

Accepted Manuscript

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PII: S0739-7240(18)30027-4

DOI: [10.1016/j.domaniend.2018.05.001](https://doi.org/10.1016/j.domaniend.2018.05.001)

Reference: DAE 6311

To appear in: *Domestic Animal Endocrinology*

Received Date: 5 August 2017

Revised Date: 7 April 2018

Accepted Date: 2 May 2018

Please cite this article as: Rodríguez-Sánchez JA, Sanz A, Ferrer J, Casasús I, Influence of post-weaning feeding management of beef heifers on performance and physiological profiles through rearing and first lactation, *Domestic Animal Endocrinology* (2018), doi: 10.1016/j.domaniend.2018.05.001.

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1 **Influence of post-weaning feeding management of beef heifers on performance and**
2 **physiological profiles through rearing and first lactation.**

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10

11 **ABSTRACT**

12 The aim of this study was to examine the effects of two post-weaning feeding
13 management approaches (FEED: 0.8 [HIGH] vs. 0.6 [MOD] kg/d target ADG) on the
14 performance of heifers of two beef breeds (BREED: Parda de Montaña [PA] vs. Pirenaica
15 [PI]) calving at 2 yr. Twenty-five heifers previously creep-fed before weaning (6 mo) were
16 assigned to two planes of nutrition from 6 to 15 mo of age. At 15 mo they were inseminated,
17 and then received similar diets until weaning of their first calf (4 mo post-calving). Several
18 parameters were measured to analyze growth and development (BW; ADG; size measures at
19 6 mo, 15 mo, calving and weaning), performance at puberty and first breeding, and dam and
20 calf performance in the first lactation (calving traits, ADG, milk yield). Metabolic (glucose,
21 cholesterol, NEFA, β -hydroxybutyrate and urea) and endocrine status (IGF-I and leptin) were
22 assessed in plasma samples collected every 3 mo from 6 mo to calving and monthly during
23 lactation. No interaction between BREED and FEED was observed. Heifers from the HIGH
24 feeding treatment had higher post-weaning ADG than those on the LOW diet. At 15 mo they
25 had greater BW, heart girth and external pelvic area, but they did not differ thereafter. All

26 heifers reached puberty at similar BW (55% mature BW) but different age. Heifers from the
27 HIGH treatment tended ($P < 0.09$) to be pubertal earlier, and PA heifers were 1.6 mo younger
28 than PI heifers ($P < 0.05$) at puberty. At the time of conception (452 ± 59 kg) and calving
29 (471 ± 51 kg) BW was above common recommendations in all groups. Calving traits and
30 performance in lactation did not differ between feeding treatments. BREED only influenced
31 birth weight; PA calves being heavier ($P < 0.05$) which resulted in a larger calf/cow BW
32 ratio, but no effect on calving difficulty or subsequent performance. Metabolic substrates and
33 hormones depended mostly on sampling date, which was related to current energy and
34 protein intake. Glucose ($P < 0.001$), cholesterol ($P < 0.001$) and IGF-I ($P < 0.05$) were greater
35 during the post-weaning phase in heifers on the HIGH diet, and persistent physiological
36 effects were observed during lactation. Age at puberty was negatively related with IGF-I ($r =$
37 -0.43 , $P < 0.001$), but not with leptin concentrations. In conclusion, regardless of breed, a
38 moderate growth rate ensured adequate heifer development and performance until the first
39 lactation, whereas no advantage was gained from enhanced post-weaning gains.

40
41 **Keywords:** beef breeds; lactation performance; metabolic and hormone profiles; pre-
42 breeding feeding strategy; replacement heifer growth

44 1. Introduction

45 To decrease the cost of rearing beef heifers, it is recommended to advance the first
46 calving to 2 yr, for which they should be bred at 15 mo and be pubertal at least 6 wk before
47 [1]. Age at puberty depends on the diets applied from 4 to 6 mo [2] and after weaning [3]. To
48 achieve this target, post-weaning growth should guarantee that heifers reach 65% of mature
49 BW at breeding [4], although other studies proposed to reduce this threshold to 50-55% [5,6].
50 Moreover, the heifers should reach 80% of mature BW [7] at first calving, with adequate

51 skeletal development. Recently, Rodríguez-Sánchez, et al. [8] indicated that beef heifers
52 calving at 2 yr should gain at least 1 kg/d either before or after weaning to prevent impaired
53 performance at calving. In mountain areas, where cow-calf pairs are housed and calves are
54 creep-fed [9], it is safer to ensure this gain before than after weaning, when heifers are turned
55 out to pasture.

56 The effects of accelerated gains of dairy heifers on adult performance have been given
57 wide attention in literature [10], but the optimal growth rates for beef cattle have yet to be
58 defined. Overnutrition during heifer development has been associated with decreased milk
59 production [11], because it hastens puberty and reduces the duration of the first allometric
60 phase of development of the mammary gland [12]. This effect has been widely studied in
61 dairy cows [13], but it is also of major interest in beef cows, since milk yield is one of the
62 main factors that determine calf weaning weight.

63 Feeding after weaning affects the metabolic and endocrine profiles, and consequently
64 development and reproductive performance [14]. This long-term effect of nutritional
65 interventions in the young calf on physiological outcomes later in life is known as metabolic
66 imprinting [15]. Moreover, since breeds can differ in growth patterns and age at puberty,
67 management should be tailored to avoid under- or over-feeding [16].

68 Parda de Montaña (PA) and Pirenaica (PI) are two beef breeds widely spread in the
69 mountain areas of Spanish Pyrenees. The first comes from the old Brown Swiss selected for
70 beef production, while the second is an autochthonous hardy breed used for beef production.
71 Although their mature BW is similar [17], other production traits differ. Parda de Montaña
72 calves are heavier at birth and, due to the greater cow milk yield [18], when not supplemented
73 they are heavier at weaning than PI calves [19]. Thereafter, PA is an intermediate-maturing
74 breed and PI a late-maturing one [20].

75 We hypothesized that accelerated gains from weaning to breeding would improve the

76 performance of beef heifers at first breeding and in their first lactation. Our second
77 hypothesis was that both breeds could be raised under the same heifer feeding program
78 designed to ensure a timely match of their requirements.

79 This study aimed to assess the effects of two feeding managements after weaning
80 designed to promote different gains (0.8 vs. 0.6 kg/d) from 6 to 15 mo of age in two breeds of
81 beef heifers (PA vs. PI) on performance, until weaning of their first calf. Heifer development,
82 performance and metabolic (glucose, cholesterol, NEFA, β -hydroxybutyrate, urea) and
83 endocrine status (IGF-I and leptin) were analyzed through their rearing phase, pregnancy and
84 lactation.

85

86 **2. Material and methods**

87 The Animal Ethics Committee of the Centro de Investigación y Tecnología
88 Agroalimentaria (CITA) approved the experimental procedures, which were in compliance
89 with the guidelines of the European Union [21] on the protection of animals used for
90 experimental and other scientific purposes.

91

92 *2.1. Animals, management and diets*

93 This study was conducted at CITA-Montañana Research Station (post-weaning phase,
94 41°43' N, 0°48' W, 225 m above sea level, mean annual temperature 15.2 ± 0.2 °C, and mean
95 annual rainfall 318 ± 63 mm) and CITA-La Garcipollera Research Station, in the mountain
96 area of central Pyrenees (gestation and lactation, 42°37' N, 0°30' W, 945 m above sea level,
97 mean annual temperature 10.2 ± 0.2 °C, and mean annual rainfall 1059 ± 68 mm).

98 Twenty-five 6-mo-old heifers, born from an autumn-calving herd (October 11 ± 10 d)
99 of 65 adult cows of PA and PI breeds, were distributed in a 2 by 2 factorial arrangement: 2
100 breeds (BREED: PA vs. PI) by 2 feeding treatments to promote different growth rates

101 (FEED: 0.8 kg/d [HIGH] and 0.6 kg/d [MOD] treatments, respectively) during the post-
102 weaning phase (from weaning at 6 mo to breeding at 15 mo). This resulted in four
103 experimental groups: PA-HIGH (7 heifers), PA-MOD (6), PI-HIGH (6) and PI-MOD (6)
104 (Fig. 1). The experiment started when the calves were weaned at 6.4 ± 0.3 mo and 238 ± 41
105 kg BW. Treatments were randomly balanced according to calf BW and age. During the
106 previous lactation phase, they were fed on their dam's milk (suckling twice daily for 30 min)
107 and had free access to a starter concentrate (Table 1), resulting in pre-weaning gains of $1.04 \pm$
108 0.18 kg/d.

