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Effects of undernutrition and hydroxytyrosol supplementation in late pregnancy on cow-calf performance, metabolic and immune status, and newborn vitality in beef herds

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Highlights:

1) Undernutrition during late pregnancy impaired cow performance and metabolic status
2) Maternal undernutrition increased calf cortisol at birth and one month of age.
3) Hydroxytyrosol supplementation improved metabolic status in undernourished dams.
4) Hydroxytyrosol supplementation during fetal phase increased calf birth weight.
5) Hydroxytyrosol may help mitigate adverse undernutrition effects on cow-calf pairs.

Abstract

In semi-extensive cattle systems, cows may be exposed to undernutrition, which may reduce the productivity of beef herds. The objective of this study was to evaluate the effects of undernutrition and supplementation with hydroxytyrosol (**HT**, polyphenol derived from olive leaves) during the last third of gestation on cow productive, metabolic and hormonal profiles, transfer of passive immunity to calves via colostrum, and birth weight and vitality of newborn calves. A total of 109 pregnant beef cows (Parda de Montaña, n = 63; and Pirenaica, n = 46) were assigned into four feeding groups following a 2 x 2 factorial design, according to feeding level (100% vs. 60% of nutritional requirements) and dietary HT supplementation (Control vs. HT; 0 and 180 mg HT/kg total mixed ration) between 28 and 40 week of gestation. At week 28, all groups (100-Control, 100-HT, 60-Control and 60-HT) were balanced in BW, **BCS** (body condition score), breed and age. Cow BW and BCS were recorded, and plasma concentrations of non-esterified fatty acids (**NEFA**), cholesterol, insulin-like growth factor 1 (**IGF-1**), glucose, fructosamine and urea were analysed every three weeks during the last third of gestation. Cow cortisol was measured in late pregnancy and shortly after parturition. Colostrum and plasma from cows and calves were analysed for immunoglobulin (**IgM, IgG**) concentrations. At birth, calf weight and a vitality test were recorded. Undernutrition in the last third of pregnancy reduced dam BW at parturition and induced fat mobilisation. Hydroxytyrosol supplementation in the 60-HT group led to a more moderate increase in NEFA and cholesterol levels, along with a lesser decline in IGF-1 levels. Hydroxytyrosol also decreased dam plasma urea levels. Calves from HT-supplemented dams were heavier at birth. Undernutrition during the last third of gestation resulted in elevated plasma cortisol levels in newborn calves, which persisted for one month, while dam cortisol levels were not affected. Plasma IgG concentrations were higher in HT-supplemented dams regardless of feeding level, and higher concentrations of IgM and IgG in colostrum were maintained throughout the first 24 hours postpartum on them. Calf vitality was only affected by calving difficulty. Overall, undernutrition during the last third of gestation had negative effects on dam BW, BCS and indicators of metabolic status, partially alleviated by HT supplementation, and increased cortisol levels in newborn. Moreover, HT supplementation increased calf birth weight and helped to maintain colostrum IgM and IgG concentration during the first 24 hours after calving.

Keywords

Maternal subnutrition; Olive polyphenols; Passive transfer immunity; Metabolism; Cattle.

Implications

Undernutrition in late gestation, a common scenario in cow-calf production systems, will impair cow performance and metabolism, with long-term repercussions on the suckler cow herds. Dietary supplementation with olive-derived polyphenols proved to be a suitable strategy to counteract some negative effects in undernourished pregnant dams, associated with intrauterine growth restriction and impaired offspring performance. This is a pioneering study about the use of HT in ruminants, done in controlled conditions, to provide essential knowledge about its potential benefits in order to apply in future management practices.

Introduction

Suckler cattle management in Spanish mountain areas is characterised by winter housing periods combined with summer grazing on natural pastures, a strategy to reduce feeding costs and optimise resource use (Casasús et al, 2002). However, in these grazing farming conditions, grass availability varies throughout the year, particularly in autumn and spring, often resulting in periods of undernutrition in cows that may affect their own performance and that of subsequent generations. The last third of gestation is a critical period, as dams must meet the increasing nutritional demands of the foetus—approximately 75% of foetal growth occurs during this period (Greenwood and Cafe, 2007)—while also preparing for the upcoming lactation.

In this scenario of extensive livestock systems with winter calving seasons, variations in grass availability can lead to undernutrition in last third of pregnancy. This can affect not only cow performance but also can have consequences in their calves and subsequent generations, resulting in long-term impacts on farm productivity. Other studies within this system have demonstrated that undernutrition in early pregnancy can have short-, medium- and long-term effects (Noya, 2020). In parallel, research groups working in different types of extensive systems have described the impact of undernutrition in late pregnancy. In undernourished Hereford beef cows, Batista et al. (2020) described greater lipid mobilisation (higher non-esterified fatty acid (**NEFA**) concentration) and reduced BW and body condition score (**BCS**) in the last third of gestation in cows that received 75% of their energy requirements compared to those that received 125%, although it did not impact on calf birth weight. However, the metabolic stress during late gestation can directly influence the transfer of passive immunity to the newborn and affect their metabolic and immune responses along the first weeks of life (Ling et al., 2018).

The use of polyphenols could be a promising strategy to enhance the metabolic adaptation to undernutrition and prevent negative effects on the newborn. Some reports have demonstrated the antioxidant properties of terpenes found in essential oils in cows (Leal et al., 2024) and the improvements in immunoglobulin concentrations in calves (Ozkaya et al., 2018). In particular, hydroxytyrosol (**HT**), a phenolic compound present in olive by-products (Rigacci and Stefani, 2016), has been studied primarily in monogastric species (Vázquez-Gómez et al., 2017; Garcia-Contreras et al., 2019; Heras-Molina et al., 2020). These studies showed contradictory results on piglet birth weight, with HT supplementation not consistently leading to increased birth weights. In a study with lipopolysaccharide-challenged broiler chickens, HT dietary supplementation has been shown to mitigate the detrimental effects of oxidative stress on body weight, body weight gain and feed conversion ratio, suggesting that HT can modulate the inflammatory response (Dias et al., 2024). Hydroxytyrosol has also been described as possessing antioxidant (Fang et al., 2023) and immunomodulatory properties (Rigacci and Stefani, 2016). The rate of production of reactive oxygen metabolites may be increased in situations of increased metabolic demand, such as late gestation and the onset of lactation (Castillo et al., 2005), and HT has been proved to react with free radicals, transforming them in non-reactive compounds (Gugliandolo et al., 2020). González-Santiago et al. (2006) showed that HT supplementation in hyperlipemic rabbits succeeded in reducing 50% of plasma total cholesterol. In fact, it inhibits lipogenesis and reduces hepatic inflammation and restores glucose homeostasis in humans (Karković Marković et al., 2019). Moreover, HT may play a role in the development of the cognitive system in humans (Zheng et al., 2015).

