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1 **i) Title page**

2 **Effects of Maternal Subnutrition during Early Pregnancy on Cow Hematological**
3 **Profiles and Offspring Physiology and Vitality in Two Beef Breeds**

4

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15

16 **ii) Abstract and keywords**

17 This experiment evaluated the effects of subnutrition during early gestation on
18 hematology in cows and on hematological, metabolic, endocrine, and vitality parameters
19 in their calves. Parda de Montaña (PA) and Pirenaica (PI) dams were inseminated and
20 assigned to either a control (CONTROL, 100% requirements) or a nutrient-restricted
21 group (SUBNUT, 65%) during the first third of gestation. Dam blood samples were
22 collected on days 20 and 253 of gestation, and calf samples were obtained during the first
23 days of life. Pirenaica dams presented higher red series parameters than PA dams, both
24 in the first and the last months of gestation. During early pregnancy, granulocyte numbers
25 and mean corpuscular hemoglobin were lower in PI-SUBNUT than in PI-CONTROL

26 cows. Calves from the SUBNUT cows did not show a physiological reduction in red
27 series values in early life, suggesting later maturation of the hematopoietic system. Poor
28 maternal nutrition clearly affected the calf endocrine parameters. Newborns from
29 dystocic parturitions showed lower NEFA concentrations and weaker vitality responses.
30 In conclusion, maternal nutrition had short-term effects on cow hematology, PI cows
31 showing a higher susceptibility to undernutrition, and a long-term effect on their offspring
32 endocrinology, SUBNUT newborns showing higher levels of IGF-1 and lower levels of
33 cortisol.

34

35 Keywords: peri-implantational period; blood; metabolic parameters; IGF-1; cortisol

36

37 **iii) Text**

38 **1. Introduction**

39 Beef cattle (*Bos taurus*) production systems have adapted to increasingly
40 extensive management by reducing feed costs. Depending on food availability, cows can
41 suffer periods of undernutrition during some phases of their production cycle, sometimes
42 concomitantly with the rearing of a calf or/and in early pregnancy. Implantation of the
43 embryo and the maternal recognition of pregnancy at approximately day 20 post-
44 conception are critical points of gestation (Spencer & Hansen, 2015). Moreover, the peri-
45 implantation period is a crucial time for embryo survival, and it could be a potentially
46 vulnerable period during which adverse programming mediated through poor maternal
47 nutrition might begin. Altered placental angiogenesis, cotyledon weight, and fetal
48 development in beef cattle (Long, Vonnahme, Hess, Nathanielsz, & Ford, 2009;
49 Vonnahme et al., 2007), cardiovascular abnormalities in mice (Watkins et al., 2008),
50 altered cardiovascular activity (Torrens et al., 2009), or suppressed behavioral reactions

51 in response to stressful conditions in ewes (Hernandez, Matthews, Oliver, Bloomfield, &
52 Harding, 2010) have all demonstrated the risk of adverse developmental programming
53 and increased chronic disease incidence attributed to peri-conceptual undernutrition
54 (Fleming, Velazquez, Eckert, Lucas, & Watkins, 2012).

55 Hematological parameters have been used routinely to monitor the health and
56 nutritional status of cattle (Strydom et al., 2008), considering that factors such as age, sex,
57 breed, stress, diet, body condition, reproductive status, ambient temperature, or altitude
58 can affect hematological profiles (Krimer, 2011). Any imbalance of hematological
59 parameters will indicate a breakdown in the homeostasis.

60 Similarly, metabolic parameters are indicators of nutritional status and ruminant
61 energy metabolism. However, these parameters can be permanently altered if some
62 stimuli, such as poor nutrition, are present during fetal programming. During this critical
63 and sensitive fetal stage, the structure, physiology, and metabolism of different organs
64 and systems can be modified (Mossa, Walsh, Ireland, & Evans, 2015), leading to
65 detrimental postnatal metabolic changes (Hoffman et al., 2017). Similarly, undernutrition
66 during fetal life could cause permanent alterations in the endocrine function in the fetus
67 to ensure fetus survival under adverse intrauterine conditions (Kiani et al., 2011; Rhind,
68 2004). For example, prenatal nutrient availability influences the ability of calves to
69 regulate plasma concentrations of glucose and insulin (Ford & Long, 2011; Long, Prado-
70 Cooper, Krehbiel, & Wettemann, 2010).

71 Inter-breed differences, which may interact with the nutritional level, must be
72 considered, since genetic differences induce changes in hematological or metabolic
73 values (Wuletaw, Wurzinger, Holt, Dessie, & Sölkner, 2011). Parda de Montaña (PA)
74 and Pirenaica (PI) are the two main beef cattle breeds that have adapted to the semi-
75 extensive system of animal husbandry in the Pyrenees mountain region (northern Spain).

76 Differences have been found between these breeds in their neuroendocrine and metabolic
77 adaptation to varied management practices (Blanco, Casasús, & Palacio, 2009; García-
78 Belenguer et al., 1996), which should be considered to choose the genotype better adapted
79 to extensive management characterized by variable food availability.

80 At present, little is known about the physiological mechanism through which
81 maternal subnutrition and breed can alter embryo hematopoiesis, metabolism, or
82 endocrine regulation in cattle. This study's hypothesis was that maternal subnutrition
83 during early pregnancy could trigger effects in dam hematological values and in offspring
84 physiology and vitality, and that the response could differ between genotypes with
85 varying baseline profiles. The aim of this study was to evaluate the effect of
86 undernutrition during the first third of gestation on the hematological parameters in the
87 peri-implantation period (day 20 after artificial insemination, AI) and in the last month of
88 gestation (day 253) in PA and PI beef cows and on hematological, metabolic, and
89 endocrine profiles and vitality of newborn calves.

90

91 **2. Materials and methods**

92 All of the procedures were approved by the Animal Ethics Committee of the
93 Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón. The care and
94 use of animals were performed in accordance with the guidelines of the European Union
95 (Directive 2010/63/E.U.) regarding the protection of animals used for experimental and
96 other scientific purposes (E.U., 2010).

97

98 **2.1. Animals and management**

99 This study was conducted at La Garcipollera Research Station in the mountainous
100 area of the central Pyrenees (northeastern Spain, 945 m a.s.l.). Seventy-four PA ($560 \pm$

101 55 kg live weight (LW); 2.73 ± 0.26 body condition score (BCS) on a 5-point scale) and
102 40 PI (579 ± 51 kg LW; 2.95 ± 0.28 BCS) multiparous cows rearing calves were
103 synchronized to estrus at 65 ± 14 days postpartum with a protocol based on a
104 progesterone-releasing intravaginal device (PRID Delta 1.55 g, CEVA, Loudéac, France)
105 and a 10 μg injection of GnRH (Busol, INVESA, Barcelona, Spain), followed 7 days later
106 by 150 μg of prostaglandin F 2α (Galapán, INVESA, Barcelona, Spain). After 9 days, the
107 PRID was removed and 500 IU of pregnant mare serum gonadotropin (Serigan,
108 Laboratorios Ovejero, León, Spain) was administered, followed 48 hours later by a
109 second injection of GnRH (10 μg). Eight hours after the final GnRH injection, the cows
110 were randomly inseminated with proven fertility sires (4 PA and 3 PI) by an expert
111 technician.

112 On the day of AI, the dams were randomly allocated to two maternal nutrition levels with
113 a total mixed ration (10.96 MJ ME/kg DM and 124 g CP/kg DM) (Table 1) during the
114 first 82 days of pregnancy. The control group (CONTROL, n = 52) was fed a diet that
115 supplied 100% of the estimated energy and protein requirements for cow maintenance,
116 lactation, and gestation (10.9 and 10.0 kg DM/cow/day for the PA and PI, respectively),
117 and the nutrient-restricted group (SUBNUT, n = 62) received 65% of their requirements
118 (7.0 and 6.4 kg DM/cow/day for the PA and PI, respectively). After this treatment phase,
119 all of the dams were fed 100% requirements until parturition. Pregnancy diagnosis was
120 performed by ultrasonography using a linear array 7.5 MHz transducer (Aloka SSD-
121 500V, Aloka, Madrid, Spain) on day 37 post-AI, and the non-pregnant cows were
122 removed from the trial thereafter. During the experiment, all of the cows and calves
123 remained in a loose housing system.

124

125 ***2.2. Measurements and blood sampling***

126 The cows were weighed and their BCS was registered by two expert technicians
127 based on the estimation of the fat covering the loin, ribs, and tailhead on day 20 post-AI,
128 and again from those who conceived from AI (n = 83) on day 82 post-AI and 1 month
129 before parturition (253 days post-AI). On days 20 and 253 post-AI, blood samples were
130 collected in EDTA tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth,
131 UK) from all of the dams via coccygeal venipuncture. The calves were weighed at birth
132 and their blood sampled once during their first days of life (between days 1 and 11) via
133 jugular venipuncture into EDTA and heparinized tubes. Samples for hematology were
134 refrigerated (4°C) and analyzed within the next 8 hours. Samples for metabolite and
135 hormone concentration were centrifuged at 3500 rpm for 20 min at 4°C immediately after
136 collection, and the plasma was harvested and frozen at -20°C until analysis.

