i) Title page

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

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ii) Abstract and keywords

This experiment evaluated the effects of subnutrition during early gestation on hematology in cows and on hematological, metabolic, endocrine, and vitality parameters in their calves. Parda de Montaña (PA) and Pirenaica (PI) dams were inseminated and assigned to either a control (CONTROL, 100% requirements) or a nutrient-restricted group (SUBNUT, 65%) during the first third of gestation. Dam blood samples were collected on days 20 and 253 of gestation, and calf samples were obtained during the first days of life. Pirenaica dams presented higher red series parameters than PA dams, both in the first and the last months of gestation. During early pregnancy, granulocyte numbers and mean corpuscular hemoglobin were lower in PI-SUBNUT than in PI-CONTROL cows. Calves from the SUBNUT cows did not show a physiological reduction in red series values in early life, suggesting later maturation of the hematopoietic system. Poor maternal nutrition clearly affected the calf endocrine parameters. Newborns from dystocic parturitions showed lower NEFA concentrations and weaker vitality responses. In conclusion, maternal nutrition had short-term effects on cow hematology, PI cows showing a higher susceptibility to undernutrition, and a long-term effect on their offspring endocrinology, SUBNUT newborns showing higher levels of IGF-1 and lower levels of cortisol.

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

iii) Text

1. Introduction

Beef cattle (*Bos taurus*) production systems have adapted to increasingly extensive management by reducing feed costs. Depending on food availability, cows can suffer periods of undernutrition during some phases of their production cycle, sometimes concomitantly with the rearing of a calf or/and in early pregnancy. Implantation of the embryo and the maternal recognition of pregnancy at approximately day 20 post-conception are critical points of gestation (Spencer & Hansen, 2015). Moreover, the peri-implantation period is a crucial time for embryo survival, and it could be a potentially vulnerable period during which adverse programming mediated through poor maternal nutrition might begin. Altered placental angiogenesis, cotyledon weight, and fetal development in beef cattle (Long, Vonnahme, Hess, Nathanielsz, & Ford, 2009; Vonnahme et al., 2007), cardiovascular abnormalities in mice (Watkins et al., 2008), altered cardiovascular activity (Torrens et al., 2009), or suppressed behavioral reactions in response to stressful conditions in ewes (Hernandez, Matthews, Oliver, Bloomfield, & Harding, 2010) have all demonstrated the risk of adverse developmental programming and increased chronic disease incidence attributed to peri-conceptional undernutrition (Fleming, Velazquez, Eckert, Lucas, & Watkins, 2012).

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

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

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

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

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

2. Materials and methods

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

2.1. Animals and management

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

55 kg live weight (LW); 2.73 ± 0.26 body condition score (BCS) on a 5-point scale) and 40 PI (579 ± 51 kg LW; 2.95 ± 0.28 BCS) multiparous cows rearing calves were synchronized to estrus at 65 ± 14 days postpartum with a protocol based on a progesterone-releasing intravaginal device (PRID Delta 1.55 g, CEVA, Loudéac, France) and a 10 μg injection of GnRH (Busol, INVESA, Barcelona, Spain), followed 7 days later by 150 μg of prostaglandin F2α (Galapán, INVESA, Barcelona, Spain). After 9 days, the PRID was removed and 500 IU of pregnant mare serum gonadotropin (Serigan, Laboratorios Ovejero, León, Spain) was administered, followed 48 hours later by a second injection of GnRH (10 μg). Eight hours after the final GnRH injection, the cows were randomly inseminated with proven fertility sires (4 PA and 3 PI) by an expert technician.

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

2.2. Measurements and blood sampling

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

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

2.3. Hematology, hormone, and metabolite assays

Unclotted (EDTA) whole-blood samples from the cows and calves were analyzed using a fluorescent flow cytometry analyzer (Sysmex XT-2000i V, Sysmex Corporation, Kobe, Japan) standardized for the analysis of bovine blood. Hematological analyses included hematocrit (HCT, expressed as a percentage), hemoglobin concentration (HGB, g/dl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin concentration (MCHC, g/dl), red blood cell count (RBC, 10^6 counts/mm^3), red blood cell distribution width (RDW, percentage), white blood cell count (WBC, 10^3 counts per mm^3, including the different leukocyte subtypes: granulocytes (GRAN), lymphocytes (LYM) and monocytes (MON)), number of platelets (PLT, 10^3 counts/mm^3), mean platelet volume (MPV, fl), platelet distribution width (PDW, fl), and plateletcrit (PCT, percentage).