After an in-depth critical analysis of available knowledge, we hypothesised that nutrient restriction during the last third of gestation would affect dam metabolism, potentially impairing body condition at calving, the transfer of passive immunity, and vitality of the newborns. Moreover, HT supplementation was expected to mitigate the principal negative impacts of nutrient restriction on cows and their newborn offspring, due to its protective role against oxidative stress, and so to be considered an important tool to help cow metabolism during critical periods and to ensure that cows reach calving in optimal condition – factors that ultimately influence overall farm productivity. To our knowledge, no effects of HT supplementation in undernourished pregnant cows and in their newborn calves have been reported Using a bovine model, this study aimed to determine the effects of undernutrition and the potential role of HT supplementation in the dam diet during the last third of pregnancy on productive, metabolic, endocrine and immunological parameters in cows and their newborn offspring. This study includes novel aspects of dietary polyphenols as a preventive solution for the negative impact of undernutrition on late gestation suckler cows and their progeny, so common in beef production systems throughout the world. Preliminary and partial results from this study were presented in abstract form (López de Armentia et al., 2024a; López de Armentia et al., 2024b; López de Armentia et al., 2024c).

Material and methods

All procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria de Aragón (**CITA**), Spain (protocol number CEEA-04 2021-09). The care and use of animals were conducted in accordance with the European Parliament and Council of the European Union on the protection of animals used for experimental and other scientific purposes (Directive 2010/63/EU).

Animals, management and diets

This study was conducted at the CITA-La Garcipollera Research Station, located in the mountainous region of the central Pyrenees (Huesca, Spain, 945 m a.s.l.). A total of 136 multiparous cows—86 Parda de Montaña and 50 Pirenaica— with an average of 193 days postpartum (minimum 55 days, median 149 days) were synchronised to oestrus and artificially inseminated at a fixed time using semen from two bulls of each respective breeders associations, following the next protocol: On day 0, cows received a progesterone intravaginal device (PRID Delta 1.55 g; CEVA, Libourne, France) and 0.1 mg of GnRH (2mL Cystoreline;CEVA, Libourne, France). On day 7, they were administered 25 mg of prostaglandin $F_2\alpha$ (5mL Enzaprost, CEVA, Libourne, France). On day 9, the PRID was withdrawn, and 500 IU of pregnant mare serum gonadotrophin (Foligon 5000 IU; Intervet, International B.V., Boxmeer, The Netherlands) was given. On day 11, a second dose of 0.1 mg of GnRH (2mL Cystoreline) was administered, followed by fixed time artificial insemination (AI) eight hours later using conventional semen. Three weeks later, two bulls of proven fertility from each breed were introduced to mate with dams of the same breed for 5 weeks to ensure a higher number of pregnancies. Natural service was used exclusively for repeat breedings. Pregnancies were confirmed by ultrasonography, with a pregnancy rate of 68.4% (93/136) after AI, and a total pregnancy rate of 83.8% (114/136) after the natural mating. A total of 109 pregnant cows were ultimately included in this study. From AI to the seventh month of gestation, all dams received a forage-based diet to meet their maintenance and gestational requirements, according to INRA (2018).

From 28 weeks of gestation to calving (40 weeks), dams received a forage-based total mixed ration (**TMR**) and were allocated to four groups, following a 2 x 2 factorial design, based on the feeding level (100% vs. 60% of maintenance and gestational nutrient requirements) and HT supplementation in the diet (Control vs. HT, corresponding to 0 and 180 mg HT/kg TMR, respectively). This resulted in the following groups: 100-Control, 100-HT, and 60-Control, 60-HT. At the beginning of the nutritional treatment (28 weeks of gestation), all groups were balanced in BW, BCS, breed and age. On average, cows had an BW of 662 ± 51 kg, a BCS of 3.6 ± 0.5 (on a scale of 1−5) and were 8.6 ± 2.25 years old. Diets were calculated according to **INRA** (National Institute for Agricultural Research, 2018) to meet dam maintenance and gestational requirements. The groups in the 100% feeding level received 10.5 kg of TMR (total mixed ration)/day, while the undernourished cows received 7.0 kg of TMR/day to meet 60% of their energy requirements. Cows were loose-housed (17.1 m^2 / cow) in group pens (13-17 cows / pen), consisting of a feeding area outdoors (9.2 m^2 / cow) and a straw-bedded area indoors (7.9 m^2 / cow). Each dietary treatment was replicated twice, with a total of eight replicate pens. Feed was provided daily at 8:00 am, distributed uniformly in the feeders of each pen. Cows remained restrained using self-locking headlocks (0.7 m / cow) for a period of 3 hour following ration administration to ensure adequate time for ration consumption. Prior to the commencement of the study, a trial was conducted to verify that this duration allowed all cows to finish their ration in 3 hours. Cows were fed with two types of TMR with a common base (Table 1). The basal TMR contained either 1.8% HT (for 100-HT and 60-HT groups) added in the form of mash, or 1.8% water (for 100-Control and 60-Control groups). To prepare the HT-enriched TMR, HT was added in the form of at 18 L/tonne of feed, with a concentration of 10 g HT/L of solution, to reach a final concentration of 180 mg HT/kg of diet. The TMR did not differ between dietary treatments, except in HT content.

The HT dose was determined based on previous evidence regarding its health-beneficial effects in other species (Rodríguez-Gutiérrez et al., 2012; Robles-Almazan et al., 2018; Vázquez-Gómez et al., 2017) and following the instructions of the company. To our knowledge, no previous experience exists with HT in ruminants, so a dose above that used in the mentioned studies was used to guarantee a sufficient dose, but still considered safe by the European Food Safety Authority (**EFSA**). Since 2011, the EFSA has approved health claim for olive oil polyphenols, recommending a daily consumption of 5 mg HT and its derivatives (e.g. oleuropein complex and tyrosol) provided by moderate amounts of extra virgin olive oil (20 g per day) as part of a balanced diet in humans. This dose is sufficient to confer health-beneficial properties including the reduction of low-density lipoproteins oxidation, an increase in high-density lipoproteins, the maintenance of normal blood pressure, and the prevention of the pro-inflammatory processes. This dose is still below the No Observed Adverse Effect Level dose for subchronic toxicity (repeated doses for 90 days) in humans (100-200 mg/kg BW) and rats (50 mg/kg BW) published by EFSA (EFSA, 2017).To ensure that the HT was being absorbed properly, dam blood samples were taken at the beginning of the experiment and 12 weeks later, detecting the presence of HT metabolites (hydroxytyrosol sulphate and homovanillic alcohol sulphate), as explained in Escalera-Moreno et al. (2025).

After calving, all cows received a diet formulated to meet 100% of their maintenance and lactation requirements, consisting of 10.5 kg/day of the Control TMR described in gestation. During lactation, cows were loose-housed (15.3 m^2 / cow) in pens (13-17 cows / pen) consisting of a feeding area outdoors (9.2 m^2 / cow) and a straw-bedded area indoors (6.1 m^2 / cow). Cows were restrained using self-locking headlocks (0.7 m / cow) for a period of 3 hour following ration administration to ensure adequate time for ration consumption, as reported in previous studies in similar conditions (Álvarez-Rodríguez et al., 2020). Calves remained in groups in fenced cubicles (1.8 m^2 / calf) bedded with a combination of straw and wood shavings, allowing cow-calf fence contact. The cows and calves could not put their heads through the fence, but they could still touch snouts with each other through it. Calves were fed only colostrum and milk from their respective mothers with a restricted twice-daily nursing system, consisting of two 30-minute periods at 7:00 and 14:30, until weaning at four months of age.

