

1 **Does nutritional status during the latter stage of pregnancy mediate the effect**
2 **of conception date on gestation length in red deer hinds? I. Voluntary food**
3 **intake of hinds during gestation**

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12 Running head: Voluntary food intake of red deer hinds during gestation

13

14 **Abstract**

15 Efficient farmed venison production under New Zealand lowland conditions requires
16 early calving to better align lactation with pasture availability. However, hinds that
17 conceive early in the breeding season have a longer gestation length than those
18 conceiving later, negating some of the gains achieved by early conception. This
19 variation in gestation length may relate to seasonal imbalances in hind nutrient
20 uptake influencing fetal growth. However, little is known about food intake cycles of
21 pregnant hinds and whether they exhibit the photoperiod-induced voluntary food
22 intake (VFI) reduction over winter seen in younger age classes and adult stags. This
23 study investigated the effect of pregnancy status on VFI of red deer hinds. In
24 addition, concentration of leptin and ghrelin circulating in the body was measured
25 throughout the study to ascertain if these hormones are indicative of hind energy
26 status. Seven pregnant (P) and seven non-pregnant (NP) hinds were housed indoors
27 in individual pens from April to November where they were offered daily an *ad libitum*

28 pelleted ration. On average, P hinds gained 75 g/day and NP hinds lost 27 g/day
29 ($P=0.02$) in autumn. Mean live weight (LW) of both groups then steadily increased for
30 the remainder of the study with no significant difference between groups. Mean body
31 condition score (BCS) change of P and NP hinds was similar in autumn and winter,
32 but whereas that of P hinds decreased in spring, that of NP hinds increased
33 ($P=0.02$). Pregnancy status of the hinds had no significant effect on mean VFI
34 throughout the trial except for the last five days before parturition when VFI of P
35 hinds decreased dramatically ($P=0.001$). VFI of both groups of hinds was
36 significantly higher in autumn ($P=0.03$) and spring ($P=0.01$) than in winter and for
37 every 0.1 MJME/kg LW^{0.75}/day increase in mean VFI during the study period,
38 gestation length decreased by 6.4 days ($r^2=0.51$; $P=0.04$). Pregnancy status had no
39 significant effect on plasma concentration of either leptin or ghrelin at any of the
40 sampling times and there was no significant association of either leptin or ghrelin
41 with VFI. However, leptin plasma concentration was positively associated with BCS
42 ($r^2=0.41$; $P=0.008$). This study showed that VFI of pregnant hinds was depressed
43 during winter and early spring, and was negatively associated with gestation length.
44 A reduction in BCS of pregnant indicated that they were in a moderate energy deficit
45 during the final third of gestation.

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47

48 **Keywords:** red deer, hind, pregnant, voluntary food intake, day length

49

50 **Implications**

51

52 Efficient farmed venison supply systems require that food availability matches
53 energy demand at all times. This study found that pregnant hinds have an
54 endogenous reduction in voluntary food intake during winter, at a time when energy
55 demand of a fetus in the last third of gestation is increasing rapidly. Fat is mobilised
56 to address the ensuing energy imbalance, with a resultant decrease in hind body
57 condition. This may influence fetal growth and may also adversely affect subsequent
58 lactation, calf growth rate and conception.

59

60 **Introduction**

61

62 To maximise the productivity of farmed venison supply systems food availability
63 needs to match energy demands at all times. For the hind and its rapidly growing
64 calf, energy demand is highest from late pregnancy through to weaning. Red deer
65 (*Cervus elaphus*) evolved in temperate regions of Europe (Whitehead, 1972) where
66 seasonal extremes in temperature and feed availability strongly influence animal
67 survival. The prevailing conditions have dictated a highly seasonal pattern of autumn
68 conception and early summer calving for survival of the species (Lincoln and
69 Guinness, 1973). Calves born very early or late in the season are less likely to
70 survive as neonates than those born at the peak of the calving period (Iason and
71 Guinness, 1985). Thus, the reproductive cycle of red deer has evolved to match
72 perfectly food availability in the temperate regions of Europe. However, under New
73 Zealand lowland farming systems, pasture quality and feed availability are often low
74 during summer and autumn (Litherland *et al.*, 2002), limiting the genetic potential for
75 calf growth. Early summer calving has resulted in a misalignment between peak

76 pasture quality in spring and the nutritional demands of a lactating hind and her
77 offspring during summer and autumn (Asher *et al.*, 1996).

78 Although significant research effort has been expended to advance the calving
79 date of red deer hinds (Asher *et al.*, 1996), such efforts appear to be partly offset by
80 robust and complex adaptations of reproductive processes in deer that have evolved
81 to ensure offspring are born at the optimal time for survival. Only a few generations
82 of red deer have been exposed to the New Zealand farming environment, so there
83 has been insufficient time for selection pressure to have modified their inherent
84 seasonality.

85 Recent research has shown that hinds conceiving early in the breeding season
86 have a longer gestation length than those conceiving late, and conversely, those
87 conceiving late in the breeding season have a shorter gestation length (Garcia *et al.*,
88 2006; Scott *et al.*, 2008a). For every 10 days change in conception date there will
89 likely be 2-4 days change in gestation length. Scott *et al.* (2008a) hypothesised that
90 a photoperiod-induced reduction in hind food intake during winter may impact on the
91 ability of early-conceiving hinds to meet the increasing energy demands of the
92 rapidly growing fetus during the last third of pregnancy. Thus, the ensuing moderate
93 energy intake imbalance between seasons mediates the observed variation in
94 gestation length. This hypothesis was based on the observations of Asher *et al.*
95 (2005a) who reported that a moderate energy intake imbalance during the last third
96 of pregnancy in red deer was compensated for by varying gestation length to ensure
97 optimal birth weight at the time of parturition.

98 Seasonal animals are assumed to maintain an appropriate body mass which
99 varies depending on circumstances such as age, reproductive status or season
100 (Kay, 1988). A model of how intake of ruminants is regulated to maintain an

101 appropriate body mass was first proposed by Montgomery and Baumgardt (1965).
102 They proposed that ruminants are able to regulate dry matter intake over a range of
103 feed digestibilities so that their energy intake remains equal to their need. Such a
104 model has since been validated in three cervid species: white-tailed deer
105 (*Odocoileus virginianus*; Ammann *et al.*, 1973), reindeer (*Rangifer tarandus*
106 *tarandus*; Ryg, 1983) and red deer (*Cervus elaphus*; Webster *et al.*, 2000; Scott *et*
107 *al.*, 2008b). As an adaptation to living in temperate zones with predictable seasonal
108 cycles of food abundance in summer and scarcity in winter, many animals exhibit
109 seasonal variations in voluntary food intake (VFI), body mass and energy
110 metabolism that do not reflect actual changes in food availability, but are a function
111 of physiological changes in response to predictors of the seasonal environment
112 (Loudon, 1994). A photoperiod-mediated reduction in VFI during 'short days' has
113 been well documented for young growing red deer of both sexes and for adult stags
114 and non-pregnant hinds (Pollock, 1975; Suttie and Simpson, 1985; Loudon *et al.*,
115 1989), but there appears to be an absence of such data for pregnant red deer hinds.

