

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

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12 Abstract

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

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

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27 Keywords

28 Beef cattle; preconception; low protein; behaviour; hormones

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34 1. Introduction

35 Exposure to low maternal protein intake (7-10%) during gestation has been reported to
36 decrease circulating reproductive, placental and growth hormones commensurate with
37 metabolite levels (Sullivan et al., 2009a; Copping et al., 2014; Hernandez-Medrano et al.,
38 2015). These hormones and growth factors are endocrine regulators of fetal growth and act
39 as epigenetic signals in the programming of *in utero* development (Sinclair et al., 2016). This
40 is of particular importance in the two-year-old calving heifer (adolescent dam), as the heifer is
41 still growing during gestation thereby competing with the fetus for nutrients (Hernandez-
42 Medrano et al., 2015). First trimester gestational heifer low protein diets have been shown to
43 affect circulating hormones post-partum and are associated with offspring milk intake during
44 the first 6 months of life (Micke et al., 2010b). In addition, lower levels of leptin and IGF1
45 were observed in offspring following nutritional restriction during the peri-conception period in
46 both cattle and sheep (de Brun et al., 2015; Micke et al., 2015). These metabolites are
47 important in the calf, as leptin regulates appetite and energy expenditure, while IGF-1
48 promotes muscle growth (Sinclair et al., 2016).

49

50 Nutritional perturbation in the period prior to conception, i.e. period of oocyte development,
51 may affect the developing embryo and fetus (Mossa et al., 2013; Copping et al., 2014;
52 Hernandez-Medrano et al., 2015), and increase birth weight in the offspring (Hernandez-
53 Medrano et al., 2015). However, the wider implications of preconception diet upon neonatal
54 behaviour and welfare in cattle remain to be evaluated. This is of particular importance for
55 the beef industry, where current protein intake recommendations for beef cows and heifers
56 prior to conception are between 9 to 16% of crude protein in dry matter (AHDB Beef & Lamb,
57 2015; 2016).

58

59 In rodents and sheep, previous studies have demonstrated that preconception dietary protein
60 restriction affects behaviour profiles in the offspring (Wright et al., 2011; Donovan et al.,
61 2013). Wright *et al.* (2011) provided evidence that increased periconceptional caloric intake,
62 but reduced protein intake, decreased anxiety in rodents. In sheep, findings vary dependent
63 upon dietary intervention, pregnancy stage and behaviour observation period. Kleemann et
64 al. (2015) provided sheep with 70% (low), 100% (standard) or 150% (high) nutritional
65 requirements (protein and energy) for 17 days prior and 6 days after insemination. When
66 released after tagging, lambs from mothers fed the low diet returned faster to their mothers
67 than those from the high diet cohort, and contacted the udder more quickly than those lambs
68 from the standard (100%) or high (150%) dietary treatments (Kleemann et al., 2015).

69 Contrary to this, a study that provided a mild maternal under-nutrition (10-15% body weight
70 reduction) between -60 to +30 days relative to conception, failed to observe any effects of the
71 dietary treatment upon ewe-lamb bonding during the first month of life (Hernandez et al.,
72 2009b). However, offspring from undernourished ewes, when in isolation, showed
73 suppressed behavioural reactions, altered behavioural laterality, reduced locomotor activity
74 and increased cortisol secretion at 4 and 18 months of age (Hernandez et al., 2009a;
75 Hernandez et al., 2010; Donovan et al., 2013).

76

77 In cattle, one report (Micke et al., 2015), suggests that maternal first trimester (0-90 days
78 post conception) diet may increase milk intake in male offspring between 94 to 191 days of
79 age. However, these researchers used the weigh-suckle-weigh technique, which is unable to
80 differentiate between offspring milk intake and heifer milk production (Sullivan et al., 2009c).
81 No conclusion on the calves' suckling behaviour could therefore be drawn. Thus, the aim of
82 this study was to investigate maternal and offspring behavioural and hormonal profiles
83 following a preconception dietary protein restriction (PreH: 18% Crude Protein (CP) vs. PreL:
84 10% CP) in nulliparous beef heifers.

85

86 2. Materials and Methods

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

90

91 2.1 Animals and Experimental Design

92 The effect of dietary protein during the preconception period on the maternal and neonatal
93 behaviour in beef cattle was evaluated using a total of 88 nulliparous heifers kept in two
94 locations within the UK (Farm A, n=44; and Farm B, n=44). Two months prior to conception
95 (conception, day 0, was taken as the day of artificial insemination (AI)), heifers were
96 randomly allocated, by weight and age, to High (PreH: 18%; n=44; 347.2 \pm 9.4kg BW) or Low
97 (PreL: 10%; n=44; 355.5 \pm 10.8kg BW; CP at the lower level of recommendation) crude
98 protein diet (Hernandez-Medrano et al., 2015). Heifers were group-fed the corresponding diet
99 and managed as two groups according to diet. At the end of the treatment period, heifers
100 were inseminated following a 5-day synchronization protocol (Hernandez-Medrano et al.,
101 2015), with semen from a single bull with known EBVs for growth and low birth weight.