Productive, metabolic and endocrine profiles of cows during gestation

Cows were weighed at 8:00 am (before feeding) every three weeks during the last third of gestation, and cow BCS (1-5 scale) was registered at the same time by two expert technicians, based on the estimation of fat cover on loin and tailhead. Calf BW (kg) and sex (male/female) were recorded at calving. Dam blood samples were collected via coccygeal venepuncture into heparinised and EDTA-coated tubes (BD Vacutainer, Becton-Dickinson and Company, Plymouth, UK), at 8:00 am (before feeding). Dam samples to analyse plasma concentrations of NEFA, cholesterol, glucose, fructosamine and urea were collected every three weeks, while samples for IGF-1 were collected every six weeks and samples for cortisol analysis were taken at 37 weeks of gestation and at day 5 postpartum. Calf blood samples for cortisol analysis were taken on day 5 and one month postpartum. All samples were centrifuged at 1 500 x g for 20 min at 4 ºC. Plasma was extracted and stored at -20ºC until analysis in an external laboratory (Laboratorio Albéitar, Zaragoza, Spain).

Urea, IGF-1 and cortisol were analysed in plasma collected in heparinised tubes, while NEFA, cholesterol, glucose and fructosamine were analysed in EDTA-coated tubes. An automatic analyser (BA 400 Led technology BioSystems, Barcelona, Spain) was used to measure the following plasma concentrations of glucose (glucose oxidase/peroxidase method; sensitivity: 0.199 mmol/L), cholesterol (cholesterol oxidase/peroxidase method; sensitivity: 0.109 mmol/L), urea (kinetic UV test; sensitivity: 0.167 mmol/L) and fructosamine (nitroblue tetrazolium method; sensitivity: 0.140 mmol/L). To that purpose, the following protocols were used, according to the manufacturer's instructions and following the methodology described at Noya et al. (2019b): Glucose concentration in plasma was determined using Glucose Oxidase/Peroxidase kit (BioSystems S.A., Barcelona, Spain; catalogue number COD 21503); cholesterol concentration was determined using Cholesterol Oxidase/Peroxidase kit (BioSystems S.A., Barcelona, Spain; catalogue number COD

21505); urea concentration was determined using the Gernon Urea Reagent kit (RAL, Técnica para el Laboratorio, S.A., Barcelona, Spain; catalogue number GN 71000) and fructosamine concentration was determined using the Fructosamina SEBQ kit (Labtest Diagnóstica S.A., Lagoa Santa, Brazil; catalogue number 97). Non-esterified fatty acids were quantified enzymatically using a Randox Laboratories Ltd. (Crumlin Co., Antrim, UK; catalogue number FA 115) commercial kit, which demonstrated a sensitivity threshold of 0.07 mmol/L. For these assays, the mean intra-assay coefficients of variation were 4.7% and 4.8% for high and low controls, respectively, while inter-assay coefficients were 4.5% and 4.3%. Insulin-like growth factor 1 and cortisol concentrations were determined using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK; catalogue numbers LKIGF1 and LKCO1, respectively). The assay sensitivities were 14.400 ng/mL for IGF-1 and 1.462 nmol/L for cortisol. Intra-assay coefficients of variation for all commercial serum controls were ≤ 10%.

Immunoglobulin concentration in colostrum and cow-calf plasma and colostrum chemical composition

Two blood samples from cows were collected via coccygeal venepuncture into EDTA-coated tubes at 37 weeks of gestation and during Period 1 (from 0 to 12h postpartum [pp]). Colostrum samples from all udder quarters were manually collected in Period 1 (0-12h pp) and in Period 2 (12-24h pp) following the methodology described by Noya et al. (2019b). All samples were stored at -20 ºC until analysis. Calf blood samples for plasma Ig concentration were taken via jugular venepuncture 48h and 1 month after birth. Blood samples were centrifuged at 1 500 × g for 20 minutes at 4ºC, and plasma was stored at -20 ºC until analysis. Plasma and colostrum immunoglobulins (**Ig**) G and M concentrations were carried out at CITA (Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain) laboratory following the methodology described at Noya et al. (2019b) and using commercial specific bovine ELISA kits (Bovine IgM ELISA Quantitation Set, Cat.No. E11-101; and Bovine IgG ELISA Quantitation Set, Cat.No. E11-118; Bethyl, Montgomery, TX, USA) which provided detection sensitivities of 1.37 ng/mL for IgM and 0.7 ng/mL for IgG. Prior to analysis, samples were diluted 1/50 000 and 1/500 000 in colostrum (IgM and IgG respectively), and 1/20 000 and 1/300 000 (IgM and IgG respectively) in plasma. All ELISA procedures followed the manufacturer's guidelines. To minimise nonspecific binding during assay procedures, 1:9 diluted gelatin from cold water fish skin (No. G7765, Merck KGaA, Darmstadt, Germany) was added to the set blocking solution. Additionally, to prevent protein sample loss, all samples were processed using low binding tubes (Protein LoBind tube 2.0 ml, Eppendorf, Hamburg, Germany). The mean intra-assay coefficients of variation were 3.3% for IgM and 3.1% for IgG, while the inter-assay coefficients of variation were 8.1% for IgM and 7.2% for IgG. Fat, protein, lactose and urea concentrations in colostrum were analysed at Asociación Interprofesional Lechera de Aragón (Zaragoza, Spain) laboratory by an infrared scan (Milkoscan 7 RM; Foss Electric Ltd., Hillerød, Denmark). Intra-assay coefficients of variation for all commercial colostrum controls were ≤ 10%. Samples were diluted to carry out the analysis: 1:4.

Evaluation of the newborn vitality

Newborn vitality test was performed immediately after parturition using a modified calf vitality test proposed by Mee (2008) and implemented by Noya et al. (2019a). The following parameters were evaluated: meconium staining around the anal area (no staining vs. stained), tongue morphology (normal vs. swollen or protruding tongue), calf general attitude (attempts to stand vs. no effort to rise), palpebral reflex (actively blinks vs. reduced or no reflex), finger suckling reflex (strong vs. weak or absent) and mucous membrane colour (bright pink vs. red/white/blue). Furthermore, calving difficulty was classified into two categories: eutocic (unassisted) vs. dystocic (assisted) parturition.

Statistical analysis

Data were analysed using the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The current response variables were recorded on individuals, thereby the animal was considered the experimental unit. Data were checked for normality of residuals using the Shapiro–Wilk test. Cow BW, BCS, metabolites and hormone concentrations (NEFA, cholesterol, IGF-1, glucose, fructosamine, urea) were analysed using a mixed linear model (**MIXED** procedure) for repeated measures with feeding level (60% vs. 100%), HT supplementation (Control vs. HT), time (week of gestation), breed (Pirenaica vs. Parda de Montaña) and dam age (< or ≥ 10 years old) and their two-way interactions (and three-way interactions that include time) as fixed effects, and cow as a random effect. In the remaining traits the models were the same but the time effect considered the comparison between 37 weeks of gestation vs. calving for cow plasma Ig concentrations, Period 1 vs. Period 2 for colostrum composition and Ig concentrations, and 48h vs. 1 month postpartum for calf plasma Ig concentrations. Calf birth BW, calf BW at 1m of age and cortisol concentrations were analysed using a GLM procedure with feeding level, HT supplementation, breed, and sex of the calf as fixed effects. Initial dam BW at 28 weeks of gestation nested within the breed was included as a covariate in all models. Least-square (**LS**) means were calculated using the Kenward-Roger adjusted degrees of freedom. The results are presented as LS means ± standard error. A chi-square test was used to examine the association between vitality test results of the newborn and the following parameters: feeding level, HT supplementation, breed, calving difficulty, calf sex, dam age. A chi-square test was also used to assess the association between calving difficulty and feeding level and HT supplementation. Fisher’s exact test was applied when expected frequencies were less than 5. Relationships between the parameters were determined using Pearson’s correlation coefficients. The considered level of significance was $P<0.05$ and $P<0.1$ was considered a tendency.

