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How does peri-implantational subnutrition affect red blood cell parameters in two beef breeds?

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Undernutrition is common in extensive beef cattle farming systems at some stages of the production cycle. A poor nutrient diet during the peri-implantation period can interfere with the correct foetal development. The aim of this study was to analyse the effects of peri-implantational undernutrition on red blood cell parameters in dams and calves of two beef breeds. Seventy-four lactating Parda de Montaña (PA) and 40 Pirenaica (PI) multiparous cows were artificially inseminated and randomly allocated to a control (CONTROL, n=52) or nutrient-restricted (SUBNUT, n=62) group, which were fed at 100 or 65% of their estimated energy requirements during the first 82 days of pregnancy, and thereafter received a control 100% diet until parturition. Red blood cell count (RBC), haemoglobin content (HGB) and haematocrit (HCT) were determined on day 19 post artificial insemination and one month before parturition for dams, and once on the first days of life (between 1 and 11) for calves. At the beginning of pregnancy, PI dams showed higher values than PA dams for RBC (6.8 vs 6.1×10^6 counts/mm^3, for PI and PA respectively, P<0.001), HGB (12.6 vs 10.8 g/dl, for PI and PA, P<0.001) and HTC (37.2 vs 32.1%, for PI and PA, P<0.001). These differences were maintained one month before parturition for RBC (6.41 vs 5.7×10^6 counts/mm^3, for PI and PA, P<0.01), HGB (11.5 vs 10.3 g/dl, for PI and PA, P<0.01) and HTC (33.2 vs 30.2%, for PI and PA, P<0.05). No differences in haematological profiles were found due to undernutrition (P>0.05). In calves, neither breed nor feeding treatment influenced the red blood series profiles (P>0.05). A negative correlation between calf age and haematological parameters was observed only in CONTROL calves (R^2=-0.37 for RBC, P=0.069; R^2=-0.47 for HGB, P<0.05; R^2=-0.52 for HTC, P<0.01), suggesting an earlier maturation of the haematopoietic system in these calves. More studies during gestation and other phases of calf development are needed to assess the effects of undernutrition during the peri-implantation period.