137 Concurrently, the cow parturition process was classified into 3 categories
138 depending on the assistance needed: unassisted, easy-pulled (assisted by hand or with a
139 rope), or hard-pulled (assisted with a fetus extractor). Newborn vitality was evaluated
140 immediately after birth via a modified calf vitality test proposed by Mee (2008a). The
141 parameters were evaluated and their categories were: meconium staining (no staining
142 around the anal area vs stained), tongue (normal vs swollen or protruding tongues), calf
143 attitude (attempts to stand vs no effort to rise), palpebral reflex (actively blinks and closes
144 eyes vs slow to blink or no reflex), finger suckling reflex (strong vs weak or absent), and
145 mucous membrane color (bright pink vs brick red or white/blue).

146

147 ***2.3. Hematology, hormone, and metabolite assays***

148 Unclothed (EDTA) whole-blood samples from the cows and calves were analyzed
149 using a fluorescent flow cytometry analyzer (Sysmex XT-2000i V, Sysmex Corporation,
150 Kobe, Japan) standardized for the analysis of bovine blood. Hematological analyses

151 included hematocrit (HCT, expressed as a percentage), hemoglobin concentration (HGB,
152 g/dl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular volume (MCV, fl),
153 mean corpuscular hemoglobin concentration (MCHC, g/dl), red blood cell count (RBC,
154 10^6 counts/mm³), red blood cell distribution width (RDW, percentage), white blood cell
155 count (WBC, 10^3 counts per mm³, including the different leukocyte subtypes:
156 granulocytes (GRAN), lymphocytes (LYM) and monocytes (MON)), number of platelets
157 (PLT, 10^3 counts/mm³), mean platelet volume (MPV, fl), platelet distribution width
158 (PDW, fl), and plateletcrit (PCT, percentage).

159 Heparin and EDTA plasma samples (according to the manufacturer's instructions)
160 were used to assess the calves' metabolic and endocrine status. An automatic analyzer
161 (GernonStar, RAL/TRANSASIA, Dabhel, India) was used to measure the blood
162 concentrations of glucose (glucose oxidase/peroxidase method, sensitivity: 1.01 mg/dl)
163 and urea (kinetic UV test, sensitivity: 1.02 mg/dl). The mean intra- and inter-assay
164 coefficients of variation for these molecules were < 5.4% and 5.8%, respectively. A
165 commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK) was used to
166 analyze the concentrations of non-esterified fatty acids (NEFA, enzymatic method,
167 sensitivity: 0.06 mmol/l). The mean intra- and inter-assay coefficients of variation were
168 5.1% and 7.4%, respectively. A solid-phase enzyme-labelled chemiluminescent
169 immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited,
170 Llanberis, Gwynedd, UK) was used to determine the plasma cortisol concentration
171 (sensitivity: 5.5 nmol/L) and insulin-like growth factor I (IGF-1, sensitivity: 20 ng/ml).
172 The mean intra-assay coefficient of variation for cortisol was 7.1% and the mean intra-
173 and inter-assay coefficients of variation for IGF-1 were 3.1% and 12.0%, respectively.

174

175 **2.4. Statistical analyses**

176 Data were analyzed with SAS and JMPPro statistical software (SAS Institute Inc.,
177 Cary, NC). Normality was confirmed by the univariate procedure ($P > 0.05$). The live
178 weights, BCS, and hematological values of the cows in the first and last months of
179 pregnancy were assessed through analysis of variance using a general linear model (the
180 GLM procedure) with dam age (5-10 years old *vs* more than 10 years old), breed (PA *vs*
181 PI), maternal nutrition (CONTROL *vs* SUBNUT), and their interactions as fixed effects.
182 In the samples collected on day 20 post-AI, the pregnancy status (pregnant *vs* non-
183 pregnant) was also included as a fixed effect. The live weights, hematological values, and
184 metabolite and hormone concentrations in the calves were assessed through analysis of
185 variance with a mixed linear model (the mixed procedure), including the type of
186 parturition (only for metabolite and hormone concentration analysis), gender (male *vs*
187 female), breed, maternal nutrition, and their interaction as fixed effects; the calf age was
188 included as a covariate and the sire used for AI was considered a random effect. The
189 relationship among the calves' hematological values and age, metabolite, and hormone
190 concentrations were determined through Pearson's correlation coefficients. The
191 association between the calves' vitality and breed, maternal nutrition, and type of
192 parturition was assessed using the F-test (the FREQ procedure).

193 All of the statistical analyses were considered significant at $P < 0.05$. Values are
194 expressed as the least square (LS) means. Multiple comparisons among treatments were
195 conducted using Tukey's test.

196

197 **3. Results and discussion**

198 In the current study, the dam age, the sire used for AI, and the pregnancy status
199 (only in the cow samples collected on day 20 post-AI) had no effect on the parameters
200 measured in the dams or calves.

201

202 ***3.1. Hematological parameters of dams on day 20 post-AI***

203 The LW, BCS, and hematological values of the dams at the third week post-AI
204 are shown in Table 2. No differences were found in the LW between breeds ($P > 0.05$) on
205 day 20 post-AI, but the BCS was lower in the PA than in the PI cows ($P < 0.001$).
206 Regarding maternal nutrition, the CONTROL and SUBNUT groups had similar LW and
207 BCS ($P > 0.05$), probably because the SUBNUT group had been undernourished during
208 only 20 days.

209 Cow hematology records were in the normal range for the adult cows (Roland,
210 Drillich, & Iwersen, 2014). The effects of dam breed, nutrition, age, and pregnancy status
211 on these data were analyzed. Breed affected most of the hematological parameters studied
212 on day 20 post-AI. Concerning the white series, the PA cows had higher values of WBC
213 than the PI ($P < 0.05$) due to a higher LYM count ($P < 0.01$). In the red series, the PI had
214 higher values of RBC, HGB, HCT, and MCV than the PA ($P < 0.001$). These results were
215 partially in concordance with those of García-Belenguer et al. (1996), who found that
216 under basal conditions, PI dams presented higher WBC, RBC, HGB, and HTC values
217 than PA dams. Both the PLT and PCT counts were higher in the PA than in the PI cows
218 ($P < 0.001$), while the MPV was higher in the PI cows ($P < 0.001$). This inverse
219 physiological relationship between PLT and MPV was also described in humans
220 (Bessman, Williams, & Gilmer, 1981), with the aim of maintaining a constant PCT value
221 (Lozano et al., 1998).

222 Maternal nutrition showed a minor effect on cow hematology values on day 20
223 post-AI, but a significant interaction between breed and maternal nutrition was observed
224 in the granulocyte counts. The values did not differ between maternal nutrition treatments
225 in the PA cows (3.3 vs 3.4×10^3 GRAN counts/ mm^3 for the PA-CONTROL and the PA-

226 SUBNUT, respectively, $P > 0.05$, standard error of the difference (s.e.d.) 0.21×10^3 ,
227 whereas the counts were higher in the PI-CONTROL than in the PI-SUBNUT (3.7 vs 3.0
228 $\times 10^3$ counts/mm³, respectively, $P < 0.05$, s.e.d. 0.31×10^3). Similarly, in an experiment
229 conducted with beef heifers in which a short-term dietary restriction (1.2 vs 0.4
230 maintenance energy requirements) was applied for 18 days, Matthews et al. (2015) found
231 no effects on the neutrophil and lymphocyte numbers. Similarly, over a longer period of
232 differential feeding during 10 weeks, Schären et al. (2016) did not observe any
233 biologically relevant effects on white blood cell populations. In the current study, there
234 was an interaction between breed and maternal nutrition in MCH, that is, no differences
235 were found between the PA-CONTROL and the PA-SUBNUT dams (17.8 vs 18.0 pg,
236 respectively, $P > 0.05$, s.e.d. 0.19), but the values were higher in the PI-CONTROL than
237 in the PI-SUBNUT (19.0 vs 18.3 pg, respectively, $P < 0.01$, s.e.d. 0.29). The RDW was
238 conditioned by maternal nutrition, with higher variability in the erythrocyte sizes in the
239 CONTROL group ($P < 0.001$). However, since the RDW values were within the reference
240 range, anisocytosis was discarded. Similar to the current results, Matthews et al. (2015)
241 found that imposing a short-term dietary restriction on beef heifers had no effects on the
242 RBC or HGB concentrations. Meacham, Warnick, Cunha, Hentges, and Shirley (1964)
243 found no differences in the HCT or HGB between bulls receiving diets with 8% vs 15%
244 crude protein over 84 days. In the current study, maternal nutrition resulted in lower PLT
245 counts and PCT in the SUBNUT dams ($P < 0.05$), in contrast to Matthews et al. (2015),
246 who found no effect on the platelet numbers from short-term dietary restrictions. Overall,
247 it is likely that in the current study, undernutrition had only a minor effect on cow
248 hematology on day 20 post-AI because it had been acting for a short time. However, a
249 clear breed-associated susceptibility to undernutrition was observed in the Pirenaica dams
250 in the first month of gestation.