Results

Productive, metabolic and endocrine profiles of cows during gestation

As the three-way interaction (feeding level × HT × time) was not significant, only the results of the significant feeding level × HT interaction observed at specific weeks are presented. Dam BW during the last third of gestation was affected by the interaction between feeding level and HT supplementation at different weeks. Differences of least square means showed that groups in the 100% feeding level (100-Control and 100-HT) were heavier at 31, 34, 37 and 40 weeks of gestation and weighed 41 kg more than groups in the 60% feeding level (60-Control and 60-HT) at 40 weeks. Moreover, 60-HT dams were heavier than 60-Control dams at 34 and 40 weeks of gestation (Fig. 1A). Cows in the 60% feeding level lost BCS along the last third of gestation, resulting in a final difference of 0.6 points compared to their 100% counterparts (Fig. 1B) at 40 weeks.

At the beginning of the study (28 weeks of gestation) no significant differences were observed in any of the metabolites or hormones studied. Cows in the 60-Control group had higher concentrations of NEFA compared to those in the 100% feeding level from 31 weeks of gestation onwards (Fig. 1D). Furthermore, 60-Control cows had higher NEFA concentration at 37 weeks of gestation ($P<0.05$) and tended to have higher concentrations at 40 weeks ($P=0.07$) compared to 60-HT. Cholesterol concentrations of cows in the 60% feeding level (60-Control and 60-HT) were higher than those observed in the 100-HT group at 34, 37 and 40 weeks of gestation. Differences of least square means showed that the 100-Control group had higher cholesterol concentrations than their 100-HT counterparts at 34 and 40 weeks. Similarly, 60-Control dams had higher cholesterol concentrations than 60-HT at 31 weeks (Fig. 1F). Insulin-like growth factor 1 values decreased as calving approached in all groups (Fig. 1C). IGF-1 concentration was higher in cows in the 100% feeding level and in 60-HT compared to 60-Control at 34 and 40 weeks of gestation. Cows in the 60% feeding level presented lower concentrations of glucose (Fig. 1E) and fructosamine (Fig. 1G) than their 100% feeding level counterparts as the study progressed and no differences were found according to HT supplementation. Fructosamine was positively correlated with glucose (r=0.46; $P<0.001$) and IGF-1 (r=0.46; $P<0.001$) and negatively correlated with NEFA (r=-0.41; $P<0.001$). Regarding the urea concentrations (Fig. 1H), cows in the 100-Control group had higher plasma urea concentrations than HT groups (100-HT and 60-HT) at 34 and 37 weeks, showing the biggest differences in 100% groups (100-Control vs. 100-HT).

Calf birth weight and cortisol concentrations in cow-calf pairs

Feeding level during the last third of gestation did not affect calf birth weight (Table 2). In contrast, maternal supplementation with HT resulted in increased calf birth weight, regardless of the feeding level. Additionally, male calves were heavier than females at birth (50.7 ± 0.7 kg and 45.5 ± 0.7 kg, respectively; $P<0.001$). Calf sex ratio was not different among groups. Cortisol concentrations are presented in Table 2. Undernutrition during the last third of gestation did not affect dam cortisol concentrations at 37 weeks of gestation or 5 days postpartum. However, it did have an effect on the offspring, as calves from dams in the 60% feeding level had higher cortisol concentrations than calves from dams in the 100% feeding level at 5 days postpartum, and this difference persisted one month later. No significant HT effect was observed in dams or calf plasma cortisol concentrations.

Immunoglobulin concentration in colostrum and cow-calf plasma and colostrum chemical composition

Maternal feeding level during late pregnancy did not influence either IgM or IgG plasma concentrations in either cows or calves. Although no effect was found on colostrum IgM concentrations, undernutrition tended to decrease colostrum IgG levels during Period 2 (72.4 ± 7.8 mg/mL vs. 57.5 ± 7.5 mg/mL, for 100% and 60% dams, respectively, *P*=0.06). Regarding HT effects, the inclusion of HT in dam diet significantly increased average IgG concentrations in dam plasma (24.8 ± 0.7 mg/mL vs. 22.7 ± 0.8 mg/mL for HT and control groups, respectively; *P*<0.05), whereas no effects were observed in dam IgM concentrations. Furthermore, as shown in Figure 2, HT supplementation in maternal diet with maintained higher colostrum concentration of both IgM (*P*<0.05) and IgG (*P*<0.05) in Period 2. However, no effects of maternal HT supplementation were observed in calf plasma immunoglobulin (IgM and IgG) concentrations. In relation to the evolution over time, all parameters were affected (Figure 3). There was an increase in IgM and a decrease in IgG concentrations from 37 weeks of gestation to calving in dam plasma (*P*<0.001). Both IgM and IgG in colostrum decreased almost threefold from Period 1 to Period 2 (*P*<0.001). In calves, plasma IgG concentrations were more than twice as high, and IgM concentrations more than four times as high, 48h after calving than one month postpartum (*P*<0.001). Calf plasma IgM concentrations 48 hours after birth were positively correlated with dam plasma IgM concentrations at both 37 weeks of gestation (r=0.56; *P*<0.001) and at calving (r=0.52; *P*<0.001). Similarly, dam plasma IgM concentrations at 37 weeks of gestation and at calving were correlated with colostrum IgM concentrations, both at Period 1 (r=0.68 and r=0.57, respectively; *P*<0.001) and at Period 2 (r=0.46 and r=0.35, respectively; *P*<0.001). Additionally, dam IgG concentrations at 37 weeks and at calving were negatively correlated with concentrations of cortisol at 37 weeks (r=-0.21 and r=-0.23, respectively; *P*<0.05).

Colostrum composition data is presented in Table 3. An interaction between feeding level and HT supplementation was observed for both fat and protein content in Period 1. HT supplementation significantly decreased fat content in the 60% feeding level (5.4% vs. 3.2% for 60-Control and 60-HT, respectively, *P*=0.008), while these differences were not significant in the 100% feeding level groups (5.0% vs. 3.9% for 100-Control and 100-HT, respectively, *P*=0.200). Similarly, HT supplementation significantly reduced the protein content in the 60% feeding level (19.1% vs. 16.1% for 60-Control and 60-HT; *P*=0.030) and in 100% feeding level (17.9% vs. 15.0% for 100-Control and 100-HT; *P*=0.046) in Period 1. Hydroxytyrosol supplementation decreased lactose content in Period 1 and increased protein and urea content in Period 2, as shown in Table 3.

Evaluation of the newborn vitality

Calving difficulty was not significantly affected by feeding level (15% vs. 26% of dystocia in dams of feeding levels 100% and 60%, respectively; *P*=0.16) or HT supplementation (18% vs. 24% of dystocia for Control and HT, respectively; *P*=0.40). The vitality of the newborn calves was affected only by calving difficulty, with no significant effects of either feeding level or HT supplementation. Dystocia reduced the proportion of calves with optimal attitude (68% for dystocic vs. 99% for eutocic parturitions; *P*<0.001), with strong finger suckling reflex (73% vs. 91%; *P*<0.05), optimal mucous membrane colour (73% vs. 94%; *P*<0.01) and non-protrusive tongue (70% vs. 98%; *P*<0.01). Neither meconium staining around the anal area nor the palpebral reflex was affected by any of the studied parameters.

