Low protein intake during the preconception period in beef heifers affects offspring and maternal behaviour

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Abstract

Maternal low protein diets prior to conception alter embryo and fetal development and are associated with detrimental outcomes in the offspring in many species. The aim of this study in beef cattle was to investigate the effect of preconception dietary protein upon maternal and offspring behaviour at birth concomitant with the associated hormonal profile. Sixty days prior to conception, nulliparous yearling heifers (n=85) were fed either a High (PreH: 18%; n=43) or Low (PreL: 10%; n=42) crude protein diet, followed by a control diet throughout gestation. After calving, each cow-calf pair was penned individually, accelerometers fitted, and each pen observed continuously via video recordings. Cows fed on the low protein diet during preconception showed an increase in standing time (P<0.01); while calves born to heifers receiving the PreL diet showed an increase in suckling time (P=0.04). These calves were also heavier at birth than calves from PreH mothers (P<0.01). In conclusion, low maternal

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dietary protein prior to conception in beef heifers modifies both offspring feeding behaviour and birth weight, and cow's standing times.

Keywords

Beef cattle; preconception; low protein; behaviour; hormones

1. Introduction

Exposure to low maternal protein intake (7-10%) during gestation has been reported to decrease circulating reproductive, placental and growth hormones commensurate with metabolite levels (Sullivan et al., 2009a; Copping et al., 2014; Hernandez-Medrano et al., 2015). These hormones and growth factors are endocrine regulators of fetal growth and act as epigenetic signals in the programming of *in utero* development (Sinclair et al., 2016). This is of particular importance in the two-year-old calving heifer (adolescent dam), as the heifer is still growing during gestation thereby competing with the fetus for nutrients (Hernandez-Medrano et al., 2015). First trimester gestational heifer low protein diets have been shown to affect circulating hormones post-partum and are associated with offspring milk intake during the first 6 months of life (Micke et al., 2010b). In addition, lower levels of leptin and IGF1 were observed in offspring following nutritional restriction during the peri-conception period in both cattle and sheep (de Brun et al., 2015; Micke et al., 2015). These metabolites are important in the calf, as leptin regulates appetite and energy expenditure, while IGF-1 promotes muscle growth (Sinclair et al., 2016).

Nutritional perturbation in the period prior to conception, i.e. period of oocyte development, may affect the developing embryo and fetus (Mossa et al., 2013; Copping et al., 2014; Hernandez-Medrano et al., 2015), and increase birth weight in the offspring (Hernandez-Medrano et al., 2015). However, the wider implications of preconception diet upon neonatal behaviour and welfare in cattle remain to be evaluated. This is of particular importance for the beef industry, where current protein intake recommendations for beef cows and heifers prior to conception are between 9 to 16% of crude protein in dry matter (AHDB Beef & Lamb, 2015; 2016).

In rodents and sheep, previous studies have demonstrated that preconception dietary protein restriction affects behaviour profiles in the offspring (Wright et al., 2011; Donovan et al., 2013). Wright *et al*. (2011) provided evidence that increased periconceptional caloric intake, but reduced protein intake, decreased anxiety in rodents. In sheep, findings vary dependent upon dietary intervention, pregnancy stage and behaviour observation period. Kleemann et al. (2015) provided sheep with 70% (low), 100% (standard) or 150% (high) nutritional requirements (protein and energy) for 17 days prior and 6 days after insemination. When released after tagging, lambs from mothers fed the low diet returned faster to their mothers than those from the high diet cohort, and contacted the udder more quickly than those lambs from the standard (100%) or high (150%) dietary treatments (Kleemann et al., 2015). Contrary to this, a study that provided a mild maternal under-nutrition (10-15% body weight reduction) between -60 to +30 days relative to conception, failed to observe any effects of the dietary treatment upon ewe-lamb bonding during the first month of life (Hernandez et al., 2009b). However, offspring from undernourished ewes, when in isolation, showed suppressed behavioural reactions, altered behavioural laterality, reduced locomotor activity and increased cortisol secretion at 4 and 18 months of age (Hernandez et al., 2009a; Hernandez et al., 2010; Donovan et al., 2013).