109 During the post-weaning phase, heifers were maintained indoors in a loose housing
110 system in straw-bedded pens. All pens assigned to heifers of each experimental group in this
111 phase were in the same barn, similar in size and environmental conditions. Fresh and clean
112 water and vitamin-mineral supplements (lick blocks) were supplied ad libitum. To achieve
113 the targeted weight gains, heifers were group-fed alfalfa hay ad libitum and 10 g (HIGH) or 4
114 g concentrate/kg BW (MOD) (44% corn, 22% barley, 15% corn gluten, 5% rapeseed flour,
115 5% soybean flour, 3% beet pulp, 3% palm oil, 3% vitamin-mineral supplements; Table 1).
116 Concentrate was provided daily at 0800 and heifers were tied up for a maximum 1 h until
117 they finished the restricted amount assigned to each one. Alfalfa hay was provided ad libitum
118 in metal feeding troughs, refilled twice daily and were long enough for all heifers in the pen
119 to eat at the same time and avoid competition.

120 When heifers reached 15 mo of age a 60-d breeding season began, during which they
121 were managed as a single group. In this phase they were fed 9 kg/head/d of a dry total mixed
122 ration (46% barley straw, 12% alfalfa hay, 18% barley, 8% sugarcane molasses, 6% soybean
123 meal, 4% cereal by-products, 4% rapeseed, 2% sunflower seed; Table 1). All heifers were
124 synchronized with an Ovsynch + progesterone releasing intravaginal device (PRID) program,
125 in which they simultaneously received 1.55 mg of progesterone in a PRID (CEVA,

126 Barcelona, Spain) and a 10 µg injection of GnRH (Busol; INVESA, Barcelona, Spain)
127 followed 10 d later by 25 mg of prostaglandin F_{2α} (Enzaprost; CEVA). The PRID was
128 removed 12 d later, and 500 IU of pregnant mare serum gonadotrophin (Foligon; Intervet,
129 Salamanca, Spain) were administered followed 48 h later by a second injection of GnRH (10
130 µg). Eight hours after the final GnRH injection, heifers were inseminated by an expert
131 technician. Three different sires for each breed were selected for their calving ease, and
132 semen from each of the 3 bulls was equally distributed in the feeding treatments per breed.

133 Heifers were checked twice daily (0700 and 1900 h) from the first AI to the end of the
134 breeding season for detecting estrus of non-pregnant heifers. They were inseminated
135 approximately 12 h after estrus detection. Return to estrus after each AI was considered as a
136 diagnostic indicator of non-pregnancy status. Pregnancy was confirmed by ultrasonography
137 (Aloka SSD-500V; equipped with a linear-array 7.5 MHz transducer; Aloka, Madrid, Spain)
138 31 d after the end of the breeding season.

139 The day of the first timed AI was used to determine age and BW at first breeding. First-
140 service fertility rate was determined as number of pregnant heifers at the first AI divided by
141 total number of heifers, and overall fertility rate was determined as the number of pregnant
142 heifers in the breeding season divided by the total number of heifers.

143 Two heifers failed to get pregnant and were removed from the experiment at the end of
144 the breeding season, which resulted in the following composition of the experimental groups
145 thereafter: PA-HIGH (6 heifers), PA-MOD (6), PI-HIGH (6) and PI-MOD (5).

146 From the confirmation of pregnancy until a month before the expected calving date for
147 each heifer, they grazed on mountain meadows (4 heifers/hectare) following the traditional
148 management system [17]. These pastures were composed primarily of grasses (*Festuca*
149 *arundinacea*, *Festuca pratensis* and *Dactylis glomerata*), legumes (*Trifolium repens*) and
150 other species (1191 kg DM/ha, Table 1). In the last month of gestation, heifers were housed

151 and fed 9 kg/animal/d of meadow hay (Table 1).

152 After calving, primiparous cows reared their calves for 4 mo. During their first
153 lactation, dams received 10 kg/animal/d of the same dry total mixed ration provided during
154 the breeding season. The diet was calculated to meet the requirements for energy and protein
155 of maintenance, growth and milk production of a cow of 490 kg BW and 6 kg daily milk
156 yield. Calves of primiparous cows had free access to suckle their dams and received no other
157 feed during the lactation period. All pens assigned to animals of each feeding treatment for
158 each phase throughout the experiment were in the same barn, similar in size and
159 environmental conditions. Water and vitamin-mineral supplements (lick blocks) were
160 supplied for ad libitum intake throughout the experiment.

161

162 *2.2. Measurements and blood sampling*

163 During the post-weaning phase, concentrate intake was recorded daily by group and
164 monthly adjusted by average group weight. Intake of alfalfa hay was recorded by pen at
165 weekly intervals. Actual daily intake in the indoor phases was calculated as feed provided
166 minus feed refused, and during the grazing season it was estimated on a monthly basis
167 considering that pasture intake had met the requirements of the heifers according to their BW,
168 ADG and month of gestation.

169 Feed samples were collected at weekly intervals and were pooled on a monthly basis
170 for chemical analyses. Samples were dried at 60 °C until constant weight and mill-ground (1
171 mm screen) and DM, ash, ether extract and CP ($N \times 6.25$) contents were determined
172 according to the Association of Official Analytical Chemists [22] (Methods 942.05, 920.39,
173 968.06). Analyses of NDF, ADF and LDF were conducted according to the sequential
174 procedure of van Soest, et al. [23]. All values were corrected for ash-free content.

175 Heifers were weighed once a week before morning feeding, without prior deprivation

176 of feed and water. Weight at key points (6, 9, 12, 15 mo, puberty onset, first breeding, calving
177 and weaning) was calculated as the average of three consecutive weights. Daily weight gains
178 (post-weaning, weaning to puberty, gestation and lactation phases) were calculated by linear
179 regression of weight against time. Calves were weighed at calving and thereafter weekly until
180 weaning at 4 mo of age to determine their ADG during lactation. At 15 mo, calving and
181 weaning, BCS was assessed by two expert technicians on a 0 to 5 scale, based on the
182 estimation of fat covering loin, ribs and tailhead.

183 Body development was studied using size measurements at 6 and 15 mo, calving and
184 weaning. Height at withers (from the highest point of the shoulder blade to the ground), rump
185 width (maximum distance between iliac tuberosities) and rump length (from the ischial
186 tuberosity to the iliac tuberosity) were recorded with a height stick. External pelvic area was
187 estimated as product of rump width and rump length. Heart girth (body circumference
188 immediately posterior to front legs) was measured with a flexible tape.

189 Calving ease was classified into two categories, i.e., assisted or unassisted. Assisted
190 calving included all types of assistance, from manual pull to a caesarean section as described
191 by Johanson and Berger [24]. Ratio of calf/cow BW was estimated to determine fetal-
192 maternal disproportion, as calf birth weight divided by cow weight at calving expressed as a
193 percentage [24].

194 Primiparous dams were milked monthly during the 4 mo of lactation, using the
195 oxytocin and machine milking technique 6 h after calf removal [25], to determine quantity
196 and composition of the milk produced daily. Milk fat and protein contents were analyzed
197 with an infrared scan (Milkoscan 4000TM; Fosselectric Ltd., Hillerod, Denmark). Energy-
198 corrected milk (ECM) yield (adjusted to 3.5% fat and 3.2% protein) was calculated as
199 described in Casasús, et al. [26].

200 Blood samples were collected weekly to determine the onset of puberty based on

201 plasma progesterone concentration. Additionally, blood samples were collected every 3 mo
202 during the post-weaning phase and gestation and monthly during lactation to determine
203 concentrations of both metabolites and hormones. Blood samples were collected before
204 morning feeding from the coccygeal vein. Samples to determine progesterone, β -
205 hydroxybutyrate, IGF-I and leptin concentrations were collected into 9 mL heparinized tubes
206 (Vacuette España S.A., Madrid, Spain). Samples to determine plasma glucose, cholesterol,
207 NEFA and urea concentrations were collected into 9 mL tubes containing EDTA (Vacuette
208 España S.A.). Blood samples were centrifuged at $1,500 \times g$ for 20 min at 4°C immediately
209 after collection, and the plasma was harvested and frozen at -20°C until analysis.

210 All measurements and samples taken at 6 mo were conducted before post-weaning diets
211 were applied.

212

213 2.3. Assays

214 The concentrations of progesterone in plasma samples were measured using an ELISA
215 kit specific for cattle (Ridgeway Science, Lydney, UK), following the manufacturer's
216 instructions. The onset of puberty was considered to occur when progesterone levels were \geq
217 1.0 ng/mL in at least 2 consecutive samples (normal estrus cycle, $\geq 14\text{ d}$; [27]). Age at
218 puberty was defined as date of collection of the first blood sample that contained $\geq 1.0\text{ ng/mL}$
219 of plasma progesterone. To ensure the continuation of estrous cycles, blood samples analyzed
220 after the attainment of puberty were confirmed by observation of at least 1 subsequent estrous
221 cycle of normal duration, based on progesterone concentration.

