Domestic Animal Endocrinology 72 (2020) 106418

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.journals.elsevier.com/domestic-animal-endocrinology

A negative energy balance during the peri-implantational period reduces dam IGF-1 but does not alter progesterone or pregnancy-specific protein B (PSPB) or fertility in suckled cows

A. Noya, I. Casasús, J.A. Rodríguez-Sánchez, J. Ferrer, A. Sanz*

Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón, Instituto Agroalimentario de Aragón – IA2 (CITA-Universidad de Zaragoza), Avenida Montañana 930, 50059 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 25 April 2019
Received in revised form 22 August 2019
Accepted 12 November 2019

Keywords:
Malnutrition
Early gestation
Pregnancy diagnosis
Metabolism
Calf performance

ABSTRACT

The aim of this study was to evaluate the effect of a negative energy balance during the first third of gestation on metabolic, endocrine, and pregnancy recognition parameters in 2 beef cattle breeds adapted to semiextensive conditions. Seventy-five lactating Parda de Montaña and 40 Pirenaica multiparous cows rearing calves were synchronized and timed artificial inseminated (TAI) on day 76 postpartum. Cows were assigned to one of 2 diets (CONTROL or SUBNUT; 100% or 65% of their requirements supplied) until day 82 of gestation. Pregnancy was diagnosed 37 d post-TAI using ultrasound. Blood samples were obtained to determine metabolic (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea) and endocrine (IGF-1) status throughout the first third of gestation and to determine the concentrations of progesterone and pregnancy-specific protein B (PSPB) in the peri-implantational period. Undernutrition affected both cow and calf performance. The CONTROL cows maintained BCS and BW, whereas SUBNUT cows had negative daily gains. The CONTROL lactating calves had higher BW gains than SUBNUT. These negative effects were more evident in the Pirenaica breed, which was more sensitive to undernutrition. The negative energy balance was reflected in the cows' metabolic profiles, with higher NEFA values and lower IGF-1 concentrations in SUBNUT cows. However, undernutrition did not affect dam pregnancy/TAI or pregnancy recognition and maintenance, confirming that during periods of undernourishment pregnant dams prioritize the allocation of dietary energy toward reproductive functions. Progesterone concentration on day 21 post-TAI (with a 4.8 ng/mL cut-off value) and PSPB on day 26 post-TAI (with a 0.57 ng/mL cut-off value) were determined as the earliest indicators to accurately establish dam pregnancy status, regardless of breed or nutrition treatment. In summary, early undernutrition affected cow performance and metabolic profiles and impaired lactating calf growth, but did not affect progesterone or PSPB concentrations or the pregnancy/TAI rate in suckled cows.

1. Introduction

Beef production systems are adapting to extensive conditions with the aim of reducing feed costs. This means that for long periods, cows will feed only on pastures or low-cost diets, which may compromise their nutritional

---

* Corresponding author. Tel.: +0034 976 713435; fax: +0034 976 716335.

E-mail address: asanz@aragon.es (A. Sanz).

0739-7240/$ – see front matter © 2019 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.domaniend.2019.106418

status and reproductive performance. For instance, undernutrition during prepartum and/or postpartum periods negatively impacts pregnancy success and reproductive efficiency [1]. It is well established that when the nutrient requirements for maintenance and lactation exceed intake, fertility, embryo quality, and viability rates are reduced. In fact, many metabolic and endocrine signals involved in reproductive processes are regulated by nutritional status [2]. A negative energy balance can impair the follicular development, the oocyte quality, or the luteinizing hormone secretion, increasing the postpartum anestrus period [3]. Undernourishment following breeding can alter oviductal and uterine support for embryo growth, negatively impacting maternal embryo recognition and pregnancy maintenance. Similarly, alterations in hormone or metabolite concentrations, induced by changes in nutritional inputs, can also affect development of the early embryo and its ability to successfully trigger maternal recognition [4].

Embryo loss is a frequent occurrence that impairs dam efficiency, representing an important source of economic loss for livestock producers [5]. Early and accurate pregnancy detection is key to improve dam reproductive performance, since it allows the reduction of days open and thus the calving interval. Direct techniques such as transrectal palpation or ultrasonography are frequently used, providing an immediate diagnosis as early as day 35 and day 26 after breeding, respectively [6]; however, accuracy requires good technician skills. Indirect techniques, based on the detection of progesterone or pregnancy-specific proteins in cow plasma or milk, or the expression of interferon tau stimulated genes (ISGs) [7], are under development, but their precision and the earliest days when they can be applied remain unclear.

Furthermore, poor nutrition effects may elicit interbreed differences, since genetic background affects metabolic [8] and endocrine status. Parda de Montaña (PA) and Pirenaica (PI) are the 2 main beef cattle breeds adapted to the semiextensive system in the Pyrenees mountain region (Northern Spain). Some interbreed differences have been reported in metabolic and hematologic profiles in response to differing managements, such as reduced granulocyte and mean corpuscular hemoglobin values in feed-restricted PI cows, but not in restricted PA cows [9], or reduced NEFA, total protein, and urea plasma concentrations in PI, but not in PA cows, with restricted nursing periods [10].

We hypothesized that a negative energy balance during the peri-implantational period could be detrimental to dam pregnancy recognition and maintenance, and although interbreed differences have been reported, reproductive functions should not have been affected by the breed, provided they are crucial for the species survival. The aims of this study are to evaluate the effect of an energy-restricted diet during early gestation on performance, metabolic (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea) and endocrine (IGF-1) status, pregnancy recognition and maintenance, and to establish the earliest day to use the pregnancy-specific protein B (PSPB) concentration as an accurate pregnancy test in PA and PI suckled cows.

2. Material and methods

All 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 on the protection of animals used for experimental and other scientific purposes [11].

2.1. Animals, management, and diets

This study was conducted at CITA-La Garcipollera Research Station, in the mountain area of the central Pyrenees (Spain, 945 m a.s.l.). Seventy-five PA (560 ± 54.8 kg body weight [BW]; 2.7 ± 0.03 body condition score [BCS] on a 5-point scale) and 40 PI (579 ± 54.9 kg BW; 2.9 ± 0.05 BCS) multiparous cows rearing a single calf were used for the study. The cows were synchronized to estrus at 65 ± 14 d 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 d later by a 150-μg injection of prostaglandin $F_{2\alpha}$ (Galapán, INVESA, Barcelona, Spain). After 9 d, the PRID was removed and 500 IU of pregnant mare serum gonadotropin (Serigan, Laboratorios Ovejero, León, Spain) was administered, followed 48 h later by a second injection of GnRH (10 μg). Eight hours after the second GnRH injection, cows were randomly timed artificial inseminated (TAI) with sires of proven fertility (4 PA and 3 PI) by an expert technician. Pregnancy diagnosis to a single AI was performed by ultrasonography using a linear-array 7.5 MHz transducer (Aloka SSD-500V, Aloka, Madrid, Spain) on day 37 ± 2.5 post-TAI.