116 Pregnancy is a dynamic state and to ensure reproductive success the energy
117 demands of the developing fetus must be met at all stages of gestation. Nicol and
118 Brookes (2007) calculated the total energy requirement for the entire pregnancy of a
119 red deer hind to be 55 MJME/kg birth weight above maintenance. During the last
120 third of pregnancy the fetal and maternal components of pregnancy gain about 70%
121 of their final mass in red deer (Adam *et al.*, 1988a), and it was estimated that the
122 additional energy requirements of pregnant above non-pregnant hinds increases
123 from 1.7 to 5.0 MJME/day during that time (Adam *et al.*, 1988b). This raises the
124 question of whether pregnant red deer hinds have reduced VFI during winter, at the
125 time when energy demands of a rapidly growing fetus are increasing.

126 The advantages of a mechanism whereby VFI matches that of food supply in
127 animals living in highly seasonal environments are well recognised. Such an
128 adaptation is thought to have evolved so that less energy is expended on foraging
129 for food during times of scarcity (Kay and Staines, 1981), but precisely how food
130 intake is regulated has yet to be fully elucidated. However, it is known that two
131 peptide hormones, leptin and ghrelin, play a major role in maintaining metabolic
132 homeostasis in mammals. Synthesis and secretion of ghrelin are regulated by
133 nutritional state; blood levels rise in anticipation of food, promoting hunger, and then
134 decrease postprandially. Plasma factors indicative of nutritional status that are
135 released at the time of food intake, such as glucose, amino acids and insulin,
136 stimulate leptin secretion and promote satiety (Gao and Horvarth, 2007). Feeding is
137 thus partly modulated by the antagonistic effects of leptin and ghrelin.

138 This study was undertaken as the first step in testing the hypothesis of Scott *et al.*
139 (2008a) that early-conceiving hinds have a longer gestation length than those which
140 conceive later because of seasonal variation in VFI, and hence of fetal growth
141 trajectory. Our aim was to quantify the daily food intake of pregnant hinds during
142 gestation and relate it to that of non-pregnant hinds during the same period. The
143 hypothesis tested was that pregnancy status does not affect the seasonal
144 depression in VFI of red deer hinds during winter.

145 A secondary objective of the study was to measure the concentrations of leptin
146 and ghrelin circulating in the body at different stages of the gestation period. It was
147 considered that plasma concentration of these hormones may be indicative of the
148 energy status of the hinds.

149

150 **Materials and Methods**

151

152 *Experimental overview*

153 The study was conducted in a single year at the AgResearch Invermay Research
154 Centre located in Mosgiel, New Zealand (latitude 45° 51' S). Pregnant (n = 8) and
155 non-pregnant (n = 8) adult red deer hinds were individually housed in a single
156 building from April to November, during which time they were fed an *ad libitum* diet of
157 deer pellets plus 5% (by weight) lucerne chaff. Food intake was monitored daily to
158 assess the effect of pregnancy status and season on voluntary food intake. In
159 addition, blood samples were collected every 4 weeks during the period of indoor
160 confinement for measurement of plasma leptin and ghrelin concentration.

161 All animal manipulations were approved by the AgResearch Invermay Animal
162 Ethics Committee (Project Number 11700), as required in New Zealand by the
163 Animal Welfare Act 1999. All procedures were conducted by fully trained staff from
164 the Invermay Agricultural Centre and in accredited facilities (NZQA accreditation
165 scheme).

166

167 *Animals and management*

168 Twenty rising-four-year-old *Cervus elaphus hippelaphus* x *C. e. scoticus* red deer
169 hinds of mixed parity were habituated to eating standard deer pellets while being
170 grazed on short pasture, at least 2 weeks before indoor confinement. Eight hinds
171 were allocated to remain non-pregnant (NP) and 12 hinds were scheduled for
172 artificial insemination on 1 April with *C. e. hippelaphus* semen to generate eight
173 pregnant (P) hinds. Treatment groups were balanced for parity and live weight.

174 NP hinds were housed indoors from 25 March until the end of the study. It was
175 considered that stress associated with becoming accustomed to indoor housing may
176 perturb the synchronised ovulation necessary for fixed-time artificial insemination;
177 therefore the 12 hinds scheduled for artificial insemination remained outdoors on
178 short pasture and were fed pellets until 7 days post-artificial insemination. From the
179 pool of twelve inseminated hinds eight were selected, on their perceived suitability
180 for indoor housing, to be housed indoors from 8 April until about 24 h post-calving.
181 The remaining four hinds were kept outdoors as 'reserves' and were fed pellets at
182 pasture until it was evident they would not be needed for the study. Pregnancy status
183 of inseminated hinds was determined by rectal ultrasound scanning on 1 May and 23
184 June using a 5 MHz linear array transducer (Aloka SSD 500; MedTel Telelectronics
185 Ltd., Auckland, NZ).

186 Hinds were calved indoors to enable accurate calculation of gestation length;
187 hinds and calves were weighed within 24 h of parturition before hind-calf pairs were
188 returned to pasture. NP hinds remained indoors until the last P hind had calved.

189

190 *Oestrous synchronisation and artificial insemination*

191 Twelve hinds received a 12-day hormone treatment to synchronise ovulation for
192 fixed-time artificial insemination. On 18 March (Day 0), hinds received an intravaginal
193 progesterone-releasing device (Eazi-breed CIDR[®] type G; Pfizer New Zealand Ltd.,
194 Mt Eden, Auckland, NZ) which was replaced by a second CIDR[®] device on Day 9.
195 The second CIDR[®] device was removed between 1000 and 1030 h on Day 12 and
196 the hinds concurrently injected with 180 i.u. equine chorionic gonadotrophin
197 (Folligon, Intervet, Lane Cove, NSW, Australia). Transcervical artificial insemination

198 began at 1800 h on 1 April, 56 h after CIDR[®] device removal, using cryopreserved
199 semen from a single stag.

200

201 *Indoor pens*

202 Sixteen indoor pens were located in a single covered, ventilated building that was
203 adjacent to outdoor exercise yards and had raceway access to a weigh-box and deer
204 handling facility (pneumatic crush). Pens (approximately 6 m²) had a concrete floor
205 covered in deep-litter sawdust and were constructed with panel walls so that visual
206 contact could be maintained between neighbouring hinds in adjacent pens. Natural
207 lighting, provided by skylights, was supplemented by artificial lights that were timed
208 to automatically switch on at sunrise and off at sunset each day. Data published by
209 the Royal Astronomical Society of New Zealand (<http://www.rasnz.org.nz/>) were
210 used to set sunrise and sunset times for the lights, with no allowance made for Civil
211 Twilight.