251 Pregnancy status ($P > 0.05$) did not have any effect, possibly due to the low
252 metabolic and nutrient requirements of the developing fetus during the first month of
253 gravidity (Dänicke et al., 2012). In fact, Mir et al. (2008) described the increase in the
254 HGB, HCT, RBC, MCV, and MCHC values in mid-gestation in crossbreed cows to
255 accommodate the higher need for oxygen consumption in advanced pregnancies. These
256 hematological values returned to lower values in late gestation due to the dilution of blood
257 that occurs as a consequence of increased plasma volume.

258

259 ***3.2. Hematological parameters of dams in the last month of pregnancy***

260 Similar to the results obtained on day 20 post-AI, the PA and PI presented similar
261 LW ($P > 0.05$) and the PI had higher BCS than the PA on day 253 of pregnancy ($P < 0.05$)
262 (Table 3). No differences were found between the CONTROL and SUBNUT groups in
263 the LW and BCS ($P > 0.05$). The differences between the groups in both parameters
264 registered after 82 days of maternal nutrition treatment (577 vs 539 kg ($P < 0.01$) and 2.9
265 vs 2.6 ($P < 0.001$) for the CONTROL and SUBNUT groups in the LW and BCS,
266 respectively) disappeared one month before parturition, probably because the 100% diet
267 received from day 82 post-AI until calving allowed the SUBNUT cows to overcome this
268 difference.

269 All of the cow hematological parameters registered in the last month of gestation
270 were within the bovine reference range (Roland et al., 2014) except for MPV, which in
271 all of the groups was higher than that referenced due to a physiological increase in the
272 last stage of gestation (Fay, Hughes, & Farron, 1983). The effects of the cow breed,
273 nutrition, and age on these data were analyzed. Regarding the breed effect, no significant
274 differences were found in the white series between the PA and the PI ($P > 0.05$). However,
275 the breed affected most of the red blood cell parameters. The PA cows had lower values

276 of RBC and HGB ($P < 0.01$) and HCT ($P < 0.05$) than the PI cows, in agreement with
277 previous observations in early pregnancy and with results obtained by García-Belenguer
278 et al. (1996). Parda de Montaña also exhibited lower MCHC ($P < 0.05$) and RDW ($P <$
279 0.01) than PI dams. No significant differences in the platelet series were observed ($P >$
280 0.05). These results confirmed that, in physiological conditions, the values of the red
281 series were higher in the PI cows, which provides evidence of inter-breed differences that
282 could imply a better adaptation to altitude conditions than the PA dams, in line with a
283 study by Bianca and Näf (1979). Blood parameters are considered important indicators
284 for measuring the adaptation of animals to altitude, which induces hematopoiesis as an
285 adaptive mechanism (Wuletaw et al., 2011).

286 Maternal subnutrition applied in the early gestation period had no long-term
287 effects on the hematological variables observed one month before calving ($P > 0.05$). This
288 lack of effect suggests that the cows were able to offset the previous differences after they
289 returned to the control diet for 171 days, and therefore their blood profiles were relatively
290 resilient to nutritional stress. It is well known that an adequate nutritional status is
291 essential to restore physiological values of the hematological parameters. In this sense,
292 Meacham et al. (1964) found lower HCT and HGB values when bulls were fed a low-
293 protein diet, but the values were restored after the bulls returned to a control diet for 100
294 days.

295

296 ***3.3. Hematological parameters of newborn calves***

297 The calves' LW and blood cell values are displayed in Table 4. The live weights
298 of the newborn calves were higher in the PA breed ($P < 0.01$) than in the PI, in line with
299 previous studies of the same breeds (Álvarez-Rodríguez, Palacio, Casasús, & Sanz,
300 2010). As expected, the male calves were heavier than the females ($P < 0.01$). However,

301 no differences were found in the calf LW at birth that could be ascribed to maternal
302 nutrition ($P > 0.05$). Accordingly, Mossa et al. (2013) did not find weight differences in
303 calves born to nutrient restricted and control heifers that were fed at 0.6 and 1.2 of their
304 requirements, respectively, during the 110 first days of gestation.

305 Regarding offspring hematology, a stress leukogram was observed in 21 calves
306 who were discarded for all subsequent analyses due to extreme granulocytosis (more than
307 10×10^3 counts/mm³) or intense lymphopenia (less than 0.2×10^3 counts/mm³) or both.
308 Neither breed (22.4% of the PA and 32.3% of the PI calves had a stress leukogram) nor
309 maternal nutrition in early pregnancy (28.6% of the CONTROL and 24.0% of the
310 SUBNUT calves, respectively) or their interaction were associated with the leukogram
311 status (F-test, $P > 0.05$). This response has been observed regularly in studies on the
312 hematology of newborn calves (Benesi et al., 2012) as a consequence of the high cortisol
313 concentrations produced by a stressful situation, such as the birthing process (Hulbert &
314 Moisés, 2016). Three pairs of twin calves were also removed from the analysis. The effects
315 of breed, maternal nutrition, and gender were therefore analyzed in the data on the
316 remaining 59 calves, which were within the bovine physiological range for calves.

317 The breed-associated differences in the red series parameters observed in the dams
318 in the current study did not occur in their newborn calves, which meant that the
319 hematological characteristics inherent to the breed are not congenital but are acquired
320 later during their postnatal life. These results agreed with those of García-Belenguer et al.
321 (1996) and Blanco et al. (2009), who did not find any differences in the white or red blood
322 cell parameters between breeds when studying PA and PI calves from 2 to 5 months of
323 age.

324 Maternal nutrition in early gestation did not influence the calf leukograms,
325 although the fetal immune system develops at the beginning of gestation, including

326 lymphoid thymus and spleen development at approximately days 42 and 55, respectively
327 (Schultz, Dunne, & Heist, 1973). However, maternal nutrition had an effect on the red
328 series parameters, with the calves from the SUBNUT treatment showing lower MCH than
329 their CONTROL counterparts ($P < 0.05$). Given that the MCHC and HGB values were
330 within the reference range, and they did not differ between the nutritional treatments, and
331 hypochromic anemia in the undernourished group was discarded (Almaguer, 2012).
332 Similarly, Dänicke et al. (2012) did not find differences in HCT, WBC, GRAN, LYM,
333 and MON values in newborn calves whose mothers were submitted to a nutritional
334 treatment during the first days of pregnancy. Hematopoiesis is a long process that starts
335 in the blood islands of the embryo yolk sac in the third week of pregnancy and is a
336 continuous process throughout gestation (Tchernia, 1989). Similarly, as the cows
337 recovered most of the values affected by undernutrition during the last 6 months of
338 gestation, the calves could also have restored their blood cell parameters if they were
339 affected.

340 A gender effect was observed in the platelet series. The female calves exhibited
341 greater values in the MPV and PDW than the male calves ($P < 0.05$). In contrast, Panousis
342 et al. (2018) found that female calves had higher red series values than males, but no
343 differences were observed in the leukocyte and platelet parameters in Holstein calves
344 sampled between 1 and 9 days of life. On the other hand, Tennant, Harrold, Reina-Guerra,
345 Kendrick, and Laben (1974) did not observe any sex-related differences in Jersey and
346 Holstein calves.

347 The correlations between calf age (days 1 to 11) and the hematological values
348 without and with regard to maternal nutrition are displayed in Table 5. No significant
349 correlation between calf age (1-11 days) and any variable of the white series was observed
350 ($P > 0.05$). However, most of the red and platelet hematological parameters were related

351 to calf age. Calf age negatively correlated with the MCH and positively correlated with
352 the RDW. Other studies have shown that the HCT, MCV, and HGB concentrations tend
353 to decrease during the first days of life (Knowles et al., 2000; Probo et al., 2012). During
354 intrauterine life, the fetus has a relatively hypoxic environment and requires larger
355 erythrocytes to compensate for this situation. During the first days of life, former
356 erythrocytes containing fetal hemoglobin are replaced by new smaller erythrocytes
357 containing hemoglobin A (Brun-Hansen, Kampen, & Lund, 2006). Thus, the decrease in
358 the HGB, HCT, MCV, and MCH values is a physiological process during the first days
359 of life, which was also described by Brun-Hansen et al. (2006) and Mohri, Sharifi, and
360 Eidi (2007). In the platelet series in the current study, both the PLT and PCT increased as
361 the days passed, but the MPV decreased. The correlation between calf age and the PLT
362 agrees with the results of Roland et al. (2014), who described that in correctly developing
363 newborns, the platelet number increases significantly during the first 2 weeks of age and
364 more slowly thereafter over the first 3 months.