Discussion

Productive, metabolic and endocrine profiles of cows during gestation

As the three-way interaction (feeding level × HT × time) was not significant, only the results of the significant feeding level × HT interaction observed at specific weeks are discussed. Although dam BW increased across all treatments during late gestation due to foetal growth (as approximately 75% of foetal growth occurs in late gestation; Greenwood and Cafe, 2007), undernutrition led to lower BW gains and a reduction in BCS. The fact that BCS remained stable in the 100% feeding level indicates that the nutritional requirements were adequately met by the diets, whereas undernourished dams (those in the 60% feeding level) had to mobilise body fat reserve to ensure the proper foetus development. These findings are consistent with previous reports, e.g. Batista et al. (2022) also found lower BW and BCS in cows subjected to feed restriction during the last three months before calving. Notably, in our experiment, 60-HT dams exhibited greater BW than 60-Control dams at 34 and 40 weeks of gestation, suggesting that HT supplementation allowed them to counterbalance the metabolic challenge of undernourished cows.

In order to meet the increasing demands of their physiological status, undernourished cows prioritised the development of the growing foetus by augmenting lipid catabolism, evidenced in the increase in NEFA concentrations. To prioritise glucose and amino acids for foetal development lipids and ketone bodies become important energy sources for the dam. Elevated NEFA concentrations in cows exposed to an energy deficit situation have been widely described in both dairy (Meikle et al., 2013; Song et al., 2021) and beef cows (Batista et al., 2020, 2022; Noya et al., 2020). Moreover, 60-HT cows exhibited lower plasma NEFA concentrations compared to 60-Control cows. Other authors have reported a reduction in plasma NEFA concentration in pigs supplemented with other polyphenols such as quercetin (Wein and Wolffram, 2014).

Cows in the 100% feeding level were expected to exhibit higher cholesterol concentrations associated with a higher nutrient intake, consistent with previous reports (Bjerre-Harpøth et al., 2014; Katica, 2019) as well as with our own previous findings in pregnant cows (Noya et al., 2020) and growing heifers (Rodríguez-Sánchez et al., 2018). However, this was not the case in the current study, in agreement with the observations of Gross et al. (2015) and Macías-Cruz et al. (2017) in feed restricted cows and ewes, respectively, which they associated with adipose tissue mobilisation. Consistent with the greater NEFA concentrations as a result of a greater fat mobilisation in undernourished cows, alterations in cholesterol metabolism may be a physiological response to ensure sufficient cholesterol supply for the production of very low-density lipoproteins to allow triglycerides to be released from the liver (Kessler et al., 2014) and avoid fat accumulation (Loor et al., 2007). Furthermore, HT supplementation was associated with lower cholesterol concentrations. Piglets born from sows supplemented with HT also exhibited reduced concentrations of total and

LDL cholesterol (Vázquez-Gómez et al., 2017). Regarding the use of polyphenols in ruminants, a study about the supplementation with resveratrol in sheep showed a decrease in cholesterol levels of supplemented sheep (Li et al., 2024). The authors pointed out that resveratrol can improve metabolic health and liver function in ewes, reducing the plasmatic cholesterol. Moreover, a study of supplementation of grape pomace in Friesian calves also showed a reduction in serum cholesterol, together with the downregulation of five genes involved in the cholesterol lipid biosynthesis pathway (Iannaccone et al., 2018). Nonetheless, further studies are warranted to better understand the mechanisms involved in cholesterol metabolism.

Similarly, plasma IGF-1 concentration, an indicator of glucose uptake, tissue growth and nutritional status (Breier et al., 1986; Ciccioli et al., 2003; Livingstone, 2013) was lower in 60-Control cows, consistent with previous reports of lower IGF-1 concentrations in undernourished cows during the first third of gestation (Noya et al., 2020). Moreover, cattle in a lower nutritional status or in a high demanding status usually show an uncoupling of the somatotropic axis, leading to lower plasma IGF-1 concentrations (Kobayashi et al., 1999; Rubio et al., 2021). Similarly, in the present study, it is likely that an uncoupling of the somatotropic axis happened in the 60% feeding groups, in response to the nutritional challenge they were experiencing. Moreover, this was mitigated by the HT supplementation in 60-HT cows, suggesting a metabolic or endocrine effect in the energy balance of these cows, and consistent with the lower NEFA mobilisation compared to their 60-Control counterparts. In a study performed by Escalera-Moreno et al. (2025) with the same cows, HT supplementation mitigated the oxidative stress and increased insulin-independent glucose uptake in blood cells (with increased gene expression of glucose transport SLC2A1/GLUT1) and an increase in long-chain fatty acid beta-oxidation. These reported metabolic changes probably improved the efficiency in obtaining energy from the diet in the 60-HT cows compared to 60-Control, reducing the uncoupling of the somatotropic axis and, therefore, reducing the drop in IGF-1 plasma concentrations.

Since plasma glucose reflects glycaemic status only at a specific, fructosamine analysis was also carried out. Concentrations of both glucose and fructosamine decreased throughout the last third of gestation in undernourished cows. In conditions of undernutrition, propionate, which depends on dietary intake, decreases, leading to a reduced rate of glucose synthesis (Lomax and Baird, 1983; Reynolds, 2005; Rodríguez-Sánchez et al., 2015), as shown in this study. Studies using supplementation with essential oils (blend from anise, cinnamon, garlic, rosemary and thyme) or tea polyphenols in small ruminants and dairy cows have described the ability of these compounds to modify rumen fermentation towards propionate, which could impact blood glucose concentrations (Oh et al., 2017; Palhares et al., 2021; Zhao et al., 2025), so HT was expected to increase glucose concentrations. The absence of this expected increase may be attributed to enhanced placental glucose transfer to the growing foetus, particularly as maternal HT supplementation was associated with increased calf birth weight (see below). Regarding the use of fructosamine in ruminants, its use as a marker of glycaemic status remains controversial. Some authors affirm that it could be a good reflection of prolonged hypoglycaemia (Mostafavi et al., 2015), while others do not support this idea (Roche et al., 2013; Megahed et al., 2018). In our study, fructosamine was negatively correlated with NEFA and positively with glucose and IGF-1, suggesting that under our experimental conditions, fructosamine was a reliable indicator of the metabolic status and energy balance of cows during late gestation.

The lower plasma urea concentrations observed at 34 and 37 weeks of gestation in HT supplemented cows suggest an effect on protein, which is unlikely to be due to an imbalanced dietary protein, given that both TMR were isoproteic. Since calves from HT supplemented dams were heavier at birth (cf. below), HT supplementation may have enhanced placental amino acid transfer to the foetus, increasing the use of nitrogen and reducing cow plasma urea. Indeed, some studies have reported a decline in urea concentrations in lactating cows supplemented with essential oils, suggesting more efficient nitrogen usage (Braun et al., 2019) in supplemented animals. This effect seems to be dose-dependent, as 100-HT cows received a higher total amount of HT included in the TMR. Nevertheless, further research is necessary to elucidate the mechanisms involved in this process.