In cattle, one report (Micke et al., 2015), suggests that maternal first trimester (0-90 days post conception) diet may increase milk intake in male offspring between 94 to 191 days of age. However, these researchers used the weigh-suckle-weigh technique, which is unable to differentiate between offspring milk intake and heifer milk production (Sullivan et al., 2009c). No conclusion on the calves' suckling behaviour could therefore be drawn. Thus, the aim of this study was to investigate maternal and offspring behavioural and hormonal profiles following a preconception dietary protein restriction (PreH: 18% Crude Protein (CP) vs. PreL: 10% CP) in nulliparous beef heifers.

2. Materials and Methods

The study was carried out under the Animal's (Scientific Procedures) Act 1986 (United Kingdom) and the protocols were approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee.

2.1 Animals and Experimental Design

The effect of dietary protein during the preconception period on the maternal and neonatal behaviour in beef cattle was evaluated using a total of 88 nulliparous heifers kept in two locations within the UK (Farm A, n=44; and Farm B, n=44). Two months prior to conception (conception, day 0, was taken as the day of artificial insemination (AI)), heifers were randomly allocated, by weight and age, to High (PreH: 18%; n=44; 347.2 ±9.4kg BW) or Low (PreL: 10%; n=44; 355.5 ±10.8kg BW; CP at the lower level of recommendation) crude protein diet (Hernandez-Medrano et al., 2015). Heifers were group-fed the corresponding diet and managed as two groups according to diet. At the end of the treatment period, heifers were inseminated following a 5-day synchronization protocol (Hernandez-Medrano et al., 2015), with semen from a single bull with known EBVs for growth and low birth weight.

Pregnancy diagnosis was carried out at 36 days and 90 days post-AI, resulting in 34 pregnant heifers: PreH (n=18) and PreL (n=16). After AI, all animals were grouped together

and kept at pasture until 2 months prior to calving when they were brought into barns and housed as one group. Four pregnant heifers lost their pregnancy during the second trimester and were withdrawn from the experiment. Before calving one heifer died suddenly and one calved earlier than expected and was not recorded, leaving 28 heifers included in the present behavioural experiment.

At calving, heifers were moved to a calving pen that was located within the group pen. Calving ease was recorded according to the assistance required during delivery (Table 1). Within 6 hours after calving, heifers were moved to individual pens where each heifer-calf pair was kept for up to 3 days. In the individual pens, heifers were fed either hay or silage ad-libitum. Individual pens had a concrete floor covered with deep straw bedding; fresh bedding was added every 24 hours. After this period in individual pens, heifer-calf pairs were transferred to a large group pen with straw bedding where they stayed for up to 3 weeks before being turn out to pasture.

2.2 Behavioural data collection

Direct visual observation after calving was used to measure birth to standing latency (minutes from birth to the first time the calf stands on its 4 feet) and birth to suckling latency (minutes from birth to the first time the calf holds teat in mouth and sucks). Additionally, each pen had a ceiling mounted Infra-Red LED CCTV camera (OBK20B: 1/3” Sony Colour, Gamut, Bristol, UK) connected to a digital video recording box (8DVRLAN, Gamut, Bristol, UK). Each camera was positioned 2.6m above the individual pens. Individual pens were identified clearly with the number of the heifer written on a white board that was visible from the camera. Cameras were set to record continuously at high quality and 30 frames/s. Video analysis was carried out by a single trained individual using The Observer® XT 11.5 (Noldus, Information Technology bv, Wageningen, The Netherlands).

Behaviour was continuously recorded from 6 hours up to 30 hours after birth. Then these 24 hours were divided in 6 hour-blocks and the initial 3 hours of each block were video analysed. Each heifer-calf pair was observed for a total of 12 hours using continuous video analysis for the behaviours described in Table 2. Self-grooming, licking calf and suckling behaviour are presented as total minutes per 12 hours of observation, while running behaviour is presented as total seconds per 12 hours. Birth to standing and birth to suckling latencies are presented as total minutes; and standing behaviour was presented as total minutes per day, number of bouts per day and mean bout duration (minutes/bout). Intra-observer reliability was 99.02% agreement for total duration for all the behaviours, and this was calculated using The Observer® XT 11.5 reliability analysis.