365 Considering maternal nutrition, negative correlations were found between calf age
366 and most of the red cell parameters (HGB, HCT, MCV, and MCH), but only in the
367 CONTROL calves. These results were consistent with the normal physiological
368 development of the calves, which showed a reduction in the red series parameters during
369 the first days of life, suggesting that in the CONTROL newborns, the bone marrow was
370 active and mature enough to start this process immediately after birth. On the contrary,
371 the lack of reduction in the red series in the SUBNUT calves could indicate some delay
372 in the newborn erythropoietic process of replacing fetal erythrocytes. The current study's
373 findings supported the idea that maternal subnutrition during the first third of gestation
374 could trigger a later maturation of the calves' hematopoietic system, although future
375 experiments will be necessary to confirm this.

376

377 **3.4. Metabolite and endocrine profiles of newborn calves**

378 The values of the plasma metabolites and hormones of the calves in their first days
379 of life are shown in Table 6 according to breed, maternal nutrition, gender, and type of
380 parturition. First, the breed had no significant effects on the calves' metabolite and
381 hormone concentrations, in line with previous studies conducted on cows and calves of
382 the same breeds (Álvarez-Rodríguez & Sanz, 2009; Rodríguez-Sánchez, Sanz, Ferrer, &
383 Casasús, 2018).

384 Maternal nutrition clearly affected the endocrine profiles, with the CONTROL
385 calves showing higher IGF-1 concentrations ($P < 0.001$) and lower cortisol values ($P <$
386 0.01) than the SUBNUT calves. Insulin-like growth factor-1 is a hormone involved in
387 muscle growth and is positively related to energy and protein intake (Blanco, Joy, Ripoll,
388 Sauerwein, & Casasús, 2011; Paradis et al., 2015), which increases its plasma
389 concentration with improved nutritional status (Rodríguez-Sánchez, Sanz, Tamanini, &
390 Casasús, 2015). Similar to previous research, in the current study, the IGF-1 concentration
391 was positively related to the circulating glucose ($r = 0.43$, $P < 0.001$), although no
392 significant differences between the maternal nutrition groups were found in glucose
393 concentrations of the offspring ($P > 0.05$). Hoffman et al. (2016) found no differences in
394 the glucose, triglyceride, and cholesterol concentrations in lambs born to poorly
395 nourished ewes. Conversely, Maresca et al. (2018) described higher glucose
396 concentrations during the first 60 days of life in calves whose mothers had received a
397 low-protein diet from mid-gestation to parturition, supporting the hypothesis of Gardner
398 et al. (2005) that maternal subnutrition during pregnancy could alter the capacity of calves
399 to regulate plasma glucose concentrations during postnatal growth. However, glucose
400 concentrations characterize nutritional status in the short term, and therefore the lack of

401 differences in the current study could indicate that the newborns received a similar diet
402 during their first days of life and their glucose metabolism was not altered. During calf
403 feeding in the first days of life, based only on the maternal colostrum and milk, the
404 CONTROL group could have taken better advantage of the nutritional resources,
405 producing higher IGF-1 concentrations that could have improved their tissue growth and
406 metabolism. Maternal undernutrition may reprogram the fetal IGF-1 system in its ability
407 to respond to acute changes in the substrate supply (Gallaher, Breier, Keven, Harding, &
408 Gluckman, 1998). Similarly, fetal IGF-1 concentration may be altered by maternal
409 nutrition during the earlier stages of development (Rhind, 2004) and the level of protein
410 intake from mid-gestation to parturition can affect calf IGF-1 at birth (Maresca et al.,
411 2018). Accordingly, other authors found a greater reduction in IGF-1 levels in the fetus
412 (Gallaher et al., 1998) and in the lamb (Hoffman, Rokosa, Zinn, Hoagland, & Govoni,
413 2014) after maternal subnutrition in sheep.

414 Poor maternal nutrition increased the circulating cortisol levels in the offspring in
415 this study. Cortisol is a hormone synthesized in the adrenal cortex. Its production
416 increases under stress conditions, and consequently it is used as an indicator of stress
417 (Möstl, Maggs, Schrötter, Besenfelder, & Palme, 2002) and animal welfare (Cook,
418 Schaefer, Lepage, & Jones, 1996). Cortisol concentrations can also reflect the nutritional
419 state of an animal (Rhind, 2004). In fact, maternal undernutrition can increase the cortisol
420 concentration in the fetus (Binienda et al., 1990) and thus in the newborn calf, in line with
421 the results of the current study. Maternal nutrient restriction can be a cause of prenatal
422 stress, modifying the hypothalamus-pituitary-adrenal function (Kapoor, Dunn, Kostaki,
423 Andrews, & Matthews, 2006). Moreover, maternal corticosteroids can induce fetal
424 growth retardation, with lower plasma IGF-1 concentrations. Any delay in fetus
425 development due to maternal undernutrition can lead to a greater fetal cortisol response

426 to undernutrition in late gestation and therefore a greater decrease in IGF-1 (Gallaher et
427 al., 1998). Accordingly, in the current study, a negative correlation between cortisol and
428 IGF-1 ($r = -0.29$, $P < 0.05$) was found in the newborn calves. In fact, the increases in the
429 circulating cortisol level in the SUBNUT calves could have contributed to many
430 metabolic changes and modifications of the immune competency of the newborns.

431 Regarding the gender effect, surprisingly, the female newborn calves presented
432 higher IGF-1 concentrations than the males ($P < 0.05$), whereas no differences were found
433 in the other metabolic or endocrine parameters according to gender ($P > 0.05$). It is known
434 that in cattle, pre- and post-pubertal plasma IGF-1 concentrations are greater in males
435 than females. Androgens indirectly increase plasma IGF-1 concentrations through
436 increasing plasma growth hormone (GH). However, other authors affirmed that higher
437 IGF-1 concentrations in males are not observed until 3 (Kerr, Manns, Laarveld, & Fehr,
438 1991) or 4 months of age (Govoni, Hoagland, & Zinn, 2003).

439 Finally, the type of parturition affected the NEFA concentrations, since the hard-
440 pulled calves presented higher concentrations than the unassisted calves ($P < 0.05$).
441 Furthermore, a tendency was observed in the cortisol concentrations, as the hard-pulled
442 calves showed greater concentrations than the unassisted calves ($P = 0.07$). Negative
443 correlations between the cortisol concentrations and lymphocyte number count ($r = -0.29$,
444 $P < 0.05$) and glucose concentrations ($r = -0.37$, $P < 0.01$) were found. Difficult calving
445 is a stressful situation that increases the plasma cortisol levels in both dams and calves
446 throughout the stimulation of the adrenocorticotrophic hormone release (Civelek, Celik,
447 Avci, & Cingi, 2008). As a consequence of the cortisol release, the plasma glucose levels
448 rise due to the increase in liver gluconeogenesis (Drackley, Overton, & Douglas, 2001).
449 In the current study, the calves from dystocic parturitions presented the highest plasma
450 cortisol concentrations, but no statistical differences in the glucose concentrations were

451 found among the groups ($P > 0.05$). Furthermore, a negative correlation was found
452 between cortisol and glucose, suggesting that although the hard-pulled calves should have
453 presented greater glucose concentrations due to their high cortisol concentrations, they
454 did not, most probably due to the low carbohydrate intake of the weakened calves. They
455 needed more time to recover and start ingesting colostrum, and milk later, in the hours
456 after birth. This ingestion delay diminished their glucose and glycogen body reserves.
457 Thus, the calves had to metabolize lipids as an alternative energy source, increasing the
458 NEFA blood concentrations in the calves from dystocic births. Accordingly, a negative
459 correlation was found between the glucose and NEFA concentrations ($r = -0.41$, $P < 0.01$).

460

461 ***3.5. Vitality test of newborn calves***

462 The relationships between the values of the calf vitality test were assessed
463 immediately after birth, and the breed, maternal nutritional, and type of parturition were
464 analyzed. First, the breed affected the finger suckling reflex, as 95% of the PI calves
465 presented a strong suckling reflex compared to 74% of the PA calves ($P < 0.05$), probably
466 due to the heavier weights at birth registered in the PA breed. This breed effect reflected
467 the higher calf/cow weight ratio at calving (0.08 vs 0.07 for the PA and PI, respectively,
468 $P < 0.05$). This ratio, used to determine the fetal-maternal disproportion, can compromise
469 the ease of calving (Johanson & Berger, 2003). In the current study, it indicates that the
470 parturition process was less troublesome in the PI than in the PA breed, and thus less
471 traumatic for the newborns. These results were in accordance with those observed in the
472 circulating cortisol concentrations in the newborn calves, although the breed difference
473 was not statistically significant.