212 Each pen was provided with a wooden food bin and a water nose-trough fitted
213 with a float valve such that water was available *ad libitum*; both were fixed to a wall
214 at a height of approximately 1 m. Faeces were removed and the sawdust raked
215 daily; all sawdust in each pen was replaced at least once per month to prevent build-
216 up of ammonia fumes from urine.

217

218 *Feeding*

219 Throughout the period of indoor confinement the diet consisted of a commercial
220 pelleted deer food (Reliance Deer Nuts, Combined Rural Traders, Yaldhurst,
221 Christchurch, NZ) containing 12.7 MJME/kg DM and 14.5% crude protein. In addition
222 to pellets, 5% by weight of the daily offer was lucerne chaff (10.5 MJME/kg DM;

223 22.9% crude protein) for adequate roughage to ensure maintenance of rumen
224 function. At 0830 h daily, hinds were released from their pens and grouped together
225 in exercise yards for two hours. During this time food not eaten (refusal) was
226 collected, weighed, discarded, and replaced with fresh rations. Hinds were initially
227 offered 0.95 kg pellets plus 0.05 kg lucerne chaff to avoid acidosis from grain
228 overload. Thereafter the food ration was adjusted to appetite daily according to the
229 rule: if the refusal was < 10% of food offered, the new ration was increased by 200 g;
230 if the refusal was > 10% of food offered, the ration remained the same as that on the
231 previous day. Once per week a sample of the residual food was collected to
232 ascertain dry matter (DM) percentage; the value obtained was used to calculate daily
233 DM intake of the hinds for that week.

234

235 *Weighing and blood samples*

236 Hinds were weighed to the nearest 0.5 kg and assessed for body condition score
237 (BCS) every fortnight. BCS was based on a 5-point scale (1 = emaciation and 5 =
238 obesity) as described by Audigé *et al.* (1998) and was assessed by visual and
239 palpation appraisal of the spine, sacrum and wings of the pelvis.

240 From 23 April until 5 November, blood samples were collected at four-week
241 intervals via jugular venepuncture into 10 ml evacuated tubes containing K₃ EDTA as
242 anticoagulant. Hinds were bled at 20 minute intervals over one hour, beginning at
243 1330 h on each collection; the samples were kept on ice until centrifuged at 4°C for
244 15 min at 2,000 g within two hours of collection. Plasma was pipetted into separate 1
245 ml aliquots for measurement of leptin and active ghrelin concentrations. In addition,
246 plasma aliquots for ghrelin analysis were acidified with 50 µl of 1 N HCl and 10 µl of
247 phenylmethylsulfonyl fluoride was added as a protease inhibitor to preserve the

248 integrity of the octanyl moiety of ghrelin, as required to measure the concentration of
249 active ghrelin. Plasma was stored at -20°C until assayed.

250

251 *Hormone assays*

252 Twenty-minute plasma samples were pooled for each animal on each sampling date
253 before assay. Samples were thawed and mixed by a vortex stirrer before 250 µl of
254 each of the 0, 20, 40 and 60 minute aliquots was pipetted into a single tube to make
255 1 ml of pooled-hour plasma aliquot. Plasma concentrations of leptin and ghrelin were
256 then measured from the pooled-hour plasma aliquots in duplicate 100 µl samples
257 using commercially available radioimmunoassay (RIA) kits. All procedures were
258 carried out in accordance with the manufacturer's protocol. The precipitate was
259 collected by centrifugation at 4°C for 25 minutes at 2,500 g and the supernatant
260 discarded. Assay tubes containing pellets were counted for 1 minute on an automatic
261 gamma counter (Wallac Wizard 1470, Perkin Elmer, Wellesley, MA, USA).

262 Plasma leptin concentrations were measured using a multi-species leptin RIA kit
263 (LINCO Research, Cat. # XL-85K, St. Charles, MO, USA). This kit has been
264 validated previously for cervids (sika deer: Suzuki *et al.*, 2004; reindeer: Soppela *et*
265 *al.*, 2008; red deer: Gaspar-López *et al.*, 2009). The antibody used in the kit was
266 raised against human leptin in guinea pigs and the protocol recommends that ng/ml
267 human equivalent (HE) is used as the unit of measure. The limit of sensitivity for the
268 multi-species leptin kit is 1.0 ng/ml HE. Intra- and inter-assay coefficients of variation
269 were 4.8 and 2.7% respectively.

270 A rat active ghrelin RIA kit (LINCO Research, Cat. # GHRA-88HK, St. Charles,
271 MO, USA) was used to measure plasma active ghrelin concentrations. The kit
272 utilises an antibody which is specific for the biologically active form of ghrelin with the

273 octanyl group on serine 3 and has a sensitivity of 7.8 pg/ml. Prior to analysing
274 experimental samples, the kit was validated for cervine plasma by demonstrating
275 parallelism to the standard curve of serially diluted cervine plasma (Figure 1). The
276 intra- and inter-assay coefficients of variation were 11.2 and 7.8% respectively.

277 (Insert Figure 1 here)

278 *Statistical analyses*

279 Data from before 27 April, while hinds were building up to an *ad libitum* food intake,
280 were not included in any of the analyses. A complete data set was available from
281 seven P and seven NP hinds.

282 Effect of pregnancy status on changes in mean live weight (LW), body condition
283 score (BCS) and VFI during specific time periods, and on plasma hormone
284 concentrations at each sampling date, were analysed by analysis of variance
285 (ANOVA), separately, fitting a term for pregnancy status. The time frames for VFI
286 analyses were normalised about date of parturition to compensate for the large
287 variation in parturition date. For NP hinds, Day 0 was taken as the mean parturition
288 date of P hinds.

289 When calculating change in VFI between seasons, mean VFI over 3 days around
290 the start and end date of the specified times was used to allow for large daily
291 variation of individual hind intake. For example, VFI for Day -200 was calculated as
292 the average VFI value of Days -201, -200 and -199. A semi-parametric linear mixed
293 model with smoothing spline was applied to the mean VFI data using REML in the
294 statistical package GenStat Version 11. Pregnancy status (Trtmnt), day of year
295 (DOY) and the interaction term (Trtmnt.DOY) were fitted as fixed effects. Individual
296 hind (ID) and the interaction ID.DOY were fitted as random model terms. The
297 covariance structure was defined by ID and ID.DOY by allowing unrestricted

298 correlation structure, and the structure formed by definition of the whole matrix. The
299 initial values for covariance matrix terms were determined by estimates from running
300 the same model but with no, or simple, covariance structure. An overall spline
301 (termed DOY in the spline model), separate treatment splines (Trtmnt.DOY) and
302 individual hind splines (ID.DOY) were also fitted as part of REML.