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

2.4. Statistical analyses

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

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

3. Results and discussion

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

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

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

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

Maternal nutrition showed a minor effect on cow hematology values on day 20 post-AI, but a significant interaction between breed and maternal nutrition was observed in the granulocyte counts. The values did not differ between maternal nutrition treatments in the PA cows (3.3 *vs* 3.4 x10^3 GRAN counts/mm^3 for the PA-CONTROL and the PA-

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

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

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

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

All of the cow hematological parameters registered in the last month of gestation were within the bovine reference range (Roland et al., 2014) except for MPV, which in all of the groups was higher than that referenced due to a physiological increase in the last stage of gestation (Fay, Hughes, & Farron, 1983). The effects of the cow breed, nutrition, and age on these data were analyzed. Regarding the breed effect, no significant differences were found in the white series between the PA and the PI ($P > 0.05$). However, the breed affected most of the red blood cell parameters. The PA cows had lower values of RBC and HGB (P < 0.01) and HCT (P < 0.05) than the PI cows, in agreement with previous observations in early pregnancy and with results obtained by García-Belenguer et al. (1996). Parda de Montaña also exhibited lower MCHC (P < 0.05) and RDW (P < 0.01) than PI dams. No significant differences in the platelet series were observed (P > 0.05). These results confirmed that, in physiological conditions, the values of the red series were higher in the PI cows, which provides evidence of inter-breed differences that could imply a better adaptation to altitude conditions than the PA dams, in line with a study by Bianca and Näf (1979). Blood parameters are considered important indicators for measuring the adaptation of animals to altitude, which induces hematopoiesis as an adaptive mechanism (Wuletaw et al., 2011).

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

3.3. Hematological parameters of newborn calves

The calves' LW and blood cell values are displayed in Table 4. The live weights of the newborn calves were higher in the PA breed (P < 0.01) than in the PI, in line with previous studies of the same breeds (Álvarez-Rodríguez, Palacio, Casasús, & Sanz, 2010). As expected, the male calves were heavier than the females (P < 0.01). However, no differences were found in the calf LW at birth that could be ascribed to maternal nutrition (P > 0.05). Accordingly, Mossa et al. (2013) did not find weight differences in calves born to nutrient restricted and control heifers that were fed at 0.6 and 1.2 of their requirements, respectively, during the 110 first days of gestation.

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

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

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

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

The correlations between calf age (days 1 to 11) and the hematological values without and with regard to maternal nutrition are displayed in Table 5. No significant correlation between calf age (1-11 days) and any variable of the white series was observed ($P > 0.05$). However, most of the red and platelet hematological parameters were related to calf age. Calf age negatively correlated with the MCH and positively correlated with the RDW. Other studies have shown that the HCT, MCV, and HGB concentrations tend to decrease during the first days of life (Knowles et al., 2000; Probo et al., 2012). During intrauterine life, the fetus has a relatively hypoxic environment and requires larger erythrocytes to compensate for this situation. During the first days of life, former erythrocytes containing fetal hemoglobin are replaced by new smaller erythrocytes containing hemoglobin A (Brun-Hansen, Kampen, & Lund, 2006). Thus, the decrease in the HGB, HCT, MCV, and MCH values is a physiological process during the first days of life, which was also described by Brun-Hansen et al. (2006) and Mohri, Sharifi, and Eidi (2007). In the platelet series in the current study, both the PLT and PCT increased as the days passed, but the MPV decreased. The correlation between calf age and the PLT agrees with the results of Roland et al. (2014), who described that in correctly developing newborns, the platelet number increases significantly during the first 2 weeks of age and more slowly thereafter over the first 3 months.