474 Maternal nutrition had no effect on any value of the calf vitality test ($P > 0.05$).
475 High dam BCS at parturition has been described as an important factor that can hinder

476 the parturition process, with negative effects on newborn vitality (Lorenz, Mee, Earley,
477 & More, 2011). Thus, the lack of maternal nutrition effects on the vitality test could be
478 explained because in the current study, no difference in the dam BCS in the last month of
479 gestation was found between the CONTROL and SUBNUT groups.

480 The type of parturition highly influenced the vitality test results. In general, the
481 parturitions required little assistance, with 53 unassisted, 3 easy-pulled, and 3 hard-pulled
482 parturitions. In the meconium staining test, the unassisted parturitions had the lowest
483 percentage of calves with stained anal areas (2, 33, and 33% for unassisted, easy-pulled,
484 and hard-pulled parturitions, respectively, $P < 0.05$). Furthermore, fewer calves with
485 swollen or protruding tongues were in unassisted than in hard-pulled parturitions (2, 33,
486 and 100%, for unassisted, easy-pulled, and hard-pulled parturitions, respectively, $P <$
487 0.05). Most of the calves from the unassisted and easy-pulled parturitions attempted to
488 stand during the calf attitude test (87, 100, and 0%, respectively, $P < 0.05$) and had a
489 strong finger suckling reflex (85, 100, and 0%, respectively, $P < 0.05$) compared to the
490 calves from the hard-pulled parturitions. Contrarily, the type of parturition did not affect
491 the palpebral test or the mucous membrane color ($P > 0.05$). These results confirmed that
492 after the dystocic births, especially in the hard-pulled parturitions when a fetus extractor
493 was used, the newborns were depressed and had weaker responses to vitality controls,
494 compromising neonatal survival. The premature rupture of the umbilical vessels
495 terminates the oxygen supply from the placenta, first causing respiratory acidosis in the
496 fetus, and if the hypoxia is severe enough, metabolic acidosis later occurs (Murray &
497 Leslie, 2013). Cyanosis of the mucous membranes is a sign of prolonged dystocia, and a
498 weak response or no response to stimulation and poor muscle tone can indicate prolonged
499 and non-compensated acidosis due to fetal hypoxia. Metabolic acidosis is the main cause
500 of suckling reflex loss (Mee, 2008b). Similar to the results of the current study, Schafer

501 and Arbeiter (1995) found that calves with lower vitality test scores had higher plasma
502 cortisol concentrations, with lower levels of lymphocytes and larger neutrophils.

503 Summarizing the main findings of this study, maternal nutrition in early
504 pregnancy had different breed-related effects on the cow hematological profiles, with the
505 Pirenaica dams showing a higher susceptibility to undernutrition. Furthermore, the results
506 suggest that it could have triggered a later maturation of the fetal hematopoietic system.
507 These cow hematological differences between the maternal nutrition groups, observed in
508 the first third of gestation, disappeared at the end of pregnancy. Few breed differences
509 were found in the neonatal calves, implying that the different hematological profiles
510 observed in the adult cows were not congenital but developed later in life. Dam
511 undernutrition definitely affected the newborn IGF-1 and cortisol concentrations.
512 Furthermore, newborn vitality was highly affected by the parturition type, as the dystocic
513 calves had weaker physiological responses. In conclusion, maternal nutrition had a short-
514 term effect on cow hematology, the PI cows showing a higher susceptibility to
515 undernutrition, and a long-term effect on offspring endocrinology, SUBNUT newborns
516 showing higher levels of IGF-1 and lower levels of cortisol.

517 The physiological mechanisms by which maternal subnutrition during the peri-
518 implantation period influenced the hematological, metabolic, and endocrine values of the
519 offspring remain unclear. Further research in this area is necessary to better understand
520 the breed-related adaptive responses coupled with the findings of the current study.

521

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529

530 **v) References**

531 Almaguer, C. (2012). Interpretación de la biometría hemática. In J. C. Jaime & D.

532 Gómez (Eds.), *Hematología: la sangre y sus enfermedades*. Mexico: McGraw
533 Hill.

534 Álvarez-Rodríguez, J., Palacio, J., Casasús, I., & Sanz, A. (2010). Does breed affect
535 nursing and reproductive behaviour in beef cattle? *Canadian Journal of Animal
536 Science, 90*, 137-143.

537 Álvarez-Rodríguez, J., & Sanz, A. (2009). Physiological and behavioural responses of
538 cows from two beef breeds submitted to different suckling strategies. *Applied
539 Animal Behaviour Science, 120*, 39-48.

540 Benesi, F. J., Teixeira, C., Leal, M. L., Lisboa, J. A., Mirandola, R., Shecaira, C. L., &
541 Gomes, V. (2012). Leukograms of healthy Holstein calves within the first month
542 of life. *Pesquisa Veterinaria Brasileira, 32*, 352-356.

543 Bessman, J. D., Williams, L. J., & Gilmer, P. R. (1981). Mean platelet volume. The
544 inverse relation of platelet size and count in normal subjects, and an artifact of
545 other particles. *American Journal of Clinical Pathology, 76*, 289-293.

546 Bianca, W., & Näf, F. (1979). Responses of cattle to the combined exposure to diurnal
547 temperature rhythm (-5 to 25°C) and to simulated high-altitude (4,000 m).
548 *International Journal of Biometeorology, 23*, 299-310.

549 Binienda, Z., Rosen, E. D., Kelleman, A., Sadowsky, D. W., Nathanielsz, P. W., &
550 Mitchell, M. D. (1990). Maintaining fetal normoglycemia prevents the increase

- 551 in myometrial activity and uterine 13, 14-dihydro-15-keto-prostaglandin F₂α
552 production during food withdrawal in late pregnancy in the ewe. *Endocrinology*,
553 *127*, 3047-3051.
- 554 Blanco, M., Casasús, I., & Palacio, J. (2009). Effect of age at weaning on the
555 physiological stress response and temperament of two beef cattle breeds.
556 *Animal*, *3*, 108-117.
- 557 Blanco, M., Joy, M., Ripoll, G., Sauerwein, H., & Casasús, I. (2011). Grazing lucerne as
558 fattening management for young bulls: technical and economic performance and
559 diet authentication. *Animal*, *5*, 113-122.
- 560 Brun-Hansen, H. C., Kampen, A. H., & Lund, A. (2006). Hematologic values in calves
561 during the first 6 months of life. *Veterinary Clinical Pathology*, *35*, 182-187.
- 562 Civelek, T., Celik, H. A., Avci, G., & Cingi, C. C. (2008). Effects of dystocia on plasma
563 cortisol and cholesterol levels in Holstein heifers and their newborn calves.
564 *Bulletin of the Veterinary Institute in Pulawy*, *52*, 649-654.
- 565 Cook, N., Schaefer, A., Lepage, P., & Jones, S. M. (1996). Salivary vs serum cortisol
566 for the assessment of adrenal activity in swine. *Canadian Journal of Animal
567 Science*, *76*, 329-335.
- 568 Dänicke, S., Kowalczyk, J., Renner, L., Pappritz, J., Meyer, U., Kramer, R., . . . Jahreis,
569 G. (2012). Effects of conjugated linoleic acids fed to dairy cows during early
570 gestation on hematological, immunological, and metabolic characteristics of
571 cows and their calves. *Journal of Dairy Science*, *95*, 3938-3953.
- 572 Drackley, J. K., Overton, T. R., & Douglas, G. N. (2001). Adaptations of glucose and
573 long-chain fatty acid metabolism in liver of dairy cows during the periparturient
574 period. *Journal of Dairy Science*, *84*, 100-112.

- 575 Directive 2010/63/EU of the European Parliament and of the Council of 22 September
576 2010 on the protection of animals used for scientific purposes, § L276 (2010).
- 577 Fay, R. A., Hughes, A. O., & Farron, N. T. (1983). Platelets in pregnancy:
578 hyperdestruction in pregnancy. *Obstetrics & Gynecology*, *61*, 238-240.
- 579 Fleming, T. P., Velazquez, M. A., Eckert, J. J., Lucas, E. S., & Watkins, A. J. (2012).
580 Nutrition of females during the peri-conceptual period and effects on foetal
581 programming and health of offspring. *Animal Reproduction Science*, *130*, 193-
582 197.
- 583 Ford, S., & Long, N. (2011). Evidence for similar changes in offspring phenotype
584 following either maternal undernutrition or overnutrition: potential impact on
585 fetal epigenetic mechanisms. *Reproduction, Fertility and Development*, *24*, 105-
586 111.
- 587 Gallaher, B., Breier, B., Keven, C., Harding, J., & Gluckman, P. (1998). Fetal
588 programming of insulin-like growth factor (IGF)-I and IGF-binding protein-3:
589 evidence for an altered response to undernutrition in late gestation following
590 exposure to periconceptual undernutrition in the sheep. *Journal of*
591 *Endocrinology*, *159*, 501-508.
- 592 García-Belenguer, S., Palacio, J., Gascon, M., Acena, C., Revilla, R., & Mormède, P.
593 (1996). Differences in the biological stress responses of two cattle breeds to
594 walking up to mountain pastures in the Pyrenees. *Veterinary Research*, *27*, 515-
595 526.
- 596 Gardner, D. S., Tingey, K., Bon, B. W. M. V., Ozanne, S. E., Wilson, V., Dandrea, J., . . .
597 . Symonds, M. E. (2005). Programming of glucose-insulin metabolism in adult
598 sheep after maternal undernutrition. *American Journal of Physiology-*
599 *Regulatory, Integrative and Comparative Physiology*, *289*, 947-954.