303 Regression lines were fitted to the mean data of each hind over the entire study
304 period to explore the relationships between plasma hormone concentration,
305 gestation length and the variables reported.

306

307 **Results**

308

309 *General*

310 One of the NP hinds did not adapt to being confined indoors and was removed from
311 the study. The remaining hinds appeared to become well habituated to indoor
312 housing conditions and took about two weeks to stabilise their *ad libitum* intake. One
313 of the P hinds lost her pregnancy somewhere between the first (1 May) and second
314 (23 June) ultrasound scan and her data were not included in the analyses. The
315 remaining seven P hinds all had an unassisted calving and produced healthy
316 singleton calves with birth weight ranging from 7.0 – 10.5 kg (mean = 9.8 kg), which
317 is within the range expected from red deer hinds grazed at pasture.

318

319 *Live weight and body condition score*

320 Mean (\pm s.e.) live weight (LW) of P and NP hinds on 27 April was 117.1 ± 6.0 kg and
321 124.1 ± 10.3 kg respectively. A number of NP hinds went through large fluctuations
322 of VFI and lost weight during an initial 'settling in' period, before regaining that

323 weight; this was not apparent in P hinds. This resulted in a difference in mean live
324 weight gain (LWG) of 102 g/day (s.e.d. 40 g/day, $P=0.02$) between P and NP hinds
325 during the first 42 days of the study. On average, P hinds gained 75 g/day while NP
326 hinds lost 27 g/day between 27 April and 8 June. There was no significant difference
327 between groups in rate of mean LW change during winter (9 June – 31 August;
328 $P=0.68$) and spring (1 September – 9 November; $P=0.72$; Table 1). Mean (\pm s.e.) LW
329 on 9 November was 146.8 ± 9.9 kg and 147.9 ± 13.9 kg for P and NP hinds
330 respectively.

331 Mean (\pm s.e.) body condition score (BCS) of P hinds on 27 April was 4.0 ± 0.6
332 while that of NP hinds was 4.2 ± 0.9 . Although BCS of both treatment groups
333 increased between 27 May and 9 November, pattern of BCS change during the
334 study differed with pregnancy status. Both P and NP hinds gained body condition
335 during autumn ($P=0.14$) and winter ($P=1.00$), but mean BCS of P hinds decreased,
336 whereas that of NP hinds increased, during spring ($P=0.02$; Table 1).

337 (Insert Table 1 here)

338 *Voluntary food intake*

339 There was considerable between-hind and between-day variation in VFI. For
340 example, one hind rarely exceeded a daily dry matter intake (DMI) of 1.6 kg while
341 another regularly ingested more than 3.5 kg. Between-day intake of individual hinds
342 often varied by more than 0.5 kg DM, with hinds on higher intakes, in particular,
343 going through 'feast and famine' cycles (Figure 2).

344 (Insert Figure 2 here)

345 Pregnancy status of the hinds had no significant effect ($P>0.05$) on mean change
346 in daily VFI during specified time periods of the study except for the last five days
347 before parturition when VFI of P hinds decreased dramatically (Table 2). This

348 relationship held when VFI was expressed as both absolute intake (MJME), or when
349 adjusted for metabolic live weight (MBW, MJME/kg LW^{0.75}).

350 (Insert Table 2 here)

351 Mean (\pm s.e.) daily VFI of hinds over three consecutive days was 0.724 ± 0.054
352 MJME/kg LW^{0.75} in early-autumn, 0.578 ± 0.029 MJME/kg LW^{0.75} in mid-winter and
353 0.686 ± 0.034 MJME/kg LW^{0.75} in late-spring. On average, daily hind intake
354 decreased by 0.146 ± 0.060 MJME/kg LW^{0.75} from autumn to winter ($P=0.029$) and
355 increased by 0.107 ± 0.035 MJME/kg LW^{0.75} from winter to spring ($P=0.009$),
356 seemingly aligned with the seasonal change in daily photoperiod (Figure 3).

357 (Insert Figure 3 here)

358 *Leptin*

359 Pregnancy status had no significant effect ($P>0.05$) on mean plasma leptin
360 concentration at any of the sampling times (Table 3) and there was no discernable
361 seasonal pattern of circulating leptin concentration.

362 (Insert Table 3 here)

363 Intake of individual hinds was not associated significantly with their plasma leptin
364 concentration. However, there was a positive relationship between BCS and
365 circulating leptin concentration ($R^2 = 0.41$, $P = 0.008$; Figure 4), such that, for every
366 unit increase in mean BCS mean circulating leptin concentration increased by 0.49
367 ng/ml human equivalent (HE).

368 (Insert Figure 4 here)

369 *Ghrelin*

370 Pregnancy status had no significant effect ($P>0.05$) on mean concentration of
371 plasma ghrelin at any of the sampling times (Table 4). Mean concentration of
372 circulating ghrelin increased from April to July and then decreased, but there was no

373 significant relationship between mean plasma ghrelin concentration and season.
374 Intake of individual hinds was not associated significantly with level of circulating
375 ghrelin.

376 (Insert Table 4 here)

377 *Gestation length*

378 Mean (\pm s.e.) gestation length of the hinds was 233.00 ± 2.32 days, with the first hind
379 calving on 13 November and the last on 2 December. There was a negative
380 correlation of gestation length with mean VFI during the study period ($R^2 = 0.51$;
381 $P=0.04$) such that for every $0.1 \text{ MJME/kg LW}^{0.75}/\text{day}$ increase in mean VFI gestation
382 length decreased by 6.1 days (Figure 5). Calf birth weight was negatively associated
383 with gestation length ($R^2=0.75$; $P=0.02$); gestation length decreased by 4.8 days for
384 every 1 kg increase in calf birth weight.

385 Gestation length was not correlated with hind live weight ($P=0.60$), BCS ($P=0.51$)
386 or circulating levels of leptin ($P=0.26$) or ghrelin ($P=0.56$).

387 (Insert Figure 5 here)

388 **Discussion**

389

390 This study has shown that pregnancy does not affect the seasonal depression in VFI
391 of red deer hinds during winter. VFI of both P and NP hinds decreased by about 20%
392 from autumn to mid-winter and then recovered to pre-winter levels by the end of
393 spring. The amplitude of change in VFI between autumn, winter and spring in the
394 present study was similar to that reported previously for non-pregnant red deer hinds
395 (Suttie and Simpson, 1985; Loudon *et al.*, 1989). Although seasonal cycles in VFI of
396 housed red deer offered *ad libitum* access to a concentrate diet have been well
397 documented for young growing deer of both sexes, adult stags and non-pregnant

398 adult hinds (Loudon, 1994; Webster *et al.*, 2000), this appears to be the first such
399 observation reported for pregnant red deer.