Calf birth weight and cortisol concentrations in cow-calf pairs

Prepartum feeding level did not affect calf birth weight, consistent with previous studies of a 70% (Redifer et al., 2023) or a 59% restriction (Hough et al., 1990). However, calves born from mothers supplemented with HT were heavier at birth than those from non-supplemented mothers. In piglet studies, Heras-Molina et al. (2020) reported lower birth weights in offspring of supplemented sows, whereas Vazquez-Gomez et al. (2017) observed higher birth weights, the latter being consistent with our findings. The increased calf birth weight observed in the HT supplemented groups in this study may be associated with the higher NEFA and lower urea concentrations found in those dams. As described above, foetal growth requires amino acids provided by some specific transporters, and changes in diet and exposure to oxidative stress can affect placental function, causing an impaired placental amino acid supply and consequences on foetal growth and development (Cleal et al., 2007; Myatt, 2010). Additionally, the lack of differences in glucose and fructosamine concentrations between HT and Control groups may indicate an increased glucose transfer to the fetoplacental unit in the supplemented dams, thereby supporting the higher birth weight of calves. Calf birth weight was also affected by sex, with males being heavier than females, as described by other authors (Villalba et al., 2000; Fiems et al., 2009).

Plasma cortisol concentrations of dams were not affected by prepartum feeding level. Interestingly, undernutrition in pregnant dams was related to higher concentrations of plasma cortisol in their newborn calves, consistent with other studies conducted during late (Hough et al., 1990) or early pregnancy (Noya et al., 2019b). In the current study, cortisol concentrations remained higher one month after calving in those calves born from undernourished dams, confirming the medium-term impact of prenatal undernutrition on calf cortisol concentrations. Studies performed in different species have described the effects of prenatal exposure to glucocorticoids extending up to the third generation (Iqbal et al., 2012; Moisiadis et al., 2017). Foetal exposure to glucocorticoids has been shown to modify the hypothalamo-pituitary-adrenal (HPA) axis function (Iqbal et al., 2012). Hence, prenatal undernutrition may have induced changes in the HPA axis function in the calves though not in the dams of the current study. Notably, although no significant differences were found in plasma cortisol concentrations of dams at calving, their values were correlated with those of calves at one month of life. This relationship supports other studies that the metabolic stress (measured by plasma cortisol concentrations) is transferred from dam to calf via placenta (Uetake et al., 2014; Arfuso et al., 2023).

Immunoglobulin concentration in colostrum and cow-calf plasma and colostrum chemical composition

The Ig concentrations in dam and calf plasma or in the colostrum were not affected by the feeding level during late gestation. Similarly, other studies found little or no differences in colostrum Ig content in 60% energy- or protein- restricted dams (Fiems et al., 2009; Hough et al., 1990) and in their calf plasma concentrations (Fiems et al., 2009; McGee and Earley, 2019). However, Shearer et al., (1992) showed that colostrum from cows gaining BCS in the previous weeks to parturition had higher Ig concentrations. In this study, undernourished cows were still on an optimal BCS which would explain the similar Ig concentration in both feeding levels and thereby a similar potential transfer of passive immunity from dams to newborn calves, regardless of pre-partum diets.

Given the immunomodulatory properties of HT (Singh et al., 2011; Persia et al., 2014), the higher plasma IgG concentrations observed in HT-supplemented dams may indicate an improvement in their immune status. The higher colostrum IgG and IgM concentrations in Period 2 (12-24h postpartum) suggest that HT may have slowed down the fall of IgM and IgG concentration in colostrum, although the mechanisms involved remain unclear. However, the higher IgM and IgG concentrations in colostrum were not associated with higher Ig concentrations in calf plasma. The capacity of calves for IgG absorption is at its maximum within the first 4-6 hours but decreases as the hours go by (Yang et al., 2025). In Period 1, the colostrum IgG concentration of all groups was high and calves from both groups had probably, calves from both groups had the chance to ingest high-quality colostrum and reach the necessary IgG plasmatic concentration.The absorption efficiency is about 65% and 17% for IgG and IgM (Ballarini, 1993), which means that calves will only absorb a small amount of the ingested Ig, but the IgG concentration in colostrum is high. The colostrum IgG concentration of the cows in this study should be sufficient to guarantee enough absorption of IgG in all calves, as the plasmatic concentrations of calves born to all groups show. Besides, it has been reported that, in addition to the systemic immune function of absorbed immunoglobulins, those that remain unabsorbed exert a local protective effect on the intestines (Aydogdu and Guzelbektes, 2018). Therefore, the elevated colostrum immunoglobulin concentration in HT-supplemented dams may have conferred gastrointestinal benefits to the calves. None of the studied fixed factors affected the calf Ig concentrations at the age of one month. Calves gradually lose the passive immunity transferred by their dams as their own immune system matures throughout the first weeks of life (Logan et al., 1973) as they begin producing endogenous IgG and IgM (Husband et al., 1972; Devery et al., 1979), with functional immune maturity generally achieved by one month of age (Barrington et al., 2001). The concentration of both studied colostral immunoglobulins (IgM, IgG) decreased from Period 1 to Period 2, highlighting the importance of ensuring that calves receive colostrum within the first hours of life.

Hydroxytyrosol supplementation decreased colostrum fat content only in undernourished animals in Period 1. Since it was such a short period of time, the difference in fat content likely did not have an effect on the calves. As reported in Escalera-Moreno et al. (2025), HT supplementation could mitigate the cellular oxidative stress (by improving the redox balance, reducing lipid peroxidation and improving mitochondrial efficiency), which could reduce the need for mobilising maternal reserves, thereby preserving body mass. In the case of protein, HT significantly reduced the protein content in both feeding groups in Period 1. Other studies on supplementation reported no significant differences in colostrum fat, protein, or lactose content in either cows (Aragona et al., 2021; Rolinec et al., 2021) or sows (Laviano et al., 2023). Our research suggests that HT supplementation may have changed the colostrum chemical composition. However, the interpretation of colostrum-related results should be approached with caution, as the total volume of colostrum could not be determined. Further research is warranted to explore these effects in greater detail.

Under our experimental conditions involving cows with high BCS, prepartum undernutrition had no observable effect on the passive transfer of immunity from dams to offspring, suggesting normal immune system development. Nonetheless, inclusion of HT in the prepartum diet of dams helped maintain higher-quality colostrum over the first 24 hours, which would be a promising tool for improving passive transfer of immunity in cows with poor immune quality colostrum. These results may be a way to follow in the further understanding into cow-calf immune status and neonatal survival.

Evaluation of the newborn vitality

Feeding level, HT supplementation, dam age, breed and sex of the calf had no effect on the vitality of the newborn calves. The only factor that affected the vitality of the calves was the calving difficulty. Dystocia reduced the proportion of calves exhibiting optimal posture, a strong finger suckling reflex, and non-protrusive tongue, among others, all of which are crucial for the colostrum intake. In the current study, the type of parturition was not affected by the feeding level, consistent with other studies (Bellows and Short, 1978; Hough et al., 1990), or by HT supplementation.