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

3.4. Metabolite and endocrine profiles of newborn calves

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

Maternal nutrition clearly affected the endocrine profiles, with the CONTROL calves showing higher IGF-1 concentrations ($P < 0.001$) and lower cortisol values ($P < 0.01$) than the SUBNUT calves. Insulin-like growth factor-1 is a hormone involved in muscle growth and is positively related so energy and protein intake (Blanco, Joy, Ripoll, Sauerwein, & Casasús, 2011; Paradis et al., 2015), which increases its plasma concentration with improved nutritional status (Rodríguez-Sánchez, Sanz, Tamanini, & Casasús, 2015). Similar to previous research, in the current study, the IGF-1 concentration was positively related to the circulating glucose ($r = 0.43$, $P < 0.001$), although no significant differences between the maternal nutrition groups were found in glucose concentrations of the offspring ($P > 0.05$). Hoffman et al. (2016) found no differences in the glucose, triglyceride, and cholesterol concentrations in lambs born to poorly nourished ewes. Conversely, Maresca et al. (2018) described higher glucose concentrations during the first 60 days of life in calves whose mothers had received a low-protein diet from mid-gestation to parturition, supporting the hypothesis of Gardner et al. (2005) that maternal subnutrition during pregnancy could alter the capacity of calves to regulate plasma glucose concentrations during postnatal growth. However, glucose concentrations characterize nutritional status in the short term, and therefore the lack of differences in the current study could indicate that the newborns received a similar diet during their first days of life and their glucose metabolism was not altered. During calf feeding in the first days of life, based only on the maternal colostrum and milk, the CONTROL group could have taken better advantage of the nutritional resources, producing higher IGF-1 concentrations that could have improved their tissue growth and metabolism. Maternal undernutrition may reprogram the fetal IGF-1 system in its ability to respond to acute changes in the substrate supply (Gallaher, Breier, Keven, Harding, & Gluckman, 1998). Similarly, fetal IGF-1 concentration may be altered by maternal nutrition during the earlier stages of development (Rhind, 2004) and the level of protein intake from mid-gestation to parturition can affect calf IGF-1 at birth (Maresca et al., 2018). Accordingly, other authors found a greater reduction in IGF-1 levels in the fetus (Gallaher et al., 1998) and in the lamb (Hoffman, Rokosa, Zinn, Hoagland, & Govoni, 2014) after maternal subnutrition in sheep.

Poor maternal nutrition increased the circulating cortisol levels in the offspring in this study. Cortisol is a hormone synthesized in the adrenal cortex. Its production increases under stress conditions, and consequently it is used as an indicator of stress (Möstl, Maggs, Schrötter, Besenfelder, & Palme, 2002) and animal welfare (Cook, Schaefer, Lepage, & Jones, 1996). Cortisol concentrations can also reflect the nutritional state of an animal (Rhind, 2004). In fact, maternal undernutrition can increase the cortisol concentration in the fetus (Binienda et al., 1990) and thus in the newborn calf, in line with the results of the current study. Maternal nutrient restriction can be a cause of prenatal stress, modifying the hypothalamus-pituitary-adrenal function (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006). Moreover, maternal corticosteroids can induce fetal growth retardation, with lower plasma IGF-1 concentrations. Any delay in fetus development due to maternal undernutrition can lead to a greater fetal cortisol response to undernutrition in late gestation and therefore a greater decrease in IGF-1 (Gallaher et al., 1998). Accordingly, in the current study, a negative correlation between cortisol and IGF-1 ($r = -0.29$, $P < 0.05$) was found in the newborn calves. In fact, the increases in the circulating cortisol level in the SUBNUT calves could have contributed to many metabolic changes and modifications of the immune competency of the newborns.

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

Finally, the type of parturition affected the NEFA concentrations, since the hard-pulled calves presented higher concentrations than the unassisted calves ($P < 0.05$). Furthermore, a tendency was observed in the cortisol concentrations, as the hard-pulled calves showed greater concentrations than the unassisted calves ($P = 0.07$). Negative correlations between the cortisol concentrations and lymphocyte number count ($r = -0.29$, $P < 0.05$) and glucose concentrations ($r = -0.37$, $P < 0.01$) were found. Difficult calving is a stressful situation that increases the plasma cortisol levels in both dams and calves throughout the stimulation of the adrenocorticotropic hormone release (Civelek, Celik, Avci, & Cingi, 2008). As a consequence of the cortisol release, the plasma glucose levels rise due to the increase in liver gluconeogenesis (Drackley, Overton, & Douglas, 2001). In the current study, the calves from dystocic parturitions presented the highest plasma cortisol concentrations, but no statistical differences in the glucose concentrations were found among the groups ($P > 0.05$). Furthermore, a negative correlation was found between cortisol and glucose, suggesting that although the hard-pulled calves should have presented greater glucose concentrations due to their high cortisol concentrations, they did not, most probably due to the low carbohydrate intake of the weakened calves. They needed more time to recover and start ingesting colostrum, and milk later, in the hours after birth. This ingestion delay diminished their glucose and glycogen body reserves. Thus, the calves had to metabolize lipids as an alternative energy source, increasing the NEFA blood concentrations in the calves from dystocic births. Accordingly, a negative correlation was found between the glucose and NEFA concentrations ($r = -0.41$, $P < 0.01$).

3.5. Vitality test of newborn calves

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

Maternal nutrition had no effect on any value of the calf vitality test ($P > 0.05$). High dam BCS at parturition has been described as an important factor that can hinder the parturition process, with negative effects on newborn vitality (Lorenz, Mee, Earley, & More, 2011). Thus, the lack of maternal nutrition effects on the vitality test could be explained because in the current study, no difference in the dam BCS in the last month of gestation was found between the CONTROL and SUBNUT groups.