During the experiment, all cows and calves remained indoors in a loose housing system. After TAI (day 0), cows were group-fed and distributed into 2 maternal nutrition treatments with a total mixed ration (10.96 MJ ME/kg DM and 124 g CP/kg DM) (Table 1) during the first 82 d of pregnancy. The control group (CONTROL, n = 53) was fed a diet that supplied 100% of the estimated energy requirements for cow maintenance, lactation, and gestation (10.9 and 10.0 kg DM/cow/d for 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/d for PA and PI, respectively) for a 580-kg beef cow producing 9 kg (PA) or 8 kg (PI) of energy-corrected milk [12]. Groups were randomly balanced according to cow BW (565 ± 60.6 and 568 ± 50.9 kg for CONTROL and SUBNUT, respectively), BCS (2.8 ± 0.27 and 2.8 ± 0.29, respectively), and postpartum period (78 ± 12.0 and 74 ± 14.6 d, respectively) at TAI. Cows were supplied water and vitamin–mineral supplements (lick blocks) ad libitum. During the experiment, suckling calves had a restricted twice-daily nursing system and their diets consisted exclusively of milk.

2.2. Animal weight, BCS assessment, and blood sample collection

Dams were weighed every 2 wk and calves were weighed on day 0, 54, and 82 of the experimental period.

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)	908 ± 5.8
CP (g/kg DM)	124 ± 10.2
NDF (g/kg DM)	466 ± 34.8
ADF (g/kg DM)	253 ± 25.1
ADL (g/kg DM)	40 ± 4.7
Ash (g/kg DM)	113 ± 15.3
ME (MJ/kg DM)	11 ± 0.4

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

The ADG was calculated by linear regression. Dam BCS was registered monthly by 2 expert technicians, based on the estimation of fat covering loin, ribs, and tailhead (using a 1–5 scale). Blood samples were collected every 2 wk for metabolic profiles; monthly for endocrine profiles; on day 14, 18, 21, 28, 42, 56, 69, and 82 post-TAI for plasma progesterone concentration; and on day 25, 26, and 28 post-TAI for PSPB concentration. Blood samples were collected before morning feeding by tail vessel puncture between the sixth and seventh coccygeal vertebrae. Samples to determine glucose, NEFA, β-hydroxybutyrate, cholesterol, and PSPB concentration were collected into 10 mL tubes containing EDTA (BD Vacutainer, Becton-Dickenson and Company, Plymouth, UK). Samples to determine urea, IGF-1, and progesterone concentration were collected into 10 mL heparinized tubes (BD Vacutainer). After bleeding, samples were centrifuged at 1,500 × *g* for 20 min at 4°C and plasma was stored at −20°C until analysis.

2.3. Assays

An automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India) was used to measure blood concentrations of glucose (glucose oxidase/peroxidase method, sensitivity: 0.056 mmol/L); β-hydroxybutyrate (enzymatic colorimetric method, sensitivity: 0.03 mmol/L); cholesterol (enzymatic colorimetric method, sensitivity: 0.256 mmol/L); and urea (kinetic UV test, sensitivity: 0.170 mmol/L). The mean intra- and interassay coefficients of variation for these compounds were <5.4% and <5.8%, respectively. Nonesterified fatty acids (NEFA, enzymatic method, sensitivity: 0.06 mmol/L) were analyzed using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). The mean intra- and interassay coefficients of variation were 5.1% and 7.4%, respectively. Insulin-like growth factor 1 (IGF-1, enzyme immunoassay, sensitivity: 20 ng/mL) was determined using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra- and interassay coefficients of variation were 3.1% and 12.0%, respectively. Plasma progesterone concentration (ELISA test, sensitivity: 0.27 ng/mL) was measured using a specific kit for cattle (Ridgeway Science, Lydney, UK). The mean intra- and interassay coefficients of variation were 8.0% and 10.4%, respectively. Pregnancy-specific protein B (PSPB) (ELISA test, sensitivity: 0.25 ng/mL) was determined using a specific bovine kit (bioPRYN, Bio Tracking Inc., Moscow, Russia). The mean intra- and interassay coefficients of variation were <5%.

2.4. Statistical analysis

All statistics were calculated using SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA). Normality of data was assessed with the Shapiro–Wilk test. Normality could not be confirmed for PSPB concentration, and therefore, it was expressed as a decimal logarithm for further analyses. The ADG of both dams and calves was analyzed using a general linear model (GLM procedure) with the breed (PA vs PI) and nutritional treatment (CONTROL vs SUBNUT) as fixed effects. In the case of cows, BW at TAI was added as a covariate, and in the case of calves, calf gender (male vs female) was added as fixed effect. Pregnancy/TAI and embryo mortality rate were analyzed using a logistic regression model (LOGISTIC procedure) considering breed, nutritional treatment, ADG during the first month of subnutrition (from TAI to ultrasound scanning day), the cow BSC at TAI, and the interval from the last calving to TAI as covariates. Embryo mortality was established in those dams that were diagnosed by ultrasonography as nonpregnant on day 37, but that presented one of these situations: (1) concentrations of progesterone on day 14 and PSPB on day 25 above the cut-off values proposed, (2) concentrations of progesterone on day 14 and PSPB on day 26 above the cut-off values, (3) concentrations of PSPB on day 25 and 28 above the cut-off values, or (4) concentrations of PSPB on day 26 and 28 above the cut-off values. Metabolites (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea), IGF-1, progesterone, and PSPB concentrations were analyzed using a mixed linear model (MIXED procedure) for repeated measures based on Kenward-Roger's adjusted degrees of freedom solution. The fixed factors were breed and nutritional treatment as the between-subject effects; sampling day as the within-subject effect; animal as the random effect (experimental unit), and the BCS at TAI as a covariate. In case of progesterone and PSPB concentrations, the pregnancy status (pregnant vs nonpregnant) was considered as a fixed effect. Pregnancy/TAI and embryo mortality rate were analyzed using a logistic regression model (LOGISTIC procedure) considering metabolites (on day 0, 14, and 28) and IGF-1 (on day 0 and 28) as covariates. The least square (LS) means of the treatments were estimated per fixed effect, and pairwise comparisons of the means were obtained by the probability of difference (PDIFF) option of the LS means procedure. Estimated cut-off values of progesterone and PSPB for diagnosing a dam as pregnant or nonpregnant were estimated using a linear logistic regression (LOGISTIC procedure), with breed and nutritional treatment as possible fixed effects. The Youden index was used to determine the sensitivity, specificity, and the cut-off value of the proposed model. Relationships among the studied parameters were determined using Pearson's correlation coefficients. The level of significance for all tests was $P < 0.05$. The results are presented as LS means ± standard error.

