


Effects of rice bran and glycerin supplementation on metabolic and productive responses of beef cows

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ABSTRACT - Fifty-seven primiparous Angus, Hereford, and crossbred cows were used to study the effect of strategic supplementation on metabolic, productive, and reproductive responses. The experiment had two periods including four phases: prepartum supplementation (phase I; 52 days), early postpartum (phase II; 43 days), pre-mating supplementation (phase III; 21 days), and the last phase including mating, gestation, and lactation until weaning (phase IV; 103 days). Phases I and II were considered as period 1, and phases III and IV were considered as period 2. During phase I (-52 ± 2 days before calving to birth), half of the cows received a supplement (S-), and the rest only grazed native swards (C-). For phase III (59 ± 2 days postpartum [DPP] until mating), cows from the previous treatments (C- or S-) were sorted in two levels of pre-mating supplementation, supplemented (-S) or not supplemented (-C), resulting in four treatment combinations (CC, CS, SC, and SS). The supplement was 1 kg dry matter/cow per day of whole rice bran and 550 mL/cow per day of crude glycerin. In period 1, cows receiving prepartum supplementation increased concentration of cholesterol, glucose, and albumin and decreased concentration of non-esterified fatty acids, beta-hydroxybutyrate, and urea. This improvement in energy balance was reflected in a higher body condition score at calving. Alternatively, in period 2, pre-mating supplementation only increased cholesterol concentration. None of the supplementation periods affected the weaning weight of calves. Prepartum, but not pre-mating, supplementation increased total pregnancy rate. A short prepartum supplementation improves pregnancy rate of primiparous cows managed under extensive production systems. However, there is no additional benefit of supplementation during the pre-mating period.

Keywords: beef cattle, grazing, native pasture, reproduction

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Introduction

In extensive pastoral production systems, based on natural grassland, pregnancy rate and weaning weights are the main limitations for cow-calf operations and, within a typical herd, primiparous beef cows are usually the category most affected (Bellows et al., 1982). The nutrient supply by the Rio de la Plata grasslands and, more specifically, in the Campos subregion (Berretta et al., 2000; Modernel et al., 2016)] during winter (~ 8.7 MJ of ME/kg DM and ~ 90 g of crude protein/kg DM; Mieres, 2004) is insufficient to meet the last third of pregnancy requirements (Ferrell et al., 1976). The shortage of nutrients that causes a negative energy balance (NEB) during late gestation (Quintans et al., 2010;

Astessiano et al., 2012) and early postpartum due to milk production (Short et al., 1990; Astessiano et al., 2013) is normally followed by a decreased reproductive outcome (Soca et al., 2013).

The negative energy balance is evidenced by changes in the concentration of some metabolites and metabolic hormones and a decrease in body condition score (BCS) of the cows. Within the metabolites and hormones, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) are increased and cholesterol, glucose, and insulin are decreased (Wettemann et al., 2003; Quintans et al., 2010; Soca et al., 2014a; Quintans et al., 2016), negatively influencing follicle growth and ovulation (Wiltbank, 1970; Sinclair et al., 2002). Cows with low BCS at calving have longer anestrus postpartum and low pregnancy rates (Wettemann et al., 2003; Soca et al., 2014b), milk production, and calf weight at weaning. This is even deeper in primiparous cows (Bellows et al., 1982), since they have an additional energy demand to continue growing (Short et al., 1990).

Short-term supplementation before calving or mating are alternatives to increase the productive and reproductive efficiency of beef cows grazing native swards (Pérez-Clariget et al., 2007; Soca et al., 2013; Quintans et al., 2016). However, since traditional concentrates are relatively expensive, it is necessary to evaluate cheaper alternatives.

Crude glycerin (12.5-14.8 MJ of ME/kg DM; Schröder and Südekum, 1999; Donkin, 2008) and whole rice bran (10.6-13.3 MJ of ME/kg DM and 134-181 g of crude protein/kg DM; Wang et al., 2012; NRC, 2016) are subproducts of the industry not used for human consumption. Notwithstanding, whole rice bran, widely used for cattle in South America to supplement poor-quality herbage (Pérez-Clariget et al., 2007; Soca et al., 2013), is a moderate-energy supplement that can be easily improved by adding crude glycerin. The new mix of supplements would not only have a better energetic value than rice bran alone (Clariget et al., 2016a), but will also decrease the unit price of the final supplement.

We hypothesized that a strategic supplementation with a mix of whole rice bran and crude glycerin before calving and before mating would improve the energy balance and productive and reproductive performance of beef cows grazing native swards. Our objective was to evaluate the effect of supplementation either during 52 days before calving or 21 days before mating on metabolic and hormonal profiles, BW, BCS, milk production and composition, calf weight, ovarian activity, and pregnancy rate of primiparous beef cows grazing *Campos* grasslands of southern South America.