Plasma and muscle responses to pre-slaughter mixing of suckler bulls

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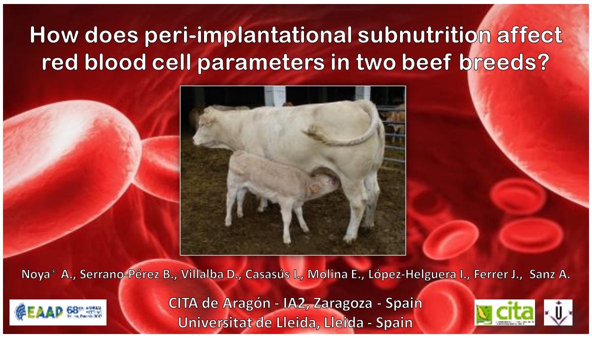
Meat colour is an important influence on the purchase decision of the consumer; 'bright red' is preferred. Dark, firm, dry beef with ultimate pH (pHu)>5.9 is typically ascribed to pre-slaughter stress but the relationship between animal interactions pre-slaughter and dark beef is uncertain. The objective was to determine the impact of mixing unfamiliar bulls, the day before slaughter, on stress-related plasma variables and beef pH and colour. Prior to mixing, Charolais-sired suckler bulls (mean (SD) live weight 671 (71.6) kg and age 17.4 (2.01) months) were housed indoors in 7 slatted floor pens (4 to 5 bulls/pen, 2.5-3.0 m^2/bull) and offered a barley-based ration and grass silage *ad libitum*. Two pens of 5 bulls were chosen as controls. From the other pens, 18 bulls were selected and moved to a single new pen (outdoors, bedded with wood chip, 6 m^2/bull) 18 h before slaughter. Bulls were then transported (45 min) without further mixing to an abattoir and slaughtered on arrival. Blood was collected for plasma preparation and muscle pH was recorded periodically post-mortem. At 48 h post-mortem, the left half of the carcass was cut at the 5/6th rib interface and muscle pH and lightness (L*) measured. Muscle was collected for measurement of glycolytic potential (GP) and drip loss (DL). Data were analysed by one-way ANOVA. Mixing increased plasma creatine kinase activity (14,556 vs 145 U/l, P<0.01) but cortisol, lactate and creatinine concentrations were not affected. Muscle GP was decreased (84 vs 179 µmol lactate equivalents/g, P<0.001) and pH at 1.5, 3, 4.5 and 6 h post-mortem increased (P<0.05) by mixing. Muscle from mixed bulls had higher pH (5.98 vs 5.55, P<0.05), was darker (lower L*; 25.5 vs 39.9, P<0.05) and had lower (P<0.05) DL (g/kg). While the maximum pH in muscle from control bulls was 5.60, six mixed bulls had muscle pH≤5.60. It is concluded that mixing of bulls increased some stress-related plasma indicators. That not all mixed bulls had high muscle pHu illustrates the complexity of the relationship between pre-slaughter stress and muscle biochemistry.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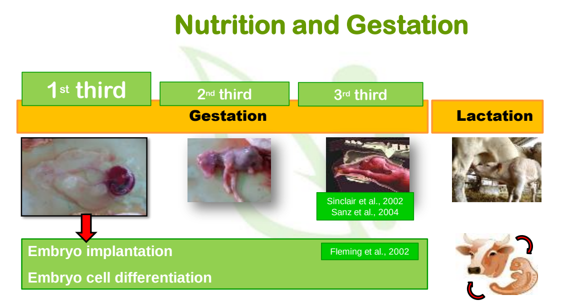
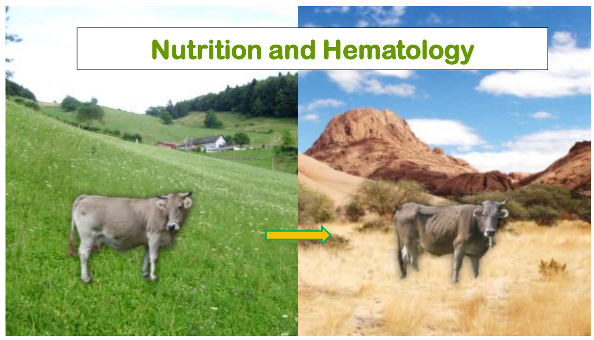
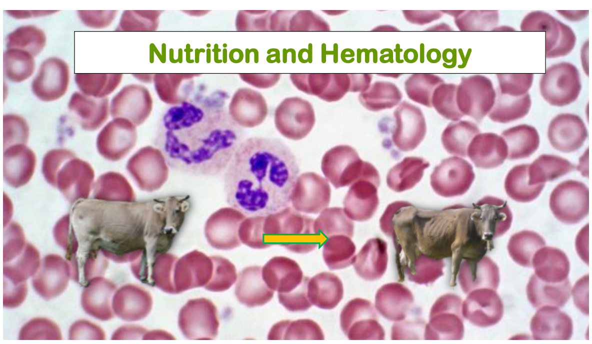
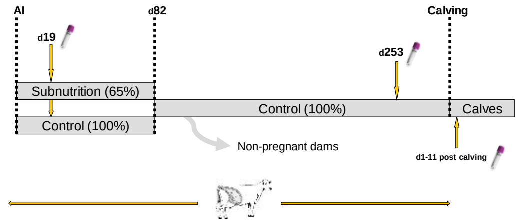

Nutrition and Gestation
1st third
2nd third
3rd third
Gestation
Lactation
Sinclair et al., 2002
Sanz et al., 2004
Embryo implantation
Fleming et al., 2002
Embryo cell differentiation

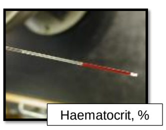

Nutrition and Hematology


Nutrition and Hematology

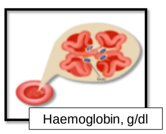

Nutrition and Hematology

Methodology




Haematocrit, %


Red blood cell counts, $10^6/mm^3$


Haemoglobin, g/dl

Results of dams

Early gestation (d 19 post AI)

	Breed		Feeding treatment	
	Parda	Pirenaica	CONTROL	SUBNUT
Red Blood Cell (10^6 counts/mm^3)	6.1 ^{b}	6.8 ^{a}	6.4	6.5
Haemoglobin (g/dl)	10.8 ^{b}	12.6 ^{a}	11.7	11.8
Haematocrit (%)	32.1 ^{b}	37.2 ^{a}	34.3	34.9
			a ≠ b, P<0.001	

Only 19 days of undernutrition

Low requirements in early gestation

Results of dams

One month before parturition (d 253 post AI)

	Breed		Feeding treatment	
	Parda	Pirenaica	CONTROL	SUBNUT
Red Blood Cell (10^6 counts/mm^3)	5.7 ^{b}	6.4 ^{a}	5.9	6.1
Haemoglobin (g/dl)	10.3 ^{b}	11.5 ^{a}	10.7	11.1
Haematocrit (%)	30.2 ^{b}	33.2 ^{a}	31.2	32.2
			a ≠ b, P<0.05	

Is Pirenaica better adapted to high mountain (altitude) conditions?