222 Plasma concentrations of glucose (glucose oxidase/peroxidase method), cholesterol
223 (enzymatic-colorimetric method), β -hydroxybutyrate (enzymatic-colorimetric method) and
224 urea (kinetic UV test) were determined with an automatic analyzer (GernonStar,
225 RAL/TRANSASIA, Dabhel, India). Protocols and reagents for glucose, cholesterol and urea

226 analyses were provided by the analyzer manufacturer (RAL, Barcelona, Spain), and reagents
227 for β -hydroxybutyrate were supplied by Randox Laboratories Ltd. (Crumlin Co., Antrim,
228 UK). Samples were run in duplicate.

229 Mean intra- and inter-assay CV for these metabolites were <5.4% and <5.8%,
230 respectively. Sensitivity was 0.056, 0.026, 0.030, 0.170 mmol/L for glucose, cholesterol, β -
231 hydroxybutyrate and urea, respectively. Plasma concentrations of NEFA were analyzed with
232 an enzymatic method using a commercial kit (Randox Laboratories Ltd.). Commercial
233 reference plasma samples (bovine precision serum; Randox Laboratories Ltd.) were used to
234 evaluate the accuracy of the analyses. Mean intra- and inter-assay CV were 5.1% and 7.4%,
235 respectively. Sensitivity was 0.060 mmol/L.

236 Circulating IGF-I concentrations were quantified with a solid-phase enzyme-labeled
237 chemiluminescent immunometric assay (Immulite; Siemens Medical Solutions Diagnostics
238 Limited, Llanberis, Gwynedd, UK). Mean intra- and interassay CV were 3.1 and 12.0%,
239 respectively. Sensitivity was 20 ng/mL.

240 Plasma leptin concentrations were determined by RIA with a multispecies commercial
241 kit (Multispecies Leptin Ria kit; LINCO Research, St. Charles, MO). Mean intra- and
242 interassay CV were 3.54 and 6.87%, respectively. Sensitivity averaged 1.30 ng/mL.

243 244 *2.4. Statistical analyses*

245 All data were analyzed as a completely randomized design with the SAS statistical
246 software package (SAS Institute Inc., Cary, NC, USA). Heifer was considered the
247 experimental unit. Data for BW, ADG, BCS, size measures (height at withers, heart girth and
248 external pelvic area), ECM yield and milk quality, metabolic (glucose, cholesterol, NEFA, β -
249 hydroxybutyrate and urea) and endocrine (IGF-I and leptin) profiles were analyzed using the
250 SAS MIXED procedure for repeated measures. Covariance structure was selected on the

251 basis of the lowest Akaike information criterion. Therefore, an unstructured covariance
252 matrix (UN) was used for analysis of repeated measures, which included BREED, FEED,
253 sampling date and their interaction as fixed effects and with heifer as the random effect in a
254 univariate linear mixed model.

255 Age and weight at puberty and at the first AI were tested with ANOVA using the GLM
256 procedure, where BREED, FEED and their interaction were fixed effects. Fertility rate at first
257 AI and at the end of the breeding period were analyzed using the GLIMMIX procedure,
258 considering BREED and FEED as fixed effects and AI sire as the random effect, with a logit
259 link and a binomial distribution.

260 Calf performance (BW at birth and weaning, and ADG) and calf/cow BW ratio were
261 tested with ANOVA using the GLM procedure, with the fixed effects of BREED, FEED, sire
262 used for AI, calf sex and their interaction. Calf sex effect was analyzed using the FREQ
263 procedure (χ^2 test). Calving assistance was analyzed using the GLIMMIX procedure,
264 considering BREED, FEED and calf sex as fixed effects and AI sire as the random effect,
265 with a logit link and a binomial distribution. The model analyzed also the effects of the length
266 of gestation, calving date and calf/cow BW ratio.

267 Pearson correlation coefficients between all variables at a given time point, and
268 between all time points for a given metabolite or hormone, were calculated using the CORR
269 procedure. Where not stated, correlations were not significant. Means were separated using
270 the LSMEANS procedure. For all tests, level of significance was $P < 0.05$ and tendencies
271 were determined if $P \geq 0.05$ and $P < 0.10$.

272 Total energy and protein intake were not tested statistically as the data recorded to
273 calculate them were registered on a group basis, and hence only absolute data are presented.

274

275 **3. Results**

276 3.1. Growth performance

277 The interaction between BREED and FEED was not significant ($P > 0.10$) at any date
278 and for any growth trait assessed; therefore, the main effects are presented separately.

279 Heifer weight at keypoints, ADG and BCS during the post-weaning phase, gestation
280 and lactation are displayed in Table 2. At the start of the study BW did not differ between
281 breeds, and since ADG were similar thereafter, they had similar weight at 15 mo, calving and
282 weaning. The BCS at 15 mo and calving were also similar between breeds, but during
283 lactation PA cows showed a slight loss of BCS whereas PI primiparous maintained it, and
284 therefore BCS at weaning was greater in PI than in PA cows ($P < 0.01$).

285 Gains during the post-weaning phase differed between both FEED treatments ($P <$
286 0.001). Consequently heifers from the HIGH treatment were heavier than those from the
287 MOD treatment at 15 mo ($P < 0.05$), and BCS also tended to be greater ($P = 0.06$). This
288 difference was compensated for during gestation, and thereafter weight and BCS at calving
289 and weaning did not differ between feeding strategies, and neither did ADG during lactation.

290 Size measurements are shown in Table 3. Heifers of both breeds had similar height at
291 withers and heart girth throughout the experiment, both being strongly and positively
292 correlated with BW ($r = 0.88$ and 0.90 , respectively, $P < 0.001$). The external pelvic area
293 tended to be greater in PA heifers at 6 mo ($P = 0.05$) and was significantly greater at 15 mo
294 ($P < 0.01$), but it did not differ at calving. This trait was also correlated with BW ($r = 0.90$, P
295 < 0.001).

296 No differences were observed between feeding strategies at 6 mo, but at 15 mo height
297 at withers tended to be greater in heifers from the HIGH feeding treatment ($P = 0.09$), and
298 they had greater heart girth ($P < 0.01$), which corresponded with their greater BW at this
299 point. They also had a larger external pelvic area ($P < 0.05$) at this point, but throughout
300 gestation all these differences were offset and values were similar at calving and at weaning.

301

302 *3.2. Productive performance*

303 Heifer performance at puberty and first breeding was not influenced by the interaction
304 between BREED and FEED and therefore results of the main effects are presented in Table 4.
305 All heifers were pubertal before the breeding season, reaching puberty at similar BW ($322 \pm$
306 38 kg), which was 55.5% of expected mature BW (580 kg for both breeds; [17]).

307 Age at puberty was different between breeds, PA heifers attaining puberty earlier than
308 PI heifers ($P < 0.05$). Heifers from the HIGH feeding treatment tended to be pubertal 1 mo
309 earlier ($P = 0.09$) than those from the MOD treatment. Consistent with this trend, a strong
310 negative correlation between age at puberty and weaning-to-puberty ADG of heifers ($r =$
311 -0.70 , $P < 0.001$) was observed. All heifers were pubertal at least 2 mo before the first AI,
312 except for one PI-MOD heifer who was pubertal only 1 mo before.

313 As shown in Table 4, BW at the first AI did not differ between breeds, despite PI
314 heifers being 35 kg lighter, most likely because of the low BW reached by PI-MOD heifers
315 (389 ± 51 kg). As expected, BW at the first AI was affected by FEED ($P < 0.05$), heifers
316 from HIGH feeding treatment being 53 kg heavier. Despite this difference, BW in all
317 experimental groups was greater than 65% of the expected mature BW (381 kg). Fertility rate
318 at the first AI and at the end of the breeding season were similar among treatments, and were
319 not influenced by AI sire.

320 Dam and calf traits in the first lactation are presented in Table 5 according to BREED
321 and FEED, since they were not affected by the interaction between these main effects. Age at
322 first calving was similar in all treatments (25.9 ± 0.9 mo). Assistance at calving was needed
323 in 36% of the primiparous cows, and mostly consisted of the use of a calving jack, caesarean
324 section not being needed in any case. The disproportion between cow and calf BW, i.e. the
325 calf/cow BW ratio, was greater in PA than in PI cows (8.1% vs. 7.1% respectively, $P < 0.05$).

326 However, differences among treatments in incidence of calving assistance related to BREED,
327 FEED, sire used for AI, gestation length, calving date, calf sex or calf/cow BW ratio were not
328 significant ($P > 0.10$).