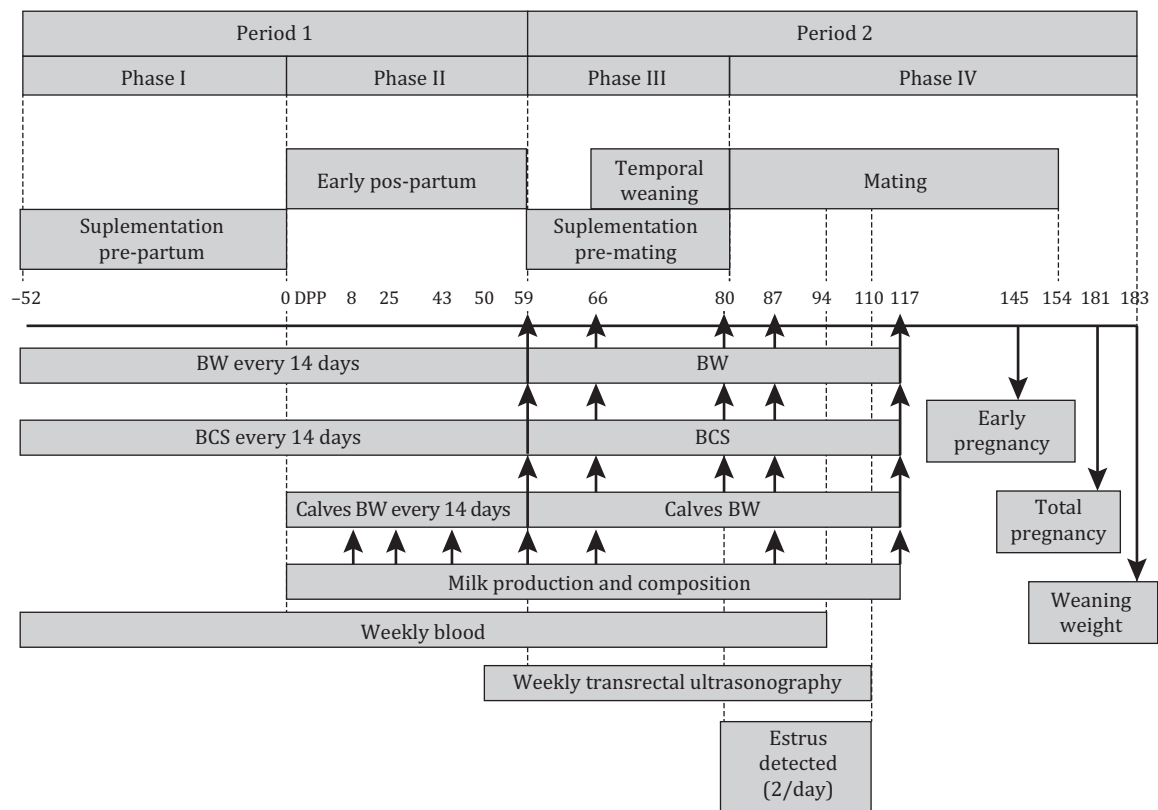
Material and Methods

The experiment was conducted at Cerro Largo, Uruguay (32° S, 54° W) according to the experimental procedures approved by the institutional Animal Experimental Committee (021130-001144-12).

The experiment had four phases (Figure 1): prepartum supplementation (phase I; 52 days), early postpartum (phase II; 43 days), pre-mating supplementation (phase III; 21 days), and the last phase that included mating, gestation, and lactation until weaning (phase IV; 103 days). For statistical purposes, phases I and II were considered as period 1, and phases III and IV were considered as period 2.

Fifty-seven Hereford (19), Aberdeen Angus (7), and crossbred (31) pregnant heifers [230±2 days of gestation and 4.8±0.1 units of BCS (scale: 1-8, in which 1 = emaciated, 8 = obese); Vizcarra et al., 1986] were stratified by BCS and calving date and randomly assigned to different treatments. Treatments were: unsupplemented (CC, n = 15), supplemented only in prepartum (SC, n = 14), supplemented only in pre-mating period (CS, n = 13), and supplemented in prepartum and pre-mating periods (SS, n = 15). To achieve this, in phase I (last 52±2 days of gestation), 29 cows were supplemented (S-), and 28 cows were unsupplemented (C-). Then, in phase III at 59±2 days postpartum (DPP), cows within each previous group (S- and C-) were randomly assigned to the pre-mating supplemented group (-S, n = 28) and pre-mating unsupplemented group (-C, n = 29) for 21 days before the onset of the breeding period.

Groups S were supplemented with 1 kg DM/cow per day of whole rice bran (139 g of CP/kg DM and estimated ME of 13.2 MJ/kg DM) and 550 mL/cow per day of crude glycerin (Alcoholes del



BW - body weight; BCS - body condition score; DPP - days postpartum.

Figure 1 - Overall management of the cows during the experiment.

Uruguay; ALUR, Uruguay; 6 g of CP/kg DM and estimated ME of 15.4 MJ/kg DM). Supplemented cows were individually penned and allowed to consume the supplement at the same time in the morning (~ 8.00 h).

During the whole experiment, all cows grazed together on the same sward, and fresh paddock was used in each experimental phase. During phase I, cows were offered an herbage allowance (HA) of 7.5 kg/100 kg of BW per day (2144 kg DM/ha; 70 g of CP/kg DM and ME of 6.1 MJ/kg DM) until calving. Thereafter, from phases II to IV, cows were offered an HA of 10 kg/100 kg of BW per day (2401 kg DM/ha; 110 g of CP/kg DM, and ME of 7.9 MJ/kg DM) until the end of the experiment. The selected HA (7.5 and 10 kg/100 kg of BW per day) are within the range used for this geographic region (Da Trindade et al., 2016).

Whole rice bran and crude glycerin were mixed daily before supplementation. The mixed supplement provided 21.4 MJ of ME and 142 g of CP. Chemical composition of whole rice bran and herbage (Table 1) were evaluated [ether extract (EE; AOAC, 1990), ash and CP (AOAC, 2007), neutral and acid detergent fibers (NDF and ADF; Van Soest et al., 1991)], and ME values were estimated using NRC (2016). Chemical composition of crude glycerin (Table 1) was evaluated [water (AOCS, 2009; Ea 8-58), ash (AOCS, 1973: Ea 2-38), glycerol (AOCS, 2012; Ea 6-51), fat (AOAC, 1980; 14.019)], and methanol concentration was analyzed.