- 600 Govoni, K. E., Hoagland, T. A., & Zinn, S. A. (2003). The ontogeny of the
601 somatotrophic axis in male and female Hereford calves from birth to one year of
602 age. *Journal of Animal Science*, *81*, 2811-2817.
- 603 Hernandez, C., Matthews, L., Oliver, M., Bloomfield, F., & Harding, J. (2010). Effects
604 of sex, litter size and periconceptional ewe nutrition on offspring behavioural
605 and physiological response to isolation. *Physiology & behavior*, *101*, 588-594.
- 606 Hoffman, M. L., Peck, K. N., Forella, M. E., Fox, A. R., Govoni, K. E., & Zinn, S. A.
607 (2016). The effects of poor maternal nutrition during gestation on postnatal
608 growth and development of lambs. *Journal of Animal Science*, *94*, 789-799.
- 609 Hoffman, M. L., Reed, S. A., Pillai, S. M., Jones, A. K., McFadden, K. K., Zinn, S. A.,
610 & Govoni, K. E. (2017). Physiology and Endocrinology Symposium: The
611 effects of poor maternal nutrition during gestation on offspring postnatal growth
612 and metabolism. *Journal of Animal Science*, *95*, 2222-2232.
- 613 Hoffman, M. L., Rokosa, M. A., Zinn, S. A., Hoagland, T. A., & Govoni, K. E. (2014).
614 Poor maternal nutrition during gestation in sheep reduces circulating
615 concentrations of insulin-like growth factor-I and insulin-like growth factor
616 binding protein-3 in offspring. *Domestic Animal Endocrinology*, *49*, 39-48.
- 617 Hulbert, L. E., & Moisé, S. J. (2016). Stress, immunity, and the management of calves.
618 *Journal of Dairy Science*, *99*, 3199-3216.
- 619 Johanson, J. M., & Berger, P. J. (2003). Birth Weight as a Predictor of Calving Ease and
620 Perinatal Mortality in Holstein Cattle. *Journal of Dairy Science*, *86*, 3745-3755.
- 621 Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal
622 programming of hypothalamo-pituitary-adrenal function: prenatal stress and
623 glucocorticoids. *The Journal of Physiology*, *572*, 31-44.

- 624 Kerr, D. E., Manns, J. G., Laarveld, B., & Fehr, M. I. (1991). Profiles of serum IGF-I
625 concentrations in calves from birth to eighteen months of age and in cows
626 throughout the lactation cycle. *Canadian Journal of Animal Science*, 71, 695-
627 705.
- 628 Kiani, A., Nielsen, M. O., Tauson, A. H., Tygesen, M. P., Husted, S. M., & Chwalibog,
629 A. (2011). Long-term effects of foetal undernutrition on intermediary
630 metabolism in growing lambs. *Archives of Animal Nutrition*, 65, 46-54.
- 631 Knowles, T. G., Edwards, J. E., Bazeley, K. J., Brown, S. N., Butterworth, A., &
632 Warriss, R. D. (2000). Changes in the blood biochemical and haematological
633 profile of neonatal calves with age. *The Veterinary Record*, 147, 593-598.
- 634 Krimer, P. M. (2011). Generating and interpreting test results: test validity, quality
635 control, reference values and basic epidemiology. In L. K. S. (Ed.), *Duncan and*
636 *Prasse's Veterinary Laboratory Medicine: Clinical Pathology*. Chichester, UK:
637 Wiley.
- 638 Long, N. M., Prado-Cooper, M. J., Krehbiel, C. R., & Wettemann, R. P. (2010). Effects
639 of nutrient restriction of bovine dams during early gestation on postnatal growth
640 and regulation of plasma glucose. *Journal of Animal Science*, 88, 3262-3268.
- 641 Long, N. M., Vonnahme, K. A., Hess, B. W., Nathanielsz, P. W., & Ford, S. P. (2009).
642 Effects of early gestational undernutrition on fetal growth, organ development,
643 and placentomal composition in the bovine. *Journal of Animal Science*, 87,
644 1950-1959.
- 645 Lorenz, I., Mee, J. F., Earley, B., & More, S. J. (2011). Calf health from birth to
646 weaning. I. General aspects of disease prevention. *Irish Veterinary Journal*, 64,
647 10.

- 648 Lozano, M., Narvaez, J., Faundez, A., Mazzara, R., Cid, J., Jou, J., . . . Ordinas, A.
649 (1998). Platelet count and mean platelet volume in the Spanish population.
650 *Medicina Clínica, 110*, 774-777.
- 651 Maresca, S., Lopez Valiente, S., Rodriguez, A. M., Long, N. M., Pavan, E., & Quintans,
652 G. (2018). Effect of protein restriction of bovine dams during late gestation on
653 offspring postnatal growth, glucose-insulin metabolism and IGF-1
654 concentration. *Livestock Science, 212*, 120-126.
- 655 Matthews, D., Waters, S. M., Diskin, M. G., Kenny, D. A., Morris, D. G., & Earley, B.
656 (2015). The effects of short term dietary restriction on haematological responses
657 and leukocyte gene expression of anovulatory and ovulatory beef heifers.
658 *Research in Veterinary Science, 98*, 145-153.
- 659 Meacham, T., Warnick, A., Cunha, T., Hentges, J., & Shirley, R. (1964). Hematological
660 and histological changes in young beef bulls fed low protein rations. *Journal of*
661 *Animal Science, 23*, 380-384.
- 662 Mee, J. F. (2008a). Managing the calf at calving time. Paper presented at the 41st
663 Annual Conference American Association of Bovine Practitioners, North
664 Carolina.
- 665 Mee, J. F. (2008b). Prevalence and risk factors for dystocia in dairy cattle: A review.
666 *The Veterinary Journal, 176*, 93-101.
- 667 Mir, M. R., Pampori, Z. A., Iqbal, S., Bhat, J. I., Pal, M., & Kirmani, M. A. (2008).
668 Hemato-biochemical indices of crossbred cows during different stages of
669 pregnancy. *International Journal of Dairy Science, 3*, 154-159.
- 670 Mohri, M., Sharifi, K., & Eidi, S. (2007). Hematology and serum biochemistry of
671 Holstein dairy calves: Age related changes and comparison with blood
672 composition in adults. *Research in Veterinary Science, 83*, 30-39.

- 673 Mossa, F., Carter, F., Walsh, S. W., Kenny, D. A., Smith, G. W., Ireland, J. L., . . .
674 Evans, A. C. (2013). Maternal undernutrition in cows impairs ovarian and
675 cardiovascular systems in their offspring. *Biology of Reproduction*, 88, 92.
- 676 Mossa, F., Walsh, S. W., Ireland, J. J., & Evans, A. C. (2015). Early nutritional
677 programming and progeny performance: is reproductive success already set at
678 birth? *Animal Frontiers*, 5, 18-24.
- 679 Möstl, E., Maggs, J., Schrötter, G., Besenfelder, U., & Palme, R. (2002). Measurement
680 of cortisol metabolites in faeces of ruminants. *Veterinary Research
681 Communications*, 26, 127-139.
- 682 Murray, C. F., & Leslie, K. E. (2013). Newborn calf vitality: Risk factors,
683 characteristics, assessment, resulting outcomes and strategies for improvement.
684 *The Veterinary Journal*, 198, 322-328.
- 685 Panousis, N., Siachos, N., Kitkas, G., Kalaitzakis, E., Kritsepi-Konstantinou, M., &
686 Valergakis, G. E. (2018). Hematology reference intervals for neonatal Holstein
687 calves. *Research in Veterinary Science*, 118, 1-10.
- 688 Paradis, F., Yue, S., Grant, J. R., Stothard, P., Basarab, J. A., & Fitzsimmons, C. (2015).
689 Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-
690 induced genes may be associated with feed efficiency in beef heifers. *Journal of
691 Animal Science*, 93, 3331-3341.
- 692 Probo, M., Giordano, A., Moretti, P., Opsomer, G., Fiems, L., & Veronesi, M. (2012).
693 Mode of delivery is associated with different hematological profiles in the
694 newborn calf. *Theriogenology*, 77, 865-872.
- 695 Rhind, S. (2004). Effects of maternal nutrition on fetal and neonatal reproductive
696 development and function. *Animal Reproduction Science*, 82, 169-181.