329 The calf sex ratio was similar among treatments ($P > 0.10$), and no differences were
330 observed between sexes in calf weight at birth (36.7 vs. 33.2 kg BW in male and female
331 calves respectively, SEM = 2.33, $P > 0.10$) or at weaning (112.2 vs. 117.9 kg BW, SEM =
332 7.31, $P > 0.10$), nor in the calf ADG during lactation (0.629 vs. 0.710 kg ADG, SEM = 0.083,
333 $P > 0.10$), neither among sires (data not shown). No other dam productive or reproductive
334 trait analyzed herein was influenced by calf sex. As shown in Table 5, PA calves were 5 kg
335 heavier at birth than PI calves ($P < 0.01$). Thereafter, calf performance was unaffected by
336 BREED or FEED treatments, showing similar gains during the 120 d of lactation ($0.669 \pm$
337 0.104 kg/d), which resulted in a similar weight at weaning (116.1 ± 12.9 kg).

338 The average milk yield of primiparous cows during lactation (Table 6) was affected by
339 the interaction between BREED and FEED ($P < 0.001$), and so was the ECM, which was
340 stable during the first three months of lactation and decreased in the last month (Fig. 2). The
341 overall milk production was unexpectedly low (5.05 vs. 5.11 kg/d in PA and PI respectively,
342 $P > 0.10$), especially in PA cows. Milk fat and protein contents were lower in PA than in PI
343 cows (3.49 vs. 4.31% fat, respectively, $P < 0.001$; 3.39 vs. 3.52% protein, respectively, $P <$
344 0.05). Finally, a FEED effect on the milk protein was observed, this content being greater in
345 HIGH than in MOD treatments (3.53 vs. 3.38%, respectively, $P < 0.05$).

346

347 3.3. Metabolic profiles

348 Estimated energy and protein intake depending on the breed and feeding management
349 are presented in Fig. 3. The profiles of different metabolites are shown in Fig. 4 according to
350 BREED and FEED, since there was no interaction between both effects, but all of them were

351 affected by sampling date ($P < 0.001$). For all metabolites, the correlations between
352 concentrations observed at 6 mo, 15 mo, calving and weaning were not significant ($P < 0.10$).

353 The greatest concentrations of glucose were observed at 6 mo (6.25 ± 0.10 mmol/L)
354 and thereafter they decreased throughout gestation to a nadir observed one week before
355 calving (3.49 ± 0.06 mmol/L). Glucose concentrations did not differ between breeds ($P >$
356 0.10). Mean values were greater in heifers from HIGH feeding management than in the MOD
357 group (4.37 vs. 4.18 mmol/L, respectively, $P < 0.001$). Glucose was positively correlated
358 with ADG and BCS ($r = 0.79$ and 0.69 , respectively, $P < 0.001$) throughout the study.

359 Regarding cholesterol, the greatest values were also observed at 6 mo (4.50 ± 0.14
360 mmol/L) and its nadir was found prior to calving (2.43 ± 0.10 mmol/L). Plasma cholesterol
361 values were lower in PA than in PI heifers (2.91 vs. 3.23 mmol/L, respectively, $P < 0.05$).
362 Besides, an interaction was observed between sampling date and FEED ($P < 0.001$). During
363 the post-weaning phase cholesterol values were greater in the HIGH feeding management,
364 they did not differ during gestation, and during lactation the highest values were observed in
365 dams from the MOD treatment. Moreover, cholesterol and glucose levels were strongly
366 correlated ($r = 0.51$, $P < 0.001$). During lactation, cholesterol concentration was positively
367 associated to milk yield of dams ($r = 0.38$, $P < 0.001$).

368 Plasma NEFA concentrations were affected by BREED (0.20 vs. 0.24 mmol/L in PA
369 and PI, respectively, $P < 0.05$), but not by FEED. They increased as gestation progressed to
370 the greatest values observed at calving (0.45 ± 0.08 mmol/L). They then showed a marked
371 drop during lactation to a nadir at weaning (0.04 ± 0.01 mmol/L), being correlated with cow
372 milk yield in the first month of lactation ($r = 0.55$, $P < 0.01$).

373 Concerning β -hydroxybutyrate, the influence of FEED depended on sampling date ($P <$
374 0.001), with greater values in the HIGH feeding treatment only during the post-weaning
375 period. In this phase, the concentrations of β -hydroxybutyrate and NEFA were negatively

376 correlated ($r = -0.51$, $P < 0.01$). Thereafter they did not differ across BREED or FEED with
377 advancing gestation and lactation.

378 Plasma urea concentration increased throughout the post-weaning phase to its greatest
379 value at 15 mo (6.5 ± 0.14 mmol/L), decreased during pregnancy to a nadir at the start of
380 lactation (3.8 ± 0.16 mmol/L) and increased again thereafter, regardless of BREED or FEED.
381 Urea concentration at 6 mo was positively related to cholesterol concentration ($r = 0.47$, $P <$
382 0.05) and negatively to age at puberty of heifers ($r = -0.58$, $P < 0.01$).

383

384 *3.4. Endocrine profiles*

385 Profiles of plasma IGF-I and leptin are presented in Fig. 5 according to BREED and
386 FEED. The concentration of both hormones was affected by sampling date ($P < 0.001$).

387 The greatest level of IGF-I was observed at the start of study (283 ± 13.1 ng/mL), and
388 individual values were associated with the pre-weaning weight gains ($r = 0.40$, $P < 0.01$).
389 Thereafter, level of IGF-I remained steady during the post-weaning period, decreased through
390 gestation to a nadir at calving (53 ± 6.9 ng/mL), and then they increased as lactation
391 progressed. Values of IGF-I did not differ between breeds but they were affected by FEED,
392 with greater values observed in the HIGH than in the MOD feeding treatment (145 vs. 120
393 ng/mL, respectively, $P < 0.05$). Heifers with higher concentrations of IGF-I had greater
394 weight gains ($r = 0.83$) and BCS ($r = 0.74$) throughout the experiment and attained puberty
395 earlier ($r = 0.43$) ($P < 0.001$ in all cases). Positive relationships were also found between IGF-
396 I and glucose ($r = 0.66$), cholesterol ($r = 0.29$) and urea ($r = 0.30$) levels during the trial, and a
397 negative relation with concentrations of NEFA ($r = -0.21$) ($P < 0.001$). At the individual
398 level, IGF-I concentrations were related across sampling times, since correlations were
399 observed between samples obtained at 6 and 15 mo ($r = 0.54$, $P < 0.001$) and up to weaning
400 of the first calf ($r = 0.63$, $P < 0.01$).

401 Circulating leptin increased through the post-weaning phase, and it was fairly stable
402 throughout the first gestation and lactation. Plasma leptin was unaffected by BREED or
403 FEED and it was not related with any other metabolite or performance trait. Further, no
404 relationship was observed among different sampling times.

405

406 **4. Discussion**

407 *4.1. Growth performance*

408 The target ADG during the post-weaning phase was achieved in both FEED treatments,
409 and consequently heifers from the HIGH treatment grew faster and at 15 mo they were
410 heavier and tended to be fatter. The similar weight gains observed in heifers of both breeds
411 throughout the study agree with previous results described in works both with heifers and
412 growing bulls [20], likely due to their similar intake capacity [26] and feed conversion
413 efficiency [28]. Considering their similar mature BW (580 kg [17]), at the time of conception
414 all heifers exceeded the minimum recommended BW to avoid future detriment to dam
415 performance, i.e. either 65% of mature BW [4] or the more restrictive 50-55%
416 recommendation [5,6]. After gestation on a common diet, heifer BW and BCS at calving
417 were similar in all treatments and in accordance with those described in PA heifers having
418 their first calving at 2 yr [8], but lighter than those of 2.5 yr-old PA primiparous [17].
419 Although the proportion of mature BW heifers at conception may have suggested excessive
420 conditioning, their weight at calving was 81% of the expected mature BW in these genotypes,
421 which roughly matched the 80% recommended by the NRC [7] for primiparous beef cows.
422 Throughout lactation all dams maintained their weight, but PA lost BCS whereas PI
423 maintained it. This would confirm the lower ability of lactating PA cattle to maintain BCS
424 compared to PI cows, observed both under grazing [17] or confinement conditions [29]. This
425 fact may be due to a different pattern of energy allocation, so that PA cows direct the energy

426 mainly for milk production whereas PI cows prioritize the accumulation or maintenance of
427 body reserves [18].