400 Pregnancy is a dynamic state and to ensure reproductive success the energy
401 demands of the developing fetus must be met at all stages of gestation. In
402 nutritionally poor environments red deer hinds may conceive successfully one year
403 but fail the next because of poor body condition at the time of the rut (Mitchell *et al.*,
404 1976). During the last third of pregnancy in red deer the fetal and maternal
405 components of pregnancy gain about 70% of their final mass (Adam *et al.*, 1988a),
406 and to meet the energy demands of the growing fetus one would expect P hinds to
407 require more food than NP hinds. Indeed, Asher *et al.* (2005a) reported that housed
408 pregnant hinds allowed *ad libitum* access to a concentrate diet increased their daily
409 VFI from about 20 MJME at Day 150 of gestation to 29 MJME at Day 210, i.e. an
410 increase of 0.15 MJME per day. It is, therefore, counterintuitive that P hinds in the
411 present study did not increase their VFI above that of NP hinds during the last third
412 of pregnancy. Between Day 130 (mid-July) and Day 210 (late October) of gestation,
413 P hinds increased their mean daily VFI by 6.0 MJME, whereas NP hinds increased
414 their mean daily VFI by 6.9 MJME during the same period. Paradoxically, P hinds
415 tended to gain more live weight than NP hinds, although eating less. This may, in
416 part, be explained by the more efficient use of nutrients during pregnancy (Brockway
417 *et al.*, 1963). Moreover, although BCS of non-pregnant hinds increased between 1
418 September and 9 November, that of pregnant hinds decreased in the same period
419 indicating a moderate energy imbalance during the last third of pregnancy. In effect,
420 hind body condition (i.e. mainly fat) served as an energy store and fat was mobilised
421 when VFI was insufficient to meet the energy demands of the fetus. Likewise,
422 pregnant Svalbard reindeer (*Rangifer tarandus platyrhincus*) have large fat reserves

423 in autumn which are used primarily during the last two months of gestation and early
424 lactation, presumably for the same reason (Tyler, 1987). It seems that the observed
425 depression in VFI during winter results in an inability of the hind to meet the
426 demands of a rapidly growing fetus through nutritional intake alone, resulting in an
427 energy shortfall which is met by body reserves during the last third of pregnancy.
428 This indicates the need for hinds to be in good body condition at the beginning of
429 winter.

430 Large variation in both VFI and LWG was a feature of the present study. Some
431 hinds went through cycles of high and low VFI, which resulted in fluctuations in live
432 weight, possibly through variation in gut fill. However, a diet high in readily available
433 carbohydrates, such as in the present study, may reduce intake due to acidosis
434 (Elam, 1976). Although the ration on offer contained 5% lucerne for roughage to
435 ensure maintenance of rumen function, greedy hinds consuming large quantities of
436 pellets may have experienced some acidosis and reduced their intake, then
437 recovered and repeated this cycle. Alternatively, these hinds may have been actively
438 regulating their intake to maintain an appropriate 'energy balance' (Scott *et al.*,
439 2008b). Other hinds consumed relatively modest amounts of food throughout the
440 study and had little variation in live weight.

441 VFI of the P hinds reached a peak about 3 weeks before parturition then
442 decreased gradually until a precipitous drop in the few days immediately preceding
443 calving. Such a depression in VFI as parturition approaches occurs also in cows and
444 sheep, and may in these species result in metabolic disorders such as ketosis and
445 hypocalcaemia (Ingvartsen and Andersen, 2000; Melendez *et al.*, 2006). There is a
446 significant negative relationship between the volume of rumen contents and the
447 volume of uterus plus other abdominal organs in sheep (Forbes, 1969). Therefore, it

448 is possible that physical size of the uterus and conceptus in the final stages of
449 pregnancy limited abdominal space available for other organs, thus restricting rumen
450 volume and VFI. However, hinds in the present study received a high quality diet of
451 pellets containing > 12.5 MJME/kg as compared with a low quality hay diet fed to
452 ewes in the study of Forbes (1969). Therefore, it is unlikely that competition for
453 abdominal space limited VFI in this study. It is worth noting, however, that
454 competition for abdominal space in the final weeks of pregnancy may possibly
455 restrict intake of hinds on low quality feed at pasture. A more plausible explanation
456 for the observed decrease in VFI of P hinds in the present study is an effect of high
457 levels of oestrogens secreted during the second half of pregnancy that reach a peak
458 during the 3 days before parturition (Tucker, 1985). Intravenous infusions containing
459 quantities of oestrogens similar to those secreted in late pregnancy depressed VFI of
460 castrated male sheep fed a concentrate diet (Forbes, 1971). In addition,
461 corticotrophin-releasing factor (CRF) has been demonstrated to decrease VFI in
462 rodents (Richard, 1993) and sheep (Ruckebusch and Malbert, 1986). Therefore, the
463 CRF-mediated increase in circulating maternal cortisol levels in the periparturient
464 period (Tucker, 1985) may also play a role in the precipitous decline in VFI that was
465 observed in the last few days preceding calving in the present study.

466 There was a 19-day spread in calving date despite all hinds conceiving to artificial
467 insemination on 1 April and having *ad libitum* access to high quality food. Asher *et al.*
468 (2005a) reported a negative correlation between duration of pregnancy and change
469 in hind live weight during late pregnancy in hinds on differing planes of nutrition.
470 They hypothesised that fetal induction of parturition is dependent on attainment of a
471 critical size, thus ensuring birth of a viable neonate. In the present study, gestation
472 length was negatively correlated with energy intake and heavier calves had a shorter

473 gestating period than lighter calves. This supports the hypothesis that variation in
474 gestation length compensates for variation in fetal growth under conditions of a
475 moderate maternal energy imbalance (Asher *et al.*, 2005a). It is interesting to note
476 that 6 out of 7 calves had a birth weight (BW) in the range 9.5-10.5 kg; the remaining
477 calf had a BW of only 7.0 kg after 245 days gestation. The hind giving birth to this
478 calf consumed approximately 0.2 MJME/ kg LW^{0.75} less than contemporaries for
479 much of the study and entered winter (8 June) with a BCS 1.1 unit less than the
480 average for P hinds on that date. It would appear that in the face of a more severe
481 energy imbalance, prolonged gestation length was unable to compensate fully for the
482 reduced fetal growth trajectory. In this instance, the calf was born at a lower birth
483 weight, as has been reported previously for red deer (*C. e. scoticus*) on the Isle of
484 Rhum, Scotland (Albon *et al.*, 1983), North American wapiti (Thorne *et al.*, 1976) and
485 red deer gestating wapiti (*C. e. roosevelti*) x red deer calves (Asher *et al.*, 2005b).