3. Results

3.1. Animal performance

No breed effect was found for dam BW during the experiment ($P > 0.05$), but PI dams had higher mean BCS than PA dams (2.7 ± 0.03 vs 2.9 ± 0.04 for PA and PI, respectively, $P < 0.001$). Cow BW and BCS were affected by an interaction between time and nutritional treatment ($P < 0.001$), BW and BCS from the second half of the experimental period being lower in the SUBNUT than in the CONTROL group, as shown in Figure 1. Throughout the experiment, cows in the CONTROL group maintained BW, whereas those in the SUBNUT group experienced a negative ADG (0.11 ± 0.031 vs −0.37 ± 0.026 kg/d, respectively, $P < 0.001$). Regarding calf performance, an interaction effect of breed and nutritional treatment influenced ADG. Calves from PA-CONTROL and PI-CONTROL groups had greater weight gains than their counterparts (0.62 ± 0.020, 0.55 ± 0.020, 0.62 ± 0.034, and 0.44 ± 0.025 kg/d for PA-CONTROL, PA-SUBNUT, PI-CONTROL, and PI-SUBNUT, respectively, $P < 0.05$). However, whereas no differences were found between CONTROL subgroups ($P > 0.05$), ADG was greater in PA-SUBNUT than in PI-SUBNUT calves ($P < 0.001$). No gender effect was found in the calf ADG (0.57 ± 0.017 vs 0.55 ± 0.017 kg/d for male and female, respectively, $P > 0.05$).

3.2. Metabolic and endocrine profiles

Plasma concentrations of glucose, NEFA, β-hydroxybutyrate, cholesterol, urea, and IGF-1, commonly associated with ruminant energy metabolism, were analyzed in order to characterize the nutritional status of suckled cows. Their profiles during the first third of gestation are displayed in Figure 2. Triple interaction effects of breed, nutritional treatment, and sampling day affected both metabolite and IGF-1 concentrations ($P < 0.05$).

Glucose concentrations fluctuated over the course of the experiment. Glucose concentrations in PI-CONTROL cows were equal to or higher than those of their PI-SUBNUT counterparts, unlike PA-CONTROL cows, which had lower values than PA-SUBNUT cows at day 56.

In general, PI had higher NEFA concentration than PA throughout the experiment (0.24 ± 0.017 vs 0.32 ± 0.024 mmol/L for PA and PI, respectively, $P < 0.05$). From the second half of the experiment, PI-SUBNUT had higher NEFA concentrations than PI-CONTROL on day 56 and 82 ($P < 0.05$), and PA-SUNBUT had higher NEFA values than PA-CONTROL from day 56 to the end of the experiment ($P < 0.05$). NEFA levels during the experiment were positively correlated with BCS at TAI, the highest correlations being observed on day 56 ($r = 0.39$, $P < 0.001$).

Few differences were found throughout the experimental period in β-hydroxybutyrate concentrations. PA-CONTROL on day 0 and 82 and PI-CONTROL on day 69 had higher values than their respective SUBNUT counterparts ($P < 0.05$).

Regarding cholesterol concentrations, no differences were found between PA-CONTROL and PA-SUBNUT cows throughout the experiment ($P > 0.05$); however, on day 28, 69, and 82 PI-SUBNUT had lower values than PI-CONTROL ($P < 0.05$). The evolution of cholesterol concentration during the experimental period was similar to that of glucose concentration, with a positive correlation on day 42 ($r = 0.33$, $P < 0.001$).

Similarly, no differences were found in urea concentrations between PA-CONTROL and PA-SUBNUT cows throughout the experimental period ($P > 0.05$), but PI-CONTROL cows had higher values than PI-SUBNUT cows on day 28, 56 ($P < 0.05$), and 69 ($P < 0.001$).

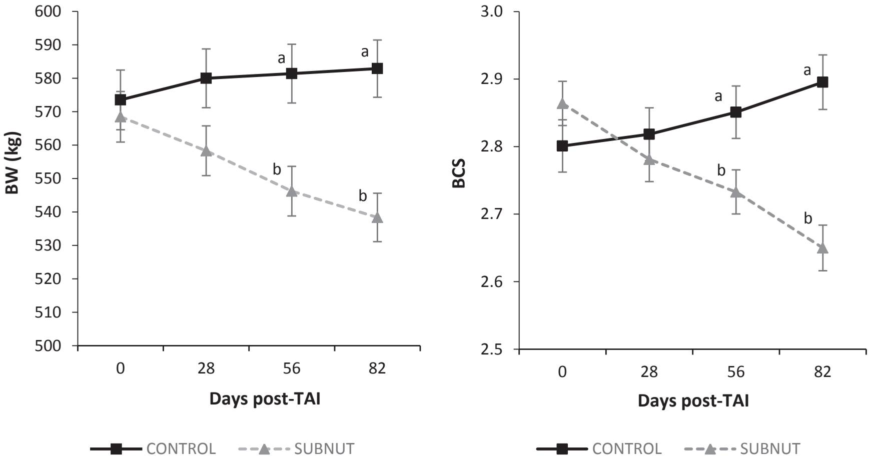


Fig. 1. Body weight (BW) and body condition score (BCS) after TAI of suckled cows according to the nutritional treatment. ^{a,b}Means at a given time with different superscripts differ significantly ($P < 0.05$); CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy.