- 697 Rodríguez-Sánchez, J. A., Sanz, A., Ferrer, J., & Casasús, I. (2018). Influence of
698 postweaning feeding management of beef heifers on performance and
699 physiological profiles through rearing and first lactation. *Domestic Animal
700 Endocrinology*, 65, 24-37.
- 701 Rodríguez-Sánchez, J. A., Sanz, A., Tamanini, C., & Casasús, I. (2015). Metabolic,
702 endocrine, and reproductive responses of beef heifers submitted to different
703 growth strategies during the lactation and rearing periods. *Journal of Animal
704 Science*, 93, 3871-3885.
- 705 Roland, L., Drillich, M., & Iwersen, M. (2014). Hematology as a diagnostic tool in
706 bovine medicine. *Journal of Veterinary Diagnostic Investigation*, 26, 592-598.
- 707 Schafer, S., & Arbeiter, K. (1995). Relations between parturition, vitality and some
708 blood parameters of the neonatal calf. *Wiener Tierärztliche Monatsschrift*, 82,
709 271-275.
- 710 Schären, M., Jostmeier, S., Ruesink, S., Hüther, L., Frahm, J., Bulang, M., . . . Breves,
711 G. (2016). The effects of a ration change from a total mixed ration to pasture on
712 health and production of dairy cows. *Journal of Dairy Science*, 99, 1183-1200.
- 713 Schultz, R. D., Dunne, H., & Heist, C. (1973). Ontogeny of the bovine immune
714 response. *Infection and Immunity*, 7, 981-991.
- 715 Spencer, T. E., & Hansen, T. R. (2015). Implantation and establishment of pregnancy in
716 ruminants. In G. R. & B. F. (Eds.), *Regulation of Implantation and
717 Establishment of Pregnancy in Mammals. Advances in Anatomy, Embryology
718 and Cell Biology*. Cham, Switzerland: Springer.
- 719 Strydom, S., Agenas, S., Heath, M., Phillips, C., Rautenbach, G., & Thompson, P.
720 (2008). Evaluation of biochemical and ultrasonographic measurements as

- 721 indicators of undernutrition in cattle. *Onderstepoort Journal of Veterinary*
722 *Research*, 75, 207-213.
- 723 Tchernia, G. (1989). Erythropoiesis and erythrocytes in children, physiology and
724 standards. *Revue du Praticien*, 39, 2111-2116.
- 725 Tennant, B., Harrold, D., Reina-Guerra, M., Kendrick, J., & Laben, R. (1974).
726 Hematology of the neonatal calf: erythrocyte and leukocyte values of normal
727 calves. *The Cornell Veterinarian*, 64, 516-532.
- 728 Torrens, C., Snelling, T. H., Chau, R., Shanmuganathan, M., Cleal, J. K., Poore, K. R., .
729 . . Green, L. R. (2009). Effects of pre□and periconceptual undernutrition on
730 arterial function in adult female sheep are vascular bed dependent. *Experimental*
731 *Physiology*, 94, 1024-1033.
- 732 Vonnahme, K. A., Zhu, M. J., Borowicz, P. P., Geary, T. W., Hess, B. W., Reynolds, L.
733 P., . . . Ford, S. P. (2007). Effect of early gestational undernutrition on
734 angiogenic factor expression and vascularity in the bovine placentome. *Journal*
735 *of Animal Science*, 85, 2464-2472.
- 736 Watkins, A. J., Ursell, E., Panton, R., Papenbrock, T., Hollis, L., Cunningham, C., . . .
737 Kwong, W. Y. (2008). Adaptive responses by mouse early embryos to maternal
738 diet protect fetal growth but predispose to adult onset disease. *Biology of*
739 *Reproduction*, 78, 299-306.
- 740 Wuletaw, Z., Wurzinger, M., Holt, T., Dessie, T., & Sölkner, J. (2011). Assessment of
741 physiological adaptation of indigenous and crossbred cattle to hypoxic
742 environment in Ethiopia. *Livestock Science*, 138, 96-104.
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746 **vi) Tables**

747

748 **Table 1.** Ingredients and chemical composition of feedstuffs used in the experiment (on
 749 an as-fed basis)

750

Ingredients, %

Alfalfa hay	25.0
Cereal straw	25.0
Crushed barley	25.0
Dehydrated alfalfa	10.0
Rapeseed meal	6.5
Citrus pulp	4.5
Soybean meal	2.5
Correctors (calcium carbonate, dicalcium phosphate, sodium chloride, vitamins, and trace elements)	1.5

Chemical composition

DM, g/kg	907.7 ± 5.8
CP, g/kg DM	124.1 ± 10.2
NDF, g/kg DM	466.2 ± 34.8
ADF, g/kg DM	253.3 ± 25.1
ADL, g/kg DM	40.3 ± 4.7
Ash, g/kg DM	113.4 ± 15.3
ME, MJ/kg DM	11.0 ± 0.4

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752 DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL,
 753 acid-detergent lignin; ME, metabolizable energy.

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1 **Table 2.** Hematological parameters of the cows on day 20 post-AI according to the breed and maternal nutrition

	<u>Breed</u>			<u>Maternal nutrition</u>			<u>Significance</u>		Breed x maternal nutrition	
	n	PA 74	PI 40	s.e.d.	CONTROL 52	SUBNUT 62	s.e.d.	Breed		Maternal Nutrition
White series	LW	575	588	10.4	586	577	10.0	ns	ns	ns
	BCS	2.7 ^b	2.9 ^a	0.05	2.8	2.8	0.05	< 0.001	ns	ns
	WBC	7.5 ^a	6.9 ^b	0.29	7.2	7.2	0.30	0.034	ns	ns
	LYM	3.6 ^a	3.0 ^b	0.2	3.2	3.4	0.19	0.003	ns	ns
	MON	0.58	0.55	0.03	0.56	0.57	0.03	ns	ns	ns
	GRAN	3.3	3.3	0.18	3.5	3.2	0.19	ns	ns	0.034
Red series	RBC	6.1 ^b	6.8 ^a	0.11	6.4	6.5	0.12	< 0.001	ns	ns
	HGB	10.8 ^b	12.6 ^a	0.18	11.7	11.8	0.18	< 0.001	ns	ns
	HCT	32.1 ^b	37.2 ^a	0.57	34.3	34.9	0.59	< 0.001	ns	ns
	MCV	53.2 ^b	55.5 ^a	0.18	54.7	54.0	0.18	< 0.001	ns	ns
	MCH	17.9 ^b	18.6 ^a	0.17	18.4	18.2	0.17	< 0.001	ns	0.008
	MCHC	33.6	33.7	0.18	33.8	33.5	0.18	ns	ns	ns
	RDW	17.0	17.0	0.16	17.3 ^a	16.7 ^b	0.17	ns	<0.001	ns
Platelet series	PLT	264.5 ^a	198.1 ^b	11.9	244.8 ^a	217.9 ^b	12.3	< 0.001	0.029	ns
	MPV	5.6 ^b	6.0 ^a	0.07	5.8	5.8	0.07	< 0.001	ns	ns
	PDW	16.1	16.2	0.06	16.1	16.1	0.07	ns	ns	ns
	PCT	0.145 ^a	0.116 ^b	0.01	0.139 ^a	0.123 ^b	0.01	< 0.001	0.025	ns

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3 ^{a-b}Means within a row with different superscripts differ significantly ($P < 0.05$); ns, not significant ($P > 0.05$).