Before pre-mating supplementation, all cows were checked for anestrus by ovarian ultrasound. Cows were determined to be in anestrus when there was non-corpus luteum in two ovarian ultrasound studies separated by nine days (Wiltbank et al., 2002).

Seven days after pre-mating supplementation began (66±2 DPP), all calves (n = 57) were fitted with a nose plate for 14 days. When supplementation finished, nose plates were removed from the calves, and two bulls were introduced for 74 days (80 to 154 DPP).

Table 1 - Chemical composition of native herbage (*Campos biome*) and supplements used during pre-partum and pre-mating phases of primiparous beef cows

Item (g/kg dry matter)	Native herbage		Supplement	
	Pre-partum	Pre-mating	Whole rice bran	Crude glycerin
Dry matter (g/kg as fed)	500	330	880	970
Ash	230	120	90	60
Crude protein	70	110	140	6
Neutral detergent fiber	620	560	190	-
Acid detergent fiber	310	270	60	-
Ether extract	20	20	170	130
Glycerol	-	-	-	770
Methanol	-	-	-	10

Pre-grazing herbage mass was determined by the double sampling method (Haydock and Shaw, 1975). Sward surface height was determined as described by Soca et al. (2007). The mean \pm standard deviation of the herbage mass and sward surface height for all phases (July to December) were 2272 ± 784 kg DM/ha and 11 ± 2 cm, respectively. In each phase, the HA was adjusted with paddock size and BW of the animals.

The BW and BCS of cows were recorded every 14 days from the beginning of the experiment (-52 ± 2 DPP) until the start of the pre-mating supplementation (59 ± 2 DPP). Thereafter, BW and BCS were recorded at the beginning of temporary weaning (66 ± 2 DPP), at the end of the pre-mating supplementation (80 ± 2 DPP), and then one week and one month after (87 ± 2 and 117 ± 2 DPP, respectively). Calves were weighed from birth to 117 ± 2 days of age at the same time as cows. Weaning weight was recorded at 183 ± 2 days of age (Figure 1).

Milk production was determined on seven occasions on two consecutive days, using a portable milk machine (DYNAMICS®, Uruguay), on 8, 25, 43, 58, 66, 87, and 117 ± 2 DPP (Figure 1). Milking was carried out according to the method described by Mondragon et al. (1983) and Quintans et al. (2010). Briefly, in the morning, cows were separated from their calves, and the udder emptied using 20 IU of oxytocin i/m (Neurofisin, Lab Fatro, Uruguay); 7 h later, cows were milked again using the same methodology and returned with their calves. The milk recovered for each cow after mechanical milking was weighed on an electronic scale (with an accuracy of ± 0.1 g), and daily production was estimated. After that, a sample of milk from each cow was obtained and sent to the laboratory for chemical analysis. Milk composition (protein, fat, and lactose) was determined using absorption of infrared radiation.

From 50 ± 2 DPP until the end of the first month of mating, ovaries were examined weekly by transrectal ultrasonography using a linear bimodal (5.0 to 7.5 MHz) transducer (Ambivision, Digital Notebook B mode, Model AV-3018V, Manufacturer AMBISEA Technology Corp., Ltd., China). Ovarian follicles and corpus luteum were identified according to the criteria described by Griffin and Ginther (1992). The resumption of ovarian activity was determined by the presence of corpus luteum in two successive ultrasound exams.

Estrus was detected twice daily (7.00 and 19.00 h) during the first month of the mating period (Figure 1); a cow was considered in estrus after accepting being mounted by the bull (Alexander et al., 1986). Pregnancy was diagnosed by transrectal ultrasonography at 145 ± 2 and 181 ± 2 DPP to determine the early and total pregnancy rates, respectively.

Weekly blood samples were taken from the coccygeal vein from the beginning of prepartum supplementation (-52 DPP) until the first two weeks of the mating period (94 DPP; Figure 1), using heparinized tubes for determinations of hormone and metabolite concentrations. Samples were centrifuged within the first hour after collection at $1,530 g$ for 15 min, and the plasma collected and stored at -20 °C until processing.

Insulin concentration was determined by an immunoradiometric assay (IRMA; Diasource, Brussels, Belgium). All samples were analyzed in one assay, the standard curve, and controls in duplicate and the samples in single. The sensitivity of the assay was 0.5 uIU/mL with intra-assay coefficients of variation for low (22.5 uIU/mL) and high (87.4 uIU/mL) controls of 16.1 and 9.7%, respectively. Glucose, total protein, albumin, urea, cholesterol, NEFA, and BHB concentrations were determined spectrophotometrically using commercial kits (glucose oxidase/peroxidase, biuret, bromocresol green; urease/salicylate; cholesterol oxidase/peroxidase, BioSystems SA, Barcelona, Spain; Wako NEFA-HR (2), Wako Pure Chemical Industries Ltd., Osaka, Japan; Ranbut, Randox, Northern Ireland, United Kingdom, respectively), with a sample volume and reagents adjusted to 96 cells and read in a Multiskan EX (Thermo Scientific, Waltham, Massachusetts, USA). Intra and inter-assay coefficients of variation for high and low controls were always less than 15%.