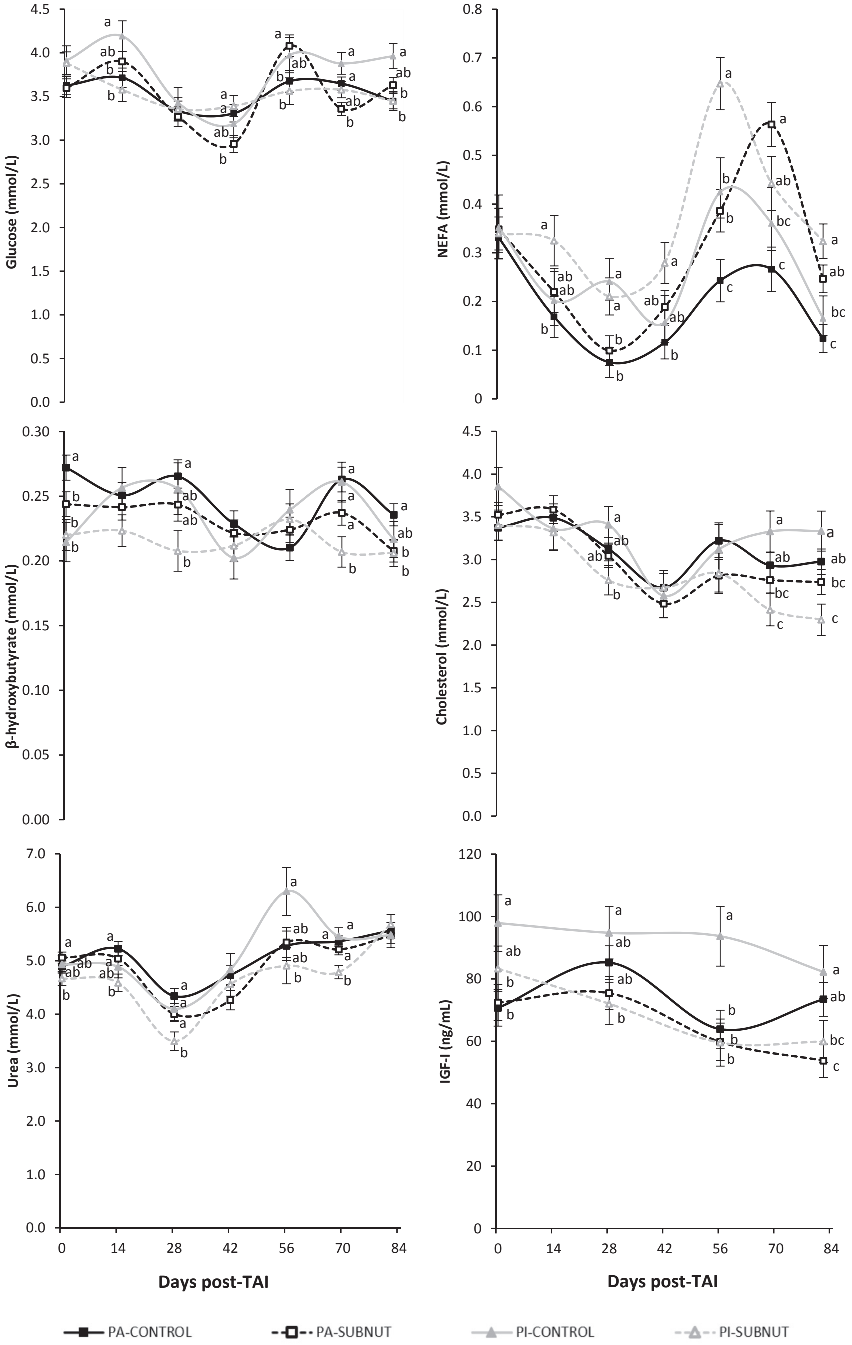
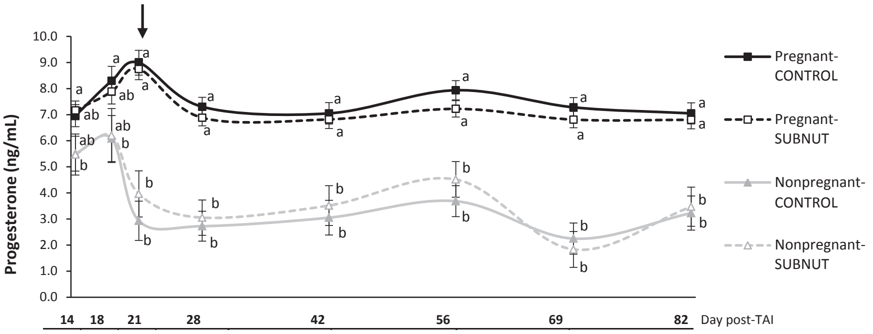


Fig. 2. Plasma concentrations of glucose, NEFA, β-hydroxybutyrate, cholesterol, urea, and IGF-1 after TAI of suckled cows according to the breed and the nutritional treatment. $^{a-c}$Means at a given time with different superscripts differ significantly ($P < 0.05$); PA, Parda de Montaña; PI, Pirenaica; 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.

In general, CONTROL groups had higher IGF-1 concentrations than SUBNUT groups (82.7 ± 4.65 vs 67.0 ± 3.99 ng/mL for CONTROL and SUBNUT, respectively, $P < 0.05$). Specifically, PA-CONTROL had higher values than PA-SUBNUT on day 82 ($P < 0.01$) and PI-CONTROL higher values than PI-SUBNUT on day 28, 56, and 82 ($P < 0.05$). A negative relationship between IGF-1 and NEFA concentration was found at AI time ($r = -0.26$, $P < 0.01$).

3.3. Progesterone and PSPB concentrations, pregnancy diagnosis, and embryo mortality

Progesterone concentrations were affected by a triple interaction among nutritional treatment, pregnancy status, and sampling day (Fig. 3), but not by breed ($P > 0.05$). No differences were found in progesterone concentration between pregnant-CONTROL and pregnant-SUBNUT dams, or



14	18	21	28	42	56	69	82	Day post-TAI
3.1	7.1	4.8	4.1	4.1	6.1	3.6	4.8	Cut-off value (ng/mL)
100	65	98	93	97	79	98	87	Sensitivity (%)
19	73	81	77	64	84	84	68	Specificity (%)

Fig. 3. Progesterone concentrations after TAI of suckled cows according to nutritional treatment and pregnancy status. ^{a,b}Means at a given time with different superscripts differ significantly ($P < 0.05$); 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; the arrow marks the earliest day for an accurate diagnosis based on progesterone concentration.

between nonpregnant-CONTROL and nonpregnant-SUBNUT throughout the experiment ($P > 0.05$). Pregnancy status affected progesterone concentration, pregnant cows having statistically higher values than their nonpregnant counterparts from day 21 to the end of the assay ($P < 0.001$). The estimated cut-off value of progesterone concentration to determine pregnancy status at each sampling day and the sensitivity and the specificity each model are presented in Figure 3. The earliest accurate cut-off value to diagnose gestation was 4.8 ng/mL on day 21 post-TAI, with an area under the curve (AUC) value of 0.93. On earlier days (14 and 18), the specificity was lower since the difference was not enough to discriminate the progesterone values from a gestational corpus luteum in a pregnant cow from a corpus luteum in the luteal phase in a nonpregnant cow (AUC = 0.66 and 0.77 for day 14 and 18, respectively). On day 28, the accuracy had slightly diminished (AUC = 0.91). Progesterone concentration from pregnant dams was quite constant from day 28 to the end of the experiment regardless of the breed and the nutritional treatment (7.1 ± 2.1 ng/mL).