Standing behaviour data was collected using an accelerometer (HOBO Pendant G Acceleration Data Logger; Onset Computer Corporation, Pocasset, MA, USA) that recorded continuously for 24 hours. Animals were fitted with an accelerometer within 6 hours after calving and subsequently removed 3 days later. The accelerometer was attached using Vet Wrap cohesive bandage (3M Products, St. Paul, MN) to the lateral side of either hind leg above the metatarsophalangeal joint (Gibbons et al., 2012). Onset HOBOware® Lite Software Version 2.2.1 (Onset Computer Corporation, Pocasset, MA) was used to download data from accelerometers which then was exported to Microsoft Excel® (Microsoft Corporation). Data was modified and edited using the Software Macro Hobo 3D Microsoft Excel® (Gibbons et al., 2012). Standing behaviour was recorded as total duration (minutes per day) and number of bouts, with mean bout frequency calculated by dividing total standing time by the number of bouts recorded.

2.3 Body weight measure, metabolite analysis and hormone assays

At birth (Day 0), calves were weighed, and jugular blood samples taken into tubes containing lithium-heparin (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Samples were stored on ice and centrifuged at room temperature (RT) at 3000 x g for 10 min. Plasma was harvested

and stored at -20°C until assayed. In parallel, maternal plasma samples were collected at birth to measure concentrations of metabolic hormones and plasma proteins as described by Sullivan et al. (2009b), and heifers were weighed within 12 hours after calving.

Hormonal concentrations were determined at the Animal Science Research Center of the University of Missouri, USA. Double antibody radioimmunoassay (previously validated in the laboratory in charge of the analysis) with inter- and intra-assay CV of <5% was used to determined plasma concentrations of IGF-1 (Lalman et al., 2000), leptin (Delavaud et al., 2000), prolactin (Lutz et al., 1991), progesterone (Pohler et al., 2015) and oxytocin (Lutz et al., 1991). In the case of serum or plasma concentrations of cortisol, they were determined via double antibody radioimmunoassay validated by Daniel et al. (2000), with inter- and intra-assay CV were < 5%. The RX-IMOLA auto analyser (Randox, County Antrim, UK) was used to quantify albumin, total protein (soluble) and NEFAs (non-esterified fatty acids) concentrations. Globulin levels were estimated by calculating the remainder of the total protein after albumin levels were accounted for.

2.4 Data presentation and statistical analyses

Data analysis was carried out on 26 pairs (heifer and calf): 12 PreH (n=8 female and n=4 male) and 14 PreL (n=3 female and n=11 male); as one calf had difficulty standing after a difficult birth (cow-calf pair were put in an individual pen and closely observed but data was not collected) and one calf was stillborn. However, for standing behaviour data was obtained from 25 heifers and 24 calves due to technical problems with the accelerometers.

Descriptive analysis was conducted using Stata/SE 12.0 (Stata Corp 2011, USA) and Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Data was analysed for normality and transformed when necessary. Linear or Poisson regression models were built for each behavioural and hormonal variable. For heifer hormonal data, diet (preH=1 and preL=2), farm (A and B), and heifer weight (at calving) were used as fixed effects. For the heifer

behavioural data, calf's sex and birth weight were added to the previous as fixed effects. For heifer weight at calving, diet (preH=1 and preL=2), farm (A and B), and heifer weight at AI were used as fixed effects. Calf hormonal and behavioural data analysis included farm, diet, calf sex and birth weight as fixed effects. Type of calving was included as a fixed effect to analyse the latencies from birth to standing and suckling; and heifer's body weight was included as a fixed effect to analyse calf's birth weight.

To further explore the association between preconception diet treatment and calving, type of calving was re-coded as unassisted (0) and assisted (1; included mild assistance & hard pull) calving. A binomial logistic regression model was built considering this as the outcome variable and diet, farm, heifer weight, calf sex and weight as fixed effects. Data is presented as mean values (±s.e.m), coefficient and standard error SE (Coefficient (SE)). Level of statistical significance was set at P=0.05.