Data from BW, BCS, milk production and composition, metabolites, and hormones were analyzed using a repeated measures analysis over time using mixed models (PROC MIXED of SAS - Statistical Analysis System, version 9.4), with pre- or postpartum days as the repetition factor, and initial values as covariates. Individual cow was considered the experimental unit. Cow and calf BW, cow BCS, milk production and composition, metabolites, and hormone concentration were analyzed in two periods as described before. In period 1 (-52 DPP until the first 43 DPP), only prepartum supplementation was considered in the model; in period 2 (59 DPP until end of experiment), both prepartum and pre-mating supplementation and the interaction between them were analyzed. The first model (period 1) included the effects of treatments prepartum, pre or postpartum days, and interaction between the two factors as fixed effects and cow as a random effect. The second model (period 2) included the effects of supplementation (supplement or not supplemented), a period of supplementation (prepartum or pre-mating), date, and their interactions as fixed effects and cow as a random effect. The statistical model used for period 1 was:

$$Y_{ijk} = \mu + c1 + T_i + D_j + TD_{ij} + \epsilon_{ijk},$$

in which μ = overall mean, $c1$ = covariate with the initial value of the variable, T_i = effect of supplementation, D_j = effect of date, TD_{ij} = effect of interaction between supplementation and date, and ϵ_{ijk} = residual error.

The statistical model used for period 2 was:

$$Y_{imjk} = \mu + c1 + T_i + P_m + TP_{im} + D_j + TD_{ij} + PD_{mj} + TPD_{imj} + \epsilon_{imjk},$$

in which μ = overall mean; $c1$ = covariate with the initial value of the variable; T_i = effect of supplementation; P_m = effect of period; TP_{im} = effect of interaction between supplementation and period; D_j = effect of date; TD_{ij} = effect of interaction between supplementation and date; PD_{mj} = effect of interaction between period and date; TPD_{imj} = effect of interaction among supplementation, period, and date; and ϵ_{imjk} = residual error.

Reproductive variables were analyzed with the second model using a generalized linear model using PROC GENMOD of SAS, with the function natural logarithm link or logit link and indicating gamma distribution (interval partum-conception) or binomial (% cycling, % early, and final pregnancy) in the model, respectively. The statistical model used was:

$$Y_{imk} = \mu + T_i + P_m + TP_{im} + \epsilon_{imk},$$

in which μ = overall mean, T_i = effect of supplementation, P_m = effect of period, TP_{im} = effect of interaction between supplementation and period, and ϵ_{imk} = residual error.

Results were presented as least square means \pm pooled standard error, and differences were considered statistically significant at $P \leq 0.05$.

Results

Supplementation affected ($P < 0.01$) BW and BCS during prepartum and early postpartum (43 DPP), but there was no interaction ($P \geq 0.55$) between supplementation and DPP. Supplemented cows were heavier ($P < 0.01$) and had greater BCS than unsupplemented cows (S-: 396 ± 2 kg, 4.5 ± 0.1 units vs C-: 388 ± 2 kg, 4.3 ± 0.1 units, BW and BCS, respectively). All cows lost BCS ($P < 0.01$) from day -52 until one week after calving, and remained low after 43 DPP. When BCS at calving was analyzed alone, group S- had greater ($P = 0.03$) BCS than cows of group C- (S-: 4.5 ± 0.1 vs. C-: 4.1 ± 0.1 units). Prepartum supplementation did not influence ($P = 0.80$) calf birth weight (S-: 35.0 ± 0.7 vs C-: 34.7 ± 0.7 kg), and no calving difficulties were observed.

Milk production in the first 43 DPP was not affected ($P = 0.94$) by prepartum supplementation (7.6 ± 0.4 kg/day for both groups), and no interaction of supplementation by DPP was found ($P = 0.99$). The means of the total content of fat, protein, and lactose in milk were not different ($P > 0.10$) between groups. No interaction between supplementation and DPP ($P > 0.10$) was found for any of the variables studied. Regardless of the treatments, the averages of total milk production of fat, protein, and lactose for both groups were: 290 ± 19 , 229 ± 11 , 383 ± 18 g/day, respectively.

Prepartum supplementation decreased ($P < 0.01$) plasma concentration of NEFA (S-: 1.01 ± 0.05 vs C-: 1.33 ± 0.05 mmol/L) and BHB (S-: 0.54 ± 0.03 vs C-: 0.76 ± 0.03 , mmol/L), and interactions between supplementation and DPP were found for both variables ($P < 0.01$). During the prepartum period, group S- maintained the initial concentration of both metabolites. Meanwhile, cows in group C- increased plasma concentrations of BHB and NEFA. After parturition, these differences disappeared (Figures 2a and 2b). Alternatively, plasma concentration of cholesterol was greater ($P < 0.01$) in S- (158.0 ± 3.3 mg/dL) than in C- cows (132.0 ± 3.3 mg/dL), and there was an interaction between supplementation and DPP ($P < 0.01$). The concentration of cholesterol in S- cows increased and remained higher ($P < 0.05$) than in C- cows until calving, while in C- cows, it did not change ($P > 0.10$; Figure 2e).