Conclusion

In conclusion, undernutrition in late pregnancy reduced dam BW and BCS, redirecting the maternal metabolic pathways towards a catabolic status, which was reflected in higher plasma concentrations of metabolites related to lipid catabolism and lower concentrations of metabolites related to carbohydrate metabolism. Furthermore, calves born from undernourished cows exhibited higher concentrations of plasma cortisol at the 5th day of life and at one month of age. Inclusion of HT in the dam's prepartum diet proved to be an effective strategy to help undernourished cows cope with the metabolic challenge and to maintain higher concentrations of IgM and IgG throughout the first 24 hours postpartum. Regarding progeny, heavier calves were born from HT-supplemented dams. To the best of our knowledge, this is the first study of supplementation with olive-derived hydroxytyrosol in beef cows during late gestation. Further research is warranted to study the impact of prepartum undernutrition and HT inclusion on the efficiency of cow-calf pairs during the following lactation and offspring rearing phases, and to determine the potential long-term repercussions on performance of beef herds.

Ethics approval

All procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Spain (protocol number CEEA-04 2021-09). The care and use of animals were conducted in accordance with the European Parliament and Council of the European Union on the protection of animals used for experimental and other scientific purposes (Directive 2010/63/EU).

Data and model availability statement

The data that supports the study findings will be available in public FAIR-compliant repositories (ZENODO and CITAREA). Until then, data are available from the authors upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process.

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Declaration of interest

None.

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Table 1

Ingredients and chemical composition of the total mixed ration (TMR) offered to cows (g/kg, unless otherwise stated)

Item	**Value**
Ingredients (as fed basis)	
Barley straw	496
Barley grain	248
Alfalfa pellet	85
Rapeseed meal	69
Sugar beet pulp	45
Soybean meal	25
Water (in Control diet) or Hydroxytyrosol mash	18
Calcium carbonate	8
Dicalcium phosphate	2
Sodium chloride	2
Vitamin-mineral premix[1]	2
Chemical composition	

DM (g/kg)	888
Ash (g/kg DM)	72
CP (g/kg DM)	111
NDF (g/kg DM)[2]	529
ADF (g/kg DM)	328
ADL (g/kg DM)	53
Ether extract (g/kg DM)	19
Starch (g/kg DM)	161

Abbreviations: TMR = Total mixed ration

[1] The vitamin-mineral premix contained (per kg of TMR): Vitamin A 6000 IU/kg, Vitamin D3 1200 IU/kg, Vitamin E 10mg/kg (all-rac-α-tocopheryl acetate), Fe 20mg/kg (ferrous carbonate), I 2mg/kg (calcium iodate), Co 0.2mg/kg (cobaltous carbonate).), Mg 30 mg/kg (manganese oxide), Zn 50 mg/kg (zinc oxide), Se 2 mg/kg (sodium selenite), citric acid 0.03 mg/kg, butyl-hydroxytoluene (BHT) 0.12 mg/kg and sepiolite 0.5 g/kg. Additionally, the cows were provided ad libitum mineral blocks, which contained Na 312 g/kg (sodium chloride), Ca 40 g/kg (calcium carbonate), Mg 1.0 g/kg (magnesium oxide), P 1.0 g/kg (dicalcium diphosphate), Zn 1 g/kg (zinc oxide), Fe 620 mg/kg (iron carbonate), Mn 445 mg/kg (manganese sulphate monohydrate), I 20 mg/kg (calcium iodate anhydrous), Se 15 mg/kg (sodium selenite), Se 0.2 mg/kg (selenomethionine-enriched yeast) and Co 15 mg/kg (cobalt acetate tetrahydrate).

[2] In vitro true digestibility of the manufactured TMR diets was 34.7 and 42.5±1.93% for Control and Hydroxytyrosol, respectively. The TMR substrates were incubated with cattle rumen inoculum at 39 º C under anaerobic conditions for 48 hours (n=9 per treatment). At the end of incubation, residues treated with neutral detergent to determine the in vitro true digestibility of the substrates as the proportion of the difference between DM incubated and the residual NDF (Gordo et al., 2023).

Table 2. Cortisol plasma concentrations of cows (37-week gestation, 5 d pp) and their calves (5d pp, 1 m pp), calf birth weight and calf BW 1 m pp.

	FL [1]			HT [2]			*P* value		
Item	100%	60%	SED	Control	HT	SED	FL	HT	FL x HT
Dam cortisol 37 week gestation (nmol/L)	11.7	11.9	1.1	12.8	10.8	1.1	0.881	0.079	0.905
Dam cortisol 5 d pp (nmol/L)	17.5	18.8	2.6	16.6	19.8	2.6	0.620	0.241	0.572
Calf cortisol 5 d pp (nmol/L)	26.0^{b}	37.0^{a}	4.3	31.5	31.6	4.3	0.014	0.985	0.191
Calf cortisol 1 m pp (nmol/L)	10.9^{b}	17.0^{a}	2.9	12.5	15.5	2.9	0.039	0.307	0.462
Calf birth weight (kg)	48.5	47.7	1.0	46.9^{b}	49.3^{a}	1.0	0.434	<0.001	0.552
Calf BW 1m pp (kg)	88.2	86.5	2.0	86.3	88.4	20	0.408	0.300	0.865

Abbreviations: FL = feeding level; HT = hydroxytyrosol supplementation; m = month; d = day; pp = postpartum

[1] Feeding level: 100 vs. 60% requirements of maintenance and gestation

[2] Supplementation with hydroxytyrosol (0 vs. 180 mg HT/kg total mixed ration per day for Control and HT respectively).

Values within a row with different superscripts differ significantly at P<0.05. No significant interactions among effects were found (P>0.05). Values are expressed as least-square (LS) means.

Table 3. Cows colostrum composition in Period 1 and Period 2.

	FL [1]			HT [2]			*P* value		
Item	100%	60%	SED	Control	HT	SED	FL	HT	FL x HT
Fat (%)									
Period 1 [3]	4.5	4.3	0.6	5.2	3.6	0.6	0.722	0.006	0.032
Period 2 [4]	4.0	4.3	0.6	4.2	4.1	0.6	0.561	0.837	0.650
Protein (%)									
Period 1	16.4	17.6	1.0	18.5	15.5	1.0	0.248	0.004	0.021
Period 2	12.1	11.7	1.0	10.8 [b]	13.0 [a]	1.0	0.645	0.032	0.182
Lactose (%)									
Period 1	2.4	2.2	0.2	2.5 [a]	2.1 [b]	0.2	0.535	0.035	0.151
Period 2	2.9	3.2	0.2	3.1	3.1	0.2	0.089	0.707	0.270
Urea (mg/L)									
Period 1	674.1	651.7	54.7	622.7	703.1	55.2	0.683	0.147	0.335
Period 2	541.4	536.3	46.4	484.5 [b]	593.3 [a]	46.5	0.912	0.021	0.145

Abbreviations: FL = feeding level; HT = hydroxytyrosol supplementation; pp = postpartum; TMR = total mixed ration

[1] Feeding level: 100 vs. 60% requirements of maintenance and gestation

[2] Supplementation with hydroxytyrosol (0 vs. 180 mg HT/kg total mixed ration per day for Control and HT respectively).

[3] Period 1: 0-12h pp

[4] Period 2: 12-24h pp

Values within a row with different superscripts differ significantly at $P<0.05$. No significant interactions among effects were found ($P>0.05$). Values are expressed as least-square (LS) means.

Figure 1. Evolution of BW, BCS and metabolic and endocrine profiles throughout the last third of gestation in beef cows, according to FL (60% vs. 100% requirements) and

HT (0 vs. 180 mg HT/kg total mixed ration per day, for Control and HT). Different letters (a,b) between treatments differ significantly (*P*<0.05).