428 Linear body measures are frequently used to complement weight as indicators of
429 growth. Both breeds showed similar height at withers and heart girth throughout the
430 experiment, indicating a similar skeletal development. However, both traits were significantly
431 greater at 15 mo in heifers from the HIGH than those from the MOD treatment, which
432 confirms their different growth pattern. Height at withers at calving was 97% of that
433 described by Álvarez-Rodríguez, et al. [30] (131 cm) for mature dry cows of the same breeds,
434 and therefore skeletal development at first calving could be considered as adequate for all
435 groups. External pelvic area at 15 mo was smaller in PI heifers, which might reflect a later
436 pelvic development, and in those of the MOD group, but these differences were offset during
437 gestation and had no further effects.

438

439 *4.2. Productive performance*

440 All the heifers attained puberty at a similar BW, which confirms that puberty is reached
441 at a critical BW around 55% of mature BW [31], irrespective of growth patterns. Freetly, et
442 al. [31] suggested that this trait was a more robust predictor of age at puberty than absolute
443 weight or age. This was also described by Grings, et al. [32] among beef heifers of three sire
444 breeds differing in potential muscularity and raised on different dietary regimes. Puberty was
445 reached before 300 d of age in 60% of the heifers, which can be considered as a precocious
446 puberty [2], and this proportion was greater in the PA (77%) than in the PI heifers (42%).
447 Weight at puberty did not differ between breeds but PA heifers attained puberty earlier, as
448 observed previously [33], which could be ascribed to the ancient origin of PA in the Brown
449 Swiss dual purpose breed, since dairy breeds reach puberty earlier than beef breeds.

450 Some studies have indicated that diets promoting greater rates of gain early after

451 weaning can increase the tendency to reach puberty at an earlier age, particularly when
452 heifers are fed high-starch, gluconeogenic diets [2]. In the current study, puberty only tended
453 to be achieved earlier in heifers from the HIGH than those from the MOD treatment, a trend
454 that was confirmed at the individual level, since rates of gain were negatively related to age at
455 puberty. The fact that differences between treatments did not reach significance could be due
456 to the limited number of animals, and also to the fact that all heifers had similar and high pre-
457 weaning gains (1.039 ± 0.176 kg/d), which were greater than those observed in previous
458 work with non creep-fed calves of both breeds [17,26]. These high gains during lactation
459 could have induced an earlier puberty, as suggested by Day and Nogueira [34]. Cardoso, et
460 al. [35] reported that a favorable nutritional status between 4 and 6.5 mo of age induced
461 functional changes in the neuroendocrine reproductive system that persisted after a period of
462 feed intake restriction between 6.5 and 9 mo of age. Furthermore, Rodríguez-Sánchez, et al.
463 [36] described a negative correlation between age at puberty and weight gains before
464 weaning, but not with ADG after weaning. Conversely, Nepomuceno, et al. [37] found that
465 enhanced nutrition during the post-weaning period was an effective method to anticipate
466 puberty, which was not influenced by pre-weaning calf supplementation. Nevertheless, all
467 heifers involved in the experiment were pubertal early enough before the first AI. One of the
468 main objectives of heifer replacement programs is to reach puberty 30 to 45 d before the
469 breeding season [4], because the fertility rate increases after the pubertal estrous [38], which
470 was achieved in the current study.

471 All primiparous cows calved at 26 mo, which is 10 mo earlier than usual in Spanish
472 beef heifers [39]. Despite the fact that weight at calving complied with NRC
473 recommendations, 36% of the primiparous cows needed assistance at calving. This rate was
474 similar to an incidence of assistance of 38% described both for beef [40] and dairy heifers
475 [24], and was not affected by any of the factors analyzed herein.

476 Regarding the offspring traits, calf weight at birth was greater in PA heifers, in
477 agreement with previous results [17], but it was not affected by FEED, as described by
478 Rodríguez-Sánchez, et al. [8]. Thereafter, offspring performance was not influenced by
479 BREED or FEED, but calf growth during lactation was lower than that reported for the
480 offspring of mature cows of both breeds [19]. Milk yield was unexpectedly low according to
481 the objective of the diet, especially in PA dams, which had similar production to PI cows
482 although it is usually lower in the latter [26]. In fact, all groups except PI-MOD had lower
483 milk production than expected, perhaps as a response to the high gains reached by these
484 groups before puberty, over 1 kg/d, whilst the PI-MOD gained 0.89 kg/d. Our results would
485 confirm that pre-pubertal gains of around 0.8 kg/d can maximize milk yield, as described in
486 dairy heifers [10], because greater pre-pubertal gains could increase deposition of mammary
487 adipose tissue and impair parenchymal development of the mammary gland [13].
488 Furthermore, Dervishi, et al. [41] indicated that the lower milk yield of beef primiparous
489 cows, which had been creep-fed as calves, was associated with a different pattern of gene
490 expression in the mammary gland, which suggested a compromised immune status during
491 lactation.

492

493 *4.3. Metabolic profiles*

494 Some differences were observed in the current study in metabolic substrates associated
495 with breed and feeding treatment in the rearing phase, but sampling date had a major effect in
496 all cases, probably because of the strong short-term effect of current energy and/or protein
497 intake. At the individual level, concentrations of the different metabolites were not related
498 over time. Metabolic imprinting was only observed in the case of glucose and cholesterol,
499 where feeding management in the juvenile period originated effects which persisted into
500 lactation.

501 Glucose concentrations decreased after weaning, because it is the main energy source
502 for the lactating calf but afterwards there is a gradual shift in the sources of physiological fuel
503 [42]. The greater values in the HIGH group were related to their greater concentrate intake in
504 this phase, which resulted in a greater ruminal production of propionate [43], which once
505 absorbed is the main source for glucose synthesis at the liver [44]. These differences were
506 still evident in the first lactation, in agreement with other authors [15,45] who found that
507 post-weaning diets differing in concentrate content resulted in different glucose and insulin
508 profiles, persisting into adulthood. The drop of glucose concentrations throughout gestation
509 to the nadir registered one week before calving could be caused by the reduced intake
510 capacity in late gestation as fetus size increases [26].

511 Circulating cholesterol was affected by an interaction between feeding treatment and
512 sampling time. In the rearing phase, the greater levels observed in the HIGH treatment were
513 associated with their greater energy intake. In contrast, the greater concentration in the MOD
514 treatment during lactation was mainly due to PI-MOD heifers, which also had the greatest
515 milk yield, indicating a mobilization of fat reserves for milk secretion, as shown by Ruegg, et
516 al. [46].

517 Plasma NEFA concentrations have been associated with the mobilization of fat tissue in
518 replacement heifers fed different diets [47], but herein they did not differ among feeding
519 treatments in any phase. In the rearing period, the greatest concentrations were observed in
520 newly weaned heifers, probably because weaning is stressful and it increases the level of
521 catecholamines, which stimulate lipolysis [48]. After breeding, values increased with
522 advancing pregnancy and peaked at calving. In lactation, dam milk yield was correlated to
523 plasma NEFA concentrations at calving, which implies that the fat mobilized was invested in
524 milk secretion. The greater values observed in PI heifers throughout the study could reflect
525 their greater reactivity to handling practices [49].

526 Plasma concentrations of β -hydroxybutyrate depend both on energy balance and
527 ruminal fermentation pattern, since both internal lipolysis and absorption of butyric acid from
528 the ruminal wall are major sources for β -hydroxybutyrate [50]. In our study, the fermentation
529 of a diet with greater concentrate content induced a greater ruminal production of butyric acid
530 in the HIGH feeding treatment [43], resulting in greater plasma β -hydroxybutyrate during the
531 rearing phase. The negative relationship observed between β -hydroxybutyrate and NEFA
532 concentrations could be explained because, although both metabolites indicate short-term
533 negative energy balance and adipose tissue catabolism, β -hydroxybutyrate can also come
534 from dietary sources [51].

535 Increased urea concentrations have been associated with a greater intake of dietary
536 protein but also to the catabolism of body protein in periods of energy shortfall [14]. In the
537 current study, circulating urea matched the pattern of estimated protein intake throughout the
538 experiment, as both are positively correlated [52]. It increased throughout the rearing phase,
539 as observed by Brickell, et al. [14] in dairy heifers. During lactation, plasma urea was below
540 7 mmol/L, a level indicated by Butler [53] as the upper threshold to avoid impairing
541 subsequent fertility in dairy cattle.

542 The positive relationship between urea concentration at 6 mo and cholesterol, and the
543 negative correlation with age at puberty confirms that puberty is hastened in heifers with
544 better nutritional status at weaning [3]. Hence, levels of cholesterol and urea registered at 6
545 mo could be helpful indicators to estimate the capacity of the heifer to attain puberty early.
546 As observed by Abeni, et al. [54] although large differences were not observed between
547 dietary treatments for some metabolites, associations at the individual level reflect a strong
548 within-animal relationship between metabolism and growth.