Plasma concentration of total protein (S-: 73.6 ± 1.1 vs C-: 74.4 ± 1.1 g/L; $P = 0.62$) was not influenced by prepartum supplementation, and there was no interaction between supplementation and DPP ($P = 0.34$; Figure 2h). As a consequence of prepartum supplementation, albumin plasma concentration increased (S-: 33.0 ± 0.5 vs C-: 31.4 ± 0.5 g/L; $P = 0.01$) and urea concentration decreased (S-: 22.4 ± 0.6 vs C-: 27.3 ± 0.6 mg/dL; $P < 0.01$). There was no interaction between supplementation and DPP ($P = 0.30$) on albumin concentration (Figure 2g). However, an interaction ($P < 0.01$) between supplementation and DPP on urea concentration was observed. In fact, the pattern of urea concentrations was greater ($P < 0.05$) during the last three weeks before calving in C- cows than in S- cows (Figure 2f). After calving, all these differences disappeared.

Cows in S- group had greater ($P < 0.01$) plasma concentrations of glucose than cows in C- group (S-: 67.4 ± 0.9 vs. C-: 62.3 ± 0.9 mg/dL), and an interaction between supplementation and DPP ($P = 0.01$) was found (Figure 2c). However, no effect of supplementation ($P = 0.15$) or interaction between supplementation and DPP ($P = 0.38$; Figure 2d) influenced plasma concentration of insulin.

At the beginning of pre-mating supplementation, all cows were on average 59 ± 2 DPP, their BW and BCS were 383 ± 3 kg and 4.2 ± 0.1 , respectively, and they were all in anestrus.

During pre-mating and mating periods (59 to 117 ± 2 DPP), prepartum, pre-mating supplementation, or their interaction did not influence ($P > 0.09$) BW or BCS (Table 2). Pre-mating supplemented cows produced 8.5% more milk than unsupplemented cows (-S: 6.5 ± 0.3 vs -C: 6.0 ± 0.3 kg/day), but these differences were not significant ($P = 0.06$). No effects of prepartum or pre-mating supplementation or their interaction were found ($P > 0.06$) on milk total content of lactose, fat, and protein. Weight of calves from 59 to 117 ± 2 days of age was not influenced by any of the supplementation periods or their interaction ($P > 0.10$); therefore, BW of calves at weaning was not affected by treatments ($P > 0.10$; Table 2).

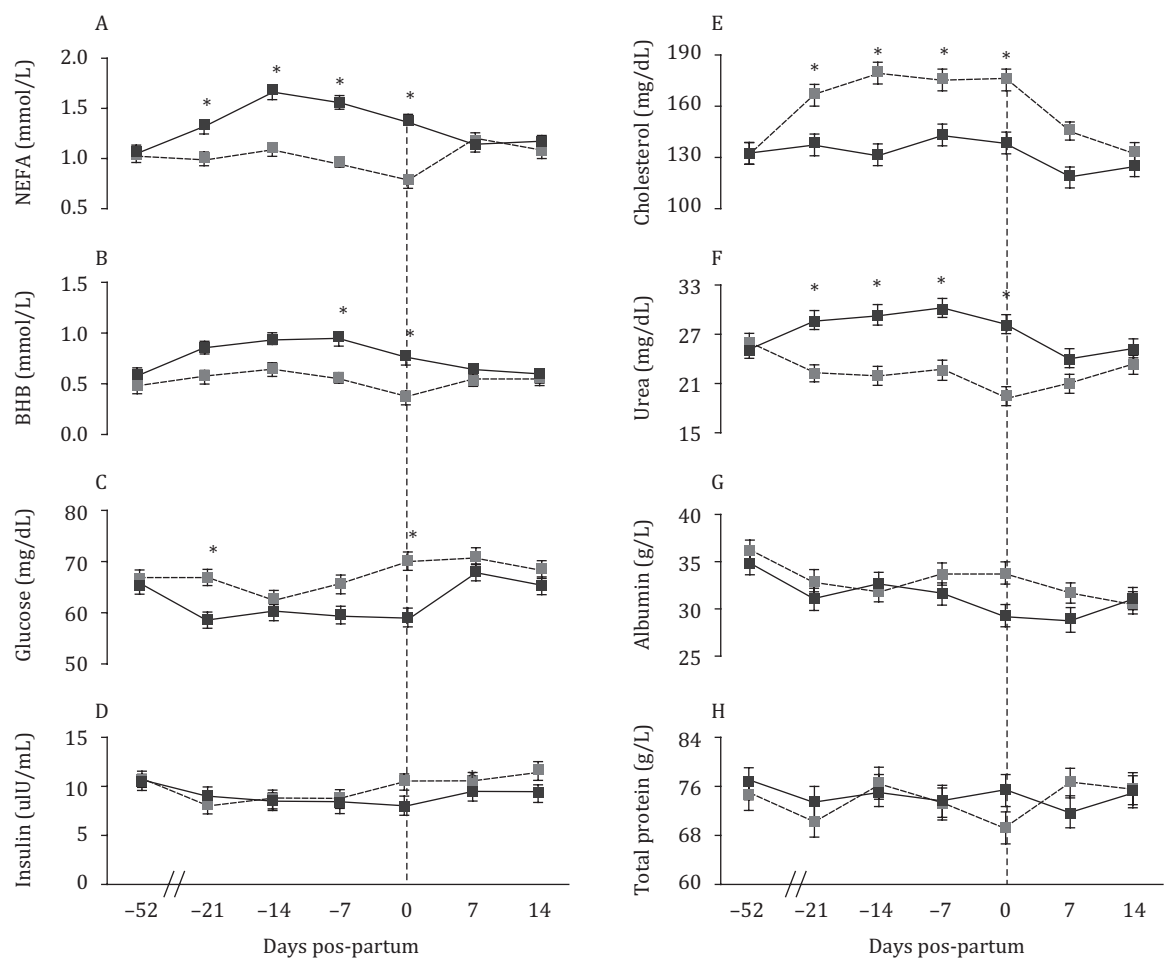
Albumin concentration during the second experimental period was greater ($P < 0.01$) for prepartum supplemented cows (31.5 ± 0.5 g/L) than for non-prepartum supplemented cows (29.6 ± 0.5 g/L).