4 LW, live weight; BCS, body condition score; WBC, white blood cells (10^3 counts/mm³); LYM, lymphocytes (10^3 counts/mm³); MON, monocytes (10^3
5 counts/mm³); GRAN, granulocytes (10^3 counts/mm³); RBC, red blood cells (10^6 counts/mm³); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%);

1 MCV, mean corpuscular volume (fl); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell
2 distribution width (%); PLT, number of platelets (10^3 counts/mm³); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%).
3 n, number; PA, Parda de Montaña; PI, Pirenaica; CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT,
4 dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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1 **Table 3.** Hematological parameters of the cows in the last month of pregnancy according to the breed and maternal nutrition

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	n	<u>Breed</u>			<u>Maternal nutrition</u>			<u>Significance</u>	
		PA 48	PI 35	s.e.d.	CONTROL 30	SUBNUT 53	s.e.d.	Breed	Maternal nutrition
	LW	634	602	11.8	611	626	12.1	ns	ns
	BCS	2.9 ^b	3.1 ^a	0.06	3.0	2.9	0.06	0.04	ns
White series	WBC	6.6	6.1	0.36	6.2	6.5	0.37	ns	ns
	LYM	3.3	3.7	0.24	3.7	3.7	0.24	ns	ns
	MON	0.43	0.48	0.05	0.46	0.46	0.05	ns	ns
	GRAN	2.5	2.2	0.31	2.3	2.4	0.32	ns	ns
	RBC	5.7 ^b	6.4 ^a	0.23	5.9	6.1	0.23	0.006	ns
Red series	HGB	10.3 ^b	11.5 ^a	0.23	10.7	11.1	0.23	0.002	ns
	HCT	30.2 ^b	33.2 ^a	1.2	31.2	32.2	1.22	0.015	ns
	MCV	53.2	52.3	1.29	53.1	52.4	1.31	ns	ns
	MCH	18.1	18.2	0.39	18.2	18.1	0.4	ns	ns
	MCHC	34.1 ^b	34.8 ^a	0.28	34.4	34.5	0.28	0.016	ns
	RDW	19.0 ^b	20.0 ^a	0.35	19.2	19.9	0.36	0.008	ns
Platelet series	PLT	250.7	256.8	31.1	259.0	248.5	31.6	ns	ns
	MPV	7.7	7.9	0.21	7.9	7.7	0.22	ns	ns
	PDW	8.6	8.8	0.5	8.7	8.7	0.51	ns	ns
	PCT	0.22	0.21	0.02	0.22	0.21	0.02	ns	ns

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4 ^{a-b}Means within a row with different superscripts differ significantly ($P < 0.05$); ns, not significant ($P > 0.05$).

5 LW, live weight; BCS, body condition score; WBC, white blood cells (10^3 counts/mm³); LYM, lymphocytes (10^3 counts/mm³); MON, monocytes (10^3
6 counts/mm³); GRAN, granulocytes (10^3 counts/mm³); RBC, red blood cells (10^6 counts/mm³); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%);

1 MCV, mean corpuscular volume (fl); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell
2 distribution width (%); PLT, number of platelets (10^3 counts/mm³); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%).
3 n, number; PA, Parda de Montaña; PI, Pirenaica; CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT,
4 dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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1 **Table 4.** Hematological parameters of the newborn calves in the first days of life according to the breed, maternal nutrition, and gender

	n	<u>Breed</u>			<u>Maternal nutrition</u>			<u>Gender</u>			<u>Significance</u>		
		PA 38	PI 21	s.e.d.	CONTROL 25	SUBNUT 34	s.e.d.	Female 32	Male 27	s.e.d.	Breed	Maternal nutrition	Gender
	LW	47.2	39.1	1.63	42.6	43.8	1.32	41.2	45.1	1.38	0.004	ns	0.007
White series	WBC	9.1	7.4	0.7	7.7	8.8	0.67	8.1	8.4	0.71	ns	ns	ns
	LYM	3.9	3.0	0.52	3.3	3.7	0.37	3.3	3.6	0.4	ns	ns	ns
	MON	0.10	0.26	0.09	0.24	0.12	0.09	0.17	0.19	0.10	ns	ns	ns
	GRAN	5.0	4.3	0.51	4.2	5.1	0.52	4.6	4.7	0.56	ns	ns	ns
Red series	RBC	8.3	8.0	0.49	8.0	8.3	0.33	8.4	7.8	0.35	ns	ns	ns
	HGB	10.8	10.3	0.72	10.5	10.6	0.47	10.9	10.2	0.5	ns	ns	ns
	HCT	34.2	31.4	2.61	32.4	33.2	1.48	34.0	31.6	1.59	ns	ns	ns
	MCV	41.3	39.5	0.94	40.8	39.9	0.88	40.3	40.4	0.60	ns	ns	ns
	MCH	13.1	12.9	0.18	13.2 ^a	12.8 ^b	0.17	12.9	13.1	0.18	ns	0.026	ns
	MCHC	31.7	32.6	0.44	32.3	32.1	0.22	32.1	32.2	0.23	ns	ns	ns
	RDW	25.1	24.6	0.57	24.4	25.3	0.54	25.0	24.7	0.57	ns	ns	ns
Platelet series	PLT	745.3	738.5	62.0	712.3	771.5	64.0	703.6	780.2	67.0	ns	ns	ns
	MPV	6.6	6.6	0.11	6.6	6.6	0.09	6.7 ^a	6.5 ^b	0.09	ns	ns	0.047
	PDW	7.8	7.7	0.22	7.7	7.8	0.2	8.0 ^a	7.5 ^b	0.22	ns	ns	0.019
	PCT	0.51	0.50	0.04	0.48	0.52	0.04	0.49	0.52	0.05	ns	ns	ns

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3 ^{a-b}Means within a row with different superscripts differ significantly ($P < 0.05$); ns, not significant ($P > 0.05$).

4 LW, live weight; WBC, white blood cells (10^3 counts/ mm^3); LYM, lymphocytes (10^3 counts/ mm^3); MON, monocytes (10^3 counts/ mm^3); GRAN, granulocytes
5 (10^3 counts/ mm^3); RBC, red blood cells (10^6 counts/ mm^3); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%); MCV, mean corpuscular volumes (fl);

1 MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell distribution width (%); PLT, number of
2 platelets (10^3 counts/mm³); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%).
3 n, number; PA, Parda de Montaña; PI, Pirenaica; CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT,
4 dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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1 **Table 5.** Correlations between the calf age (days 1 to 11) and their red and platelet hematological variables, without and with regard to maternal
2 nutrition

	Red series							Platelet series			
	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	PDW	PCT
<u>All calves</u>											
Corr.	-0.09	-0.16	-0.17	-0.25	-0.27	0.03	0.27	0.62	-0.39	-0.16	0.55
Sign.	ns	ns	ns	ns	0.041	ns	0.043	<0.001	0.004	ns	<0.001
<u>CONTROL calves</u>											
Corr.	-0.37	-0.47	-0.52	-0.59	-0.47	0.31	0.22	0.69	-0.31	-0.12	0.63
Sign.	ns	0.017	0.008	0.002	0.019	ns	ns	<0.001	ns	ns	0.001
<u>SUBNUT calves</u>											
Corr.	0.06	-0.01	0.01	-0.13	-0.24	-0.15	0.35	0.61	-0.48	-0.21	0.54
Sign.	ns	ns	ns	ns	ns	ns	0.043	<0.001	0.006	ns	0.001

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5 ns, not significant ($P > 0.05$).

6 RBC, red blood cells (10^6 counts/ mm^3); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%); MCV, mean corpuscular volume (fl); MCH, mean
7 corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell distribution width (%); PLT, number of platelets (10^3
8 counts/ mm^3); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%).

9 CONTROL, 100% fed group; SUBNUT, 65% fed group; Corr., Pearson's coefficient correlation; Sign., significance.

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1 **Table 6.** Metabolic and endocrine profiles of the newborn calves in their first days of life according to the breed, maternal nutrition, gender, and
 2 type of parturition

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	<u>Breed</u>			<u>Maternal nutrition</u>			<u>Gender</u>			<u>Parturition</u>				<u>Significance</u>				
	n	PA 38	PI 21	s.e.d.	CONTROL 25	SUBNUT 34	s.e.d.	Female 32	Male 27	s.e.d.	Unassisted 52	Easy- pulled 3	Hard- pulled 4	s.e.d.	Breed	Maternal nutrition	Gender	Parturition
Glucose		108.2	107.7	4.83	111.5	104.3	4.69	109.4	106.4	5.32	114.2	106.2	103.3	11.15	ns	ns	ns	ns
Urea		27.0	20.3	2.78	23.1	24.2	2.49	23.8	23.5	2.85	23.4	25.4	22.1	5.94	0.06	ns	ns	ns
NEFA		0.3	0.3	0.03	0.3	0.3	0.03	0.3	0.3	0.04	0.2 ^b	0.3 ^{ab}	0.4 ^a	0.08	ns	ns	ns	0.04
IGF-I		85.6	82.2	10.90	106.1 ^a	61.7 ^b	10.40	98.0 ^a	69.8 ^b	11.81	80.9	58.9	111.9	24.75	ns	0.0001	0.02	ns
Cortisol		41.9	33.7	12.71	29.0 ^b	46.5 ^a	5.89	38.7	36.9		28.1	28.0	57.2	14.13	ns	0.005	ns	0.07

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6 ^{a-b}Means within a row with different superscripts differ significantly ($P < 0.05$); ns, not significant ($P > 0.05$)

7 Glucose, (mg/dl); Urea, (mg/dl); NEFA, non-esterified fatty acids (mmol/l); Cortisol, (nmol/l); IGF-1, insulin-like growth factor 1 (ng/ml).

8 n, number; PA, Parda de Montaña; PI, Pirenaica; CONTROL, calves whose mothers were fed 100% of their nutritional requirements from day 0 to day 82 of

9 pregnancy; SUBNUT, calves whose mothers were fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the

10 difference. Hard-pulled, fetus extractor was used in parturition; Easy-pulled, hand or rope assistance was used in parturition; Unassisted, no assistance in

11 parturition.

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