3. Results

3.1. Effect of preconception diet on heifers

3.1.1. Body weight, circulating hormones and behaviour

There was no association between preconception dietary treatment and heifer body weight before artificial insemination (AI), at AI or at calving (Table 3). Similarly, circulatory concentrations of cortisol, IGF-1, leptin, progesterone (P4), prolactin, and oxytocin in the heifers as measured immediately after calving (i.e. within 30-45 minutes) were not associated with preconception dietary treatment (Table 3). Other blood metabolites (NEFA, albumin, total protein) circulatory concentrations were not affected by preconception dietary treatment (Table S.1). In line with the physiological measures, there was no association between the preconception dietary treatment and calf- and self-grooming behaviours in the heifers (Table 3). Heifers from both groups spent a similar amount of time self-grooming and licking their calves (Table 3). However, Heifers in the preL group stood longer than those in preH group, when controlled for farm, calf's sex and weight (Table 3). There was no

significant association with the other two standing behaviour variables: number of standing bouts and average standing bout duration (Table 3).

3.1.2 Calving Assistance

All experimental animals were primiparous heifers; therefore, continuous monitoring at parturition was necessary both from an animal welfare and economic point of view. Almost half of heifers (n=12/26 heifers) calved without assistance, with those remaining requiring either mild assistance (pulled by hand; n=6/26) or assistance where the calf was pulled with calving chains with reasonable force (hard pull; n=8/26). After controlling for farm, heifer weight and calf sex and weight, no significant association was observed between preconception dietary treatment and calving assistance type (Odds ratio 5.55; P = 0.22).

3.2 Effect of preconception diet on calves

3.2.1 Body weight, circulating hormones and behaviour

In contrast to heifers, a significant positive association between birth weight and maternal preconception dietary treatment was observed in calves. Calves from PreL heifers were heavier (P<0.005; Table 4) compared to calves from the PreH heifers, this after controlling for heifer's weight, farm and calf's sex (Figure 1). There was no association between maternal diet and circulatory concentrations of leptin, cortisol or IGF1 in the calf (Table 4).

3.2.2 Behaviour

The latencies from calving to standing up and to first suckling event were recorded for all experimental heifer-calf pairs. These latencies were not significantly associated with maternal diet. The latencies of standing (Coefficient: -0.04 (SE: 0.02); P=0.05) and suckling (Coefficient: 0.63 (SE: 0.29); P=0.04) after birth were, however, positively associated with hard pull calving. Calves experiencing dystocia (i.e. those needing severe calving assistance) took longer to stand up (238.9 ±89 min) and to suckle (328.0 ±89 min) than those without assistance (Standing: 71.8 ±9.5 min; Suckling: 114.0 ±17.7 min). There was a

significant positive association between suckling time and maternal diet. In the 30 hours after birth, calves born to PreL heifers spent more time suckling (Coefficient: 14.82 (SE: 6.62); P=0.036) than those from PreH heifers (Figure 1). Running in calves was not significantly different between diet treatments (data not shown here).

4. Discussion

This study is the first to show that a low protein diet during the preconception period affected both neonatal offspring and dam behaviour in cattle. Calves from heifers receiving the low protein preconception diet showed increased suckling times and were heavier than calves from mothers that received the high protein diet. Standing behaviour time was also increased in heifers that received the low protein diet which could be associated with the increase suckling behaviour of their offspring (Stehulova et al., 2013). Hormone concentrations measured at calving in heifers are within similar ranges to previous reports (Sullivan et al., 2009b; Micke et al., 2015), with no effect in this study of dietary treatment.

Previous studies suggest that heifers fed a low protein diet in the first trimester had higher milk production between the first and sixth month after parturition (Sullivan et al., 2009c; Micke et al., 2015). Increased suckling behaviour, as observed in the current study, and in lambs from undernourished mothers (Kleemann et al., 2015) provides a stimulatory effect on milk production and could thus account for the increased milk production. In sheep, weight loss prior to conception altered not only leptin plasma profiles, but also leptin receptors in the maternal liver during pregnancy (de Brun et al., 2015). Low protein diet during pregnancy or unspecific undernutrition may alter maternal leptin levels, which in turn affect the development of fetal hypothalamic pathways and may also cause leptin resistance in the offspring (Orozco-Solis et al., 2009; Stevens et al., 2010). In the present study, changes in leptin serum levels were not observed which may be due to a low number of animals.