Meanwhile, pre-mating supplementation increased ($P = 0.04$) plasma cholesterol concentration (-S: 162 ± 4 vs. -C: 149 ± 4 mg/dL; Table 2).

No interaction of prepartum supplementation and date was observed for any of the variables studied during pre-mating and mating periods. On the contrary, interaction of pre-mating supplementation and date affected plasma concentration of BHB ($P = 0.01$), NEFA ($P < 0.01$), and insulin ($P = 0.01$). Indeed, -S cows maintained ($P > 0.1$) plasma concentration of NEFA, increased ($P < 0.05$) plasma concentration of insulin, and decreased ($P < 0.05$) concentrations of BHB in relation to -C cows (Figure 3).

There was only a triple interaction amongst prepartum, pre-mating supplementation, and DPP ($P = 0.03$) on plasma concentration of cholesterol. Cows supplemented in both periods had the greatest ($P < 0.05$) plasma concentration of cholesterol at 80 ± 2 DPP (CC: 156.8 ± 9.6 ; CS: 156.5 ± 10.3 ; SC: 153.7 ± 10.0 and SS: 201.0 ± 10.4 mg/dL).

No interaction ($P > 0.10$) between prepartum and pre-mating supplementation on reproductive variables was found. Prepartum supplementation did not increase the percentage of cycling cows in the first month of the mating period ($P = 0.08$) and early pregnancy rate ($P = 0.09$), but increased ($P = 0.02$) total pregnancy rate (Table 2). Alternatively, pre-mating supplementation did not affect any of the reproductive variables studied ($P > 0.10$).



BHB - beta-hydroxybutyrate; NEFA - non-esterified fatty acids.
Day 0 = partum.
Differences between treatments are indicated by * when $P \leq 0.05$.

Figure 2 - Concentrations of insulin and metabolites in primiparous beef cows unsupplemented (■) and supplemented for 52 ± 2 days pre-partum with whole rice bran and crude glycerin (■).

Discussion

The hypothesis that a strategic supplementation with a mix of whole rice bran and crude glycerin supplement before calving and before mating would improve the energy balance and productive and reproductive performance of beef cows grazing native swards was accepted. Prepartum and pre-mating supplementation improved energy balance compared with that observed in unsupplemented cows. This was reflected by an increase in plasma concentrations of cholesterol, glucose, and insulin and a decrease in BHB, NEFA, and urea concentrations. This improved hormonal and metabolite milieu resulted in a better BCS at calving (prepartum supplementation) and a small increase in milk production (pre-mating supplementation). However, only prepartum supplementation improved reproductive performance by increasing total pregnancy rate.

It is well established that cows must be fed adequately before and after calving to achieve optimal reproductive and productive performance (Perry et al., 1991). This is even more important with heifers because they must cover their pregnancy demands over their own growth requirements (Bellows et al., 1982; Short et al., 1990; Lalman et al., 2000). In the present study, prepartum unsupplemented primiparous cows grazing native grassland could not meet their energy requirements;

Table 2 - Productive, metabolic, and reproductive performance of primiparous beef cows unsupplemented (C-) or supplemented with whole rice bran and crude glycerin (S-) for 52 days pre-partum and unsupplemented (-C) or supplemented with whole rice bran and crude glycerin (-S) for 21 days pre-mating

Item	Pre-partum		Pre-mating		SE	P-value		
	n	C- 28	S- 29	-C 29		-S 28	Pre-partum	Pre-mating
Productive								
Body weight (kg)		395	402	397	393	4	0.18	0.67
Body condition score (scale: 1-8)		4.2	4.3	4.3	4.2	0.1	0.09	0.17
Milk production (kg/day)		6.1	6.3	5.9	6.4	0.2	0.42	0.06
Fat (g/day)		196	222	205	213	9	0.06	0.54
Protein (g/day)		192	195	188	198	6	0.65	0.19
Lactose (g/day)		299	308	293	314	10	0.53	0.11
BW weaning calves ¹ (kg)		158	161	159	160	2	0.56	0.42
Metabolic								
NEFA (mmol/L)		0.39	0.39	0.38	0.40	0.02	0.77	0.38
BHB (mmol/L)		0.51	0.52	0.52	0.52	0.02	0.78	0.93
Glucose (mg/dL)		62.2	63.4	62.8	62.8	0.8	0.29	0.95
Insulin (uIU/mL)		9.7	10.2	9.8	10.1	0.4	0.42	0.57
Cholesterol (mg/dL)		151	160	149	162	4	0.16	0.04
Urea (mg/dL)		12.4	11.8	12.3	11.9	0.4	0.26	0.50
Albumin (g/L)		29.6	31.5	30.3	30.8	0.5	<0.01	0.43
Total protein (g/L)		77.3	77.3	77.4	77.2	1.1	0.99	0.90
Reproductive								
Cycling (%) ²		57	79	67	72	9	0.08	0.69
Early pregnancy (%) ³		37	61	52	46	10	0.09	0.67
Total pregnancy (%) ⁴		61	90	80	77	8	0.02	0.82
Calving-conception interval (d)		105	104	100	108	3	0.93	0.11

NEFA - non-esterified fatty acids, BHB - beta-hydroxybutyrate; DPP - days postpartum.