486 Mean plasma leptin concentration varied between 1.84 and 2.35 ng/ml HE in the
487 present study, a range similar to that reported previously by researchers using the
488 multi-species leptin RIA kit on cervid plasma (Suzuki *et al.*, 2004; Soppela *et al.*,
489 2008; Gaspar-Lopez *et al.*, 2009). Leptin is secreted primarily by white adipose
490 tissue and a positive relationship has been reported between body fatness and
491 circulating leptin levels in both monogastric (Morgan and Mercer, 2001; Mustonen *et*
492 *al.*, 2005; Klok *et al.*, 2007) and ruminant (Delavaud *et al.*, 2000; Suzuki *et al.*, 2004)
493 species. This was also the case in the present study, suggesting that level of
494 adiposity plays a dominant role in determining the concentration of leptin circulating
495 in red deer. Previous studies (Bocquier *et al.*, 1998; Soppela *et al.*, 2008) have found
496 that leptin secretion is modulated by daily photoperiod in ruminants, independently of

497 food intake, body fatness and gonadal feedback, but no such relationship was found
498 in the present study.

499 Photoperiod is also reported to have an effect on sensitivity of the hypothalamus
500 to leptin in seasonal mammals, which become leptin resistant during 'long days' and
501 leptin sensitive during 'short days' (Rousseau *et al.*, 2003; Adam *et al.*, 2006; Zieba
502 *et al.*, 2007). Such a mechanism enables fat deposition during summer and
503 mobilisation during winter as occurred in the P hinds of the present study. Pregnancy
504 has also been reported to alter sensitivity of the hypothalamus to leptin in rats, with
505 leptin unable to suppress VFI in pregnant rats, as it does in non-pregnant animals
506 (Grattan *et al.*, 2007; Ladyman *et al.*, 2009). Thus, despite elevated plasma leptin
507 concentration, pregnancy in rats is associated with hyperphagia and increased fat
508 mass. There was no evidence of such leptin resistance during pregnancy in the
509 present study; VFI of pregnant hinds did not increase above that of NP hinds.
510 Moreover, BCS decreased in the last third of pregnancy, as did circulating leptin
511 concentration.

512 Pregnancy status had no significant effect on plasma ghrelin concentration and
513 there was no association between food intake and level of circulating ghrelin.
514 However, it is noted that there was a trend for both food intake and ghrelin
515 concentration to be lower in P than NP hinds from June until the end of the study.
516 Ghrelin is thought to play a minor role in modulating long-term seasonal body weight
517 cycles, but acts predominantly as a short-term regulator of feeding by playing a
518 pivotal role in the initiation of feeding. This role has been firmly established in
519 monogastric species (Wren *et al.*, 2000; Tschöp *et al.*, 2000; Nakazato *et al.*, 2001),
520 but is less certain in ruminants. In cows, plasma ghrelin concentration decreased 1 h
521 after feeding before recovering to pre-feeding levels (Hayashida *et al.*, 2001). In

522 sheep, it has been shown to increase immediately prior to, as compared with an hour
523 before, a scheduled meal and then decline rapidly during feeding (Sugino *et al.*,
524 2002). However, changing the feeding pattern modified time of ghrelin increase and
525 the authors considered that the observed increases may have been mediated by a
526 conditioned behavioural response, rather than hunger. In the present study, hinds
527 were allowed *ad libitum* access to food and water, and the blood sampling regimen
528 began at the same time on each occasion (1330 h). Food rations were changed at
529 about the same time each day (0830 – 1030 h) and hinds invariably began eating as
530 soon as they were returned to their pens. Therefore, it is possible that blood
531 sampling took place at the nadir of ghrelin secretion, masking a possible difference
532 between P and NP hinds.

533 There was no significant effect of season on circulating levels of ghrelin. This is in
534 agreement with Harrison *et al.* (2008) who found that mean levels of circulating
535 endogenous ghrelin were not different between LD and SD. However, the possibility
536 that the present samples were obtained at the nadir of daily ghrelin concentrations
537 means that any seasonal effects may have been masked by the time of sampling.

538

539 **Conclusions**

540

541 This study supports the hypothesis that pregnancy status has no significant effect on
542 the photoperiod-mediated depression in VFI of red deer hinds during winter. It is
543 inferred that pregnant hinds are unable to overcome an endogenous cycle of VFI
544 and therefore cannot increase their VFI to meet the energy demands of a rapidly
545 growing fetus in the last third of gestation. Instead, the extra energy required to
546 support the pregnancy is attained through mobilisation of the hinds' body energy

547 reserves, i.e. fat. This indicates the importance of ensuring that pregnant hinds are in
548 good body condition at the start of winter, and that high quality food is available
549 throughout gestation.

550

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757

758 **Table 1** *Mean change in hind live weight and body condition score during specific time*

759 *periods.*

760

Time period	Treatment		s.e.d.	P-value
	Pregnant	Non-pregnant		
Autumn:				
LW (g/day)	75	-27	39.7	0.02
BCS (units/day)	0.014	0.005	0.0038	0.14
Winter:				
LW (g/day)	108	123	35.9	0.68
BCS (units/day)	0.002	0.002	0.0014	1.00
Spring:				
LW (g/day)	230	207	61.1	0.72
BCS (units/day)	-0.001	0.006	0.0019	0.02

761

762
 763 **TABLE 2** *Mean change in hind daily voluntary food intake (Intake change) over specified*
 764 *time periods during the study as calculated from regression analysis of the predicted mean*
 765 *daily voluntary food intake. Data have been normalised around days from calving (Day 0 =*
 766 *day of parturition) to compensate for the wide variation in calving dates. Day 0 for non-*
 767 *pregnant hinds was taken as the mean parturition date of pregnant hinds.*

768

Time period (days before parturition)	Intake change (MJME/kg LW ^{0.75} /day)		s.e.d.	P-value
	Pregnant	Non-pregnant		
	200-150	-0.20		
150 -100	0.00	0.04	0.067	0.56
100-50	0.05	0.11	0.054	0.28
50-20	0.07	-0.00	0.084	0.45
20-5	-0.07	-0.04	0.123	0.79
5-0	-0.24	0.17	0.094	0.001

769

770
771

Table 3 Mean plasma leptin concentration at each sampling date.