549

550 *4.4. Endocrine profiles*

551 Animal growth is controlled primarily by the somatotrophic axis, formed by growth
552 hormone, insulin and the IGF-I. Under diets formulated for high rates of gain (1.2 kg/day)
553 through the first year of life, Govoni, et al. [55] observed that IGF-I increased particularly
554 from 200 to 300 d of age in Hereford heifers and plateaued afterwards. In the current
555 experiment, however, the greatest values of IGF-I were observed at 6 mo, associated with
556 high gains during lactation, and then they decreased with advancing age. Concentrations of
557 IGF-I reached a nadir at calving, which could be associated with parturition stress [56], and
558 they increased thereafter, as described in other works [57].

559 There were no differences between genotypes in their plasma IGF-I levels, as reported
560 in previous work for suckling cows [29] and growing bulls [28] of the same breeds, which
561 was associated with their similar growth potential. Heifers from the HIGH treatment showed
562 greater values of IGF-I throughout the study, and at the individual level there was a strong
563 correlation among concentrations in samples collected over time. This would confirm the
564 theory of Reis, et al. [15], who reported that concentrate supplementation in early life had a
565 long-term impact on the mRNA expression of hepatic IGF-I, but they could not find
566 equivalent translation into circulating IGF-I concentrations.

567 The within-animal relationships observed between IGF-I, weight gains and age at
568 puberty have also been described in dairy heifers [14], confirming that IGF-I is an important
569 metabolic mediator involved in the onset of puberty in beef cattle [58]. Similarly, the
570 correlations of IGF-I with glucose and cholesterol reflect the bond of this growth factor with
571 energy balance.

572 Circulating leptin increased through the post-weaning phase, which was associated with
573 fat deposition, because leptin is a key metabolic signal synthesized by fat cells in white
574 adipose tissue that communicates information about body energy reserves and nutritional
575 status [59]. There were no differences between breeds, which agrees with their similar BCS.

576 Leptin was not influenced by FEED, and at the individual level it was not related with gains
577 or with age at puberty. Greater gains during juvenile development can induce adipose tissue
578 accretion and enhance the synthesis and release of leptin, which signals the central nervous
579 system of the availability of enough nutritional reserves to support the pubertal transition
580 [60]. Therefore, the lack of effects observed here implies that nutritional status was adequate
581 in both treatments. Similarly, Cooke, et al. [58] did not find that greater leptin concentrations
582 hastened the puberty attainment of beef heifers, and concluded that it served as a permissive
583 signal that allows puberty to occur, but with a secondary role to that of IGF-I. The
584 peripubertal rise in plasma leptin reported by Garcia, et al. [61] most likely reflects fat
585 deposition but would not be a mandatory condition for the attainment of puberty, which can
586 occur over a wide range of plasma leptin concentrations [62].

587

588 In conclusion, both post-weaning growth rates (0.8 and 0.6 kg/d over a 9-mo rearing
589 phase) allowed animals to surpass the threshold weights recommended for beef heifers both
590 at conception (15 mo) and at calving (2 yr) in both breeds. Moreover, a moderate growth rate
591 was sufficient to ensure metabolic and hormone profiles that were adequate for heifer
592 development and performance until the first lactation, whereas no advantage was gained from
593 a higher feeding level.

594

595 **Acknowledgements**

596 Appreciation is expressed to the staff of CITA Research Center and Drs. C. Tamanini
597 and N. Govoni from Università degli Studi di Bologna for technical support. Spanish
598 Ministry of Economy and Competitiveness (INIA RTA 2010-057, RTA 2013-059 and RZP
599 2015-001), European Regional Development Fund and Research Group Funds of

600 Government of Aragon (A11) funded this research. The first author received a grant from
601 INIA-Government of Spain.

ACCEPTED MANUSCRIPT

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784

785 **Table 1.** Nutrient composition of the feedstuffs provided in the different phases.

	Starter concentrate ¹	Rearing concentrate ²	Alfalfa hay ²	Total mixed ration ³	Grazed pasture ⁴	Meadow hay ⁵
DM, g/kg	894	900	851	897	242	883
ME, MJ/kg DM	15.1	15.2	9.2	9.6	10.5	11.3
CP, g/kg DM	166	147	98	103	197	84
NDF, g/kg DM	214	252	462	595	553	646

786 ¹Pre-weaning phase.787 ²Post-weaning phase.788 ³Breeding season (60 d) and Lactation (120 d).789 ⁴Gestation (from confirmation of pregnancy until a month before the expected calving date).790 ⁵Last month of gestation.

791

792 **Table 1.** Weights, ADG and BCS of heifers throughout the study according to breed
 793 (BREED) and feeding management (FEED) applied in the post-weaning period (6 to 15 mo).

Item	BREED		FEED		SEM	P-value	
	PA ¹	PI ¹	HIGH ¹	MOD ¹		BREED	FEED
<i>n</i> ²	13 (12)	12 (11)	13 (11)	12 (12)			
<i>Weight, kg</i>							
6 mo	247	229	235	241	17.6	0.30	0.76
15 mo	441	410	452 ^x	400 ^y	21.3	0.15	0.02
Calving	477	464	480	461	21.9	0.58	0.43
Weaning	479	475	489	465	20.2	0.84	0.28
<i>ADG, kg/d</i>							
Post-weaning	0.737	0.700	0.814 ^x	0.624 ^y	0.034	0.27	<0.001
Gestation	0.040	0.102	0.030	0.111	0.040	0.58	0.32
Lactation	0.017	0.083	0.071	0.030	0.073	0.39	0.59
<i>BCS (0-5)</i>							
15 mo	4.1	4.2	4.3	3.9	0.22	0.76	0.06
Calving	2.7	2.8	2.8	2.7	0.11	0.60	0.56
Weaning	2.6 ^b	2.8 ^a	2.8	2.7	0.06	0.008	0.34

794 ^{a,b}LSMeans within a row with different superscripts differ significantly among breeds ($P <$
 795 0.05).

796 ^{x,y}LSMeans within a row with different superscripts differ significantly between feeding
 797 managements ($P < 0.05$).

798 ¹PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
 799 target ADG.

800 ²n = heifers per treatment in the post-weaning phase (heifers per treatment during gestation
 801 and lactation).

802 **Table 2.** Size measures of heifers throughout the study according to breed (BREED) and
 803 feeding management (FEED) applied in the post-weaning period (6 to 15 mo).

Item	BREED		FEED		SEM	<i>P-value</i>	
	PA ¹	PI ¹	HIGH ¹	MOD ¹		BREED	FEED
<i>n</i> ²	13 (12)	12 (11)	13 (11)	12 (12)			
<i>Height at withers, cm</i>							
6 mo	100.9	100.3	100.8	100.4	2.24	0.81	0.87
15 mo	121.0	120.1	122.4	118.8	2.08	0.65	0.09
Calving	128.1	127.4	128.6	126.9	1.84	0.73	0.37
Weaning	128.1	128.0	128.1	128.0	1.85	0.97	0.99
<i>Heart girth, cm</i>							
6 mo	136.0	132.9	133.7	135.2	3.62	0.40	0.69
15 mo	178.5	173.5	181.2 ^x	170.8 ^y	2.99	0.10	0.002
Calving	177.1	176.1	178.4	174.8	2.72	0.73	0.22
Weaning	177.1	175.8	178.1	174.8	2.56	0.64	0.23
<i>External pelvic area, dm²</i>							
6 mo	11.4	10.1	10.6	10.9	0.67	0.05	0.62
15 mo	21.7 ^a	19.0 ^b	21.4 ^x	19.3 ^y	0.93	0.009	0.04
Calving	24.5	24.0	24.5	24.5	1.05	0.63	0.75
Weaning	25.3	24.6	25.1	25.1	1.03	0.47	0.76

804 ^{a,b}LSMeans at a given age with different superscripts differ significantly between breeds ($P <$
 805 0.05).

806 ^{x,y}LSMeans at a given age with different superscripts differ significantly between feeding
 807 managements ($P < 0.05$).

808 ¹PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
 809 target ADG.

810 ²n = heifers per treatment in the post-weaning phase (heifers per treatment during gestation
 811 and lactation)

812 **Table 3.** Reproductive performance of heifers at breeding according to breed (BREED) and
 813 feeding management (FEED) applied in the post-weaning period (6 to 15 mo).