¹ Age of weaning calves: 183±2 days.

² Cycling was defined by the presence of corpus luteum in two or more ultrasound studies at seven-day intervals until the first month of mating period (110±2 DPP).

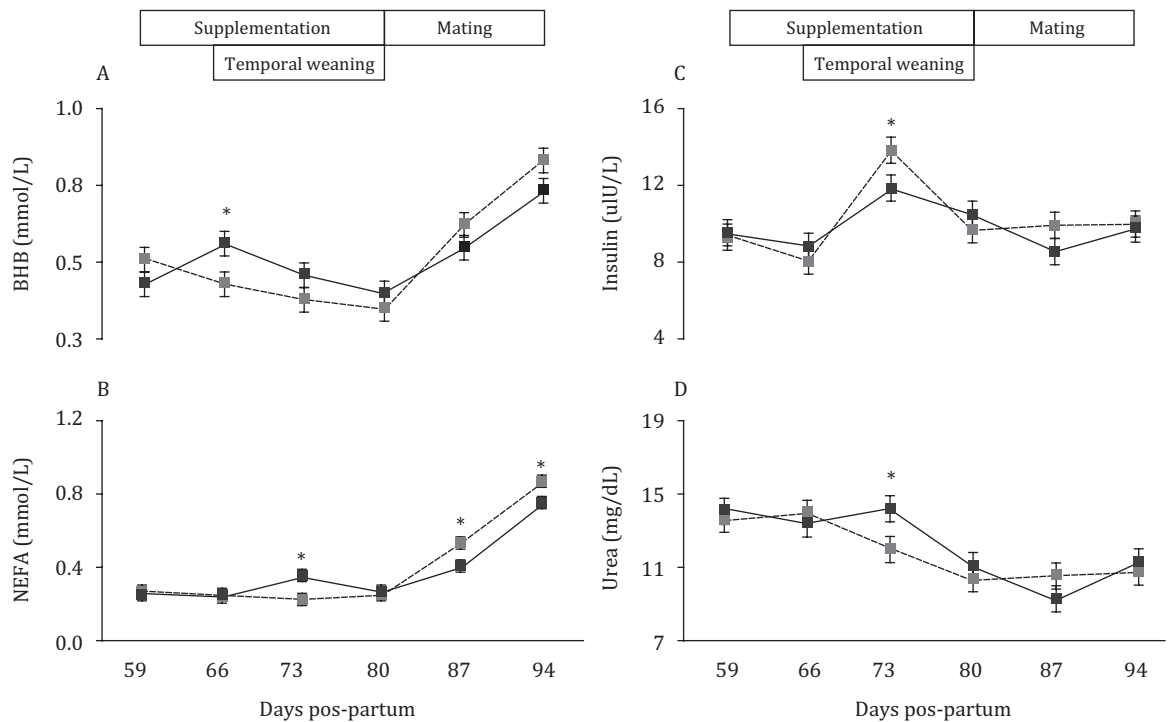
³ Early pregnancy was considered in the first 35 days of mating period (115±2 DPP).

⁴ Total pregnancy was considered which occurred during the 74 days of mating period (154±2 DPP).

therefore, they suffered a NEB. Cows had to mobilize body lipid and protein reserves to cover their energy demands, which was reflected in an increase of plasma concentration of NEFA, BHB, and urea (Guedon et al., 1999; Holcomb et al., 2001; Reynolds et al., 2003; Dann et al., 2005; Quintans et al., 2010; Soca et al., 2013).

For this motive, we reasoned that supplementation in late gestation would be a logical alternative to cover the cows' pregnancy energy requirements making the use of less necessary reserves, and then if any spare, it would be used to improve reproduction. In fact, the increase of energy and nutrient intake by the prepartum supplemented cows contributed to reduce the NEB. Supplementation avoided energy coming from catabolism, as was reflected by the better plasma concentration of glucose. Therefore, catabolic activity decreased, reflected by a reduction in NEFA, BHB, and urea plasma concentration, and the anabolic activity increased, indicated by an improved plasma concentration of albumin and cholesterol. The cows were less dependent from energy coming from body reserves, which reflected on a lighter decrease in BCS at calving and higher total pregnancy rate compared with unsupplemented cows.

Body condition score at calving, which is a consequence of prepartum nutrition (Wettemann et al., 2003), is the primary factor determining the length of postpartum anestrus and the probability of pregnancy (Hess et al., 2005; Montiel and Ahuja, 2005; Soca et al., 2013). Indeed, more prepartum supplemented cows were pregnant at the end of the breeding period. Hunter (1991) suggested that the prepartum nutrition plan affects the development of follicles that mature in the subsequent breeding season, and this could be because folliculogenesis takes 80 to 100 days (Britt, 1991). It is also possible that the prepartum nutrition plan affects oocyte quality (Krisner, 2004), size, and steroidogenic capacity of the corpus luteum, and uterine function through mechanisms that cause extended anestrus (Lucy, 2003), all of these mechanisms resulting in an improved reproductive outcome.



BHB - beta-hydroxybutyrate; NEFA - non-esterified fatty acids.

Day 0 = partum.

Differences between treatments are indicated by * when $P \leq 0.05$.

Only hormones and metabolites with interaction treatment by days postpartum are presented.

Figure 3 - Concentrations of BHB, NEFA, insulin, and urea in primiparous beef cows unsupplemented (■) and supplemented for 21 days pre-mating with whole rice bran and crude glycerin (■).