Various studies have described possible long-term modifications of offspring development, health and welfare following maternal nutrient restriction during periconception (reviewed by Sinclair et al., 2016). In sheep, following a comparable dietary period intervention, cognitive behaviour appears unaffected (Abecia et al., 2015) despite effects on long-term locomotor behaviour (Donovan et al., 2013). In cattle, suckling behaviour characteristics (duration and events) are established by the third day (Lidfors et al., 1994), suckling bouts show consistency within lactation, and are initially lead by the calf and then by the cow around day 30 (Stehulova et al., 2013). Previous findings showed an effect of PreL preconception dietary treatment on milk production during the first and sixth months after calving (Sullivan et al., 2009c; Micke et al., 2015). Although long term effects were not studied, findings suggest that PreL preconception dietary treatment may affect suckling behaviour in beef cattle from birth up to six months of age.

Changes in the preconception period may not only have effects on the appetite of calves, it also alters cow's behaviour pattern. Total standing time was associated with preconception dietary treatment: PreL heifers showed longer total standing time than PreH heifers. Calves from PreL heifers showed increased suckling time, which may have induced a longer standing time for nursing. Alternatively, postnatal nursing has been shown to be primarily initiated by the cow and only later by the calf (Tucker, 2017), thus preconceptional diet may primarily impact maternal nursing behaviour shortly after parturition. This would be in line with studies in beef and dairy cows, during early days post-calving, showing intense maternal care and protectiveness towards the calf (Jensen, 2012; Stehulova et al., 2013). It is possible, therefore, that changes in standing time may also reflect a greater maternal care, however, this was not fully explored in the present study.

Protein restriction during the first trimester of gestation can increase placental development thereby enabling adequate delivery of nutrients to the fetus (Perry et al., 1999). In heifers, Hernandez-Medrano et al. (2015) reported that a low protein diet (10%CP) prior to

conception affected fetal development. Furthermore, the low protein diet caused a rise in blood flow to the mid-uterine artery at 210 days post conception, possibly increasing nutrient availability to the pregnant uterine horn and the developing placenta in this group (Hernandez-Medrano et al., 2015). The preconception dietary protein restriction in this study may have resulted in increased blood flow to the feto-placental unit enabling enhanced nutrient supply. This may have led to increased availability of nutrients to the PreL fetus during later stages of gestation due to this enhanced placental development; resulting in increased birth weight in these calves.

The observed effects upon maternal behaviour and offspring weight and behavioural changes associated with the PreL diet suggests that uterine environment may be modified by maternal intake before the implantation period as previously reported in sheep and rodents (Stevens et al., 2010; Kleemann et al., 2015). Previous findings, however, suggest that compensatory mechanisms exist within this progeny, as weight differences apparent at birth may not be apparent at 6 months of age (Micke et al., 2010a; Miguel-Pacheco et al., 2017). This does not however infer that differences in physiology and importantly production traits do not persist (Micke et al., 2010a). In addition, previous studies have observed differences in hormone levels following preconception dietary interventions dependent upon fetal sex (Hernandez-Medrano et al., 2015; Micke et al., 2015). The fact that we did not observe such endocrine effects could be due to the low number of animals in the study following a <40% heifer conception rate. This led to the inability to compare between sexes as reported by Micke et al. (2015).

5. Conclusions

In the present study, we report that maternal dietary protein restriction during the preconception period (i.e. 60 days prior to conception), increases offspring birth weight and suckling behaviour, and maternal standing behaviour. These findings may indicate a compensatory mechanism post-partum that supports neonatal survival: increased appetite.

The observed effects may result from perturbations to the fetus during a critical period of development in utero. Longitudinal studies are needed to investigate the long-term effect of these neonatal behavioural changes on the performance and welfare of the offspring, and the effects on maternal behaviour budgets and welfare.

