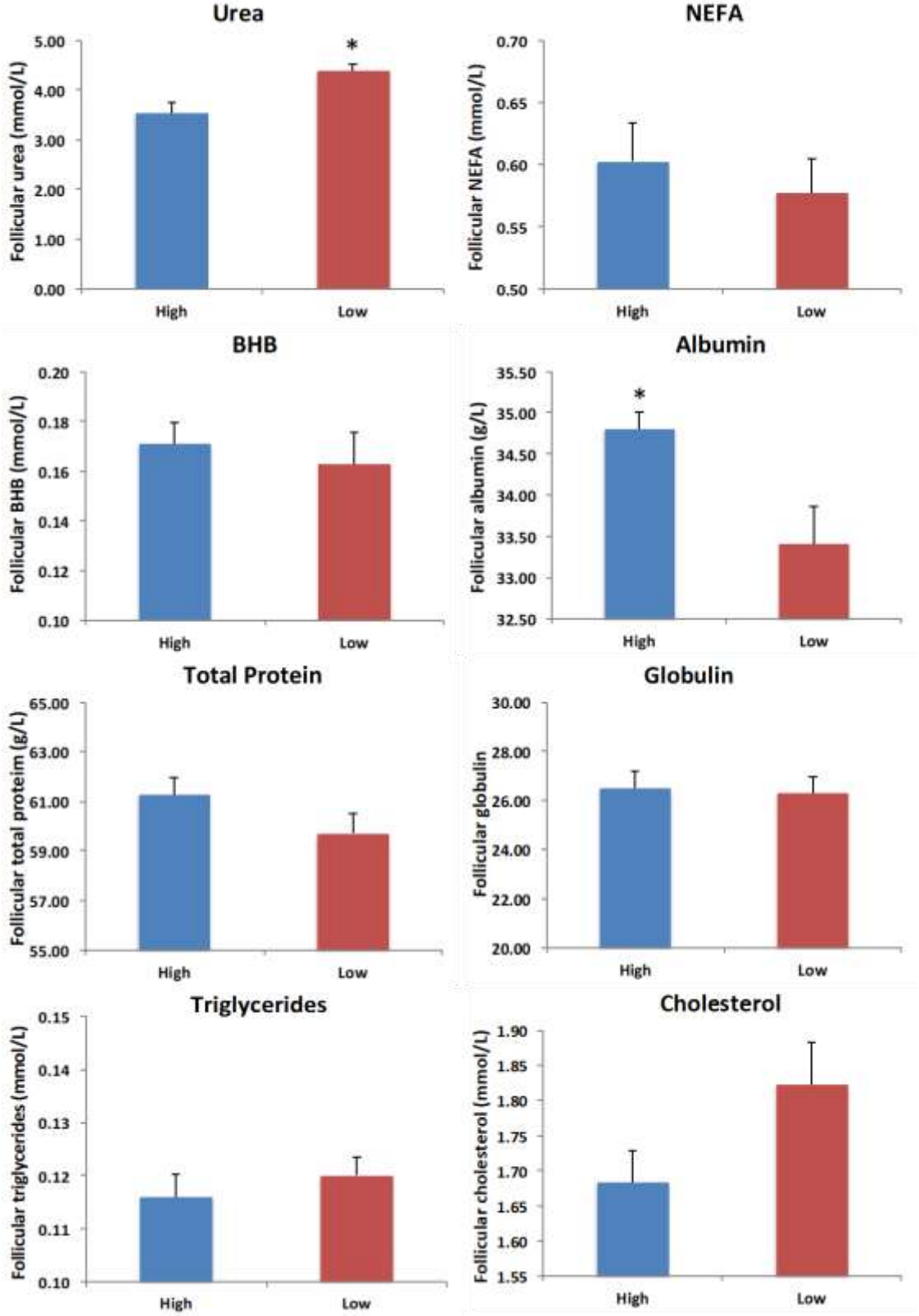
Figure 46. Average (±s.e.m.) antral follicle count by size category and by oestrous cycle stage at slaughter from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low group.*



4.3.4 Follicular fluid metabolites

There was a dietary effect upon circulating urea and albumin levels. Follicular urea concentrations were higher in heifers on the low diet than in heifers from the high dietary group (Fig 47). While, follicular albumin concentrations were lower in heifers from the low protein diet than in heifers on the high diet (Fig 47). No other serum metabolite was affected by diet.