Results of calves

(d 1 – d 11after birth)

	Breed		Feeding treatment	
	Parda	Pirenaica	CONTROL	SUBNUT
Red Blood Cell (10^6 counts/mm^3)	8.3	8.0	8.0	8.3
Haemoglobin (g/dl)	10.8	10.3	10.5	10.6
Haematocrit (%)	34.2	31.4	32.4	33.2
				P> 0.05

The physiological red blood cell differences observed in adults must be acquired after birth

Results of calves

Correlations

CALF AGE (control calves)	R^2	P value
Red blood cell counts	-0.37	0.069
Haemoglobin level	-0.47	<0.05
Haematocrit	-0.52	<0.01

Results

Calves (d 1 – d 11 after birth)

Relatively hypoxic environment

Physiological anaemia of the newborn calf

- Bigger foetal eritrocytes
  ↑ RBC counts and Haematocrit
- Higher haemoglobin content

- Smaller corpuscular volume
  ↓ RBC counts and Haematocrit
- Lower haemoglobin content

Conclusions

BREED

- Breed had a great effect on red blood cell parameters of **adult cows**:
  Red blood cell, haematocrit and haemoglobin content: Pirenaica > Parda de Montaña.
- This breed effect might not be congenital but **acquired during life**:
  No differences observed in calves during the first days of life.

NUTRITIONAL TREATMENT

- The undernutrition applied had **no effect** on the red series profiles:
  neither in cows (third week of pregnancy or one month before calving)
  nor on newborn calves values.
- However, control calves experimented physiological changes earlier than undernourished calves, suggesting an earlier **development of the haematopoietic system**.

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How does peri-implantational subnutrition affect red cell parameters in two beef cattle breeds?

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ABSTRACT

Undernutrition is common in extensive beef cattle farming systems at some stages of the production cycle. A poor nutrient diet during the peri-implantation period can interfere with the correct foetal development. The aim of this study was to analyse the effects of peri-implantational undernutrition on red series profiles in dams and calves of two beef breeds. Seventy-four lactating Parda de Montaña (PA) and 40 Pirenaica (PI) multiparous cows were artificially inseminated and randomly allocated to a control (CONTROL, n=52) or nutrient-restricted (SUBNUT, n=62) group, which were fed at 100 or 64% of their estimated energy requirements during the first 82 days of pregnancy, and thereafter received a control 100% diet until parturition. Red blood cell counts (RBC), haemoglobin content (HGB) and haematocrit (HCT) were determined on day 19 post AI and one month before parturition for dams, and once on the first days of life (between 1 and 11) for calves. Pirenaica dams showed higher values than PA dams, both at the start and at the end of pregnancy; but no differences in haematological profiles were found due to undernutrition. In calves, neither breed nor feeding treatment influenced the red series profile. A negative correlation between calf age and haematological parameters was observed only in CONTROL calves, suggesting an earlier maturation of the haematopoietic system in these calves. More studies during gestation and other phases of calf development are needed to assess the effects of undernutrition during the peri-implantation period.

Keywords: haematological parameters, undernutrition, early gestation.

1. INTRODUCTION

Beef cattle production systems have adapted to increasingly extensive conditions with a reduction in feed costs. Depending on food availability, cows can suffer periods of undernutrition in some phases of their production cycle, sometimes concomitantly with the rearing of a calf and with an early stage of pregnancy. Peri-implantation is a crucial time for embryo development, and could be a potentially vulnerable period, during which adverse programming mediated through poor maternal nutrition might initiate (Fleming et al., 2012).

A reduced nutrient intake, either because of poor diet composition or low quantity, will affect cattle haematological profiles, i.e. a low protein diet affects different haematological variables, as haematocrit, haemoglobin or serum protein content (Meacham et al., 1964; O'kelly and Seifert, 1969). Interbreed differences must be considered in this regard (García-Belenguer et al., 1996), which may interact with nutritional level. Parda de Montaña (PA) and Pirenaica (PI) are two beef cattle breeds adapted to semi-extensive system of animal husbandry in the Pyrenees mountain region.

The aim of this study was to evaluate the effect of undernutrition during the peri-implantation period on red blood cell parameters in PA and PI beef cattle at the start and end of gestation, and in their newborn calves.