A triple interaction effect of nutritional treatment, pregnancy status, and sampling day affected the PSPB concentration (Fig. 4). No differences were found between breeds ($P > 0.05$) neither between pregnant-CONTROL and pregnant-SUBNUT dams, nor between nonpregnant-CONTROL and nonpregnant-SUBNUT dams throughout the experiment ($P > 0.05$). Pregnancy status affected PSPB concentration on day 26 and 28, with higher values in pregnant than in nonpregnant dams ($P < 0.001$). No statistical differences were found on day 25 between pregnant-CONTROL and nonpregnant-SUBNUT dams ($P > 0.05$). The estimated cut-off value to diagnose pregnancy status according to PSPB concentration, its sensitivity, and its specificity are displayed in Figure 4. For pregnancy diagnosis at day 25, a 0.76 AUC value was obtained, but no cut-off value was proposed because of the overlap between pregnant and nonpregnant PSPB values. On day 26 and 28, the AUC values were 0.88 and 0.93, respectively, but no significant differences were found between these logistic models ($P > 0.05$). Thus, the first cut-off value obtained to diagnose pregnancy was 0.57 ng/mL on day 26 post-TAI. Concerning only pregnant dams, PSPB concentration increased over time ($P < 0.001$), with no breed or nutritional treatment effect ($P > 0.05$). A negative relationship was found between PSPB and progesterone concentrations in pregnant dams throughout the experiment. Specifically, the PSPB concentrations on day 26 were negatively related to progesterone concentrations on days 14 ($r = -0.41$, $P < 0.01$), 21 ($r = -0.29$, $P < 0.05$), 28 ($r = -0.37$, $P < 0.01$), 56 ($r = -0.45$, $P < 0.001$), and 82 ($r = -0.29$, $P < 0.05$), among others. Concentration of PSPB was also negatively correlated with IGF-1 on day 28 post-TAI ($r = -0.40$, $P < 0.001$).

The pregnancy rate obtained by ultrasonography 37 d post-TAI was 77% (89/115), with no breed (73% vs 85%, for PA and PI) or nutritional treatment (71% vs 82%, for CONTROL and SUBNUT) effect ($P > 0.05$). The ADG during the first month of the experiment, the cow BSC at TAI, the calving to TAI interval, or the metabolite concentrations had not a significant effect on pregnancy rate ($P > 0.05$). Neither IGF-1 on day 0 was related with the fertility rate ($P > 0.05$); however, IGF-1 concentration on day 28 had a negative relationship with fertility rate ($P < 0.01$), the probability to be pregnant decreasing by 2.2% for each extra point of IGF-1.

Embryo mortality rate, diagnosed in 8 dams (8/97 possibly pregnant cows, according to their progesterone and PSPB concentrations), was not related to breed (5/60 PA and 3/37 PI) or nutritional treatment (2/40 CONTROL and 6/57 SUBNUT, $P > 0.05$). The ADG during the first month of the experiment, the cow BSC at TAI, the calving to TAI interval, metabolite, and IGF-1 concentrations had not a significant effect on embryo mortality rate ($P > 0.05$).

4. Discussion

4.1. Animal performance

Nutritional restriction at 65% of cows' requirements over 82 d reduced BCS and BW throughout the study with no difference between breeds, indicating that the estimated requirements, specifically calculated for each breed,

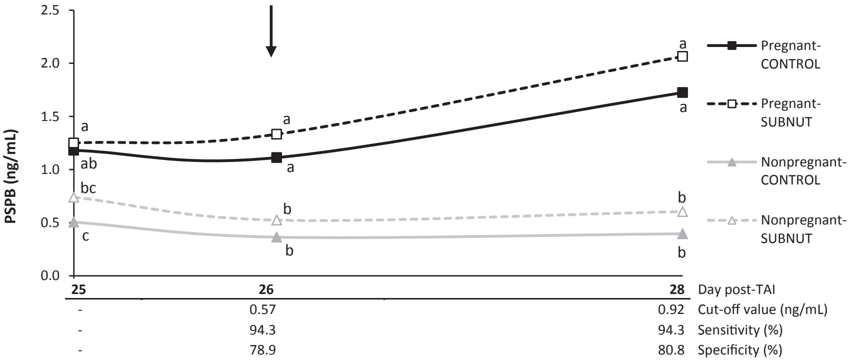


Fig. 4. Pregnancy-specific protein B (PSPB) concentrations after TAI of suckled cows according to nutritional treatment and pregnancy status. $^{a-c}$Means at a given time with different superscripts differ significantly ($P < 0.05$); CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; the arrow marks the earliest day for an accurate diagnosis based on PSPB concentration.

were well adjusted. The lower calf gains observed in SUBNUT groups resulted from the negative effects of feed restriction on dam milk yield and its protein concentration [13]. However, whereas no differences were found between CONTROL subgroups, PA-SUBNUT calves had higher gains than PI-SUBNUT calves, suggesting that nutritional restriction in PI dams may more severely impair milk yield and/or composition.

4.2. Metabolic and endocrine profiles

Circulating glucose is an indicator of energy balance that shows a strong dependence on the current energy and protein intake at a given time [14]. In our study, CONTROL groups had higher or equal values than SUBNUT groups in most of cases. Similarly, Richards et al [15] found lower glucose concentrations in restricted cows compared to cows fed at maintenance after 30 wk.

A negative energy balance increases plasma NEFA concentration as a consequence of fatty acid release from adipose tissue. In the current study, SUBNUT cows had higher NEFA concentrations than CONTROL cows from the second half of the experiment. Pirenaica cows had higher NEFA concentrations than PA breed, which was related with their higher BCS during the experiment.

Ketogenesis increases blood glucose concentrations when glucose becomes scarce and glycolysis falls to very low levels [16]. In the current study, despite the greater fat tissue mobilization in SUBNUT cows, few differences were found in β-hydroxybutyrate concentration between CONTROL and SUBNUT groups. This implies that β-hydroxybutyrate, which is the predominant circulating ketone body, was not the main energy source used by SUBNUT groups. The mobilization of NEFA from adipose tissue is not associated with concomitant increases in their oxidative metabolite (β-hydroxybutyrate) [10].

Cholesterol is related to glucose concentration [17], with both metabolites indicating a positive energy balance. Accordingly, a positive relationship between them was found in our study. Furthermore, PI-CONTROL had greater cholesterol concentrations than PI-SUBNUT, whereas no differences were found between PA subgroups, highlighting the greater sensitivity to undernutrition of the PI breed.

Blood urea is a good indicator of the protein status of the animal, directly related to degradable protein intake, but also to the catabolism of body protein in periods of energy shortfall [18]. Blood urea concentrations have long been known to reflect inefficient utilization of dietary CP by ruminants [19]; that is, blood urea concentration increases in a cow fed excess dietary protein. In our experiment, CONTROL groups had equal or higher urea concentrations than SUBNUT groups, mostly in PI breed, reflecting their greater CP intake.