102

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

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

110

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

119

120 2.2 Behavioural data collection

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

131

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

142

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

155

156 2.3 Body weight measure, metabolite analysis and hormone assays

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

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

163

164 Hormonal concentrations were determined at the Animal Science Research Center of the
165 University of Missouri, USA. Double antibody radioimmunoassay (previously validated in the
166 laboratory in charge of the analysis) with inter- and intra-assay CV of <5% was used to
167 determined plasma concentrations of IGF-1 (Lalman et al., 2000), leptin (Delavaud et al.,
168 2000), prolactin (Lutz et al., 1991), progesterone (Pohler et al., 2015) and oxytocin (Lutz et
169 al., 1991). In the case of serum or plasma concentrations of cortisol, they were determined
170 via double antibody radioimmunoassay validated by Daniel et al. (2000), with inter- and intra-
171 assay CV were < 5%. The RX-IMOLA auto analyser (Randox, County Antrim, UK) was used
172 to quantify albumin, total protein (soluble) and NEFAs (non-esterified fatty acids)
173 concentrations. Globulin levels were estimated by calculating the remainder of the total
174 protein after albumin levels were accounted for.

175

176 2.4 Data presentation and statistical analyses

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

182

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

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

194

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

201

202 3. Results

203 3.1. Effect of preconception diet on heifers

204 3.1.1. Body weight, circulating hormones and behaviour

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

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

218

219 3.1.2 Calving Assistance

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

227

228 3.2 Effect of preconception diet on calves

229 3.2.1 Body weight, circulating hormones and behaviour

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

235

236 3.2.2 Behaviour

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

244 significant positive association between suckling time and maternal diet. In the 30 hours after
245 birth, calves born to PreL heifers spent more time suckling (Coefficient: 14.82 (SE: 6.62);
246 $P=0.036$) than those from PreH heifers (Figure 1). Running in calves was not significantly
247 different between diet treatments (data not shown here).

248

249 4. Discussion

250 This study is the first to show that a low protein diet during the preconception period affected
251 both neonatal offspring and dam behaviour in cattle. Calves from heifers receiving the low
252 protein preconception diet showed increased suckling times and were heavier than calves
253 from mothers that received the high protein diet. Standing behaviour time was also increased
254 in heifers that received the low protein diet which could be associated with the increase
255 suckling behaviour of their offspring (Stehulova et al., 2013). Hormone concentrations
256 measured at calving in heifers are within similar ranges to previous reports (Sullivan et al.,
257 2009b; Micke et al., 2015), with no effect in this study of dietary treatment.

258

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

270

271 Various studies have described possible long-term modifications of offspring development,
272 health and welfare following maternal nutrient restriction during periconception (reviewed by
273 Sinclair et al., 2016). In sheep, following a comparable dietary period intervention, cognitive
274 behaviour appears unaffected (Abecia et al., 2015) despite effects on long-term locomotor
275 behaviour (Donovan et al., 2013). In cattle, suckling behaviour characteristics (duration and
276 events) are established by the third day (Lidfors et al., 1994), suckling bouts show
277 consistency within lactation, and are initially lead by the calf and then by the cow around day
278 30 (Stehulova et al., 2013). Previous findings showed an effect of PreL preconception dietary
279 treatment on milk production during the first and sixth months after calving (Sullivan et al.,
280 2009c; Micke et al., 2015). Although long term effects were not studied, findings suggest that
281 PreL preconception dietary treatment may affect suckling behaviour in beef cattle from birth
282 up to six months of age.

283

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

295

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

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

307

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

321

322 5. Conclusions

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

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

331

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341

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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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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

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

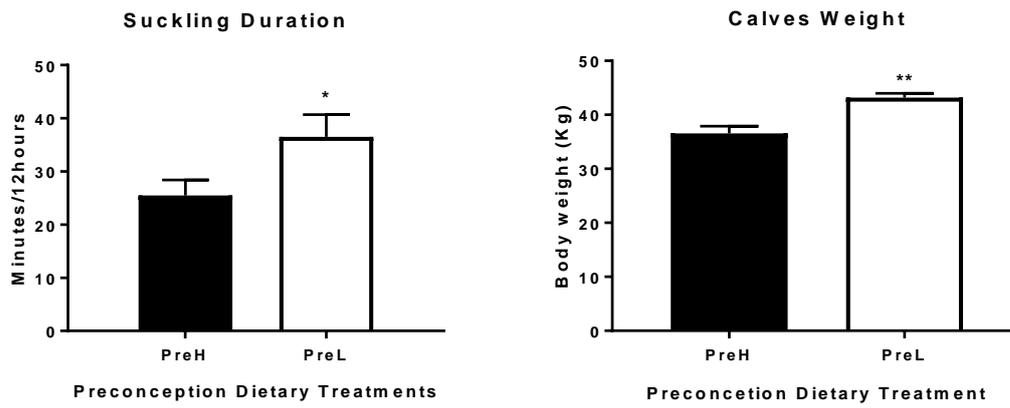
482 AI= Artificial Insemination

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484 Figure captions

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

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