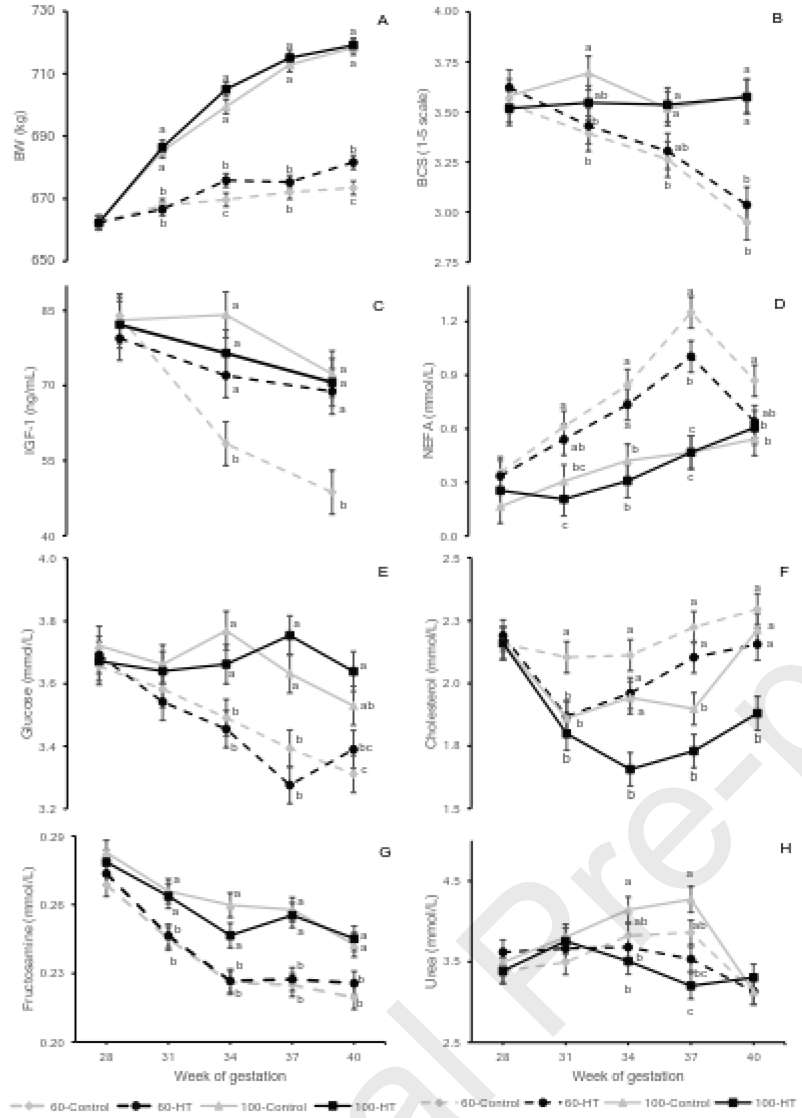
Abbreviations: BCS = body condition score; NEFA = non-esterified fatty acids; FL = feeding level; HT = hydroxytyrosol supplementation

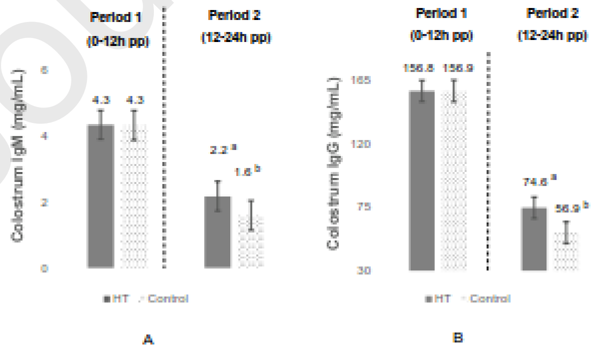
Figure 2. Cow colostrum Ig M (A) and Ig G (B) concentrations in Period 1 (0-12h pp), and Period 2 (12–24 h pp) according to HT (**HT**,0 vs. 180 mg HT/kg total mixed ration per day, for Control and HT) in dam diet during the last third of gestation. Different letters (a,b) between treatments differ significantly (*P*<0.05).

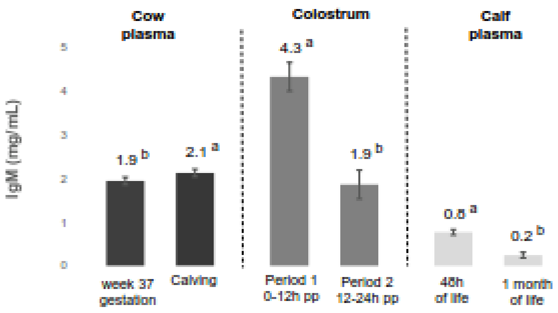
Abbreviations: Ig = immunoglobulin; pp = postpartum; HT = hydroxytyrosol supplementation

Figure 3. Mean Ig M (A) and Ig G (B) concentrations in dam plasma, colostrum and calf plasma throughout the time. Values with different letters (a,b) between cow, colostrum or calf samples differ significantly (*P*<0.05).

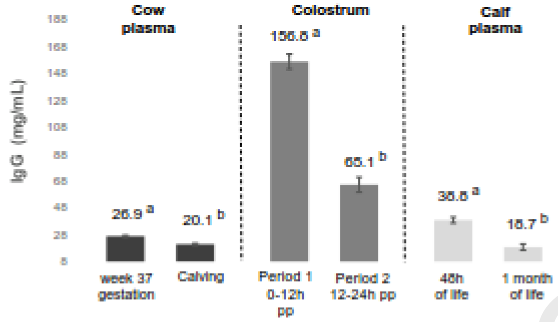
Abbreviations: Ig = immunoglobulin; pp=postpartum


A
BW (kg)
650
670
690
710
730
B
BCS (1-5 scale)
2.75
3.00
3.25
3.50
3.75
4.00
C
IGF-1 (ng/mL)
40
55
70
85
D
NEFA (mmol/L)
0.0
0.3
0.6
0.9
1.2
E
Glucose (mmol/L)
3.2
3.4
3.6
3.8
4.0
F
Cholesterol (mmol/L)
1.5
1.8
2.0
2.3
2.5
G
Fructosamine (mmol/L)
0.20
0.23
0.26
0.29
H
Urea (mmol/L)
2.5
3.5
4.5
28
31
34
37
40
Week of gestation
60-Control
60-HT
100-Control
100-HT


Period 1
(0-12h pp)
Period 2
(12-24h pp)
Colostrum IgM (mg/mL)
0
2
4
6
4.3
4.3
2.2 a
1.6 b
HT
Control
A
Period 1
(0-12h pp)
Period 2
(12-24h pp)
Colostrum IgG (mg/mL)
30
75
120
165
156.8
156.9
74.6 a
56.9 b
HT
Control
B


IgM (mg/mL)
5
4
3
2
1
0
Cow plasma
Colostrum
Calf plasma
1.9 b
2.1 a
4.3 a
1.9 b
0.8 a
0.2 b
week 37 gestation
Calving
Period 1 0-12h pp
Period 2 12-24h pp
48h of life
1 month of life

A


IgG (mg/mL)
180
160
140
120
100
80
60
40
20
0
Cow plasma
Colostrum
Calf plasma
26.9 a
20.1 b
156.6 a
65.1 b
36.6 a
16.7 b
week 37 gestation
Calving
Period 1 0-12h pp
Period 2 12-24h pp
48h of life
1 month of life

B