In the current study, PI-CONTROL dams had the highest IGF-1 concentrations, whereas PI-SUBNUT concentrations were similar to that obtained in PA groups. The differences between PA-CONTROL and PI-CONTROL cows contrast with other experiments where IGF-1 differences between these breeds were not found [10,18]. Nutrient intake is positively related to IGF-1 concentration [20], and accordingly, IGF-1 concentration was higher in CONTROL than in SUBNUT cows, with negative correlation between NEFA and IGF-1 concentration. At parturition, 6 mo after the nutrient treatment was finished, calves born from CONTROL cows had also higher IGF-1 blood concentration than those from SUBNUT cows [9], highlighting the maternal-embryo crosstalk and its role in embryonic and fetal development.

4.3. Progesterone and PSPB concentrations, pregnancy diagnosis, and embryo mortality

Progesterone plays a central role in the establishment of uterine receptivity to the embryo and drives conceptus elongation through molecular changes induced in the endometrium [21]. A negative energy balance is detrimental for the early growth of ovarian follicles, and after ovulation, progesterone secretion of the corpus luteum can be reduced [22]. In the current study, nutritional treatment did not affect progesterone concentration between pregnant dams, allowing for the maintenance of pregnancy in both CONTROL and SUBNUT cows. On the contrary, other studies have described an inverse relationship between energy intake and systemic progesterone concentration.

High energy intake increases metabolic rate and the blood flow through the liver, resulting in an increased clearance rate of progesterone [23,24]. Accordingly, Nolan et al [25] found 25% lower progesterone concentrations in heifers fed a high versus a low-energy diet.

In our experiment, day 21 was determined to be the earliest accurate day to diagnose pregnancy status based on progesterone concentration, with a 4.8 ng/mL cut-off value and both high sensitivity and specificity values. In agreement with our results, Otavă et al [26] found that in pregnant cows, the progesterone levels increased continuously up to day 21 postfertilization and established the progesterone levels between days 18 and 24 as an indirect method for pregnancy diagnosis. Similarly, Humblot [27] established a combination of <3.5 ng/mL on day 0 and >5 ng/mL on days 21–24 as criteria to diagnose a dam as pregnant.

Pregnancy-specific protein B, formerly known as pregnancy-associated glycoprotein 1 [28], is a glycoprotein synthesized by the binucleate trophoblastic cells of the bovine placenta [29]. Unlike progesterone, PSPB is a specific pregnancy signal induced as a result of the presence of a conceptus [30]. According to Humblot [27], PSPB concentrations rise from day 15 to 35 to reach 2 to 3 ng/mL at this stage, the critical period for maternal recognition of pregnancy taking place between days 15 and 18 of gestation [28]. The earliest day when the PSPB pregnancy test can yield accurate and consistent results remains unclear, with estimates ranging from day 24 postconception [30], 25 [31], 28 [32], to day 30 [33]. Nevertheless, PSPB clearance from circulation during the postpartum period is extremely slow [34], involving the persistence of high peripheral PSPB concentrations in postpartum cattle. In the current study, the day 25 blood sample was taken on day 100.8 ± 13.5 after parturition, consistent with the manufacturer's instructions (more than 73 d since last calf). However, residual PSPB concentrations in nonpregnant dams on day 25 did not permit the determination of an accurate cut-off value to diagnose pregnancy. The low metabolic rates of beef compared to dairy cattle might have delayed the clearance of the residual PSPB from the last gestation. The PSPB concentration on day 26 yielded a 0.57 ng/mL cut-off value, with both high sensitivity and specificity and similar accuracy to that from day 28. This suggests that in our conditions, day 26 was the earliest day to diagnose pregnancy, regardless of the nutritional treatment or breed.

Surprisingly, the PSPB concentration was negatively correlated with progesterone values from the critical period of days 15–18 until day 82 post-TAI. We hypothesized that higher PSBP concentration may compensate for lower progesterone production, due to the response by the trophoblastic cells to establish a stronger maternal-embryo cross-talk to permit maternal recognition and ensure the maintenance of gestation. Humblot et al [35] found negative but nonsignificant correlations between circulating progesterone on day 24 and PSPB on day 24, 26, and 30–35 and therefore concluded that there was no relationship between them in pregnant animals. Similarly, López-Gatius et al [36] discounted any potential involvement of progesterone with pregnancy-associated glycoproteins from the placenta or vice versa. However, Ayad et al [37] observed that pregnancy-associated glycoproteins tended to be higher in pregnant females with higher progesterone concentrations. Additional research is needed to determine the role of PSPB in the maternal recognition of a viable conceptus and in pregnancy maintenance, which is not yet fully understood.

In the current study, 77% of cows were pregnant at the TAI, a higher pregnancy rate compared with other studies using similar synchronization protocols [18,38,39], regardless of the breed or the nutritional treatment, probably because of their optimal BCS at TAI. Nutrition determines cow BW and BCS, which underpin fertility rate in postpartum cows [40]. In the current study, despite the SUBNUT group being in a negative energy balance after TAI, these cows' optimal BCS at TAI allowed the conception and maintenance of gestation. Keady et al [41] found similar fertility between a control group fed with ad libitum grass silage as the sole diet and a group supplemented with 5 kg/d of concentrate during late gestation. Contrastingly, Perry et al [42] found that post-AI supplementation improved pregnancy success, and Fontes et al [43] reported an increased pregnancy failure rate associated to a nutrient restriction during early gestation. Metabolite concentration during the first month of the experiment and IGF-1 concentration on day 0 were not related with the pregnancy/TAI rate. Surprisingly, lower IGF-1 concentrations on day 28 were associated with higher pregnancy success. High plasma IGF-1 concentration at TAI has been described as a useful predictor of reproductive success in cattle [23]. Taylor et al [44] reported that cows with plasma IGF-1 values greater than 50 ng/mL at first service exhibited a fivefold increase in likelihood of conception, and Moyes et al [45] found that plasma IGF-1 concentrations in pregnant cows were numerically higher than those of nonpregnant cows after conception; however, these differences were not significant until 15 wk postconception. On the other hand, Falkenberg et al [46] found no significant differences in IGF-1 concentration between cows that conceived at the first AI, in later services, or in cows that did not become pregnant. In the current study, no IGF-1 effect on pregnancy/TAI rate was found at day 0. At that moment, all cows had an optimal BCS and the IGF-1 concentration of all groups was above the threshold before reproduction is adversely affected [47], which implies that IGF-1 concentration did not determine the reproductive performance. From day 0 onward, due to the nutritional treatment, IGF-1 concentration in SUBNUT cows started to decrease, specifically in PI breed. Although the differences in pregnancy/TAI rate between CONTROL and SUBNUT cows were not significant, 57% of pregnant dams belonged to SUBNUT group, while 58% of nonpregnant dams belonged to CONTROL group. That could be the reason why on day 28 pregnant dams (most from SUBNUT group) had lower IGF-1 concentration than nonpregnant dams (most from CONTROL group). Our hypothesis is that despite these lower values at the onset of pregnancy, according to Moyes et al [45], IGF-1 concentration of pregnant dams increases above that of nonpregnant dams as gestation proceeds.