Our results are in agreement with those of other authors who reported benefits on reproduction when primiparous (Wiley et al., 1991; Soto et al., 2001) or multiparous (Quintans et al., 2016) cows were well fed or supplemented before calving. However, Stalker et al. (2006) did not find a positive impact of prepartum supplementation on pregnancy rates. These authors explained the lack of response of supplementary nutrition prepartum on reproductive outcome due to BCS at calving. They used mature cows in very good condition score (~5.0 units of BCS), whereas in our experiment, we used primiparous cows with at least 10% less BCS (~0.5 units less). In the same line, Scarsi (2012) worked with primiparous cows and also reported no differences in reproductive performance when cows were supplemented before calving. However, in that case, the BCS at calving was not responsible for the lack of response, since their cows had about 10% less BCS than ours, 4.5 vs. 4.1 in the present study and in Scarsi (2012), respectively. These results suggest that the window of BCS where prepartum supplementation exerts an effect on reproduction is narrow and should be considered when doing prepartum supplementation.

Our results are also in agreement with those of Clariget et al. (2016b) and Astessiano et al. (2013), who reported no benefits on reproduction when cows were supplemented before or during mating. Unlike our study, those authors worked with cows with less than 50 DPP when cows would be allocating the consumed energy to milk production and not to reproductive functions (Short et al., 1990). However, our results do not agree with those obtained by Carrere et al. (2005), Soca et al. (2005, 2013), Do Carmo (2006), and Claramunt (2007), who observed higher percentages of early and total pregnancy rate on supplemented cows compared with control cows. In the previous studies, the cows had similar DPP to those in our work (61 vs. 59 days, respectively), but they had lower BCS at the onset of the treatment (3.5 vs. 4.2 units, respectively). Since their control cows had lower early and total pregnancy rates than ours (33 vs 52 and 63 vs 80%, respectively), it could be considered that our cows were already in good herbage condition (availability herbage more than 2000 kg DM/ha; HA: 10 kg/100 kg of BW per day; CP: 110 g/kg) to have an increment in pregnancy rate by adding any extra feed. Indeed, pre-mating supplementation only increased milk production by 8.5%, but there was no effect on calf weight gain or cow BCS or BW.

We expected that an additional supplementation during the pre-mating period will boost the energy supply to the cow and increase the reproductive outcome attained by the prepartum supplementation alone; however, the interaction between supplementation periods did not affect the variables studied. Although pre-mating supplementation improved energy balance, indicated by a decrease on plasma concentration of NEFA, BHB, and urea and by a rise in cholesterol (Ndlovu et al., 2007), the impact was smaller than prepartum supplementation. It is possible that in our experiment, differently from that of Soto et al. (2001), who reported an increased pregnancy rate only when cows were supplemented pre- and postpartum, our second supplementation was not accurate enough to do so. Within the possible interfering factors, a higher allowance of herbage than the prepartum (7.5 or 10 kg/100 kg of BW per day), physiological status of the cow (late pregnancy or lactating cow), and duration of supplementation periods (52 or 21 days) could explain, at least partially, the different effects between both supplementation regimens.

An additional advantage of short supplementation period of less than 75 days before calving is that it seems not to influence calf birth weight (Wiley et al., 1991; Bellows et al., 2001; Soto et al., 2001; Alexander et al., 2002; Scarsi, 2012; Quintans et al., 2016). Indeed, in the present experiment, calves' weight at calving was not influenced by supplementation, and no difficulties at calving were observed. On the other hand, long prepartum supplementation of 100 days or more before calving increases calf birth weight (Corah et al., 1975; Perry et al., 1991; Radunz et al., 2010) and could induce calving difficulties (Gunn et al., 2014). Long supplementation periods have more chance to result in calf dystocia than shorter ones, since good nutrition can increase the size and function of the placenta (Rasby et al., 1990), which has a high correlation with the size of the calves (Echternkamp, 1993).

Postpartum supplementation had greater impact on milk production than the prepartum supplementation as has been reported before by Wiley et al. (1991) and Lalman et al. (2000). However, the increase in milk production in our experiment was less than 10% and was not reflected in an

increased weaning weight of the calves. The reason might be that in our experiment, supplementation was not started until 59 days postpartum, and it has been reported that after the first 60 days of age, daily weight gain of calves does not depend only on milk production (Neville Jr. et al., 1962). Indeed, the correlation between calf weight gain and dam milk production decreased from 0.74 in the first 60 days of life to 0.63 in the next 60 days.

Conclusions

In extensive pastoral system production, pregnancy rate and weaning weights are the primary limitations of the cow-calf operation that might be mitigated with a strategic supplementation. However, the evaluation of cheap feeds such as crude glycerin and rice bran, which are not used for human nutrition, is desirable. Mixing them as a supplement and offering them to cows in small quantities (0.4 kg/100 kg of BW) and in a strategic moment (last two months of gestation) increases total pregnancy rate with no additional effect to supplement those cows during the pre-mating period.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.M. Clariget, G. Quintans and R. Pérez-Clariget. Formal analysis: J.M. Clariget and O. Bentancur. Funding acquisition: R. Pérez-Clariget. Investigation: J.M. Clariget. Methodology: J.M. Clariget and C.R. López-Mazz. Supervision: A. Álvarez-Oxiley, C.R. López-Mazz and R. Pérez-Clariget. Writing-original draft: J.M. Clariget and R. Pérez-Clariget. Writing-review & editing: G. Quintans and G. Bancho.

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