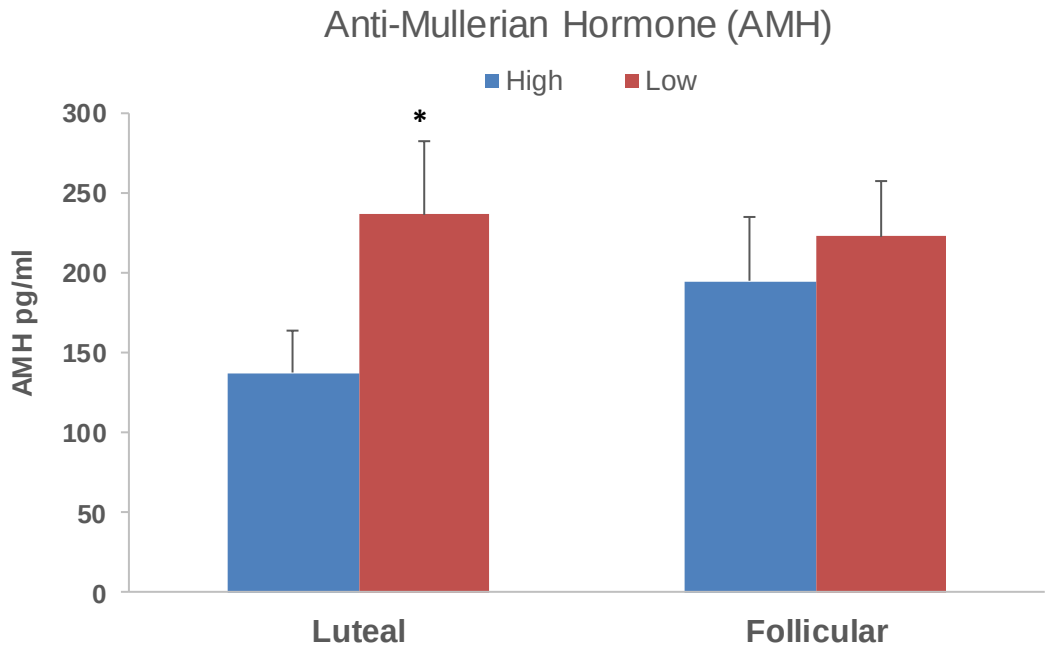
Figure 47. Average (±s.e.m.) follicular urea, NEFA, BHB, albumin, total protein, globulin, triglycerides and cholesterol concentrations at slaughter from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low group.*



4.3.5 Anti-Mullerian Hormone (AMH)

There was an effect of diet upon circulating AMH levels, especially during the luteal phase. AMH was lower in heifers from the high protein group (Fig 48).

Figure 48. Average (±s.e.m.) circulating AMH concentrations at slaughter from heifers that received a high (14% CP) or low (10%CP) protein diet 60 days prior slaughter. ** Indicates a significant difference when comparing High vs Low group.*



5. Conclusions

- Nutrient intake during early-gestation may increase dystocia in nulliparous beef heifers calving at 2 years of age when combined with altered diet in mid gestation;

- Increased protein for 60d in 2yo nulliparous beef heifers increased levels of circulating progesterone and enhanced ovarian follicular development. This effect was sufficient to alter embryo survival indicating that protein supplementation may be required to maximise fertility if dietary protein intake is below 14%CP.

- Milk intake of the calf may be increased by a low protein diet in early gestation which may result in increased growth during lactation in combination with increased IGF1 levels. There was, however, no effect on growth rate long term.

- There were significant effects upon calving to conception interval of diet prior to calving commensurate with effects upon leptin and progesterone production. Heifers calving in BCS of 2.5 or less only attained sufficient progesterone measures to indicate ovarian cyclicity at 3mths post calving. There was a trend for heifers that had received the high protein diets to return to cyclicity earlier than their low protein counterparts as measured by leptin and progesterone 3mths post calving

- There was no effect of preconception or first trimester diet upon birthweight of progeny in the F1 generation.

- Increased protein (14%CP vs 10%CP) for 60d prior to slaughter in 2yo nulliparous beef heifers increased carcass quality.

6. Acknowledgments

We gratefully acknowledge the input of Dr Bob Robinson and Dr Jennifer Edwards in the completion of section 3.4.& 4.3. This research into bovine follicular dynamics was completed by Jennifer Edwards as part of her PhD candidacy which was funded by BBSRC. We also gratefully acknowledge the contribution of Dr Robin Flynn in the examination of immunoglobulin in the colostrum. We particularly wish to thank Dovecote Park, the Farmers and their staff for their unstinting support. Dr Hernandez-Medrano was funded by an AHDB fellowship grant.

7. References

Anthony, R. V. et al. 1986. Fetal growth of beef calves. II. Effect of sire on prenatal development of the calf and related placental characteristics. Journal of Animal Science 62: 1375-1387.

Bellows, R. A., R. E. Short, and G. V. Richardson. 1982. Effects of sire, age of dam and gestation fed level on dystocia and postpartum reproduction. Journal of Animal Science 55: 18-27.