In our study, an 8% embryo mortality rate was reported. Although fertility rates are usually high in beef cattle, pregnancy outcome may decrease due to embryo losses, which can account for up to 29%–39% of pregnancies after fertilization, most of them between day 8 and 16 after insemination [48]. Nutritional and metabolic status of the cow can affect embryonic development and survival [2]. In beef heifers, Dunne et al [24] found that a short-term (2 wk) reduction in energy intake after AI severely reduced embryo survival rates by 41%, but Doyle et al [23] reported no effect of postinsemination plane of nutrition. In the current study, SUBNUT cows had higher embryo loss rates than their CONTROL counterparts, but the difference was not significant, probably due to the low incidence of embryo losses. Therefore, more studies are needed to assess the impact of the negative energy balance on embryo mortality in adult beef cows.

Therefore, in our study, undernutrition during the first third of pregnancy did not impair the cow reproductive performance and allowed to establish and maintain the gestation. A 65% energy restricted diet was a severe feed restriction, reflected in most of the metabolites and IGF-1 SUBNUT cow profiles. Nevertheless, at the beginning of the experiment, all cows had an optimal BCS to face this nutritional challenge. Animals with lower BCS and a worse metabolic status at the beginning of the study would possibly have obtained a worse reproductive performance. Fernández-Foren et al [49] affirmed that initial body reserves determine the endocrine response to undernutrition. As in our experiment, undernourished animals with optimal initial BCS developed compensatory mechanisms against adverse environmental factors, counteracting the negative effects caused by a food restriction on reproduction. However, it is interesting to highlight that in our study, although the reproductive performance was not initially affected by undernutrition, an altered maternal environment compromised the fetal programming with long-term consequences in the newborns [9].

5. Conclusions

A restrictive diet during the first 82 d after TAI induced a negative energy balance in suckled cows, reflected in higher NEFA and lower IGF-1 concentrations, which affected dam performance and impaired calf growth. These negative effects were more evident in the PI breed, which was more sensitive to feed restriction. Undernutrition did not affect dam pregnancy recognition, maintenance of gestation, or pregnancy/TAI rate, confirming that pregnant dams cope with undernourishment by prioritizing the allocation of dietary energy toward reproductive functions.

CRediT authorship contribution statement

A. Noya: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **I. Casasús:** Methodology, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Writing - original draft, Writing - review & editing. **J.A. Rodríguez-Sánchez:** Methodology, Investigation, Project administration, Writing - review & editing. **J. Ferrer:** Resources, Methodology, Data curation, Funding acquisition, Investigation, Project administration, Writing - review & editing. **A. Sanz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Acknowledgments

The authors wish to thank the technical staff at CITA de Aragón and La Garcipollera Research Station for their technical support, Dr J.L. Alabart and Dr B. Serrano-Pérez for their assistance with the pregnancy-specific protein B (PSPB) and progesterone assays, and Dr D. Villalba for his statistical advice. This work was supported by the Spanish Ministry of Science, Innovation and Universities and the European Regional Development Funds under Grant INIA RTA 2013–00059-C02 and INIA RZP 2015–001 and the Government of Aragon under the Grant Research Group Funds (A14_17R). A. Noya received a grant from INIA Government of Spain. Conflict of interest: none.

References

[1] Diskin MG, Mackey DR, Roche JF, Sreenan JM. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim Reprod Sci 2003;78:345–70.
[2] Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvão KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim Reprod Sci 2004;82-83:513–35.
[3] Sanz A, Bernués A, Villalba D, Casasús I, Revilla R. Influence of management and nutrition on postpartum interval in Brown Swiss and Pirenaica cows. Livest Prod Sci 2004;86:179–91.
[4] Block J, Hansen PJ, Loureiro B, Bonilla L. Improving post-transfer survival of bovine embryos produced in vitro: actions of insulin-like growth factor-1, colony stimulating factor-2 and hyaluronan. Theriogenology 2011;76:1602–9.
[5] Diskin MG, Kenny DA. Managing the reproductive performance of beef cows. Theriogenology 2016;86:379–87.
[6] Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszweski MA, Kraemer DC. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology 2006; 66:1034–41.
[7] Serrano-Pérez B, Rizos D, López-Helguera I, Molina E, Garcia-Ispierto I, López-Gatius F. Progesterone supplementation during the pre-implantation period influences interferon-stimulated gene expression in lactating dairy cows. Ann Anim Sci 2019;19:713–24.
[8] Wuletaw Z, Wurzinger M, Holt T, Dessie T, Sölkner J. Assessment of physiological adaptation of indigenous and crossbred cattle to hypoxic environment in Ethiopia. Livest Sci 2011;138:96–104.
[9] Noya A, Serrano-Pérez B, Villalba D, Casasús I, Molina E, López-Helguera I, Sanz A. Effects of maternal subnutrition during early pregnancy on cow hematological profiles and offspring physiology and vitality in two beef breeds. Anim Sci J 2019;90:857–69.
[10] Álvarez-Rodríguez J, Palacio J, Sanz A. Metabolic and luteal function in winter-calving Spanish beef cows as affected by calf management and breed. J Anim Physiol Anim Nutr 2010;94:385–94.
[11] Union E. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official J Eur Union 2010;276:33–79.
[12] NRC. Nutrient requirements of beef cattle. 7th ed. Washington DC: The National Academies Press; 2000. p. 232.
[13] Walker G, Dunshea F, Doyle P. Effects of nutrition and management on the production and composition of milk fat and protein: a review. Aust J Agric Res 2004;55:1009–28.
[14] Rodríguez-Sánchez J, Sanz A, Ferrer J, Ripoll G, Casasús I. First calving performance and physiological profiles of 2 yr old beef heifers according to their prebreeding growth. Can J Anim Sci 2017; 97:488–98.
[15] Richards MW, Wettemann RP, Schoenemann HM. Nutritional anestrus in beef cows: concentrations of glucose and Nonesterified fatty acids in plasma and insulin in serum. J Anim Sci 1989;67: 2354–62.
[16] Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999; 15:412–26.

[17] Ndlovu T, Chimonyo M, Okoh A, Muchenje V, Dzama K, Raats J. Assessing the nutritional status of beef cattle: current practices and future prospects. Afr J Biotechnol 2007;6:2727–34.

[18] Rodríguez-Sánchez JA, Sanz A, Ferrer J, Casasús I. Influence of postweaning feeding management of beef heifers on performance and physiological profiles through rearing and first lactation. Domest Anim Endocrinol 2018;65:24–37.

[19] Broderick GA, Clayton MK. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 1997;80:2964–71.