2. MATERIAL AND METHODS

2.1. Animal management

Lactating 74 PA (560 ± 55 kg live weight (LW); 2.73 ± 0.26, in a 5 point scale, body condition score (BCS)) and 40 PI (579 ± 51 kg LW; 2.95 ± 0.28 BCS) multiparous cows were synchronized to estrus and inseminated at 66 ± 10.9 days postpartum with proven fertility sires (4 PA and 3 PI, respectively). On the day of artificial insemination (AI), dams were randomly allocated to two feeding treatments with a total mixed ration (10.96 MJ ME/kg DM and 124 g CP/kg DM) during the first 82 days of pregnancy. The control group (CONTROL, n= 52) was fed a diet that supplied 100% of cow estimated energy requirements for maintenance, lactation and gestation (10.9 and 10.0 kg DM/cow/day for PA and PI, respectively), whereas the nutrient-restricted group (SUBNUT, n=62) received 64% of their requirements (7.0 and 6.4 kg DM/cow/day for PA and PI, respectively). After this treatment phase, all dams were fed at 100% requirements until parturition.

2.2. Blood sample collection

Blood samples were collected into EDTA tubes from all dams by coccygeal venipuncture at day 19 post AI (peri-implantation) and in those who conceived to AI, again one month before parturition (253 day post AI, pre-calving). Calf samples were collected once during the first days of life (between day 1 and 11) by jugular venipuncture. All blood samples were maintained under refrigeration (4^{o}C) and were analysed in the next 8 hours.

2.3. Haematology

Unclotted whole-blood samples were analysed using a fluorescent flow cytometry analyzer (Sysmex XT-2000 i V, Sysmex Corporation, Kobe, Japan) standardized for the analysis of bovine blood. The red cell parameters studied were: red blood cell count (RBC, 10^6 counts/mm^3), haemoglobin level (HGB, g/dl) and haematocrit (HCT, %).

2.4. Data collection and statistical analysis

Data were analysed with SAS and JMPro statistical software (SAS Institute Inc., Cary, NC). Haematological values were analysed through analysis of variance with a general linear model (GLM) with age, pregnancy status, gender of the calf, breed, feeding treatment and their interactions as fixed effects. Sire of AI was considered an aleatory effect. The relationship between age of calves and haematological values was determined through Pearson’s correlation coefficients. All statistical analyses were considered significant at the P value < 0.05. Values are expressed as the LS mean ± standard error.

3. RESULTS AND DISCUSSION

All red series haematological values were within the bovine reference range for adults and calves (Brun-Hansen et al., 2006; Roland et al., 2014). No interaction between fixed effects was observed.

3.1. Haematological parameters of dams in early gestation

On day 19 post AI, only the breed had a significant effect on red blood cell parameters in early pregnancy (Table 1). Pirenaica had higher values for RBC, HGB and HCT than PA (P<0.001 in all variables). These results agree with those of García-Belenguer et al. (1996), and suggest that, in standard conditions, values of the red series parameters are greater in PI cows.

In this period, feeding treatment had no effect on these parameters (P>0.05), which suggests that the nutrient restriction applied to the SUBNUT group did not trigger immediate deficiencies in specific elements involved in the haematopoiesis process, like iron, folate or vitamin B12 (Brun-Hansen et al., 2006; Roland et al., 2014; Sertoglu et al., 2015).

This lack of effect of undernutrition could be ascribed, on one hand, to the short duration of underfeeding at the time of sampling (19 days), and on the other hand, to the fact that metabolic and nutritional requirements in early gestation were still low.

Table 1. Red cell parameters of dams on day 19 post-AI, according to breed and feeding treatment.

	Breed		Feeding treatment		Significance	
	PA	PI	CONTROL	SUBNUT	Breed	Feeding treatment
RBC	6.1 ± 0.09	6.8 ± 0.11	6.4 ± 0.1	6.5 ± 0.09	***	ns
HGB	10.8 ± 0.14	12.6 ± 0.17	11.7 ± 0.16	11.8 ± 0.15	***	ns
HCT	32.1 ± 0.44	37.2 ± 0.53	34.3 ± 0.51	34.9 ± 0.48	***	ns

RBC, red blood cells (10^6 counts/mm^3); HGB, haemoglobin content (g/dl); HCT, haematocrit (%).
ns, not significant (P>0.05); *, P<0.05; **, P<0.01; ***, P<0.001; PA, Parda de montaña; PI, Pirenaica

3.2. Haematological parameters of dams one month before parturition

One month before parturition, the feeding treatment applied in the peri-implantational phase had no effects on the haematological variables (P>0.05) (Table 2). Again, the only differences were attributed to the breed. Consistent with the previous observations at the beginning of pregnancy, and the results obtained by García-Belenguer et al. (1996), PA presented lower values for RBC and HGB (P<0.01), and also for HCT (P<0.05), than did PI cows.