Date	Leptin concentraton (ng/ml HE)		s.e.d.	P-value
	Pregnant	Non-pregnant		
23 Apr	1.88	2.31	0.335	0.23
21 May	1.90	2.09	0.274	0.49
17 Jun	1.94	1.84	0.224	0.68
16 Jul	2.07	2.35	0.491	0.59
13 Aug	2.11	2.12	0.372	0.97
10 Sep	2.33	2.27	0.383	0.89
8 Oct	2.09	2.26	0.385	0.66
5 Nov	1.95	2.20	0.390	0.53

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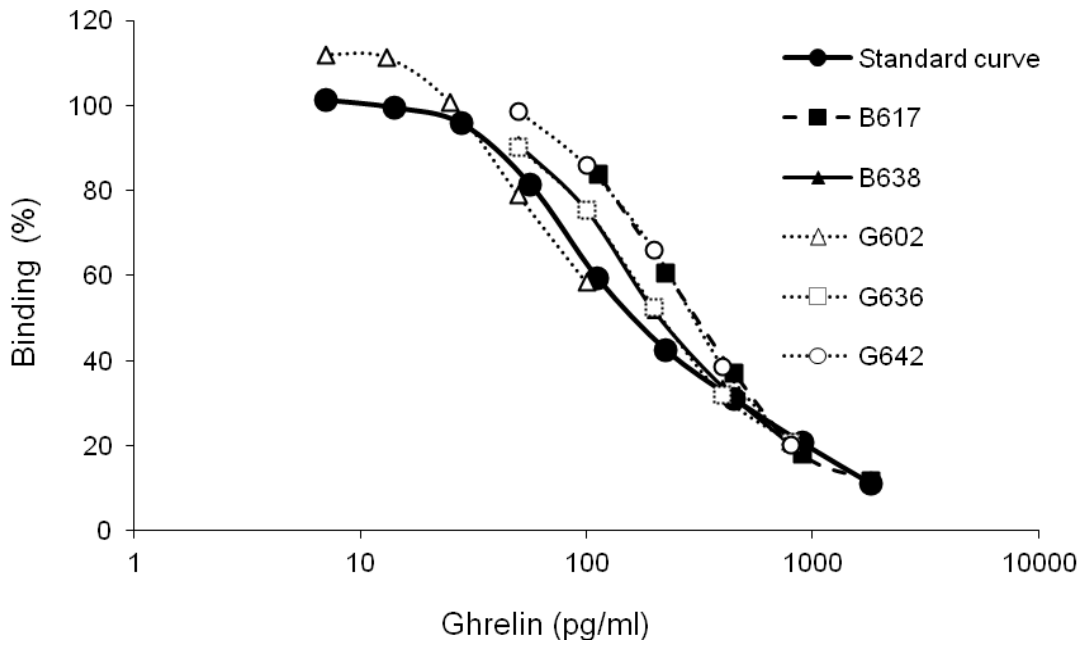
773

774 **Table 4** *Mean plasma ghrelin concentration at each sampling date.*

Date	Ghrelin concentration (pg/ml)		s.e.d.	P-value
	Pregnant	Non-pregnant		
23 Apr	137	123	31.9	0.67
21 May	204	225	40.5	0.61
17 Jun	209	309	49.0	0.08
16 Jul	260	360	87.6	0.29
13 Aug	225	276	47.7	0.31
10 Sep	223	309	46.0	0.09
8 Oct	198	248	38.9	0.22
5 Nov	222	308	84.7	0.34

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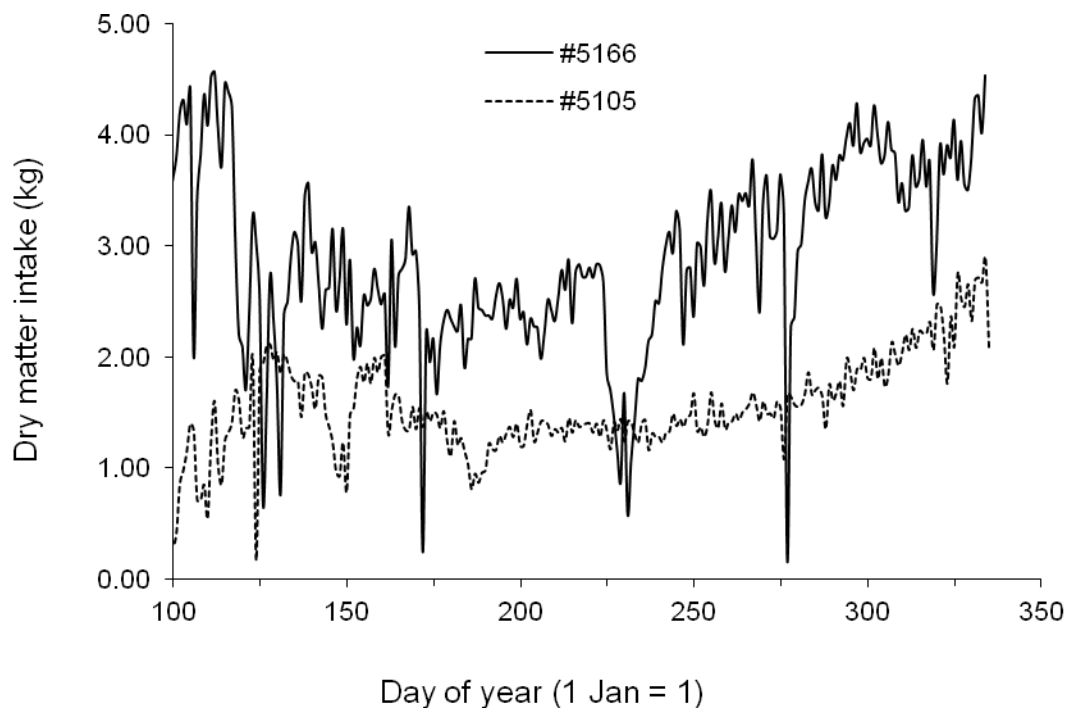


777

778 **Figure 1** Parallelism between percentage binding for serial dilutions of cervine plasma from

779 5 hinds and the standard curve generated from the rat active ghrelin RIA kit.