[20] Paradis F, Wood KM, Swanson KC, Miller SP, McBride BW, Fitzsimmons C. Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. BMC Genomics 2017;18:632.

[21] Martins T, Pugliesi G, Sponchiado M, Cardoso BO, Gomes NS, Mello BP, Celeghini ECC, Binelli M. Supplementation with long-acting progesterone in early diestrus in beef cattle: I. Effect of artificial insemination on onset of luteolysis. Domest Anim Endocrinol 2019;67:63–70.

[22] Sanz A, Casasús I, Bernués A, Revilla R. Reinicio de la actividad folicular en vacas nodrizas sometidas a diferentes niveles de alimentación antes y después del parto. ITEA Vol Extra 2001;22:727–9.

[23] Doyle DN, Lonergan P, Diskin MG, Pierce KM, Kelly AK, Stanton C, Waters SM, Parr MH, Kenny DA. Effect of dietary n-3 polyunsaturated fatty acid supplementation and post-insemination plane of nutrition on systemic concentrations of metabolic analytes, progesterone, hepatic gene expression and embryo development and survival in beef heifers. Theriogenology 2019;127:102–13.

[24] Dunne LD, Diskin MG, Boland MP, O'Farrell KJ, Sreenan JM. The effect of pre- and post-insemination plane of nutrition on embryo survival in beef heifers. Anim Sci 2016;69:411–7.

[25] Nolan R, O'Callaghan D, Duby RT, Lonergan P, Boland MP. The influence of short-term nutrient changes on follicle growth and embryo production following superovulation in beef heifers. Theriogenology 1998;50:1263–74.

[26] Otavă G, Cernescu H, Mircu C, IGNA V. Pregnancy diagnosis in cow using progesterone measurements. Lucrări stiinłifice medicină veterinară 2007:95–8.

[27] Humblot P. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 2001;56:1417–33.

[28] Hopper RM. Bovine reproduction. Ames, IA: John Wiley & Sons; 2015. p. 320–5.

[29] Almería S, Serrano-Pérez B, López-Gatius F. Immune response in bovine neosporosis: protection or contribution to the pathogenesis of abortion. Microb Pathog 2017;109:177–82.

[30] Garth Sasser R, Ruder CA, Ivani KA, Butler JE, Hamilton WC. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol Reprod 1986;35:936–42.

[31] Green JC, Volkmann DH, Poock SE, McGrath MF, Ehrhardt M, Moseley AE, Lucy MC. A rapid enzyme-linked immunosorbent assay blood test for pregnancy in dairy and beef cattle. J Dairy Sci 2009;92:3819–24.

[32] Northrop EJ, Rich JJJ, Rhoades JR, Perry GA. Comparison of two bovine serum pregnancy tests in detection of artificial insemination pregnancies and pregnancy loss in beef cattle. PLoS One 2019;14:e0211179.

[33] Romano JE, Larson JE. Accuracy of pregnancy specific protein-B test for early pregnancy diagnosis in dairy cattle. Theriogenology 2010;74:932–9.

[34] Kiracofe GH, Wright JM, Schalles RR, Ruder CA, Parish S, Sasser RG. Pregnancy-specific protein B in serum of postpartum beef cows. J Anim Sci 1993;71:2199–205.

[35] Humblot F, Camous S, Martal J, Charlery J, Jeanguyot N, Thibier M, Sasser RG. Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows. J Reprod Fertil 1988;83:215–23.

[36] López-Gatius F, Garbayo JM, Santolaria P, Yániz J, Ayad A, Sousa NMd, Beckers JF. Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Domest Anim Endocrinol 2007;32:29–42.

[37] Ayad A, Sousa NM, Sulon J, Hornick JL, Watts J, Lopez-Gatius F, Iguer-Ouada M, Beckers JF. Influence of progesterone concentrations on secretory functions of trophoblast and pituitary during the first trimester of pregnancy in dairy cattle. Theriogenology 2007;67:1503–11.

[38] Sanz A, Macmillan K, Colazo M. A review of the ovarian synchronization programs based on the use of gonadotrophin releasing hormone and prostaglandin F2α for dairy and beef heifers. Itea-Inf Tec Econ Ag 2019;115:326–41.

[39] Schmitz W, Kramer M, Erhardt G, Gauly M, Driancourt M-A, Holtz W. Pregnancy rate after fixed-time artificial insemination of suckled beef cows subjected to a cosynch protocol with either buserelin or hCG as ovulation inducing agent. Livest Sci 2017;206:141–7.

[40] D'Occhio MJ, Baruselli PS, Campanile G. Influence of nutrition, body condition, and metabolic status on reproduction in female beef cattle: a review. Theriogenology 2019;125:277–84.

[41] Keady T, Mayne C, Fitzpatrick DA, McCoy MA. Effect of concentrate feed level in late gestation on subsequent milk yield, milk composition, and fertility of dairy cows. J Dairy Sci 2001;84:1468–79.

[42] Perry GA, Larimore EL, Perry BL, Walker JA. Grazing behavior of drylot-developed beef heifers and the influence of postinsemination supplementation on artificial-insemination pregnancy success. Prof Anim Sci 2015;31:264–9.

[43] Fontes PLP, Oosthuizen N, Ciriaco FM, Sanford CD, Canal LB, Pohler KG, Henry DD, Mercadante VRG, Timlin CL, Ealy AD, Johnson SE, DiLorenzo N, Lamb GC. Impact of fetal vs. maternal contributions of Bos indicus and Bos taurus genetics on embryonic and fetal development. J Anim Sci 2019;97:1645–55.

[44] Taylor VJ, Cheng Z, Pushpakumara PG, Beever DE, Wathes DC. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Vet Rec 2004;155:583–8.

[45] Moyes TE, Stockdale CR, Humphrys S, Macmillan KL. Differences in plasma concentration of insulin-like growth factor-1 between pregnant and non-pregnant dairy cows. Reprod Fertil Dev 2003;15:22.

[46] Falkenberg U, Haertel J, Rotter K, Iwersen M, Arndt G, Heuwieser W. Relationships between the concentration of insulin-like growth factor-1 in serum in dairy cows in early lactation and reproductive performance and milk yield. J Dairy Sci 2008;91:3862–8.

[47] Velazquez MA, Spicer LJ, Wathes DC. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domest Anim Endocrinol 2008;35:325–42.

[48] Dunne LD, Diskin MG, Sreenan JM. Embryo and foetal loss in beef heifers between day 14 of gestation and full term. Anim Reprod Sci 2000;58:39–44.

[49] Fernández-Foren A, Sosa C, Abecia JA, Vázquez MI, Forcada F, Meikle A. Dietary restriction in sheep: uterine functionality in ewes with different body reserves during early gestation. Theriogenology 2019;135:189–97.