Cafe, L. M., D. W. Hennessy, H. Hearnshaw, S. T. Morris, and P. L. Greenwood. 2006. Influences of nutrition during pregnancy and lactation on birthweights and growth to weaning of calves sired by Piedmontese and Wagyu bulls. Australian Journal of Experimental Agriculture 46: 245-255.

Echternkamp, S. E. 1984. The relationship between prepartum systemic estrone sulfate concentrations and calf birth weight in beef cows. Journal of Animal Science 59: 368.

Hernandez-Medrano, J. H. et al. 2015. Gestational Dietary Protein Is Associated with Sex Specific Decrease in Blood Flow, Fetal Heart Growth and Post-Natal Blood Pressure of Progeny. PLoS ONE 10: e0125694.

Johnson, S. K., G. H. Deutscher, and A. Parkhurst. 1988. Relationships of pelvic structure, body measurements, pelvic area and calving difficulty. Journal of Animal Science 66: 1081-1088.

Kindahl, H., B. Kornmatitsuk, K. Konigsson, and H. Gustafsson. 2002. Endocrine changes in late bovine pregnancy with special emphasis on fetal well-being. Domestic Animal Endocrinology

Fourth International Conference on Farm Animal Endocrinology 23: 321-328.

McGee, M., M. Drennan, and P. Caffrey. 2006. Effect of age and nutrient restriction pre partum on beef suckler cow serum immunoglobulin concentrations, colostrum yield, composition and immunoglobulin concentration and immune status of their progeny. Irish J Agr Food Res 45: 157 - 171.

Mee, J., D. Berry, and A. Cromie. 2008. Prevalence of, and risk factors associated with, perinatal calf mortality in pasture-based Holestin-Friesian cows. animal 2: 613 - 620.

Micke, G. C. et al. 2010a. Dystocia in 3-year-old beef heifers; Relationship to maternal nutrient intake during early- and mid-gestation, pelvic area and hormonal indicators of placental function. Animal Reproduction Science 118: 163-170.

Micke, G. C. et al. 2010b. Heifer nutrition during early- and mid-pregnancy alters fetal growth trajectory and birth weight. Animal Reproduction Science 117: 1-10.

Rasby, R. J., R. P. Wettemann, R. D. Geisert, L. E. Rice, and C. R. Wallace. 1990. Nutrition, body condition and reproduction in beef cows: Fetal and placental development, and estrogens and progesterone in plasma. Journal of Animal Science 68: 4267-4276.

Shah, K. D., T. Nakao, H. Kubota, and T. Maeda. 2007. Relationship of prepartum plasma concentrations of estrone sulfate and estradiol- 17B with the weight of the calf and placental parameters in Holstein-Friesian cows. Animal Science Journal 78: 489-494.

Sullivan, T. M. et al. 2009. Dietary protein during gestation affects circulating indicators of placental function and fetal development in heifers. Placenta 30: 348-354.

8. Appendix

Appendix 1. Nutrient and dry matter analysis of diets supplied at farm A and B 60d prior to conception.

Farm		A						B	
Nutrient/Dry Matter Analysis		Mar-13		Apr-13		May-13		March-May 2013	
		Low[a]	High[b]	Low[c]	High[d]	Low[e]	High[f]	Low[1]	High[2]
Dry matter	**% (Volume)**	125.1	122.9	121.7	115.7	120	114.2	173.3	170.6
Protein	**%**	10.2	15.5	10.6	25.5	9	23.8	10.9	19.5
Fat	**%**	2.5	3.7	2.4	5.9	2.6	6	2.7	3.3
NDF	**%**	53.3	52.9	38.1	37.8	40.7	40.3	39.1	38.7
ME_RUM MJ	**MJ/kg**	8.9	9	11	11.3	10.5	10.8	12.2	12.4
Calcium	**%**	0.6	0.8	0.4	0.8	0.3	0.7	0.5	0.6
Phosphorus	**%**	0.4	0.4	0.4	0.5	0.3	0.4	0.3	0.3
CAL:PHOS	**NIL**	1.8	1.9	1	1.6	1	1.8	1.8	1.8
NA	**%**	0.1	0.2	0.1	0.3	0	0.2	0.2	0.2
K	**%**	1.7	1.9	1.2	1.8	0.4	1.1	1.3	1.6
CL	**%**	0.2	0.3	0.2	0.4	0.1	0.3	0.8	0.7
MG	**%**	0.2	0.2	0.1	0.3	0.1	0.3	0.3	0.3
S	**%**	0.1	0.2	0.2	0.3	0.1	0.2	0.3	0.3
VIT A	**IU/g**	0	1.5	0	4.2	0	4.1	0	0
VIT D3	**IU/g**	0	0	0	0	0	0	0	0
VIT E	**mg/kg**	9	1.6	26.3	4.5	25.9	4.4	15.4	9.2
FE	**mg/kg**	241.6	276.5	144.9	248.3	149.7	251.5	219.1	225.8
ZN	**mg/kg**	27.8	37.4	31.2	58.1	23.4	50.3	24	34.3
MN	**mg/kg**	119.2	123.9	67.1	82.8	24.2	41.7	56.8	56.6
CU	**mg/kg**	5.9	9	6.5	15.1	5.3	13.8	5.1	5.8
SE	**mg/kg**	0.1	0.1	0.1	0.3	0.1	0.3	0.1	0.2
MO	**mg/kg**	10.9	11.5	5.5	7.6	0.4	2.7	2	2.1
CO	**mg/kg**	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.2
I	**mg/kg**	0.5	0.5	0.2	0.4	0.1	0.3	0.3	0.3