Item	BREED		FEED		SEM	<i>P-value</i>	
	PA ¹	PI ¹	HIGH ¹	MOD ¹		BREED	FEED
n	13	12	13	12			
Weight at puberty,	321	325	321	326	15.68	0.81	0.75
Age at puberty, mo	9.1 ^b	10.7 ^a	9.4	10.5	0.62	0.01	0.09
ADG 6 mo-puberty	0.99	0.82	1.067 ^x	0.757 ^y	0.089	0.07	0.002
% MBW ² at puberty	55.4	56.0	56.1	55.3	0.03	0.81	0.75
Weight at first AI,	458	423	467 ^x	414 ^y	22.08	0.12	0.02
Age at first AI, mo	15.7	15.8	15.8	15.8	0.14	0.55	0.99
Fertility at first AI,	31	50	31	50		0.32	0.33
Fertility ³ , %	92	92	85	100		0.91	0.18

814 ^{a,b}LSMeans at a given age with different superscripts differ significantly between breeds ($P <$
 815 0.05).

816 ^{x,y}LSMeans at a given age with different superscripts differ significantly between feeding
 817 managements ($P < 0.05$).

818 ¹PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
 819 target ADG.

820 ²MBW = mature BW.

821 ³Fertility rate in a 60-d breeding season.

822 **Table 5.** Performance of primiparous dams in the first calving and lactation according to
 823 breed (BREED) and feeding management (FEED) applied in the post-weaning period (6 to
 824 15 mo).

Item	BREED		FEED		SEM	<i>P-value</i>	
	PA ¹	PI ¹	HIGH ¹	MOD ¹		BREED	FEED
n	12	11	11	12			
<i>Cow</i>							
Age 1 st calving, mo	26.0	25.8	25.8	26.1	0.39	0.61	0.39
Calving assistance, %	58	10	18	55		0.13	0.13
Calf/Cow BW ratio, %	8.1 ^a	7.1 ^b	7.4	7.1	0.42	0.04	0.42
<i>Calf</i>							
Male/Female ratio	8/4	5/6	6/5	6/6		0.21	0.99
Birth BW, kg	38.0 ^a	33.0 ^b	35.4	35.6	1.68	0.01	0.90
Weaning BW, kg	119.0	114.5	118.7	113.8	5.28	0.36	0.41
ADG, kg	0.675	0.675	0.694	0.655	0.041	0.99	0.39

825 ^{a,b}LSMeans within a row with different superscripts differ significantly between breeds ($P <$
 826 0.05).

827 ¹PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
 828 target ADG.

829

830 **Table 6.** Milking performance in the first lactation of heifers according to breed (BREED)
 831 and feeding management (FEED) applied in the post-weaning period (6 to 15 mo).

Item	BREED				SEM	<i>P-value</i>		
	PA ¹		PI ¹			BREED	FEED	BREED × FEED
	HIGH ¹	MOD ¹	HIGH ¹	MOD ¹				
n	6	6	6	5				
Yield, kg/d	5.51 ^a	4.60 ^b	4.36 ^b	5.86 ^a	0.58	0.84	0.33	<0.001
Protein content, %	3.44 ^{ab}	3.34 ^b	3.62 ^a	3.42 ^b	0.12	0.049	0.02	0.45
Fat content, %	3.56 ^b	3.42 ^b	4.51 ^a	4.11 ^a	0.35	<0.001	0.15	0.50

832 ^{a,b}LSMeans within a row with different superscripts differ significantly among experimental
 833 groups ($P < 0.05$).

834 ¹PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
 835 target ADG.

836 **List of Figures**

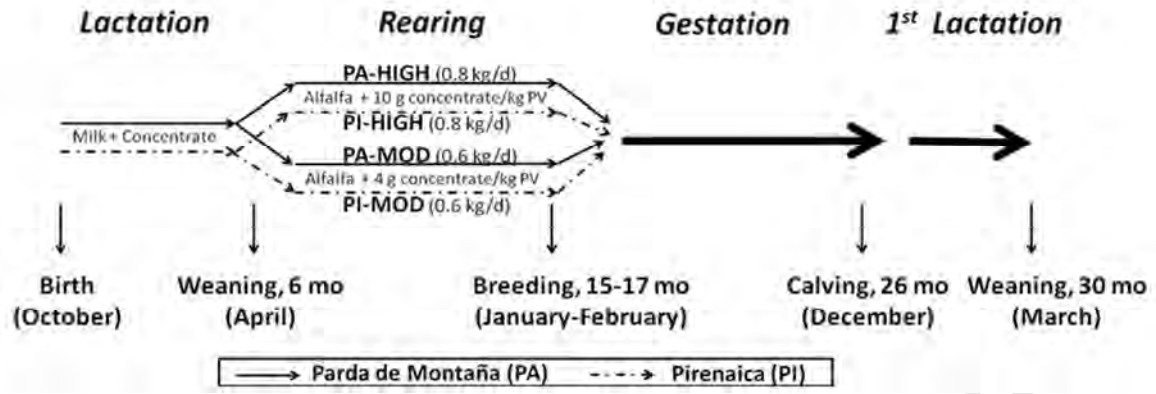
837 **Figure 1.** Experimental design with the post-weaning diets and the target ADG for each
838 breed (BREED) and feeding management (FEED).

839 **Figure 2.** Energy Corrected Milk (ECM) yield throughout the first lactation of beef heifers
840 according to breed and feeding management applied in the post-weaning period (6 to 15 mo).
841 PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d
842 target ADG; ^{a,b}LSMeans at a given month with different superscripts differ significantly
843 among treatments ($P < 0.05$).

844 **Figure 3.** Estimated energy and protein intake by heifers during the experiment according to
845 breed and feeding management applied in the post-weaning period (6 to 15 mo). PA = Parda
846 de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d target ADG.

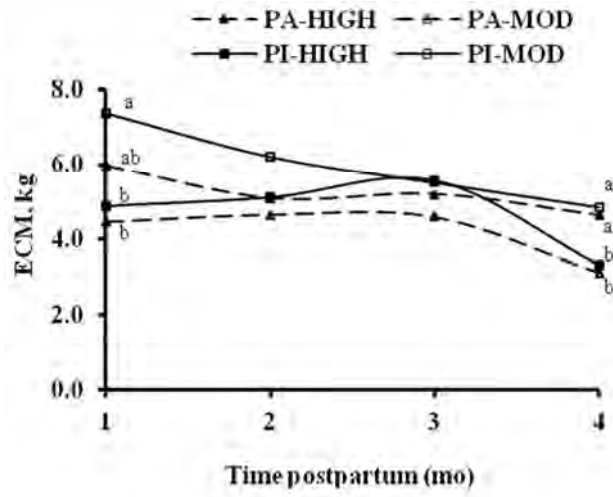
847 **Figure 4.** Plasma concentrations of glucose, cholesterol, NEFA, β -hydroxybutyrate and urea
848 in beef heifers according to breed and feeding management applied in the post-weaning
849 period (6 to 15 mo). PA = Parda de Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG;
850 MOD = 0.6 kg/d target ADG; ^{a,b}LSMeans at a given age with different superscripts differ
851 significantly among breeds ($P < 0.05$); ^{x,y}LSMeans at a given age with different superscripts
852 differ significantly among feeding managements ($P < 0.05$).

853 **Figure 5.** Plasma concentrations of IGF-I and leptin in beef heifers according to breed and
854 feeding management applied in the post-weaning period (6 to 15 mo). PA = Parda de
855 Montaña; PI = Pirenaica; HIGH = 0.8 kg/d target ADG; MOD = 0.6 kg/d target ADG;
856 ^{a,b}LSMeans at a given age with different superscripts differ significantly among breeds ($P <$
857 0.05); ^{x,y}LSMeans at a given age with different superscripts differ significantly among
858 feeding managements ($P < 0.05$).