These results would confirm that, in physiological conditions, PI has higher values of red series parameters than PA, perhaps because Pirenaica is a hardy beef breed, which could be better adapted to altitude conditions than PA (Bianca and Näf, 1979).

Table 2. Red cell parameters of dams one month before parturition, according to breed and feeding treatment.

	Breed		Feeding treatment		Significance	
	PA	PI	CONTROL	SUBNUT	Breed	Feeding treatment
RBC	5.7 ± 0.16	6.4 ± 0.21	5.9 ± 0.21	6.1 ± 0.16	**	ns
HGB	10.3 ± 0.26	11.5 ± 0.36	10.7 ± 0.36	11.1 ± 0.28	**	ns
HCT	30.2 ± 0.83	33.2 ± 1.14	31.2 ± 1.12	32.2 ± 0.86	*	ns

RBC, red blood cells (10^6 counts/mm^3); HGB, haemoglobin content (g/dl); HCT, haematocrit (%).
ns, not significant (P>0.05); *, P<0.05; **, P<0.01; ***, P<0.001; PA, Parda de montaña; PI, Pirenaica

3.3. Haematological parameters of newborn calves

Calf red blood counts did not show any differences due to either breed or dam feeding treatment in early pregnancy (P>0.05). It is remarkable that the breed differences in RBC, HGB and HCT observed in adult animals did not occur in newborn calves, which could mean that these characteristics inherent to the breed are acquired after birth. These results agree with García-Belenguer et al. (1996) and Blanco et al. (2009), who did not find significant differences in red blood cell counts between breeds, studying PA and PI calves of 2 to 5 months of age.

Considering the feeding treatment of their mothers, only calves from CONTROL dams showed a significant negative correlation between calf age and HGB (R^2 = -0.47, P<0.05) and HCT values (R^2 = -0.52, P<0.01). Red blood cell counts also tended to decrease with age (R^2 = -0.369, P = 0.069). These relationships could be explained because in the first days of life, foetal erythrocytes are replaced by the newly formed calf erythrocytes, smaller than former ones (Roland et al., 2014). In a normal development, the calf experiments a reduction in red series parameters, known as physiological anaemia of the newborn (Tennant et al., 1974; Jaime Perez, 2012). During the intrauterine life, the foetus has a relative hypoxic environment, and needs more and bigger erythrocytes to compensate this situation. In the first days of life, red cells containing foetal haemoglobin will be replaced by new smaller red cells with haemoglobin A (Brun-Hansen et al., 2006). Hence, the lack of decrease of the red series values of the SUBNUT calves could suggest a delay in the newborn erythropoiesis process to replace foetal erythrocytes. We hypothesize that in control newborns, bone marrow is active and mature enough to start this process immediately after birth.

Table 3. Red cell parameters of calves on first days of life.

	Breed		Feeding treatment		Significance	
	PA	PI	CONTROL	SUBNUT	Breed	Feeding treatment
RBC	8.3 ± 0.3	8.0 ± 0.4	8.0 ± 0.3	8.3 ± 0.3	ns	ns
HGB	10.8 ± 0.4	10.3 ± 0.6	10.5 ± 0.5	10.6 ± 0.4	ns	ns
HCT	34.2 ± 1.6	31.4 ± 2.1	32.4 ± 1.6	33.2 ± 1.4	ns	ns

RBC, red blood cells (10^6 counts/mm^3); HGB, haemoglobin content (g/dl); HCT, haematocrit (%).
ns, not significant (P>0.05); *, P<0.05; **, P<0.01; ***, P<0.001; PA, Parda de montaña; PI, Pirenaica

4. CONCLUSIONS

Values of RBC, HGB and HCT were strongly linked to the breed, being higher in PI in comparison with PA dams. These breed-dependent red cell profiles are not congenital, they must be acquired during the development of the calf, as breed did not affect calves profiles at birth. The undernutrition applied during the first third of pregnancy did not affect these red cell parameters in dams, neither in the first weeks of pregnancy nor one month before parturition. However, subnutrition could affect the precocity of development of haematopoietic system, as CONTROL calves suffered some physiological changes in red series parameters earlier than their counterparts. More studies are needed to quantify all possible effects of the restricted diet on the evolution of haematological parameters in cow-calf pairs.

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