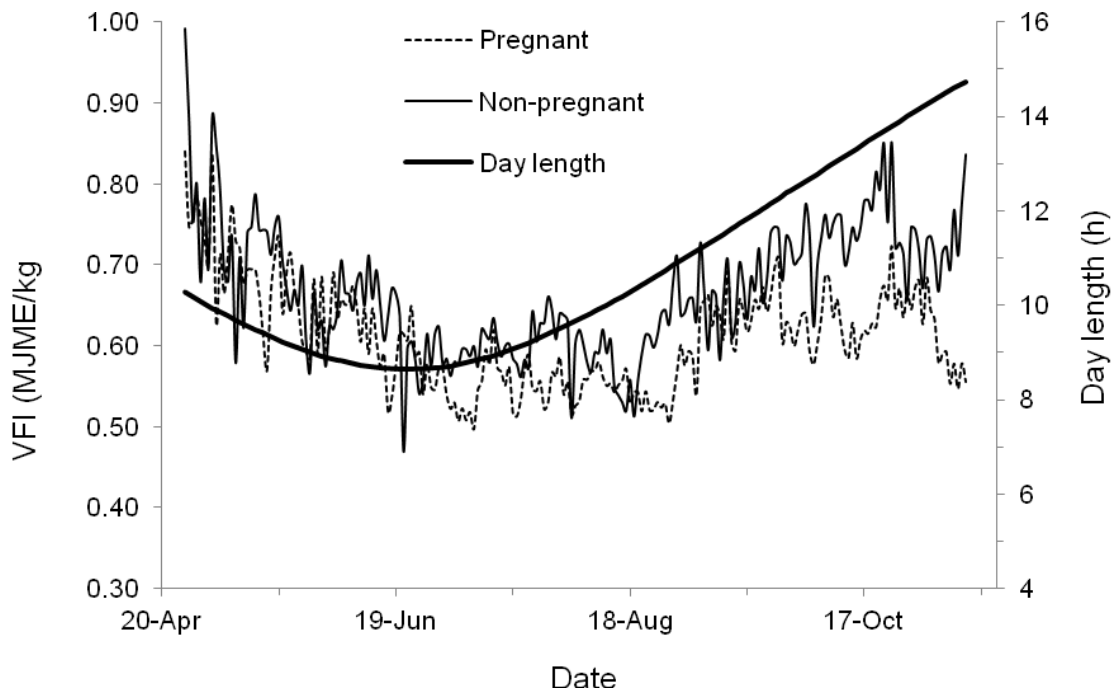
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782

783 **Figure 2** Examples of daily dry matter intake (kg) from two individual hinds demonstrating a
784 high mean intake with large daily variation (#5166) and a low mean intake with little daily
785 variation (#5105).

786



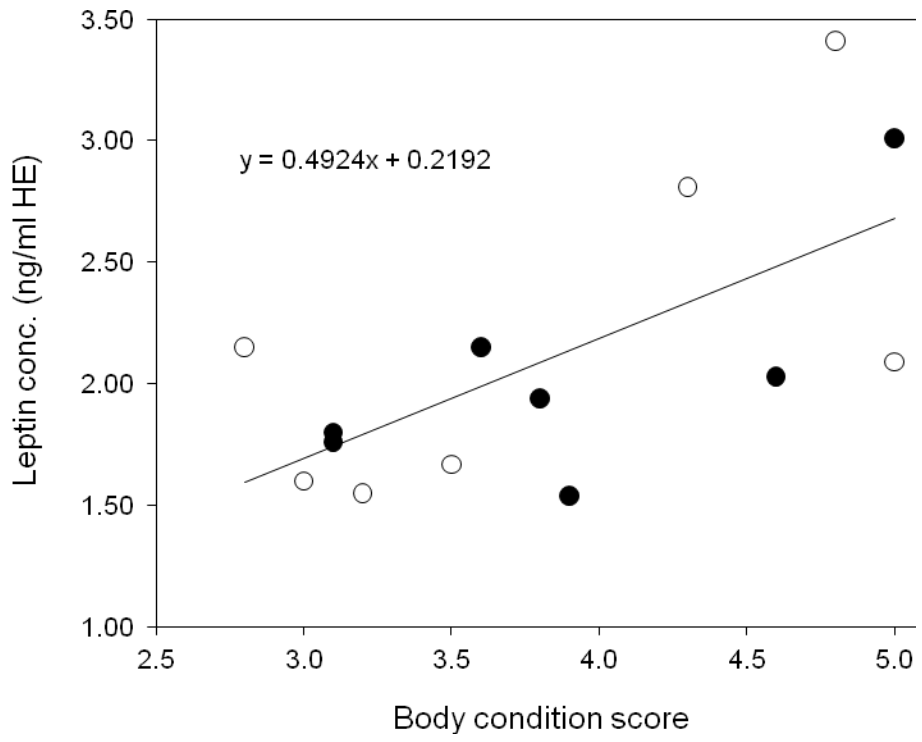
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788 **Figure 3** Predicted mean daily voluntary food intake of pregnant and non-pregnant hinds

789 relative to day length (hours between sunrise and sunset) during indoor feeding.

790

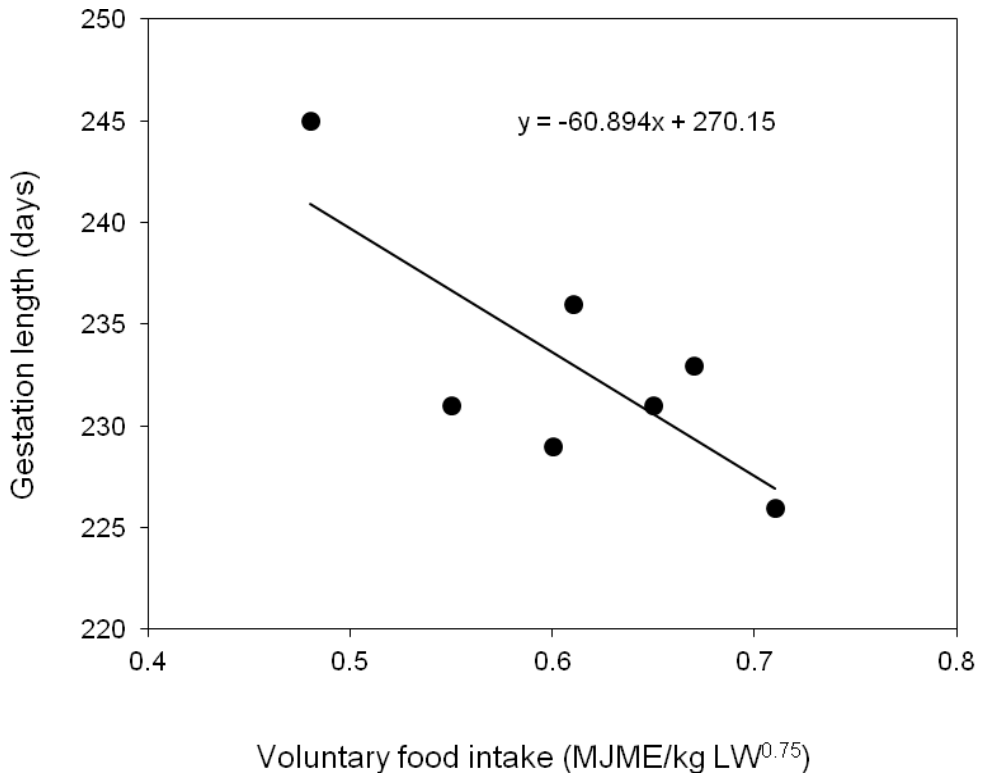
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792

793 **Figure 4** Regression of mean body condition score (BCS, 1 = emaciated, 5 = obese) with
794 mean concentration of circulating leptin (ng/ml HE) of pregnant (solid circles) and non-
795 pregnant (open circles) hinds.

796



797

798 **Figure 5** Regression of mean voluntary food intake (MJME/kg LW^{0.75}) during the study
799 period with gestation length (days).

800