The type of parturition highly influenced the vitality test results. In general, the parturitions required little assistance, with 53 unassisted, 3 easy-pulled, and 3 hard-pulled parturitions. In the meconium staining test, the unassisted parturitions had the lowest percentage of calves with stained anal areas (2, 33, and 33% for unassisted, easy-pulled, and hard-pulled parturitions, respectively, $P < 0.05$). Furthermore, fewer calves with swollen or protruding tongues were in unassisted than in hard-pulled parturitions (2, 33, and 100%, for unassisted, easy-pulled, and hard-pulled parturitions, respectively, $P < 0.05$). Most of the calves from the unassisted and easy-pulled parturitions attempted to stand during the calf attitude test (87, 100, and 0%, respectively, $P < 0.05$) and had a strong finger suckling reflex (85, 100, and 0%, respectively, $P < 0.05$) compared to the calves from the hard-pulled parturitions. Contrarily, the type of parturition did not affect the palpebral test or the mucous membrane color ($P > 0.05$). These results confirmed that after the dystocic births, especially in the hard-pulled parturitions when a fetus extractor was used, the newborns were depressed and had weaker responses to vitality controls, compromising neonatal survival. The premature rupture of the umbilical vessels terminates the oxygen supply from the placenta, first causing respiratory acidosis in the fetus, and if the hypoxia is severe enough, metabolic acidosis later occurs (Murray & Leslie, 2013). Cyanosis of the mucous membranes is a sign of prolonged dystocia, and a weak response or no response to stimulation and poor muscle tone can indicate prolonged and non-compensated acidosis due to fetal hypoxia. Metabolic acidosis is the main cause of suckling reflex loss (Mee, 2008b). Similar to the results of the current study, Schafer and Arbeiter (1995) found that calves with lower vitality test scores had higher plasma cortisol concentrations, with lower levels of lymphocytes and larger neutrophils.

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

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

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vi) Tables

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

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

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

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

		Breed			Maternal nutrition			Significance		
		PA	PI	s.e.d.	CONTROL	SUBNUT	s.e.d.	Breed	Maternal Nutrition	Breed x maternal nutrition
	n	74	40		52	62				
	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
White series	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

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

LW, live weight; BCS, body condition score; 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 (10^3 counts/mm^3); RBC, red blood cells (10^6 counts/mm^3); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%);

MCV, mean corpuscular volume (fl); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell distribution width (%); PLT, number of platelets (10^3 counts/mm^3); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%). 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, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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

		Breed			Maternal nutrition			Significance	
		PA	PI	s.e.d.	CONTROL	SUBNUT	s.e.d.	Breed	Maternal nutrition
	n	48	35		30	53			
	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
Red series	RBC	5.7^{b}	6.4^{a}	0.23	5.9	6.1	0.23	0.006	ns
	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

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

LW, live weight; BCS, body condition score; 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 (10^3 counts/mm^3); RBC, red blood cells (10^6 counts/mm^3); HGB, hemoglobin concentration (g/dl); HCT, hematocrit (%);

MCV, mean corpuscular volume (fl); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell distribution width (%); PLT, number of platelets (10^3 counts/mm^3); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%). 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, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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

		Breed			Maternal nutrition			Gender			Significance		
		PA	PI	s.e.d.	CONTROL	SUBNUT	s.e.d.	Female	Male	s.e.d.	Breed	Maternal nutrition	Gender
	n	38	21		25	34		32	27				
	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

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

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 (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);

MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dl); RDW, red cell distribution width (%); PLT, number of platelets (10^3 counts/mm^3); MPV, mean platelet volume (fl); PDW, platelet distribution width (fl); PCT, plateletcrit (%).

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, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; s.e.d., standard error of the difference.

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

	Red series							Platelet series			
	RBC	**HGB**	**HCT**	**MCV**	**MCH**	**MCHC**	**RDW**	**PLT**	**MPV**	**PDW**	**PCT**
All calves											
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
CONTROL calves											
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
SUBNUT calves											
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

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

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

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

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

	Breed			Maternal nutrition			Gender			Parturition				Significance			
	PA	PI	s.e.d.	CONTROL	SUBNUT	s.e.d.	Female	Male	s.e.d.	Unassisted	Easy-pulled	Hard-pulled	s.e.d.	Breed	Maternal nutrition	Gender	Parturition
n	38	21		25	34		32	27		52	3	4					
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

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

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).

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