a= Haylage ad lib +4kg Barley; b= Haylage ad lib +4kg Rumigan; c= Haylage ad lib +6kg Barley; d= Haylage ad lib +6kg Rumigan; e= Straw ad libitum +6kg Barley; f= Straw ad libitum +6kg Rumigan

1= Grass silage + 4kg Barley; 2= Grass silage + 1.5 Barley + 2.5 Rumenco

Appendix 2. Nutrient and dry matter analysis of diet supplied at Farm C after conception.

POST-CONCEPTION DIET – TRIAL		From AI up to 90d	
Nutrient/Dry Matter Analysis		Low	High
		FG	FG+Pellets
Dry matter	**% (Volume)**	28.7	31.2
Protein	**%**	10.0	14.0
Fat	**%**	19.2	
NDF	**%**	54.6	53.6
ME_RUM MJ	**MJ/kg**	9.92	10.1
Calcium	**(g/kg DM)**		1.14
Phosphorus	**(g/kg DM)**		8.52
CAL:PHOS	**NIL**		
NA	**(g/kg DM)**		2.84
K	**(g/kg DM)**		10.23
CL	**(g/kg DM)**		
MG	**(g/kg DM)**		3.41
CU	**(g/kg DM)**		28.4
Ash	**(g/kg)**	7.9	
Oil-A	**(g/kg)**	19.25	
Sugar	**(g/kg)**	102.5	
Nitrate Nitrogen	**%**	0.1	
Buffering Capacity	**meq/kg**	382.5	

FG =Fresh grass

Appendix 3. Nutrient and dry matter analysis of the feed provided during pregnancy on Farms A and B.

Farm		A					B	
Nutrient/Dry Matter Analysis		May-13	Jul-13	Aug-13	Sep-13	Jan-Feb 2014	Jul-13	Calving
		FG	FG	FG	FG	Hay + Silage	FG	Silage
Dry matter	**% (Volume)**	236.0	201.0	150.0	193.5	0.8	330.0	504.0
Protein	**%**	19.0	18.8	18.1	20.9	6.6	9.0	11.9
D value	**%**	74.0	70.0	75.0	63.0	54.8	62.0	69.0
Fat	**%**							
NDF	**%**	36.4	39.2	40.2	45.2	72.1	62.5	53.7
ME_RUM MJ	**MJ/kg**	11.5	10.9	12.0	9.9	8.8	9.7	11.0
Ash	**(g/kg)**	76.0	78.0	44.0	93.0	57.0	67.0	63.0
Oil-A	**(g/kg)**	30.0	26.0		27.0		48.0	33.0
Sugar	**(g/kg)**	182.0	136.0	146.5	63.5	52.0	103.0	29.0
Nitrate Nitrogen	**%**	0.2	0.1		0.2		0.1	
Buffering Capacity	**meq/kg**	424.0	414.0		442.0		370.0	
NCGD	**g/kg**			58.5		69.0		

FG =Fresh grass

Appendix 4. Nutrient and dry matter analysis of the feed provided two months before slaughter at farm D

	Protein Level	
Ration as fed (Dry weight)	**Low**	**High**
Chopped Straw (kg/day)	1.3	-
Oat Feed (kg/day)	2	-
Fruit (kg/day)	0.27	-
Citrus pulp (kg/day)	0.37	-
Bread (kg/day)	1.7	-
Lime. Flour (kg/day)	0.11	-
Beef minerals (kg/day)	0.11	-
Yeast (kg/day)	0.04	-
Grass silage (kg/day)	1.5	5.1
Maize silage (kg/day)	4.2	-
Poundon Pre-Mix (kg/day)	-	3.3
Apple pulp (kg/day)	-	1.8
Feed grade urea (kg/day)	-	0.099
Diet composition (g/kg DM)		
DM (g/kg)	436	431
ME (MJ/kg DM)	11.8	11.2
Crude protein (g/kg DM)	104	145
Oil (g/kg DM)	41.4	40
NDF (g/kg DM)	320	395
Starch (g/kg DM)	302	145
Sugar (g/kg DM)	59	113
Long Roughage (%)	49.2	49.3