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468 Table 1. Classification of calving ease in heifers based on Mee et al. (2008)

Calving Category	Description
Unassisted	No assistance needed, heifer calved in her own.
Mild/slight assistance	Calving assistance consisted in mild manual pull of the calf without position correction needed.
Hard pull (Severe assistance)	Calving assistance consisted in hard calf pull requiring at least two-person force with or without correction of calf position.
Veterinary assistance	Surgery required

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470

471 Table 2. Behavioural definitions used on the video analysis, adapted from Jensen (2011)

Behaviour	Description
Heifer	
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.
Licking calf	Muzzle in contact with any body part of the calf.
Calf	
Running	Galloping, jumping, bucking or rear kicking.
Suckling	Head is under the heifer's belly in the udder area.

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473

474 Table 3. Body weight, circulating hormones at calving, and post-calving behaviour in heifers.
475 Behaviours were measured during the 6 hours post-calving in heifers that received a high
476 (18%CP; PreH; n=12) or low (10%CP; PreL; n=14) protein diet during the preconception period
477 (-60 days to AI, conception day).

Variables	Treatment		Coefficient	SE	P
	PreH	PreL			
Body Weight (kg)					
Before treatment	317.9	360.4	-37.89	19.54	0.07
At AI	396.3	444.5	1.31	5.88	0.82
At calving	534.0	585.7	6.98	14.27	0.63
Hormones at Calving					
P4 (ng/ml)	0.5	0.4	-0.04	0.08	0.65
Leptin (ng/ml)	3.2	3.2	0.00	0.05	0.93
IGF1 (ng/ml)	65.5	73.8	7.42	7.18	0.30
Cortisol (ng/ml)	17.9	21.5	0.25	0.22	0.26
Oxytocin (pg/ml)	491.7	463.3	-0.07	0.07	0.37
Prolactin (ng/ml)	20.3	20.6	0.00	0.00	0.87
Behaviour at Calving					
Licking calf (min/12hr)	39.9	45.6	8.53	8.65	0.32
Self-Grooming (min/12hr)	28.3	15.8	-9.83	5.77	0.09
Standing duration (min/24hr)	811.4	910.4	167.23	62.43	**0.01**
Standing frequency (bouts/24hrs)	15.8	11.4	-0.07	0.15	0.65
Standing bout duration (min/bout)	59.0	115.0	-0.02	0.01	0.17

For diet preH diet was the reference
AI= Artificial Insemination

478

479 Table 4. Birth weight and circulatory hormone concentrations at calving in calves whose
480 mothers received either a high (18%CP; PreH) or low (10%; PreL) protein diet during the
481 preconception period (-60 days to AI, conception day).

Independent Variables	Treatment		Coefficient	SE	P
	PreH	Pre L			
Body weight (Kg)	36.6	43.2	4.92	1.57	0.005
Hormones at calving					
Leptin (ng/ml)	3.4	3.1	-0.19	0.23	0.416
IGF1 (ng/ml)	51.6	45.7	-14.67	10.79	0.188
Cortisol (ng/ml)	66.4	69.2	0.40	0.31	0.210
Behaviour at Calving					
Running (sec/12hr)	90.5	73.0	2.73	2.34	0.256
Standing duration (min/24hr)	241.0	253.6	-6.14	26.84	0.821
Standing frequency (bouts/24hrs)	21.0	21.0	0.04	0.09	0.621
Standing bout duration (min/bout)	11.8	12.3	-0.79	1.60	0.628
Birth to suckling latency (min)	113.5	244.5	0.11	0.31	0.721
Birth to standing latency (min)	70.5	179.4	-0.01	0.02	0.697

482 AI= Artificial Insemination

483

Figure captions

Figure 1. Suckling duration and body weight significant differences in calves born to heifers that received a high (PreH) or low (PreL) protein diet during the preconception period (-60d to conception, AI (Artificial Insemination)). PreH: n = 12; PreL n=14. Mean + SEM. * P <0.05; ** P<0.005