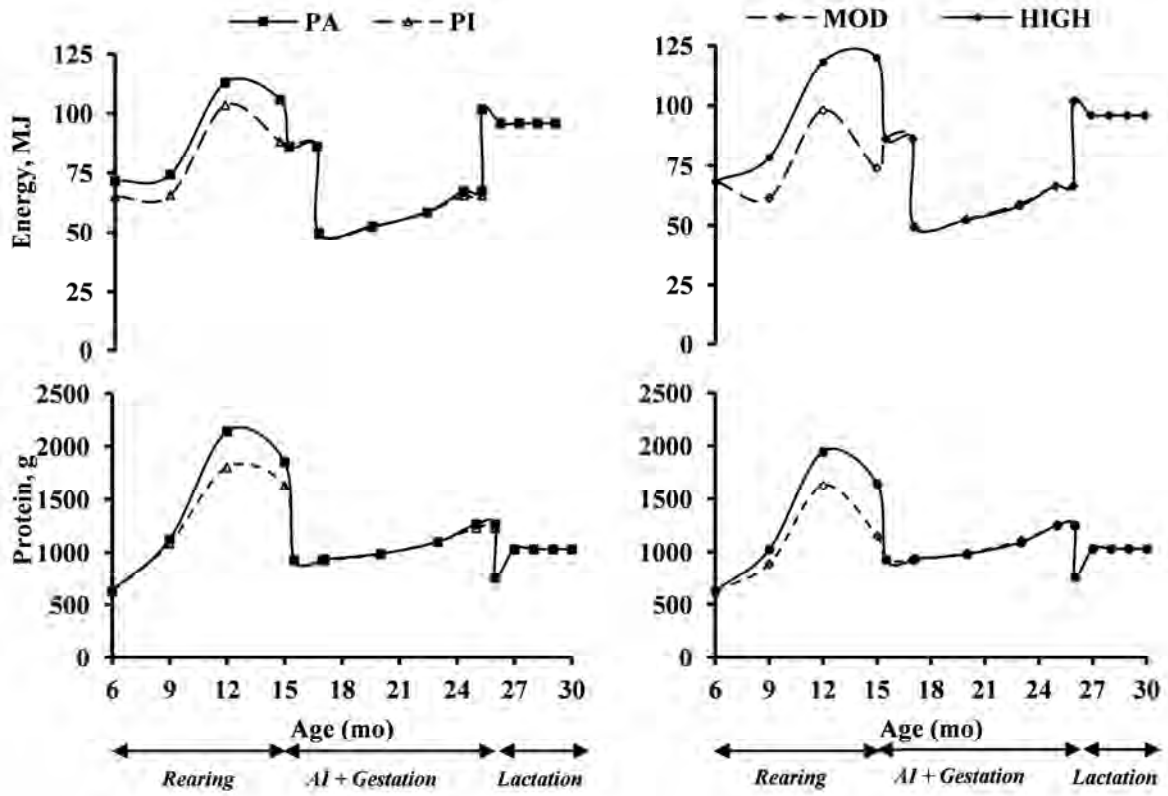
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860 **Figure 2.**



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862 **Figure 2.**



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864 **Figure 3.**

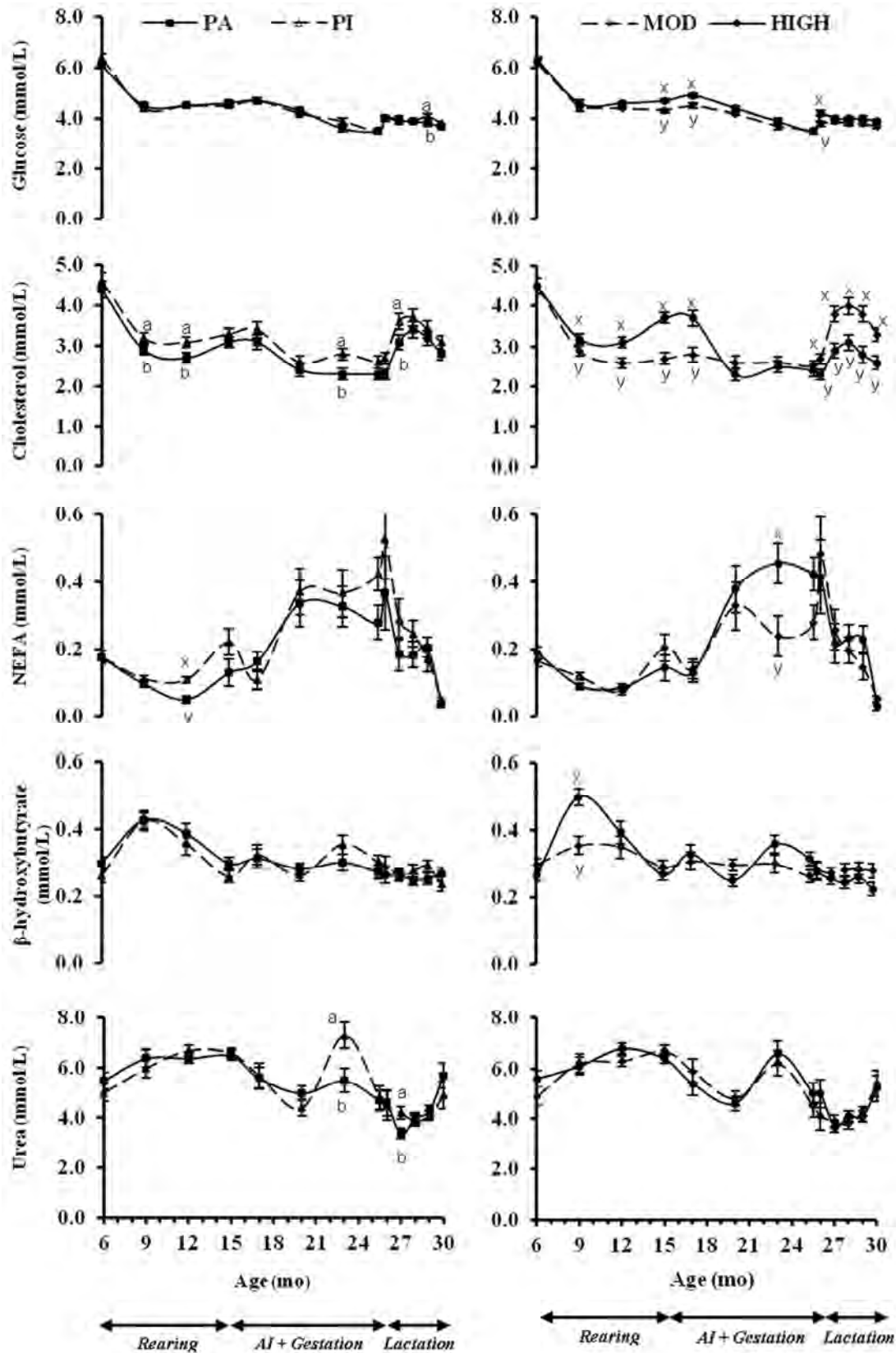
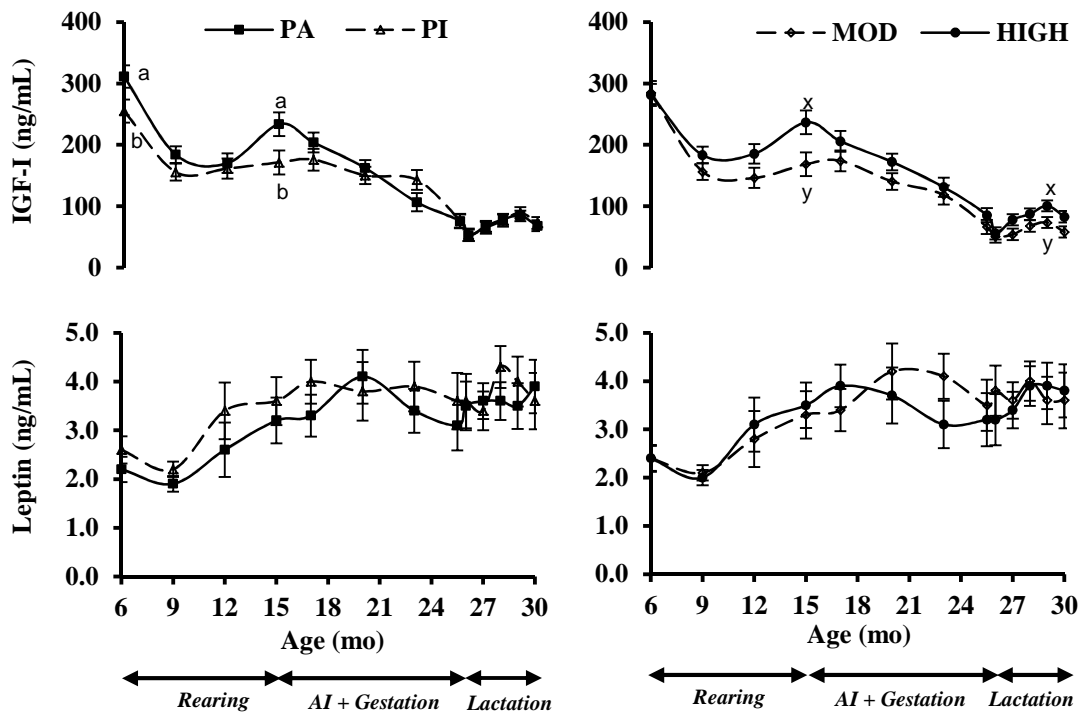
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Figure 4.

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Figure 5.

Highlights

- Feeding either high or moderate energy diets allowed for adequate body growth before breeding season and calving.
- Puberty was reached at the same weight, but different ages between breeds, but all heifers were pubertal at least 1 month before the breeding season.
- Breed has more influence on first calving performance than the two pre-breeding feeding levels evaluated in the study.
- Neither metabolic profiles nor IGF-1 or leptin levels differed between breeds with feeding management.